MOVEMENT BEHAVIOURS AND INFLAMMATION IN CHILDREN

AN ASSESSMENT OF MOVEMENT BEHAVIOURS AND INFLAMMATION IN CHILDREN WITH A CHRONIC INFLAMMATORY DISEASE

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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

Descriptive Note

McMaster University MASTER OF SCIENCE (2022) Hamilton, Ontario

TITLE:	**An assessment of movement behaviours and inflammation in children with a chronic inflammatory disease**
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NUMBER OF PAGES:	**xvii, 106**

LAY ABSTRACT

The purpose of this study was to assess how physical activity, sedentary time, and inflammation are associated. Inflammation is an important part of our immune system that protects us from infection and disease; however, when inflammation goes unchecked, it can cause serious chronic inflammatory disease. We were interested in understanding if children with a chronic inflammatory disease had different levels of physical activity and sedentary time, or different levels of inflammation than healthy children with no medical conditions. We were also interested in understanding if physical activity or sedentary time were related to levels of inflammation in children with a chronic disease. We asked boys and girls between 7 and 17 years old to visit our lab twice. Some of our participants had a medical condition, including chronic kidney disease, cystic fibrosis, juvenile idiopathic arthritis, or inflammatory bowel disease and type 1 diabetes. We also invited a group of children who had no medical condition to participate. During their first visit, we measured their weight, height, pubertal status and gave them a physical activity monitor to wear for 7 days before coming back for their second visit. At their second visit we took a small blood sample that we used to measure immune proteins called cytokines, these proteins act like messengers to tell the immune system what to do. Some of them make inflammation worse, and others help to bring down levels of inflammation. We found that children who had a chronic inflammatory disease participated in less physical activity but had similar levels of sedentary time compared with healthy children. We also found that there were no differences in inflammation between children with a chronic disease and healthy children. Lastly, we found that physical activity and sedentary time were not related to inflammation levels. Although we did not find a relationship between physical activity and inflammation, we know that physical activity has many

beneficial cardiorespiratory and mental health effects. As such, it is still of interest to uncover any potential effects movement behaviours may have on health outcomes.

ABSTRACT

Children with chronic inflammatory disease (CID) are at an increased risk for health complications including mental health issues, cancer, and cardiovascular disease. These complications have been linked to elevated levels of pro-inflammatory cytokines and lifestyle behaviours including low physical activity, and high sedentary time. Physical activity may represent a simple and effective strategy to modulate inflammation and subsequently improve health outcomes. However, the link between cytokines and movement behaviours in children with a CID remains poorly understood. Indeed, no studies to date have examined the link between a broad complement of inflammatory markers and patterns of movement behaviours in children with a CID. Therefore, the objectives of this study were to: (1) examine movement behaviours and inflammation in children with either cystic fibrosis (CF), juvenile idiopathic arthritis (JIA), inflammatory bowel disease (IBD), and type 1 diabetes (T1DM), (2) examine movement behaviours and inflammation in children with a CID compared with healthy controls and (3) examine the association between movement behaviours and inflammation profiles in children with a CID. We hypothesized that (1) children with a CID will have comparable movement profiles and inflammation, (2) children with a CID will have lower physical activity levels and higher levels of pro-inflammatory cytokines relative to healthy counterparts, and (3) children with a CID who are more physically active and engage in less sedentary time will have less inflammation. Boys and girls with a CID and controls wore an ActiGraph GT3X accelerometer around the waist during waking hours for 7 days. Outcomes of interest included, sedentary time, time spent in light physical activity (LPA), moderate-to-vigorous PA (MVPA), and total PA (TPA), determined using Evenson cut-points. After one week a fasted blood sample was collected to determine serum cytokines (TNFα, IL-23, IL-1β, IL-12, IL-6, IL-17, TGFβ, IL-10) by multiplex assays and C-reactive protein by enzyme linked immunosorbent

assay. A total of 132 participants (47% girls; age: 13.3±2.8 years), including JIA (N=27), IBD (N=21), CF (N=14), T1DM (N=18) and healthy controls (N=52), completed the study. Physical activity and inflammatory profiles were comparable between CF, JIA, IBD and T1DM groups. Children with a CID participated in 13.3 fewer mins/day [95% confidence interval 6.9, 101.2] (MVPA ($F(1,113)=11.015$, $p=0.001$) of MVPA relative to healthy controls and had comparable cytokine profiles. Physical activity did not predict inflammation in children with a CID. However, we know that physical activity has many beneficial cardiorespiratory and mental health effects. As such, it is still of interest to uncover any potential effects movement behaviours may have on our immune system.

ACKNOWLEDGEMENTS

I will be forever grateful for the ability to conduct research in such a warm welcoming environment. Over the past few years I have learned an incredible amount and I would like to take this opportunity to thank all of those who have helped me along the way.

I would like to express my deepest appreciation to Dr. Joyce Obeid, thank you for believing in my ability to return to research after a stint out of school. You have helped me develop important research skills that I will take with me into my future career. You have supported me in many academic and personal endeavours over the past two years, all while continuing to flourish in your own career. Your empathy and compassion are what sets you apart from other supervisors and I cannot thank you enough for all that you have taught me.

To my committee members, Dr. Brian Timmons, and Dr. Nikhil Pai, thank you for your support and guidance through an odd (mostly) zoom-navigated process. To the students, post-docs and faculty who have helped me through a strange COVID-19 thesis, Dr. Bhanu Sharma, Maddy Byra, Elyse Letts, Sam Morin, Mahnoor Akram and Randy Calotti. It has been a pleasure getting to know each one of you and I am so thankful that we were able to spend some time in person together. A special thank you to Maddy Byra, for welcoming me on to the CHAMPION team – but more notably being a great friend.

A special thanks to my friends, thank you for your unwavering support and understanding through this time. You have been patient and understanding of what I do and how it impacts the amount of time you are able to spend with me. When we do have precious time together, you are supportive, encouraging and uplifting.

Finally, my sincerest thanks to my mom, dad, and sister. My path to this has been a winding one and you have been patient and supportive of me during my failures along with my successes. Dad, your love for science, accomplishments and teaching is aspirational and what I could only hope to achieve in a lifetime. I would not be who I am or where I am, today without all of you.

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LIST OF ABBREVIATIONS AND SYMBOLS:

APC	Antigen-presenting cell
BMI	Body mass index
CF	Cystic fibrosis
CID	Chronic inflammatory disease
CKD	Chronic kidney disease
CRP	C reactive protein
CTL	control
IBD	Inflammatory bowel disease
IFNγ	Interferon gamma
IL	Interleukin
JIA	Juvenile idiopathic arthritis
LPA	Light physical activity
MVPA	Moderate-vigorous physical activity
T1DM	Type 1 diabetes melitus
TGFβ	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
TPA	Total physical activity

DECLARATION OF ACADEMIC ACHIEVEMENT

Authors of funding grants for this project: JO, BWT

Conceived and designed the research: JO, BWT, MB, EB

Participant recruitment and testing: JO, RC, MB, EB

Post-visit bloodwork: JO, MBjelica, EB

ELISAs: EB

Accelerometer cleaning and analyses: EB, JO

Analyzed and interpreted data: EB, JO

Written report: EB

Revision of report: JO, EB

Chapter 1: Introduction

1.0 CYTOKINES

1.1 Overview of Cytokines

Cytokines are endogenous non-structural proteins that have multiple sites of origin throughout the human body[1]. They are implicated in degenerative processes with aging, antigen response and recognition, as well as disease pathology and autoimmunogenicity[2,3]. These small proteins regulate the immune system, acting as intercellular messengers to activate signalling cascades and confer pro- and anti-inflammatory effects both locally and systemically[4,5]. Inflammatory processes are an important function of the immune system; however, when cytokines become dysregulated, these processes can result in autoimmunity and disease. To date, there has been a considerable amount of research that investigates cytokines and their respective functions as therapies against human disease. More recently cytokines have been validated as important non-traditional biomarkers in several pediatric diseases including, but not limited to, Cystic Fibrosis (CF), Juvenile Idiopathic Arthritis (JIA), Inflammatory Bowel Disease (IBD), Type 1 Diabetes (T1DM), Chronic Kidney Disease (CKD), and Idiopathic Nephrotic Syndrome[2,4,6–13]. For example, in one study on steroid susceptibility as a treatment for children with Idiopathic Nephrotic Syndrome, researchers found that interleukin (IL) -1β, IL-6 and IL-8 were accurate biomarkers for predicting whether a child would respond to steroid treatment[6]. Given the ubiquitous nature of cytokines, and the role of cytokine dysregulation in multiple pediatric diseases, identifying ways that cytokines can be modulated may serve as a strategy to improve outcomes in chronic inflammatory disease (CID) and predict responsiveness to treatment.

1.2 Types of Cytokines

Cytokines are generally grouped by functional classes defined by their predominant function, such as differentiation, inflammation, angiogenesis, and viral pathogenesis[3,4]. Pro-inflammatory cytokines primarily function to increase inflammation as a protective host mechanism. However, a hyper-inflammatory response can occur when pro-inflammatory cytokines spill over from tissue into serum. This can result in undue harm such as the chronic low-grade systemic inflammation commonly reported in CID[1,14,15]. Conversely, anti-inflammatory cytokines act in concert with pro-inflammatory cytokines to regulate and dampen the inflammatory human immune response. A strict functional cytokine dichotomy does not exist because most cytokines are pleiotropic and confer both pro- and anti-inflammatory effects[3,5]. Autoimmunity and auto-inflammatory conditions are often a result of an imbalance between anti-inflammatory and pro-inflammatory mechanisms. As such, cytokines are considered to be relevant pharmacologic biomarkers that can also be manipulated for countering disease[5,16,17].

1.3 Role of Cytokines in Innate and Adaptive Immunity

Innate immunity is a human's first line of defence against pathogens because it is non-specific and quick to react[18]. When an innate inflammatory response begins to mount, IL-1, IL-6 and TNF-α are generated in preparation for microbial defense[19]. Secondarily, any local foreign material is presented by antigen presenting cells (APCs) as an initiation signal for the adaptive immune response.

Adaptive immunity reacts with more specificity to threats and is slower to mount a response than the innate branch of the immune system. Once cellular components of the adaptive immune system recognize an antigen via an APC, subsequent cytokine involvement mediates and

determines specific T-cell subsets for activation. For example, commitment to the Type 1 helper T lymphocytes (Th1) lineage occurs in the presence of IL-12, while Type 2 helper T lymphocytes (Th2) differentiation occurs in the presence of IL-4, STAT6, GATA3, IL-5, IL-9, and IL-13. These lineage commitments combined are important drivers for humoral and antibody IgE-mediated immunity[19]. Importantly, specific cytokines can activate different Th subsets and drive inflammatory responses, thereby highlighting their complex and central role in the immune response.

1.4 Cytokine Role in Pathology

Dysregulated or imbalanced cytokines have been implicated in disease pathology. For the purposes of this thesis, I will focus on the role of select cytokines in a subset of pediatric chronic inflammatory conditions, including CKD, CF, JIA, IBD, and T1DM. I will also draw on evidence from other CIDs in human and mouse models examining the impact of cytokines on important health-related outcomes. Overall, cytokine dysregulation has been linked to a breadth of symptoms including joint pain, abdominal pain, diarrhea, constipation, fatigue, and immobility[2,15,20–25]. Moreover, studies that have investigated the role of cytokines in disease pathology in pediatric populations often report significant growth challenges, like height stunting[26], that may be partly attributable to cytokine-mediated increases in inflammation[26–28].

1.5 Selected Cytokines and Chronic Inflammatory Disease

1.5.1 Tumor Necrosis Factor Alpha

Tumor Necrosis Factor alpha (TNF-α) is a pro-inflammatory cytokine primarily produced by macrophages and monocytes during acute inflammation. TNF-α can be a potent tumour killing

causative agent or it can cause chronic systemic inflammation. High TNF-α levels and its associated inflammation are implicated in a range of autoimmune diseases and pathological processes[29]. For example, TNF-α is produced by adipocytes and is higher in adults with obesity. This increase in expression is positively correlated with weight, waist circumference, and measures of insulin resistance[30]. Furthermore, in mice TNF-α expression has a direct role in obesity-linked insulin resistance, whereby neutralizing TNF-α causes a significant increase in peripheral uptake of glucose in response to insulin[31]. Elevated circulating levels of TNF-α are linked with rapid loss of kidney function (as defined by declining glomerular filtration rate) in adult patients with CKD, and TNF-α is an independent marker of CKD progression[10]. Similarly, nephron injury in pediatric CKD has been partially attributed to TNF-α, as seen in Figure 1[19,23]. Elevated levels of TNF-α have been found in synovium of adult patients with rheumatoid arthritis and children with JIA[21]. Elevated TNF-α contributes to the erosion and destruction of cartilage and bone in these patients[32]. TNF-α has also been linked to uveitis in children with JIA[21]. In children with IBD, elevated TNF-α has been linked to mucosal dysregulation[33]. Moreover, TNF-α contributes to β-cell toxicity and the pathogenesis of insulin resistance in adults with T1DM. High levels of TNF-α have also been found in the sputum of adult patients with CF[24,34], and are associated with impaired secretory gland function[2]. Taken together, these data from children and adults highlight the negative role of TNF-α across multiple CID.

Despite these largely negative effects, when regulated properly in healthy individuals, TNF-α causes increased leukocyte activation and degranulation that helps defend against pathogens[19]. This beneficial effect makes it difficult to simply mute the function of this cytokine, as downregulation may increase susceptibility to infections[35]. Nevertheless, TNF-α-supressing

medication is commonly used in the treatment of autoimmune diseases, including JIA and IBD. While effective at managing these conditions, these anti-TNF-α treatments also suppress the beneficial host immune defenses the cytokine confers (leukocyte activation, degranulation, and vasodilation)[35]. It may therefore be beneficial to manage these diseases with an approach that is more delicate, rather than entirely pharmacologically blunting the effect of TNF-α[27].

Figure 1. Detrimental cellular pathology of TNF-α.

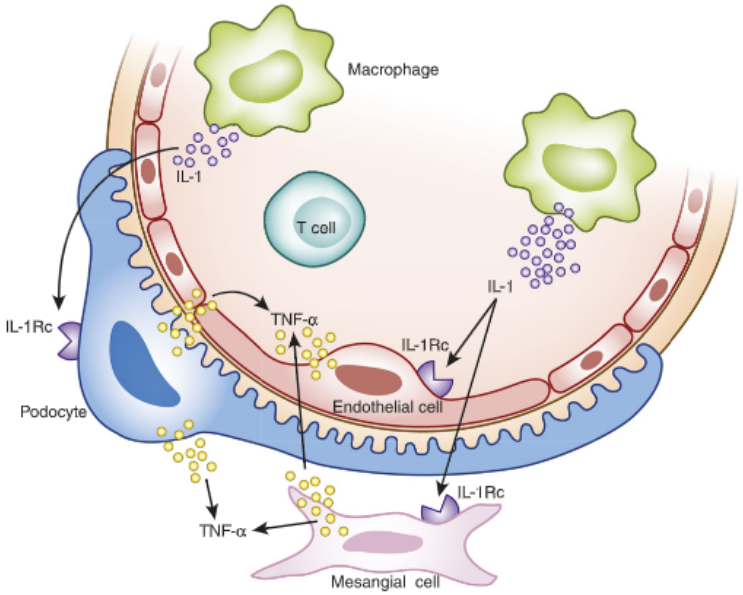

Figure note: An example of the negative effects of TNF-α. TNF-α and IL-1 associated nephron damage adapted from Holdsworth 2015. "Innate macrophages produce IL-1, which binds to IL-1Rc on intrinsic renal cells (endothelial cells, podocytes, and mesangial cells) to produce injurious TNF-α that amplifies T effector cell responses resulting in crescent formation and glomerular injury".

1.5.2 Interleukin-23

IL-23 is a heterodimeric pro-inflammatory cytokine that is part of the IL-6 superfamily. IL-23 is involved in tissue immunoregulatory inflammatory processes. IL-23 mediates appropriate differentiation of naïve CD4+ T-cells (adaptive immune branch) into various helper T cells subsets

and regulates the functions of different effector cells. IL-23 is widely implicated in the regulation of mucosal inflammation in the gut in IBD, rheumatoid arthritis, and spondyloarthritis[15,36]. IL-23 has been positively correlated with clinical symptoms including joint pain, and spondyloarthropathies in adult Crohn's disease and subsets of arthritis[37]. Elevated levels of IL-23 have also been observed in adult patients with CF undergoing pulmonary exacerbation[38]. Additionally, blood serum levels of IL-23 and TNF-α are elevated with onset of T1DM in children and adults[39,40]. IL-23 also contributes to cancer causing processes and acts as a bridge between our innate and adaptive immune system. The IL-23/IL-17 pathway is heavily implicated in inducing CD4+ T-cells to become highly pathogenic helper T-cells; a process that contributes to development of autoimmune diseases including rheumatoid arthritis and spondylarthritis, though the exact mechanisms are unclear[36]. Indeed, both innate and adaptive immune cells involved in the IL-23/IL-17 axis that express IL-23 receptors become pathogenic after they are exposed to IL-23[15,36]. This is still being investigated and it is not yet understood exactly how this may contribute to development and progression of chronic inflammatory disease. Despite not yet knowing the exact mechanism of IL-23 pathogenesis, it is important to measure in conjunction with axis cytokines IL-17 and IL-12 to better understand inflammatory pathways.

1.5.3 Interleukin-12

IL-12 is a heterodimeric pro-inflammatory cytokine implicated in tissue inflammation processes[14,15]. It is derived from dendritic cells and plays an important role in connecting the innate and adaptive components of the immune system, much like IL-23[15]. IL-12 is derived from the same heterodimeric subunit as IL-23 and IL-27, and contributes to pathology in IBD and other autoinflammatory conditions[15]. Experiments conducted in mice and humans have demonstrated

that blocking IL-12 confers protective benefits. Specifically, Ustekinumab is a therapeutic antibody used in the treatment of Crohn's Disease that targets IL-12 and IL-23 and has been shown to confer resistance against IBD autoimmune-induced symptoms such as bowel irregularities[15]. Importantly, the marked decrease in symptoms with IL-12 and IL-23 blockade in adults with IBD were sufficient to induce clinical remission in 53.1% of responders on a 12 week trial and 48.8% of responders in an 8 week trial, compared to 35.9% of patients on placebo[15]. These results have made IL-12 a target for therapeutics in autoimmunity and auto-inflammatory disorders. Unfortunately, researchers found that targeting IL-12 did not consistently reverse or maintain disease state, and exacerbated disease status and symptoms in the longer term. While more long-term follow up is needed, this effect has largely been attributed to IL-23 interacting with IL-12, highlighting the need for reliable long term therapeutic strategies to manage cytokines associated with disease symptomology. To date, there are a lack of studies on the relationship between IL-12 and symptoms of JIA, CF, and T1DM.

