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A cross- sectional study of stress biomarkers and their associations with post-trauma complaints, and how those associations are moderated by early life adversity

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Abstract

Chronic musculoskeletal pain results in significant personal, economic, and social burden. Early identification and intervention in those people with acute pain that are likely to transition into a state of chronicity can prevent the onset of chronic pain before it emerges and becomes resistant to treatment. This study investigated the potential stress biomarkers associated with acute pain and disability and how those associations are influenced by early life adversities.

Stress level was determined according to the plasma level of stress biomarkers (cortisol, BDNF, TGFB1) and self-report measures of stress following musculoskeletal traumatic events. The magnitude and direction of associations of cortisol and BDNF with self-reported stress markers provided supportive evidence for further exploration of cortisol and BDNF as acute stress biomarkers. The results of the study also supported the moderating role of adverse childhood experiences on the associations between self-reported distress and stress biomarkers.

Keywords

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Chapter 1

1   Introduction

The main rationale of this thesis is based on the multidimensional (biological, psychological and social) conceptualizations of chronic pain. Understanding the effects of the bio-psycho-social variables and their associations in the development of chronic pain could help clinicians to identify the patients who are likely at risk of developing chronic pain related conditions after having a non-catastrophic musculoskeletal injury. Early recognition of the people who are at high risk could help clinicians to offer adequate and appropriate patient care. This could in turn reduce what can amount to enormous suffering and cost related to chronic pain conditions. To aid in the understating about the complex, multifactorial nature of chronic pain, this chapter provides an overview of the suggested mechanisms that are involved in the development of chronic pain. It starts by exploring the personal, social and financial impacts of chronic pain. The possible role of stress markers in the acute pain setting and how it can shape the development of chronic physical and psychological conditions are also discussed. The possible moderating effects of adverse childhood experiences on the associations between stress biomarkers and pain-related cognitions are also included in this chapter.

1.1 Prevalence and significance of chronic musculoskeletal pain

The personal and social burden of chronic pain is increasingly being recognized. Chronic non cancer pain is the most common sequela of non-catastrophic musculoskeletal injuries. The International Association for the study of pain defined chronic pain as persistent or periodic pain experienced almost every day for a period of about six month (Phillips, 2009). In many cases the onset of that pain can be traced back to a trauma such as a sports injury, a workplace injury, aging, a car crash, surgery or even a chronic condition, such as arthritis or diabetes. About 1 in 5 individuals in Canada live with some forms of chronic pain (Moulin, Clark, Speechley, & Morley-Forster, 2002) and pain accounts for 80% of all physician visits (Gatchel, Robert J.; Peng, Yuan Bo; Peters, Madelon L.; Fuchs, Perry N.; Turk, 2007). Current best estimates following acute traumatic neck pain indicate that about 50% will continue to report persistent problems even after 1 year (Carroll et al., 2008). The economic impact of chronic pain is larger than other health conditions as disability tends to peak in middle age, adversely affecting adults during their peak productivity years (Gaskin & Richard, 2012; Phillips, 2009). It is estimated that the cost of chronic pain in North America ($560 to $635 billion) is greater than the costs of heart disease ($309 billion), neoplasm ($243 billion) and diabetes ($127 billion) (Gaskin & Richard, 2012 ; Gatchel, McGeary, McGeary, & Lippe, 2014).

"Chronic Pain" is considered as one of the major public health problems of the industrialized world (Gatchel et al., 2014). Approximately 60% of people in Canada who

are diagnosed with chronic pain eventually lose their job (Lynch, 2011). In Sweden the loss of production due to absence from work as a result of chronic pain accounts for 91% of the total socioeconomic costs (Phillips, 2009). A study by Dennis (2006) reported about 1 million cumulative days of sick leave annually in Denmark as a result of chronic pain (Eriksen, Sjøgren, Bruera, Ekholm, & Rasmussen, 2006; Phillips, 2009). In Canada, the mean sick leave due to chronic pain is 28.5 days per year (Phillips, 2009; Lynch, 2011). A Canadian study (STOP-PAIN) estimated direct (drug treatment) and indirect costs (lost labor time) relating to chronic pain and it was $1,462 per patient per month in Canada (Guerriere et al., 2010).

Much of this burden is due to the high level of activity limitations among people with chronic musculoskeletal pain. Chronic pain is a "silent epidemic" as there is too little awareness of the real prevalence of chronic pain and it is impossible to count the costs of reduced quality of life, job loss, ineffective and inadequate management of pain and increased rates of mental disorders (Sessle, 2012). However the problem is not confined to the economic burden. It can also lead to enormous suffering and reduced quality of life. Chronic pain and musculoskeletal disorders have been associated with poorest quality of life indices (Sprangers et al., 2000).Several studies have found that health related quality of life (HRQL) in patients with chronic non cancer pain is within the lowest range when compared to their age and sex matched healthy controls. In a study of over 150 patients attending a chronic pain clinic in Denmark, there were markedly reduced Medical Outcome Study- short form (SF- 36), Psychological General well-being Scale (PGWB), Hospital Anxiety and Depression Scale (HADS) scores in patients with chronic pain

compared to normal population which are indicative of major physical, social and psychological impairments (Becker et al., 1997).People with chronic musculoskeletal pain report worse health related quality of life in comparison to patients with end-stage cancer (Fredheim et al., 2008). Researchers have shown that chronic pain can lead to depression and other mood disorders, sleep disturbances, chronic fatigue syndrome and overall decrease in physical and mental functioning (Ashburn & Staats, 2017;Phillips, 2009). A survey conducted on 85,088 people from Europe, USA, Africa, Asia and the Middle East found that people with a history of chronic neck or low back pain had 2.3 times higher odds for mood disorder, 2.2 times more for anxiety disorders and 1.6 times more odds for developing substance abuse disorder in comparison with people who did not endorse any chronic pain symptoms (Demyttenaere et al., 2007). It is alarming that the rate of committing suicide is much higher among chronic pain patients compared to the general population. Suicide rates remain significantly higher even when mental disorders are controlled (Lynch, 2011).

The problem of chronic pain is compounded by consistent findings that there are no obvious findings on routine diagnostic imaging that can explain the pain symptoms. This has led to experiences of stigma, scrutiny and alienation amongst many dealing with chronic pain (Rhodes, McPhillips-Tangum, Markham, & Klenk, 1999).

Despite the high prevalence, there is by comparison relatively little guidance to support treatment decisions available to clinicians. The mechanisms to explain the progression to chronic pain after an acute musculoskeletal traumatic event also remain elusive. It is

necessary to develop more integrated and explanatory pathways to predict and prevent the development of chronic pain.

1.2 Available treatment resources of chronic pain

Proper diagnosis and management of chronic pain still remains ambiguous. Several treatment options have been evaluated for the management of chronic pain including pharmacotherapy (Rosenblum, Marsch, Herman, & Russell, 2008), psychological therapy (Eccleston, Morley, & Williams, 2013) and physical therapy (Alami et al., 2011). However no one approach alone proves to serve the best in terms of chronic pain management as the development of chronic pain from acute trauma is not straightforward or well defined. Available evidence on non-pharmacological approaches to chronic pain generally indicate a small effect (Teasell et al., 2010a). Even many of the pharmacological agents provide temporary relief of pain and small effects and there are controversies regarding the effectiveness, safety and abuse liability of long term use of those agents (Rosenblum et al., 2008;Chang & Compton, 2013). Despite some progress in creating opioids with lower abuse potential, scientific and anecdotal evidence of fatal consequences from chronic opioid use remain (Centre for Addiction and Mental Health Prescription opioid Policy Framework -Canada, 2016).

Several reasons have been cited for the long term sufferings of people with chronic pain such as poorly equipped pain clinics with long wait lists, under diagnosis of the problem and lack of knowledge regarding the mechanisms to explain the development of chronic pain (Lynch, 2011).

The introduction of the biopsychosocial model in the management of chronic pain offered several breakthrough clinical approaches (Gatchel, 2013). According to this model the development of chronic pain is the result of complex interactions between biological, psychological and environmental factors (Gatchel et al., 2013). This multidimensional, well accepted model has suggested that managing chronic pain through biological or psychological means in isolation was not sufficient to prevent the progression from acute to chronic pain or to improve outcomes in chronic pain. Rather clinicians should focus on the tailored approach based on individual's specific needs (Gatchel et al., 2014; Sessle, 2012). Although there have been some improvements in our understanding of the mechanism of chronic pain, considerable gap in knowledge regarding clarifications of the mechanisms, etiology, and pathogenesis of most chronic pain conditions still exist. While the relationships between biological markers and acute self-reported psychological distress was explored to some extent by prior research studies, the environmental and social counterpart of this model received relatively less attention (Sessle,2012). One of the motivations of this thesis is to achieve a deeper understanding of the rich interactions between biological, psychological and social factors that will help to improve the current treatment for acute, and by extension, chronic pain.

1.3 Prognostic factors in the development of chronic pain following acute trauma

Recent efforts in the field of chronic pain research have endorsed an approach of early identification and intervention in those people with acute pain that are likely to transition

into a state of chronicity, attempting to prevent the onset of chronic pain. Numerous psychosomatic investigations have dealt with the etiology and dynamics of chronic pain syndromes. A growing body of literature has agreed that the development of chronic pain involves complex and dynamic interactions of biological, psychological and environmental factors (van Hecke, Torrance, & Smith, 2013; McLean, Clauw, Abelson, & Liberzon, 2005). However the exact mechanism that can explain the development of chronic pain after an acute episode is not yet fully understood. A number of models or frameworks have been developed to attempt to explain the reasons why a subset of people experience persistent pain following an acute traumatic event while the majority of patients recover. Different pain models explored several risk / prognostic factors that include biological, cognitive and environmental elements (Walton & Elliott, 2017; Leeuw et al., 2007; McLean et al., 2005). Currently the best available evidence indicates that the most consistent predictors of chronic pain are largely cognitive in nature, including high ratings of pain intensity or disability, fear, catastrophizing, and low expectations of recovery (Walton et al., 2013a). However, recent large pragmatic clinical trials intended to specifically target negative cognitions have found no added benefit over a single session of advice and education or standard treatment (Lamb et al., 2012; Jull, Kenardy, Hendrikz, Cohen, & Sterling, 2013). While it would seem that a propensity to rate the experience as more terrible or distressing in the acute stage of injury is temporally associated with longer term outcome, the results of these intervention trials would suggest the mechanisms to explain these associations are not yet understood. It is worth exploring the knowledge gaps regarding acute stress markers and to integrate biological, psychological and social markers to identify major intervention targets.

The following section will provide a detailed overview of the acute stress markers and their potential roles in the progression from acute pain to chronic pain conditions.

1.4   Stress markers

This section provides an overview of the different biological, psychological and social stress markers that have been proposed by previous researchers to explain the possible mechanism of chronic pain development. The proposed role of those biomarkers in various stress related maladies are also discussed. At first, a brief description of "stress" in general is provided.

1.4.1   Stress

The term "Stress" was introduced by Selye (1936) as the body's nonspecific adaptive response to any demand. He compared stress reaction with alarm process that warns us about imbalance in homeostasis. According to McEwen (2003) a stressor is a real or implied threat to homeostasis. Stressors can be pleasant or unpleasant; real or perceived; physiological or psychological (H Selye, 1936; Selye, 1973; Russell et al., 2012). Selye (1973) gave an example to clarify the idea of stress. When a mother realizes that her son has some devastating disease, she experiences emotional stress that may require a shift in what she considers 'normal' to cope with the perceived threat. If she finds later that the diagnostic report was wrong and her son is completely fine that incident of extreme joy is also considered as a stress in that it leads to another shift in homeostatic status to another state of 'normal'. In these two situations the stressors are completely different yet their response could be exactly same. In order to re-establish the normal environment, our body

produces several reactions. However, if the exposure to the stressors continues for a prolonged period of time the stress response becomes maladaptive which has been associated with various pathophysiology including chronic musculoskeletal pain (Louw, Diener, Butler, & Puentedura, 2011; Poleshuck et al., 2009), Post traumatic stress disorder (McFarlane, Atchison, & Yehuda, 1997), Fibromyalgia (Gupta & Silman, 2004), Diabetes (Byung-Wan et al., 2010), Depression (Yehuda, Halligan, Golier, Grossman, & Bierer, 2004), Cardiovascular disease (Schwartz et al., 2015; Dimsdale, 2008) and poor pregnancy outcome (Latendresse, 2009). The maladaptive stress pathway and its role in the experience of acute pain will be explored in this research.

1.4.2   Biological stress markers

When the human body encounters a real or perceived threat to homeostasis it activates a complex range of responses including endocrine, metabolic, nervous and immune systems. Activation of the stress response ensures survival in the presence of adverse stimuli. One common pathway is activation of the autonomic nervous system (ANS). It provides a rapid response through both sympathetic and parasympathetic systems. Sympathetic system response is referred to as the classic "fight, flight or freeze" response (Canon, 1929). Neurotransmitters that are released by the neurons of ANS are epinephrine, norepinephrine and acetylcholine (McCorry, 2007). Sympathetic adrenomedullary circuit, noradrenergic neurons and parasympathetic system also have role in body's adaptation process (S. M. Smith & Vale, 2006). These responses include some physical and psychological phenomena such as increased blood flow to the muscle, increased cardiovascular tone, increased blood pressure, increased blood sugar and fat deposition to order to supply the

body with extra energy, the blood clotting system speeds up to prevent extra blood loss, increased muscle tension to provide extra speed and strength, increased respiratory rate, increased awareness, improved cognition and euphoria, decreased feeding and appetite (Carrasco & Van De Kar, 2003; Smith & Vale, 2006; McCrory, De Brito, & Viding, 2010;Canon, 1929).

One of the objectives of this thesis was to explore the role of biological stress markers in acute post-traumatic pain. Three stress biomarkers were specifically explored in my study, namely cortisol, brain-derived neurotrophic factor (BDNF), and transforming growth factor beta1 (TGFB1) owing to their recognized involvement in stress pathways. The detailed descriptions of these three biomarkers are discussed in the following sections.

1.4.2.1   Cortisol

***General description and mechanism of action*:**

Cortisol is a steroid hormone that is essential for maintaining homeostasis of the human body. Removal of the adrenal gland from body can be fatal if glucocorticoid is not administered externally (McEwen, 2006; McEwen & Wingfield, 2003).

The main three glands that play a vital role in the initiation of the stress response are the paraventricular nucleus of the hypothalamus, the anterior lobe of the pituitary gland and the adrenal gland. Collectively they are known as Hypothalamic- Pituitary -Adrenal (HPA) axis. The HPA axis is the major stress system pathway in humans (Carrasco &Van De Kar, 2003; Hans Selye, 1973; Smith & Vale, 2006; Tsigos & Chrousos, 2002). Upon

encountering a hostile environment or threat, the HPA neuroendocrine cascade initiates the release of corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) in the hypothalamus. Hypophysiotropic neurons of the paraventricular nucleus of the hypothalamus synthesize and secrete CRH. CRH binds with the corticotrophin-releasing hormone receptor-1 (CRHR1) and stimulates the anterior pituitary gland to release adrenocorticotrophic hormone (ACTH) into the systemic circulation. ACTH binds with the melanocortin type 2receptor (MC2-R) in the adrenal cortex and acts on the Zona fasciculata. Zona fasciculata is the second of the three layers of the adrenal cortex, in order they are Zona glomerulosa, Zona fasciculata and Zona reticularis. This binding action to the mid layer of the adrenal gland triggers the synthesis and secretion of glucocorticoid (cortisol in primates and corticosterone in rodents). Glucocorticoids play a prominent role to regulate the magnitude and duration of HPA axis activation. Glucocorticoid acts on the hypothalamus and pituitary to suppress CRH and ACTH production in a negative feedback cycle, thus downregulating these hormones once the stressor has been eliminated (Tsigos & Chrousos, 2002; Smith & Vale, 2006;Tasker & Herman, 2011; Rivier & Vale, 1983; McEwen & Wingfield, 2003). This negative feedback loop is essential to ensure the return of the HPA axis to homeostasis when the organism is no longer challenged (Canon, 1929).

Cortisol is a potent glucocorticoid hormone and a key biomarker of HPA axis which received considerable attention in biomedical and clinical research. The HPA axis not only promotes adaptation to stressors, but also leads to pathophysiology when it is dysregulated. One of its many functions is to stimulate gluconeogenesis (production of glucose) in the early hours of fasting. Cortisol also aids in the metabolism of fat, protein and carbohydrate.

Cortisol prevents sodium loss from the cells and accelerates the rate of potassium excretion, helping to regulate the body's PH balance. Cortisol also prevents the release of pro-inflammatory cytokines from macrophages, helper T-cells and related immune cells that promote inflammation. While exposure to a stressor results in release of certain inflammatory cytokines namely Tumor Necrosis Factor (TNF), IL-1b, and IL-6, HPA axis activity and cortisol suppress the further release of these pro-cytokines (Tian, Hou, Li, & Yuan, 2014). However, several studies reported higher level of pro-inflammatory cytokines in chronic stressful conditions (Wolkow, Aisbett, Reynolds, Ferguson, & Main, 2015; Tian et al., 2014). It has been proposed that chronic stressful condition results in glucocorticoid receptor resistance (GCR). GCR is responsible for up regulation of inflammatory cytokines which can lead to serious health consequences (Cohen et al., 2012).

The influence of stressful conditions on cortisol production and its proposed role in the development of several pathological conditions including chronic pain:

The features of dysregulated HPA axis function includes fatigue, malaise, abnormal metabolism, impaired sleep and the presence of widespread pain which are also the main characteristics of several chronic pain disorders (Chrousos, 2004). This was one of the main reasons for which cortisol received much attention in chronic pain research.

Animal studies have proposed the role of HPA axis dysfunction in the development of chronic pain and inflammation. Persistent stress has been cited as the main reasons for the HPA axis dysregulation in most animal studies (Tanriverdi, Karaca, Unluhizarci, &

Kelestimur, 2007). For example, rats that were exposed to stressful conditions during pregnancy via application of a stressor such as restraint or dexamethasone injection gave birth to offspring with increased basal plasma corticosterone level (Blackburn-Munro, 2004). Maternal separation for 6 hours daily throughout the first 3 weeks of life resulted in a hypercortisolism state in animal studies (Plotsky & Meaney, 1993; Blackburn-Munro, 2004). In human studies, the possible dysregulation of HPA axis after stressful events and the development of pathological conditions continue to be examined. Although there are sufficient evidence to support that cortisol is implicated in the pathophysiology of different pain related disorders, research has yet to identify the exact mechanism involved in the dysregulation of HPA axis (Chrousos & Kino, 2007).

