EXERCISE, INFLAMMATION, AND DEPRESSION

THE EFFECTS OF HIGH-INTENSITY INTERVAL TRAINING ON MENTAL HEALTH AND THE ACUTE INFLAMMATORY RESPONSE

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A Thesis Submitted to the School of Graduate Studies in Partial Fulfillment of the Requirements for the Degree Master of Science

MASTER OF SCIENCE (2017) McMaster University
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TITLE: The effects of high-intensity interval training on mental health and the acute inflammatory response.

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NUMBER OF PAGES: x, 77

ABSTRACT

Mental illness is on the rise in university students, with approximately one third of all students experiencing depression. One way that depression can arise is through chronic stress, a common experience for university students. Exercise has been shown to buffer the effects of chronic stress and improve depression. One hypothesis suggests that exercise improves depression by decreasing pro-inflammatory cytokines at rest; however, it is unclear what role exercise intensity plays. Prior work has shown that over 6 weeks of training, HIIT buffered the development of depressive symptoms, but increased stress, anxiety, and resting-state pro-inflammatory cytokines IL-6 and TNF-a. However, it is unknown how HIIT influenced the acute inflammatory response, and if adaptations in this response can improve mood. The aim of this study was to examine the effect of HIIT on mood and both resting state and acute inflammation over a training period of 9 weeks. Fifty-two sedentary university students (63% females), aged 18-30 (*M*±*SD*: 19.9±2.2 years) were randomized into one of two groups: 1) high-intensity interval training (HIIT), consisting of exercise three times per week, or 2) sham-exercise (controls), who were instructed to remain sedentary during the intervention. The sham-exercise group was given a cover story that they were part of an "acute" exercise group, and thus believed they were also assigned to an exercise condition. Mood was tracked weekly using surveys for depression (BDI-II), anxiety (BAI), and stress (PSS). Levels of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β) were measured using serum draws at rest and immediately after the completion of a peak power output test at the beginning, middle, and end of the training period. Both groups decreased in depression and anxiety across

the nine-week training period ($p < 0.001$), and decreased in stress during the first three weeks of training ($p = 0.07$), suggesting that HIIT exercise does not improve mood to a greater extent than a sham-exercise condition. Interestingly, at the end of training, HIIT had higher levels of IL-6 compared to controls ($p = 0.03$), and this may be why one reason why the HIIT group did not improve mood over controls. Despite an increase in overall IL-6 levels at rest, nine weeks of HIIT attenuated the acute pro-inflammatory response to exercise compared to controls, who experienced an elevated IL-6 response ($p = 0.04$) and TNF-α response ($p = 0.003$). Taken together, these results suggest that HIIT exercise impacts the inflammatory response both at rest and in response to maximal exercise, but it may not be an effective intensity of exercise for improving mental health outcomes in sedentary individuals.

ACKNOWLEDGEMENTS

First I would like to thank my supervisor Dr. Jennifer Heisz for the opportunity to complete my Master of Science degree under her guidance in the NeuroFit Lab. Her encouragement helped me to tackle challenges successfully, and develop a passion for research.

I would also like to thank my committee, Dr. Steven Bray and Dr. Dawn Bowdish, for their insight on my project, which helped to expand my knowledge base and synthesize a thesis that covers a broad range of topics in the field of psychoneuroimmunology and exercise.

Additionally, thank you to my labmates in the NeuroFit Lab for your uncanny ability to help me solve problems, and most importantly- your friendship. To the McMaster Kinesiology Graduate Body, thank you for your family vibe and positive energy. The past two years a part of this community has made my experience even more fulfilling!

To my parents, Marty, friends, and family, thank you for your unconditional love and positive encouragement; I could not have completed this journey without your support. Mom and Dad, thank you for being my biggest supporters and superb role models. I love you very much.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BAI	Beck Anxiety Inventory
BDI-II	Beck Depression Inventory- II
BDNF	Brain-derived neurotropic factor
CNS	Central nervous system
GR	Glucocorticoid receptor
HIIT	High-intensity interval training
HPA-axis	Hypothalamic-pituitary-adrenal axis
HR	Heart Rate
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IFN-α	Interferon-alpha
IQR	Interquartile range
MCT	Moderate continuous training
MDD	Major depressive disorder
NSAID	Non-steroidal anti-inflammatory drug
η_p^2	Partial eta-squared
PSS	Perceived Stress Scale
RER	Respiratory Exchange Ratio
RPE	Rating of Perceived Exertion
SAM- axis	Sympatho-adrenal-medullary axis
T_H1	T-helper-1 cell
T_H2	T-helper-2 cell
TNF-α	Tumor necrosis factor alpha
VO_2 peak	Maximum oxygen consumption

DECLARATION OF ACADEMIC ACHIEVEMENT

E.M. Paolucci's Role:

- Contributed to study concept, design, and measurement selection
- Contributed to ethics amendment
- Led participant recruitment
- Preparation of lab settings and materials
- Trained and supervised volunteers who assisted with data collection
- Responsible for data collection, analysis, and interpretation

Role of JJH:

- Obtained study funding
- Assisted with ethics amendment
- Assisted with study concept, design, and measurement selection
- Assisted with data analysis

INTRODUCTION

Overview

University students face a multitude of unique challenges that can cause chronic stress and depression (Beiter et al., 2015). Exercise may be a promising approach to help manage these stressors (Rimmele et al., 2007) and reduce depression (Mead et al., 2009) because of its anti-inflammatory effects (Eyre & Baune, 2012; Gleeson et al., 2011). However, it is unclear whether all forms of exercise are equally beneficial. Previous work has investigated low-to-moderate intensity exercise (Rethorst et al., 2013), but the benefits of higher-intensity exercise are unclear. The present study examined the effects of high-intensity interval training (HIIT) on mood and pro-inflammatory cytokines. The ultimate goal is to refine the prescription of exercise for mental health within a university student population.

In the following sections, I review: 1) the prevalence of depression amongst both general and student populations, 2) current treatments for depression, 3) the pro-inflammatory mechanism for stress-induced depression, 4) pro-inflammatory cytokines associated with depressive symptoms, clinical depression, and anxiety, 5) cytokines as a potential epiphenomenon of clinical depression, and 6) how exercise can positively impact both stress and pro-inflammatory cytokines.

Challenges of living with depression

Approximately 12% of all Canadians will experience an episode of major depression, and it is generally more common in women than men (Patten et al., 2006). The criteria for diagnosis constitutes having a depressed mood or loss of pleasure in daily

activities for more than two weeks (*DSM 5*, 2013). In addition, five of the following nine symptoms must be experienced nearly every day: irritable or depressed mood, decreased interest or pleasure, significant weight change or change in appetite, change in sleep, change in activity, fatigue or loss of energy, guilt or worthlessness, concentration, or suicidality (*DSM 5*, 2013). Although diagnoses can be made effectively with these criteria, one key problem for developing treatments is that depression can be caused by various pathophysiological mechanisms (Nutt et al., 1999; Rosenblat, Cha, Mansur, & McIntyre, 2014), making it difficult to target with pharmaceuticals.

In addition to mood disturbances, people with depression face an abundance of other daily challenges, such as frequent sleep disturbances (Breslau, Roth, Rosenthal, & Andreski, 1996) and poorer sleep quality (Hall et al., 2000). Depression also negatively impacts one's social life, as people with higher levels have fewer positive social interactions and more negative interactions on a daily basis (Steger & Kashdan, 2009). Depression is also more prevalent in people who are unemployed or experiencing chronic diseases (Patten et al., 2006), further adding to daily challenges.

Prevalence of depression among students

According to the National College Health Assessment-II done in 2016, depression has been on the rise in Canadian university students since 2013 ("American College Health Association-National College Health Assessment II: Ontario Canada Reference Group Executive Summary Spring 2013," 2013; "American College Health Association-National College Health Assessment II: Ontario Canada Reference Group Executive Summary Spring 2016," 2016) . Indeed, approximately 30% of students experience

depression at some point during university (Ibrahim, Kelly, Adams, & Glazebrook, 2013), which is higher than the prevalence of 12% within the general population (Patten et al., 2006). The most serious outcome of depression is suicide, which is the second most common cause of death within young adults aged 15-34 (Statistics-Canada, 2015), thus indicating the critical need to discover effective treatments for depression.

Current approaches to treatment and etiology of depression

The initial pathophysiological cause of depression was thought to involve serotonin. Specifically, the *Serotonin Hypothesis* posits that individuals with lower functioning serotonin pathways are more likely to develop depression (Cowen & Browning, 2015). Serotonin is a neurotransmitter in the brain responsible for mood regulation (Martinowich & Lu, 2008), and in healthy amounts, rewards optimistic thoughts and behaviour while limiting negative patterns of thinking (Cowen & Browning, 2015). When serotonin's precursor tryptophan is depleted, it reduces the amount of available serotonin, which can lead to the development of negative thinking patterns and rumination (Cowen & Browning, 2015). Selective serotonin reuptake inhibitors (SSRIs) were created as a pharmaceutical response to increase levels of serotonin within the brain (Nutt et al., 1999). SSRIs block the receptors that clear serotonin from neural synapses, and consequently increase the synaptic concentration of serotonin in the brain (Nutt et al., 1999). Indeed, this drug improves mood and reduces depressive symptoms after approximately three weeks (Nutt et al., 1999), and is currently the most common pharmaceutical treatment for depression.

However, approximately one-third of patients taking SSRIs do not respond and thus, experience no relief from their depressive symptoms (Fava & Davidson, 1996). Interestingly, these same non-responders also have higher levels of resting-state pro-inflammatory cytokines such as IL-6 and TNF-α (Lanquillon, Krieg, Bening-Abu-Shach, & Vedder, 2000; O'Brien, Scully, Fitzgerald, Scott, & Dinan, 2007). Pro-inflammatory cytokines are large proteins released by white blood cells that amplify the body's immune response. This elevation in non-responders suggests that pro-inflammatory cytokines may also be implicated in the development of depression. Although increased pro-inflammatory cytokines can reduce tryptophan to low levels (Cowen & Browning, 2015), individuals with high inflammation who take SSRIs still experience symptoms of depression (Fava & Davidson, 1996; O'Brien et al., 2007). This suggests that pro-inflammatory cytokines act through other pathophysiological mechanisms to produce depressive symptoms.

One alternative pathway is related to chronic stress, which can elevate resting-state levels of pro-inflammatory cytokines and depressive symptoms (Miller & Raison, 2016; Raison, Capuron, & Miller, 2006; Rosenblat et al., 2014). This is known as *stress-induced depression* (Bartolomucci & Leopardi). This proposed pathway stems from the observation that both acute and chronic stressors are important predictors of depression (Kendler, Karkowski, & Prescott, 1999). Acute psychological stress induces a pro-inflammatory response that elevates cytokine levels (Steptoe, Hamer, & Chida, 2007), and this inflammatory stress response is more pronounced in patients with Major Depressive Disorder (MDD) (Pace et al., 2006). Moreover, chronic stress is listed as a

key cause of non-resolving, elevated, resting-state inflammation in patients with depression (Haroon, Raison, & Miller, 2012). Importantly, chronic psychological stress is common amongst university students (Beiter et al., 2015), which may increase their risk of developing stress-induced depression. Additionally, a greater proportion of female students reported high levels of stress from academics (Beiter et al., 2015), suggesting that female students are more susceptible to developing depression through this pathway.