1.5.4 Interleukin-1 beta

IL-1β is a pro-inflammatory cytokine that responds to infection and injury, and has a role in pathogen resistance[1,41]. However, uncontrolled upregulated IL-1β levels have been correlated with symptoms in T1DM, JIA, IBD, CKD, CF and neuroinflammatory disorders[36,39,40,44]. IL-1β-mediated neutrophilia has been positively correlated with persistent mucous production and obstruction in adult patients with CF[44]. Elevated expression of IL-1β have been found in biopsy samples of colonic mucosa from symptomatic IBD patients with Crohn's and Ulcerative colitis, as well as in serum samples in adults and children with IBD[45,46]. Similarly, in adults with CKD, elevated IL-1β has been correlated with increased inflammation, decreased glomerular filtration

rate, increased inflammation scores, and higher urine albumin-to-creatinine ratio, all of which indicate worsening disease status[23]. Additionally, blood serum levels of IL-1β, are elevated at the onset of T1DM disease in adults and chilren[39,40 46]. In T1DM, IL-1β elevation is damaging because IL-1β auto-stimulation contributes to β-cell toxicity and the pathogenesis of insulin resistance[2]. Increased levels of IL-1β in the blood occur by a failed on/off mechanism, in which IL-1β secretion is not controlled or down-regulated properly. This leads to excess secretion of IL-1β from blood monocytes, and ultimately, systemic inflammation. Elevated IL-1β serum levels have been observed in patients with JIA, Familial Mediterranean Fever, and neuroinflammatory conditions; however, the relationship between elevated IL-1β and disease progression is not well characterized in these conditions[1,43,47]. Blocking IL-β activity in mice can cause a nearly immediate reversal of disease mechanisms that cause diabetic retinapothy[1,43]. Despite the clinical relevance of IL-1β, there appear to be no studies that evaluated the effects of IL-1β modulation on health outcomes in children with CID.

1.5.5 Interleukin-6

IL-6 originates from T cells, B cells, monocytes and polymorphonuclear leukocytes. One of its major anti-inflammatory functions is to inhibit TNF-α and IL-1 production by macrophages[5]. However, it can also induce T-cell growth, cytotoxic T-cell differentiation, and osteoclast differentiation, often leading to pro-inflammatory processes. IL-6 contributes to antigen-specific immune and inflammatory responses that are involved in causing bone and cartilage damage[3,48]. As such, IL-6 overproduction is implicated in rheumatoid arthritis and systemic JIA[49]. Additionally IL-6 is elevated and positively correlated with disease severity and specifically in secretory gland impairment adults with CF, renal fibrosis in adults with CKD, and gut mucosal

inflammation in those with Crohn's disease[24,34,46,50]. The role of IL-6 in T1DM development and progression are unclear[51]. Implementing an anti-IL-6 therapy such as Tocilizumab, while effective in limiting symptoms, leaves patients vulnerable to severe bacterial and viral infections[20].

1.5.6 Interleukin -17a

Interleukin-17a (IL-17) is expressed by T helper 17 cells and play key roles in human homeostasis regulation and host defense[52]. IL-17 is predominantly protective, guarding against pathogens, in particular bacteria and fungi, at epithelial and mucosal barriers[53]. Despite its integral role in host protection, IL-17 can contribute to excessive pro-inflammatory cytokine expression. This chronic inflammation can lead to tissue damage and has been linked to many autoimmune diseases[38,52,54,55]. For example, IL-17 has been found to be elevated in draining lymph nodes in patients with CF compared to controls[56,57]. There is evidence to suggest that IL-17 contributes to activation of the IL-17/IL-22 pathway in pediatric T1DM, contributing to the inflammation of islet cells of the pancreas[55]. Children with JIA have elevated joint synovial IL-17 correlated with polyarticular joint tenderness compared to patients with osteoarthritis or rheumatoid arthritis[54] and IL-17 is elevated in inflamed intestinal mucosa in adults with IBD[58]. While presence of IL-17 in inflamed tissues in IBD patients was once thought to be solely detrimental, recent evidence suggests that the this observation may indicate a protective effect[59]. This observation is bolstered by adoptive transfer experiments in mice that demonstrated a more aggressive colitis presentation in IL-17a deficient mice[60]. It is unclear whether the role of IL-17 in health and disease states is predominantly inflammatory, neutral, or anti-inflammatory in nature.

1.5.7 Transforming growth factor beta

Transforming growth factor beta (TGFβ) regulates cell proliferation, differentiation, death, cytoskeletal organization, adhesion and migration[61]. TGFβ is a family of proteins expressed in many cell lines[62], and TGFβ receptors are ubiquitous across most immune cells[63]. Given its diverse cellular impacts, TGFβ is fundamental in development and regulation of multicellular organisms. The numerous effects of TGFβ are depicted in Figure 2. Key among these is the ability of TGFβ to promote deposition of extracellular matrix and epithelial-mesenchymal transition[64,65], which is implicated in many disease processes[62]. This pathway is specifically thought to contribute to disease progression of kidney fibrosis and renal failure in adult patients with T1DM and CKD[22,66]. Exploiting this pathway to develop a treatment has been explored for T1DM, Marfans, cancer, cardiovascular disease, asthma and rheumatoid arthritis[22]. Dysregulated TGFβ has been associated with a hypertrophic chondrocyte phenotype in JIA, that may explain local joint growth disturbances[67]. TGFβ is also hypothesized to be a relevant target for treatment of CF due to its involvement in lung fibrosis, inflammation and injury; however, the mechanism of TGFβ in CF is unclear[12]. Additionally, TGFβ has been positively correlated with spontaneous colitis in mouse models and is observed in the intestines of child and adult IBD patients[64]. TGFβ can inhibit pro-inflammatory cytokine synthesis, thus making it an anti-inflammatory and immunosuppressive cytokine as well as a pro-inflammatory cytokine[5]. This duality, along with its broad impacts on the cellular environment pose a challenge in creating viable treatment options[68].

Figure 2. The numerous effects of TGFβ.

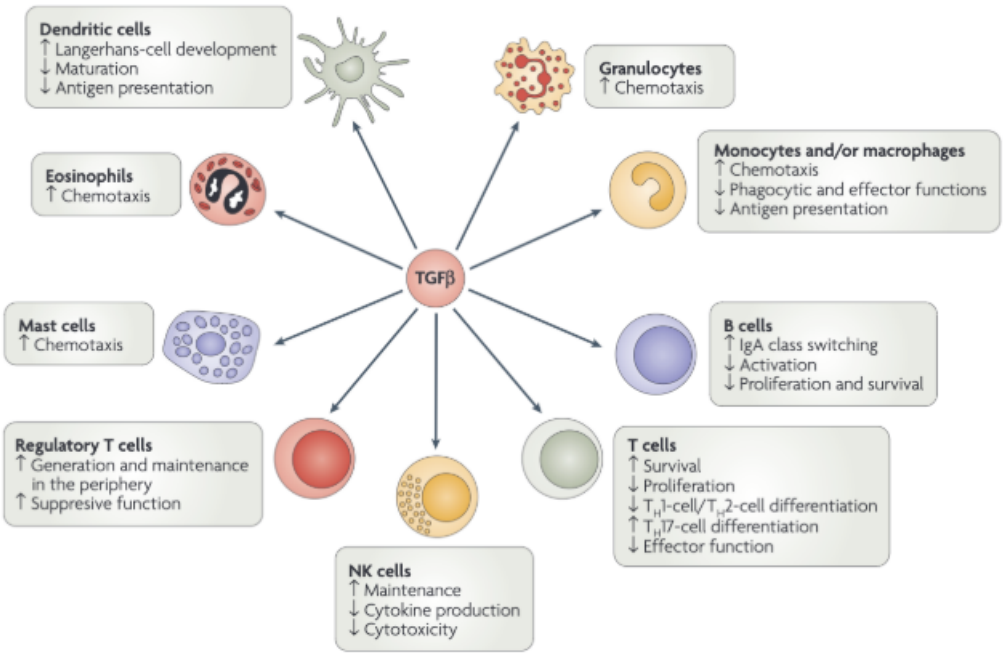

Figure note: adapted from Rubtsov & Rudensky 2007.

1.5.8 Interluekin-10

IL-10 is known for its anti-inflammatory properties. It is expressed by both cells of the innate (macrophages and dendric cells) and adaptive (T-cells) branches of the immune system. IL-10 contributes to the downregulation of excessive inflammatory immune responses by macrophage inhibition, via major histocompatibility complex class II and costimulatory molecule B7-1/B7-2, to limit production of pro-inflammatory cytokines IL-1, IL-6, IL8, TNF-α, IFN-γ and IL1β[69]. Loss of function via IL-10 receptor mutations has been associated with development of spontaneous enterocolitis in mice[70]. Defective or insufficient levels of IL-10 were found to contribute to pathogenesis in a systemic JIA-like mouse model[71]. Similarly, mice lacking IL-10 develop more severe kidney inflammation and fibrosis than control mice[72], while administration of IL-10

reduced fibrosis in a rat model of CKD[73]. Moreover, compared to healthy controls adult patients with CF have reduced levels of IL-10[24,34]. Without IL-10's ability to inhibit pro-inflammatory cytokines, pro-inflammatory cytokine activity becomes more dominant, thereby contributing to increased systemic inflammation. Interestingly, the delivery of IL-10 probiotics in murine models of Crohn's disease showed promising effects on preventing mucosal inflammation[70]. Collectively, these findings suggest that IL-10 plays a critical role in balancing pro-inflammatory processes, and as such, optimizing levels of IL-10 levels may lead to improved outcomes for those with a CID.

1.5.9 C-reactive Protein

C-reactive protein (CRP) is a widely-established, and routinely used as a clinical marker of acute inflammation as its plasma concentration increases during inflammatory states, such as myocardial infarctions, acute rheumatic flares, and intestinal flares [74–76]. CRP is part of the innate host immune response, and as such responds rapidly to infection and intrusion[76]. Traditionally CRP was thought of as a pro-inflammatory molecule, however, increasing evidence suggests it is pleiotropic and induces both anti- and pro-inflammatory effects[76]. CRP is a widely accepted indicator of inflammation in pediatric and adult patients with CF, CKD, IBD, JIA, and T1DM[77,78]. In a study of newly diagnosed pediatric IBD patients, common place laboratory markers such as hemoglobin, platelet count, albumin level, erythrocyte sedimentation rate and CRP were normal, indicating that these markers do not always correspond to disease pathology[79]. Importantly, cytokines may be elevated in sub-clinical disease states when CRP is not, due to its transient nature and isoforms[78]. Taken together, this suggests value in measuring CRP together with multiple inflammatory markers to gather a more robust picture of inflammatory processes.

1.6 Summary of CID and Associated Cytokines

Chronic inflammation in children and adults with CID is at least partially attributable to cytokine dysregulation, and is associated with a host of negative outcomes, as summarized in Table 1 below. Among these negative outcomes are impaired growth, pain, fibrosis, and debilitating symptoms[4,26,67]. Conversely, some cytokines downregulate inflammation and confer protective benefits, and in some cases, these may be the same cytokines that can also cause inflammation. Given their central role in disease processes, cytokines are valuable therapeutic targets. Importantly, measuring a wide array of cytokines, rather than single markers in isolation, may provide more comprehensive insight into a patient's health status. Current therapies are often targeted to be cytokine-specific, to blunt functions of cytokine activity that cause negative and/or uncomfortable symptoms. However, these therapies can also result in blunting beneficial actions of cytokines and may do more harm in the long term. This highlights the need to identify alternative avenues for regulating inflammation. Physical activity may be a viable alternative avenue and/or an adjunct therapy to trialled pharmaceutical treatments that can modulate cytokines and is discussed in detail below.

	JIA	IBD	CF	CKD	T1DM
TNF-α	• +ve relationship with joint inflammation and cartilage damage[21]	• +ve relationship with gut mucosal dysregulation[33]	• -ve relationship with secretory gland functioning*[24,34]	• -ve relationship with kidney function[10,19,23]	• Implicated in pathogenesis of insulin resistance*[2] • Elevated with disease onset*[2]
IL-23	• Found in joint synovial fluid[36]	• +ve relationship with gut mucosal dysregulation • +ve relationship with IBD associated spondyloarthropathies [37]*	• Elevated with pulmonary exacerbation*[38]	• N/A	• Elevated with onset of disease[39,40]
IL-12	• +ve relationship with joint immobility[80]	• +ve correlation with symptom exacerbation[15]	• N/A	• N/A	• N/A
IL-1β	• No relationship with increased joint inflammation or immobility[81] but elevated in serum	• +ve correlation with symptom exacerbation[15] • Elevated with disease onset[15]	• +ve correlation with persistent mucous production and obstruction*[44]	• -ve relationship with kidney function (decreased GFR)[23]	• Elevated with disease onset[39,40] • Positively correlated with β-cell toxicity and insulin resistance[39,40 46]
IL-6	• JIA > controls[49]	• +ve relationship with gut mucosal irregularity*[24,34,46,50]	• +ve relationship with disease severity[24,34,46,50]	• +ve correlation with disease severity and renal fibrosi[24,34]	• Not well defined

	• Positively relationship with pain[3,48]				
IL-17	• +ve relationship with active disease[54]	• Conflicting evidence for relationship[59,60]	• +ve relationship with CF exacerbation[57]	• Minimal evidence	• -ve relationship with pancreatic islet function[55]
TGFβ	• +ve trend with disease severity[67] • +ve relationship with hypertrophic chondrocyte phenotype[67]	• +ve correlation with spontaneous colitis**[62] • Elevated in Crohn's Disease[62]	• +ve trend with lung fibrosis*[12]	• +ve relationship with kidney fibrosis*[60]	• +ve relationship with disease progression and renal fibrosis*[22]
IL-10	• Deficiency relationship to pathogenesis**[71]	• +ve association with prevention of mucosal inflammation**[70]	• CF<controls*[24,34] • -ve relationship with inflammation*[24,34]	• +ve relationship with reduced renal fibrosis**[68]	• -ve relationship with renal fibrosis**[67,68]
CRP	• +ve relationship with joint inflammation and disease flare[74–76]	• +ve relationship during acute intestinal flares[74–76]	• +ve relationship during disease flare[77,78]	• +ve relationship with disease progression[77,78]	• +ve relationship with disease flare[77,78]

Table 1: Summary of cytokines and CID. Table note: Associated observations listed per condition. N/A = no studies available. *= adult study only, **=mouse model study only.

1.7 Movement behaviours, cytokines and inflammation

1.7.1 Physical activity

Physical activity is a behaviour described as "any bodily movement produced by skeletal muscles that results in substantial increase over the resting energy expenditure"[82,83], and is an important and well-established predictor of health across the lifespan[84,85]. Low levels of physical activity have been linked with increased cardiovascular risk, obesity, and greater inflammation[28,82]. Conversely, in adults, those who are more physically active have lower levels of resting cytokine concentrations (CRP, IL-6, IL-18, TNFα), indicative of less inflammation[28]. Given its role in health promotion, measurement of physical activity is becoming more common in research and clinical practice[84,86].

1.7.2 Sedentary behaviour

Sedentary behaviour is any waking behaviour with energy expenditure that is below light physical activity[87,88]. Activities such as sitting, reclining, laying down or watching television are characterized as sedentary behaviour[87]. It has been suggested that sedentary behaviour has independent effects from physical activity. One study reported independent relationships between physical activity and sedentary time with cardiorespiratory fitness[89] . However, others argue that sedentary time is on a spectrum of movement behaviour. In other words, sedentary behaviours and physical activity are dependent on one another[90]. In either case, few studies exist that evaluate the relationship between inflammation and sedentary behaviour. One study found that reducing sedentary time is associated with improved levels of CRP in women with newly diagnosed type 2 diabetes[91]. The Maastricht study found that sedentary behaviour and physical activity is associated with low-grade inflammation as measured by a composite score of CRP, IL-6, IL-8, TNFα, serum

amyloid A and soluble intercellular adhesion molecule-1 in patients with type 2 diabetes[92]. It is not clear if decreasing sedentary time can lead to a positive influence on these biomarkers. Collectively, both physical activity and sedentary behaviour, may be important in the modulation of inflammation; however, there is minimal literature that evaluates the relationship between sedentary time on cytokines in healthy children or children with a chronic disease.

1.7.3 Measurement of movement behaviours

Physical activity and sedentary time, collectively known as movement behaviours, can be assessed using subjective questionnaire methods, validated against more rigorous measures of energy expenditure, including doubly-labelled water or indirect calorimetry. These criterion standards are cumbersome and expensive, while questionnaires are limited by low reliability and specificity, as well as high levels of reporter bias[93]. Over the last two decades, accelerometery has become a preferred, device-based measure of physical activity and sedentary time that overcomes many of the aforementioned limitations[86]. With appropriate calibration and consideration for variables such as minimum wear time and the type of activity being measured, accelerometers accurately and reliably record movement as accelerations over a specified period of time[84,94,95].

Accelerometers are capable of recording and extracting many domains of activity including intensity, frequency, and volume of movement. More importantly the high-resolution free-living activity data collected from these accelerometers allows researchers to link movement behaviours to important health-related outcomes, including markers of inflammation in children with CID[86,96–98]. As interest in exercise as medicine and the implications of movement for the immune system grows, the studies investigating the relationship between movement behaviours and immune response are also increasing[28,99,100].

1.8 Movement behaviours modulating cytokine response in healthy populations

There is growing evidence to suggest that specific episodes of physical activity may modulate innate components of our immune systems. Acute exercise, or a single episode of structured physical activity, elicits an inflammatory response in healthy children, increasing levels of IL-6, TNF-α, IL-1β , and IL-1ra[7]. Chronic exercise, or repeated bouts of structured physical activity, can increase gene expression of IL-10 and TNF-α in children[101] . Additionally in a study assessing the effect of continuous and intermittent exercise (as forms of physical activity) it was found that IL-6 increased significantly with moderate-intensity continuous exercise in healthy children with a mean age of 13.9 ± 2.1 years[102]. It has not been established how or if these transient changes contribute to long-term changes in resting values, but there is some preliminary evidence that suggest being more physically active leads to a decrease of the odds of having high CRP in adults[103].