Fibromyalgia is a common pain disorder which is characterized by chronic widespread pain, fatigue, anxiety and poor sleep (Tanriverdi et al., 2007). The involvement of HPA axis in the development of fibromyalgia has been increasingly documented by previous research. However, there are inconsistencies regarding the exact role of HPA axis or cortisol in this condition. There is evidence that in Fibromyalgia the concentration of circulating cortisol is increased which reflects the HPA axis dysfunction (Bote, Garca, Hinchado, & Ortega, 2012). However, Tanriverdi and colleagues found the opposite, a reduced level of plasma cortisol in a cohort of people with fibromyalgia (Tanriverdi et al., 2007) . The alteration of HPA axis function was also documented in conditions like chronic pelvic pain. The associations between CPP and HPA axis dysfunction was found to be mediated by chronic depression (Wingenfeld et al., 2009) suggesting a possibly complex interaction between pain, mood, and stress activity that may explain the apparently

opposing findings from the fibromyalgia literature. Increased plasma cortisol was also observed in conditions like Chronic migraine and temporomandibular joint disorder (Peres et al., 2001; Korszun et al., 2002). In contrast, lower cortisol concentration was observed in chronic musculoskeletal pain, whiplash syndrome, chronic fatigue syndrome and post-traumatic stress disorder (Yehuda & Seckl, 2011; Gaab et al., 2005; Heim, Ehlert, Hanker, & Hellhammer, 1998; Generaal et al., 2014). Park and Ahn found lower cortisol awakening response (CAR) in patients with Complex regional pain syndrome who reported frequent attacks of pain compared to patients who reported lower frequency of spontaneous pain attacks (Park & Ahn, 2012).

From the above-mentioned discussion, it can be interpreted that the cortisol concentration and HPA axis activity are highly variable in previous studies. The variability in the results regarding cortisol and chronic pain may be due to the demographic differences of the participants, the methods used for the study, the stage of the specific disease, the inclusion of potential effect modifiers or interaction variables, and the interpretation of the results (Tanriverdi et al., 2007).

Several researchers have anticipated the role of several demographic variables, early life stress, recent stress and social disadvantages in the alterations of HPA axis function in at least a subset of population (Essex et al., 2011; Dowd, Simanek, & Aiello, 2009). All these factors should be evaluated while interpreting the role of cortisol in the development of chronic pain. There is increasing evidence that childhood adversities (e.g. experience of abuse, parental separation, household breakdown, loss of security) lead to persistent changes in the HPA axis regulation (McGowan, 2013). However, the findings are

inconsistent throughout the studies. Some studies suggested higher level of salivary and hair cortisol concentration in people who reported childhood adversities (Schreier, Enlow, Ritz, Gennings, & Wright, 2015). Rats who encountered parental separation showed increased corticosterone production. There is also evidence of increased salivary cortisol response in people who lost their parents in early age (Luecken, LJ &Appelhans, 2006). In contrast, some studies found decreased salivary cortisol concentration in people with history of adverse parenting and childhood maltreatment in compared to people who have not reported such adversities (Kawai et al., 2017).

The alterations of HPA axis activity by several sociodemographic factors was also investigated by recent research. Lower socioeconomic status, lower income, and education level all have been cited as risk factors for the development of chronic pain conditions (Oliver van Hecke, Torrance, & Smith, 2013; Udom, Janwantanakul, & Kanlayanaphotporn, 2016). It has been proposed that the influence of these variables to the development of chronic pain may be mediated by the dysregulation of HPA axis activity (Ulirsch et al., 2015).

Although cortisol measures have contributed much to the literature; the role of the HPA axis and cortisol following acute stressful event is still not well understood. To the best of our knowledge, this is the first study which will explore the acute reactivity of plasma cortisol level immediately after non-catastrophic musculoskeletal trauma. The modifying effects of the demographic variables and childhood adverse experiences on the acute stress response will also be explored to provide an insight about the mechanism of chronic pain development following acute traumatic event.

1.4.2.2   **The brain-derived neurotrophic factor (BDNF)**

*General description and mechanism of action***:**

One of the most abundant neurotrophins of the mammalian central nervous system is BDNF (Li et al., 2005). BDNF belongs to the family of Nerve growth Factor (NGF) which promotes the survival and maintenance of brain function (Binder & Scharfman, 2004). BDNF is considered an essential protein that acts on the neurons of the central and peripheral nervous systems and helps in survival and growth of the existing neurons. It also ensures growth and differentiation of the new neurons and synapses (Generaal et al., 2016; Daskalakis, De Kloet, Yehuda, Malaspina, & Kranz, 2015). The role of BDNF is well recognized in central nervous system development, maintenance and adult neuroplasticity which is essential for wellbeing(Carbone & Handa, 2013; M. A. Smith, Makino, Kvetnansky, & Post, 1995). BDNF protein is active at the connections between nerve cells (synapses), where cell-to-cell connection occurs. The synapses can change themselves over time in response to experience, which is known as synaptic plasticity. The BDNF protein helps regulate synaptic plasticity. Synaptic plasticity is important to maintain learning and memory. The brain-derived neurotrophic factor (BDNF) is one of the most studied neurotrophins of the central nervous system in the development and maintenance of brain function. BDNF plays an important role in neurogenesis, the process by which new neurons are developed in the brain. Previous researchers showed that mice who were BDNF gene deficient suffered developmental anomalies and also died soon after birth (Kucera, Lee, Loring, & Jaenisch, 1995).

BDNF is synthesized in the endoplasmic reticulum as a small precursor protein, pro BDNF. Pro BDNF undergoes two cleavage steps to form mature BDNF, which remain stored in secretary vesicles. Upon neuronal stimulation, BDNF is released from the synapse. It binds with at least 2 receptors to exert its effects. One is a low affinity nerve growth factor receptor (commonly known as p75) and the other one is a high-affinity protein kinase receptor known as tropomyosin related kinase B (TrKB). The biological role of p75 is not clear yet. Most of the effects to maintain neuronal integrity and survival mediated by BDNF are thought to occur following binding to TrKB receptor (Carbone & Handa, 2013;Daskalakis et al., 2015; Kucera et al., 1995;Barrett, 2000).

The influence of stressful conditions on BDNF expression and its proposed role in the development of several pathological conditions including chronic pain:

Brain derived neurotrophic factor disturbances and stressors have been shown to induce different life long adverse health consequences both independently and in interactions. Considerable evidence (mostly animal studies) suggest that both the early life stress and recent stress has the potential to alter the BDNF expression and this can lead to various hazardous health consequences (Daskalakis, De Kloet, Yehuda, Malaspina, & Kranz, 2015; Elzinga et al., 2011).

Although limited in numbers, studies have reported an association between BDNF expression and the development of chronic pain related conditions in humans. However the results of these studies are inconsistent. The precise mechanism underlying this association has not been fully understood to date.

It has been observed that the rats with a history of frequent maternal separation during childhood displayed lower BDNF level (D. Liu, Diorio, Day, Francis, & Meaney, 2000). This hypothesis was supported by similar findings in human studies later. Several studies reported lower BDNF level associated with early and recent life stressors and lower BDNF level has been implicated in the development of several negative health states such as depression and bipolar disorder (Elzinga et al., 2011; Fernandes et al., 2015). It has been proposed that altered BDNF expression along with environmental adversities may result in the development of depressive disorders. As the pathophysiology of depression share many similarities with chronic pain it was hypothesized that there may be a role for BDNF in the development of chronic pain (Maletic, 2009). Moreover, BDNF appears to play an important role in central sensitization (Generaal et al., 2016). Central sensitization is a condition in which the nervous system goes through a wind up process and gets regulated in a continuous state of high reactivity (Latremoliere & Woolf, 2010). Central sensitization is thought to be associated with fibromyalgia, low back pain, whiplash headache and osteoarthritis. The influential role of BDNF in the central sensitization makes it a novel target to prevent chronic pain development (Nijs et al., 2015).

It is interesting to note that cortisol has emerged as an important mediator of BDNF expression. Recent research has shown that stress induced increase in glucocorticoid level leads to reduced BDNF in the hippocampus (Lakshminarasimhan & Chattarji, 2012). According to recent research, high BDNF and low cortisol is essential for the neuronal maintenance and synaptic integrity. This Glucorticoid - BDNF equilibrium should be maintained throughout the life in order to properly regulate stress (Daskalakis et al., 2015).

1.4.2.3 **Transforming growth factor beta 1 (TGFB1)**

*General description and mechanism of action***:**

TGFB1 is a polypeptide that belongs to the cytokine family. Three isoforms exist in mammals (TGF-beta 1, beta 2 and beta 3). Among them, TGFB1 has widely been regarded as an injury related cytokine. It performs many cellular functions, including cell growth, cell proliferation, cell differentiation and apoptosis. It also plays an important role in the immune system of our body (Gomes, Sousa, & Romão, 2005). In humans it is encoded by TGFB1 gene. Most of the immune cells or leukocytes secrete TGFB1 and most of the cells have receptors for TGFB1. Hence, it is likely that this growth factor must be properly regulated to maintain homeostasis and prevent disease (Khalil, 1999). TGFB1 is produced in cells such as platelets, macrophages, B-lymphocytes and T-lymphocytes, fibroblasts, osteoblasts and osteoclasts, astrocytes, and microglial cells. The mechanism of regulation of TGFB1 is complex. TGF beta is produced in a latent form that must be activated to produce its biologically active form (Khalil, 1999) .

*The variation in TGFB1 expression and its proposed role in the development of several pathological conditions including chronic pain***:**

Increase or decrease of TGFB1 level has been found to be associated with the development of several chronic conditions such as chronic obstructive pulmonary disease, Rheumatoid arthritis, Systemic lupus erythematosus, Osteoarthritis, chronic kidney disease and atherosclerosis (Celedón et al., 2004; Shen, Li, & Chen, 2014; Blobe,Schiemann,& Lodish, 2000). To date, no studies have evaluated TGFB1 in the development of chronic

musculoskeletal pain. Although very limited in number, few studies have suggested the potential role of TGFB1 in normal nociceptive pain processing as well as in the development of pathological pain conditions. TGFB1 has got some important neuroprotective effects. It has the capability to minimize the damage to the neurons following peripheral nerve injury (Echeverry et al., 2009; Lantero, Tramullas, Díaz, & Hurlé, 2012). It reduces pro-inflammatory cytokines induced nerve lesion. All these actions are helpful to prevent the development of neuropathic pain (Echeverry et al., 2009). Therefore, TGFB1 was hypothesized to have some potential role as an intervention target in pain management (Lantero et al., 2012).

Mice deficient of TGFB1 showed increased neuronal cell death whereas over expression of TGFB1 protects against neurodegeneration in acute and chronic injury paradigm (Brionne, Tesseur, Masliah, & Wyss-Coray, 2003). This result is in line with some human studies that found the up regulation of TGFB1 following cerebral ischemia to prevent further neurodegeneration (Dhandapani & Brann, 2003). It has been suggested to play an important role to reduce the damage induced by a wide range of insulting agents including hypoxia, ischemia, oxidative damage etc. (Dhandapani & Brann, 2003). Therefore TGFB1 can be an attractive candidate to explore in the acute traumatic condition to get an idea about the preventive strategies of TGFB1 against chronic pain development following acute trauma.

1.4.3   Psychological variants of stress markers

The ability of psychological factors to facilitate the development of chronic pain is well established by resent research. The acceptance of psychological factors and their correlations to the onset and outcomes of acute pain episodes is increasing. Variables such as attitudes, perceptions, mood state, social factors and work appear to interact with pain behavior and are collectively called psychosocial factors. It is necessary to explore these psychological variables and their associations with other stress markers to understand the cognitive, emotional, and behavioral manifestations of pain.

Existing evidence suggest that the most consistent psychological predictors of chronic pain development are pain catastrophizing and self-reported psychological distress (Walton et al., 2013a; McLean et al., 2005; Quartana et al., 2010). Most of the current research has agreed that there is a significant relationship between excessive fear of pain or pain catastrophizing following acute trauma and the development of chronic pain and disability. Pain catastrophizing is defined as the tendency to magnify or exaggerate the threat or seriousness of pain sensation (Chaves & Brown, 1987). People who catastrophize become so overwhelmed with pain related fear or worry that they cannot distract their attention from pain. The overall literature suggests that exaggerated psychological responses to acute pain are maladaptive and likely to intensify the pain experience and delay recovery. High level of catastrophizing after acute trauma should be considered a risk factor for chronic pain development (Quartana et al., 2009; Innes, 2005). These associations can be understood through the fear avoidance model of chronic pain. The fear avoidance model of chronic pain is one of the most influential models of chronic pain development. The fear

avoidance model described the role of excessive fear of pain or catastrophizing and avoidance behavior in the progression from acute to chronic pain related conditions. In the acute phase of trauma, avoidance of some activities may be beneficial. However, prolonged avoidance of activities due to catastrophic beliefs about the pain outcome and fear of re injury may lead to chronicity, disuse and disability (Vlaeyen & Linton, 2012).

Self-reported psychological distress following acute trauma was also cited as a prognostic factor for the development of chronic pain conditions (Walton et al., 2013a). It has been reported that as many as one third of people after having a traumatic event experience some forms of psychological distress (Innes, 2005). Despite a growing body of literature suggesting the role of psychological factors in the development of chronic pain, these factors are not completely understood and evaluated following acute traumatic events. The association of dysregulated HPA axis function in the development of psychological distress and pain catastrophizing was supported by recent research(Walton, Macdermid, Russell, Koren, & Uum, 2013). Inclusion of psychological and physiological stress markers is a unique aspect of my research study.

1.4.4 The influence of social stress markers (early life adversities) on the development of chronic pain conditions

Research studies have predominantly focused on the effects of adverse childhood experiences as it has emerged as an important indicator of adult health and wellbeing (De Bellis & Zisk, 2014; Vincent J Felitti & Anda, 2010). According to WHO World Mental Health Survey, physical abuse, sexual abuse and exposure to family violence was reported

by 5- 11%, 1-2% and 4-8% respondents, respectively, of people surveyed from 21 countries (Kessler et al., 2010). Patients with chronic widespread pain are more likely to report adverse childhood experiences than those who do not report such adversities (Davis&Luecken, 2005). In North America there is increasing evidence that abused women report more pelvic pain, multiple somatic symptoms and more lifetime surgeries compared to non-abused women (DA, Leserman, Nachman, & Al, 1990).

Previous researchers often mentioned the history of childhood adversities as a potential venue to look for to understand the development of chronic pain related conditions (Lampe et al., 2017; Linton, Lardén, & Gillow, 1996). Sexual abuse history has been found to be associated with chronic musculoskeletal pain (Linton et al., 1996). It was suggested that people with sexual abuse history may have diminished ability to cope with the pain. As a result, they reported higher level of distress and that may contribute to the persistence of pain (Linton et al., 1996). Several studies have reported the associations of childhood physical, sexual and emotional abuse and the development of fibromyalgia in adulthood (Häuser, Kosseva, Üceyler, Klose, & Sommer, 2011; Walker et al., 1997). Similar patterns of associations were reported between abuse and post-traumatic stress disorder (Lang et al., 2008). Elevated rates of childhood trauma were also reported by a sample of patients with chronic low back pain (Linton, 1997). It has been suggested that the individuals who report childhood physical or sexual abuse are 4 to 5 times more likely to have chronic pain problems in adulthood (Linton, 1997). The high frequency of childhood abusive history among people with chronic pain conditions make it a potential candidate to include in the routine assessment of chronic pain related disorders (Linton et al., 1996). As with most

such relationships, the evidence is not consistent and may be confounded by recall bias. There is evidence that childhood sexual abuse did not have any role in the development of chronic pain (Lampe et al., 2017). Raphael et al. (2001) documented no associations between childhood abuse and pain in adulthood when childhood abuse history was determined by court documented cases. Interestingly, they found positive associations between abuse history and chronic pain when they used self-reported retrospective data to evaluate the abuse history (Raphael, Widom, & Lange, 2001). It has also been proposed that childhood victimization is associated only with pain of psychogenic origin rather than pain of clear organic origin (Adler, Zlot, Hürny, & Minder, 1989).

It is clear from the above discussion that the results regarding the influence of childhood adversities on chronic pain development in adulthood are variable throughout the literature. Moreover, most of the studies focused on specific types of abuse (either physical or sexual) to explore its role in the development of chronic pain. The current thesis will explore the modifying role of childhood adversities (physical, sexual and emotional abuse, neglect, household dysfunction) on the acute stress markers.

The existing literature regarding the relationship between childhood victimization and stress system regulation remain inconclusive. There is increasing evidence that childhood adversities lead to persistent changes in the HPA axis regulation via epigenetic mechanism (McGowan, 2013). *Epigenetics* refers to the reversible regulation of various genomic functions without changing the underlying DNA sequence. Rodent models have provided support to the idea that early life stress contribute to chronic disorders via epigenetic mechanisms (Moffitt & Tan, 2013). Though the idea of epigenetic plasticity is in its infancy

in human studies, it has been proposed that the history of childhood abuse could cause permanent stress system dysregulation which makes the abused population more vulnerable to stressors and more likely to develop chronic pain following acute trauma (Heim, Plotsky, & Nemeroff, 2004; McLean et al., 2005).

Besides cortisol and HPA axis, some other biomarkers such as BDNF and TGFB1 have expressed themselves as potential avenue via which early life stress may influence the development of chronic pain conditions. A recent rodent study found epigenetic changes in BDNF expression in rats that experience childhood maltreatment. The study observed that the rats that were exposed to maltreatment by stressed caretakers showed methylation of BDNF DNA and which resulted in reduced BDNF gene expression in the prefrontal cortex. They also reported altered BDNF DNA methylation in the offspring of those maltreated females. Interestingly, their offspring had not been exposed to any childhood adversities. This may indicate the transmission of altered genes throughout generations (Roth, T.L., Lubin, F.D., Funk, A. J., Sweatt, 2009). In another animal study, two groups of primates were reared in two different conditions were examined. The group that was exposed to adversities in early life showed no significant correlation with cortisol following stressful condition in adulthood. On the other hand, the group reared in normal environment showed strong correlation with cortisol (Smith, Batuman, Trost, Coplan, & Rosenblum, 2002).

From this narrative overview of stress biomarkers, psychological markers, and early life adversity it can be concluded that there are several outstanding questions about the mechanisms underlying stress, the involvement of childhood adversities and the experience

of acute or chronic pain. I believe that the conflicting findings from some previous stress biomarker work may be due to the lack of control for potentially important social and early life experiences in the subjects. Also, most such work has been conducted in animals with relatively little done in humans under natural conditions. However, the promising results of these animal studies indicate that they are worthy to explore in human studies too. As a preliminary step to unfold the mechanism by which abuse affects chronic pain, the current study will explore the associations between pain, psychological distress, physiological biomarkers, and early life adversities in a sample of people following acute musculoskeletal trauma.

Chapter 2

2 Methodology

2.1 Introduction

Chronic musculoskeletal pain has been described as a "silent epidemic" of the modern, civilized world (Sessle, 2012). It is the most common cause of severe long term physical disability and is frequently accompanied by psychological co-morbidities such as depression, distress or anxiety (Bair, Robinson, Katon, & Kroenke, 2003). Chronic pain affects millions of people around the world. It has been estimated that one of every five North American adults has chronic pain at any one time (Moulin et al., 2002)and pain accounts for 80% of all physician visits (Gatchel, Robert J.; Peng, Yuan Bo; Peters, Madelon L.; Fuchs, Perry N.; Turk, 2007).