A central tenet of *stress-induced depression* is the relationship between the stress response, allostasis, and allostatic load. Allostasis refers to the regulation of the acute and chronic stress responses, which keeps them in healthy equilibrium (Dhabhar, 2014). The acute physiological stress response (i.e., the "fight or flight" response) allows individuals to respond immediately to their environment, and is essential for survival (Dhabhar, 2014; McEwen, 2003). When a threatening stressor in the environment is detected by our brain, our body triggers a rapid physiological response, activating our sympatho-adrenal-medullary (SAM) axis which releases adrenaline (Smeets, 2010; Ulrich-Lai & Herman, 2009). This fight-or-flight response is characterized by symptoms of rapid breathing and heart rate (Charmandari, Tsigos, & Chrousos, 2005). It also produces an acute pro-inflammatory response which increases circulating pro-inflammatory cytokines (Kim, Na, Myint, & Leonard, 2016; Steptoe et al., 2007). In general, this acute inflammatory response aids in proper immune system functions such as infection recovery and healing of tissue (Nathan, 2002; Woodroofe, 1995). A second, slower stress response is activated by the hypothalamic-pituitary-adrenal (HPA) axis, which releases glucocorticoids such as cortisol (Smeets, 2010; Ulrich-Lai & Herman, 2009). Once cortisol binds to

glucocorticoid receptors (GR) in the hippocampus, a negative feedback loop is activated to shut off the HPA-axis (Pariante & Lightman, 2008; Rosenblat et al., 2014). This signals the end of the stress response. As a result, both cortisol and adrenaline will resolve back to baseline within a relatively short period, ranging from a few minutes to a couple of hours (Dhabhar, 2014). Importantly, when these glucocorticoids bind to their receptors, they produce an anti-inflammatory response that will decrease circulating pro-inflammatory cytokines, allowing inflammation to return to baseline levels (Kim et al., 2016). This is a short-term stress response that keeps our stress system functioning properly, and our immune system primed for environmental threats.

Allostatic load refers to the chronic activation of the stress response. It is defined as the negative physiological consequences of a stress response lasting for several hours each day for multiple weeks or months (Dhabhar, 2014; McEwen, 1998, 2000). Chronically, elevated levels of cortisol causes desensitization of the GRs, which then prevents cortisol from binding to shut down the HPA-axis. This in turn inhibits the anti-inflammatory response (Kim et al., 2016; Pariente, 2008). Consequently, the physiological stress response becomes dysregulated. The HPA-axis becomes hyperactive; it produces high levels of circulating cortisol (Pariante & Miller, 2001; Raison & Miller, 2003), which elevates pro-inflammatory cytokines (Kim et al., 2016). Critically, individuals with MDD experience HPA-axis hyperactivity, and have elevated levels of cortisol and pro-inflammatory cytokines (Gillespie & Nemeroff, 2005; Nemeroff & Vale, 2004; O'Brien et al., 2007; Pariante & Lightman, 2008), thereby providing evidence for the link between the stress and immune systems in the etiology of depression.

Pro-inflammatory cytokines can bind to receptors in the hippocampus and amygdala (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Eyre & Baune, 2012; Miller, Maletic, & Raison, 2009), and can influence the brain through multiple pathways. In a healthy individual, the hippocampus has a large amount of GRs that act as a negative feedback loop to shut off the HPA-axis, and thus aid in regulating the stress response (Sapolsky, 2001; Sapolsky, Krey, & McEwen, 1986; Young, Haskett, Murphy-Weinberg, Watson, & Akil, 1991). However, elevated pro-inflammatory cytokines inhibit GR function in the hippocampus, and thus decrease GR sensitivity and perpetuate high levels of pro-inflammatory cytokines (Eyre & Baune, 2012; Kim et al., 2016; Rosenblat et al., 2014). If the GRs become insensitive, this can lead to chronically high levels of cortisol that can cause atrophy of hippocampal neurons (Sapolsky, 2001; Sapolsky et al., 1986; Young et al., 1991). Elevated pro-inflammatory cytokines can also impair hippocampal neurogenesis by reducing levels of brain-derived neurotrophic factor (BDNF) that is needed to support neural growth and development (Eyre & Baune, 2012; Rosenblat et al., 2014). Hippocampal damage is associated with the development of depressive symptoms and further dysregulation of the HPA-axis, which perpetuates the cycle of chronic stress (Kim et al., 2016; McEwen, 1999; Sapolsky, 2001; Sapolsky et al., 1986; Young et al., 1991).

The hippocampus also has neurons that communicate with the amygdala and prefrontal cortex, which are involved in emotional regulation and inhibition, respectively (Drevets, 2001; Wager, Davidson, Hughes, Lindquist, & Ochsner, 2008). As a consequence, damage to the hippocampus can result in dysregulation of both the

amygdala and prefrontal cortex to cause more global deficits in mood regulation (Drevets, 2001; Warner‐Schmidt & Duman, 2006).

Although the direct mechanism for the link between hippocampal damage and depression is not yet known, elevated neuroinflammation can affect metabolism of neurotransmitters such as serotonin, and causing imbalances that impair mood (Cowen & Browning, 2015; Eyre & Baune, 2012; Rosenblat et al., 2014). Circulating cytokines in the CNS also bind to receptors in the brain's immune cells called microglia, which in turn produce more pro-inflammatory cytokines (Haroon et al., 2012) and amplify levels of cytokines in the brain (Frank, Baratta, Sprunger, Watkins, & Maier, 2007), perpetuating these dysfunctions in mood regulation.

Pro-inflammatory cytokines implicated in depressive symptoms

Temporary increases in circulating cytokines can impact functioning of the hippocampus and its associated neurotransmitter pathways to produce sickness behaviours (e.g., social withdrawal, fatigue, and loss of appetite) (Beck, Steer, & Brown, 1996; Dantzer, 2001; Reichenberg et al., 2001; Shakhar & Shakhar, 2015; Suarez, Lewis, Krishnan, & Young, 2004; Wright, Strike, Brydon, & Steptoe, 2005). Two main cytokines involved in the pro-inflammatory response to stress and related to the development of sickness behaviour are TNF-α and IL-1β (Dantzer, 2001; Dantzer et al., 2008). In animal models, it has been shown that injecting healthy mice with TNF-α and IL-1β will induce sickness behaviour (Bluthe et al., 1994). Though in the short-term, sickness behaviour provides an evolutionary advantage by preventing the spread of disease through social withdrawal (Shakhar & Shakhar, 2015), chronically these

symptoms can lead to the development of depressive symptoms (Dantzer et al., 2008; Rosenblat et al., 2014). This is typically induced by chronic elevations of pro-inflammatory cytokines, such as heightened levels of TNF-α at rest (Kim et al., 2016). Sickness behaviour symptoms overlap with symptoms of depression including loss of appetite and social withdrawal (Beck et al., 1996; Dantzer et al., 2008). Another cytokine implicated in the development of depressive symptoms is IL-6, which is produced by leukocytes. At elevated levels, IL-6 is predictive of the subsequent development of depressive symptoms in humans (Gimeno et al., 2009). However, infusion of IL-6 did not induce depressive symptoms within mouse models (Brebner, Hayley, Zacharko, Merali, & Anisman, 2000), thus IL-6 may serve as a marker of depressive symptoms and is thought to contribute to the chronic systemic inflammation that is implicated in the development of these symptoms.

Human studies that examine the effects of transient increases in peripheral cytokines on mood give further evidence for a causal role of pro-inflammatory cytokines in the manifestation of depressive symptoms (Reichenberg et al., 2001; Suarez et al., 2004; Wright et al., 2005). After exposure to an acute immune challenge (such as a viral or bacterial infection), increases in circulating levels of TNF-α and IL-6 are seen, and are correlated with increases in negative mood (Wright et al., 2005) and depressive symptoms (Reichenberg et al., 2001; Suarez et al., 2004). Additionally, a higher expression of TNF-α post-immune challenge is associated with higher depressive symptom scores (Suarez et al., 2004), suggesting that increased cytokine expression may have a dose-response relationship with depressive symptoms. Indeed, similar cytokine profiles have been found

in patients with diagnosed depressive disorders, which will be highlighted in the following section. Moreover, cancer patients treated with cytokines interleukin-2 and/or interferon-alpha (IFN-α) developed significantly more depressive symptoms after the first month of treatment compared to baseline (Capuron et al., 2002), a key finding supporting the causal link between increased inflammation and the occurrence of depressive symptoms.

Interestingly, the link between elevated pro-inflammatory cytokines and depressive symptoms may explain why patients with chronic inflammatory conditions such as psoriasis report experiencing depressive symptoms at a higher rate compared to healthy controls (Devrimci‐Ozguven, Kundakci, Kumbasar, & Boyvat, 2000). When psoriasis patients are treated with anti-TNF-α drugs for their inflammatory condition, it has been found that their depressive symptoms will also improve (Menter et al., 2010; Tyring et al., 2006). This indicates that elevated TNF-α may play a role in the emergence of pre-clinical depressive symptoms.

There have also been sex differences reported in the immune system, where in general, females have a greater amount of circulating T-Helper-2 (T_H2) cells (Fairweather, Frisancho-Kiss, & Rose, 2008; Giron-Gonzalez et al., 2000; Klein, Bird, & Glass, 2001), which produce anti-inflammatory cytokines both peripherally and in the CNS (Aloisi, Ria, & Adorini, 2000; Nicholson & Kuchroo, 1996). Generally, males have a higher count of T-Helper-1 (T_H1) cells (Fairweather et al., 2008; Giron-Gonzalez et al., 2000; Klein et al., 2001), which produce an pro-inflammatory response both peripherally and in the CNS (Aloisi et al., 2000; Nicholson & Kuchroo, 1996). This suggests that men

may be at a greater risk of developing depressive symptoms through a pro-inflammatory mechanism.

Pro-inflammatory cytokines implicated in clinical depression

Analogous to the trends seen with cytokines and depressive symptoms, elevated TNF-α and IL-6 profiles are also found in individuals with mood disorders such as major depressive disorder (MDD) (Dowlati et al., 2010; O'Brien et al., 2007). Increased mitogen-stimulated IL-1β levels in individuals with chronic depression, known as dysthymia, were also correlated with increased severity of depressive symptoms (Anisman, Ravindran, Griffiths, & Merali, 1999). This suggests that IL-1β may also be a marker of clinical depression. Indeed, chronic inflammatory conditions such as inflammatory bowel disease and rheumatoid arthritis also have a high comorbidity with MDD (Dickens, McGowan, Clark-Carter, & Creed, 2002; Fuller‐Thomson & Sulman, 2006; Graff, Walker, & Bernstein, 2009), adding further support to the hypothesis that elevated pro-inflammatory cytokines are implicated in the development of depressive disorders. Critically, the acute IL-6 inflammatory response to a psychosocial stress is also elevated in patients with MDD (Pace et al., 2006). This indicates individuals with MDD may have enhanced immune reactivity to stressful events, and adds further support for the link between stress and elevated levels of inflammation.

Moreover, there have also been sex differences reported in the cytokine profiles of patients with MDD, where female patients had lower concentrations of TNF-α and IL-6 than male patients (Kim et al., 2007). Thus, a greater proportion of males may experience cytokine-induced MDD. This sex difference in MDD patients was compared to healthy

controls who did not differ in IL-6 or TNF-α (Kim et al., 2007). Overall these results indicate that levels of pro-inflammatory cytokines may manifest differently in males and females during the development of MDD.

Pro-inflammatory cytokines as a potential epiphenomenon of depression

Although there is accumulating evidence for the cytokine-depression hypothesis, there is also evidence that elevated cytokines might simply be an epiphenomenon of depressive symptoms and MDD rather than a causal factor (de Beaurepaire, 2002; Schiepers, Wichers, & Maes, 2005). Three key findings support the notion that while elevated cytokines might occur simultaneously with the development of depression, they may not cause its development. First, if cytokines play causal role in the development of depression, we would expect cytokine levels to normalize before depression resolves. However, cytokines such as TNF-α, IL-1β, and IL-6 do not consistently return to normal levels even when depressive symptoms have abated (Anisman et al., 1999; Hannestad, DellaGioia, & Bloch, 2011; Kubera et al., 2000).

Second, if cytokines caused the development of depression, anti-inflammatory medications, such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs), that decrease circulating levels of cytokines would also have antidepressant effects. Yet, the evidence is mixed. Corticosteroids which exhibit anti-inflammatory effects have been shown to exacerbate the development of depression rather than resolve it (Brown & Chandler, 2001; Ciriaco et al., 2013). While some studies who treated patients with MDD with NSAIDs reported successful reductions of inflammatory markers and improvements in depressive symptoms compared to placebo groups (Abbasi,

Hosseini, Modabbernia, Ashrafi, & Akhondzadeh, 2012; Müller et al., 2006), other studies have shown that NSAID exposure can increase the likelihood of developing treatment-resistant depression and exacerbate symptoms (Browning, 1996; Gallagher et al., 2012; Warner-Schmidt, Vanover, Chen, Marshall, & Greengard, 2011).