In adults, Phillips and colleagues (2017) conducted a study sampled from a primary care setting. Using device-based measures, they found that replacing sedentary time with increasing amounts of MVPA correlated with favourable inflammatory profiles, including decreased IL-6, complement 3, leptin and white blood cell concentrations. Increasing time spent sedentary was related to detrimental inflammatory profiles, reflected by higher concentrations of serum CRP, TNF-α, IL-6, leptin, and white blood cells[104]. In this study, only substituting MVPA for sedentary time had a significant effect, but there may be reason to speculate that there are different intensities and patterns of physical activity that can serve to modulate inflammatory profiles and health indicators in children.

There is some conflicting evidence in healthy children that suggests physical activity substituted for sedentary time may have a negative effect on cytokine responses. In a study that modelled the theoretical impact of reallocating a specific amount of sedentary time with physical activity it was found that replacing sedentary time with 10 minutes of VPA per day (regardless of how it was accumulated) was beneficially associated with CRP[88]. However, in this same study replacing sedentary time with MPA was associated with increased CRP. It is difficult to be confident in these findings given the small sample sizes, a lack of review of a broader subset of cytokines and/or disease status. More recently, in a study of children in social isolation during COVID-19, sedentary behaviour was linked with higher levels of pro-inflammatory markers IL-17 , IFN-γ, leptin, TNF-α and IL-2, while children who were less sedentary had higher levels of IL-10 (anti-inflammatory marker)[105]. Though limited, these data suggest that physical activity and sedentary time may have meaningful effects on the immune system.

In 2010, Warnberg and colleagues reported that the biomarkers CRP, IL-6, IL-1β, and TNF-α can be used to estimate inflammation in children and as such have the potential to capture a more complete picture of immune modulation by physical activity[100]. While the relationship between movement behaviours and modulation of inflammatory profiles has been explored in healthy populations, there is no comparable investigation in clinical pediatric populations. More recent studies have shifted focus to the effects of physical activity on cytokine profiles in children with CID[27,28,99,100,106–108], and will be explored in the section below.

1.9 Movement behaviours and inflammation in children with a chronic inflammatory disease

Although the prevalence of children with a CID is increasing, and physical activity may provide potential therapeutic benefits, very few studies have examined the relationship between

immune markers and movement in pediatric CIDs. It is well established that children with CIDs engage in more sedentary time and less physical activity than their healthy counterparts[108]. It is also widely accepted that being less physically active and having higher levels of sedentary time put children at greater risk for many comorbidities including mental health issues, cardiovascular problems, weight gain and obesity[109]. Importantly, it has been established that children with CIDs may benefit from increasing physical activity and decreasing time spent sedentary[109].

The limited existing studies examining cytokine levels and inflammation in childhood tend to focus on children with cardiovascular conditions and/or obesity, with acute or chronic exercise as an intervention rather than habitual physical activity. With acute exercise, children with CID demonstrate an initial increase in IL-6[7,28]. Children with CF who participated in acute exercise demonstrated increases of IL-6 and TNF-α after ten 2-minute bouts of cycling[110]. Similar significant increases in IL-6 were observed in children with T1DM in response to acute exercise[110]. In contrast, a single bout of exercise (20 min exercise at 70% of max heart rate) in children with JIA did not increase serum IL-6 concentrations but did transiently elevate calprotectin. This collectively demonstrates that acute exercise influences inflammatory responses in children with CIDs.

The limited existing data also appear to support a link between habitual physical activity and inflammatory markers in children with CIDs. For example, in a meta-analysis of 11 randomized controlled trials conducted in children with obesity by Han and colleagues, structured physical activity of various intensities (chronic exercise), was associated with a significant reduction of CRP levels. In a sample of 115 children who were overweight, there was a trend towards a negative association between physical activity and IL-6[111]. In children with JIA, physical activity

may be modulating dysregulated cytokine responses, such as IL-6, and in fact balancing them to such an extent that may provide relief of symptoms[27,106]; however, compared to healthy children, minimal data linking markers of inflammation and physical activity in patients with chronic disease were found.

The existing literature suggests that acute and chronic exercise, a form of physical activity, affect inflammatory markers. However, to our knowledge, no studies to date have examined a comprehensive subset of cytokines that are linked to more than one CID. Additionally, many of these studies only explore the impact of a single bout of exercise or an exercise training program, rather than free-living movement behaviours. These gaps have limited our understanding of the relationship between movement behaviours and inflammation more broadly and across multiple diseases.

1.10 Summary

It is well established that nearly all CIDs have complex innate immune dysregulation that is at least partially attributed to cytokine dysregulation. Physical activity may modulate these detrimental immune responses; however, the link between cytokines and physical activity in children with CIDs is poorly understood. Limited research in healthy children and adults suggest that physical activity may be able to modulate cytokine dysregulation; however, existing evidence is limited by only examining a small subset of cytokines. Moreover, despite some established preliminary links between movement behaviours and inflammatory outcomes, these relationships have yet to be investigated in children with CID.

Chapter 2: Study Objectives and Hypothesis

To address the existing gaps in the literature, the objectives and hypotheses of this study are:

1. To assess the differences movement behaviours and inflammation between children with CKD, CF, JIA, IBD, and T1DM.
    a. We hypothesize that children with CKD, CF, JIA, IBD, and T1DM will exhibit comparable physical activity levels and cytokine profiles.
2. To examine movement behaviours and inflammation in children with a CID (CKD, CF, JIA, IBD, and T1DM combined) compared with healthy controls (CTL).
    a. We hypothesize that children with a CID will have lower levels of physical activity, higher levels of pro-inflammatory cytokines, and lower levels of anti-inflammatory cytokines relative to CTL.
3. To determine the association between movement behaviours and inflammation in children with a CID.
    a. We hypothesize a negative relationship between physical activity and inflammation in children with a CID

Chapter 3: Methods

3.1 Recruitment

The data collected in this study is under the umbrella of a larger study in the Child Health and Exercise Medicine Program (CHEMP), **C**ardiovascular **H**ealth in children with a chronic infl**AM**matory condition: role of **P**hysical activity, fitness, and inflammat**ION** (the CHAMPION study). Eligible participants with a CID were recruited during their regular clinic visits at McMaster Children's Hospital. A member of the clinic staff initially introduced the study. Upon demonstrated interest from the family and/or child, a researcher was available to discuss the study and answer any questions. Appropriate consent and/or assent were obtained for further contact. Our control group was recruited via community-based sampling from the local Hamilton community via posters, on-line/social media advertisements, and by word of mouth.

3.2 Eligibility Criteria

To be eligible for this study children with a CID were between the ages of 7-17 years at the time of recruitment. In our experience, 7 years is the youngest age for valid and reliable aerobic fitness measures used in the larger CHAMPION project; while 17 years of age is the upper age limit for most patients treated at McMaster Children's Hospital. They must have had a confirmed single diagnosis of CKD, CF, JIA, IBD, or T1DM, to limit mixed effects of multimorbidity. Patients were diagnosed for at least 1 year prior to enrollment in our study, to give sufficient time to acclimate to treatment regimens and establish a stable baseline. Cytokine measurements are extremely sensitive and immune variability and suppression that can result from a hospitalization event and/or new medications may be disruptive to these measurements. As such, patients who

had medications changes in the previous month or had been hospitalized in the 3 months prior to a potential visit were excluded.

Children recruited for the CTL group were eligible for this study if they were between 7-17 years of age and did not have any suspected or diagnosed medical condition. They could not have been participating in a medical program and could not have a sibling with a medical condition, to avoid the introduction of home environmental influences. At least 30% of the pediatric population is overweight or obese and as such it is important to include children who fit this criterion in our study to ensure that our sample was representative of the general pediatric population[112]. As such, there were no exclusions made in either the control or CID group based on body mass index (BMI) to avoid bias. As per the rationale described in the experimental group, participants in the control group were excluded if they were on any medications or had been hospitalized 3 months prior to a potential visit.

3.3 Study Visits

Participants completed two study visits. Please note that only methods relevant to this specific sub-study are described below. Study visits were run by me and another member of the CHAMPION study team. The visits took place at the CHEMP laboratory located within the McMaster Children's Hospital. The first visit consisted of providing the participant and family with an overview of the study. Consent was obtained from adolescents over the age of 16 years, and parental consent in addition to child assent was obtained from younger children. Participant eligibility was confirmed in person at the beginning of the session by administering the CHEMP lab activity and medical questionnaires. Next, anthropometric measures were taken. At the end of the first visit participants were outfitted with an accelerometer and instructed to wear the device

for the next 7 days. The second visit consisted of the participant returning to the CHEMP lab for fasting blood work and returning the accelerometer. This study was approved by the Hamilton Integrated Research Ethics Board (#14-659). Detailed descriptions of the study measurements and outcomes are provided below.

3.4 Measurements

3.4.1 Anthropometric Assessments

At the start of the first visit children were asked to complete a series of anthropometric measures including standing height, weight, and body composition. All measures were completed without shoes and with minimal/light clothing. Standing height (cm) and sitting height (cm) were measured using a wall-mounted calibrated stadiometer (Harpenden Stadiometer 2109, CMS Weighing Equipment LTD, London, UK), while weight (kg) was measured using a digital scale (Tanita BWB-800, Tokyo, Japan). Each measurement was performed a minimum of two times, or until at least two of the measurements are within 0.3 cm for height measurements, and 0.1 kg for weight to ensure accurate reporting. Height and weight data were used to calculate BMI as [weight (kg)/height (m)^2] and converted into percentiles based on the CDC growth charts[113]. Sitting height was used to measure maturity offset, by calculation of estimated years from peak height velocity (YPHV)[114]. An InBody machine (InBody 570 Cerritos, California, USA) was used to evaluate body composition (body fat percentage) by bioelectrical impedance analysis.

3.4.2 Pubertal Stage Assessment

Tanner staging was completed for boys and girls as a self-reported method to approximate pubertal development[115]. For girls, the Tanner stage for breast development was used and for boys

the pubic hair scale. Participants were given a folder with a series of 5 illustrated images of either pubic hair stages or breast development in a private setting and instructed to circle the number they believe best represents them. Children that self-report Tanner 1 are classified as pre-pubertal, Tanner 2 as early pubertal, Tanner 3 as pubertal, Tanner 4 as late-pubertal, and Tanner 5 as post-pubertal.

3.4.3 Physical Activity

At the end of the study visit participants were provided with a small (3.8x3.7x1.8cm), lightweight (27 g) accelerometer (ActiGraph GT3X, ActiGraph, Florida, USA) to wear around their waist, to quantify habitual physical activity for 7 consecutive days. The participant and parent were shown how to wear the device during their study visit and received an instructional pamphlet on how to wear the device for future use. During this period of wear, the participant was asked to fill out a logbook where they recorded any times the device was taken off and put back on. The participants were asked to return their accelerometer on their second visit. They also received a pre-paid envelope to mail the accelerometer and logbook back to CHEMP, if necessary.

Accelerometer data was collected at 30 Hz, downloaded in 3-sec epochs and validated using ActiLife, as previously described by our team[116]. Only participants with accelerometer data for $\geqslant$ 10 hours per day on $\geqslant$ 3 days were included in this analysis[95]. With this criteria, the reliability of accelerometer readings is estimated to be 90% in children without a chronic health condition[95]. Specific movement outcomes measured included average daily minutes of total physical activity (TPA), activity by intensity including light physical activity (LPA), moderate (MPA), vigorous (VPA), moderate-to-vigorous physical activity (MVPA), and sedentary time, as defined by Evenson et al. cut-points[117].

3.4.4 Blood Collection

At the end of the week of accelerometer data collection, participants returned to the laboratory for a second visit between 7:30 and 10:00 AM. Participants were asked to fast for at least 10 hours before the study visit. Upon arrival to the laboratory, participants were asked to rest for 10 minutes in a quiet and private room, and a blood sample was collected by venipuncture in the antecubital fossa region of the arm by a trained member of our research team. The blood sample included 2 x 10-mL EDTA-coated tube for plasma, and 1 x 10-mL uncoated tube for serum. EDTA-coated tubes were stored on ice and processed immediately, while serum samples were left to clot for 30 min at room temperature. Both samples were centrifuged at 2,000 rcf for 20-min at 4ºC. Both plasma and serum were stored in 0.5 mL aliquots at -80°C for later cytokine analysis. Only serum was used for the analyses in this thesis.

We quantified serum levels of IL-6, IL-12, IL-23, TNF-α, IL-1β, TGF-β, IL-17, IL-10 and CRP. This was done using two custom multiplex kits: 1. a human 14-plex kit and assay for IL-1β, IL-6, IL-10, IL-12p70, TNF-α, Il-23, and IL-17, and 2. a TGF-β multiplex panel. All multiplex analyses were completed by Eve Technologies, and all samples were measured in duplicate. Multiplex analyses were run on Millipore 96-well plates using Luminex instruments. CRP was analyzed in-house using enzyme linked immunosorbent assay kits (ELISA, R&D high-sensitivity human CRP). All samples were analyzed in duplicate, and included immunoassay controls (low, medium, and high concentrations) to verify accuracy. ELISA samples were re-run if the coefficient of variation (CV) of the sample was greater than 10%. The CV was calculated was:

$$CV\ (\%) = \frac{\text{standard deviation of duplicate}}{\text{average of duplicate}} \times 100$$

The intra-assay CV (within plate, or CV of duplicates on each plate) and inter-assay CV (between plates, or CV of samples run on multiple plates) are reported in Table 2 below. Blood marker values were standardized by creating a z-score using the formula:

$$\text{z-score} = \frac{cytokine\ concentration - mean\ cytokine\ concentration}{standard\ deviation\ of\ cytokine}$$

Table 2: Intra-assay and Inter-assay precision of Immunoassays.

Cytokine	Intra-assay precision (%CV)	Inter-assay precision (%CV)
IL-1β	4.5 (3.7-5.3)	11.8 (7.7-15.9)
IL-6	5.6 (4.6-6.7)	14.2 (12.7-15.7)
IL-10	7.0 (5.7-8.3)	15.4 (12.6-18.5)
IL-12p70	5.3 (4.3-6.3)	11.4 (9.6-13.2)
TNF-α	4.1 (3.4-4.9)	13.9 (11.5-16.3)
IL-23	5.0 (4.0-5.9)	15.4 (11.4-19.4)
IL-17	5.2 (4.3-6.2)	15.9 (13.6-18.2)
TGF-β1	4.3 (3.6-5.2)	4.9 (3.3-5.5)
CRP	6.9 (3.9-8.9)	10.3 (6.3-14.3)

Table note: CVs are presented as mean CV with a 95% confidence interval. All cytokines were run on Millipore 96-well plates, except CRP which was run on an R&D high sensitivity ELISA. Intra-assay calculations were based on 112 samples for all analytes except for CRP which was based on 80. Inter-assay precision was based on 8 samples for all analytes, except for CRP which was based on 12.

3.5 Experimental Visit Controls

Several experimental controls were implemented to ensure that the measured cytokines were representative of the participant's inflammatory profile and not influenced by acute factors. First, only participants with no medication changes in the last month and no hospitalizations for the last

3 months were recruited to participate. Second, we confirmed that participants had not experienced any acute illness in the 2 weeks leading up to their blood sample. The cytokine measurements are extremely sensitive, and a current or ongoing infection could alter the results of the cytokine assay[118]. Participants were asked to reschedule sessions if they were experiencing any viral illness, infection, or symptoms of illness of any kind. Rescheduled sessions were booked at least 2 weeks post-illness, to ensure sufficient time for cytokines to return to baseline[119]. Third, it is well documented that fatty foods and strenuous exercise and can transiently increase levels of inflammatory markers[120]. As such, in addition to a 10-h fast, participants were asked to avoid consuming any fast food and avoid participating in atypical strenuous activities for 24-h prior to their blood sample.

3.6 Statistical analyses

The statistical analyses conducted for this project are summarized in Table 3. This is a cross-sectional study; therefore, all analyses and relationships represent a single time point. To compare inflammatory and physical activity profiles across diagnoses, a one-way analysis of covariance (ANCOVA) was performed for each disease group by each cytokine and physical activity outcome. For this analysis, the group was the factor (JIA vs. CKD vs. CF vs. T1DM vs. IBD) and age was included as a covariate. Similarly, for objective 2, one-way ANOVA and ANCOVA were conducted with groups (CID vs. CTL) as the factor and age as a covariate. Pearson correlations were run to assess co-linearity between anthropometric, movement outcomes and inflammation outcomes. Anthropometric measures that positively correlated with movement or inflammation outcomes were included as covariates in the analyses.

To determine the association between movement behaviours and inflammation in objective 3, a series of analyses were performed. First, a principal component analysis (PCA) was executed on 9 inflammatory inputs. PCA is a dimension reduction technique that creates a composite score of multiple variables that load on one another, in this case inflammatory variables. The suitability of PCA was assessed prior to analysis using a correlation matrix to assess variance of each component and the Kaiser-Meyer-Olkin (KMO) measure. PCA was deemed appropriate if the following criteria were satisfied:

1) Correlation coefficients for each variable were greater than 0.3;
2) KMO values were greater than 0.6 or above. The KMO indicates sample adequacy and is an index between 0-1 that determines linear relationships between variables.
3) Bartlett's test of sphericity must be satisfied for individual correlation coefficients from step 1 are kept in the analyses but close to 0.3. In this case, bartlett's test of sphericity is satisfied if statistically significant values of $p<0.05$ and thus confirms that the data is factorizable for PCA.

Next, a series of criteria were verified to determine the final number of components from the PCA that were retained. An assessment of eigenvalues was done to ensure that they were greater than one. An eigenvalue of less than one indicates that a component explains less variance than an individual variable would, and therefore should not be retained. A visual inspection of the scree plot inflection point was used to determine the appropriate number of components that should be retained[121]. Each component was then used as the dependent variable in linear regression models to understand if physical activity or sedentary time predict inflammation.

Prior to running regression models, linearity was confirmed for each outcome using scatterplots of each activity outcome (SED, LPA, MVPA) against inflammation. Each inflammation component was then run through a multiple linear regression. The dependent variable for each regression was an inflammation component, the movement outcomes were entered stepwise as independent variables, and sex and age were included as covariates. SPSS version 27 was used for the analyses. All data are presented as mean ± standard deviation, and significance was set at $p<0.05$, unless otherwise stated. All figures were created in GraphPad Prism (Prism 9 Version 9.3.1), and where possible, individual participant data were plotted to illustrate the range of responses seen in our sample.

Table 3. An outline of statistical analyses for the outlined objectives.