Several studies have shown that chronic musculoskeletal pain results in significant personal, economic, and social burdens. The estimated cost for chronic pain in Canada is $1462 per patient per month and in North America is $635 billion per year (Gaskin & Richard, 2012; Guerriere et al., 2010).The impact of chronic pain should not be evaluated in economic terms solely. Chronic musculoskeletal pain patients report worse health related quality of life in comparison to palliative cancer patients (Fredheim et al., 2008).

Despite the high prevalence, there is by comparison relatively little guidance to support treatment decisions available to clinicians. Current guidelines endorse the use of

polypharmacy in addition to non-pharmacological management including physical therapy, stress management strategies, and psychological interventions, but what evidence exists to support such approaches generally indicates a small effect (Teasell et al., 2010).

Recent efforts in the field have endorsed an approach of early identification and intervention in those people with acute pain that are likely to transition into a state of chronicity, attempting to prevent the onset of chronic pain before it emerges and becomes resistant to treatment. The incidence of chronic problems following musculoskeletal injury has been reported to range from approximately 20 to 50% depending on the conditions (Kongsted, Kent, Hestbaek, & Vach, 2017; Rosenbloom et al., 2016). For example, current best estimates following acute traumatic neck pain indicate that about 50% will continue to report persistent problems 1 year later, and about 30% will report severe pain and/or disability (Carroll et al., 2009; Sterling, 2011). Identifying mechanisms that can explain the development of chronic pain has therefore been identified as a high priority research area by experts in the field (Walton et al., 2016).The natural progression of mechanistic research should then be the identification of therapeutic approaches to prevent the onset of chronic pain.

Development of chronic pain is a complex interplay involving biological, psychological and social factors (McLean et al., 2005). Currently the best available evidence indicates that the most consistent predictors of chronic pain are largely cognitive in nature, including high ratings of pain intensity or disability, fear, catastrophizing, and low expectations of recovery (Walton et al., 2013a). However, recent large pragmatic clinical trials intended to specifically target negative cognitions have found no added benefit over a single session

of advice and education or standard treatment (Lamb et al., 2012; Jull, Kenardy, Hendrikz, Cohen, & Sterling, 2013). While it would seem that a propensity to rate the experience as more terrible or distressing in the acute stage of injury is temporally associated with poorer outcome, the results of these intervention trials would suggest the mechanisms to explain these associations are not yet understood.

Several stress biomarkers namely cortisol, Brain-derived neurotrophic factor (BDNF) and Transforming growth factor beta 1(TGFB1) received attention in recent biomedical and clinical research as having potential roles in the development of chronic pain. Cortisol is a steroid hormone. It is considered as the major stress hormone of the human body. It is produced in humans by the zona fasciculata of the adrenal cortex within the adrenal gland. It is released in response to any kind of stressful situation (Smith & Vale, 2006). A growing body of literature have supported the hypothesis that the stress system (HPA axis) dysfunction is associated with the pathogenesis of chronic pain following acute trauma (Chrousos & Kino, 2007).BDNF is a member of the neurotrophin family of the growth factor. Neurotrophic factors are found in the brain and the periphery(Lee & Kim, 2010). BDNF is an important protein that acts on the neurons of the central and peripheral nervous systems and helps in growth, differentiation and survival of the neurons (Generaal et al., 2016). BDNF has been demonstrated as a novel therapeutic target in the treatment of chronic pain due to its potential role in neuroplasticity and central sensitization (Nijs et al., 2015). TGFB1 (Transforming growth factor beta 1) is a polypeptide that belongs to the cytokine family. It performs many cellular functions, including cell growth, cell proliferation, cell differentiation and apoptosis. It also plays an important role in the

immune system. Most immune cells or leukocytes secrete TGFB1 (Khalil, 1999).TGFB1has shown to be involved in nociceptive pain processing. Hence, it was hypothesized to have some potential role as an intervention target in pain management (Lantero et al., 2012). However, the exact role of cortisol, BDNF and TGFB1 in the development of chronic musculoskeletal pain following acute trauma continues to be examined. We believe that deeper understanding of the rich interactions between biological, psychological and social factors will help to improve the current treatment for acute, and by extension, chronic pain.

Adverse childhood experiences (e.g. experiencing or witnessing abuse, early parentification, poverty, parental incarceration or separation) appear to be more commonly reported among patients with chronic health conditions and may function as an important moderator of the association between negative affect, stress, and recovery from subsequent musculoskeletal trauma in adulthood. Davis and Luecken found that people who reported abusive childhood experiences are more likely to develop chronic pain conditions in their adulthood (Davis & Luecken, 2005). Early childhood adversity has been endorsed as a potentially valuable avenue for exploration in the search for mechanisms to explain the development of chronic conditions such as musculoskeletal pain (Linton et al., 1996), Posttraumatic stress disorder (Lang et al., 2008) , Fibromyalgia (Häuser et al., 2011), Chronic pelvic pain (Hu, Link, McNaughton-Collins, Barry, & McKinlay, 2007), and low back pain (Linton, 1997). Linton (1997) found that those who reported childhood physical or sexual abuse were 4 to 5 times more likely to have chronic pain problems in adulthood (Linton, 1997). However, as with the association between negative cognitions and chronic

pain, the mechanisms to explain the link between adverse childhood events and the genesis of chronic pain remain unclear. Work from the lab of Walton and others has recently found that stress biomarkers (e.g. cortisol) appear to show associations with both negative posttraumatic cognitions and acute pain intensity, and may function to influence the course of recovery following trauma. To the best of our knowledge, the moderating effect of childhood adversities on the associations between these biological and psychological stress markers following acute trauma has yet to be explored.

2.2 Purpose

The main objectives of the study were to:

1) Explore differences in key stress biomarkers and self-rated pain intensity in a sample of people with acute musculoskeletal injuries when grouped by age, sex, body mass index (BMI), income level, education and medication history.

 2) Explore the associations between major stress biomarkers (cortisol, brain-derived neurotrophic factor (BDNF) and transforming growth factor beta1 (TGFB1)) and pain severity and distress in the acute setting of pain.

3) Identify potential moderating effects of adverse childhood experiences on the associations between stress biomarkers and pain-related cognitions.

2.3   Methods and Materials

2.3.1   Study Design

This was a cross-sectional exploratory study.

2.3.2   Participants

Eligible participants were those who had experienced an acute, non-catastrophic musculoskeletal injury within 1 week of presenting to the urgent care centre at St. Joseph's hospital (London Ontario, Canada). Participants were initially approached by their primary clinician (either physician or emergency care nurse) who obtained consent to be contacted by the research assistant for more information, full eligibility screening, and to obtain informed consent if they were willing and eligible to participate. Participants received 30 Canadian dollars as compensation for their participation in this part of the study. Full inclusion criteria were: aged 18 years or older, able to speak and understand English, and presented for an acute injury affecting the musculoskeletal system that did not require surgery or hospitalization. Exclusion criteria included those with cognitive impairments that interfered with ability to follow detailed instructions, those with neuromuscular disorders that impaired mobility (e.g. stroke, multiple sclerosis), any active malignancies within the past 5 years, and active systemic inflammatory or autoimmune conditions (e.g. rheumatoid arthritis, systemic lupus erythematosus). Finally, those who had been diagnosed with a concussion or were hospitalized overnight at any point in the prior 6

months, those under the influence of drugs or alcohol at the time of presentation to the department, or those with no fixed address were excluded from this part of the study.

2.3.3   Procedure (sample and data collection)

After obtaining consent, participants were provided an intake and screening form and brief questionnaire to gather more information at the time of presentation. Questions such as "Are you pregnant or lactating" or "Are you taking any antibiotics currently" were highlighted in the intake and screening form. The above mentioned two criteria are not exclusion criteria for the study. However it is helpful to know about these kinds of additional information to better understand the sample characteristics. Among all participants, single patient was pregnant. As none of the stress biomarkers were out of the normal reference range for that participant, they were retained for this study. Additional metadata were collected at that time (e.g. age, sex, current medication use, and pregnancy/post-partum status if applicable). Two vials of antecubital blood were then collected by a trained phlebotomist that were immediately stored in a clinic freezer before being transported to a laboratory for centrifugation and storage at -80C until extracted for assay.

Participants were discharged from the department with a set of questionnaires for capture of more detailed experiences after the traumatic event. These questionnaires captured a number of constructs including additional metadata (height, weight, income, working status, educational status, compensation/litigation involvement), Injury specifics (mechanism, area injured, time since onset), and a series of standardized questionnaires

(described below). Participants were asked to complete the forms within 24 hours of discharge.

2.3.4   Predictor (Independent) variables

2.3.4.1   **Demographic factors**

Age, sex**,** BMI, education, income, medication usage all these factors are used as independent variables to test the hypothesis of this study.

2.3.4.2   **The traumatic injuries distress scale (TIDS)**

The Traumatic Injuries Distress Scale (Walton, Krebs, et al., 2016) is a 12-item questionnaire designed to measure 3 different areas of trauma-related distress (negative affect, uncontrolled pain, and intrusion/hyper arousal) following acute musculoskeletal injuries. The TIDS is intended to capture affective vulnerabilities of the participants following acute trauma. The scale is available in 3 languages: English, French and Spanish. Walton and colleagues have found elevated scores on the TIDS to be associated with slower recovery 3 or 6 months later (Walton, Krebs, et al., 2016).

2.3.4.3   **The adverse childhood experiences questionnaire (ACE)**

The Adverse Childhood Experiences (ACE) questionnaire is a 10-item self-report tool that asks respondents to recall adverse events experienced in the first 18 years of life. High level of childhood adversities were found to be associated with a 4 to 12-fold increased risk of alcoholism, drug abuse, depression and suicidal attempt; a 2 to 4-fold increased risk of

smoking and poor health habits; and a 1.4 to 1.6-fold increased risk of severe obesity (Felitti et al., 1998). This is one of the widely used instruments to explore the cumulative stress experienced during childhood. It has been used in pediatrics, mental health and other health settings (Murphy et al., 2014).

ACE can provide retrospective reports about childhood abuse (physical, sexual and emotional), neglect and household dysfunctions. Each question is rated dichotomously: yes / no. The ACE questionnaire has been shown to be adequately reliable and valid to identify potentially vulnerable individuals with childhood adversities (Murphy et al., 2014; Felitti et al., 1998). In the current study, the ACE questionnaire was used to divide the data into two groups (one group who have endorsed at least one variety of childhood abuse and the other group who have not endorsed any of them). The differences between these two groups in the interplay between stress markers in the acute pain setting were explored.

2.3.4.4   **The acute stress disorder scale (ASDS)**

The ASDS scale (Bryant, Moulds, & Guthrie, 2000) is a 19-item screening tool intended to screen for Acute Stress Disorder (ASD) using criteria defined in the Diagnostic and Statistical Manual of Mental Disorder (*DSM-IV*; 4[th]ed; American Psychiatric Association, 1994). The term "acute stress disorder" refers to the stress reaction that occurs within the first month after having a traumatic event. People with acute stress disorder are more likely to develop post traumatic stress disorder later in life (Edmondson, Mills, & Park, 2010;Harvey & Bryant, 2002). It is the most widely used self- report questionnaire to diagnose "acute stress disorder" (Edmondson et al., 2010).

The scale comprises 5 dissociative, 4 re-experiencing, 4 avoidance and 6 arousal symptoms. The items are scored on a 5 point scale (1= not at all, 2=mildly, 3=medium, 4= quite a bit, 5= very much). The total ASDS is scored by summing the scores of all the items. The total ASDS score correctly identified 91% of people who developed subsequent PTSD (Bryant et al., 2000). Despite of having high specificity in diagnosing PTSD, it has showed lack of sensitivity in PTSD diagnosis (Bryant et al., 2000; Harvey & Bryant, 2002).

2.3.4.5 The brief pain inventory (BPI) - short form

The brief pain inventory (C S cleeland, 1994) is a self-administered questionnaire that was initially developed to measure pain in cancer patients (C S cleeland, 1994). However, it is also commonly used as an adequately valid and reliable measure of severity and interference from non-cancer pain (Tan, Jensen, Thornby, & Shanti, 2004;Keller et al., 2004). The BPI scale is easy to administer, available in many languages and easily accessible (Tan et al., 2004). The BPI is very efficient in evaluating and identifying pain intensity and associated disabilities in patients reporting lower back pain and arthritis (Keller et al., 2004) and chronic non-malignant pain (Tan et al., 2004). It provides a quick means of measuring pain intensity and the degree to which pain interferes with living and general activities.

This 11-item self-report tool includes 4 items capturing pain intensity and other 7 items reporting pain interference. The pain intensity subscale asks about worst pain, best (least) pain, and average pain over a period of about 24 hours and current level of pain to measure

the severity of pain. Each of the items is graded from no pain (0) to extreme pain (10) for a scale range of 0 to 40. The 7 items pain interference subscale assesses how pain interferes with activities of daily living such as general activity, mood, walking ability, work, relation with other people, sleep and enjoyment of life. These 7 items are anchored with "does not interfere" to "completely interferes" for a range from 0 to 70. Pain interference subscale should be interpreted as 2 different domains -Physical interference and affective interference. The item demonstrating sleep interference should be interpreted separately (Walton, Beattie, Putos, & MacDermid, 2016). Current study used this scale to explore the pain intensity and pain interference scores of people who underwent a traumatic musculoskeletal injury.

2.3.5   Outcome (Dependent) variables

2.3.5.1   **Cortisol**

*Assay technique***:**

Human plasma concentrations of cortisol were determined by radio immunoassay with a commercially available kit (DetectX Cortisol Immunoassay kit; Arbor Assays, Michigan, USA). Total cortisol was quantified according to the manufacturer's protocol. The assay sensitivity was ascertained at 1.73pg/mL and the limit of detection was determined as 45.4pg/mL. The concentration of cortisol was calculated using software available with most plate readers.

2.3.5.2   **BDNF & TGFB1**

*Assay technique***:**

Levels of brain-derived neurotropic factor (BDNF) and transforming growth factor beta 1 (TGFB1) were measured using multiplexed biomarker immunoassay kits according to manufacturers' instruction (BDNF: Human Magnetic Luminex Assay, R&D Systems, Inc., Minneapolis, USA, TGFB1: Milliplex MAP TGFB1 Magnetic Bead Single Plex Kit, EMD Millipore Corporation, Missouri, USA). A Bio-Plex^{TM} 200 readout System was used (Bio-Rad Laboratories, Hercules, CA), which utilizes Luminex® xMAP^{TM} fluorescent bead-based technology (Luminex Corp., Austin, TX). Levels were automatically calculated from standard curves using Bio-Plex Manager software (v.4.1.1, Bio-Rad). Plasma BDNF and TGBF1 protein levels were expressed in picograms per millilitre (pg/mL).

2.4   Data analysis

All variables were examined for missing values, outliers, normality of distribution, and homogeneity of variance. At first, subject characteristics (age, sex, BMI, Income, education) were evaluated descriptively (mean/ median, SD, range, frequency). Independent variables were demographic factors, baseline ASDS (total score, distress subscale, dissociation subscale), BPI (pain intensity subscale, pain interference subscale, physical interference subscale, affective interference subscale, sleep interference subscale), TIDS (total score, uncontrolled pain, negative affect and hyper arousal subscales) and ACE

(those with/without at least 1 adverse experience endorses). The dependent variables on the hypothesis being tested included stress biomarkers (Cortisol, BDNF and TGFB1).

2.4.1 Handling missing values and outliers

Where data were missing, we excluded the participant just for that analysis. Box plots and scatter plots were generated to identify outliers. As correlations can be influenced by extreme data, outliers (those that were more than 3 times the interquartile range) were removed from this exploratory analysis. Data were removed cautiously at this preliminary stage of research as outliers may indicate an interesting but rare subset of people. The rich dataset including metadata offered ample opportunity to scrutinize each outlying data point by exploring other characteristics of that participant. If that person showed proportionate and consistent values in other related variables that were theoretically understandable then that outlier was kept in the data set.

2.4.2 Data Normality

Shapiro-Wilk's normality test was performed and a histogram was plotted to check the normality of each variable. Stress biomarker variables were positively skewed initially. To ensure the normality of the data square root transformation of variables were performed. All the dependent variables were approximately normally distributed (according to the Shapiro-Wilk test) after doing the square root transformation. Assumptions of normality were not violated for most of the variables as all the p values were more than 0.05. Normal Q-Q plot also indicated the normality of the data. However due to nature of the data and

limited sample size normality could not be assumed for few self-reported variables. To ensure the robustness of the results, non-parametric tests were performed in those variables.

2.4.3   Test for homogeneity of variance

Equality of variance was evaluated using Levene's test. Where it was violated, a correction factor was applied to avoid alpha error.

2.4.4   Data coding

2.4.4.1   **Age**

Age was provided in years. Median age (40 years) was used as the cut-off point. Participants were divided into two groups according to their age; 40 or under (group =1) and above 40 (group =2).

2.4.4.2   **Sex**

About 25 males and 46 females completed the study. Sex was coded as: male= 1, female=2.

2.4.4.3   **BMI**

Median value (26.1) was used as the cut off point for BMI. BMI was coded as 2 groups: 26.1 kg/m^2 or under (group= 1), above 26.1 kg/m^2 (group=2).

2.4.4.4 **Medication usage**

There were 27 people who were taking anti-anxiety / anti-depressant medications at the time of injury and 49 people who did not provide such history. Medication history was coded as: not taking any anti-anxiety / anti-depression medications =0, taking anti-anxiety/ anti depression medications=1. These classes of drugs were considered the most relevant considering the primary evaluations related to post-traumatic stress and affective interference.

2.4.4.5 **Income**

There were 8 ranges provided in the questionnaire: < \$ 20,000= 1; \$20000-\$40000= 2; \$ 41000- \$60000= 3; \$ 61000- \$ 80000= 4; \$ 81000- \$100000= 5; \$101000-\$150000= 6; \$151000- \$200000= 7; >\$200000= 8. Income history was coded as $\leq$ CDN \$100,000 total household per year (group= 1) and income > 100,000 total household per year (group=2).

2.4.4.6 **Education level**

There were 7 levels in the questionnaire: did not finish school, high school, community college, trade school, university undergraduate degree, master's degree, and doctorate. Education was coded as Low education (group 1) = Trade school, community college, or no post-secondary education and High education (group 2) = university undergraduate degree or higher.

2.4.4.7 **Adverse childhood experiences**

ACE questionnaire was used to determine the presence or absence of childhood adverse history. Total 52 people endorsed at least one component of ACE questionnaire and 22 people indicated that they did not have such adversities in their childhood. These two groups were coded as 1 (who had adverse childhood history) and 0 (who did not have such histories) respectively.

2.4.5 Statistical tests (Hypothesis testing)

2.4.5.1 **Hypothesis 1**

There will be a significant difference in stress markers in the acute pain setting when the sample is split according to age, sex, BMI, education level, income level, medication usage, and adverse childhood experiences.

Stress level was determined according to the plasma level of stress biomarkers (cortisol, BDNF, TGFB1) and also the self-report measures of stress following musculoskeletal traumatic event. At first the sample is split into two groups according to age, sex, BMI, education level, income level, medication usage, and adverse childhood experiences. Then, Independent samples t-test was performed to explore differences between each group. As some of the self-report questionnaires were not normally distributed Mann- Whitney U-test was used.

