Inflammatory markers, physical activity and exercise tolerance in the adult cystic fibrosis population

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ABSTRACT

Background & objective

Adults with cystic fibrosis (CF) were assessed to establish whether a relationship exists between inflammation (systemic and/or pulmonary), physical activity and/or exercise tolerance, following in-hospital treatment for an acute exacerbation, and whether these factors can predict for time to next pulmonary exacerbation. In addition to this, demographic information was collected to establish if age, sex, lung function, and/or body mass index is related to the primary study outcomes.

Methods

Adults with CF were included following hospitalisation for a pulmonary exacerbation and were followed up for 12 months. Inflammatory markers were measured immediately post discharge via sputum and plasma concentrations of interleukin-6, interleukin-8 and tumour necrosis factor-α. Physical activity was monitored for 7 days post discharge via a Sensewear armband. Exercise tolerance was measured at this same time point via six-minute walk test (6MWT), modified shuttle test-25 (MST-25) and isometric quadriceps strength. Statistical analyses included Shapiro-Wilk's test and Q-Q plots to determine normal distribution, T-tests, Pearson's correlational analyses and one-way MANOVAs.

Results

Thirty-two adults with CF (18 (56%) male, aged 28.8 ± 8.8 years, FEV_1 59.4 ± 23.0% predicted) were prospectively recruited via a sample of convenience. Physical activity negatively correlated with plasma inflammation ($r = -0.48$, $p < 0.01$), and positively with disease severity via FEV_1 ($r = 0.45$, $p < 0.05$) and body mass index ($r = 0.39$, $p < 0.05$). Body mass index also negatively correlated with sputum inflammation ($r = -0.51$; $p > 0.01$). No

associations were found between plasma cytokines and measures of exercise tolerance (six minute walk distance (6MWD), MST-25, quadriceps strength). 6MWD and MST-25 had low and moderate positive correlations respectively with disease severity in both FEV_1 ($r = 0.48$, $p = 0.005$; $r = 0.79$, $p < 0.001$) and FEV_1 % predicted ($r = 0.43$, $p < 0.05$; $r = 0.66$, $p < 0.001$). Male participants had significantly greater quadriceps strength than females (t (30) = 3.779, $p = 0.001$). Quadriceps strength did not correlate with either 6MWD ($r = 0.22$, $p > 0.1$) or MST-25 ($r = 0.35$, $p > 0.01$). There was no significant relationship between time to re-exacerbation and any inflammatory marker, or any measure of physical activity or exercise tolerance (all $p > 0.05$).

Conclusion

Increased physical activity levels following exacerbation in adults with CF is associated with lower levels of systemic inflammation, however, is unrelated to pulmonary inflammation. Both systemic and pulmonary inflammation are unrelated to measures of exercise tolerance (aerobic nor strength related). Time to next pulmonary exacerbation is not related to post-discharge inflammation, physical activity levels or exercise tolerance. MST-25 was found to be a stronger predictor of FEV_1 compared to 6MWD. No associations were found between sex and physical activity and/or aerobic exercise tolerance.

Key words

Cystic fibrosis, inflammation, physical activity, exercise tolerance.

STATEMENT OF ORIGINALITY

This work has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made in the thesis itself.

Signed_____ 18/7/19

Kate Burton

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LIST OF ABBREVIATIONS

Abbreviation	Description
6MWD	Six minute walk distance
6MWT	Six minute walk test
BMI	Body mass index
BW	Body weight
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane regulator
COPD	Chronic obstructive pulmonary disease
ELISA	Enzyme-linked immunosorbent assay
FEV_1	Forced expiratory volume in one second
IL-6	Interleukin-6
IL-8	Interleukin-8
IV	Intravenous
MANOVA	Multivariate analysis of variance
METs	Metabolic equivalents
MST-25	Modified shuttle test-25 Level
Q-Q	Quantile-quantile
TNF- α	Tumour necrosis factor alpha

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DECLARATION OF INTEREST

The author reports no conflict of interest. The author alone is responsible for the content of writing this document.

WORK ARISING FROM THESIS

Acknowledgement of Papers included in this Thesis (all papers included are co-authored)

Included in this thesis are papers in *Chapters 4 and 5* which are co-authored with other researchers. My contribution to each co-authored paper is outlined at the front of the relevant chapter. The bibliographic details/status for these papers including all authors, are:

Publications during candidature

Peer reviewed journal article

Burton K, Morris NR, Reid D, Smith D & Kuys S (2019). Increased physical activity post-exacerbation is associated with decreased systemic inflammation in cystic fibrosis – An observational study. Accepted for publication in *Physiotherapy Theory and Practice*, DOI: 10.1080/09593985.2019.1566942 (Copyright is owned by the publisher, Taylor and Francis). Incorporated in Chapter 4.

- SK and DR assisted with study design conception.
- SK assisted with HREC application.
- DR & DS provided guidance with physical sample processing.
- SK provided assistance with statistical analyses.
- SK, NRM DR & DS provided guidance for writing and reviewed the analyses and manuscript drafts.
- SK & NRM provided guidance and reviewed the response to reviewers.