Objective	Independent and dependent variable(s)	Hypothesis	Statistical Analysis
To compare movement behaviours and inflammation across diagnoses	IV: JIA, CKD, CF, T1DM, IBD DV: movement behaviours OR serum cytokine levels	Movement behaviours and inflammation values will be comparable between CIDs	Analysis of variance (ANOVA)
To examine movement behaviours and inflammation in children with a CID compared with CTL	IV: Disease status (CTL vs. CID) DV: movement behaviours OR serum cytokine levels	Children with a CID will have lower levels of physical activity and higher levels of pro-inflammatory cytokines relative to CTL.	Analysis of covariance (ANCOVA)
To determine the association between movement behaviours and inflammation in children with CID	IV: SED, LPA, MVPA and age DV: serum cytokine levels	Children who are more physical active will have decreased inflammation	PCA & Multiple linear regression

3.7 Sample Size Requirement

The cross-sectional CHAMPION study was designed to ensure adequate sample size was obtained to conduct a regression analysis with 9 predictors. This sub-study requires 4 predictors; therefore we will need 54 participants to conduct the analyses. This follows Harris's formula[122], stating that a minimum number of participants should exceed the number of predictors by at least 50, in this case 54. As an absolute minimum, 10 participants per predictor can be used (n=40). In this analysis we have included 4 predictors in our regression model thus involving fewer predictors than the CHAMPION study, and as such Harris's calculation is sufficient to conduct this sub-analysis with an expected 80% power at an α level of 0.05[122].

Chapter 4: Results

4.1 Descriptive Participant Characteristics:

A total of 132 individuals (48% female) that were CTL (N=52) or had a CID (N=80) agreed to participate in our study. Of these, 114 (46% female) completed the physical activity portion of the study and 118 (45% female) had complete inflammatory data. Of the total participants, 100 participants (46% female; CTL= 44, CID=56) had both complete sets of physical activity and inflammation data. Sample sizes broken down by CID and CTL are presented in Tables 4-5. We were unable to recruit enough participants for an analysis of the CKD group (n=2), as such this group was not included in the following results.

4.1.1 Anthropometric characteristics of children with CF, JIA, IBD and T1DM

Our participants from the CID group who had either CF, JIA, IBD and T1DM had significant differences in age, height, YPHV and body fat percentage, as described in Table 2. Children with CF were on average 2.8 years [95% confidence interval: -0.55, 0.34] younger than children with IBD (p=0.013), 13.7 cm [-26.4, -1.1] shorter than children with IBD (p=0.27) and 13.8 cm [-26.9, -0.8] shorter than children with T1DM (p=0.03). Children with CF were also less developed, with a YPHV on average -2.02 years [-0.4, 0.05; p=0.04] less than those with IBD. Children with JIA were in the highest weight percentile, and also presented with the highest body fat percentage. More specifically, children with JIA had 11% [3.2, 19.0] higher body fat percentage than those with CF (p=0.002), 9% [1.9, 15.6] higher than IBD (p=0.007), and 8% [0.6, 15.1] more than T1DM (p=0.048).

Table 4: Descriptive characteristics of CF, JIA, IBD or T1DM participants.

	CF	JIA	IBD	T1DM	F
N (Female)	14 (6)	27 (18)	21 (11)	18 (10)	0.764
Age (years)	11.4 ± 2.5* (7.3 - 15.0)	12.7 ± 3.1 (7.1 - 17.1)	14.2 ± 1.9* (10.4-17.8)	13.7 ± 2.7 (9.0-18.2)	3.734+
Height (cm)	145.1 ± 14.2* (120.7-172.0)	154.1 ± 14.5 (129.0 - 171.7)	158.9 ± 13.2* (139.2-180.7)	159.0 ± 13.5* (134.8-178.5)	3.412+
Height Percentile	46.1 ± 29.1 (1.6-99.0)	66.1 ± 24.7 (16.1-98.3)	45.7 ± 27.6 (1.4-94.6)	60.5 ± 19.3 (24.2-97.1)	3.539
Weight (kg)	39.9 ± 12.4 (21.80-73.0)	52.4 ± 17.1 (29.0-91.9)	49.2 ± 12.8 (30.9-74.4)	52.5 ± 14.7 (28.6-94.0)	2.571
Weight Percentile	54.2 ± 26.7 (10.6-94.2)	72.0 ± 25.0* (11.1-99.8)	44.4 ± 28.5* (0.9-93.3)	62.1 ± 25.6 (11.7-97.1)	5.452+
Body Fat (%)	15.8 ± 7.0* (3.7-32.6)	26.9 ± 8.7* (10.2-42.8)	18.2 ± 8.9* (5.6-34.5)	19.3 ± 9.2* (4-35.9)	6.393+
BMI Percentile	56.8 ± 27.2 (10.0-91.8)	70.0 ± 27.6* (1.2-99.1)	45.0 ± 31.9* (1.3-85.1)	57.4 ± 26.5 (9.0-98.3)	3.048+
YPHV (years)	-1.3 ± 2.0* (-4.1-2.8)	-0.28 ± 2.3 (-4.6-2.3)	0.73 ± 2.0* (-2.7-4.6)	0.55 ± 2.4 (-3.6-5.0)	2.982+
Tanner Stage	2 (1-4)	2 (1-5)	3 (1-5)	3 (1-5)	2.262

Note: All data are presented as mean ± SD and (range), except Tanner stage that is presented as median and range. YPHV = years from peak height velocity. * indicates a significant difference between indicated CID groups, $p<0.05$. + indicates a significant main effect of group, $p<0.05$.

4.1.2 Anthropometric characteristics of children with a CID and CTL

Our participants with a CID were on average comparable in age, weight, height and YPHV to CTL. There were significant differences in sex distribution, percent body fat, and height percentile between groups. The CID group had a higher proportion of females (56% vs. 35%), and an average of 21.2 ± 9.6% body fat compared to 16.6 ± 9.0% in the CTL group ($p=0.008$).

Table 5: Descriptive characteristics of CID and CTL participants.

	ALL	CTL	CID	F
N (Female)	132 (63)	52 (18)*	80 (45)*	6.096+
Age (years)	13.1 ± 2.9 (7.1-18.3)	13.2 ± 2.8 (7.5-18.3)	13.1 ± 2.8 (7.1-18.2)	0.052
Height (cm)	156.6 ± 15.4 (120.4-186.0)	159.4 ± 16.4 (120.4-186.0)	154.9 ± 14.6 (120.7-180.7)	2.713
Height Percentile	60.4 ± 26.5 (1.4-99.8)	67.2 ± 25.5* (2.0-99.8)	56.0 ± 26.0* (1.4-99.8)	5.854+
Weight (kg)	50.4 ±1 6.0 (21.3-94.0)	51.9 ± 17.2 (21.3-91.9)	49.4 ± 15.2 (21.8-94.0)	0.802
Weight Percentile	61.1 ± 26.2 (0.9-99.9)	63.7 ± 23 (13.6-99.9)	59.4 ± 28.1 (0.9-99.8)	0.854
Body Fat (%)	19.3 ± 9.6 (3.7-44.0)	16.6 ± 9.0* (3.8-44.0)	21.2 ± 9.6* (3.7-42.8)	7.259+
BMI Percentile	57.5 ± 27.3 (1.2-99.3)	56.4 ± 23.6 (16.6-99.3)	58.3 ± 29.6 (1.2-99.1)	0.160
YPHV (years)	0.26 ± 2.4 (-4.6-5.3)	0.67 ± 2.6 (-4.4-5.3)	-0.01 ± 2.3 (-4.6-5.0)	2.505
Tanner Stage	3 (1-5)	3 (1-5)	3 (1-5)	0.590

Table Note: All data are presented as mean ± SD and (range) except Tanner stage that is presented as median and IQR. YPHV = years from peak height velocity. *Indicates a significant difference between CTL and CID groups, $p<0.05$. + indicates a significant main effect of group, $p<0.05$.

Descriptive note on analysis of outcomes:

1) Complete summary tables of physical activity and inflammatory outcomes can be found in Appendix 1.
2) ANOVA tables are summarized in the text below. The complete ANOVA tables can be found in Appendix 2.

4.2 COMPARISONS OF MOVEMENT BEHAVIOURS AND INFLAMMATION BETWEEN CF, JIA, IBD AND T1DM

4.2.1 Children with CF, JIA, IBD or T1DM have comparable movement profiles:

There were significant differences between CID groups for time spent sedentary and in LPA ($F(3,67)=3.564$, $p=0.019$ and ($F(3,67)=4.819$, $p=0.004$, respectively). A Tukey's post-hoc test revealed that this was driven by children with IBD. Specifically, children with IBD spent 49.0 [95% confidence interval: 7.2, 90.7] more minutes per day being sedentary than those with JIA ($p=0.015$), as seen in Figures 3 and 4. Children with IBD also participated in lower LPA than children with CF and JIA ($p<0.05$), as seen in Figures 4 and 5. An ANCOVA adjusting for age a sex determined that there were no significant differences between groups for SED or LPA. There were no other significant differences between groups for SED and LPA. There were no statistically significant differences between CID groups for MPA ($F(3, 67) = 2.317$, $p = 0.084$), VPA ($F(3, 67) = 0.291$, $p = 0.83$), MVPA ($F(3, 67) = 0.892$, $p = 0.450$), or TPA ($F(3, 67) = 1.310$, $p = 0.279$).

Figure 3: Heat map of mean z-scores of daily movement behaviours of children with CF, JIA, IBD and T1DM.

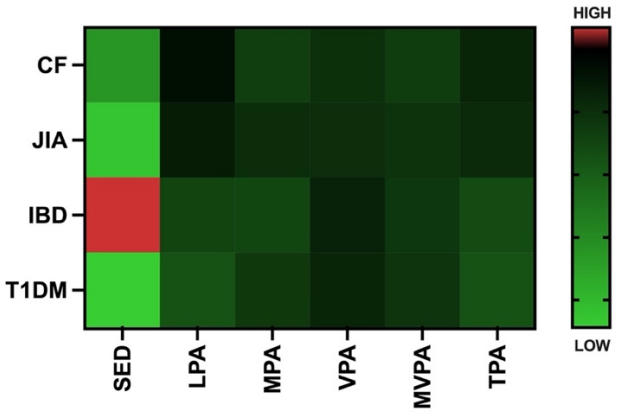

Figure note: The lighter the shade of green indicates a lower number of minutes spent in the respective movement outcome. Dark shades of green indicate closer to the mean. Black indicates the mean and as the gradient shifts to red, this indicates the most minutes spent in that movement

behaviour. Brightest green indicates the lowest z-score, and brightest red indicates the highest z-score.

Figure 4: Heat maps of movement behaviour outcome z-scores by participant for each CID

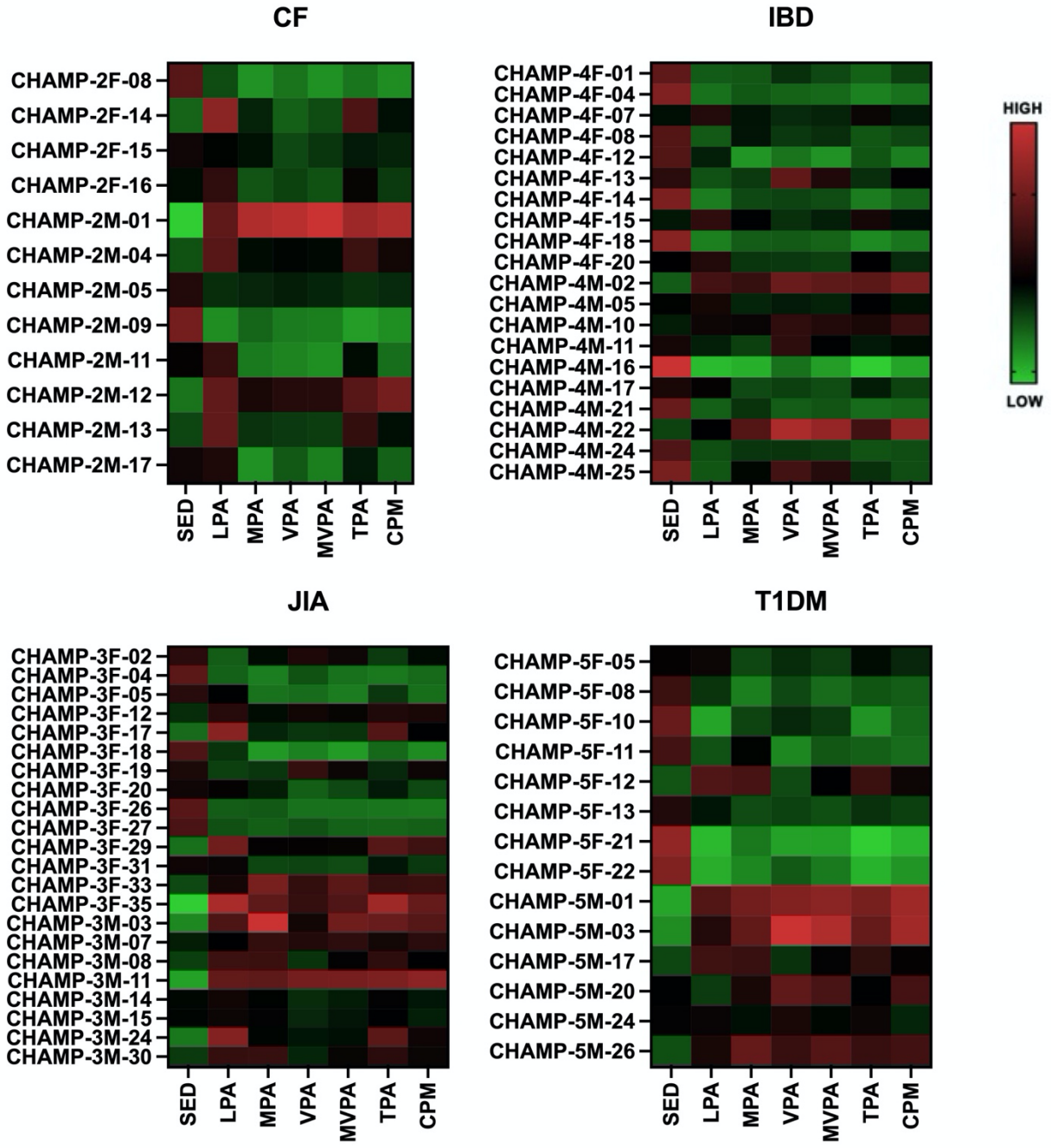

Figure note: Each row is a participant, and each column is a movement outcome. The lighter the shade of green indicates a lower number of minutes spent in the respective movement outcome. Dark shades of green indicate closer to the mean and more minutes spent in that behaviour. Black

indicates the mean and as the gradient shifts to red, this indicates the most minutes spent in that movement behaviour. Brightest green indicates the lowest z-score, and brightest red indicates the highest z-score.

Figure 5: Time spent participating in movement behaviours by CID group.

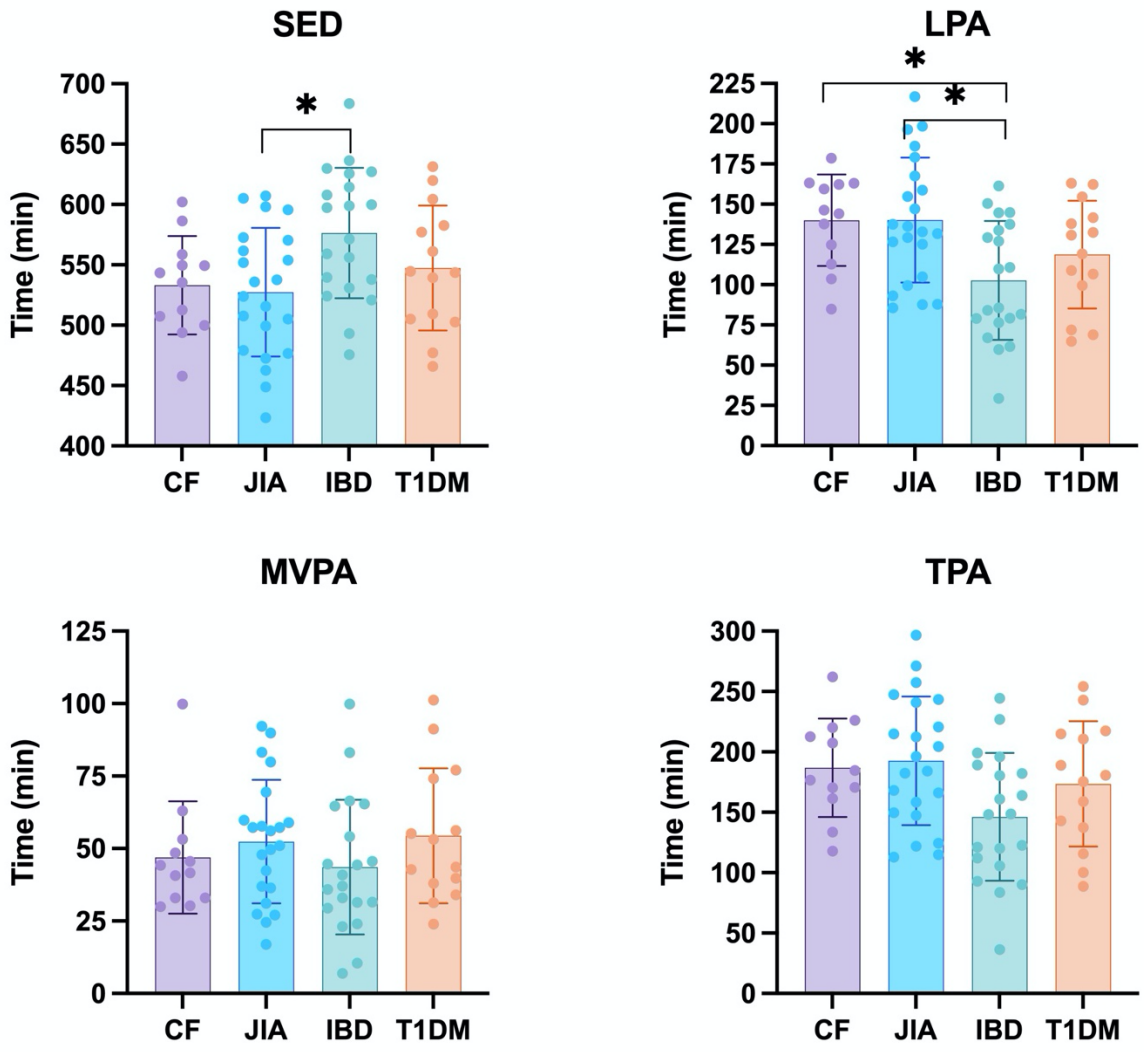

Figure note: The bars represent mean values, error bars are the standard deviations, and each dot represents a single participant in the CID group. * Indicates a significant difference between the denoted CID, $p<0.05$.