Third, cytokine-inhibitor medications that reduce circulating levels of pro-inflammatory cytokines are not consistently effective at treating depression. Although anti-TNF-α medication decreases depressive symptoms in psoriasis patients (Menter et al., 2010; Tyring et al., 2006), it is not effective in treating depression in patients with MDD (Raison et al., 2013). Also, Tocilizumab, an IL-6 inhibitor drug, did not improve depressive symptoms in a group of patients with rheumatoid arthritis (Traki et al., 2014).

More research is needed to clarify the role that cytokines play in depressive symptoms. Although cytokines are associated with depression and seem to be involved in the pathophysiology of depressive symptoms, it is unclear why reducing cytokines levels would not also reduce depressive symptoms in all populations. There may be specific subgroups with depressive symptoms that respond to anti-inflammatory or anti-cytokine treatments.

Pro-inflammatory cytokines implicated in anxiety

In humans, another main correlate with stress and depression is anxiety (Beiter et al., 2015). Anxiety has been shown to be highly comorbid with depression, and patients who suffer from both disorders often have more severe depression (Sherbourne & Wells, 1997). Additionally, for those experiencing pre-clinical depression, anxiety increases the likelihood of a new depressive episode (Sherbourne & Wells, 1997). Similar to

depression, anxiety is more prevalent among women (O'Donnell et al., 2016; Watterson, Williams, Lavorato, & Patten, 2016), putting women at a greater risk of developing an anxiety or mood disorder. In animal models, chronic stress has been shown to produce anxiety (Vyas, Pillai, & Chattarji, 2004), which may be due to an increase in pro-inflammatory cytokines (Donev, 2014; Leonard & Myint, 2009). However, little is known about the link between anxiety and inflammation.

Since depression and anxiety are highly correlated it can be difficult to examine the pathophysiology separately, specifically with respect to how inflammation affects both disorders (Donev, 2014). In fact, similar to the effect on depressive symptoms, when humans were injected with a lipopolysaccharide that induced a pro-inflammatory response, it resulted in increased anxiety symptoms (Reichenberg et al., 2001). Other studies have reinforced a positive relationship between the pro-inflammatory cytokines TNF-α and IL-6 and anxiety in both clinical (Arranz, Guayerbas, & De la Fuente, 2007; Maes et al., 1998), and healthy populations (Pitsavos et al., 2006). One study showed that levels of IL-6 are higher in anxious patients independent of depression (O'Donovan et al., 2010), adding further support that elevated cytokines are also linked to anxiety. However, some studies have found no link between TNF-α, IL-6, and anxiety symptoms in a clinical population (Vogelzangs, Beekman, De Jonge, & Penninx, 2013). Thus, the inflammatory profile of patients with anxiety remains unclear, and more research is needed to determine these profiles in anxious populations.

Exercise and Chronic Stress

Individuals who experience stress-induced depression are in need of treatments that can target the stress response and inflammatory pathways. By mitigating stress through regular physical exercise, one might be able to reduce their pro-inflammatory cytokine levels, and mitigate the development of depressive symptoms (Eyre & Baune, 2012). This will be the focus of the following sections.

Exercise may mitigate stress-induced depression by increasing an individual's threshold to stress and minimizing the pro-inflammatory response that follows. Exercise is a controllable and acute physiological stressor that will stimulate the SAM-axis stress response by releasing both epinephrine and norepinephrine (Dhabhar, 2014; Howley, 1975; Kappel et al., 1991; Kjaer, Christensen, Sonne, Richter, & Galbo, 1985). At moderate-to-high intensities (60-80% of VO_2 peak), levels of cortisol will also be released; triggering the body's stress response (Hill et al., 2008). By partaking in exercise, an individual may be able to prime their acute stress response to work effectively, honing the body to use allostasis efficiently and reduce their allostatic load (Dhabhar, 2014). Potentially, a person who engages in regular physical exercise may become more resilient to stress (Rimmele et al., 2007). In a study done with trained and untrained men, both groups were asked to complete the Trier Stress Test, which is a psychological stressor that involves completing a math test and verbal presentation (Rimmele et al., 2007). Throughout and after the test, trained men with higher fitness levels had a lesser change in cortisol and heart rate compared to untrained men (Rimmele et al., 2007). This signifies that the trained men had a lower biological stress response to the psychological

stress, and suggests that exercise may help to regulate the acute stress response, possibly by increasing the activation threshold for psychological stressors. Furthermore, in mouse models, exercise has been shown to attenuate the effects of chronic, uncontrollable stress, and prevent the onset of depression (Liu et al., 2013). Exercise has also been shown to buffer the effects of oxidative stress, thereby preventing the development of anxiety in mice (Salim et al., 2010). This protective effect against the development of mental illness has been attributed to the anti-inflammatory effects of exercise (Liu et al., 2013), which can lower resting-state levels of pro-inflammatory cytokines (Gleeson et al., 2011; Liu et al., 2013).

Exercise and Depression

For patients who are non-responders to anti-depressant medications and exhibit high levels of inflammation (O'Brien et al., 2007), exercise is a promising anti-inflammatory treatment that is low-cost and includes a multitude of health benefits with minimal side-effects. One main cytokine involved in exercise is IL-6, which is released by skeletal muscle during and immediately after strenuous exercise (Pedersen & Febbraio, 2008), and can inhibit levels of TNF-α in systemic circulation (Starkie, Ostrowski, Jauffred, Febbraio, & Pedersen, 2003). Therefore, while IL-6 generally serves a pro-inflammatory role systemically, it may also act through an anti-inflammatory pathway in an acute exercise setting. Indeed, IL-6 levels can be elevated up to 100 times baseline levels following an intense exercise session (Fischer, 2006), suggesting that high-intensity exercise may have greater inhibitory effects on TNF-α than moderate-intensity exercise, and thus could produce more potent improvements in depression.

However, little research has been done on the effects of high-intensity exercise within a clinically depressed population.

Indeed, moderate-intensity exercise has been extensively studied in the literature and shown to be an effective treatment for depression in clinically depressed patients (Mead et al., 2009; Medina, Jacquart, & Smits, 2015), including those who are resistant to SSRIs (Rethorst et al., 2013). This effect is even greater in those with high levels of TNF-α (Rethorst et al., 2013; Schuch, Dunn, Kanitz, Delevatti, & Fleck, 2016), indicating that exercise might be a more effective treatment for these individuals specifically.

However in non-clinical, otherwise healthy populations, few studies have examined how exercise affects the inflammatory pathway to alleviate depressive symptoms. While there is evidence from separate studies that exercise training reduces resting-state levels of inflammation (Ostrowski, Rohde, Asp, Schjerling, & Pedersen, 1999; Petersen & Pedersen, 2005; Thompson et al., 2010) and improves depressive symptoms (Mead et al., 2009; Medina et al., 2015; Nabkasorn et al., 2006), few studies have examined both factors in tandem. Some of the only studies to examine inflammation and depressive symptoms together within a healthy population evaluated the effects of exercise withdrawal on mood and inflammation (Kop, Weinstein, Deuster, Whittaker, & Tracy, 2008; Poole, Hamer, Wawrzyniak, & Steptoe, 2011). Both of these studies recruited regularly active individuals and examined the effects of withdrawing exercise for two weeks compared to a group that continued to exercise (Kop et al., 2008; Poole et al., 2011). Both studies reported a decrease in mood for the exercise withdrawal group (Kop et al., 2008; Poole et al., 2011); however, the changes in pro-inflammatory cytokines

yielded mixed results. One study found that IL-6 decreased in the exercise withdrawal groups, and the authors posited that this was due to reduced release from the skeletal muscle from lack of exercise (Poole et al., 2011). TNF-α also showed no change. In contrast, the other study found no significant changes in IL-6 after two-weeks of exercise withdrawal (Kop et al., 2008). It is important to note that these findings can only be attributed to halting exercise, and not directly to exercise training.

Recent literature has demonstrated that during a single strenuous exercise test, exercise training can influence the acute cytokine response that occurs post-exercise (Gokhale, Chandrashekara, & Vasanthakumar, 2007). Split into two groups, trained and untrained men completed a single, strenuous exercise session. The athlete's training sessions consisted of three, 20-minute running sessions separated by two rest intervals of three minutes each, and the non-athletes partook in three 10-minute exercise sessions (Gokhale et al., 2007). The completion of the exercise was defined as either maximal exhaustion or completion of the exercise session (Gokhale et al., 2007). Levels of TNF-α and IL-6 were measured before and after the exercise session (Gokhale et al., 2007). Athletes showed a smaller change in cytokine levels compared to non-athletes, even though the athletes exercised for a greater duration on the test (Gokhale et al., 2007). This suggests that greater fitness and exercise training may attenuate the pro-inflammatory response that occurs after exercise. Whether the acute inflammatory response is affected by a negative mood state is still unclear. Two other studies have examined the acute inflammatory response to exercise but in patients with MDD compared to healthy controls (Boettger et al., 2010; Hallberg et al., 2010). While one study found that there

was no difference in cytokine reactivity between depressed and healthy groups (Boettger et al., 2010), another found that healthy controls had higher IL-6 reactivity (Hallberg et al., 2010). Although these studies give information on how one exercise session can influence the acute pro-inflammatory response, changes in cytokine reactivity in relation to exercise training and negative mood states have yet to be studied across a long-term exercise intervention. We predict that exercise training in sedentary individuals will attenuate the acute TNF-α and IL-6 response, to transform the acute cytokine profile to look similar to that of a trained individual (Gokhale et al., 2007). With training, the lower amounts of circulating pro-inflammatory cytokines after exercise might reduce the negative effects of elevated pro-inflammatory cytokines on the CNS. Thus, attenuation in the acute inflammatory response may also buffer the development of depressive symptoms.

A recent study conducted by our group examined the effects of implementing an exercise-training program on depressive symptoms and inflammation in sedentary healthy adults attending university (Paolucci; Submitted 2017 to Biological Psychiatry). Participants were randomized into a high-intensity interval training (HIIT), moderate-intensity continuous training (MCT), or a no-exercise control condition for six weeks. Before and after the six-week intervention, levels of depressive symptoms (BDI-II) along with highly correlated mood levels of anxiety (BAI) and stress (PSS) were measured using self-report surveys. Levels of pro-inflammatory cytokines IL-6, TNF-α, and IL-1β were measured using a serum blood draw at pre and post-testing. Depressive symptoms, anxiety, and stress increased for controls, and remained the same for the MCT group at

post-testing. Interestingly, HIIT showed no change in depressive symptoms, but had increases in anxiety and stress together. These increases in negative mood were paired with increases in IL-6 and TNF-α for both HIIT and controls (Paolucci, Submitted). Critically, pro-inflammatory cytokines IL-6 and TNF-α were lower in the MCT group compared to HIIT and controls after the six-week intervention, suggesting that moderate-intensity exercise may be more protective than HIIT against the development of depressive symptoms by reducing resting-state levels of pro-inflammatory cytokines.

However, limitations in the study design prevent a clear understanding of how HIIT impacts pro-inflammatory cytokines in humans (Paolucci, Submitted). Although HIIT showed elevated levels of IL-6 at post-test, it may be due to the greater release from skeletal muscle as a result of the greater exercise intensity. Only one blood draw was taken at rest during post-testing, which does not provide information on the acute IL-6 response after exercise. Thus, without blood draws taken from before and after exercise, we cannot ascertain if HIIT provides any benefits to the acute pro-inflammatory response.

The present study aims to replicate our previous findings from the HIIT group over a longer training period of nine weeks. This extended training period will help elucidate if HIIT needs a longer training time in order to have a positive effect on anxiety and stress. HIIT is intense, and can elicit a strong physical stress response and symptoms similar to anxiety such as wobbliness in legs and a pounding heart (Beck, Epstein, Brown, & Steer, 1988; Beck & Steer, 1990). For sedentary individuals these feelings might be mistaken for anxiety. With further training and fitness adaptations, perhaps sedentary individuals would have a longer period to normalize these symptoms and experience positive effects

on mood. The experimental group consisted of university students, who are at a greater risk of experiencing chronic stress across the school term and developing depressive symptoms (Beiter et al., 2015). Similar to our previous study, post-testing occurred the week before the students final examination period, which has been shown to be a stressful time for students (Steptoe, Wardle, Pollard, Canaan, & Davies, 1996). In addition, a more detailed investigation on how HIIT impacts both mood and inflammation was conducted by measuring mood weekly, and measuring the acute inflammatory response to the maximal fitness tests at pre, mid, and post-testing.