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- SK and DR assisted with study design conception.
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Conference and invited speaker seminars related to work arising from this thesis

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CHAPTER 1 INTRODUCTION

Cystic fibrosis (CF) is the most common life-threatening genetic recessive condition affecting Caucasian Australians (1). One in 25 people are asymptomatic carriers for the defective gene, and one in almost 3000 Australians are affected by CF (2). CF is a progressive disease affecting a number of organ systems, in particular the lungs and gastrointestinal system. As the disease progresses people with CF require more intensive health care such as home based therapy and medications, more frequent and prolonged hospital admissions for respiratory exacerbations and, in around half of all cases, lung transplantation (3, 4); with the major cause of death being progressive lung disease (5).

A hallmark of CF is systemic inflammation in response to chronic infection in the airways (6). On a cellular level there is reduced function of a protein, the CF transmembrane conductance regulator (CFTR), which is involved in the transport of chloride ions (5). The consequence of this is mucus plugging, predisposition to chronic infection and neutrophil-dominated inflammation in the lungs (7). Additionally, pancreatic insufficiency is present in at least 80% of cases (5). It has been long established that individuals with CF have elevated circulating levels of inflammatory markers even under resting conditions (8), namely increased interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α (8-10), likely multi-faceted in origin (5, 10, 11).

At present, there is limited evidence regarding the relationship between physical activity, exercise tolerance and inflammation in adults with CF, hence the focus of this research program. Currently exercise tolerance in this patient cohort is thought to be reduced as a result of the underlying issues associated with the disease, namely malnutrition, increased

basal energy expenditure and hypoxaemia (12), and, of course, inflammation (13). In the wider literature "physical activity" refers to the amount of activity an individual actually *undertakes* in their daily life whereas "exercise tolerance" refers to the amount of exercise an individual is *capable of doing* (14).

In healthy adults differing levels of physical activity have been shown to be associated with changes in inflammatory state (15-18). Maximal exercise (for example running a marathon) has been found to produce a short-term, pro-inflammatory response in healthy adults (16, 19). However, sustained exercise training (for example endurance running training over a 9 month period) has been found to produce an anti-inflammatory effect in healthy adults (20, 21). Participation in regular gentle exercise and physical activity has been shown to reduce resting levels of inflammatory markers (17). Regular exercise at a mild to moderate intensity in healthy adults is also associated with reduced incidences of type II diabetes, asthma, chronic obstructive pulmonary disease (COPD), cancer, rheumatoid arthritis, and obesity (22). Similarly, in other chronic disease patient populations, physical activity has been shown to be linked to inflammatory state. This is true for individuals with COPD (23), type II diabetes (24), metabolic syndrome (25), and those with human immunodeficiency virus and/or acquired immunodeficiency syndrome (26).

Maintaining physical activity is fundamental to the management of CF (27), and participation in individually-tailored structured exercise, as a component of an inpatient physiotherapy program, is an expectation for adults with CF following a pulmonary exacerbation (28). The benefits of physical activity and exercise in adults with CF include improved sputum clearance (27), increased muscle hypertrophy (29), improved bone mineral density (30) and enhanced insulin sensitivity (29), accompanied by improvements in

lung function (12). In addition, the capacity to undertake physical activity has been identified as an independent predictor of morbidity and mortality in adults with CF (31), and it has been demonstrated that improvements in physical activity are associated with improvements in both lung function and exercise tolerance (32).

Regular exercise is a key component of high levels of habitual physical activity. It is known from the paediatric CF literature that exercise is associated with increases in systemic levels of TNF-α and IL-6 (13). As children with CF get older and move into adolescence, a lower exercise tolerance is associated with a higher mortality rate, a steeper decline in pulmonary function and a greater increase in systemic inflammatory markers (31), however little is known regarding the relationship between exercise tolerance and inflammation in adults with CF.

The relationship between raised inflammatory markers and decreased exercise tolerance is thought to be an important one. Elevated inflammatory markers can contribute to muscle wasting (10), and in turn may affect exercise tolerance. Moreover, the prescription of maximal exercise may have a detrimental effect on the person with CF by further increasing the levels of inflammatory markers (8).

The relationship between physical activity, exercise tolerance and inflammation in adults with CF remains unclear. The purpose of this research was to establish if a relationship exists between inflammatory markers, physical activity and/or exercise tolerance following a 10-14-day course of intravenous (IV) antibiotics in hospital for an acute respiratory exacerbation. The results of this research will provide clinicians with the information to define the potential benefits of physical activity on levels of inflammation in adults with CF. From this, an evidence-based approach can be applied, and exercise programs for CF

patients can be individualised so as to optimise the inflammatory response. As airway inflammation plays a pivotal role in the progression of CF pulmonary disease, both systemic inflammatory markers (i.e. those in the blood) and markers that reflect "lung inflammation" (i.e. markers in sputum) could be used to monitor disease progression and the pulmonary response to physical activity and exercise. This would be extremely valuable in routine clinical assessment of the appropriateness of an individual's exercise program (33).

1.1 Aims and significance

The primary aim of this research program was to investigate if a relationship exists between inflammatory markers (both systemic and pulmonary), physical activity levels and exercise tolerance in adults with CF following hospitalisation for an acute exacerbation. Understanding any potential relationship between physical activity, exercise tolerance and chronic inflammation and establishing proof of concept is essential if physiotherapists are to provide an evidence-based approach to physical activity prescription for adults with CF.

Secondary aims were as follows:

- Explore whether inflammation, physical activity and/or exercise tolerance can predict for time to next pulmonary exacerbation
- Determine if body mass index (BMI) is related to inflammation in adults with CF
- Investigate the relationships between age, sex and disease severity in relation to inflammation, physical activity and exercise tolerance.