4.2.2 Children with CF, JIA, IBD or T1DM have comparable inflammatory profiles:

There were no statistically significant differences between CID groups for all serum cytokines measured, as seen in Figure 6. Complete ANOVA tables are provided in Appendix 1.

Figure 6: Serum cytokine level by CID group.

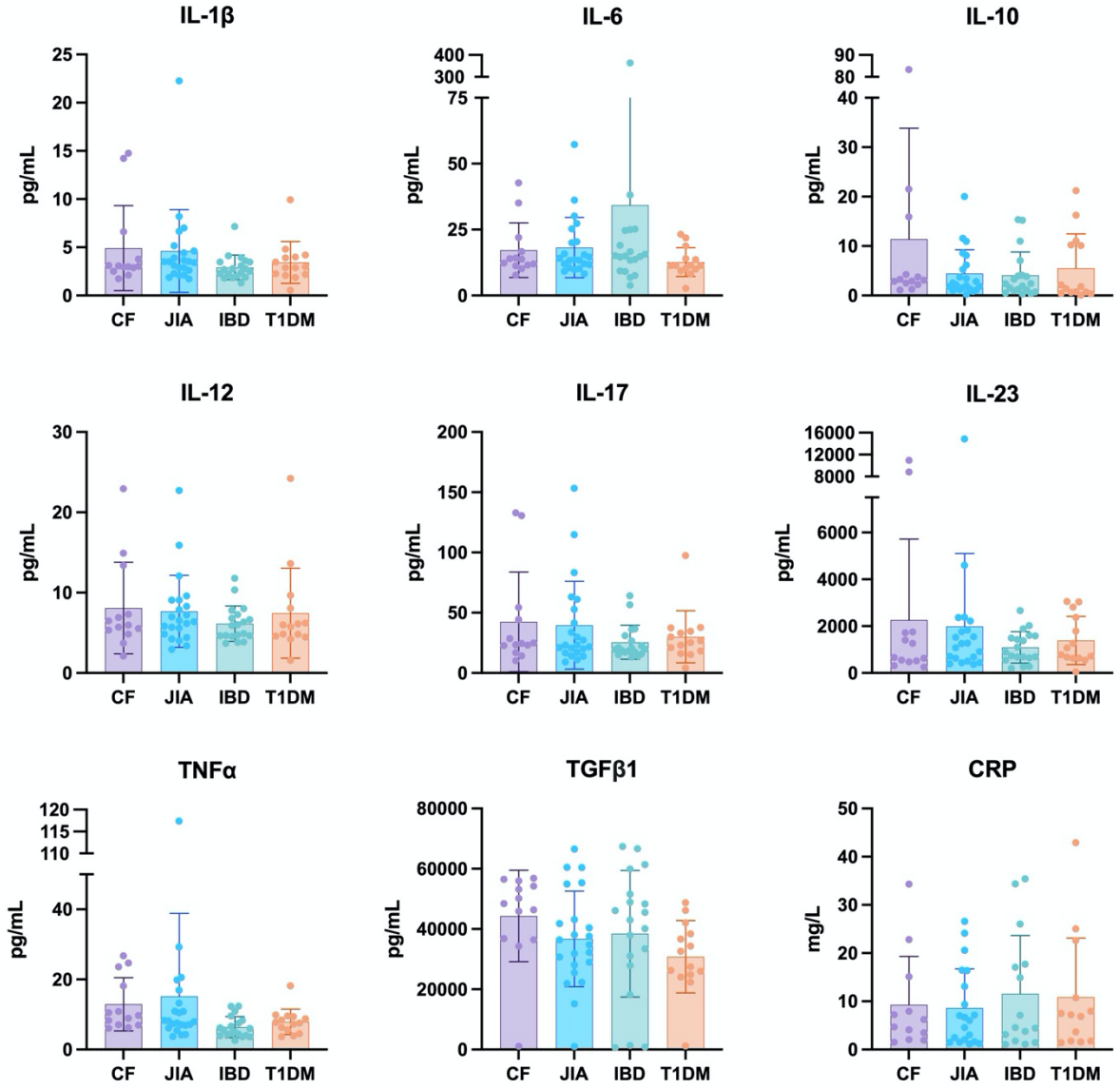

Figure Note: The bars represent mean values, error bars are the standard deviations, and each dot represents a single participant in the CID group. There were no significant differences between CID groups for each inflammatory marker.

4.3 COMPARISONS OF MOVEMENT BEHAVIOURS AND INFLAMMATION IN CHILDREN WITH A CHRONIC INFLAMMATORY DISEASE AND CONTROLS

4.3.1 Children with a CID are less active than CTL:

Children with a CID participated in 13.3 [95% confidence interval 6.9, 101.2] (MVPA ($F(1,113)=11.015$, $p=0.001$) fewer mins/day of MVPA than CTL as depicted in Figure 7. A separate assessment of the component parts of MVPA revealed that both MPA ($F(1,113)=0.208$, $p=0.003$) and VPA ($F(1,113)=7.983$, $p=0.006$), were lower in CID compared to CTL. There were no statistically significant differences between CID and CTL groups for SED, LPA or TPA.

Figure 7: Bar plots of movement behaviours for CID vs. CTL

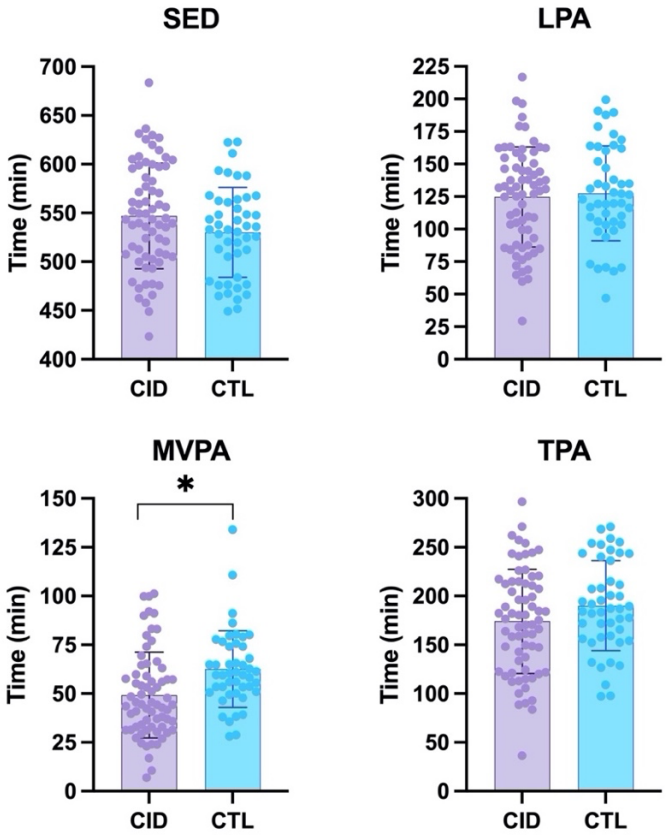

Figure note: Time spent participating in movement behaviours by CID group. The bars represent mean values, error bars are the standard deviations, and each dot represents a single participant in the CID group. * Indicates a significant difference between the denoted CID, $p<0.05$.

4.3.2 Children with CID have comparable inflammation to CTL:

There were no statistically significant differences between CID and CTL groups for serum cytokine levels. However, children with a CID were trending towards having higher levels of CRP (10.0 ± 10.3 mg/L) compared to CTL (6.3 ± 9.8 mg/L; $F(1,98)=3.162$, $p=0.078$), as depicted in Figure 8.

Figure 8: Serum cytokine level by CID and CTL group.

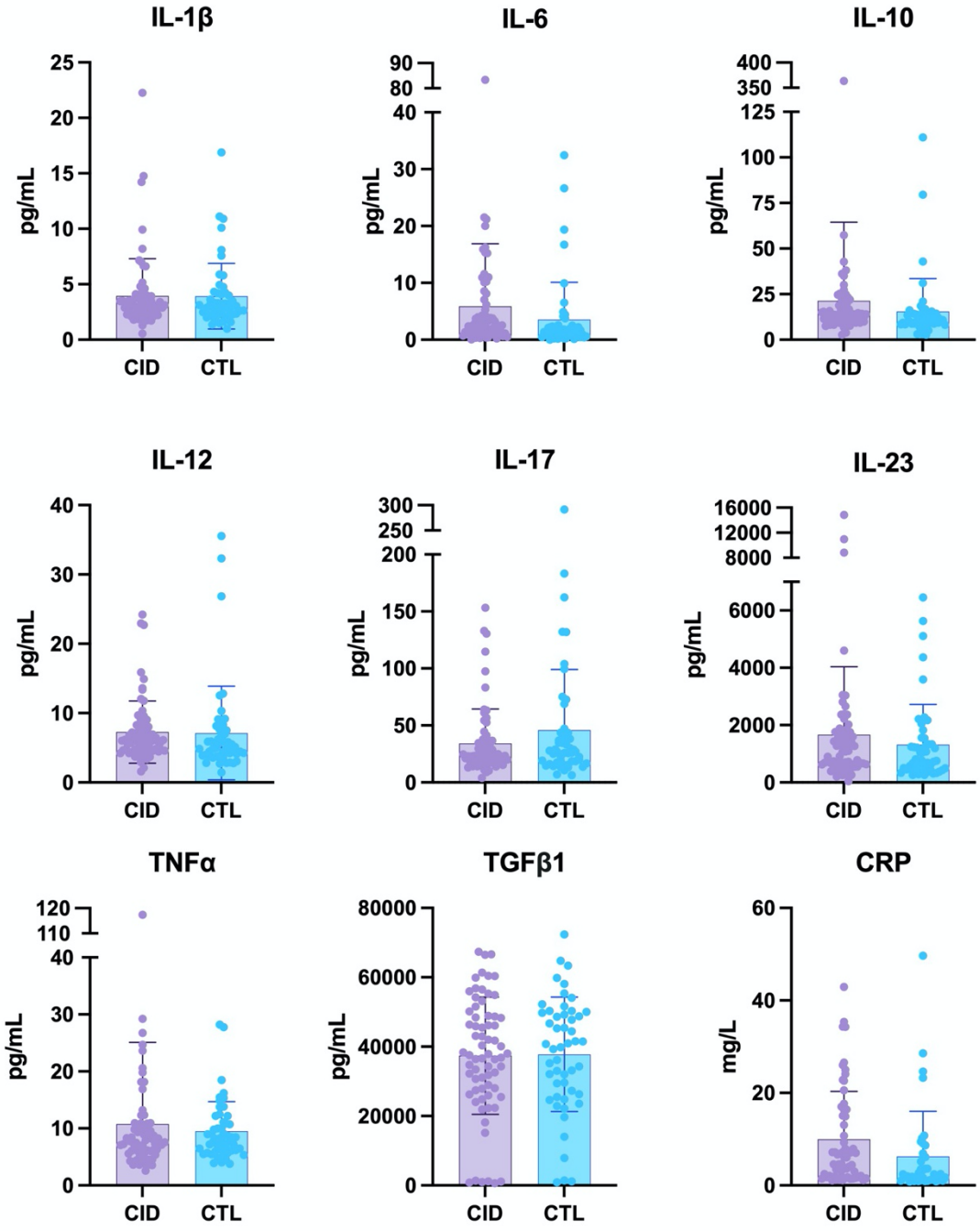

Figure note: The bars represent mean values, error bars are the standard deviations, and each dot represents a single participant in the group. There were no significant differences between CID and CTL groups for each inflammatory marker.

4.4 RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND INFLAMMATION IN CHILDREN WITH CHRONIC INFLAMMATORY DISEASE

4.4.1 Movement behaviours do not prediction inflammation in children with a CID

A principal component analysis (PCA) was run on 9 cytokines that measured participant inflammation on 68 participants with a CID. The correlation matrix showed that all variables had at least one correlation coefficient greater than 0.3 and the overall Kaiser-Meyer-Olkin (KMO) measure was 0.81 with individual KMO measures all greater than 0.7. This falls under the classification of 'middling' to 'meritorious' as per Kaiser[123]. Bartlett's test of sphericity was statistically significant ($p<0.001$). PCA revealed two components that had eigenvalues greater than one and explained 50.2% and 14.6% of the total variance. Visual inspection of the scree plot in Figure 9 also indicated that two components should be retained[121]. The two-component solution explained 64.8% of total variance. The first component-based score included IL-17a, IL-1β, IL-23, IL-12, Il-6 and TNFα. The second score included CRP, TGFβ-1 and IL-10. These two scores were used as dependent variables in linear regression models to understand if physical activity predicts inflammation.

Figure 9: Scree plot.

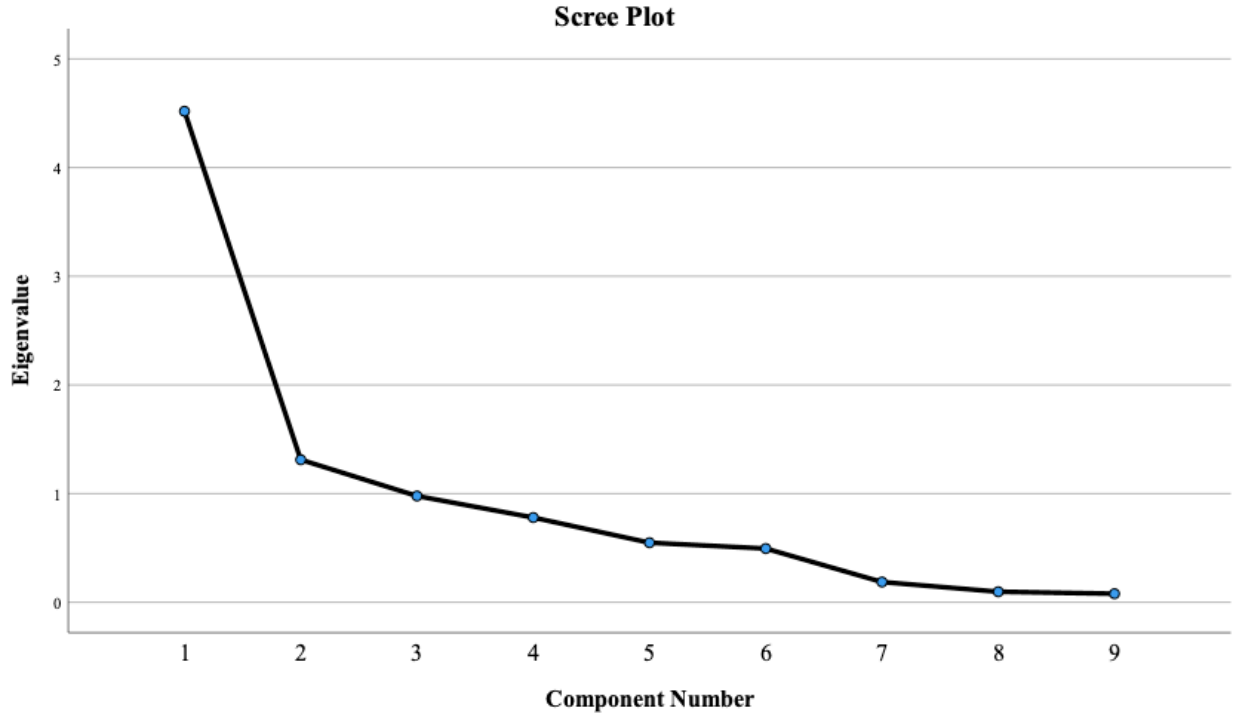

Figure note: A scree plot of 9 eigenvalues for inflammatory variables. The inflection point on the graph indicates how many components to retain; in this case, 2 principal components were retained.

Model 1: Physical activity does not predict component 1 based inflammation:

A linear regression was run to understand the relationship between movement behaviours (SED, LPA, MVPA) and inflammation (component 1: IL-17a, IL-1β, IL-23, IL-12, Il-6 and TNFα). There was homoscedasticity and normality of the residuals. Although overall model 1 was trending ($F(4,47)=2.205$, $p=0.084$) and accounted for 17% of variation, this was largely driven by age ($p=0.016$). For every year increase in age there is a 0.216 increase in component 1 inflammation. Neither sedentary time nor physical activity predicted inflammation component 1. Complete Model 1 results are reported in Table 6.

Table 6: Regression for Component 1 Inflammation

	(Component 1 Inflammation: IL-17a, IL-1β, IL-23, IL-12, IL-6 and TNFα)			
	Model 1a	Model 1b	Model 1c	Model 1d
SED	-0.004 (0.003)	-0.007 (0.009)	-0.016 (0.021)	-0.008 (0.02)
LPA	--	-0.004 (0.12)	-0.013 (0.23)	0.007 (0.023)
MVPA	--	--	-0.010 (0.021)	-0.007 (0.020)
Age	--	--	--	0.216* (0.086)
R^2	0.041	0.043	0.048	0.170
Adjusted R^2	0.021	0.001	-0.017	0.093
N	48	48	48	48

Table note: Relationship between physical activity, sedentary time, and component.1 inflammation. Stepwise regression models were used to enter SED (1a), LPA (1b), MVPA (1c), and age (1d). Values reported are unstandardized B coefficients, with standard errors in parentheses . *indicates statistically significant observations $p<0.05$.

Model 2: Physical activity does not predict component 2 based inflammation:

A linear regression was run to understand the relationship between movement behaviours (SED, LPA, MVPA) on inflammation (component 2: CRP, TGFβ-1 and IL-10). There was homoscedasticity and normality of the residuals. Overall model 2 was not significant ($F(4,47)=0.818$, $p=0.512$), suggesting that physical activity did not predict inflammation component 2. Complete Model 2 results are reported in Table 7.

Table 7: Regression for Component 2 inflammation.

	(Component 2 Inflammation: CRP, TGFβ-1 and IL-10)			
	Model 2a	Model 2b	Model 2c	Model 2d
SED	-0.002 (0.003)	0.011 (0.010)	-0.013 (0.023)	-0.014 (0.023)
LPA	--	0.017 (0.013)	-0.007 (0.025)	-0.009 (0.027)
MVPA	--	--	-0.026 (0.022)	-0.026 (0.023)
Age	--	--	--	-0.022 (0.099)
R^2	0.005	0.042	0.070	0.071
Adjusted R^2	-0.017	-0.001	0.006	-0.016
N	48	48	48	48

Table note: Relationship between physical activity, sedentary time, and component 2 inflammation. Stepwise regression models were used to enter SED (2a), LPA (2b), MVPA (2c), and age (2d). Values reported are unstandardized B coefficients, with standard errors in parentheses . *indicates statistically significant observations $p<0.05$.