Based on the findings of our prior study, we hypothesized that mood would decline across the term for university students, as the control group in the aforementioned study (Paolucci, Submitted) had significant increases in depressive symptoms, anxiety, and stress at the end of the term. We also predicted that with extended training, HIIT would buffer the emergence of depressive symptoms, anxiety, and stress due to a longer time to acclimatize to the exercise protocol. This would suggest that for sedentary individuals, a longer training period is needed to observe positive mood benefits. In terms of pro-inflammatory cytokine changes, we hypothesized that HIIT would increase resting-state levels of pro-inflammatory cytokines due to increased skeletal muscle release (Fischer, 2006), but attenuate the acute cytokine response. If the acute response is attenuated, it would suggest that HIIT transforms the acute cytokine response from a sedentary pattern into a trained pattern (Gokhale et al., 2007). This study will help elucidate how mood changes across the term for university students and whether HIIT is able to positively impact mood across the school term. It will also reveal whether HIIT improves mood

through a specific pro-inflammatory cytokine mechanism, by influencing either resting-state levels or the acute response.

METHOD

Participants

A total sample size of 18 participants was required to have 80% power to detect significant between group differences given a large effect size (as derived from our previous study; (Paolucci, Submitted). However, our previous study had ~18 participants per group, thus we aimed to replicate this sample size to detect changes in pro-inflammatory cytokines as well. Fifty-two healthy young adults (63% females) aged 18-30 (*M*±*SD*: 19.9±2.2 years) were recruited from McMaster University and began pre-testing. All participants provided written informed consent. Participants were included if they were a student at McMaster University and partook in no more than one hour of moderate-to-vigorous physical activity per week prior to joining the study. This ensured that participants were not meeting the Canadian Physical Activity Guidelines of 150 minutes of moderate-to-vigorous exercise per week (Tremblay et al., 2011), and were partaking in less exercise time than involved in the HIIT protocol for this study. Participants were stratified by sex, and randomly assigned to one of two groups: 1) *high-intensity interval training* (HIIT) group (n=24, 63% females), or 2) *sham-exercise* group (*control)* (n = 22, 64% females) (Table 1). The Hamilton Integrated Research Ethics Board approved this study.

Procedure

Pre, Mid, and Post-Intervention Assessments

The pre, mid, and post-intervention assessments occurred during weeks 1, 6 and 11. These assessments consisted of the completion of mood surveys, followed by a peak power output test with blood samples drawn at rest and immediately following the exercise test.

Mood surveys. Participants completed the Beck Depression Inventory-II (BDI-II) (Beck et al., 1996), the Beck Anxiety Inventory (BAI) (Beck et al., 1988; Beck & Steer, 1990), and Perceived Stress Scale (PSS) (Roberti, Harrington, & Storch, 2006), to assess levels of depressive symptoms, anxiety, and stress.

Aerobic fitness. All participants completed a peak power output (VO_2 peak) test on a digital cycle ergometer (Lode Groningen, The Netherlands), using a metabolic cart (MEDISOFT ExpAir Software VO_2 System). The exercise test started at an initial workload of 50W for the first 30 seconds, followed by a graded increase in workload of 1 W/2 sec (on average 30W/min). Participants were instructed to maintain a cycling cadence of 70-90 revolutions per minute (rpm). Criteria for test failure consisted of either volitional exhaustion or when the cycling cadence dropped below 60 rpm. The respiratory exchange ratio (RER) was recorded to determine whether VO_2 peak was actually achieved (RER $\geq$ 1.10) (Edvardsen, Hem, & Anderssen, 2014). Relative VO_2 peak (ml/kg/min) achieved on the test was used as a measure of fitness levels across the intervention. To assess adaptations in workload, maximum wattage achieved on the peak power output test were recorded. Maximum wattage and heart rate from the peak power

output test at pre-test were used to determine the appropriate individual training intensity for each participant in the HIIT group.

Pro-inflammatory cytokines. Levels of IL-6, TNF-α, and IL-1β were measured using serum samples drawn from our participants both at rest and 5 minutes after the completion of the peak power output exercise tests. Following collection, the blood tubes were clotted for 1 hour and centrifuged at 4000 rpm for 10 minutes at 4°C. The supernatant was then collected to obtain serum and aliquoted into cryovials for storage at -80°C until analysis. Serum cytokines (IL-6, TNF-α, IL-1β) were measured using the Milliplex MAP 60K cytokine panel as per manufacturer's protocol (EMD Millipore). The average of duplicate measures of serum cytokines was reported.

Exercise Intervention

The *HIIT group* sessions consisted of 20 minutes of intervals on a stationary cycle ergometer, alternating between a 60 second sprint at 80% of their maximum wattage and ~90-95% maximum heart rate, and 60 seconds of active rest cycling at 30% of their maximum wattage (≥ 50W). Training sessions had a three-minute warm up, and two-minute cool down while cycling on the stationary ergometer at 50W. To ensure exercisers were cycling at a high-intensity, Borg's Rating of Perceived Exertion (RPE, scale 6 to 20)(Borg, 1982), heart rate (HR), and workload were tracked during each exercise session. Workload was adjusted across the intervention to ensure that participants reached the target HR zone during the exercise training sessions. The HIIT group completed training three times per week, for nine weeks (weeks 2-10), achieving a total of 27 exercise sessions.

The *sham-exercise group* remained sedentary for nine weeks, except for three peak power output tests at pre, mid, and post-testing. Critically, the sham-exercise participants were given a cover story that they were part of an "acute" exercise group, consisting of the three peak power output tests. They were told the aim was to see how long the effects of one bout of exercise would last over several weeks, and were instructed to remain sedentary in between each test. Thus, they were under the impression that they were also assigned to an exercise group, rather than to a no-exercise group.

Statistical Analysis

Data were initially screened for outliers; they were identified as Q1-(1.5*IQR) or Q3+(1.5*IQR) from weekly scores, and were removed from the analysis for all variables except for the cytokine difference scores. To conserve data, only extreme outliers identified as Q1-(3*IQR) or Q3+(3*IQR) were removed for the cytokine difference scores. All inflammatory markers were log transformed to achieve a normal distribution. Effect sizes are provided as partial eta-squared (η_p^2) values (Table 7, Appendix B).

Manipulation Checks. To ensure that the HIIT intervention improved in fitness, a 2 x 3 (group x time) repeated measures ANOVA was conducted on the VO_2 peak scores, controlling for sex. A post-hoc analysis was conducted using a one-way repeated measures ANOVA to analyze the simple main effects of time.

Mood. To assess changes in mood across the term, a 2 x 2 x 11 (group x sex x time) repeated measures ANOVA was conducted on depressive symptoms, anxiety, and stress scores. Due to sex differences in depression (Patten et al., 2006), anxiety (O'Donnell et al., 2016; Watterson et al., 2016) and stress (Beiter et al., 2015), sex was

included as a factor to examine if there were sex differences across the term. For tests in which sphericity was violated, the Greenhouse-Geisser values for the ANOVA were reported.

Resting-state pro-inflammatory cytokine levels. To examine changes in the pro-inflammatory cytokines, 2 x 2 (group x time) repeated measures ANCOVAs were conducted comparing mid and post-test for IL-6, IL-1β, and TNF-α separately. To account for a wide range of variance in individual levels of cytokines at baseline (Table 4), we controlled for pre-test scores to control against variance in their individual baseline scores. Sex was also included as a covariate as there is evidence of differences in pro-inflammatory cytokine profiles in males and females (Kim et al., 2007; Rosen, Ham, & Mogil, 2017). A post-hoc analysis was conducted using one-way ANOVAs, with the same covariates, to examine the simple main effects of group and time.

Acute inflammatory response. A 2 x 2 (group x time) repeated measures ANCOVA was used to assess differences in the acute cytokine response at pre, mid, and post-testing. To examine the acute change in pro-inflammatory cytokines from before (resting-state) to immediately after exercise we calculated a difference score for IL-6, IL-1β, and TNF-α. There is a large amount of variance in cytokine levels both at rest and after exercise (Table 4), and the difference score allowed the acute changes to be assessed on an individual basis. The amount of inflammation released with exercise is impacted by intensity and duration (Fischer, 2006), and people with higher fitness can work harder and longer on the peak power output test, which could potentially produce greater amounts of inflammation after exercise. To control for improvements in the HIIT group on the peak

power output test across the intervention, the change in maximum workload from pre to post-test was included as a covariate. Other covariates included in the model were the acute change score at pre-test, and sex. A post-hoc analysis was conducted using one-way ANOVAs, with the same covariates, to examine the simple main effects of group and time.

Maximum Workload. We assessed changes in maximum workload achieved on the peak power output tests using a 2 x 3 (group x time) repeated measures ANOVA, controlling for sex. We also examined the relative improvements in maximum workload achieved by the HIIT group compared to controls using the percent change score for maximum workload changes from: pre-to-mid, mid-to-post, and total change. This will help elucidate if changes in the inflammatory response were similar to changes in maximum workload during pre, mid, and post-testing. To evaluate group differences in maximum workload improvements, one-sample paired *t* tests were run to test the difference of the change scores from 0 (i.e., no change).

RESULTS

Adherence

Forty-six participants (63% females) completed the intervention. Six participants dropped out (4 = control, 2 = HIIT), four did not specify a reason, one cited personal reasons, and one person dropped out due to a prior chronic injury. Adherence to the HIIT intervention was 100% for participants who completed post-testing, attending a total of 27 exercise sessions. For the two dropout participants from the HIIT group, one

completed 20 sessions before withdrawing due to a prior chronic injury, and the other completed 4 sessions before dropping-out for personal reasons. Table 1 provides demographic information by group for the participants who completed the intervention.

Demographics

Table 1. *Demographic characteristics by group*

	n	HIIT M (SD)	n	Control M (SD)
Sample Size (n)	24	Total	22	Total
Sex	15	Females	14	Females
Age (years)	24	20.3 (2.6)	22	19.6 (1.7)
BMI (kg /m^2)	24	23.6 (4.6)	22	21.9 (3.4)
Baseline VO_2 peak (ml_{O2}/kg/min)	24	29.3 (5.2)	20*	29.7 (6.4)
Baseline Max Workload (W)	24	186.8 (43.9)	22	185.0 (55.3)

*note: outliers removed using Q1-(1.5*IQR), or Q3+(1.5*IQR) from weekly scores.

Manipulation Checks

The HIIT group increased in aerobic fitness compared to the control group (Figure 1), as indicated by a significant linear contrast group x time interaction for VO_2 peak [$F(1,38) = 4.02$; $p = 0.05$; $\eta_p^2 = 0.10$]. The simple main effects of time were analyzed by running a one-way repeated measures ANOVA on the pre, mid and post values for each group, controlling for sex. There was a significant simple linear contrast, as shown by a

strong main effect of time for the HIIT group [$F(1,21) = 7.48$; $p = 0.01$; $\eta_p^2 = 0.26$], indicating that the HIIT group improved in fitness across the intervention. There was no simple main effect of time for the control group [$F(2,32) = 0.45$; $p = 0.66$; $\eta_p^2 = 0.03$], demonstrating no improvements in fitness across the intervention. Average RER values achieved on the VO_2 peak test indicate that the majority of participants reached their VO_2 max based on the RER cutoff of ≥ 1.10, at pre and post-testing, but not at mid-test (Table 2 and Table 3).