It was hypothesised that:

- There will be an inverse relationship between pulmonary and systemic inflammatory markers and physical activity levels and/or exercise tolerance in adults with CF following completion of inpatient hospital treatment for an exacerbation; i.e. adults with CF with increased levels of inflammatory markers will have reduced levels of physical activity and/or exercise tolerance post-discharge.

- Individuals with increased systemic and/or pulmonary inflammatory markers and reduced physical activity and/or exercise tolerance will predict for time to next pulmonary exacerbation (independent of disease severity). Pulmonary exacerbation is defined as any or all of the following symptoms (34), resulting in the need to commence a new course of IV or inhaled antibiotics:
    - Increased cough
    - Increased sputum production
    - Shortness of breath
    - Chest pain
    - Loss of appetite
    - Loss of weight
    - Decline in pulmonary function tests

- Individuals with decreased physical activity levels and/or exercise tolerance will have a shorter time to next pulmonary exacerbation (defined as above), independent of lung disease severity

Individuals were recruited immediately post-inpatient IV antibiotic treatment for an exacerbation. Measures of exercise tolerance, physical activity levels and inflammation were obtained within the first week following hospitalisation for an acute infective exacerbation.

This research program comprised three (3) stages that were explored in one (1) study, from which two (2) separate but related reports were written.

Stage 1: Determine the relationship between inflammatory markers (IL-6, IL-8 and TNF-α in both blood plasma and sputum) and physical activity, as per data collected by the Sensewear® armband (Model MF; Bodymedia, Inc., Pittsburgh, PA, USA) in the first week following discharge from hospital in adults with CF following a pulmonary exacerbation. Evaluate if time to next pulmonary exacerbation (as defined above) is related to inflammatory markers and/or physical activity levels.

Stage 2: Determine the relationship between inflammatory markers (IL-6, IL-8 and TNF-α in both blood plasma and sputum) and exercise tolerance, as measured by 6 Minute Walk Test (6MWT), Modified Shuttle Test-25 (MST-25), and isometric quadriceps strength testing. Evaluate if time to next pulmonary exacerbation (as defined above) is related to inflammatory markers and/or exercise tolerance.

Stage 3: Investigate the interrelationship between inflammatory markers, physical activity levels, exercise tolerance, BMI, age, sex and severity of lung disease (FEV_1 % predicted).

1.2 Thesis structure

This thesis comprises six chapters.

Chapter 2 presents the background to this thesis; detailing the underlying pathophysiology of inflammation in CF and the impact on physical activity and exercise tolerance. The rationale for exercise in the setting of chronic inflammation across the lifespan is described in detail and potential differences based on sex, age and disease severity are outlined. This chapter also provides a background to the principles of measurement of physical activity and exercise tolerance, and gaps in the current literature are highlighted.

Chapter 3 presents the methods used in the study included in this research program. The two reports were generated from the one study, so detailed methods pertaining to both reports are presented together in this chapter. The study design, setting, participants, ethical approvals, procedures, outcome measures and statistical analyses used are outlined.

Chapter 4 includes a published manuscript entitled "Increased physical activity post-exacerbation is associated with decreased systemic inflammation in cystic fibrosis – An observational study". This report, together with the report outlined in chapter 5, forms the basis of the research program of this thesis.

This research program comprises a single-site observational cohort study which recruited 32 participants from one major CF centre in south east Queensland, Australia. Participants were recruited prior to hospital discharge following an inpatient admission for a pulmonary

exacerbation. Both systemic and pulmonary inflammatory markers were measured via blood plasma and sputum; physical activity was monitored for 7 days post-discharge via a tri-axial accelerometer and exercise tolerance was measured via 6MWT, MST-25 and isometric quadriceps strength testing. Participants were followed-up for 12 months to determine time to next pulmonary exacerbation.

In Chapter 4, the primary aim of the thesis is partially addressed. Systemic and pulmonary inflammatory markers were examined in relation to physical activity levels in the first 7 days following discharge from hospital. The secondary aim of time to next pulmonary exacerbation was explored in relation to inflammation and physical activity. In addition, the interrelationship between age, sex, BMI and disease severity are explored in relation to inflammation and physical activity.

Chapter 5 is presented as a manuscript prepared but not yet submitted for peer review. This report, titled "Increased exercise tolerance post-exacerbation is not associated with decreased systemic inflammation in cystic fibrosis – An observational study" together with Report 1 outlined in chapter 4, forms the research program of this thesis. In Chapter 5, data from this research program provided the opportunity to address the primary aim of the thesis to completion. Systemic and pulmonary inflammatory markers were examined in relation to exercise tolerance measures (6MWD, MST-25, isometric quadriceps strength) in adults with CF following an inpatient admission for a pulmonary exacerbation. The secondary aim of time to next pulmonary exacerbation was explored in relation to inflammation and exercise tolerance. In addition, the interrelationship between age, sex, BMI and disease severity are explored in relation to inflammation and exercise tolerance.

Chapter 6 provides a final discussion for this thesis. A summary of the findings of the reports included in this thesis are presented and the clinical implications of the combined results of the study are discussed. This chapter also presents limitations of the study and provides recommendations for future research.

CHAPTER 2 BACKGROUND

This chapter will present an overview of the aetiology of cystic fibrosis and the common presentations relating to disease severity, highlighting the effects of inflammation to the individual with CF and how this is measured within this research program. Physical activity and exercise tolerance (both aerobic and skeletal muscle strength-based) are explored in relation to CF, including the measurement of same. Finally, the influence of age, sex and disease severity on physical activity and exercise tolerance are presented.