2.4.5.2 **Hypothesis 2**

There will be significant correlations between biological and psychological markers of stress following acute traumatic events.

Pearson's correlation coefficient (r) and Spearman's Rho (rho) were used to explore the correlations between biological (cortisol, BDNF, TGFB1) and psychological (TIDS, ASDS, BPI) stress markers in the acute pain setting. Then, multiple regressions were performed to find out the proportion of variance of dependent variables that could be explained by independent variables after controlling for any relevant confounding demographics identified from hypothesis1. Preliminary analyses were performed to ensure that there was no violation of assumptions of regression (normality, linearity, multicollinearity and homoscedasticity).

2.4.5.3 **Hypothesis 3**

The history of adverse childhood experiences will moderate the correlations between acute stress biomarkers and self-reported distress.

To test the hypothesis about the moderating effects of adverse childhood experiences, the correlations between acute stress markers were generated when the sample is split in two groups according to the scores of the ACE questionnaire. Significant correlations between variables were plotted by scatter plots to visualize and compare the associations between ACE and No ACE groups. All statistical tests were 2-tailed tests conducted at $p \leq 0.05$, unless otherwise indicated.

2.4.6   Bootstrap resampling

A bootstrap resampling method was used to identify the 95% confidence interval of each of the significant correlations. A sample size of 1000 was used to test the hypothesis. Those correlation point estimates (Pearson's r) that differed between ACE groups to an extent that the point estimate of one was outside the 95%CI of the other were considered significantly different associations.

2.4.7   Sample size estimation

Sample size was estimated assuming a medium effect size (Pearson's correlations of 0.3 to 0.5) for our primary analyses, with a desired power of 80% to observe the effect while accepting a 5% alpha error rate. G*power software version 3 was used for the calculation (Faul, Erdfelder, Lang, & Buchner, 2007). As a result, a sample size of 84 subjects was targeted.

Raw data from the ongoing study were analyzed using SPSS software version 23 (IBM corp., USA).

Chapter 3

3 Results and Discussions

3.1 Results

3.1.1 Sample characteristics

Within our recruitment timeline, 76 participants completed the study. A total of 691 patients were approached to participate of which 518 refused or were unable to comply with the requirements of the study. Of the remaining173 who consented in the urgent care department and provided blood, saliva and partial baseline data, 76 completed and returned all baseline data and formed the sample for this study. The breakdown of total participation is given below:

```
┌─────────────────────────────────────────────────────────────┐
│ Urgent Care Center of St. Joseph Hospital, London, Ontario  │
└─────────────────────────────────────────────────────────────┘
                              ↓
┌─────────────────────────────────────────────────────────────┐
│ Patients who reported non- catastrophic musculoskeletal injuries │
└─────────────────────────────────────────────────────────────┘
                              ↓
┌─────────────────────────────────────────────────────────────┐
│ Assessed for eligibility and informed consent (n= 691)      │
└─────────────────────────────────────────────────────────────┘
                              │        ┌──────────────────────────────┐
                              │──────→ │ Unable to participate (n=518) │
                              │        │ 1) Some were not eligible     │
                              ↓        │ 2) Some refused to participate│
                                       └──────────────────────────────┘
┌─────────────────────────────────────────────────────────────┐
│ Consented to participate ( n=173)                           │
└─────────────────────────────────────────────────────────────┘
                              ↓
              ┌─────────────────────────────────────┐
              │ Completed the study (n= 76)         │
              └─────────────────────────────────────┘
```
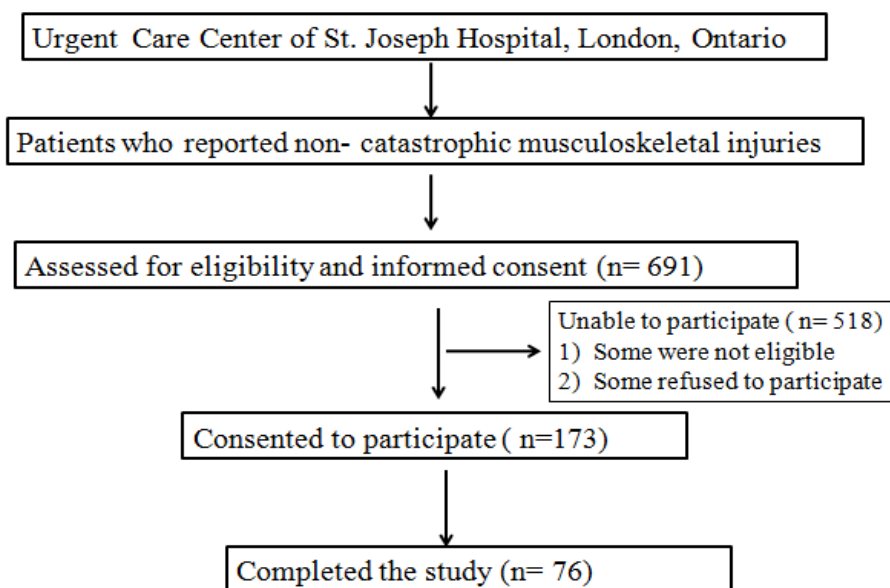
Table 3.1 provides the participant characteristics. The sample was primarily female (64.8%) of median 40 years of age. Modal education level was university undergraduate degree (28%) and 60% were employed full time. There was a bimodal distribution of income, where both $41,000 - 60,000 and $101,000-150,000 salary range were reported by 24.3% of the sample. The most common etiologies were fall on level ground and being hit by an object (15.1% each). The modal body regions injured were ankle (25%), knee (13.2%) and wrist (13.2%).

Table 3.2 provides details from the self-report tools and descriptive statistics of the stress biomarkers (cortisol, BDNF, TGFB1). Some of the self-report data and plasma biomarkers were not normally distributed. Hence, median and range are reported for them in table 3.2.

Table 3.1: Demographic data of participants.

Characteristics		Value
Age (median, range)		40 (18 to 66)
Sex (no. female %)		46 (64.8%)
Height in meters (mean, range)		1.6 (1.4 to 1.9)
Weight in kg (mean, range)		76.2 (43.6 to 136.3)
BMI (kg/m^2)	Median	26.1
	SD	5.7
	Range	15.8 to 43.6
Total household income level per year (number of people, percentages)	<$20000	6 (8.1%)
	$20000- $40000	9 (12.2%)
	$41000- $60000	18 (24.3%)
	$61000- $80000	8 (10.8%)
	$81000-$100000	11 (14.9%)
	$101000- $150000	18 (24.3%)
	$150000-$200000	3 (4.1%)
	> $200000	1 (1.4%)
Level of education (n (%))	Did not finish school	2 (2.7%)
	High school	19 (25.3%)
	Community college	15 (20.0%)
	Trade school	7 (9.3%)
	University undergraduate degree	21 (28.0%)
	Master's degree	8 (10.7%)
	Doctorate	2 (2.7%)
	Others	1 (1.3%)
Employment status (n (%))	Full-time	45 (60.0%)
	Part-time	12 (16.0%)
	Off work due to injury	2 (2.7%)
	Not employed for pay	16 (21.3%)
Current anti-anxiety or anti-depressant medications history (n (%))	People who were taking medications[1]	27 (35.5%)
	People who were not taking medications[1]	49 (64.5%)
Primary cause of the symptoms (n (%))	Motor vehicle accident	4 (5.5%)
	Pedestrian accident	1 (1.4%)
	Fall while on the level ground	11(15.1%)
	Fall down a hill or stairs	6 (8.2%)
	Fall from a height	1 (1.4%)
	Hit by an object	11 (15.1%)
	Hit by another person(not while playing)	1 (1.4%)
	An awkward lift or twist	10 (13.7%)
	Hit by another person (while playing)	2 (2.7%)
Part of body injured (n (%))	Neck	6 (7.9%)
	Shoulder	10 (13.2%)
	Elbow	4 (5.3%)
	Hand and Wrist	10 (13.2%)
	Lower back	7 (9.2%)
	Hip	1 (1.3%)
	Knee	10 (13.2%)
	Foot and Ankle	19 (25.0%)

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[1]: *Medications considered were anti-depressant or anti-anxiety drugs.*

Table 3.2: Descriptive statistics of dependent and independent variables.

Tool	Subscales	Median (range)
Adverse childhood effect questionnaire (ACE)	People who have no history of early life adversity (29.7%)	0
	People who endorsed at least 1 component of the questionnaire (70.3%)	3 (1 to 8)
Acute stress disorder scale (ASDS)	ASDS scale (Total, median(range))	95, 26 (19 to 67)
	Dissociation subscale (Total, median(range))	25, 6 (5 to 17)
	Distress subscale (Total, median(range))	70, 20 (14 to50)
Brief pain inventory-short form (BPI)	Pain intensity subscale(Total, median(range))	40, 18.5 (3 to 32)
	Pain interference subscale(Total, median(range))	70, 29.5 (3 to 67)
	Physical interference subscale(Total, median(range))	30,16.5 (2 to30)
	Affective interference Subscale (Total, median(range))	30, 4(0 to 18)
	Sleep interference subscale (Total, median(range))	10, 4 (0 to 10)
Traumatic injuries distress scale (TIDS)	Uncontrolled pain subscale(Total, median(range))	8, 3 (0 to 8)
	Negative Affect subscale (Total, median(range))	12, 2(0 to 12)
	Hyper arousal subscale(Total, median(range))	4, 0 (0 to 3)
	Total score of TIDS(Total, median(range))	24,6 (0 to 17)
Biomarkers of stress		Reference range (pg/mL)	Median pg/mL (range)
	Cortisol	20,000 to 2,50,000[1]	89,285 (21,270 to 3,59,60)
	BDNF	8000 to 46,000[2]	2,034.1 (422.08 to 10625.22)
	TGFB1	1000 to 33,000 [3]	20,313.5 (5428.9 to 59,122.7)

[1]. *Microcomputer Software Review Enzlab - - Elsevier Biosoft Synthetic Peptides in Biology and Medicine Book Reviews Textbook of Clinical Chemistry*, 1986

[2].Polacchini et al., 2015

[3].Kyrtsonis et al., 1998

3.1.2 Hypothesis testing

3.1.2.1 **Hypothesis 1: Exploring the influence of demographic variables on stress markers**

Table 3.3 shows the mean differences in stress biomarkers (cortisol, BDNF, TGFB1) when the sample is split according to age, sex, BMI, education level, income level, medication usage, and adverse childhood experiences. The only significant differences were in cortisol (t (56) = 2.09; p = 0.04) and BDNF (t (54) = -1.92; p = 0.05) between the high and low BMI groups. The high BMI group showed lower cortisol (279.9 vs.329.9 pg/mL) and higher BDNF (52.2 vs.43.3 pg/mL) compared to the low BMI group. Differences between all other groups and all TGFB1 comparisons did not reach statistical significance.

Table 3.3: Means (SD) of biomarkers split by key personal-level variables in the acute setting of pain.

Variable		BDNF (pg/mL)	Cortisol (pg/mL)	TGFB1 (pg/mL)
Age	40 or under (n = 36)	47.6 (18.1)	304.1 (97.7)	146.6 (40.9)
	Over 40 (n=35)	47.0 (19.6)	315.9 (86.8)	138.2 (37.4)
Sex	Male (n = 25)	47.3 (22.2)	308.7 (77.8)	132.2 (33.1)
	Female (n = 46)	47.4 (16.7)	310.5 (100.2)	148.1 (41.5)
BMI	$\leq$26.1 kg/m^2 (n = 31)	**43.3 (15.0)**	**329.9 (105.7)**	139.9 (40.6)
	>26.1 kg/m^2 (n = 31)	**52.2 (19.5)**	**279.9 (73.8)**	149.4 (41.6)
Income[1]	Group 1 (n = 48)	49.7 (20.1)	311.8 (96.2)	147.8 (39.6)
	Group 2 (n = 22)	44.1 (14.2)	316.5 (89.6)	139.4 (48.4)
Meds[2]	No (n = 49)	47.3 (18.4)	320.4 (107.7)	139.7 (41.9)
	Yes (n = 27)	49.8 (19.4)	299.5 (57.2)	157.2 (40.0)
Education[3]	Low (n = 37)	46.3 (17.6)	316.2 (71.8)	139.0 (38.3)
	High (n = 38)	49.9 (19.7)	310.3 (112.3)	151.7 (44.5)
ACE[4]	None (n = 22)	48.8 (21.4)	293.5 (71.2)	144.3 (37.3)
	At least 1 (n = 52)	48.1 (17.7)	309.4 (80.0)	146.0 (43.8)

Bold: Differences between groups significant at the p ≤ 0.05 level
1. Income Group 1 = CDN $100,000 or <CDN$100,000 total household per year
Income Group 2= > CDN$100,000 total household per year.
2: Medications considered were anti-depressant or anti-anxiety drugs.
3: High education = university undergraduate degree or higher
 Low education = Trade school, community college, or no post-secondary education.
4: ACE = Adverse Childhood Experiences questionnaire.

Table 3.4 includes the differences in self-report measures of stress as measured by Acute

stress disorder scale(ASDS) following traumatic musculoskeletal injury when the sample

is split according to age, sex, BMI, education level, income level, medication usage, and

adverse childhood experiences. None of the differences were statistically significant.

Table 3.4: Means (SD) of scores of the Acute Stress Disorder Scale (ASDS) and subscales split by key personal-level variables in the acute setting of pain.

Variable		Total ASDS Score	Distress Subscale	Dissociation Subscale
Age	40 or under (n = 36)	26.7(8.7)	20.4 (7.2)	6.4(2.3)
	Over 40 (n = 35)	30.3 (11.5)	22.6 (8.7)	7.6 (3.4)
Sex	Male (n = 25)	28.2 (11.3)	21.5 (8.8)	6.6 (2.9)
	Female (n = 46)	28.6 (9.6)	21.4 (7.6)	7.2(2.9)
BMI	≤ 26.1 kg/m² (n = 31)	26.6 (9.3)	20.3 (7.4)	6.3(2.7)
	>26.1 kg/m² (n = 31)	29.1 (9.4)	21.5(6.8)	7.4 (2.9)
Income[1]	Group 1 (n = 48)	28.0 (8.6)	21.3 (6.8)	6.7 (2.4)
	Group 2 (n = 22)	30.4(13.4)	22.5 (10.3)	7.8(3.7)
Medication[2]	No (n = 48)	28.2 (8.7)	21.3 (7.1)	6.8(2.5)
	Yes (n = 23)	29.1 (12.6)	21.8 (9.2)	7.2 (3.4)
Education[3]	Low (n = 35)	27.5 (8.6)	20.7 (6.9)	6.7 (2.4)
	High (n = 35)	29.7 (11.5)	22.4 (8.7)	7.2 (3.2)
ACE[4]	None (n = 20)	28.7(10.4)	21.8 (8.2)	6.9 (2.9)
	At least 1 (n = 49)	28.5 (10.1)	21.4 (7.8)	7.1 (2.9)

1. Income Group 1 = CDN $100,000 or <CDN$100,000 total household per year
Income Group 2= > CDN$100,000 total household per year
2: Medications considered were anti-depressant or anti-anxiety drugs.
3: High education = university undergraduate degree or higher
 Low education = Trade school, community college, or no post-secondary education.
4: ACE = Adverse Childhood Experiences questionnaire.

Table 3.5 shows the differences in self-report measures of stress as measured by the Brief Pain Inventory (BPI) following traumatic musculoskeletal injury when the sample is split according to age, sex, BMI, education level, income level, medication usage, and adverse childhood experiences. The only significant difference was in pain intensity by education, where the low education group reported significantly higher pain intensity than the high education group (t (65) =2.04, p =0.04).

Table 3.5: Mean (SD) of scores of the Brief Pain Inventory (short form) scale and subscales Split by key personal-level variables in the acute setting of pain.

Variable	Pain intensity Subscale	Pain interference Subscale	Physical interference Subscale	Affective interference	Sleep interference Subscale
Age					
40 or under (n = 34)	18.4(7.3)	28.6 (16.3)	16.3 (7.7)	4.8 (4.9)	3.6 (2.9)
Over 40 (n = 32)	17.6(7.8)	29.0 (16.6)	15.6 (7.9)	5.3 (4.7)	3.9(3.6)
Sex					
Male (n = 23)	18.5 (7.6)	30.5 (16.2)	15.6 (6.7)	6.0(5.4)	4.2 (3.1)
Female (n = 43)	17.8 (7.5)	27.9 (16.5)	16.2(8.3)	4.5(4.4)	3.5 (3.3)
BMI					
$\leq$ 26.1 kg/m^2 (n = 27)	18.0 (7.3)	29.3(15.0)	16.3(6.9)	5.4 (4.5)	3.7 (3.1)
>26.1 kg/m^2 (n = 27)	18.8 (6.6)	30.9 (16.3)	16.8 (7.8)	5.3 (4.8)	4.2 (3.2)
Income[1]					
Group1 (n = 51)	18.4 (6.9)	30.8 (16.6)	16.9 (8.2)	5.2 (4.8)	4.2(3.1)
Group 2 (n = 18)	17.6 (8.6)	28.7(16.1)	15.1 (6.1)	5.7 (4.9)	3.8 (3.5)
Medication[2]					
No (n = 48)	18.6 (7.6)	29.4 (15.4)	15.9 (7.3)	5.3 (4.5)	3.9 (3.1)
Yes (n = 24)	17.2 (6.7)	30.7 (18.5)	17.3(8.5)	4.9 (5.3)	4.1(3.5)
Education[3]					
Low (n = 35)	**20.1 (6.9)**	33.5 (16.7)	18.0(7.8)	6.1 (5.0)	4.5 (3.2)
High (n = 35)	**16.5 (7.3)**	26.8 (15.5)	15.1(7.3)	4.4 (4.5)	3.6 (3.2)
ACE[4]					
None (n = 20)	17.3 (8.3)	29.0 (14.9)	17.4 (7.8)	4.2 (3.8)	3.4 (3.1)
At least 1 (n = 49)	18.7 (6.9)	30.5(17.2)	16.2 (7.8)	5.7 (5.2)	4.3 (3.3)

Bold: Differences between groups significant at the p < 0.05 level
1. Income Group 1 = CDN $100,000 or <CDN$100,000 total household per year
Income Group 2= > CDN$100,000 total household per year
2: Medications considered were anti-depressant or anti-anxiety drugs.
3: High education = university undergraduate degree or higher
Low education = Trade school, community college, or no post-secondary education.
4: ACE = Adverse Childhood Experiences questionnaire.

Table 3.6 represents the differences in self-report measures of stress as measured by Traumatic Injuries Distress Scale (TIDS) following traumatic musculoskeletal injury when the sample is split according to age, sex, BMI, education level, income level, medication usage, and adverse childhood experiences. Those with income ≤ CDN $100,000 total household per year scored higher on the negative affect subscale than those with income >$100,000 (t (72) =2.19; p =0.03). Those taking medications for anxiety or depression scored higher on the total TIDS score: t (72) = -3.47; p <0.01, the uncontrolled pain: t (72) = -3.05; p <0.01and negative affect: t (73) = -3.24; p <0.01 subscales. Those with lower education scored higher on the total score: t (62) = 2.56; p =0.01, negative affect subscale: t (55) = 2.53; p =0.01 and hyper arousal subscale: t (52) = 2.97; p < 0.01.