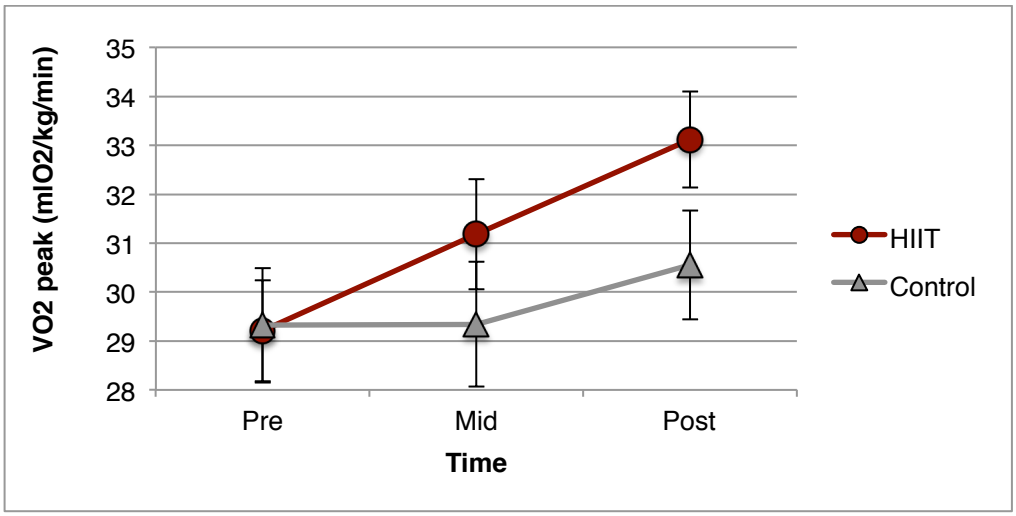

Figure 1. VO_2 peak values across time. (Covariate sex = 1.66.)

Table 2: Average RER values achieved at VO_2 peak

	Pre M(SD)	Mid M(SD)	Post M(SD)
HIIT	1.25 (0.12)	1.03 (0.20)	1.16 (0.14)
Control	1.27 (0.09)	1.07 (0.21)	1.18 (0.17)

Table 3: Percentage of participants who achieved an RER ≥ 1.10

	Pre	Mid	Post
HIIT (%)	85	46	83
Control (%)	96	60	82

Mood Changes Across the Term

Depressive symptoms and anxiety decreased across the term as shown by a significant main effect of time [depression: $F(4.48, 156.79) = 10.89$; $p < 0.001$; $\eta_p^2 = 0.24$; anxiety: $F(5.43, 157.59) = 9.49$; $p < 0.001$; $\eta_p^2 = 0.25$]. Follow-up within-subjects contrasts revealed a significant linear main effect of time for both measures [Depressive symptoms: $F(1,35) = 12.97$; $p = 0.001$; $\eta_p^2 = 0.27$; anxiety: $F(1,29) = 28.63$; $p < 0.001$; $\eta_p^2 = 0.50$), showing that both decreased over time (Figure 2A and 2B). Stress also decreased across the term, but this was displayed by a marginal main effect [$F(4.81,168.35) = 2.01$; $p = 0.07$; $\eta_p^2 = 0.06$]. Follow-up within-subjects contrasts revealed a significant cubic main effect of time for stress [$F(1,35) = 8.50$; $p = 0.006$; $\eta_p^2 = 0.20$] that was driven by a decrease in stress during the first three weeks of the intervention (Figure 2C). There was no significant between-subjects effect of sex on depressive symptoms [$F(1,35) = 2.54$; $p = 0.12$; $\eta_p^2 = 0.07$]. Anxiety [$F(1,29) = 4.00$; $p = 0.06$; $\eta_p^2 = 0.12$] and stress [$F(1,35) = 3.913$; $p = 0.06$; $\eta_p^2 = 0.10$] both had a marginally significant between-subjects effect of sex. On average, females had marginally higher

levels of anxiety than males (M ± SD; 5.2 ± 3.6 vs. 2.8± 3.2), and marginally higher levels of stress than males (19.2 ± 6.6 vs. 14.7 ± 6.8).

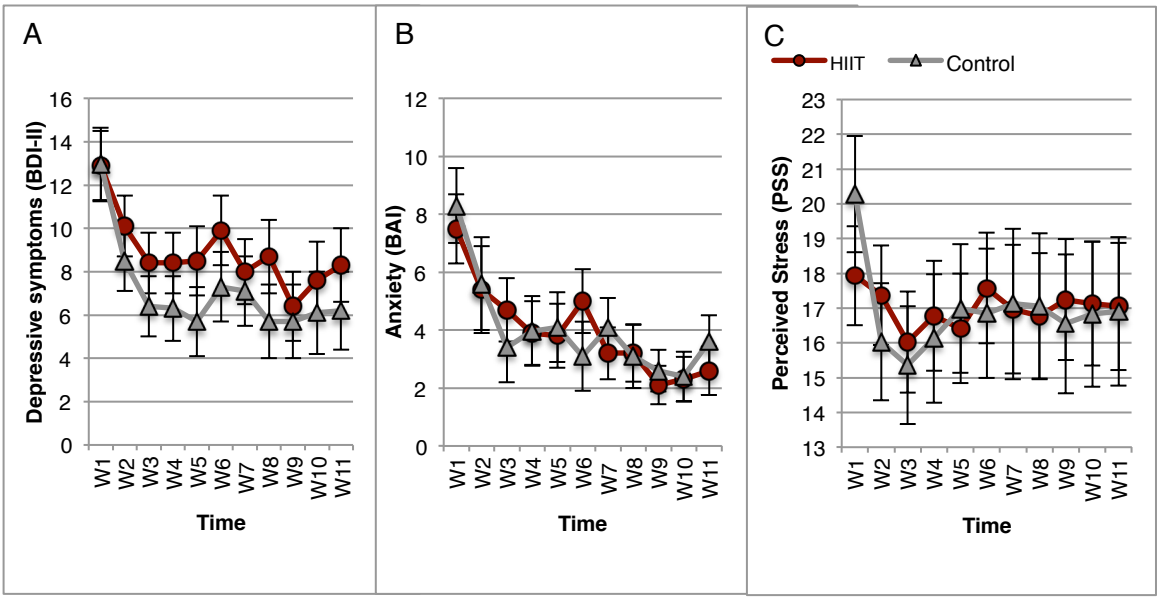

Figure 2. Main effect of time for: depressive symptoms, anxiety and stress across 11 weeks. W1= pre-test, W6 = mid-test, W11= post-test.

Resting-state pro-inflammatory cytokines

There were higher levels of resting-state IL-6 at post-test for the HIIT group than the control group, as indicated by a significant group x time interaction [$F(1,36) = 8.11$; $p = 0.007$; $\eta_p^2 = 0.18$] (Figure 3A). Post-hoc analyses revealed that HIIT and controls did not differ in resting-state IL-6 at mid-test [$F(1,37) = 0.44$; $p = 0.51$; $\eta_p^2 = 0.01$] (0.58±0.54 vs. 0.61±0.69). However, there was a simple main effect of group at post-test [$F(1,38) = 5.26$; $p = 0.03$; $\eta_p^2 = 0.12$], demonstrating that HIIT had higher resting-state IL-6 levels than controls at the end of the intervention (0.67±0.49 vs. 0.45±0.67). In addition, IL-6 did not change for the HIIT group from mid to post [$F(1,20) = 2.55$; $p = 0.13$; $\eta_p^2 = 0.11$] (0.63±0.52 vs. 0.67±0.49). Control levels of IL-6 were also unchanged

from mid to post [$F(1,14) = 0.22$; $p = 0.65$; $\eta_p^2 = 0.02$] (0.61±0.69 vs. 0.44±0.85). Although there were no significant differences for IL-1β [$F(1, 36) = 0.84$; $p = 0.37$; $\eta_p^2 = 0.02$] and TNF-α [$F(1,37) = 0.64$; $p = 0.43$; $\eta_p^2 = 0.02$], we observed trends similar in direction to the IL-6 changes (Figures 3B and 3C). Table 4 provides the log-transformed cytokine concentrations from before (resting-state) and after the peak power output test at pre, mid, and post-testing. The raw cytokine concentrations at both resting-state and after exercise for pre, mid, and post-tests are displayed in Table 6 (Appendix B).

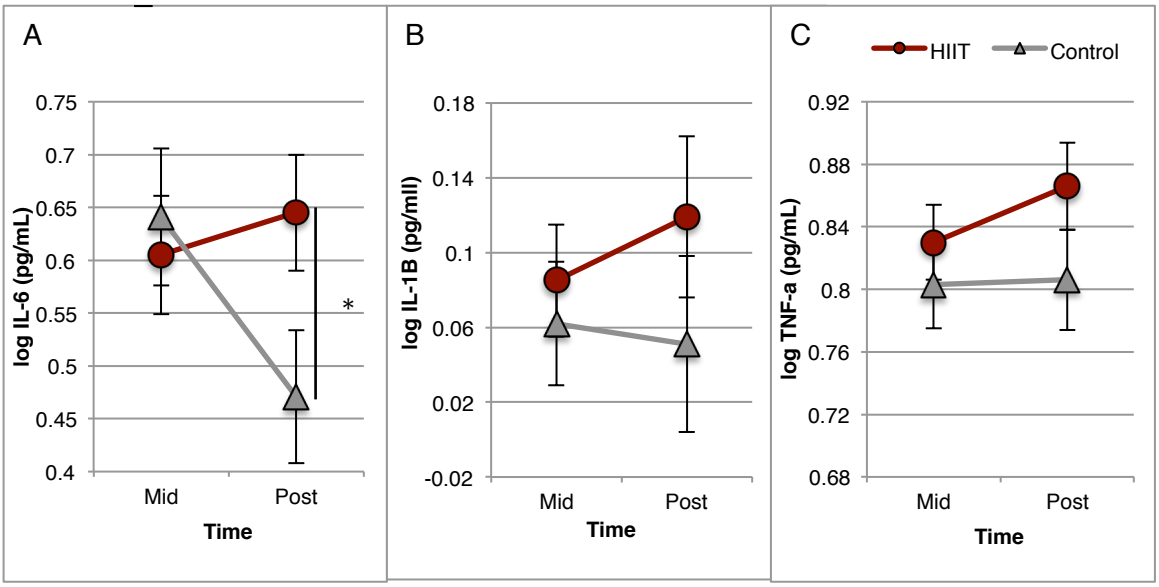

Figure 3. Resting-state levels of pro-inflammatory cytokines (IL-6, IL-1β, TNF-α) at mid and post-testing. Figure 3A, *$p = 0.007$ (Covariates: pre-test = 0.58, sex = 1.63). Figure 3B (Covariates: pre-test = 0.10, Sex = 1.62). Figure 3C (Covariates: pre-test = 0.84, sex = 1.63).

Table 4. *Means (standard deviations) of log transformed pro-inflammatory cytokine values, at rest (before ex.) and immediately after exercise (after ex.), at pre, mid, and post-testing.*

		Pre M(SD)		Mid M(SD)		Post M(SD)	
		Before	After	Before	After	Before	After
log IL-1β (pg/ml)	HIIT	0.12 (0.42)	0.23 (0.38)	0.14 (0.43)	0.17 (0.43)	0.17 (0.47)	0.24 (0.42)
	Control	0.04 (0.45)	0.13 (0.46)	-0.001 (0.48)	0.02 (0.48)	0.05 (0.55)	0.14 (0.54)
log IL-6 (pg/ml)	HIIT	0.59 (0.57)	0.71 (0.52)	0.59 (0.54)	0.67 (0.53)	0.67 (0.49)	0.72 (0.48)
	Control	0.67 (0.77)	0.71 (0.79)	0.61 (0.69)	0.64 (0.70)	0.45 (0.83)	0.61 (0.76)
log TNF-α (pg/ml)	HIIT	0.83 (0.28)	0.87 (0.27)	0.82 (0.28)	0.87 (0.29)	0.88 (0.28)	0.95 (0.26)
	Control	0.88 (0.35)	0.90 (0.29)	0.82 (0.31)	0.83 (0.33)	0.80 (0.28)	0.91 (0.27)

Acute Inflammatory response

At post-test after nine weeks of training, there were significant group x time interactions for the acute cytokine responses of IL-6 [$F(1, 27) = 8.83$; $p = 0.006$; $\eta_p^2 = 0.25$] (Figure 4A), and TNF-α [$F(1, 30) = 6.99$; $p = 0.01$; $\eta_p^2 = 0.19$] (Figure 4B).