2.1 Aetiology of CF

Cystic fibrosis is a hereditary recessive disease caused by a genetic mutation that results in an abnormality in the cystic fibrosis transmembrane conductance regulator, an anion channel located in the epithelial cell wall (5) (Figure 2.1). This abnormality affects nearly all the exocrine glands in the body, but primarily affects the respiratory and gastrointestinal systems (5). Individuals with CF can be either homozygous or heterozygous regarding their cystic fibrosis transmembrane conductance regulator mutations (5). This abnormality results in impaired sodium and chloride transport across the cell wall leading to retention of viscous secretions within the organ lumen (5) (Figure 2.1). This in turn leads to an ongoing cycle of infection and inflammation (5).

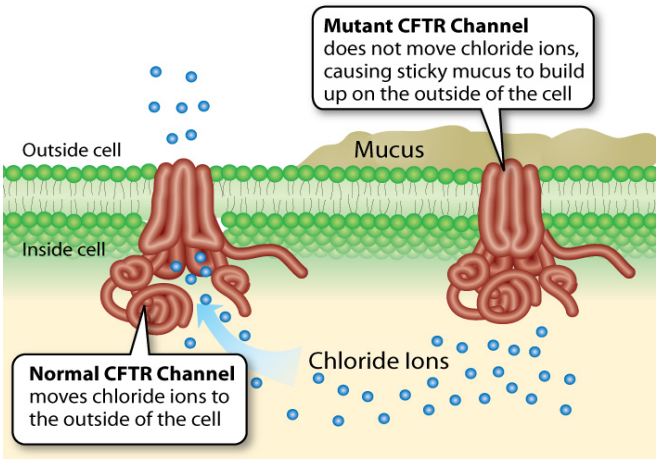

Figure 2.1 Cystic fibrosis transmembrane conductance regulator (35)

Due to improved treatment and life expectancy, approximately 50% of people in Australia with CF are adults (36). The median age of death for Australians with CF has steadily increased since 1998, and is now reported to be 27.3 years (Figure 2.2) (36). With mortality rates decreasing by 2% every year (37), the median survival of individuals with CF born after the year 2000 is predicted to exceed 50 years (37).

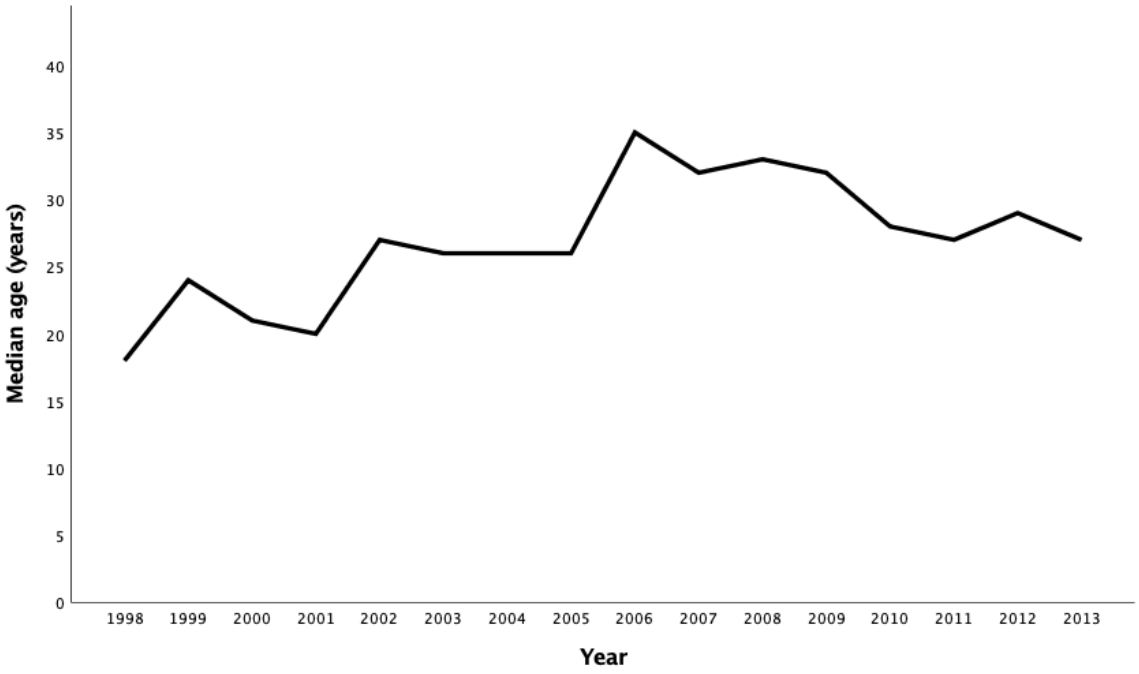

Figure 2.2 Median age of death of the Australian CF population (2013) (36)

2.2 Severity of CF

Disease severity in CF is quantified in terms of FEV_1% predicted (38), expressed as a percentage of predicted values for an individual's height, age and sex according to standardised equations (39). This rating is determined by environmental, genetic and/or stochastic factors (40, 41), and helps to predict survival (42, 43). Disease severity in people with CF is characterised as one of four categories: normal, mild, moderate and severe (44).