Chapter 5: Discussion

5.1 Summary of Findings

To our knowledge, this is the first study to investigate activity by intensity in conjunction with a broad subset of cytokines in children with a CID. As hypothesized physical activity, sedentary time, and inflammation were comparable between children with CF, JIA, IBD, and T1DM. Moreover children with a CID were less active than their CTL peers, as hypothesized. However, to our surprise, there were no significant differences in inflammation between CID and CTL. We further used multiple regression to assess the relationship between movement behaviours and inflammation and we found that movement behaviours were not a significant predictor of inflammation. These findings and their implications will be addressed in detail below.

5.1.1 Children with CF, JIA, IBD or T1DM have comparable movement and inflammatory profiles:

We observed that children with CF, JIA, IBD or T1DM participated in comparable sedentary time and physical activity levels. With the exception that, children with IBD participated in significantly more sedentary time compared to children with JIA, and less LPA than children with CF or JIA. However, an ANCOVA adjusting for age and sex determined that SED and LPA were no longer significant. Children with IBD were the oldest and most developed. We know that children become less active as they get older[124]. Using a cross-sectional study design, researchers in the U.S. found that with both self-reported measures of physical activity and accelerometer-based measures 42% of children between ages of 6-11 years meet the recommended 60 min/day of physical activity, yet only 8% of adolescents 12-19 years old achieve the same goal.[124] In Canadian children, 46% of younger children (ages 5-11 years) met the MVPA guidelines while only 30% of older youth (ages 12-17 years) spent 60 minutes per day in MVPA[125]. The observed

age difference in the IBD group (14.2 ± 1.9 vs. other CIDs 12.6 ± 2.9 years) likely explains the increased amount of time spent sedentary and decrease in LPA relative to other CID groups.

In addition to an age difference, it is plausible that the diagnosis of IBD may lead to physical inactivity. A prospective multi-centre cross-sectional study revealed that adult IBD patients significantly reduced their physical activity levels after an IBD diagnosis, even in remission[126]. A unique feature of IBD that sets the diagnosis apart from JIA, CF and T1DM is bowel urgency. Bowel urgency has been cited as the most notable symptom impacting quality of life in those with IBD[127]. A cross-sectional survey was developed to better understand fears and barriers of men and women (mean age 40.4 years) with IBD in participating in day-to-day activities. It was found that participants declined to participate in sports/physical exercise due to the following IBD symptoms: bowel urgency 38%, fear of urge incontinence 36%, increased stool frequency 24%, and blood in stool 10%[127]. This evaluation has not been done in the pediatric population, however it was found that even in youth aged 5-15 years that there is a discordance in their perception of disease status[128]. This meaning that, they may self-regulate or adjust their behaviour to participate in less activity despite clinical remission. Our participants had low disease status at the time of recruitment; however, we cannot rule out that these symptoms or fear of symptoms may have been present that consequently contributed to lower LPA and higher SED.

In addition to our movement behaviour findings, we hypothesized and found that inflammation between children with CF, JIA, IBD or T1DM was comparable across all 9 cytokines of interest. While each condition varies in symptomology, they are also united by innate immunopathology that may explain the comparable inflammatory profiles[2]. Elevated levels of TNFα, IL-6, CRP have been positively associated with an increase in disease onset, mucosal

inflammation and pain in CF, JIA, IBD and T1DM[74,76]. IL-17, IL-23, IL-12, IL-1β and TGF-β1 are implicated in pathogenic disease processes in all 4 conditions[15,36,50,52,55,59]. IL-10's anti-inflammatory presence is measurable during times of innate inflammation in all 4 conditions as well[5,40,65,101]. Moreover, evidence linking mitochondrial dysfunction as a pathway for dysfunctional cytokine responses has been associated with these chronic inflammatory diseases in recent years[129]. Aside from innate immunity commonalities, participants were recruited during a time where their disease was low or well-controlled therefore, it is perhaps unsurprising that they are likely to be more comparable during this stable period as compared to during a disease flare.

5.1.2 Children with a CID are less active than CTL yet have comparable inflammation

Children with a CID participated in significantly less MVPA than their healthy peers, and this was driven by reduced levels of both MPA and VPA in CID. It is known that children with CIDs tend to be less active and more sedentary[107,126,130]. Given that disease activity was low among our participants at the time they completed the study, it is important to consider other reasons that might explain this observation. Contributing factors to this decrease in physical activity are complex and can likely be partially explained by a collection of linked factors, such as: a child's psychosocial challenges (individual mood, external social interactions) and mental health status. We know that individuals with chronic disease experience greater levels of mood disorders such as anxiety and depression[131]. We also know that these mood disorders effect an individual's capacity to participate in physical activity and that greater levels of physical activity are associated with improved depressive and anxiety symptoms[132,133]. The notion that patients with chronic disease experience greater levels of stress is not new and in patients with IBD dates back to 1955[134]. In addition to the powerful effects of stress, lower physical activity levels are directly associated

with higher disease-specific psycho-social stressors in children with chronic disease[135,136]. In children with JIA lower physical activity has been associated with increased pain and pain related stress as measured by the JAQQ psychosocial domain survey[137]. Lower physical activity has also been associated with IBD related stressors[135] and lower quality of life in children with T1DM[136,138]. Children with a CID are also influenced by external social factors, for example being viewed as a child with a medical condition. We did not measure mental health challenges and psychosocial factors, however combined they may partially explain our finding that children with a CID participate in less MVPA relative to CTL.

Another possible explanation for this finding is that children with a CID may have side effects from their medication. Commonly prescribed medications such as corticosteroids, immunomodulators and biologic agents have a host of potential side effects such as increased risk of infection, weight gain, nausea, and diarrhea[139]. These side effects may make children with a CID less likely to participate in physical activity.

Importantly, there is evidence to suggest that children in remission still do not participate in very high levels of physical activity[140] and that mental health status in combination with unpleasant medication side effects and disease specific psychosocial challenges may at least partially explain this observation. In addition to this it should be noted that, more than half of the children in both CID and CTL did not meet the MVPA guidelines of 60 mins/day. Our CTL group participated in 62.6 ±19.6 mins/day of MVPA, while CID participated in 49.2 ± 21.9 mins/day of MVPA. In a Canadian assessment of healthy youth between the ages of 5-17 it was found that children participate in an average of 63 mins/day of MVPA. Therefore, it is of note that children with a chronic disease participate in less MVPA relative to our CTL and the Canadian reference

range, implying that having a chronic condition, regardless of disease status or activity, may be in part responsible for this observation.

We hypothesized that children with a CID would exhibit more inflammation than the CTL group, however a comparison revealed that there were no significant differences between groups. This is not likely explained by age, sex, weight, or height differences as these were also comparable between groups. Interestingly, some of the cytokine concentrations observed in the CTL population were high relative to the literature in healthy participants. For example, our CTL had an average TNFα of 9.5 ± 5.2 pg/mL (range: 3.8-28.2 pg/mL), children with a CID were on average 10.8 ± 14.3 pg/mL (2.6 – 117.3 pg/mL). TNFα levels measured by multiplex analyses have previously been reported in those <45 years as 3.2±4.04 pg/mL (0.93-26.8)[141]. In another cohort of healthy children age 4 to 17 years, average TNFα measured by ELISA was 3.9 pg/mL (IQR 4.5-6.2)[142]. We also observed elevated anti-inflammatory cytokine IL-10 relative to the literature, with commonly reported values of 1.32 ± 3.06 pg/mL (0.01-19.8 pg/mL)[141] while mean values in the current study were 15.6 ± 18.0 pg/mL (2.7-111.0 pg/mL) for CTL and 21.4 ± 43.1 pg/mL (2.7-363.5 pg/mL) for CID. However, one study using multiplex assays reported IL-10 values of 7.9 (7.5-8.1) pg/mL in 15-year-old healthy girls[143]. Observed values for IL-6 were more comparable to the literature, with one study reporting IL-6 concentrations of 8.5 (0.1-9.6) pg/mL in 15 year old healthy girls[143], and our values for CTL were 3.6±6.5 (0.03-83.3) and CID were 5.9±11.0 (0.1-83.3). Yet a more recent study in healthy children aged 6-8 years reported values of 0.9 pg/mL[144]. Therefore, it is possible that the reason we saw comparable inflammatory profiles is that our CTL group had abnormally high or inflated cytokine values, while our children with CID may have had tempered inflammation values, as their disease status was controlled or low at the time of their visit. Importantly, although some cytokine values were high they did not exceed normal

physiological ranges. Nevertheless, given a departure from some published cytokine concentrations, it is important to evaluate the accuracy of our inflammatory measurements.

The observation that CTL children had higher levels of cytokines relative to the literature could be due to assay error. Cross-reactivity (or cross-talk) is a known challenge in multiplex assays that can lead to overamplification of some signals when assessing for cytokines in the same laminar plate well, leading to higher reported concentrations[145]. However, the sensitivity and specificity of the multiplex assay we selected was comparable to other immune-assay platforms and all standards that were used had less than 5% cross-reactivity[146]. We did not run comparative ELISA plates for each cytokine for a number of reasons: time, cost, efficiency, lack of serum volume, and more importantly a lack of comparability[145]. A common criticism of multiplex is that results between multiplex and ELISA are not the same. Multiplex assays were developed for high throughput micro-array type problems using recombinant antibodies to simultaneous assess analyte concentrations while ELISAs were developed for individual analyte assessment. Using ELISAs for multiple analyte analyses introduces the potential for large inter-assay error. Thus, it is true that the two assays are not comparable. Moreover, there is a consortium of large molecule assay specialists that endorse the use of multiplex and simultaneously condemn the comparison of ELISA to multiplex[147]. In other words, they will likely not be comparable, but that is to be expected as they have different indications. ELISAs are preferred in individual or few analyte investigations but multiplex analyses have been widely accepted in monitoring more complex multi-factorial immune disease[148]. Naturally this technological advancement has caused disruption in how we measure analytes that are integral to human health[149]. Until multiplex and ELISA assays can be optimized to reconcile absolute values, absolute concertation measurement should be less emphasized than the ratio of measurement. For example, if concentrations of an analyte measured

by ELISA are 1:2 in a healthy population, we would expect that the values will be 1:2 as measured by multiplex, but not necessarily the same concentration. Work is currently being called for to create clinical reference ranges based on multiplex assays[149].

Another potential avenue for unreliable cytokine measurements is the duration of time in which the serum was frozen and the number of freeze thaw cycles. The longer a sample remains frozen and with increasing number of freeze thaw cycles the lower the reliability of an analyte measured[146]**.** Our samples did not exceed the number of freeze thaw cycles (3) that would reportedly cause variance in observations; however we did have a large range of freezer time storage that may be responsible for contributing to the measures observed[150], ranging from as little as 2 and up to 9 years. Thus, we conclude that if there are any erroneous inflammation measurements the multiplex assay is likely not the source, but age of the sample may be.

Lastly, viral or bacterial infections can inflate some transient measures of inflammation such as CRP, IL-6 and TNFα[4,78,119,151]; however, high cytokine values were not isolated to a few children and it is unlikely that all of our participants were acutely ill at the time of visit. Given consideration of these factors, if there is an inaccuracy in our findings, the most likely culprit is freezer storage duration.

5.1.3 Movement behaviours do not prediction inflammation in children with a CID

Contrary to our hypothesis, we found that physical activity was not a significant predictor of inflammation. Physical activity and sedentary behaviour did not predict inflammation component 1 (IL-17a, IL-1β, IL-23, IL-12, IL-6 and TNFα) or inflammation component 2 (CRP, TGFβ-1 and IL-10). Each inflammatory component in our model had statistical and biological rationale. While PCA was used to group these cytokines together, this does not exclude biological

meaning. Biologically, we can infer that component 1 has a strong pro-inflammatory[36,143] presence while component 2 may be more anti-inflammatory with the inclusion of IL-10[70]. It is possible that physical activity truly may not modulate or predict inflammation but given the modulatory capacity of acute exercise on cytokines this seemed surprising. There is evidence to suggest that more frequent physical activity is associated with lower odds of having elevated CRP in healthy adult men and women[103]. Moreover, in a study modelling the effects of swapping sedentary behaviour out for various intensities of physical activity it was found that replacing sedentary time with 10 minutes of VPA per day (regardless of how it was accumulated) was beneficially associated with CRP[88]. However, in this same study replacing sedentary time with MPA was associated with increased CRP[103]. This model was computational and there is minimal observational evidence directly linking physical activity to innate cytokine modulation. Perhaps one of the few studies that has uncovered a relationship between movement behaviours and inflammation is one recently conducted on 390 boys and girls ages 6-8 years[144]. In this Finnish population it was reported that sedentary time was directly associated with CRP and that VPA was inversely associated with CRP and IL-6 in children with higher body fat percentage[144]. Nevertheless, there has been more focus on examining the relationship between structured exercise bouts (acute or chronic), rather than free-living physical activity, and inflammation. In part, longitudinal research is needed and called for by researchers dedicated to the study of movement behaviours and physical activity[88].

It is hard to assess what ought to be measured to accurately represent a true profile of inflammation. We chose to assess 9 cytokines that are implicated in innate components of chronic inflammatory disease however there are other cell types (Th17 cells, NK cells etc.)[57,59,71] upstream of serum cytokine concentrations that may capture a more thorough picture of inflammation in

chronic disease states. Therefore, it may be helpful to modify and refine how inflammation is defined in future investigations. There are likely mechanisms, yet to be revealed that contribute to an overall inflammation profile. Perhaps the inclusion of platelet count, neutrophil count and specific lymphocytes[152] such as Th17 cells that are implicated in chronic long-term inflammation and included in other systemic inflammation indices could be of value to add to an even more robust composite inflammation measure. Given this, it is possible that physical activity could predict other markers of inflammation outside of the cytokines measured in this study.

Another consideration are the limitations in accelerometry and movement behaviour analyses. We measured movement behaviours for 7-days; however, longer wear time may better represent a child's habitual physical activity than 7 days as we know that increased wear time leads to greater reliability[95]. Perhaps a more conceptual question is are we capturing habitual movement accurately? While accelerometry is validated against gold standard physical activity measurement techniques, it is possible that our processing and analyses may not capture habitual movement wholistically. For example, we found that physical activity volume by intensity did not predict inflammation but perhaps the type of physical activity (e.g. leisure vs. chores or school)[132] or frequency or pattern of the activity may predict inflammation[132]. One recent study conducted in adults revealed that the relationship between high sensitivity CRP and physical activity depends on the setting of physical activity[153]. Researchers found that adults that participated in less leisure time physical activity had 12% higher CRP levels compared to those participating in more leisure time[153]. We did not assess the mode or setting that children acquired their physical activity. Healy et al. reported that breaks from sedentary time were beneficially associated with decreases in CRP, independent of total sedentary time in a cohort of 2935 adults aged 20-79 years[154]. A UK study found that the odds of having hypertriglyceridemia (an immune-mediated cardiometabolic risk

factor) was significantly higher in children aged 10-14 years who engaged in more prolonged sedentary bout per day[155]. These findings highlight the importance of analyzing activity patterns (volume vs. bouts) not just in relation to health outcomes such as mortality risk reduction outcomes, but also in relation to inflammatory markers. Mode and frequency dimensions of movement behaviours were not addressed in this study but merit future consideration.

5.2 Limitations:

First, one of the more notable limitations of this study was the participants disease status. More specifically, our participants with a CID were intentionally recruited into the larger CHAMPION study at a time when they were not in flare or having active disease symptoms, as such this underrepresents the spectrum of inflammation a child with a CID may experience. The larger CHAMPION study has longer and more involved study visits where a child participates in exercise, questionaries, and cardiovascular measures. This visit can take up to 4 hours and there are obvious ethical and logistical challenges with having a child come in for this long complement of protocols during a chronic disease flare. Due to this study design limitation, we were not able to measure inflammation and physical activity during a flare for the current study.

Another existing limitation of the study design is that we were not able to successfully recruit enough children with CKD to make meaningful insights about the comparability of their movement behaviours or inflammation to other CID groups. There are typically fewer children in clinic with CKD than have no comorbidities and are in a controlled state of disease, thereby making it difficult to recruit children with this condition.

An existing design limitation inherited from the parent study was our measurement of movement behaviours. We only recorded movement for 7 days and only analyzed activity by

duration and intensity. One of the primary reasons for not analyzing additional dimensions of physical activity was due to sample size limitations. With a larger sample, two areas of consideration could be measuring physical activity for a longer duration of time and evaluating other dimensions of physical activity such as mode or type of activity and frequency of performing the activity. There are also notable general limitations of the accelerometry. For example, some children find the unit to be bulky and cumbersome to wear despite its small size. As a result, non-compliance is an issue that directly effects our measured wear-time of the device. In our case, 10 children were excluded from our analyses due to non-compliance.

Another notable limitation was our blood sampling protocol. Our serum samples had a variety of freezer storage time which is not ideal for comparison purposes. Ideally samples would be drawn, spun down, and analyzed within a year of storage. Furthermore, we only had one blood sample at one time point; more blood draws over a fixed time period may provide a more comprehensive understanding of temporal changes in inflammation. An example of such a schedule could be drawing blood once per week for 4 weeks while a child is also simultaneously equipped with an accelerometer. Lastly our study only estimated inflammation from serum samples derived from a peripheral blood sample. This is a logical first step in inflammation assessment; however, acquiring a tissue sample would be likely more informative as skeletal muscle is an immune regulator. It reasonable to hypothesize that cytokine concentrations along with other cell types such as Th17, and NK cells likely have different composition in this tissue as this has been seen in mice and adult studies[156]. It is plausible that a muscle-derived profile would be closely associated with a movement profile than a cytokine profile derived from a systemic sample. However, invasive procedures such as blood draws are already difficult to acquire in a

pediatric population, therefore, acquiring an even more invasive procedure such as a tissue biopsy would likely be a greater challenge in this population.

5.3 Novelty of Findings:

We observed two novel findings. First, we observed that inflammation was comparable between children with CF, JIA, IBD and T1DM. It is interesting to consider that across 9 cytokines measured values between 4 conditions are comparable. Traditionally these 4 conditions are viewed as being entirely different diseases, with very different symptom profiles. Although these conditions manifest in very different ways, it could be that uncovering more details regarding similarities between chronic inflammatory diseases at the molecular level may provide a target for therapeutic design. This is just scratching the surface of trying to understand the immunology that unites these conditions.