Post-hoc analysis of the simple main effects revealed that the acute IL-6 response was marginally higher in HIIT than controls at mid-test, indicated by a simple main effect of group [$F(1,29) = 3.14$; $p = 0.09$; $\eta_p^2 = 0.10$] (0.06±0.08 vs. 0.01±0.10). However, at post-test controls had a marginally higher acute IL-6 response than HIIT (0.15±0.10 vs. 0.07±0.11) displayed by a simple main effect of group [$F(1,30) = 3.35$; $p = 0.08$; $\eta_p^2 = 0.10$]. Follow-up analysis of the simple main effects of time revealed that the HIIT group displayed no change from mid to post in the acute response of IL-6 [$F(1,14) = 0.11$; $p = 0.75$; $\eta_p^2 = 0.01$] (0.06±0.08 vs. 0.08±0.10) despite an increase in workload of 6.24%

during the peak power output test (Table 5). Conversely, the control group significantly increased their acute IL-6 response from mid to post (0.00±0.10 vs. 0.16±0.10) as demonstrated through a simple main effect of time [$F(1,10) = 5.83$; $p = 0.04$; $\eta_p^2 = 0.37$], though they did not significantly increase their maximum workload (Table 5).

Post-hoc analysis revealed that the acute TNF-α response significantly increased in the control group (0.0±0.07 vs 0.11±0.08) as demonstrated by a simple main effect of time [$F(1,12) = 14.14$; $p = 0.003$; $\eta_p^2 = 0.54$]. This was compared to no change in the HIIT group from mid to post [$F(1,15) = 0.40$; $p = 0.54$; $\eta_p^2 = 0.03$] (0.05±0.05 vs. 0.10±0.08). However, there was no difference in the acute TNF-α response between HIIT or controls at mid-test [$F(1,32) = 2.56$; $p = 0.12$; $\eta_p^2 = 0.07$] (0.05±0.05 vs. 0.01±0.08) or at post-test [$F(1,33) = 2.48$; $p = 0.13$; $\eta_p^2 = 0.07$] (0.07±0.10 vs. 0.11±0.08). A similar but non-significant trend was observed for IL-1β [$F(1,26) = 1.34$; $p = 0.26$; $\eta_p^2 = 0.05$] (Figure 4C).

Figure 5 displays the raw change score values for IL-6, TNF-α, and IL-1β at pre, mid, and post-testing. Similar attenuation patterns can be seen for the HIIT group compared to controls for both IL-6 (Figure 5A) and TNF-α (Figure 5B). The raw IL-1β scores do not demonstrate a similar pattern as the log-transformed scores (Figure 5C), likely due to the IL-1β changes being non-significant.

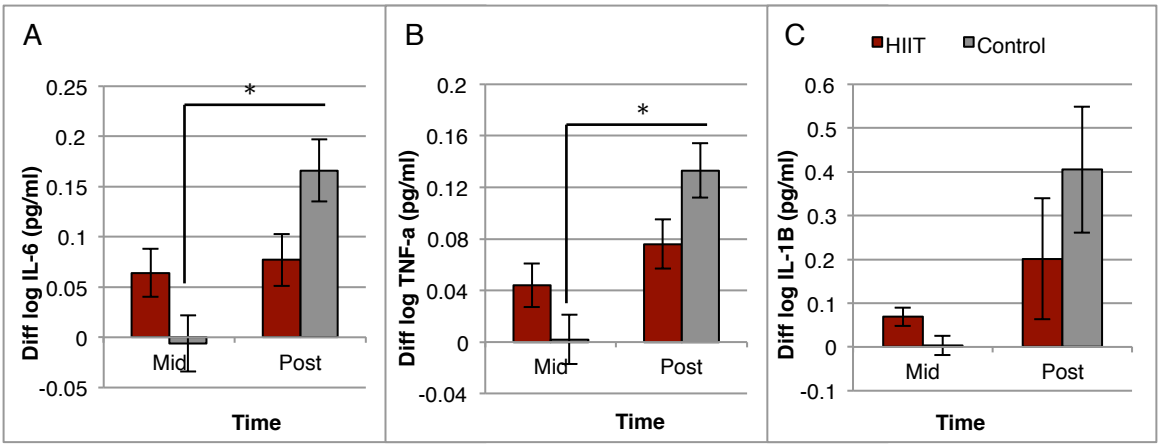

Figure 4. The log transformed acute change in pro-inflammatory cytokines from rest to after exercise (IL-6, TNF-α, IL-1β), at mid and post-testing. Figure 4A, **p* = 0.006 (Covariates: Pre-test= 0.075, Sex = 1.59, Change in maximum workload = 19.15). Figure 4B, **p* = 0.01 (Covariates: Pre-test = 0.05, Sex = 1.60, Change in maximum workload = 18.63). Figure 4C (Covariates: Pre-test = 0.07, Sex = 1.61, Change in maximum workload = 16.74).

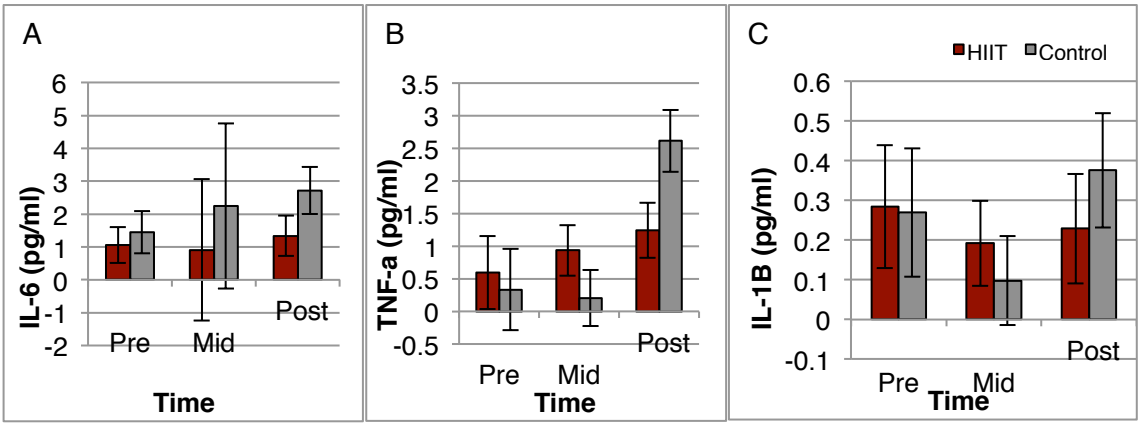

Figure 5. The raw change scores in pro-inflammatory cytokines from rest to after exercise (IL-6, TNF-a, IL-1β), at pre, mid, and post-testing. Figure 5A (Covariates: Sex = 1.59, Change in maximum workload = 19.16). Figure 5B (Covariates: Sex =1.60, Change in maximum workload = 18.60). Figure 5C (Covariates: Sex = 1.61, Change in maximum workload = 16.73).

Maximum Workload

The HIIT group significantly increased in the maximum workload achieved compared to the control group, as supported by a significant group x time interaction [$F(1.60, 68.90) = 19.92$; $p < 0.001$; $\eta_p^2 = 0.32$] (Figure 6).

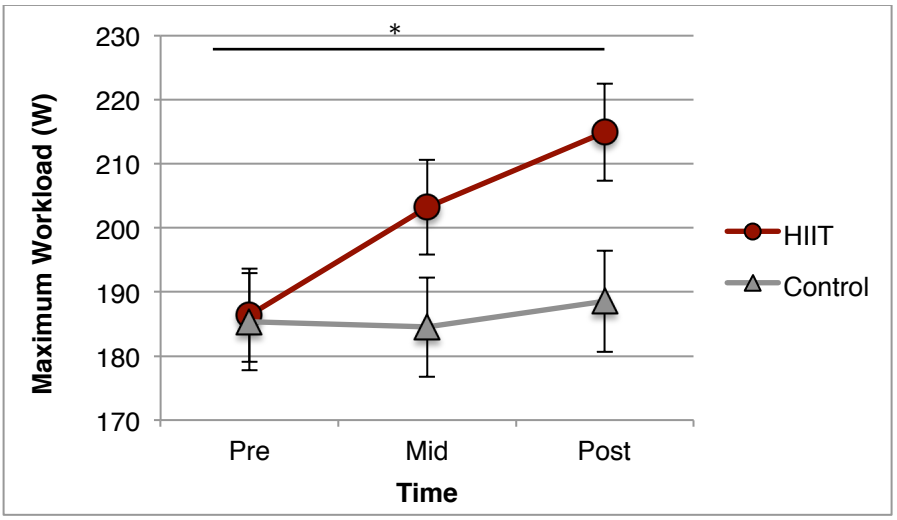

Figure 6. Maximum workload achieved on the peak power output test at pre, mid, and post-tests. *$p < 0.001$, (Covariate: Sex = 1.63).

Table 5 lists the percent change improvements in maximum workload for the HIIT and control groups. From pre-test to mid-test, the HIIT group improved their maximum workload by 9.67% [$t(24) = 5.47$; $p < 0.001$], and by 6.24% from mid to post-test [$t(23) = 6.21$; $p < 0.001$]. Overall across the entire intervention, the HIIT group improved the max workload achieved on their peak power output test by 15.3% [$t(22) = 8.93$; $p < 0.001$]. The control group did not show any significant relative improvements in maximum workload (Table 5).

Table 5. *Percent change improvements in maximum workload*

	Pre-Mid (%)	Mid-Post (%)	Total change (%)
HIIT	9.67*	6.24*	15.3*
Control	-0.13	1.59	2.02

*$p < 0.05$; one-sample two-tailed t-test indicating there was a significant difference from 0.

DISCUSSION

The purpose of this study was to evaluate the effects of HIIT on mental health, resting-state inflammation, and the acute pro-inflammatory response after exercise. Overall there was no effect of HIIT on anxiety and stress, which replicated our previous study (Paolucci, Submitted). Although we expected that HIIT would buffer the development of these symptoms with extended training, this was not observed. Instead, HIIT had elevated resting-state levels of IL-6 compared to controls at post-test. That said, there was evidence of physiological adaptations with training such that the HIIT group increased in their workload tolerated at maximal exertion yet experienced a reduction in inflammation immediately following the exercise. This suggests that there are physiological adaptations to the immune system from HIIT training; however, these changes may not be related to mood.

Mood. All participants were university students, and their levels of depressive symptoms, stress, and anxiety decreased over time as they progressed through their academic semester. This was contrary to our prediction that negative mood would peak during exams at the end of the term. Findings in past literature demonstrated that stress increased within two or three weeks of the final examination period compared to two or

three months earlier in the school term (Steptoe et al., 1996), supporting the common notion that the examination period is the most stressful time of the school term. Yet, our findings suggest that the beginning of the school term can also be a period of heightened stress for students. Thus, resources offered by universities to help students cope with stress and mental health issues should also be available at the beginning of the school term.

The observation that these three mood outcomes declined similarly across the term replicate the robust finding that depression, anxiety, and stress are highly correlated (Bayram & Bilgel, 2008; Beiter et al., 2015). Females also had marginally higher levels of stress and anxiety compared to males, adding further support for the sex differences reported in the literature (Beiter et al., 2015; O'Donnell et al., 2016; Steptoe et al., 1996; Watterson et al., 2016). However, improvements in mood across the first three weeks contrasts our previous findings, in which controls worsened in mood across the term (Paolucci, Submitted).Our prior exercise intervention was only six weeks in duration (Paolucci, Submitted) which corresponded to weeks 4 to 11 in the current study, therefore we did not capture the initial decline in depression, anxiety and stress seen in the current study. Previous research at other universities showed that one-third of undergraduate students surveyed at the beginning of the school term and then one year later during their second year (Andrews & Wilding, 2004) entered university with mild case depression and anxiety, but were symptom-free one year later (Andrews & Wilding, 2004). In our study, the average depression score for both groups at intervention onset was approximately 13 (Figure 2A) which is approaching mild depression according to the BDI-II criteria (Beck

et al., 1996). Accordingly, students in our study may have experienced a similar improvement in depressive symptoms and anxiety symptoms across the term.

Furthermore, there was no effect of HIIT exercise on mood over and above that of the control group. This replicates our previous finding that the HIIT and control conditions were not as effective at buffering stress and anxiety compared to MCT (Paolucci, Submitted). While MCT has been repeatedly shown to improve mental health and mood (Dunn, Trivedi, Kampert, Clark, & Chambliss, 2005; Mead et al., 2009; Medina et al., 2015), HIIT does not appear to be as effective in improving these outcomes (Paolucci, Submitted). HIIT can produce symptoms similar to anxiety such as shortness of breath and a pounding heart (Beck et al., 1988; Beck & Steer, 1990) that might mitigate its ability to buffer anxiety and stress in sedentary individuals who are not accustomed to the exercise. Moderate-intensity exercise is less strenuous and may be viewed as less stressful compared to HIIT, and thus may be more effective at buffering the development of negative mood.