- Normal = FEV_1 90% of predicted and above
- Mild = FEV_1 70 - 90% of predicted
- Moderate = FEV_1 40 - 70% of predicted
- Severe = FEV_1 below 40% of predicted

As people with CF age their lung function declines (36), and the majority of adults (both male and female) with CF in Australia suffer from mild to moderate lung function impairment (36).

2.3 Inflammation and CF

All individuals with CF display exaggerated levels of inflammation, demonstrated systemically via pathology, and specific to the lungs via radiology (45). Individuals with CF experience inflammation in a number of different organs (for example, the bowel), however it is the inherent inflammation within the lungs that characterises CF (46).

As the person with CF ages the inflammatory response escalates resulting in the destruction of parenchymal tissue, a deterioration in pulmonary function, and, eventually, respiratory failure and death (47). Dysfunction occurs within the process of cystic fibrosis transmembrane conductance regulator cell-signalling (47), giving rise to a number of

abnormal inflammatory responses (namely increases in pro-inflammatory pathways and decreases in anti-inflammatory pathways), regardless of bacterial infection (47).

Distinguishing between innate abnormalities in inflammatory responses and inflammation as a result of infection represents an ongoing challenge for researchers and clinicians (47). Whilst this may be the case both systemic and pulmonary inflammation represent a substantial challenge for people with CF with increased levels of morbidity and mortality associated with elevated inflammatory markers. Preventing any further increases in inflammation and subsequent parenchymal destruction (48) is thought to be of significant benefit to the individual with CF (49).

Associated with the immune response within CF, monocytes and macrophages release cytokines (regulatory proteins such as interleukins or growth factors) (48). Cytokines are responsible for cell-signalling (50). Excess pro-inflammatory cytokines, namely IL-6, IL-8 and TNF-α are chronically produced by epithelial cells in CF, promoting clinical manifestations of this inflammatory disease (10, 50). This excess production of cytokines is seen more prevalently in the airways, even in the absence of bacteria (10).

2.3.1 Current anti-inflammatory therapies

Anti-inflammatory therapy is commonly used in the treatment of adults with CF, including oral and inhaled corticosteroid therapy, and immunomodulators such as high-dose ibuprofen and azithromycin (51). However, this therapy is not without its own significant treatment burden. Adverse effects of corticosteroids include glucose intolerance and

growth impairment (50). In fact inhibiting inflammation may even impair an individual with CF's innate immune function, giving rise to the potential for infection-related adverse events (51).

Azithromycin is thought to have a combined mechanism of action via modulation of bacterial pro-inflammatory effects at the airway epithelium, and alteration of the biofilm surrounding *Pseudomonas aeruginosa* (52). Around 70% of adults with CF in Australia are colonised with pseudomonas (53). A 2009 systematic review evaluating the efficacy and safety of azithromycin in both adults and children with CF demonstrated an overall significant increase in FEV_1 of 3.53% ($p = 0.05$) (54), however no measures of change in inflammatory markers were reported due to differing study designs and study periods. The risk of gastrointestinal side effects (nausea and diarrhoea) was found to be 72% higher with azithromycin use compared to normal management without the use of azithromycin ($p < 0.01$) (54). Of note this review included only four studies (2 adult, 2 paediatric) and excluded 37 studies, with a total number of 368 people with CF and a mean age of 18.5 years. In ex-vivo models examining CF epithelial cells, azithromycin has been shown to decrease TNF-α levels (55), however to date this has not been studied in the in-vitro model in CF, most likely because withholding this beneficial medication from an individual in need of anti-inflammatory therapy would be considered unethical. More recent studies have investigated the use of inhaled glutathione (an antioxidant in the airway epithelium) (56, 57) and N-acetylcystine (a glutathione precursor) (58) as pulmonary anti-inflammatory agents, however to date no significant anti-inflammatory effect has been established (51).

Finding an innovative non-pharmacological method of reducing inflammation and maintaining a reduction in inflammation in adults with CF without impairment of host

defence would be beneficial. Particularly in light of increasing life expectancies and increasing co-morbidities over the lifespan of this clinical population. Additionally, present data indicates that emerging therapies such as CFTR modulator therapies (i.e. Ivacaftor) do not appear to reduce airway inflammation (59), demonstrating the ongoing need for anti-inflammatory therapies in combination (51). Given the known beneficial anti-inflammatory effects of regular physical activity in the healthy population (20, 21), it is worth investigating the relationship between physical activity and inflammation in adults with CF.

2.3.2 Measurement of inflammation

Inflammation in CF is commonly measured on a systemic basis (in the blood plasma), however it can also be measured on a pulmonary basis (sputum or bronchoalveolar lavage samples) (33). Speculation over whether systemic markers are sensitive enough to detect a meaningful change in pulmonary disease continues, considering that the CF inflammatory response to infection is largely contained to the lungs (13). Systemic measurement of inflammation in CF is considered an adjunct to, rather than a substitute for, direct measurement of airways inflammation, as to date the relationship between the two demonstrates a relatively poor correlation (38). It would be reasonable to suggest that both should be measured.

Plasma biomarkers

Pro-inflammatory markers can be measured on a systemic basis (i.e. in blood plasma), a testing mechanism that is more commonly available in laboratories worldwide (33).

Inflammation in blood plasma is measured by examining the expression or activity of pro-inflammatory mediators (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-9, IL-19, and TNF-α) by way of enzyme-linked immunosorbent assay (ELISA) analysis kits (47). It is recommended that studies examining systemic inflammation in CF assay for IL-6 and TNF-α (9).