Second, we observed that physical activity does not predict inflammation, which is curious and subject to limitations. However, this finding has not been reported before in this population and sets the stage for several follow-up research questions.

5.4 Future Directions:

This study was a first step towards an understanding of movement behaviour and inflammation, and their interaction, in children with a CID. Future work should include measurements during a flare and expanding our measures of movement behaviours and inflammation to be more comprehensive, while also including measures of psychosocial factors that may influence these associations.

Capturing movement behaviours and inflammation during a period of flare or uncontrolled disease status will likely be more insightful since it would encompass larger variability in the outcomes of interest. Future studies might consider to recruiting healthy participants, children with a CID in flare and children with a CID that is controlled. Use of clinical disease status scales such a physician's global assessment (PGA) may be helpful in this recruitment process. Depending on the condition PGAs are typically a well-accepted and commonly used scale for the evaluation of treatment response in children and adults[157].

In conjunction with recruitment changes, movement behaviour analyses conducted for longer periods of time may yield a better representation of habitual movement and as such will likely be more easily analyzed to reveal activity patterns. Future physical activity analyses should consider other dimensions of physical activity such as frequency and mode not addressed in this study. It is still unclear if the pattern of activity accumulation is important for health outcomes, and specifically for inflammatory profiles, in the pediatric population. Interestingly, a recent longitudinal study from Dunton et al. revealed that as healthy girls became older, dispersion in their activity throughout a day decreased[158]. This indicates that girls take fewer intermittent active breaks throughout the day than boys of equivalent age, which merits further exploration in the context of inflammation. It is also important to quantify and understand what type of activity a child is participating in. Do children acquire most movement via school or chore related activities or do they acquire activity via leisure activity? Understanding these dimensions of physical activity in addition to volume and intensity will lead to a more complete picture of movement behaviours in this population of children with a CID.

Expanding on our inflammation measures it would be useful to contextualize cytokine findings by establishing number and function of circulating peripheral immune cells in conjunction with cytokine concentrations. Future work should consider measuring important T-cell subsets implicated in these conditions such as Th17 cells. Additionally, measuring these immune correlates not only in serum but also in synovial fluid and/or tissue, when possible, would yield a better representation of active concentrations.

Lastly, other lifestyle behaviours and participant characteristics that may be relevant predictors of both movement behaviours and inflammation, such as mental health status, nutrition, sleep, socio-economic status, and quality of life were not considered in this regression model. It is possible that these other factors are greater predictors of inflammation than physical activity. As such future studies should aim to incorporate expanded recruitment techniques, more robust measures to define habitual physical activity and inflammation, as well as psycho-social parameters.

5.5 Study Implications and Conclusions

We have shown that physical activity and inflammation are comparable between children with CF, JIA, IBD and CF. This may imply that children with these conditions are more alike than their individual symptomology would suggest. This cross-sectional study is an important first investigation into the relationships between movement behaviours and inflammation in children. Children with a CID may have other important commonalities such as adaptive immune markers, frequency of movement behaviours and psychosocial challenges that can help us better treat their conditions. These similarities are a clue into better understanding chronic inflammatory disease.

We have also confirmed that children with a CID participate in less physical activity than CTL. Given that inflammation was comparable between CID and CTL this implies that there is may be other inflammatory outcomes we should assess to better understand differences between healthy children and children with a chronic disease. We need to understand the factors that cause a child with a CID to be less active and what can be done to encourage increased movement in this population.

These findings led us to investigate the relationship between movement behaviours and inflammation. Children with a CID undergo many difficult pharmaceutical treatments and consequently it would be a reprieve to have a treatment as simple as physical activity help modulate their condition. We hypothesized that physical activity may modulate inflammation however, we did not find this to be the case. We did see an age effect whereby an increase in age was associated with an increase in inflammation component 1: IL-17a, IL-1β, IL-23, IL-12, IL-6 and TNFα. It will be important to monitor this age effect in the same children through time. The CHAMPION-2 study is a longitudinal extension of this study's parent study, CHAMPION-1 and has the potential to explore these relationships over time.

Although we did not find a relationship between physical activity and inflammation, we know that physical activity has many beneficial cardiorespiratory and mental health effects. As such, it is still of interest to uncover any potential effects physical activity may have on the immune system in CID. Further work is needed to choose how to best represent inflammation and physical activity in cross-sectional and longitudinal studies. This can be done by creating a study design with multiple measures of both inflammation and movement outcomes while also recruiting children in active disease flare. With broadened investigations into the relationship between

physical activity and inflammation longitudinally, this line of investigation may serve to uncover relationships that can only be observed in a lifetime.

Chapter 6: References

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APPENDIX 1:

A detailed summary of anthropometric, physical activity and inflammatory ANOVAs and ANCOVAs. Please note that post-hoc tests are only included for significant ($p<0.05$) ANOVA outcomes.

One-way ANOVAs between CID groups: CF, JIA, IBD, and T1DM

An original ANOVA table is provided. Tukey's Post-hoc tests are provided for variables that were significant with $p<0.05$ in the P-value column of the ANOVA table in TS1.

TS1: A one-way ANOVA conducted on CF, JIA, IBD and T1DM for anthropometric variables.

	ANOVA	SS	df	MS	F	P value
Sex	Between Groups	.576	3	.192	.764	.518
	Within Groups	19.111	76	.251		
	Total	19.688	79			
Age	Between Groups	78.525	3	26.175	3.734	.015
	Within Groups	532.773	76	7.010		
	Total	611.298	79			
Weight (kg)	Between Groups	1678.133	3	559.378	2.571	.060
	Within Groups	16535.974	76	217.579		
	Total	18214.108	79			
Height	Between Groups	1985.486	3	661.829	3.412	.022
	Within Groups	14740.678	76	193.956		
	Total	16726.164	79			
YPHV	Between Groups	42.125	3	14.042	2.982	.037
	Within Groups	357.834	76	4.708		

	Total	399.959	79			
PercentBF	Between Groups	1420.789	3	473.596	6.393	.001
	Within Groups	4963.669	67	74.085		
	Total	6384.458	70			

TS2: TUKEY'S POST HOC TESTS for Age, Height, YPHV and Percent BF.

				Mean Difference	Std. Error	Sig.	Lower Bound	Upper Bound
Age	Tukey HSD	CF	JIA	-1.38291	.87199	.393	-3.6734	.9076
			IBD	-2.86048*	.91353	.013	-5.2601	-.4608
			T1DM	-2.33310	.94349	.072	-4.8115	.1453
		JIA	CF	1.38291	.87199	.393	-.9076	3.6734
			IBD	-1.47757	.77036	.229	-3.5011	.5460
			T1DM	-.95019	.80566	.642	-3.0665	1.1661
		IBD	CF	2.86048*	.91353	.013	.4608	5.2601
			JIA	1.47757	.77036	.229	-.5460	3.5011
			T1DM	.52738	.85045	.925	-1.7066	2.7614
		T1DM	CF	2.33310	.94349	.072	-.1453	4.8115
			JIA	.95019	.80566	.642	-1.1661	3.0665
			IBD	-.52738	.85045	.925	-2.7614	1.7066
Height	Tukey HSD	CF	JIA	-8.96376	4.58667	.215	-21.0120	3.0845
			IBD	-13.74048*	4.80521	.027	-26.3628	-1.1182
			T1DM	-13.85635*	4.96280	.033	-26.8926	-.8201
		JIA	CF	8.96376	4.58667	.215	-3.0845	21.0120
			IBD	-4.77672	4.05211	.642	-15.4208	5.8673
			T1DM	-4.89259	4.23780	.657	-16.0244	6.2392
		IBD	CF	13.74048*	4.80521	.027	1.1182	26.3628
			JIA	4.77672	4.05211	.642	-5.8673	15.4208

			T1DM	-.11587	4.47341	1.000	-11.8666	11.6349
		T1DM	CF	13.85635*	4.96280	.033	.8201	26.8926
			JIA	4.89259	4.23780	.657	-6.2392	16.0244
			IBD	.11587	4.47341	1.000	-11.6349	11.8666

				Mean Difference	Std. Error	Sig.	Lower Bound	Upper Bound
YPHV	Tukey HSD	CF	JIA	-.99842	.71463	.505	-2.8756	.8788
			IBD	-2.02014*	.74868	.042	-3.9868	-.0535
			T1DM	-1.83491	.77323	.091	-3.8660	.1962
		JIA	CF	.99842	.71463	.505	-.8788	2.8756
			IBD	-1.02172	.63134	.375	-2.6801	.6367
			T1DM	-.83648	.66027	.587	-2.5709	.8979
		IBD	CF	2.02014*	.74868	.042	.0535	3.9868
			JIA	1.02172	.63134	.375	-.6367	2.6801
			T1DM	.18524	.69698	.993	-1.6456	2.0161
		T1DM	CF	1.83491	.77323	.091	-.1962	3.8660
			JIA	.83648	.66027	.587	-.8979	2.5709
			IBD	-.18524	.69698	.993	-2.0161	1.6456

BF (%)	Tukey HSD	CF	JIA	-11.08590*	3.00385	.002	-19.0001	-3.1716
			IBD	-2.31930	3.17378	.884	-10.6813	6.0427
			T1DM	-3.50952	3.38607	.729	-12.4308	5.4118
		JIA	CF	11.08590*	3.00385	.002	3.1716	19.0001

		IBD	8.76660*	2.59781	.007	1.9222	15.6110
		T1DM	7.57637*	2.85327	.048	.0589	15.0939
	IBD	CF	2.31930	3.17378	.884	-6.0427	10.6813
		JIA	-8.76660*	2.59781	.007	-15.6110	-1.9222
		T1DM	-1.19023	3.03166	.979	-9.1777	6.7973
	T1DM	CF	3.50952	3.38607	.729	-5.4118	12.4308
		JIA	-7.57637*	2.85327	.048	-15.0939	-.0589
		IBD	1.19023	3.03166	.979	-6.7973	9.1777

The mean difference is significant at the 0.05 level. Confidence intervals are 95%.

TS3: A one-way ANOVA conducted on CF, JIA, IBD and T1DM for movement and inflammation variables.

SED	Between Groups	28074.745	3	9358.248	3.564	.019
	Within Groups	168060.074	64	2625.939		
	Total	196134.819	67			
LPA	Between Groups	18292.659	3	6097.553	4.819	.004
	Within Groups	80988.510	64	1265.445		
	Total	99281.169	67			
MPA	Between Groups	726.704	3	242.235	2.317	.084
	Within Groups	6689.742	64	104.527		
	Total	7416.446	67			
VPA	Between Groups	158.260	3	52.753	.291	.831
	Within Groups	11585.447	64	181.023		
	Total	11743.708	67			
MVPA	Between Groups	1294.566	3	431.522	.892	.450
	Within Groups	30975.105	64	483.986		
	Total	32269.670	67			
TPA	Between Groups	24991.859	3	8330.620	3.216	.029
	Within Groups	165763.106	64	2590.049		
	Total	190754.965	67			

CPM	Between Groups	131977.487	3	43992.496	1.310	.279
	Within Groups	2149845.092	64	33591.330		

IL-1β	Between Groups	45.709	3	15.236	1.371	.260
	Within Groups	711.241	64	11.113		
	Total	756.950	67			
IL-6	Between Groups	501.908	3	167.303	1.413	.247
	Within Groups	7575.519	64	118.367		
	Total	8077.427	67			
IL-8	Between Groups	5392.159	3	1797.386	3.506	.020
	Within Groups	32809.634	64	512.651		
	Total	38201.793	67			
IL-10	Between Groups	4666.002	3	1555.334	.830	.482
	Within Groups	119959.927	64	1874.374		
	Total	124625.929	67			
IL-12p70	Between Groups	37.464	3	12.488	.611	.610
	Within Groups	1308.337	64	20.443		
	Total	1345.800	67			
IL-17A	Between Groups	3223.977	3	1074.659	1.183	.323
	Within Groups	58130.961	64	908.296		
	Total	61354.937	67			
IL-23	Between Groups	508140947.236	3	169380315.745	.812	.492

	Within Groups	13356328401.380	64	208692631.272		
	Total	13864469348.616	67			
TNFα	Between Groups	963.809	3	321.270	1.607	.196
	Within Groups	12792.018	64	199.875		
	Total	13755.826	67			
TGF β-1	Between Groups	1269465202.891	3	423155067.630	1.517	.219
	Within Groups	17857236520.049	64	279019320.626		
	Total	19126701722.939	67			
CRP (mg/L)	Between Groups	90.806	3	30.269	.273	.845
	Within Groups	6209.254	56	110.880		
	Total	6300.059	59			

TS4: Tukey's post hoc tests from a One way ANOVA conducted on CF, JIA, IBD and T1DM for movement and inflammation variables.

SED	Tukey HSD	CF	JIA	5.56124	18.38992	.990	-42.9484	54.0709
			IBD	-43.41328	18.71163	.104	-92.7715	5.9450
			T1DM	-14.38844	20.15925	.891	-67.5653	38.7884
		JIA	CF	-5.56124	18.38992	.990	-54.0709	42.9484
			IBD	-48.97452*	15.83218	.015	-90.7372	-7.2118
			T1DM	-19.94968	17.51936	.667	-66.1629	26.2636
		IBD	CF	43.41328	18.71163	.104	-5.9450	92.7715
			JIA	48.97452*	15.83218	.015	7.2118	90.7372
			T1DM	29.02484	17.85676	.372	-18.0784	76.1281
		T1DM	CF	14.38844	20.15925	.891	-38.7884	67.5653
			JIA	19.94968	17.51936	.667	-26.2636	66.1629
			IBD	-29.02484	17.85676	.372	-76.1281	18.0784

LPA	Tukey HSD	CF	JIA	-.08560	12.76613	1.000	-33.7606	33.5894
			IBD	37.40031*	12.98946	.027	3.1362	71.6644
			T1DM	21.32140	13.99438	.430	-15.5935	58.2363
		JIA	CF	.08560	12.76613	1.000	-33.5894	33.7606
			IBD	37.48591*	10.99057	.006	8.4946	66.4772
			T1DM	21.40700	12.16179	.302	-10.6738	53.4878

	IBD	CF	-37.40031*	12.98946	.027	-71.6644	-3.1362
		JIA	-37.48591*	10.99057	.006	-66.4772	-8.4946
		T1DM	-16.07891	12.39602	.568	-48.7776	16.6198
	T1DM	CF	-21.32140	13.99438	.430	-58.2363	15.5935
		JIA	-21.40700	12.16179	.302	-53.4878	10.6738
		IBD	16.07891	12.39602	.568	-16.6198	48.7776

One-way ANOVAs and ANCOVAs between CTL and CID:

TS5: ANOVA table results for anthropometric measures between CID and CTL.

		SS	df	MS	F	Sig.
Sex	Between Groups	1.475	1	1.475	6.096	.015
	Within Groups	31.457	130	.242		
	Total	32.932	131			
Age	Between Groups	.421	1	.421	.052	.820
	Within Groups	1054.237	130	8.110		
	Total	1054.658	131			
WeightAverage	Between Groups	205.082	1	205.082	.802	.372
	Within Groups	33248.776	130	255.760		
	Total	33453.857	131			
Height	Between Groups	636.792	1	636.792	2.713	.102
	Within Groups	30518.597	130	234.758		
	Total	31155.389	131			
APHV	Between Groups	9.869	1	9.869	6.381	.013
	Within Groups	201.055	130	1.547		
	Total	210.924	131			
YPHV	Between Groups	14.356	1	14.356	2.505	.116
	Within Groups	744.890	130	5.730		
	Total	759.245	131			
Waist	Between Groups	4.409	1	4.409	.046	.830

	Within Groups	12062.853	127	94.983		
	Total	12067.262	128			
Hip	Between Groups	.075	1	.075	.001	.982
	Within Groups	15775.891	107	147.438		
	Total	15775.966	108			
PercentBF	Between Groups	630.465	1	630.465	7.259	.008
	Within Groups	10334.883	119	86.848		
	Total	10965.348	120			

TS6: ANCOVA table results between CF, JIA, IBD and T1DM adjusted for age and sex.

Dependent Variable	SS	Df	MS	F	P value	Partial eta-squared
SED	7763.179	2	3881.589	2.492	.093	.092
LPA	2963.604	2	1481.802	2.306	.110	.086
MVPA	1130.897	2	565.448	1.438	.247	.055
TPA	7078.569	2	3539.284	2.401	.101	.089
IL-1beta	69.919	2	34.960	2.883	.065	.105
IL-6	787.664	2	393.832	2.966	.061	.108
IL-10	2332.334	2	1166.167	.500	.610	.020
IL-12	74.295	2	37.148	2.230	.118	.083
IL-17	5323.016	2	2661.508	2.815	.070	.103
IL-23	28695133.767	2	14347566.883	2.220	.120	.085
TNFalpha	916.341	2	458.170	1.842	.169	.070
TGFbeta1	526589829.021	2	263294914.510	.873	.424	.034
CRP	55.148	2	27.574	.264	.769	.012

SS = Type III Sum of Squares, Df = degrees of freedom, MS = Mean Square, and F = F statistic

TS7: ANCOVA table results between CID and CTL adjusted for age and sex.

Dependent Variable	SS	Df	MS	F	P value	Partial eta-squared
SED	9051.399	1	9051.399	6.244	.014	.054
LPA	771.297	1	771.297	1.307	.255	.012
MVPA	3807.232	1	3807.232	9.749	.002	.081
TPA	7924.637	1	7924.637	5.646	.019	.049
IL-1beta	.269	1	.269	.026	.872	.000
IL-6	180.850	1	180.850	2.065	.153	.018

IL-10	760.095	1	760.095	.629	.430	.005
IL-12	3.923	1	3.923	.126	.723	.001
IL-17	4333.030	1	4333.030	2.475	.118	.021
IL-23	3231270.639	1	3231270.639	.792	.375	.007
TNFalpha	100.493	1	100.493	.776	.380	.007
TGFbeta1	38556626.273	1	38556626.273	.141	.708	.001
CRP	356.193	1	356.193	3.435	.067	.035

SS = Type III Sum of Squares, Df = degrees of freedom, MS = Mean Square, and F = F statistic

APPENDIX 2:

Data sheets collected during study visits for CHAMPION-1.