However, in this current study HIIT also did not buffer depression scores, which contrasts our previous findings where HIIT buffered the development of depressive symptoms relative to controls (Paolucci, Submitted). The improvements in mood for the control group may have diminished any buffering effect that would have been comparatively seen in the HIIT group. Due to the improvements in the control group, it is difficult to ascertain the extent to which the HIIT protocol itself was responsible for improving mood within the exercise group.

One potential reason for the lack of mood difference between our HIIT and control groups may have been due to the cover story that the sham-exercise group was provided in this study compared, to a no-contact control group used in the prior study(Paolucci, Submitted). In our previous study, we used a typical no-contact control group who were told that they were assigned to a no-exercise control group (Paolucci, Submitted). However, knowing that they could not partake in an exercise intervention that they signed up to complete may have worsened their mood (Paolucci, Submitted). The current control group differed as they were told that they were part of an "acute" exercise group with the goal of understanding how long the benefits of one maximal bout of exercise (the peak power output tests) would last over a period of several weeks. They repeated these exercise bouts at three time points (pre, mid, and post during the nine-week intervention). This cover story may have created a placebo effect that reduced depressive symptoms and anxiety, given the widely-held belief that exercise improves mood (Desharnais, Jobin, Côté, Lévesque, & Godin, 1993). A similar effect was found when examining young adults who participated in a 10-week exercise intervention (Desharnais et al., 1993). In this study, one group was told that exercise would improve their psychological wellbeing, but nothing was said to the second group (Desharnais et al., 1993). Similar to our study, the group with the cover story significantly improved in psychological well being along with self-esteem (Desharnais et al., 1993), suggesting that the perception that exercise improves well being may also improve mental health.

Further supporting the notion that exercise may have placebo effects, it has been shown that perceived fitness is associated with positive personality and mood variables,

whereas aerobic fitness is not (Plante, Lantis, & Checa, 1998). Additionally, people with higher perceived fitness score lower on the depression scale (Plante et al., 1998), indicating that perceived increases in fitness by our sham-exercise group might have resulted in mood improvements. Further evidence supporting a potential placebo effect in our control group comes from a recent meta-analysis, which compared the effect size for placebo groups and exercise training groups in interventions that examined the psychological benefits of exercise (Lindheimer, O'Connor, & Dishman, 2015). They found that the mean effect size for psychological improvements within the placebo group was approximately half of the mean effect size for improvements observed in exercise training groups (Lindheimer et al., 2015). Thus, the placebo effect may account for approximately half of the observed psychological improvements during exercise training (Lindheimer et al., 2015). In the studies examined, those that measured anxiety and depression, and used low-intensity exercise reported the greatest placebo effects (Lindheimer et al., 2015). Although the control group in our study partook in maximal intensity exercise tests, overall they completed very little exercise over the nine weeks in comparison to the HIIT group. It follows that the low volume of exercise, and the anxiety and depression measures may have further driven the placebo effect in our control group.

Moreover, mood may have improved in both HIIT and controls due to other factors associated with participating in an intervention-based research study, such as increased social support. During the intervention, participants exercised with up to two other students, and had a research student supervising each exercise session, which encouraged socializing between participants. Although the control group did not exercise,

they believed that they were also part of an exercise group. The controls also came in weekly for blood draws every Friday and interacted with the researchers and other participants. These weekly interactions with the researchers and other participants may have increased their sense of belonging to a group and perceived social support. It has been shown that increased social support is associated with greater levels of physical activity (Christensen, Schmidt, Budtz-Jørgensen, & Avlund, 2006; Treiber et al., 1991), especially in women (Janisse, Nedd, Escamilla, & Nies, 2004; Sallis, Calfas, Alcaraz, Gehrman, & Johnson, 1999), who composed approximately two-thirds of our participant population. In turn, increased social support has been shown to protect mental health (Ozbay, Fitterling, Charney, & Southwick, 2008), and in undergraduate students, increased perceived social support was associated with lower scores of depression and anxiety (Zimet, Dahlem, Zimet, & Farley, 1988). Thus, if participants felt supported by participating in the study, it may have provided unexpected mood benefits that may not have been seen if each participant instead exercised in isolation during the intervention.

Interestingly, the frequent reporting of mood each week may have influenced participants' responses, especially among those with a preconceived belief that exercise "should" improve their mood each week (Desharnais et al., 1993). In a similar study using weekly reporting of depressive symptoms over a 12-week intervention, moderate-dose and low-dose aerobic exercise groups along with the stretching-control group all yielded improvements in depression across time (Dunn et al., 2005). Perhaps frequent reporting during interventions increases the probability that participants become de-sensitized to the survey, and makes week-to-week changes less sensitive, thus

contributing to the gradual improvements found over time. A potential solution is for future studies to employ less frequent reporting of mood to increase sensitivity to changes across an intervention. Overall it appears that our HIIT and control group may have improved in mood through potential psychological mechanisms associated with being in an exercise intervention, rather than due to the exercise training itself.

Resting-state cytokines. Due to the relationship between elevated levels of inflammation and depression (Rosenblat et al., 2014), pro-inflammatory cytokines were examined to see if they displayed similar patterns to the mood changes. It was expected that with the extended exercise-training period, there would be improved adaptations in the HIIT group for both mood and the acute inflammatory response, despite elevations in pro-inflammatory cytokines at rest. Consistent with this prediction, resting-state IL-6 remained higher for HIIT than controls, which supports our previous findings that HIIT elevates resting-state IL-6 (Paolucci, Submitted). This may be due to the release of IL-6 by the skeletal muscle during HIIT exercise (Pedersen & Febbraio, 2008) causing resting-state levels to remain elevated across the exercise intervention. This effect was also found in the exercise withdrawal study, where exercisers had higher levels of resting-state IL-6 compared to those who stopped exercising for two weeks (Poole et al., 2011), and implies that two weeks without exercise might be enough time to see IL-6 levels decline after a multi-week training period. Likewise, the lack of exercise training in the control group may be a reason why resting-state levels of IL-6 were lower at post-test.

No significant changes occurred in resting-state levels of TNF-α or IL-1β, but both showed directional trends similar to IL-6, indicating that these pro-inflammatory

cytokines may have reacted similarly to HIIT. Exercise has been shown to elevate TNF-α and IL-1β (Ostrowski et al., 1999; Petersen & Pedersen, 2005); however, they are not elevated to the same extent as IL-6 (Fischer, 2006; Ostrowski et al., 1999; Petersen & Pedersen, 2005). Thus, TNF-α and IL-1β may not have been captured as significantly elevated during resting-state measures taken at post-testing. In our previous study, HIIT was also shown to elevate TNF-α at rest (Paolucci, Submitted); however, this was not replicated in the current study, possibly due to lower scores on the anxiety and depression scales at post-test, which are generally associated with lower levels of TNF-α. The lack of change in IL-1β corroborates the findings in our previous study (Paolucci, Submitted), thus IL-1β might not be as sensitive of a biomarker for anxiety and depressive symptoms in a non-clinical population.

As there is a relationship between elevated resting-state inflammation and a negative mood state (Rosenblat et al., 2014), it follows that the elevated IL-6 levels resulting from skeletal muscle release might mitigate HIIT's ability to improve depression and anxiety symptoms to a greater extent than the control group alone. Indeed, although not significant, TNF-α and IL-1β were both trending as higher in the HIIT group at post-testing compared to the control group. In our previous study, HIIT also increased resting-state levels of TNF-α (Paolucci, Submitted). Thus, elevations in circulating IL-6 and TNF-α from HIIT training might prevent HIIT from reducing depressive symptoms through the neuroendocrine inflammatory mechanism.

Max workload and the acute cytokine response. As expected, with training the HIIT group increased the maximum workload achieved on their peak power ouput test

over time, whereas the control group showed no improvement across the intervention. After nine weeks of training, the acute responses of IL-6 and TNF-α were significantly greater for controls compared to HIIT. Critically, the HIIT group displayed no change in the acute response despite a 6.24% increase in workload during the peak power output test. This demonstrates that HIIT may attenuate the acute cytokine response to exercise in our previously sedentary individuals, as they become more trained and more fit. These results match the findings of the cross-sectional study displaying that trained individuals have an attenuated cytokine response compared to untrained individuals (Gokhale et al., 2007).

A similar but non-significant trend was observed for IL-1β (Figure 4C), further supporting the notion that IL-1β may not be a sensitive biomarker within a non-clinical population. According to the manufacturer's multiplex protocol (EMD Millipore; Billerica, Massachusetts), the minimum detectable concentration for IL-1β is 0.14 pg/ml. It is possible that the small and irregular fluctuations in the IL-1β raw scores seen in Figure 5C are due to random variations as a result of the difficulty in detecting IL-1β at low concentrations. In contrast, the minimum detectable concentrations for IL-6 and TNF-α are 0.11 pg/ml, and 0.16 pg/ml respectively, which are much smaller than the raw score changes in IL-6 and TNF-α depicted in Figure 5A and 5B. Thus, the changes in IL-6 and TNF-α appear to be more robust than IL-1β.

Alternatively, it is also possible that the adaptations seen in the acute cytokine response may be due to day-to-day variations in the cytokine concentrations. One study examined the daily variation in plasma IL-6 in healthy older adults, and found that while

daily variation in IL-6 within an individual is relatively low, variations in IL-6 between individuals is high (Picotte, Campbell, & Thorland, 2009). This could explain why large standard deviations are seen within our sample (Table 4 and Appendix B: Table 6). Additionally, Picotte's results indicate that any changes in IL-6 less than 0.32 pg/ml may be due to normal fluctuations in IL-6 concentrations between trials, and not attributed to an intervention such as exercise (Picotte et al., 2009). Although the day-to-day variation of IL-6 and TNF-α in younger adults has not been examined, it is possible that changes in the acute cytokine response may have been influenced by this random daily variation. However, for IL-6 we did see changes in cytokines that were larger than 0.32 pg/ml (Picotte et al., 2009)(Figure 5A), suggesting that these adaptations may be due to HIIT training. Overall these results support the hypothesis that exercise attenuates the acute pro-inflammatory response of IL-6 and TNF-α after exercise, suggesting that HIIT is inducing physiological adaption of the acute inflammatory response, although it does not appear to have an effect on mood.

Limitations

One limitation of this study is that participants were not able to fast for blood draws due to the taxing nature of the maximal exercise-test with draws occurring before and after exercise in the afternoon. Thus, pro-inflammatory cytokines could have been influenced by variations in our participants' diets (Giugliano, Ceriello, & Esposito, 2006). Despite the un-fasted blood draws, all three pro-inflammatory cytokines examined in this study showed similar trends for each cytokine measure, and replicated cytokine results from our previous study in which participants fasted for 12 hours prior to the blood draw

(Paolucci, Submitted). This may signify that participants' diets did not have a major influence on our blood marker measures. To help control for influences of diet that could alter resting-state levels, the difference scores were calculated from rest to after exercise to ascertain the cytokine reactivity after exercise, regardless of baseline.

Secondly, the average RER values for the HIIT and control groups at mid-testing appear to be just below the cutoff point for a maximal VO_2 peak test ($\geq$1.1; Table 2). Approximately half of participants reached their physiological max on the peak-power output test (Table 3) at mid-test. This may have been due to a calibration error in the metabolic chart, or participants not pushing themselves as hard as on the pre and post-tests. However, HIIT participants still showed an improvement in maximum workload from pre to mid-test (Table 5), suggesting that the HIIT group worked harder and improved performance on the peak power output test despite some not reaching the physiological threshold for maximum exertion. Controls achieved a similar maximum workload on all three peak-power output tests, suggesting that they performed similarly on the test each time.

Third, although there were adaptations in the acute cytokine response, perhaps nine weeks of training was still not long enough to see adaptations in resting-state levels as a result of the attenuated acute response. Future studies should examine the effects of long-term HIIT interventions on the pro-inflammatory profile of sedentary individuals.