Table 3.6: Mean (SD) of scores of the Traumatic Injuries Distress Scale (TIDS) scale and subscales Split by key personal-level variables in the acute setting of pain.

Variable	Total	Uncontrolled pain Subscale	Negative Affect Subscale	Hyper arousal Subscale
Age 40 or under (n = 36)	5.8 (4.1)	2.9 (2.0)	2.8 (2.5)	0.4 (0.7)
Over 40 (n = 35)	5.8 (4.3)	3.1 (2.4)	2.6 (2.2)	0.2 (0.5)
Sex Male (n = 25)	5.5 (4.3)	2.6 (1.9)	2.7 (2.7)	0.4 (0.8)
Female (n = 45)	5.9 (4.1)	3.1 (2.3)	2.7 (2.1)	0.2 (0.5)
BMI ≤ 26.1 kg/m^2 (n = 31)	6.1 (3.6)	3.2 (2.0)	2.7 (2.0)	0.2 (0.4)
>26.1 kg/m^2 (n = 30)	5.6 (4.3)	2.6 (2.1)	2.8 (2.7)	0.4 (0.8)
Income[1]Group 1 (n = 52)	6.4 (4.2)	3.1(2.1)	**3.2 (2.5)**	0.3(0.7)
Group 2 (n = 22)	4.9 (3.5)	2.8 (2.1)	**1.9 (1.8)**	0.3(0.6)
Medication[2]No (n = 48)	*4.8(3.8)*	*2.4(1.9)*	*2.2 (2.2)*	0.3(0.6)
Yes (n = 27)	*7.9(3.7)*	*3.9(2.1)*	*3.9 (2.1)*	0.3(0.6)
Education[3] Low (n = 37)	**7.1 (4.6)**	3.3 (2.2)	**3.4(2.8)**	*0.5 (0.8)*
High (n = 38)	**4.7 (3.0)**	2.6 (1.9)	**2.1(1.5)**	*0.1 (0.4)*
ACE[4]None (n = 22)	4.7 (3.8)	2.4 (2.2)	2.3 (1.9)	0.3 (0.6)
At least 1 (n = 52)	6.5 (4.0)	3.2 (2.0)	3.0 (2.5)	0.3(0.7)

Bold: Differences between groups significant at the p < 0.05 level
Italic: Differences between groups significant at the p < 0.01 level
. 1. Income Group 1 = CDN $100,000 or <CDN$100,000 total household per year
Income Group 2= > CDN$100,000 total household per year
2: Medications considered were anti-depressant or anti-anxiety drugs.
3: High education = university undergraduate degree or higher
Low education = Trade school, community college, or no post-secondary education.
4: ACE = Adverse Childhood Experiences questionnaire.

3.1.2.2   Hypothesis 2: Exploring the correlations between stress markers following acute musculoskeletal injury

Table 3.7 provides correlations between biological and psychological markers of stress following an acute stressful event. The association between cortisol and different self-report measures of stress were significant at the p < 0.05 level (table 7). Cortisol showed significant correlations with ASDS- total scores, ASDS- distress sub scale, BPI-pain intensity, BPI- pain interference, BPI- affective and sleep interference sub scale scores and TIDS- hyper arousal scores. BDNF was correlated negatively with BPI-physical interference subscale scores. TGFB1 showed no significant correlations with the provided scale scores in the acute setting of pain.

In the next step, Hierarchical regression was performed to assess the ability of self-report measures of stress to predict the variance in cortisol and BDNF after controlling for BMI. BMI was used as a continuous variable here. Table 3.8 contains the regression results. BPI-pain intensity scores and TIDS- hyper arousal scores explained 13% and 8% variance in cortisol respectively after controlling for BMI. 7% of the variance of BDNF was explained by BPI-physical interference scale score after controlling the confounding effects of BMI.

Table 3.7: Simple bivariate associations between key independent variables and stress biomarkers in the acute stage of injury.

	Cortisol (pg/mL)		BDNF (pg/mL)		TGFB1 (pg/mL)	
	r (p)	95% CI	r (p)	95% CI	r (p)	95% CI
ASDS-total subscale[1]	**0.26 (0.04)**	**0.03 to 0.45**	-0.19 (0.14)	-0.38 to 0.03	0.03 (0.83)	-0.24 to 0.28
ASDS-distress subscale[1]	**0.28(0.03)**	**0.05 to 0.49**	-0.19(0.13)	-0.41 to 0.02	0.02 (0.87)	-0.23 to 0.26
ASDS-dissociation subscale[2]	0.13(0.30)	-0.13 to 0.37	-0.10 (0.41)	-0.31 to 0.10	0.20 (0.87)	-0.23 to 0.26
BPI-pain intensity subscale[1]	*0.34(<0.01)*	**0.15 to 0.51**	-0.03 (0.81)	-0.26 to 0.22	0.07 (0.60)	-0.17 to 0.31
BPI-pain interference subscale[1]	**0.28(0.02)**	**0.08 to 0.46**	-0.14 (0.27)	-0.34 to 0.07	0.01 (0.94)	-0.25 to 0.27
BPI-physical interference subscale[1]	0.24 (0.06)	0.02 to 0.44	**-0.25 (0.04)**	**-0.45 to -0.03**	0.01 (0.96)	-0.22 to 0.23
BPI-affective interference subscale[1]	**0.26(0.03)**	**0.08 to 0.46**	-0.13(0.31)	-0.36 to 0.09	- 0.05 (0.69)	-0.28 to 0.19
BPI-sleep interference subscale[2]	**0.26(0.03)**	**0.03 to 0.47**	-0.03 (0.80)	-0.28 to 0.23	0.07 (0.56)	-0.21 to 0.34
TIDS-total score subscale[2]	-0.13 (0.30)	-0.37 to 0.13	0.01 (0.89)	-0.21 to 0.24	0.12 (0.34)	-0.12 to 0.34
TIDS-uncontrolled pain subscale[2]	0.09 (0.94)	-0.26 to 0.24	-0.02 (0.86)	-0.24 to 0.21	0.16 (0.19)	-0.06 to 0.38
TIDS- negative affect subscale[2]	-0.15 (0.21)	-0.43 to 0.13	0.03 (0.78)	-0.19 to 0.26	0.03 (0.78)	-0.20 to 0.25
TIDS- hyper arousal subscale[2]	**-0.26 (0.03)**	**-0.49 to -0.01**	0.15 (0.21)	-0.10 to 0.40	0.17 (0.17)	-0.07 to 0.39

Bold: Correlation is significant at the p < 0.05 level
Italic: Correlation is significant at the p < 0.01 level
ASDS: Acute stress disorder scale, BPI (short form): Brief pain inventory, TIDS: Traumatic injuries distress scale.
1: Pearson's Correlation (r) is performed as the variables are approximately normally distributed.
2: Spearman's Rho (rho) is performed as the data are not normally distributed.

Table 3.8: Percentage of variance of cortisol and BDNF explained by self-reported stress scores after controlling for BMI.

DV	IV	R^2	Δr^2	F	ΔF	p (change in F)
Cortisol	ASDS- total score	0.09	0.04	2.62	2.10	0.15
Cortisol	ASDS- distress	0.10	0.05	2.62	2.42	0.12
Cortisol	BPI - pain intensity	0.17	0.13	1.92	7.33	**<0.01**
Cortisol	BPI- pain interference	0.08	0.05	2.02	2.47	0.12
Cortisol	BPI- affective interference	0.08	0.04	2.05	1.99	0.16
Cortisol	BPI- sleep interference	0.08	0.04	2.06	2.45	0.12
Cortisol	TIDS- hyper arousal	0.11	0.08	1.82	4.81	**0.03**
BDNF	BPI - physical interference	0.13	0.07	3.36	4.14	**0.04**

3.1.2.3 Hypothesis 3: Exploring the moderating effects of adverse childhood experiences on the correlations between biological and psychological variables in the acute pain setting.

The data were split according to adverse childhood experience endorsements into ACE and NO ACE groups to explore potential differences in the magnitude and direction of correlations between self-report tools and biomarkers of stress. There were differences in the patterns of significant associations between the two groups. In the ACE group cortisol showed significant positive correlations with BPI- pain intensity (r= 0.36), pain interference (r = 0.36), physical interference (r = 0.35) and affective interference scores (r = 0.31). In contrast, people who belong to no ACE group did not show such associations. BDNF also showed significant negative associations with ASDS- total scores (r = -0.29) and distress scores (r = -0.31) in ACE group only but not in the NO ACE group. The associations between cortisol and ASDS- total scores (r =0.47) and distress (r = 0.49) were significant in the NO ACE group only. BPI- sleep interference sub scale scores are positively correlated with Cortisol (r = 0.53) and TGFB1 (r= 0.46) in the NO ACE group only. The differences in correlation coefficient were significantly different between groups in the cortisol x BPI Physical Interference, cortisol x BPI Affective interference, and cortisol x BPI sleep interference, BDNF x ASDS- total, BDNF x ASDS- distress, TGFB1 x BPI sleep interference evaluations by virtue of point estimates for one group that lay outside the 95% CI of the other group.

All the significant associations are plotted in scatter plots to visualize the patterns of associations between biological and psychological markers of stress in ACE and No ACE group (Figures 3.1 to 3.9).

Table 3.9, 3.10 and 3.11 include the correlations between cortisol, BDNF, TGFB1 and self-reported measures of stress in the acute pain setting when the data is split according to the history of adverse childhood effects.

Table 3.9: Simple bivariate associations between key independent variables and cortisol in the acute stage of injury when the sample is split into two groups according to the history of childhood adversities.

	ACE (n=44)		NO ACE(n=19)	
	Cortisol (pg/ml) r(p)	95 % CI	Cortisol (pg/ml) r(P)	95% CI
ASDS-total[1]	0.23(0.15)	-0.07 to 0.48	**0.47* (0.05)**	**-0.10 to 0.81**
ASDS- distress[1]	0.25 (0.12)	-0.05 to 0.53	**0.49*(0.04)**	**-0.02 to 0.79**
ASDS dissociation[1]	0.17 (0.28)	-0.15 to 0.49	0.29 (0.22)	-0.18 to 0.67
BPI- pain intensity[1]	**0.36*(0.02)**	**0.16 to 0.56**	0.28(0.25)	-0.13 to 0.61
BPI-pain interference[1]	**0.36 *(0.02)**	**0.12 to 0.46**	0.14 (0.57)	-0.34 to 0.50
Physical interference subscale[1]	**0.35*(0.02)**	**0.08 to 0.57**	0.01(0.96)	-0.50 to 0.41
Affective interference subscale[1]	**0.31* (0.04)**	**0.07 to 0.51**	0.06(0.79)	-0.35 to 0.48
Sleep interference subscale[2]	0.19(0.21)	-0.09 to 0.46	**0.53* (0.02)**	**0.11 to 0.78**
TIDS total score[2]	-0.12 (0.45)	-0.37 to 0.18	-0.13 (0.57)	-0.61 to 0.26
Negative affect[2]	-0.17 (0.25)	-0.44 to 0.14	-0.24 (0.29)	-0.67 to 0.22
Uncontrolled pain[2]	0.02 (0.90)	-0.26 to 0.31	-0.01 (0.98)	-0.52 to 0.47
Hyperarousal[2]	-0.23 (0.12)	-0.47 to 0.09	-0.29 (0.19)	-0.64 to 0.17

Bold: Correlation is significant at the $p \leq 0.05$ level
ASDS: Acute stress disorder scale, BPI (short form): Brief pain inventory, TIDS: Traumatic Injuries Distress Scale.
1: Pearson's Correlation (r) is performed as the variables are approximately normally distributed.
2: Spearman's Rho (rho) is performed as the data are not normally distributed.
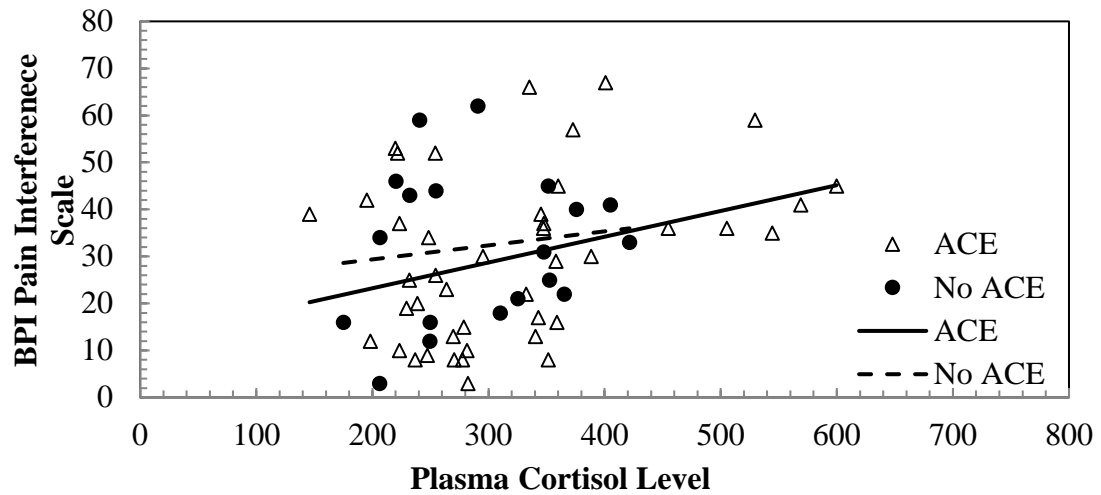
Figure 3.1: Scatter plot showing the linear relationships between plasma cortisol and BPI-pain interference sub scale scores in ACE group and No ACE group. The correlation between biological and psychological markers of stress is significant at the p <0.05 level in ACE group.

Figure 3.2: Scatter plot showing the linear relationships between plasma cortisol and BPI-physical interference sub scale scores in ACE group and No ACE group .The correlation between biological and psychological markers of stress is significant at the p <0.05 level in ACE group.

Figure 3.3: Scatter plot showing the linear relationships between plasma cortisol and BPI-Affective interference sub scale scores in ACE group and No ACE group. The correlation between biological and psychological markers of stress is significant at the p <0.05 level in ACE group.
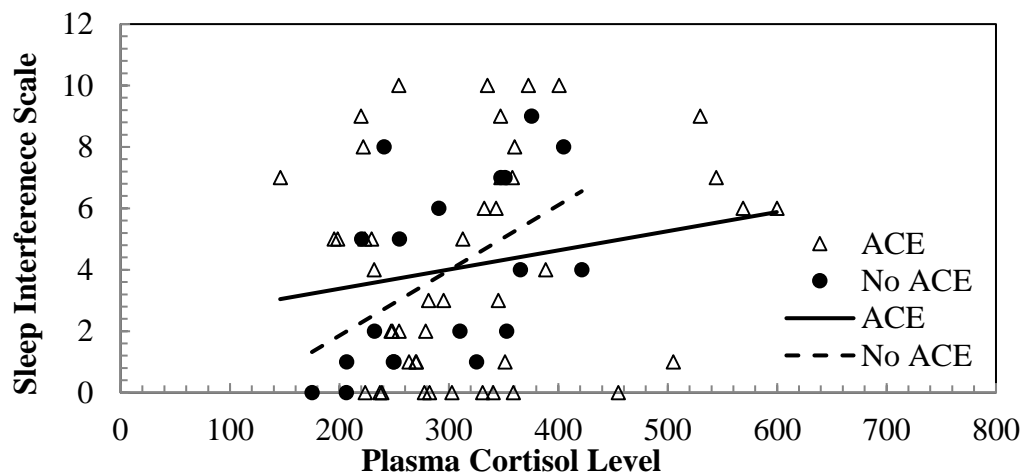
Figure 3.4: Scatter plot showing the linear relationships between plasma cortisol and BPI-sleep interference sub scale scores in ACE group and No ACE group. The correlation between biological and psychological markers of stress is significant at the p <0.05 level in No ACE group.

Figure 3.5: Scatter plot showing the linear relationships between plasma cortisol and ASDS scale scores (total) in ACE group and No ACE group. The correlation between biological and psychological markers of stress is significant at the p ≤ 0.05 level in No ACE group.

Figure 3.6: Scatter plot showing the linear relationships between plasma cortisol and ASDS distress sub scale in ACE group and No ACE group. The correlation between biological and psychological markers of stress is significant at the p <0.05 level in No ACE group.

Table 3.10: Simple bivariate associations between key independent variables and BDNF in the acute stage of injury when the sample is split into two groups according to the history of childhood adversities.

	ACE (n=46)		NO ACE(n=18)	
	BDNF(pg/ml) r(p)	95 % CI	BDNF(pg/ml) r(P)	95% CI
ASDS-total[1]	**-0.29 (0.04)**	**-0.51 to -0.01**	0.08 (0.75)	-0.27 to 0.48
ASDS-distress[1]	**-0.31 (0.04)**	**-0.49 to -0.04**	0.06 (0.81)	-0.29 to 0.47
ASDS dissociation[1]	-0.19 (0.19)	-0.48 to 0.11	0.25 (0.31)	-0.31 to 0.81
BPI- pain intensity[1]	-0.03 (0.84)	-0.35 to 0.27	-0.01 (0.98)	-0.44 to 0.49
BPI-pain interference[1]	-0.13 (0.39)	-0.41 to 0.14	-0.17 (0.50)	-0.53 to 0.18
Physical interference subscale[1]	-0.19 (0.19)	-0.42 to 0.03	-0.31 (0.21)	-0.62 to 0.03
Affective interference subscale[1]	-0.16 (0.29)	-0.45 to 0.15	-0.05 (0.85)	-0.46 to 0.36
Sleep interference subscale[2]	-0.12 (0.44)	-0.39 to 0.24	0.08 (0.75)	-0.25 to 0.45
TIDS total score[2]	0.08 (0.58)	-0.17 to 0.34	-0.39 (0.10)	-0.82 to 0.17
Negative affect[2]	0.09 (0.51)	-0.18 to 0.34	-0.29 (0.28)	-0.81 to 0.28
Uncontrolled pain[2]	0.01 (0.92)	-0.24 to 0.26	-0.35 (0.15)	-0.76 to 0.27
Hyperarousal[2]	0.21 (0.14)	-0.08 to 0.47	-0.04 (0.86)	-0.57 to 0.49

Bold: Correlation is significant at the p < 0.05 level
Italic: Correlation is significant at the p < 0.01 level
BDNF: Brain Derived Neurotrophic Factor.
ASDS: Acute stress disorder scale, BPI (short form): Brief pain inventory, TIDS: Traumatic Injuries Distress Scale.
1: Pearson's Correlation (r) is performed as the variables are approximately normally distributed.
2: Spearman's Rho (rho) is performed as the data are not normally distributed.