Assent and consent forms

Child Health & Exercise Medicine Program

Study ID: CHAMP - _ _ - _ _
Date: _ _ - _ _ _ - _ _

The CHAMPION Study
Visit #1 – Data Collection Sheet

1) Participant Information

Date of Birth: __ __ - __ __ __ - __ __ __ __

Medical Information: ☐ Allergies: _____

☐ Injuries: _____

☐ Exercise Restrictions: _____

Resting Blood GLU: _____ ☐ N/A
(See p.4 for guidelines)

2) Anthropometry (Measured by: ___)

Tanner: _____

Weight (kg): _____/_____
(within 0.1 kg)

Height (cm): _____/_____
(within 0.3 cm)

Sitting Hgt (cm): _____/_____
(within 0.3 cm)

Waist (cm): _____/_____/_____
(within 0.5 cm)

Hip (cm): _____/_____/_____
(within 0.5 cm)

InBody % BF: _____ ☐ Old BIA ☐ New BIA
*Ensure participant has voided bladder
*Rest for 2-3 minutes before InBody

3) Pulmonary Function (**CF ONLY;** Measured by: ____)

☐ N/A # of attempts: _____ 3 Best Trial #s: _____

_____

_____

4) Grip Strength (Measured by: ___)

	Dominant	Order	Trial #1	Trial #2	Trial #3
Right	☐				
Left	☐				

**Trial values should be within 3 kg by hand.*

5) Motor Function

☐ BOT-2 Short Form completed at Visit #1.

If no, reason: _____

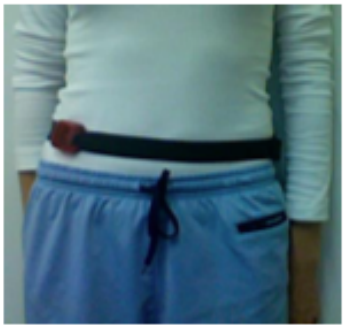
Data Entry Initials: _____

Page 1 of 4

Child Health & Exercise Medicine Program

HOW DO I WEAR THE ACTIVITY MONITOR?

The activity monitor must be worn around your waist, over your **RIGHT HIP BONE**, like in the picture below. You can wear the monitor on top or underneath your clothes.




WHEN DO I WEAR THE ACTIVITY MONITOR?

You should wear the activity monitor at **ALL times for the next 7 days, except when you're doing water activities**. You can wear the activity monitor when playing sports, even contact sports like hockey and football. You can also wear the activity monitor when you're sleeping. The only time you should **NOT** wear the activity monitor is during water activities like showers, baths, or swimming.

HOW DO I KNOW THE ACTIVITY MONITOR IS WORKING?

There is a small light on the front of the device. **This light will NOT be flashing when the activity monitor starts recording**. If you notice that the light is flashing when you are wearing the belt, that means there is a problem. Please call or e-mail Maddy at 905-521-2100 ext. 73517 or champion.mac1@gmail.com.

WHAT DO I RECORD IN THE ACTIVITY LOG BOOK?

1. The time you wake up in the morning
2. Any time that you took the activity monitor off during the day
3. The time you get into your bed at night (even if you're not asleep yet)
4. If you had a special or different day from usual.

If you have to take the activity monitor off during the day for any reason, we ask that you record:

1. The time you took the belt off
2. The time you put the belt back on
3. The reason you took the belt off

If you have any questions or concerns, please do not hesitate to contact Maddy at 905-521-2100 ext. 73517 or champion.mac1@gmail.com. Thank you!

2021 – Version 2

Child Health & Exercise Medicine Program

ACTIVITY LOG BOOK:

In addition to wearing the activity monitor for the next 7 days, we ask that you keep this log to monitor the times you woke up and went to sleep, as well as any time the activity monitor was put on or taken off. This will help us to understand your regular physical activity. Please return this log along with your activity monitor when you are done wearing it.

Event	Example	DAY 1 Day: _____ Date: _____	DAY 2 Day: _____ Date: _____	DAY 3 Day: _____ Date: _____	DAY 4 Day: _____ Date: _____	DAY 5 Day: _____ Date: _____	DAY 6 Day: _____ Date: _____	DAY 7 Day: _____ Date: _____
Wake up time	8:02 AM							
Times the device may have been taken off and put back on and reason(s) (ex. nap, swimming, shower, etc)	4:45 pm – 5:27 pm (Nap)							
	7:10 pm – 7:37 pm (Shower)							
	-							
	-							
Time you went to bed	10:19 pm							
Did you do anything special or different from usual today? If it was a special/different, please describe why (for example: went on a field trip, wasn't feeling well, stayed home from school).		☐ No, it was a normal day ☐ Yes. Please describe why it was special:	☐ No, it was a normal day ☐ Yes. Please describe why it was special:	☐ No, it was a normal day ☐ Yes. Please describe why it was special:	☐ No, it was a normal day ☐ Yes. Please describe why it was special:	☐ No, it was a normal day ☐ Yes. Please describe why it was special:	☐ No, it was a normal day ☐ Yes. Please describe why it was special:	☐ No, it was a normal day ☐ Yes. Please describe why it was special:

ID: _____

ACCELEROMETER #: _____

PLEASE SEE THE LAST PAGE FOR DETAILED INSTRUCTIONS

McMaster University Health Sciences

Hamilton Health Sciences
EXEMPLARY CARE • ACADEMIC EXCELLENCE

PARENT CONSENT FORM

Title of Study: Cardiovascular Health in children with a chronic inflAMmatory condition: role of Physical activity, fItness, and inflammatiON

Local Principal Investigator: *Dr. Brian W. Timmons (PhD), Pediatrics*

Principal Investigator: *Dr. Joyce Obeid (PhD), Pediatrics*

Co-Investigators: *Dr. Maureen MacDonald (PhD), Kinesiology*

Dr. Tania Cellucci (MD), Pediatrics

Dr. Lehana Thabane (PhD), Clinical Epidemiology & Biostatistics

Funding Source: **Heart & Stroke Foundation**

INTRODUCTION

Your child is being invited to participate in a research study conducted by Dr. Brian Timmons and colleagues because they are healthy. In order to decide whether or not you want to be a part of this research study, you should understand what is involved and the potential risks and benefits. This form gives detailed information about the research study, which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate. Your child will be asked to sign another form to confirm that they agree to participate. Take your time to make your decision.

WHY IS THIS RESEARCH BEING DONE?

We know that heart disease can start in childhood. But because children hardly ever get heart attacks, so-called risk factors can be measured to guess which children might eventually develop heart disease. We are also learning that children growing up with a medical condition may be at even greater risk for heart disease, although we have a lot more to learn. This is a problem that we will begin to examine in this project. We don't know if the problem is because of their medical condition or because of lifestyle factors, such as not getting enough exercise, or a combination of both. In this project, we will study some of the most common diseases that children get: cystic fibrosis, juvenile arthritis, kidney disease, inflammatory bowel disease, and type 1 diabetes. Doctors need to know if their patients are at risk for getting heart disease. This could stop heart disease or at least reduce its impact because we may be able to intervene with the right therapy at the right time. By studying these patients together, we will quickly learn a lot about heart health for these children who are now living longer than ever before.

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of this study is to compare heart health in children diagnosed with a medical condition and healthy children. We also want to see if heart health in our patients is related to physical activity, fitness, and inflammation. Inflammation is when the body's immune system doesn't work properly and this is probably not good for heart health. If we find that any of these factors are related to heart health, then we can design studies to test whether changing them will be good for health in our patients.

WHAT WILL MY CHILD'S RESPONSIBILITIES BE IF THEY TAKE PART IN THE STUDY?

If you volunteer to participate in this study, we will ask your child to visit the Child Health & Exercise Medicine Program laboratory at the McMaster Children's Hospital on two occasions, separated by 1 or 2 weeks, to perform the following things:

- **Visit #1:** This visit will take a total of 3 hours and can be scheduled at your convenience, in either the evening, after school, or on weekends. During this visit, we will measure the following things:
    1) **Body composition assessment**: To determine how much muscle and fat is in your child's body, we will have them stand on a special machine. There is a small electric current that is passed through their body but they will not feel it because it is so small.
    2) **Aerobic fitness test**: This test requires your child to ride on our stationary bicycle for about 12 minutes – during the test it will feel like they are riding up a hill and the hill is gradually getting steeper and steeper. During the whole test, they will breathe through a mouthpiece connected to a machine that tells us how much oxygen the body is consuming. This mouthpiece feels like a mouth guard they might wear during sports or a snorkel. We use this information to determine aerobic fitness, because more fit children can use more oxygen during this test. They will also have their blood pressure measured throughout the test using an inflatable cuff on the upper arm.
    3) **Muscle strength test**: During this test your child will be asked to do a series of kicking exercises and we will measure their muscle power during the exercise.
    4) **Questionnaires**: Between fitness tests when your child is resting, we will have them and you fill out some questionnaires that tell us about their physical activity and the issues they see as important for getting or not getting enough exercise. We will also ask you to complete a few questionnaires to better understand your background and your child's medical history. Some of these questionnaires will ask about how your child feels, and may seem personal. We ask that you complete these to the best of your knowledge. All of your answers will be kept strictly confidential. You should also

know that you can choose not to answer any questions that make you feel uncomfortable, this will not affect your child's participation in the study.

5) **Physical activity assessment**: Before leaving the laboratory, we will give your child a small pager-like device to wear for the next 7 days in a row – this device monitors physical activity. They can take it off only if they are going to get wet (like in the bathtub or swimming) and at bedtime. We request that you write down in a diary, that we will provide, the times that it is taken off and put back on. We will ask you to return the monitor at your second visit. At that time, we will have a few questions to ask you and your child about any issues that might have arisen while wearing the device for the 7 days.

- **Visit #2:** This visit will take about 3.5 hours to complete. We will ask that your child come in to the lab in a fasted state for this visit (no food or drink for 10 hours), so we will schedule this visit in the morning either before school or on the weekend. During this visit we will measure the following things:

1) **Cardiovascular health:** We will measure the health of the main blood vessel in your child's neck. This test requires your child to lie on a bed for about ~~15~~ 25 min for the measurements to be taken. This will be followed by an assessment of how well your child's blood vessels work. To do this, we will ask them to lie on a bed and we will place a blood pressure cuff on their arm, this will be inflated for 5 minutes and then deflated, and we will take images of how the blood vessel in their arm reacts to the blood pressure cuff. This assessment has been shown to be reliable in adults, but we don't know as much about its reliability in children. To test this, we will ask your child to rest for 20 minutes on the bed and we will repeat this assessment a second time. Doing the test 2 times will let us compare the images of the blood vessels in your child's arm to make sure we are getting the same results. In between these measurements, we will look at heart health. We will ask your child to remove their shirt and put on a gown. Images of the heart will be taken while they are lying down using a special camera (i.e., ultrasound) placed on the chest.
2) **Blood sample:** A small, 40 mL (about 2.5 tablespoons) blood sample will be taken to allow us to measure some cells in the blood that have been linked with cardiovascular health.
3) **Motor skills test**: This test will take about 15 minutes and will help us assess your child's motor skills by asking them to drawing, balancing, running, and ball-throwing tasks.

- **Visit #3 (optional, 1 hour):** This visit is optional for your child and is not required to be part of the CHAMPION study. If you agree to have your child participate, we will ask that your child returns to the lab in a fasted state (no food or drink for 3 hours) within a week of visit #2. At this visit we will again measure how well your child's blood vessels work to see if the assessment gives different results on a different day. To do this, we will ask your child to rest quietly for 10 minutes on a bed. A blood pressure cuff will then be placed on their arm and will be inflated for 5 minutes and then deflated. During this process, pictures will be taken of the blood vessels in your child's arm to see how it reacts to the blood pressure cuff.

Visit #3 is optional, and not required to participate in the CHAMPION study. Do you consent to having your child participate in Visit #3?
☐ Yes, my child will participate in Visit #3. ☐ No, my child is not interested in participating in Visit #3.

WHAT ARE THE POSSIBLE RISKS AND DISCOMFORTS?

There are no risks or discomforts to measuring body composition. The tests of fitness and muscle power require an all-out effort so people generally feel tired after this test. The test of aerobic fitness also requires your child to keep pedaling until they can no longer keep going. This test measures fitness by seeing how hard the heart, lungs, and muscles can work together. There is a small chance (about 3 in 10,000) that your child may faint after the test of aerobic fitness. However, young people tend to recover very quickly. We will give your child lots of rest before the next test. Wearing the physical activity monitors should not pose any risks or discomforts for your child, nor should completing the questionnaires. The assessment of cardiovascular health is done with the same ultrasound machine that doctors use to take a picture of a baby during a pregnancy. Your child may experience some discomfort when the blood pressure cuff is inflated, but this will be very minor and temporary (until the blood pressure cuff is deflated). An experienced investigator will collect a blood sample during your child's second visit. A small bruise may appear where the needle goes through the skin. While it is very rare, there is also a chance that your child may feel light-headed after the blood sample. We will have snacks and water on hand to minimize the risk of this happening, but taking this amount of blood will have no major negative effects.

HOW MANY PEOPLE WILL BE IN THIS STUDY?

We are asking a total of 180 children and adolescents to participate in this study. Your participation is voluntary.

WHAT ARE THE POSSIBLE BENEFITS FOR MY CHILD AND/OR FOR SOCIETY?

We cannot promise any personal benefits to you or your child from their participation in this study. We will make each visit fun and enjoyable for your child. You will learn about your child's physical activity and fitness. This information could provide us with information on areas that your child can work on improving. Your participation will be very important for us to learn how best to address physical activity, fitness, and heart health in young children with chronic disease and to design programs that can help in these areas of health.

WHAT INFORMATION WILL BE KEPT PRIVATE?

All of your child's information will be stored in locked filing cabinets under the supervision of Dr. Brian Timmons for 10 years. We will supervise access to your child's information by other people in our group, only if necessary. Your child will be assigned a subject number used to identify them. Records identifying your child will be kept confidential. If the results of the study are used in a presentation, your child's identity will remain confidential.

CAN PARTICIPATION IN THE STUDY END EARLY?

If you and your child volunteer to be in this study, you or your child may withdraw at any time with no prejudice. The investigator may withdraw you from this research if circumstances arise which warrant doing so.

WILL MY CHILD BE PAID TO PARTICIPATE IN THIS STUDY?

We will provide your child $50 as reimbursement for their participation in this study. If you quit the study for personal reasons, we will change the amount for the time completed. If you choose to quit because of a complication from the study, we will give you the full amount. We will pay for your parking expenses at the McMaster Children's Hospital. We will also provide you with a 1-page report of the findings and what they mean.

IF I HAVE ANY QUESTIONS OR PROBLEMS, WHOM CAN I CALL?

If you have any questions about the research now or later, or if you think you have a research-related injury, you can contact Joyce Obeid at our research office at 905-521-2100 extension 73517 (Daytime) or at 905-928-5538 in the evenings, you can also contact Dr. Brian Timmons directly at 905-521-2100 extension 77218 or 77615.

If you have any questions regarding your rights as a research participant, you may contact Deborah Mazzetti (Manager) at the Hamilton Integrated Research Ethics Board at 905-521-2100 extension 42013.

CONSENT STATEMENT

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction and to the satisfaction of my son and/or daughter. I agree to allow my child to participate in this study entitled: *"Cardiovascular Health in children with a chronic inflAMmatory condition: role of Physical activity, fItness, and inflammatiON: The CHAMPION Study"*. I understand that I will receive a signed copy of this form.

Would you like to be contacted by Dr. Timmons or a member of the Child Health & Exercise Medicine Program research team with information about future studies other than the one described in this consent form? Any future studies would be approved by the Research Ethics Board, and would require you to sign a new consent form. **Please note we will only contact you if your child is eligible for a maximum of 2 times per year.
☐ Yes, please contact me. ☐ No, please do not contact me.

_____

Name of Participant (child's name)

_____

Name of Legally Authorized Representative

_____ _____

Signature of Legally Authorized Representative Date

Consent form administered and explained in person by:

_____

Name and title

_____ _____

Signature Date

SIGNATURE OF INVESTIGATOR:

In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent for their son and/or daughter to participate in this research study.

_____

Name and title

_____ _____

Signature of Investigator Date

FUTURE RESEARCH

At the end of the study, we may wish to store leftover sample for use in a future study. We will not store your child's sample longer than 10 years. All records identifying your child will remain confidential. Information about your child will not be released. If the results of the study are published, your child's identity will remain confidential.

CONSENT STATEMENT FOR STORAGE OF SAMPLES (BLOOD)

I have read the preceding information thoroughly. I have had the opportunity to ask questions, and all of my questions have been answered to my satisfaction and to the satisfaction of my son and/or daughter. I agree to have my child's blood stored so it can be used for future research studies approved by the Research Ethics Board other than the one described in this information form.

_____

Name of Participant (child's name)

_____

Name of Legally Authorized Representative

_____ _____

Signature of Legally Authorized Representative Date

Consent form administered and explained in person by:

_____

Name and title

_____ _____

Signature Date

SIGNATURE OF INVESTIGATOR:
In my judgement, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent for their son and/or daughter to store blood.

_____

Name and title

_____ _____

Signature of Investigator Date

PHOTO, AUDIO AND VIDEO RELEASE FORM

I, _____, hereby give McMaster University's Faculty of Health Sciences my permission to take and use any photographs, movie films, audio or video tapes made of my child, (name) _____, taken on (date) _____, and I consent to the reproduction of same in any proper manner whatsoever for possible publication and I hereby waive any rights that I may have in such photographs, movie films and audio or video tapes or reproductions of same.

I hereby release McMaster University's Faculty of Health Sciences, its employees, agents, servants and attending physicians from all actions, causes of actions, claims and demands arising out of such consent.

NOTICE OF COLLECTION OF PERSONAL INFORMATION

By taking my photograph, whether by still photograph, film or video and/or taping my voice, I acknowledge that McMaster University is collecting my personal information as defined by the Freedom of Information and Protection of Privacy Act of Ontario (RSO 1990).

The personal information is collected under the authority of The McMaster University Act, (1976). The information is used for public relations purposes of the Faculty of Health Sciences including, but not limited to, publications, websites and materials promoting McMaster University. Personal information will not be used for any unrelated purpose without prior consent. This information is protected and is being collected pursuant to section 39(2) and section 42 of the Freedom of Information and Protection of Privacy Act of Ontario (RSO 1990). Questions regarding the collection or use of this personal information should be directed to the Manager, Public Relations, Faculty of Health Sciences.

I, _____, represent that I am the parent or guardian of the minor named above and that I have the legal authority to execute the foregoing consent and release, and hereby approve the foregoing, and waive any rights in the premises.

Name of Participant	Signature of Participant	Date
Name of Parent/Guardian	Signature of Parent/Guardian	Date
Name of Witness	Signature of Witness	Date