Sputum biomarkers

Current understanding of inflammatory profiles within the CF lung has come primarily from bronchoalveolar lavage samples (60). From these samples, cytokine levels, namely interleukins and tumour necrosis factor, can be examined through the process of cell culture (47). Cell culture in this instance involves the dispersal of cells from sputum samples in an artificial environment to enable the measurement of specific inflammatory markers (61). It has been recommended that IL-6 and IL-8 be assayed when measuring pro-inflammatory cytokines in human CF sputum samples (47). Further details regarding the cytokines used in this program of research (IL-6, IL-8 and TNF-α) will be discussed next.

Cytokine measurement

Inflammation is measured by examining the expression or activity of pro-inflammatory mediators (IL-1β, IL-6, IL-8, and TNF-α) using ELISA analysis kits (47). In this research program IL-6, IL-8, and TNF-α will be measured to examine both systemic and airway inflammation in CF. Other pro-inflammatory cytokines such as IL-1β have also been found in high concentrations in bronchoalveolar lavage samples taken from people with CF (62).

The role of each inflammatory cytokine explored within this research program is as follows:

- IL-6 is a pro-inflammatory cytokine (63), however it is also considered an anti-inflammatory myokine – a cytokine produced by muscle and elevated as a result of

muscle contraction (63, 64).

- IL-8 is a chemokine (65) – a specific type of cytokine that chemically attracts cells (i.e. neutrophils) to sites of inflammation (66) which has been found to be present in high concentrations in the airways of individuals with CF with a significant inflammatory burden (50).
- TNF-α is a pro-inflammatory cytokine (67) and, together with IL-1β, is one of the first cytokines present in high numbers in response to sepsis (68). TNF-α and IL-1β stimulate the production of IL-6 (68).

The role and action of these cytokines in response to exercise is expanded upon in Chapter 2.5.

2.4 Physical activity, exercise and CF

The relationship between inflammation, physical activity and exercise in adults with CF is the topic of this thesis, however before exploring this it is important to have an understanding of the relationship between physical activity, exercise and CF. Physical activity encompasses any daily habitual activity that results in energy expenditure (69, 70). Exercise is a subset of physical activity, one that is structured, planned and repetitive in its goal of physical fitness (70). Often used interchangeably, it is important to distinguish that physical activity is not synonymous with exercise.

Exercise tolerance, also referred to as exercise capacity, is defined as the maximum amount of physical exertion an individual can sustain under testing conditions (71). Exercise tolerance is measured as an indicator of exercise capacity and is important to measure in

adults with CF as has been shown to predict mortality (72). Measures of exercise tolerance should reflect both aerobic and strength-based capacity. These concepts in relation to adults with CF will be discussed.

2.4.1 Physical activity and CF

The benefits of physical activity and exercise in adults with CF include improved sputum clearance (27), increased muscle hypertrophy (29), improved bone mineral density (30) and enhanced insulin sensitivity (29), accompanied by improvements in lung function (12, 73). The effects of exercise amongst adults with CF have been established to improve morbidity and quality of life (74), and as such exercise is considered to be a cornerstone of physiotherapy treatment and maintenance for individuals with CF (27).

Physical activity levels in adults with CF appear to be comparable with that of their non-CF peers (75). A Belgian study, conducted amongst adults with CF (n = 64) aged 25 $\pm$ 5 years with mild to moderate lung disease (FEV_1 72 $\pm$ 18% predicted) indicated that the number of steps (approximately 9,500 on average) and time spent performing mild intensity physical activity is comparable between adults with CF and healthy adults (76). However higher intensity levels of physical activity, for example metabolic equivalents (METs) ≥ 3.0, appeared on average 44% lower in adults with CF (76).

A recent Australian study investigating physical activity levels demonstrated that adults with CF engaged in a median of approximately 30 minutes of moderate to vigorous physical activity each day, across all disease severity levels and this was related to aerobic exercise

tolerance (77). Across the CF lifespan, for individuals with normal, mild, moderate and severe lung disease physical activity levels appear directly related to exercise tolerance (78) and skeletal muscle strength (76).

2.4.2 Exercise tolerance and CF

Exercise tolerance refers to the maximum amount of physical exertion a subject can sustain under replicable testing conditions (71). Exercise tolerance in individuals with CF is complex given varying rates of disease progression (79). Factors such as lung function (reduced FEV_1) (31), nutritional status (31) and the underlying inflammatory state (79) have been shown to influence exercise tolerance (79). Exercise tolerance is recognised as an independent predictor of mortality amongst individuals with CF (72).

Many intrinsic factors are thought to contribute to exercise tolerance in adults with CF, including basal metabolic rate, however to date there appears to be no inherent differences in metabolism in response to exercise in individuals with CF (80-82). Additionally, it appears that in both children and adults with CF, reduced muscle mass (as opposed to metabolic dysfunction) appears to be the underlying cause of differences found in exercise tolerance (83-85). Werkman et al. (81) demonstrated that adolescents (aged 12 - 18 years) with mild CF who were clinically stable had no intrinsic metabolic constraints or abnormalities in oxygenation in response to exercise, when compared with healthy age-matched control subjects. Similar findings came out of a study examining 15 adults with stable CF who performed a maximal cycling test (80). Again, compared to healthy age-matched controls, no metabolic constraints nor abnormalities in muscle oxidative metabolism in response to

exercise were found (80). However, adults with an FEV_1 < 40% predicted were excluded, so it is unknown if intrinsic metabolic constraints or abnormalities in muscle oxidative metabolism exist in adults with severe CF-related lung disease. Therefore, in theory no intrinsic limitations to exercise exist for adults with mild to moderate CF based on metabolism alone, however the same remains unknown in adults with severe CF.