Figure 3.7: Scatter plot showing the linear relationships between plasma BDNF and ASDS total score   in ACE group and No ACE group. The correlation between biological and psychological markers of stress is significant at the p <0.05 level in ACE group.
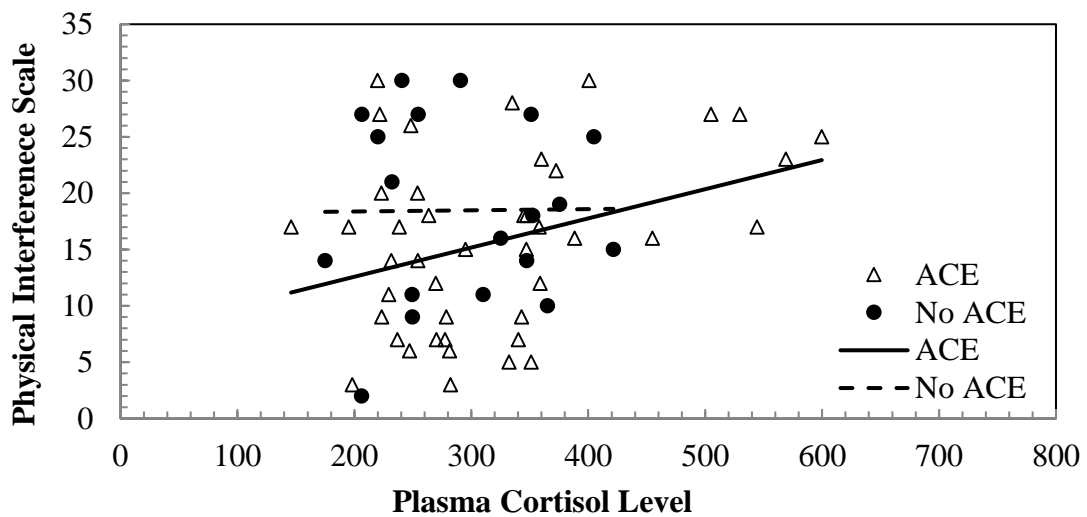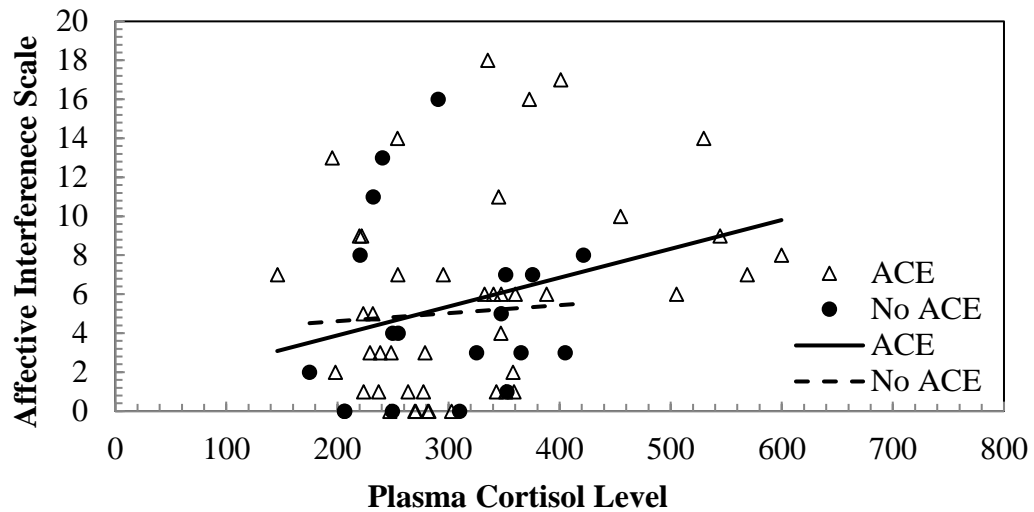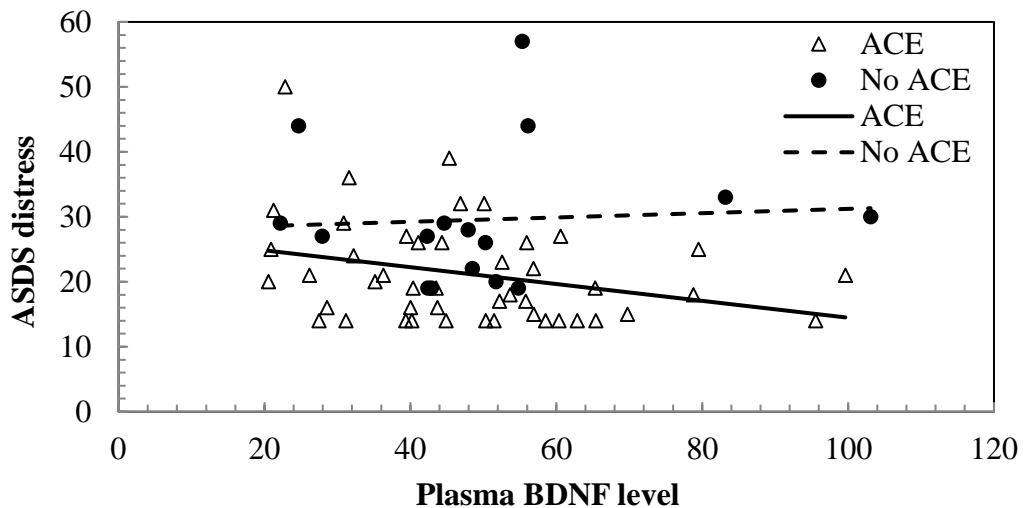

Figure 3.8: Scatter plot showing the linear relationships between plasma BDNF and ASDS -distress sub scale scores in ACE group and No ACE group .The correlation between biological and psychological markers of stress is significant at the p <0.05 level in ACE group.
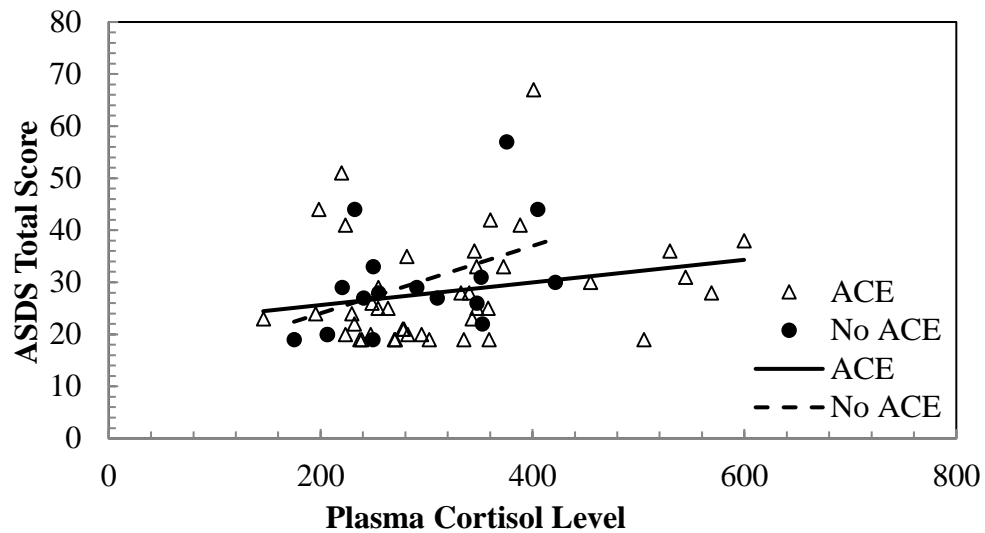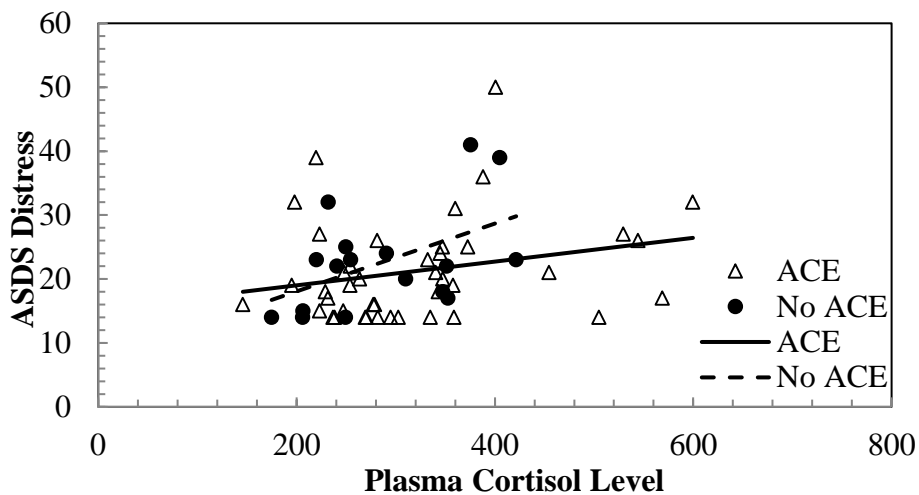
Table 3.11: Simple bivariate associations between key independent variables and TGFB1 in the acute stage of injury when the sample is split into two groups according to the history of childhood adversities.

	ACE (n=45)		NO ACE(n=18)	
	TGFB1(pg/ml) r(p)	95 % CI	TGFB1(pg/ml) r(p)	95% CI
ASDS-total[1]	-0.09 (0.56)	-0.36 to 0.16	0.37 (0.16)	-0.39 to 0.81
ASDS-distress[1]	-0.10 (0.50)	-0.36 to 0.15	0.31 (0.13)	-0.42 to 0.78
ASDS dissociation[1]	-0.02 (0.86)	-0.27 to 0.19	0.18 (0.46)	-0.65 to 0.69
BPI- pain intensity[1]	0.03 (0.87)	-0.27 to 0.32	0.18 (0.47)	-0.29 to 0.63
BPI-pain interference[1]	-0.05 (0.71)	-0.36 to 0.24	0.19 (0.43)	-0.22 to 0.56
Physical interference subscale[1]	-0.07 (0.62)	-0.34 to 0.20	0.19 (0.43)	-0.15 to 0.57
Affective interference subscale[1]	-0.08 (0.58)	-0.35 to 0.20	0.06 (0.79)	-0.44 to 0.47
Sleep interference subscale[2]	-0.02 (0.91)	-0.34 to 0.30	**0.46 (0.05)**	**-0.06 to 0.77**
TIDS total score[2]	0.12 (0.41)	-0.14 to 0.37	0.22 (0.38)	-0.35 to 0.70
Negative affect[2]	0.04 (0.78)	-0.24 to 0.31	0.15 (0.56)	-0.36 to 0.61
Uncontrolled pain[2]	0.16 (0.28)	-0.13 to 0.41	0.28 (0.25)	-0.28 to 0.71
Hyperarousal[2]	0.18 (0.22)	-0.12 to 0.46	0.28 (0.25)	-0.29 to 0.71

Bold: Correlation is significant at the $p \leq 0.05$ level
TGFB1: Transforming Growth Factor Beta 1.
ASDS: Acute stress disorder scale, BPI (short form): Brief pain inventory, TIDS: Traumatic Injuries Distress Scale.
1: Pearson's Correlation (r) is performed as the variables are approximately normally distributed.
2: Spearman's Rho (rho) is performed as the data are not normally distributed.
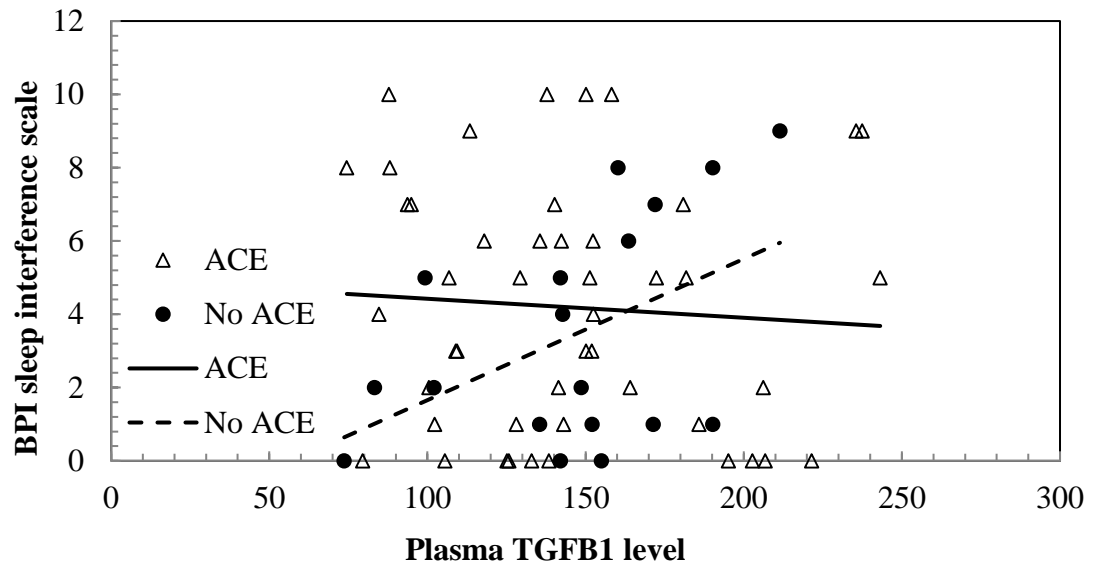
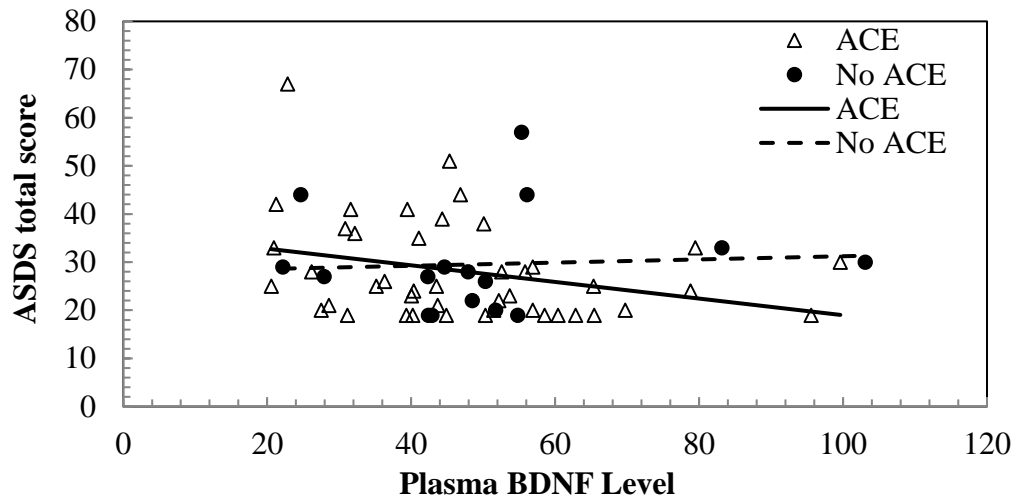**Figure 3.9: Scatter plot showing the linear relationships between plasma TGFB1 and BPI -sleep interference sub scale scores in ACE group and No ACE group. The correlation between biological and psychological markers of stress is significant at the level p ≤ 0.05 in No ACE group.**

3.2 Discussions

This study examined the role of stress biomarkers in acutely traumatized patients to identify the major stress markers that have the potential to serve as an intervention target to prevent the development of chronic pain. The magnitude and direction of associations of cortisol and BDNF with self-reported stress markers provided supportive evidence to support further exploration of cortisol and BDNF as acute stress biomarkers. However, these results need replication. The influence of the demographic variables on the acute stress markers was investigated. It was found that BMI, education, income and medication usage were all associated with the level of different stress markers (both biological and psychological). The results of the study also supported a moderating role of adverse childhood experiences on the relationships between biological and psychological stress markers following acute trauma.

All of the major findings of the current study are discussed in the following section in context of the existing literature.

3.2.1 Demographic factors (age, sex, BMI, education, income level, medication usage) that influence the level of stress following acute non catastrophic musculoskeletal trauma.

3.2.1.1 **Stress biomarkers and demographics**

The current study found no significant differences in stress biomarkers between high and low age groups. This finding is consistent with previous studies that reported no differences in salivary and urinary cortisol levels between different age groups (Ceccato et al., 2015; Ceccato et al., 2014). However, some other studies reported significant associations between older age and stress biomarkers. They reported increased level of salivary cortisol level (Nicolson, Storms, Ponds, & Sulon, 1997) and decreased level of plasma BDNF in older age groups (Lommatzsch et al., 2017). It has also been showed that serum TGFB1 level was higher in low age groups in compared to adults (Okamoto et al., 2005).It is worth noting that, in all these cases the differences in stress biomarkers level between age groups were evaluated in healthy people. To the best of our knowledge, no other studies have evaluated the age related differences in stress biomarkers in people who survived a non-catastrophic musculoskeletal injury. Previous researchers have recommended that in order to find the age related differences in cortisol level, it is necessary to include participants who are more than 70 years of age in the sample (Nicolson et al., 1997; Sapolsky & Plotsky, 1990). Due to the nature of the data, the present study used 40 as a cut off score for age groups and the age range of this sample was 18 to 66

years. This could be a reason for which no differences in stress markers between two age groups were observed in the present study.

Many previous studies included the differences in stress biomarkers between males and females (Larsson, Gullberg, Råstam, & Lindblad, 2009; Kajantie & Phillips, 2006). This is in contrast with the current study that found no significant differences in any stress biomarkers between males and females. However, the result was consistent with the study that reported no significant differences in salivary cortisol level in males and females of different age groups (Nicolson et al., 1997). Another study found no significant differences in plasma BDNF level between males and females (Lommatzsch et al., 2017). According to Paris and colleagues, the differences in cortisol level in males and females following acute stressful condition depends on the types of the stress exposure (Paris et al., 2011). He found higher cortisol level in men who survived a motor vehicle collision in comparison to their female counterparts. On the other hand, in response to stressor like noise exposure women showed higher cortisol level than men.

There were significant differences in biological markers (cortisol and BDNF) when the sample was split by BMI. Previous studies have reported lower level of cortisol in obese people (Odeniyi, Fasanmade, Ogbera, & Ohwovoriole, 2015 ; Travison T O'Donnell A Araujo A Matsumoto A McKinlay J, 2007 ; Champaneri et al., 2013; Walker, Soderberg, Lindahl, & Olsson, 2000). Travison et al. (2007) found a negative association between cortisol concentration and all of their body composition parameters. This is consistent with the findings of the present study. In contrast, the level of plasma BDNF was higher in the high BMI group compared to the low BMI group which is supported by several other

studies (Pillai et al., 2012; Liu et al., 2016; Fang et al., 2016). Many previous studies have explored BDNF and BMI in people with major depressive disorder, Schizophrenia and other mood disorders (Pillai et al., 2012). To the best of our knowledge, this is the first time the level of BDNF was explored in high and low BMI people following a traumatic musculoskeletal injury.

3.2.1.2 **Psychological stress markers and demographics**

The current study added to a growing body of literature that suggests "socioeconomic status" as one of the non -modifiable risk factors for the development of chronic pain conditions (van Hecke, Torrance, & Smith, 2013; Blyth, 2008; Poleshuck & Green, 2008). Self-reported measures of stress were different in groups split according to the education and income level of participants. The low education group reported greater pain intensity after having a traumatic event. The level of overall acute distress as measured by TIDS total score following trauma was also higher in the low education group. Several studies reported the possible role of low education status in the development of chronic conditions (Udom, Janwantanakul, & Kanlayanaphotporn, 2016 ; van Hecke et al., 2013; Dionne, 2001). To date however the mechanism has been unclear. This current study at least sheds some light on this question, suggesting that lower education may lead to greater traumatic distress.