Fourth, cortisol was not analyzed in this study, thus conclusions about stress-reactivity and the effects of exercise on the physiological stress system cannot be formed at this time. However, stress was measured in order to ascertain if the student population

was undergoing stress across the school term, which showed elevated levels at the beginning of the term that decreased within the first three weeks. Lastly, stress was not elevated near the examination period at the end of the term, suggesting that our student sample may not have been undergoing the same chronic stress as our previous study (Paolucci, Submitted). This may have influenced the buffering effect of HIIT on stress-induced depressive symptoms, as these students did not appear at a high risk of developing it.

Future Directions

Further studies are required to examine whether HIIT can buffer the effects of stress-induced depressive symptoms and anxiety in a sample of students who are experiencing greater levels of stress across the school term. It should also be examined if moderate-intensity exercise produces the same physiological adaptations in cytokine reactivity. Subsequently, cortisol and other markers of the HPA-axis (Dhabhar, 2014) should be analyzed to ascertain if there is an interaction between stress-reactivity and immune-reactivity in response to exercise training. This additional knowledge can help elucidate which physiological pathways exercise training elicits in order to improve mood. Additionally, this study reveals important information about the use of HIIT to improve mental health outcomes, as it appears similarly effective in improving mood as other alternative psychological mechanisms, such as social engagement. It will be important for future work to examine the synergistic effects of HIIT and social engagement to unpack the combined and isolated effect of each on mood.

Conclusion

Although there is strong belief that exercise benefits mood, we were unsuccessful in finding support for HIIT to improve mental health outcomes over and above a sham exercise group. HIIT was also accompanied by higher levels of pro-inflammatory cytokine IL-6 and may have mitigated its mood boosting effects, given the link between inflammation, depressive symptoms, and anxiety. Still, there was an adaptation to maximal exercise such that the maximum workload tolerated increased while the acute inflammatory response to exercise decreased, suggesting that HIIT was able to induce physiological change but did not seem to modulate mood. Overall, more research is needed to examine the exercise intensity that is most effective in improving mental health outcomes such as depression, anxiety, and stress. This work suggests that HIIT is not the most effective prescription for mental health. For individuals with elevated levels of inflammation, who may be at a greater risk of developing anxiety or depression, moderate-intensity exercise appears to be a more effective prescription than HIIT to improve these mental health outcomes.

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Appendix A: Study Materials

Beck Depression Inventory-II

Beck Anxiety Inventory

Perceived Stress Scale

Borg Rating of Perceived Exertion (RPE)

Beck Depression Inventory-II

BDI-II

Name: _____ Date: _____ Mood Id: _____

Please read each group of statements carefully, then pick out the **one statement** in each group which best describes the way you have been feeling during the **past two weeks including today!** Circle the number beside the statement you have picked. **Do not** leave any statements blank.

If several statements in the group seem to apply equally well, circle the largest number for that group of statements. Be sure that you do **not** mark more than one statement for Item 16 (change in sleeping pattern and Item 18 (change in appetite).

1. **Sadness**
   - 0 I do not feel sad.
   - 1 I feel sad much of the time.
   - 2 I am sad all the time.
   - 3 I am so sad or unhappy that I can't stand it.

2. **Pessimism**
   - 0 I am not discouraged about my future.
   - 1 I feel more discouraged about my future than I used to be.
   - 2 I do not expect things to work out for me.
   - 3 I feel my future is hopeless and will only get worse.

3. **Past Failure**
   - 0 I do not feel like a failure
   - 1 I have failed more than I should have.
   - 2 As I look back, I see a lot of failures.
   - 3 I feel that I am a total failure as a person.

4. **Loss of Pleasure**
   - 0 I get as much pleasure as I ever did from the things I enjoy.
   - 1 I don't enjoy things as much as I used to.
   - 2 I get very little pleasure from the things I used to enjoy.
   - 3 I can't get any pleasure from the things I used to enjoy.

5. **Guilty Feelings**
   - 0 I don't feel particularly guilty.
   - 1 I feel guilty over many things I have done or should have done.
   - 2 I feel quite guilty most of the time.
   - 3 I feel guilty all of the time.

6. **Punishment Feelings**
   - 0 I don't feel I am being punished.
   - 1 I feel I may be punished.
   - 2 I expect to be punished.
   - 3 I feel I am being punished.

7. **Self Dislike**
   - 0 I feel the same about myself as ever.
   - 1 I have lost confidence in myself.
   - 2 I am disappointed in myself.
   - 3 I dislike myself.

8. **Self Criticism**
   - 0 I don't criticize or blame myself more than usual.
   - 1 I am more critical of myself than I used to be.
   - 2 I criticize myself for all my faults.
   - 3 I blame myself for everything bad than happens.

9. **Suicidal thoughts and Dying**
   - 0 I don't have any thoughts of killing myself.
   - 1 I have thoughts of killing myself, but I would not carry them out.
   - 2 I would like to kill my self.
   - 3 I would kill myself if I had the chance.

10. **Crying**
    - 0 I don't cry any more than I used to.
    - 1 I cry more than I used to.
    - 2 I cry over every little thing.
    - 3 I feel like crying but I can't.

11. **Agitation**
    - 0 I am no more restless or wound up than usual.
    - 1 I feel more restless or wound up than usual.
    - 2 I am so restless or agitated that it's hard to stay still.
    - 3 I am so restless or agitated, I have to keep moving or doing something

12. **Loss of Interest**
    - 0 I have not lost interest in other people or activities.
    - 1 I am less interested in other people or things than before.
    - 2 I have lost most of my interest in other people or things.
    - 3 It's hard to get interested in anything.

13. **Indecisiveness**

(Please turn over and complete the other side.)

0 I make decisions about as well as ever.

1 I find it more difficult to make decisions than usual.

2 I have much greater difficulty in making decisions than I used to.

3 I have trouble making any decisions.

14. **Worthlessness**

0 I do not feel I am worthless.

1 I don't consider myself as worthwhile or useful as I used to.

2 I feel more worthless compared to other people.

3 I feel utterly worthless.

15. **Loss of Energy**

0 I have as much energy as ever.

1 I have less energy than I used to have.

2 I don't have enough energy to do very much.

3 I don't have enough energy to do anything.

16. **Change in Sleeping Pattern**

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.
2b I sleep a lot less than usual.

3a I sleep most of the day.
3b I wake up 1-2 hours early and can't get back to sleep.

17. **Irritability**

0 I am no more irritable than usual.

1 I am more irritable than usual.

2 I am much more irritable than usual.

3 I am irritable all the time.

18. **Changes in Appetite**

0 I have not experienced any changes in my appetite.

1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.

2a My appetite is much less than usual.
2b My appetite is much greater than usual.

3a I have no appetite at all.
3b I crave food all the time.

19. **Concentration Difficulty**

0 I can concentrate as well as ever.

1 I can't concentrate as well as usual.

2 It's hard to keep my mind on anything for very long.

3 I find I can't concentrate on anything.

20. **Tiredness or Fatigue**

0 I am no more tired or fatigued than usual.

1 I get more tired or fatigued more easily than usual.

2 I am too tired or fatigued to do a lot of the things I used to do.

3 I am too tired or fatigued to do most of the things I used to do.

21. **Loss of Interest in Sex**

0 I have not noticed any recent changes in my interest in sex.

1 I am less interested in sex than I used to be.

2 I am much less interested in sex now.

3 I have lost interest in sex completely.

Supplemental Questions:

22. Approximately how long has your emotional distress being going on?

1 Less than 2 weeks

2 2-3 weeks

3 1-2 months

4 3-5 months

5 6-12 months

6 More than 1 year

23. Do you feel worse in the morning and better as the day progresses?

1 Yes

2 No

24. Do you find that good things that happen don't make you feel any better?

1 Yes

2 No

25. Do you find that good things do make you feel better at least temporarily?

1 Yes

2 No

26. Have you had period lasting several days or weeks in which you felt very happy, elated (or "high"), had tremendous energy, and were much more active than usual?

1 Yes

2 No

Beck Anxiety Inventory

BAI

Name: _____ Date: _____ ID Number: _____

Below is a list of common symptoms of anxiety. Please read each item in the list carefully. Indicate how much you have been bothered by each symptom during the **PAST WEEK, INCLUDING TODAY** by filling in the circle in the column next to each symptom. Do not fill in more than one circle for each symptom and do not leave any symptom blank.

		Not at all	Mildly it did not bother me much	Moderately it was very unpleasant but I could stand it	Severely I could barely stand it
1	Numbness or tingling	○	○	○	○
2	Feeling hot	○	○	○	○
3	Wobbliness in legs	○	○	○	○
4	Unable to relax	○	○	○	○
5	Fear of the worst happening	○	○	○	○
6	Dizzy or lightheaded	○	○	○	○
7	Heart pounding or racing	○	○	○	○
8	Unsteady	○	○	○	○
9	Terrified	○	○	○	○
10	Nervous	○	○	○	○
11	Feelings of choking	○	○	○	○
12	Hands trembling	○	○	○	○
13	Shaky	○	○	○	○
14	Fear of losing control	○	○	○	○
15	Difficulty breathing	○	○	○	○
16	Fear of dying	○	○	○	○
17	Scared	○	○	○	○
18	Indigestion or discomfort in abdomen	○	○	○	○
19	Faint	○	○	○	○
20	Face flushed	○	○	○	○
21	Sweating (not due to heat)	○	○	○	○

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name _____ Date _____

Age _____ Gender (*Circle*): **M F** Other _____

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?	**0**	**1**	**2**	**3**	**4**
2. In the last month, how often have you felt that you were unable to control the important things in your life?	**0**	**1**	**2**	**3**	**4**
3. In the last month, how often have you felt nervous and "stressed"?	**0**	**1**	**2**	**3**	**4**
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	**0**	**1**	**2**	**3**	**4**
5. In the last month, how often have you felt that things were going your way?	**0**	**1**	**2**	**3**	**4**
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	**0**	**1**	**2**	**3**	**4**
7. In the last month, how often have you been able to control irritations in your life?	**0**	**1**	**2**	**3**	**4**
8. In the last month, how often have you felt that you were on top of things?	**0**	**1**	**2**	**3**	**4**
9. In the last month, how often have you been angered because of things that were outside of your control?	**0**	**1**	**2**	**3**	**4**
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	**0**	**1**	**2**	**3**	**4**

Borg Rating of Perceived Exertion

6	No exertion at all
7	Extremely light
8	
9	Very light
10	
11	Light
12	
13	Somewhat hard
14	
15	Hard (heavy)
16	
17	Very hard
18	
19	Extremely hard
20	Maximal exertion

APPENDIX B: TABLES

Table 6: Raw means (standard deviations) of pro-inflammatory cytokine values, at rest (before ex.) and immediately after exercise (after ex.), at pre, mid, and post-testing.

Table 7: Effect Size Ranges for Partial Eta-Squared (η_p^2)

Table 6: *Raw means (standard deviations) of pro-inflammatory cytokine values, at rest (before ex.) and immediately after exercise (after ex.), at pre, mid, and post-testing.*

		Pre M(SD)		Mid M(SD)		Post M(SD)	
		Before	After	Before	After	Before	After
IL-1β (pg/ml)	HIIT	2.18 (2.40)	1.43 (2.38)	2.20 (2.48)	2.34 (2.47)	2.57 (2.79)	2.77 (2.76)
	Control	1.88 (2.63)	2.36 (2.92)	1.86 (6.21)	1.94 (2.60)	2.71 (4.81)	3.27 (5.87)
IL-6 (pg/ml)	HIIT	7.97 (9.10)	9.53 (10.24)	7.64 (9.35)	8.65 (9.49)	8.04 (8.39)	8.77 (8.98)
	Control	10.68 (9.67)	11.81 (10.18)	10.11 (13.00)	12.08 (21.32)	8.25 (8.71)	10.34 (10.48)
TNF- α (pg/ml)	HIIT	8.26 (5.65)	9.01 (5.60)	8.15 (5.62)	9.19 (6.27)	9.36 (6.82)	10.59 (6.31)
	Control	10.07 (7.84)	9.63 (5.47)	8.53 (6.68)	9.06 (7.66)	7.68 (4.93)	9.62 (5.44)

Table 7: Effect Size Ranges for Partial Eta-Squared (η_p^2) (Miles & Shevlin, 2001)

Effect Size	Small	Medium	Large
Partial eta-squared	0.01	0.06	0.14