Both aerobic exercise and resistance training are recommended for adults with CF in order to maintain health and wellbeing (28). Therefore, when discussing exercise tolerance in individuals with CF it is important to distinguish between aerobic exercise tolerance and strength-based exercise tolerance (i.e. skeletal muscle strength). These are exercise-training specific measures; however, both are valuable indicators of health and fitness amongst adults with respiratory disease (86).

2.4.2.1 Aerobic exercise tolerance

Aerobic exercise, also known as cardiovascular exercise, targets the heart and lungs as it requires the use of free oxygen to meet the body's energy demands during exercise (87). Common examples of aerobic exercise include running, jogging, walking, swimming and cycling (87). Higher levels of aerobic fitness (VO_{2peak}) have been shown to be associated with a significantly lower risk of dying (independent of FEV_1) in adults with CF (72).

This was echoed more recently in a French study in 2014 in which 102 adults with CF aged from 17 to 67 years, separated into two groups based upon disease severity, underwent cardiopulmonary exercise testing using an incremental cycle ergometer protocol (79). In those adults with severe lung disease bronchial obstruction played a dominant role in an individual's ability to work to maximal exercise tolerance. Whereas in those with mild to

moderate lung disease, the major limitation to exercise tolerance was excessive hyperventilation (79). Abnormalities in both gas exchange and ventilatory response were also seen in both groups, demonstrating the complexity of mechanisms involved in exercise tolerance in adults with CF (79).

2.4.2.2. Skeletal muscle strength

Adults with CF have been found to have significantly weaker skeletal muscle strength than their age-matched controls (88); typically between 25 to 35% reduction in quadriceps muscle strength (76, 88, 89). This is an understandable consequence of their reduced lean muscle mass (88), however several other factors are thought to also contribute to decreased muscle strength in CF. This includes most predominantly disuse, but also includes factors such as the effects of inflammation, hypoxaemia and oxidative stress, diminished levels of anabolic hormones, the prolonged effect of corticosteroids, and malnutrition (90). All these factors have the potential to affect even the most treatment-diligent individual, due to the nature of recurrent infections and exacerbations that is CF (91, 92).

Skeletal muscle strength is known to be related to physical activity levels and aerobic exercise tolerance in adults with CF (76, 93). Quadriceps strength has been found to have a strong positive relationship with time spent in mild to moderate physical activity intensity ($r = 0.61$, $p = 0.007$) in an adult CF population ($n = 19$, aged 25 ± 6 years) with mild to moderate lung disease (FEV_1 $69 \pm 25\%$ predicted) (93). Interestingly, quadriceps strength was not found to be directly related to daily step count in this same study (93). In a study of 64 adults with CF (age 26 ± 8 years) Troosters et al. similarly found that the level of physical activity had a modest relationship with quadriceps muscle strength ($r = 0.48$). Specifically, time spent in moderate ($p = 0.03$) and vigorous ($p = 0.02$) intensity physical activity was

correlated with quadriceps muscle strength (76).

Additionally, Troosters et al. found that increased quadriceps strength was positively correlated with exercise tolerance (r = 0.56); in this case, the distance walked in a 6MWT (76). Participants in this study performed a maximal incremental exercise test (cycling at 20 watts/min^{-1} to the point of exhaustion) and completed two 6MWT, while wearing a Sensewear armband (76). As none of the above studies included participants older than 35 years in age, nor included individuals with severe CF-related lung disease, little is currently known regarding the relationship between quadriceps strength and physical activity in the older adult with CF, or those with severe CF-related lung disease.

From the available evidence it would appear that measures of strength-based fitness have the same relationship with physical activity as that of aerobic fitness measures. This message, however, needs to be interpreted with caution as there is currently only one study pertaining to this topic and further investigation is needed to clarify the relationship between strength-based exercise tolerance and physical activity in adults with CF. Further differences in physical activity levels and exercise tolerance in CF related to age, sex and disease severity are explored below.

2.4.3 Physical activity, exercise and ageing in CF

Limited evidence presently exists in regard to the relationship between physical activity, exercise tolerance and age in individuals with CF. Median life expectancy for individuals with CF in Australia is currently 35.6 years (44), with 50% of the Australian CF population aged 18 years or older (2), and 20% of the Australian CF population aged 30 years or older (2). In a

longitudinal study examining 212 children and adolescents with CF (aged 7-17 at baseline) who were followed for up to nine years it was demonstrated that habitual physical activity (as measured by a subjective self-reported scale) increased at a mean rate of 0.28 ± 0.03 hours.day^{-1} per year, which translated to an increase of ~17 minutes per day (73). In a second longitudinal study following 149 children and adolescents with CF (aged 13.29 ± 1.24 years at baseline) it was found that exercise tolerance (VO_{2max}) is equivocal to that of healthy, age-matched peers at age 12, but this then declines by 20% during adolescence (31).