The same pattern of result was also observed in people with income ≤ CDN\$100,000 total household per year. The mean score of this income group was higher on the Negative Affect subscale of the TIDS compared to those with income > CDN \$100,000 total

household per year, which is indicative of more detachment or depressive symptoms. This finding is consistent with other studies that also reported the role of perceived income inadequacy as one of the causative factors in the development of chronic pain (Jordan, Thomas, Peat, Wilkie, & Croft, 2008). According to the results of the current study, people with income ≤ CDN$100,000 total household per year and comparatively low education level report greater post-traumatic distress, which in turn may predispose them to greater risk of chronic pain.

The presence of psychological co-morbidities such as depression and anxiety with chronic pain conditions is well established by research (Holmes, Christelis, & Arnold, 2013; Banks, Sara M.; Kerns, 1996; Hu et al., 2007) . However, the acute stress reaction in people who have already diagnosed with depression or anxiety has not been explored to our knowledge. This study hypothesized greater level of perceived stress in people who had depression or anxiety disorders at the time of the traumatic event based on the findings of previous research (Clauw & Chrousos, 1997; Breslau, GC, Andreski, & Peterson, 1991). The findings of the study supported the hypothesis. Participants who self-reported current use of anti- anxiety or anti- depressant drugs had higher level of overall distress, a lower sense of pain control, and greater affective impairment as obtained by TIDS- total scale scores, Negative affect sub scale scores and Uncontrolled pain subscale scores in comparison with the scores of people who did not provide such medication history. While the mechanisms are unclear from this study, these results suggest that it will be important to control for pre-existing psychological comorbidity in future studies of trauma, stress and pain.

3.2.2 Potential stress biomarkers and their associations with self-report measures of stress

The current study investigated the relationships between perceived stress and biomarkers of stress in people who experienced a non-catastrophic musculoskeletal injury. The positive correlations of cortisol with self-reported post traumatic distress can be considered as supportive evidence for cortisol as an acute stress biomarker. Self-reported psychological distress, perceived pain intensity and disabilities associated with pain showed statistically significant positive associations with cortisol.

The current study found that self-reported pain intensity score predicted 13% of the variance in cortisol after controlling for the confounding effects of demographic variable ($p<0.01$). Walton et al. (2013b) found a significant positive correlation between pain catastrophizing and hair normalized salivary cortisol in participants who underwent a traumatic injury. Positive associations between salivary cortisol and heightened pain intensity/pain catastrophizing were also evident in the experimental pain setting (Quartana et al., 2010Jones, Rollman, & Brooke, 1997). Perceived high pain intensity in the immediate aftermath of trauma has been suggested as one of the consistent predictors of chronic pain development (Walton et al., 2013a; Quartana, Campbell, & Edwards, 2009; Bortsov et al., 2014). As cortisol showed positive relation with higher pain intensity and previous research supported the idea that the stress system is capable of influencing pain processing (McLean et al., 2005); regulation of cortisol following trauma may hold promise as an intervention target to prevent the development of chronic conditions.

However, this relationship should be investigated further to reach any definitive conclusion considering the limited sample size and cross-sectional nature of the study.

In the current study, self-reported hyper arousal subscale score predicted 8% of the variance in cortisol after controlling for the confounding effects of demographic variable ($p < 0.05$). Hyper arousal is referred to as a state which is characterized by difficulty to fall asleep and wakefulness because of enhanced physical, emotional and cognitive arousal (Kay & Buysse, 2017).Hyper arousal after trauma was suggested as a strong indicator of chronic conditions like Post traumatic stress disorder (PTSD) , Whiplash associated disorder(WAD) and Chronic pain (Andersen, Elklit, & Vase, 2011; Liedl & Knaevelsrud, 2008 ; Liedl et al., 2010). It has been demonstrated by a longitudinal study that the people who identified "acute hyper arousal " as a prominent symptom following a traumatic event are more likely develop chronic PTSD symptoms than who included hyper arousal as a less prominent one (Schell, T. L., Marshall, G. N., & Jaycox, 2004). Interestingly, PTSD and recovery from trauma also appeared to be dependent on each other (Sterling, Hendrikz, & Kenardy, 2010). Further longitudinal studies are necessary to elucidate the mechanism of associations between hyper arousal and the PTSD and their subsequent role in the development of chronic pain.

One of the new additions of this study was to explore BDNF as an acute stress marker after a traumatic event. BDNF showed significant negative association with physical interference subscale score. In this study, self-reported physical disability as a result of trauma explained 7% of the variance in BDNF after controlling for the confounding effects of demographic variable ($p < 0.05$). It has been reasonably well established that exercise

and physical activity can lead to increased BDNF levels in the brain (Vaynman, Ying, & Gomez-Pinilla, 2004; Ding et al., 2004; Schmolesky, Webb, & Hansen, 2013) that has potentially important neuroprotective function. According to Nijs and colleagues , high BDNF level may reduce the likelihood of chronic pain development due to its crucial role against maladaptive neuroplasticity which is the reason underlying many chronic pain conditions (Nijs et al., 2015). The current study suggests an association between self-rated interference in physical function and plasma BDNF level, but the clinical importance of this association requires further exploration.

The current study also explored the role of TGFB1 as an acute stress biomarker. TGFB1 was found not to be associated with any of the self-reported values. This is in contrast to previous findings. Previous studies regarded TGFB1 as potential biomarker for conditions like temporomandibular joint tenderness and osteoarthritis(Slade et al., 2011;Shen, Li, & Chen, 2014). However no studies had investigated the role of TGFB1 in the acute pain condition. It is probable that the role of TGFB1 may become more prevalent in chronic pain conditions rather than in the acute pain setting. This relationship should be explored further to reach a definitive conclusion.

3.2.3 The moderating role of adverse childhood experiences on the associations between biological and psychological indictors of stress in the acute pain setting

Considerable amount of research studies have focused on the effects of adverse childhood experiences as it has emerged as an important indicator of adult health and well-being(De

Bellis & Zisk, 2014; Vincent J Felitti & Anda, 2010). The current study investigated the role of adverse childhood experiences (ACE) in the acute pain setting to understand its interplay with other stress markers in acute pain and distress.

People who have endorsed at least one component of the ACE scale were considered as the "ACE group" in this study. In the ACE group, cortisol showed significant positive correlations with the BPI pain intensity score and almost all of the subscale scores of BPI. Previous studies reported a relationship between heightened perceived pain intensity and childhood adversities (Drevin et al., 2015; Sansone,Watts, & Wiederman, 2013).Existing evidence suggests that the most consistent predictors of chronic pain development are high initial pain intensity, self-reported psychological distress and activity interference(Walton et al., 2013a ; McLean et al., 2005). It is noteworthy that all of these variables (as measured by BPI- pain intensity subscale, physical and affective interference subscale) were positively correlated with cortisol only in the people who endorsed adverse childhood experiences. This association maybe partially explained by the dysregulation of biological stress system in people with ACE. Earlier research suggested that early life adversity increases endocrine and autonomic responses to stress (Anacker, O'Donnell, & Meaney, 2014). It has also been proposed that childhood adversities lead to persistent HPA axis dysregulation via an epigenetic mechanism (McGowan, 2013) that may preferentially influence the stress-response pathways. Therefore it can be proposed that people who experienced childhood adversities may react to subsequent stressful events differently than those with no such exposure, likely due to a complex interplay of both psychological and physiological processes. However, this result should be interpreted with caution because

of the limited sample size, exploratory, cross-sectional nature of the study and retrospective measure of the adverse childhood experiences.

BDNF was negatively correlated with ASDS- total score and ASDS- distress subscale score in the ACE group only. People who develop acute stress disorder following a traumatic event are more likely to develop PTSD in later life(Harvey & Bryant, 2002). From the results of the current study, high BDNF may protect against acute stress disorder, or acute stress disorder may lower levels of BDNF. Lower BDNF level was associated with higher ASDS scores only in the subsample who had history of adverse childhood experiences. This is in line with the studies that suggested the stressful childhood experiences can reduce the BDNF level in a subset of the population (Vollmayr, Faust, Lewicka, & Henn, 2001; Daskalakis, De Kloet, Yehuda, Malaspina, & Kranz, 2015). Lower BDNF level has also been implicated in the development of several chronic conditions such as depression, bipolar disorder, chronic widespread pain (Elzinga et al., 2011; Fernandes et al., 2015 ; Caumo et al., 2016). It has been shown that direct infusion of BDNF resulted in improvement of symptoms despite of high level of circulating corticosterone in a study that investigated stressed rodents (Lakshminarasimhan & Chattarji, 2012). These findings point to a possible interplay between cortisol, BDNF and psychologically perceived stress level in the acute pain setting that may suggest a new target for intervention to prevent chronic pain.

There were only 19 people on the No ACE group, leading to wide confidence limits for many correlations and very few significant associations beyond ASDS- total score x cortisol and ASDS- distress subscale score x cortisol. No other psychological markers

were related to cortisol in the no ACE group. BDNF showed no significant correlations with any of the stress variables. The Sleep interference subscale of the BPI was positively correlated with cortisol and TGFB1 in the No ACE group only. There are inconsistencies regarding the role of cortisol in sleep disturbances. Some studies found increased level of evening cortisol and increased cortisol awakening response in people with sleep disturbances (Kumari et al., 2009). Others reported decreased cortisol awakening response with sleep disturbances (Backhaus, Junghanns, & Hohagen, 2004) . This study found that increased plasma cortisol is associated with increased self-reported sleep disturbances but data on the cortisol awakening response is not yet available. Sleep disturbances are frequently associated with cytokines namely Interleulin-1(IL- 1) and Tumor necrosis factor (TNF) and it has been hypothesized that other cytokines may play a role in the development of sleep related maladies(Krueger, 2008). To the best of our knowledge this is the first study that reported an association between TGFB1 and sleep interference. The current study revealed that those who scored higher on the BPI sleep interference subscale had higher level of TGFB1. In the ACE group, cortisol and TGFB1 showed no associations with sleep interference scale scores. The mechanisms to explain these findings are unclear but it would appear that early life adversity moderates the association between self-reported distress and stress biomarkers that should be considered in the design of future studies in this area.

Chapter 4

4   Conclusions and Recommendations

This study supported the promising role of cortisol and BDNF as acute intervention targets following trauma for the prevention of chronic pain development. It can also be proposed from the results of the study that that people who experienced childhood adversities may react to subsequent stressful events differently than those with no such exposure, likely due to a complex interplay of both psychological and physiological processes which in turn may predispose to chronic pain development. However, several limitations of this study should be taken into consideration when interpreting the results.

4.1   Limitations and future directions

The study had a small sample size. According to my findings, if I wanted to fully explore the association between cortisol and negative affect in people with no adverse childhood experiences (correlation magnitude of 0.24), desiring 80% power and accepting a 5% alpha error rate, 99 subjects would be required (Faul, Erdfelder, Lang, & Buchner, 2007). Replicating the results using a larger sample is necessary. Generalizability of these findings may be limited due to the specific setting of the study along with the small sample size. This study only focused on the people who survived a minor traumatic event.

As the study was cross sectional in nature, it was not able to indicate any causal relationship. More information is needed to reach any conclusions based on the results of

the study. In the future, similar research should be conducted with follow up at a regular basis to determine the complex nature of the biological and psychological markers of pain.

Childhood adverse effects were assessed retrospectively by ACE questionnaire. Though the ACE questionnaire is a widely used and reliable tool to diagnose early life adversities, the chance of memory bias is not to be ignored. Furthermore, the participants were categorized dichotomously. The participants who scored 1 to 8 were considered as ACE group. It is possible that the people who scored higher (4 to 8), perceived stressful conditions differently than the people who had moderate (1 to 3) childhood adverse experiences. Different types of maltreatment (physical, sexual and emotional abuse, parental separation, death of a parent in childhood) could have different influences on stress biomarkers that were not examined separately in the present study. All these factors should be evaluated separately to find out the modifying role of childhood adversities on stress markers. This needs to be addressed in the future research.

The impact of daily life stressors was not assessed in the current study. Current life stressors may influence the biomarkers which is a direction for additional research in the future.

In this study, the serum cortisol level was determined in those people who presented to the emergency department between 10 am to 5 pm with a history of non-catastrophic musculoskeletal injuries. After obtaining consent, blood was drawn to measure stress biomarkers level. Cortisol secretion varies throughout the day. The diurnal secretion of cortisol and the resulting variation in serum cortisol concentration made the interpretation of a single cortisol value of the current study problematic. There may be variations in

cortisol concentration in people who showed up in the emergency department at different times of day. Venipuncture itself can results in the rise of cortisol level. All these factors should be kept in mind while evaluating the results of the current study.

This study used serum BDNF level which may not be a direct measure of central BDNF level, although a strong correlation has been observed between central and peripheral BDNF level in animal studies (Karege, Schwald, & Cisse, 2002). An interesting topic for the future studies could be the evaluation of the correlations between central and peripheral BDNF level in humans.

By considering the preliminary stage of this study, caution was taken while identifying outliers and removing them. It is possible that some outliers on a specific scale may represent an interesting subset of the population which could not be explored at this time, due to the limited sample size. However, the figures represented the relationships between stress markers (included in the result section) confirmed evenly distribution of scores. It is possible that few existing outliers could affect the relations dramatically. Hence, Additional work is warranted before interpreting the results of the study.

4.2 Conclusions

In summary, the current study provided tentative evidence for plasma cortisol and BDNF as acute stress biomarkers that appear to be associated with some aspects of post-traumatic pain and distress. Cortisol in particular was correlated with several perceived stress scores. The influence of demographic variables on both biological and psychological stress

markers provided supportive evidence to be considered in the design of future trauma-related pain studies. The associations between the stress markers in people who endorsed a history of childhood adversities provided preliminary support for the modifying effects of the adverse childhood effects on individuals' reactions to a stressful condition in adulthood. These findings point to a possible interplay between stress biomarkers, psychologically perceived stress level, demographic and socio-economic status and early life stress in the acute pain setting that may suggest a new target for intervention to prevent chronic pain. These associations need to be explored further to better understand the influence of acute distress on pain and the subsequent development of chronic problems.

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Appendices

Appendix A: Ethics approval form

Western Research

Western University Health Science Research Ethics Board
HSREB Delegated Initial Approval Notice

Principal Investigator: Dr. Dave Walton
Department & Institution: Health Sciences\Physical Therapy, Western University

Review Type: Expedited
HSREB File Number: 106140
Study Title: Modeling post-traumatic pain and recovery: The SYMBIOME longitudinal cohort study
Sponsor: Canadian Institutes of Health Research

HSREB Initial Approval Date: November 17, 2015
HSREB Expiry Date: November 17, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Other	Instructional pamphlet for biomarker collection	2014/11/28
Instruments	Intake questionnaires package	2014/12/05
Instruments	Follow-up questionnaires package	2014/12/05
Instruments	Intake questionnaire package	2015/10/29
Instruments	Screening form	2015/10/29
Instruments	Follow-up forms	2015/10/06
Advertisement	flyer	2015/11/02
Other	Crisis management protocol (received for information only)	2015/10/29
Recruitment Items	consent to contact form	2015/05/29
Western University Protocol		2015/11/15
Letter of Information & Consent		2015/11/15

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer to Contact for Further Information: Erika Basile ___ Nicole Kaniki ___ Grace Kelly ___ Mina Mekhail _✓_ Vikki Tran ___

This is an official document. Please retain the original in your files

Appendix B: Letter of information and consent form

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version: February 2017
Principal Investigator: Dr. David Walton
Funding source: Western internal funding, CIHR bridge grant, Canadian Pain Society

Letter of Information

Modeling recovery after traumatic injuries: A Longitudinal Databanking project

Dear Sir/Madam,

Thank you for your time in reviewing this letter of information and for considering participation in our study. Please be sure to read this letter in its entirety and have any questions you may have answered to your satisfaction before consenting to participate.

Why am I being invited to participate?

You are being invited to participate because you have indicated that you are a male/female at least18 years old and are seeking care from emergency, medical or rehabilitation services for a recent accident or injury to your muscles, bones or ligaments, or because you have responded to one of the posted advertisements for this study.

You are not eligible for this study if any of the following apply to you. Please tell the research coordinator if any of these apply:

1. Severe gingivitis, periodontal disease, active dental caries (tooth decay), or any other active oral condition
2. Actively undergoing cancer treatment
3. You have a neuromuscular disorder (e.g. stroke, multiple sclerosis, ALS or 'Lou Gehrig's Disease')
4. You have an autoimmune disorder (e.g. rheumatoid or psoriatic arthritis, scleroderma, lupus)
5. You have severe heart, lung, kidney or liver disease that affects your ability to function in every day life
6. You currently have stomach ulcers, Celiac or Inflammatory Bowel Disease (Ulcerative colitis or Crohn's Disease)

What is this study about?

Initials:_____   Page 1 of 9

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We are trying to understand the process of recovery over the 6 months following a traumatic injury, and to identify things (factors) that may explain why people differ in how they recover after these events. We will be collecting information including the nature of your injury, your biology, psychology, and past experiences all in the same period. Our goal is to not only improve understanding of *how* people recover following different types of injuries, but what factors influence that recovery. By identifying important factors we will start to work on developing new ways to treat those factors and eventually improve the likelihood of successful recovery for people injured in the future.

What will I be asked to do?

If you agree to participate, you will be provided with a package that includes almost all of the data collection instruments that you will be asked to complete on your own at home starting at least 48 hours after your injury. The procedures include questionnaires for you to complete and different vials into which you will provide saliva and a stool sample. Once collected, the samples can be stored in your home freezer until a member of the research team comes to pick them up. The questionnaires will be repeated 1, 2, 3, 6 and 12 months after you enter the study and the biological sampling will be repeated after 3 and 6, and 12 months. After the 12thmonth, your participation in the study will be complete. Below you will find more detailed information on the types of data instruments in this study.

1. A set of questionnaires that will ask you about a variety of different things. These include: i) your age, sex, work and educational status, ii) the nature of your injury (type of injury, when it occurred, how long ago it occurred, a brief description of the injury itself), iii) your medical and legal involvement (if any), iv) experiences from your childhood, including bullying and home environment, v) recent stressors you may have experienced, vi) the stress you have experienced as a result of your injury, vii) the type and amount of symptoms and interference you have experienced as a result of your injury.

2. **Drool/Saliva (part 1)** – You will receive 3 specialized test tubes with sterile cotton swabs in each.You will start on a day that is convenient to you within 3 days of completing your questionnaires. A pamphlet explaining all procedures is included with the instruments. This pamphlet should be read in its entirety. The tubes with the cotton swabs are to be used 3 times during the same day – once immediately upon waking, again 20-30 minutes after waking, and again mid-afternoon between 2pm and 4pm. This will require you to chew the cotton swab for about 10 seconds before returning it to the test tube, sealing it and placing it in your freezer.

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3. **Drool/Saliva (part 2):** You will receive a specialized test tube into which you will spit or drool a small amount of saliva BEFORE your nightly (bedtime) routine, before brushing but at least 2 hours after eating. Once completed, this and the other samples can be stored in your residential freezer until retrieved by a member of the research team.
4. **Serum:** A trained phlebotomist will draw 2 vials of blood from the vein on the front of your elbow or forearm.

The following two components are optional.

5. **Stool:** This is an optional part of the study. You will provide a sample of stool using a specialized, sterile tube with a Q-tip type cotton swab. This will simply require you to twirl the end of the swab in a piece of used bathroom tissue, sealing it in the test tube and placing in your freezer. Only a small sample is required, and this can be collected at any time of day.
6. **Hair:** This is an optional part of the study for which you will be compensated if you choose to participate. As long as you have at least 3cm of hair on your head, we will cut approximately 100 hairs from the back of your scalp in a manner that minimizes any obvious physical change in your hair style using sterile scissors. This will be done by a member of the research team, and will be repeated with saliva and blood collection.

We are collecting saliva samples in order to analyze the levels of specific proteins, which we are calling "biomarkers", that are typically present in the body and that may change during times of stress. Specifically, these are classed broadly as the stress hormone *cortisol*, the gonadal hormone *testosterone*, and immune or inflammatory markers that are referred to as '*cytokines*'. Stool samples, on the other hand, will provide us with specific information regarding the different bacterial populations that inhabit your intestines. The types of bacteria in your intestines may be influenced as a result of significant stressors, such as trauma or injury. We will be looking to see if any major shifts in the types of bacteria occur in your system as you are recovering. There is some research that suggests certain genes play a role in the speed and effectiveness of recovery from an injury. The blood will allow us to look at very specific genes to help us understand what is driving different recovery trajectories. At this stage we are planning to target specific genes and explore them only for their potential role in your pain and recovery. None of these analyses will indicate the current existence or future potential of any disease states. However, your data will be included as part of 'databank' for ongoing research by our or other researchers for currently unplanned research questions. These may involve sequencing your entire genome. Finally, from your hair we will be able to determine the

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presence of different hormones that have been stored in your hair from the time before your injury.

It is important to understand that everyone's body is different and it's currently difficult to say what is 'good' or 'bad' in these analyses. For that reason, these tests should not be considered diagnostic of any specific diseases or conditions.

Once all samples have been collected, contact the research team at Western University. These samples will then be retrieved from you by a member of the research team at a day, time and location that is convenient for you. A subset of the questionnaires will be completed again at 1, 2, 3, 6 and 12 months after you enter the study(approximately 10 minutes to complete). The biological samples (saliva, blood, stool) will be collected at 3, 6 and 12 months. After the 12th month, your participation in the study is complete.

What are the risks and benefits of participating?

There are no immediate anticipated benefits to you from participating in this study. However, if our predictions are correct and we are able to identify dysfunction in key systems that can explain at least part of the pain experience, this may open new avenues to treatment that may have benefit to you or others in the future.

All participants may receive a final report of the study in which the results (using only group data) will be presented. If you wish to receive this report, you will need to indicate this on the consent form and include contact information to which the report should be sent. Those participants who wish to receive their own individual results will be required to contact the Lead Researcher Dr. David Walton directly to make that request. His contact information can be found at the end of this letter. Keep in mind that the data associated with this study is not a medical record and shouldn't be used as such. We will keep the Master List that links your name with your ID number for 6 months after your completion of the study after which it will be shredded for confidentiality and privacy protection reasons. This means that we will not be able to provide your individual results beyond 1 year from your injury.

The risks to participation are minimal and are largely inconveniences due to time. The salivette (saliva collection tube with cotton swab) samples must be performed at three separate times throughout a single day which maybe a mild disruption toyour daily routine for that day. Improper collection and handling of stool samples MAY pose a risk of bacterial contamination/infection, however, if carefully performed (including washing your hands afterwards), this risk is quite minimal.The blood will be drawn using a standard protocol by a

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trained phlebotomist that you have likely experienced before in a doctor's office or the Red Cross. Completion of the questionnaires may lead to some people experiencing emotional distress, especially those that ask you to recall and reflect upon childhood experiences if yours were not positive. We have provided suggestions for managing emotional distress, should you experience it, at the end of this letter and in the forms package itself.

We will do everything in our power to ensure your data, including the biological specimens and your questionnaires, are kept secure and confidential. However, we cannot guarantee against a data breach regardless of how good our physical and virtual security is. Your data will be stored with only a random ID number in order to mitigate any potential risk, with the master list containing your identifying information stored separately on the campus of Western University in London. Nonetheless the risk of data breach or loss is possible and we want to ensure you're aware of this.Should this happen you will be quickly informed.

Will I be compensated for my participation?

You have different options for the degree to which you wish to participate in this study. The minimum level of participation is to complete the paper forms, saliva, and blood draw. This would be done once when you enter the study, then at 1, 2, 3, 6 and 12 months later. Each follow-up will likely take about 45 minutes of your time, and you will receive $30 total for participating in this level of the study. The hair and stool are optional components, and for each one you will receive an additional $15 ($30 for both). We recognize that collecting these samples is no small commitment, but can be completed in its entirety in a single day and a total anticipated time commitment of approximately 1 hour at each collection period. Out of respect for your time, you will be thereforereimbursed a minimum of $180total for participating in each phase of this study (intake and 1, 2, 3, 6, and 12 months). If you complete the two additional components you are eligible for an additional $30 per session, up to an additional $120 for the entire study ($300 total reimbursement).

Who will have access to my information?

A unique randomly-generated 6-digit ID number will appear on all forms belonging to you for the sole purpose of keeping your data anonymous while allowing the team to connect the data you providefor analysis. Once transcribed, all survey data are stored on the secure, password protected and firewalled server of Western University and the paper forms are shredded. Your biological samples (blood, hair, saliva, stool if applicable) are stored using separate barcodes in a secured scientific freezer that is physically located inside Victoria Hospital (London Health Sciences Centre) that is only accessible by approved people. Western University's REB and

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representatives from Lawson's Quality Assurance and Education Program will have access to participant's data to ensure that it is following the proper laws and regulations.Outside of these groups, your specific information will not be shared with anyone without your express written consent to do so.

Note some of the tools to be completed are meant to measure severity of symptoms related to depression or anxiety. *IF* your responses lead to a score that is suggestive of either significant depression or anxiety, your family doctor will be contacted to inform him/her of the results of the scale and what they may mean. It will ultimately be up to your family physician to decide how and when he/she should follow up with you if at all.

Data will be retained in anonymous form indefinitely as an ongoing database, but any information that connects your personal or contact information to your data will be destroyed 5 years after you complete the study. This means that there may be additional research conducted in the future about which we do not currently know that may use some or all of your data and for which you may not be able to be contacted for consent. If you should wish to have your data *removed* from the database within 5 years from your participation in this study, you will need to contact the lead researcher Dr. David Walton whose contact information can be found below and request it. You will *not* be required to provide an explanation.

Voluntary participation

Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time. If you choose to withdraw from the study, you may request to have your contributions to that point removed, at any time up until 6 months after you are done the study. Withdrawal from the study or refusal to participate is your decision, and may be done without the requirement of explanation on your part. Withdrawal will in no way affect your current or future relationship with any of the research team or clinicians associated with the study.

We request that if you move at any point during your participation in this study, you please let the research team know by emailing

What if I want more information?

You may contact the lead researcher, Dr. David Walton, at Western University (London, Canada) if you require any further clarification. His contact information can be found below. If

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you have any questions about your rights as a research participant or the conduct of the study you may contact the Office of Research Ethics at   or by email at   . You are encouraged to keep this letter of information for your own records.

If you wish to receive a summarized copy of the results of this study and/or your individual results, you may leave your email address on a separate sheet. The sheet will be held by the research coordinator, and the email addresses will only be used to provide the results, after which the list will be destroyed.

We thank you in advance for considering participation in this study. *You do not waive any legal rights by signing this consent form.*

Sincerely,

David Walton BScPT, PhD
Lead Researcher
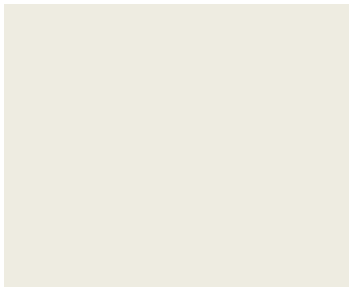
Co-researchers:
Ruth Lanius MD PhD
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Greg Gloor PhD
Walter Siqueira DDS, PhD
Eric Wong MD
Doug Fraser MD, PhD
Melanie Colombus
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Marnin Heisel MD, PhD
Joshua Lee PhD (c)
Sadia Siraj MSc (c)
Gordon Good
Joy MacDermid PT PhD
Lynn Cooper
Jordan Miller PT PhD (c)
Siobhan Schabrun PT PhD
James Elliott PT PhD
Paul Phares

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Information and Resource Sheet

The information in this study will help us and other health professionals identify those things that may influence whether someone is likely to recover or not, and will lead to new ways to deal with acute injuries.Personal injury can sometimes be hard to deal with, both physically and emotionally. If you find that you are experiencing emotional problems as a result of your injury, you should first know that this is not uncommon and can usually be addressed through talking with someone you trust or a trained professional. If you personally find your emotions are difficult to manage, do not be ashamed. It is most often better to address them early rather than assume they will go away on their own. You may have found that answering some of the questions in this booklet has stirred emotions in you that you didn't even know were there. In any of these cases you might find it helpful to speak with someone about these challenges, potentially including a close family member, friend, confidant, or healthcare provider.

If you would like to give permission for your healthcare provider to contact us to find out more about your participation in this study, please feel free to either contact us directly or have your doctor contact us. The lead researcher is Dr. David Walton,

You might find contacting some of the following helpful if you are experiencing emotional difficulties:

1. A Supportive Family Member or Friend

2. A Member of the Clergy (Rabbi, Priest, Nun, Minister, Pastor, Imam, etc.)

3. Your Family Doctor or Other Healthcare Provider

4. Telephone Support Lines (London and District Distress Centre; available 24 hours/day):
   Distress Line
   Crisis Response Line

5.South West Community Care Access Centre (CCAC)

6. Community Social Workers (in your neighbourhood hospital, clinic, or community centre)

7. Psychiatrists or Psychologists (in your neighbourhood hospital, clinic, or in private practices)

8. Telehealth Ontario (available 24 hours/day)

9.Emergency Care from Your Local Emergency Care Facility, including:

University Hospital:
Victoria Hospital:
St. Joseph's Health Care:
St. Joseph's Urgent Care Clinic:

10. In the Event of an Emergency or Crisis:

11. The Canadian Mental Health Association also includes several resources on their website that may be of help: http://www.cmha.ca/mental-health/find-help/

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November 15, 2015

Consent form

Modeling recovery from traumatic injuries

Principal Investigator: Dr. David M. Walton PT PhD

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction. I also consent to being contacted by the Lead Researcher in the case any of my scale scores suggest possible problems with depression or anxiety.

Please indicate the level of study participation to which you are consenting by placing a check in the appropriate circle:

○ Paper forms, saliva and blood only (paper forms at 1, 2, 3, 6, and 12 months, approximately 20 minutes each, saliva and blood at 3, 6, and 12 months. $30 compensation per period, max $180)

○ (Optional): Provide stool at intake, and 3, 6, and 12 months (additional $15 per period, max $60)

○ (Optional): Hair provided at intake, and 3, 6, and 12 months (additional $15 per period, max $60)

_____
Participant name (print)

_____   _____
Participant signature   Date

_____
Person obtaining consent (print)

_____   _____
Signature of person obtaining consent   Date

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Appendix C: Traumatic Injuries Distress Scale (TIDS)

ID No.: _____   Date: _____

TIDS

RATE THE EXTENT TO WHICH YOU HAVE BEEN BOTHERED BY THE FOLLOWING SYMPTOMS <u>SINCE YOUR ACCIDENT</u>:	Never	Occasionally	Often or All of the Time
1. Difficulty maintaining your concentration	0	1	2
2. Difficulty thinking about anything other than the pain	0	1	2
3. A feeling of being overwhelmed by pain or other symptoms	0	1	2
4. Flashbacks of the accident while you're awake that feel very real	0	1	2
5. Feeling 'wound up', agitated or scared when in a place that reminds you of the accident (e.g. In a car, at work or on a slippery surface)	0	1	2
6. Frustration at your inability to control your pain	0	1	2
7. Loss of motivation to get up and start a new day	0	1	2
8. Pain that lasts an entire day, without really easing	0	1	2
9. Loss of interest in your appearance	0	1	2
10. Difficulty doing the things that you would normally enjoy	0	1	2
11. Feeling 'numb' or disengaged, as if you were watching the world through a window	0	1	2
12. Anger directed at others	0	1	2

Appendix D: Acute Stress Disorder Scale (ASDS)

ID No.: _____   Date: _____

<div align="center">ASRQ</div>

This questionnaire asks you about your reactions to the accident/injury (called 'trauma') you recently experienced.

1. Did the experience frighten you?

   ○ Yes or ○ No

Please answer each of these questions about how you felt since the event. Circle one number beside each question to indicate how you felt.

	Not at all	Mildly	Medium	Quite a bit	Very much
1. During or after the trauma, did you ever feel numb or distant from your emotions?	1	2	3	4	5
2. During or after the trauma, did you ever feel in a daze?	1	2	3	4	5
3. During or after the trauma, did things around you ever feel unreal or dreamlike	1	2	3	4	5
4. During or after the trauma, did you ever feel distant from your normal self or like you were watching it happen from outside?	1	2	3	4	5
5. Have you been unable to recall important aspects of the trauma?	1	2	3	4	5
6. Have memories of the trauma kept entering your mind?	1	2	3	4	5
7. Have you had bad dreams or nightmares about the trauma?	1	2	3	4	5
8. Have you felt as if the trauma was about to happen again?	1	2	3	4	5
9. Do you feel very upset when you are reminded of the trauma?	1	2	3	4	5
10. Have you tried not to think about the trauma?	1	2	3	4	5
11. Have you tried to not talk about the trauma?	1	2	3	4	5
12. Have you tried to avoid situations or people that remind you of the trauma?	1	2	3	4	5
13. Have you tried not to feel upset or distressed about the trauma?	1	2	3	4	5
14. Have you had trouble sleeping since the trauma?	1	2	3	4	5
15. Have you felt more irritable since the trauma?	1	2	3	4	5
16. Have you had difficulty concentrating since the trauma?	1	2	3	4	5
17. Have you become more alert to danger since the trauma?	1	2	3	4	5
18. Have you become jumpy since the trauma?	1	2	3	4	5
19. When you are reminded of the trauma, do you sweat or tremble or does your heart beat fast?	1	2	3	4	5

Appendix E: Brief Pain Inventory (BPI) Scale

ID No.: _____   Date: _____

BPI

1. Please rate your pain by marking the box best describes your pain **at its worst** in the last 24 hours

0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain

2. Please rate your pain by marking the box beside the number that best describes your pain **at its least (best)** in the last 24 hours

0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain

3. Please rate your pain by marking the box beside the number that best describes your pain **on average**

0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain

4. Please rate your pain by marking the box beside the number that describes how much pain you have **right now**

0	1	2	3	4	5	6	7	8	9	10
No Pain										Extreme Pain

5. Circle the number that describes how much, **during the past 24 hours**, pain has interfered with your:

A. General Activity

0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

B. Mood

0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

C. Walking Ability

0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

D. Normal Work (includes both work outside the home and housework)

0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

E. Relations with other people

0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

F. Sleep

0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

G. Enjoyment of Life

0	1	2	3	4	5	6	7	8	9	10
Does Not Interfere										Completely Interferes

Oct 2015

Appendix F: Adverse Childhood Experiences (ACE) Questionnaire

ID No.: _____   Date: _____

The next questionnaire will ask you about experiences from your youth. This is intended to provide us with a more complete picture of all things that may have affected you while growing up, especially as it relates to different stressors in your life. As with all such information, this will be kept in strictest confidence.
While you were growing up, <u>during your first 18 years of life</u> (circle Yes or No for each):

1. Did a parent or other adult in the household **often or very often**... • Swear at you, insult you, put you down, or humiliate you? or • Act in a way that made you afraid that you might be physically hurt?	Yes No
2. Did a parent or other adult in the household **often or very often**... • Push, grab, slap, or throw something at you? or • **Ever** hit you so hard that you had marks or were injured?	Yes No
3. Did an adult or person at least 5 years older than you **ever**... • Touch or fondle you or have you touch their body in a sexual way? or • Attempt or actually have oral, anal, or vaginal intercourse with you?	Yes No
4. Did you **often or very often** feel that ... • No one in your family loved you or thought you were important or special? or • Your family didn't look out for each other, feel close to each other, or support each other?	Yes No
5. Did you **often or very often** feel that ... • You didn't have enough to eat, had to wear dirty clothes, and had no one to protect you? or • Your parents were too drunk or high to take care of you or take you to the doctor if you needed it?	Yes No
6. Were your parents **ever** separated or divorced?	Yes No
7. Was your mother or stepmother: • **Often or very often** pushed, grabbed, slapped, or had something thrown at her? or • **Sometimes, often, or very often** kicked, bitten, hit with a fist, or hit with something hard? or • **Ever** repeatedly hit at least a few minutes or threatened with a gun or knife?	Yes No
8. Did you live with anyone who was a problem drinker or alcoholic or who used street drugs?	Yes No
9. Was a household member depressed or mentally ill, or did a household member attempt suicide?	Yes No
10. Did a household member go to prison?	Yes No

Oct 2015

Curriculum Vitae

Sadia Siraj

Education:

Masters of science Health and Rehabilitation Sciences (Physical Therapy) University of Western Ontario, London, ON	2015 - present
Bachelor of Medicine and Bachelor of Surgery (MBBS) Sher-E-Bangla Medical College University of Dhaka, Dhaka, Bangladesh	2006 - 2011

Academic Awards:

Western Graduate Research Scholarship	2015 - 2017
Bangladesh Higher Secondary Education Board merit scholarship	2005
Bangladesh secondary Education Board merit scholarship	2003
Junior scholarship award	2001
Award from the Family Planning Institute for work excellence, Sher- E-Bangla Medical College Hospital	2014

Job Experiences:

Graduate Research Assistant, Health and Rehabilitation Sciences. University of western Ontario, London, Ontario, Canada.	Sept. 2015 - present
Graduate Teaching Assistant, Health and Rehabilitation Sciences. University of western Ontario, London, Ontario, Canada.	Sept. 2016 - Apr.2017
Intern Doctor, *Sher-E-Bangla Medical College Hospital*, Barisal, Bangladesh.	Nov. 2011- Nov. 2012
Medical Officer, Family Planning Institute, Sher-E-Bangla Medical College, Bangladesh.	Jan. 2013- Apr. 2013

Medical Officer, *Marie Stops Maternity Centre*, Jordan Road, Barisal, Bangladesh.   June 2013 – Sept. 2013

Residential Medical Officer, *Jhilmil Hospital*, Begunbari, Chunkutia Chourasta, South Keranigonj, Dhaka, Bangladesh.   Oct. 2013 – Dec. 2013

Professional Memberships:

Bangladesh Medical and Dental Council   2013- present

Breast Cancer Awareness Society (BCAS)   2006 - 2012

Bangladesh Diabetic Association   2011 - 2013

Bangladesh Red Crescent Society   2011 - 2013