In adults, a French study examined the relationship between age and exercise tolerance in 102 adults with CF (aged 17-67) with moderate to severe lung disease. Participants underwent cardiopulmonary exercise testing as per a standardised incremental cycling protocol and age was not found to be related to aerobic exercise tolerance ($r = -0.11$, $p = 0.29$) (79).

Additionally, one Australian study has explored age as a potential factor relating to physical activity and exercise tolerance amongst adults both in the inpatient environment and in the first month after discharge from hospital following an acute exacerbation of CF (94). Twenty-four adults (63% male) aged from 18-48 with an FEV_1 of 34-97% predicted, wore a Sensewear armband for one week during admission, and again for one month following discharge home after 10-14 days of inpatient IV antibiotic treatment (94). Participants also completed an MST-15 during their inpatient stay (94). Age was shown to be unrelated to both physical activity level (METs ≥ 3) and exercise tolerance (94).

Limited conclusions can be drawn from these four different studies due to the small number of participants, varying participant samples and varying measures of physical activity and exercise tolerance. How age, physical activity and exercise tolerance relates to inflammation in adults with CF remains unknown at present. Further investigation into the relationship between age, physical activity, exercise tolerance and inflammation in adults with CF is warranted to better understand this potential relationship.

2.4.4 Physical activity, exercise tolerance and sex differences in CF

Some differences have been observed for physical activity levels and exercise tolerance between males and females with CF at particular time points across the lifespan. In prepubescent individuals with CF, no difference exists between males and females for physical activity levels (95). A disparity appears to exist between males and females with CF following the onset of puberty whereby males demonstrate greater physical activity levels on both accelerometer counts and activity diary reporting ($p < 0.05$) than their female counterparts (94, 95).

Emerging evidence appears to suggest that this difference in physical activity levels between the sexes in individuals with CF following the onset of puberty continues into adulthood (96). Physical activity levels (as measured by Sensewear armband) have been shown to be lower in adult women than men with CF (1.6 vs 1.8 METs) (96). This has also been seen in an Australian adult CF population in the immediate post-exacerbation period where males were found to have greater time spent performing physical activity at a level of METs ≥ 3 than their female counterparts, which approached statistical significance ($p = 0.055$) (94). This latter study demonstrated no relationship between sex and aerobic

exercise tolerance (94). Sex differences were also found for adults with CF in regard to meeting physical activity guidelines. Approximately one-third of females (38%) met physical activity guidelines, completing more than 30 minutes a day of moderate-vigorous physical activity compared to more than two-thirds (69%) of males (77). Based on this evidence in the adult CF population the potential difference between sexes should be further explored as an influencing variable in physical activity levels and exercise tolerance in adults with CF.

From adolescence through to adulthood females have been shown to have lower exercise tolerance than males, irrespective of lung function (97). A large American study examined 110 participants with CF (aged 7 to 35 years), stratified into three groups based upon mild, moderate or severe lung disease, who completed a structured incremental cycle ergometer task to exhaustion (97). Males demonstrated a significantly greater mean VO_{2peak} than females (males 36.9 $\pm$ 11.4 ml/kg/min; females 31.0 $\pm$ 7.4 ml/kg/min, $p < 0.001$) (97). Similarly, increased variance in VO_{2peak} can also be explained by female sex (partial $R^2 = 0.05$, $p = 0.005$) (76).

This difference between the sexes is thought to be an interplay of a number of factors, namely genetic (i.e. morphology) and hormonal profiles (95, 98). One factor which may influence exercise tolerance is quadriceps muscle strength. However, no difference has been found in relation to sex when comparing quadriceps strength in adults with CF. In a group of 64 adults with CF (mean age of 26 $\pm$ 8 years) with predominantly mild to moderate CF related disease (mean FEV_1 65 $\pm$ 19% predicted), no difference was found between males and females in regards to quadriceps strength (76).

2.4.5 Physical activity, exercise and disease severity in CF

Physical activity has been shown to be related to disease severity in adults with CF (76, 77, 99), and has demonstrated a moderate to strong positive relationship with exercise tolerance (79, 100-102). Troosters et al. demonstrated that the number of steps taken each day measured via Sensewear armband had a significant positive correlation with FEV_1 ($r = 0.39$, $p = 0.08$) (76). Similarly, time spent in moderate to vigorous physical activity correlated positively with FEV_1 in litres at study recruitment ($r = 0.4$, $p = 0.004$) and at 12-month outpatient follow-up ($r = 0.4$, $p = 0.006$) (77). Interestingly, no relationship was found between disease severity categories and physical activity levels ($X^2 = 1.89$, $p = 0.6$) (77). However, participants in this latter study were recruited from an outpatient population, were stable, and were excluded if requiring IV antibiotics in the preceding four weeks. Similarly in an Australian study of 64 adults with CF (mean age 28 ± 7 years, mean FEV_1 68 ± 20% predicted), accumulating 30 minutes a day of moderate to vigorous physical activity in bouts of at least 10 minutes was shown to be an independent predictor of a decreased decline in FEV_1 over a 3 year follow-up period ($\beta = 0.081$, SE of $\beta = 0.033$, $p = 0.018$) (99). A remaining gap in the evidence concerns physical activity levels in the immediate discharge period following exacerbation for adults with CF of varying disease severity.

Current evidence regarding the relationship between aerobic exercise tolerance and disease severity and between skeletal muscle strength and disease severity has demonstrated differing results. Variance in aerobic exercise tolerance (VO_{2peak}) can be explained by disease severity in adults with CF (partial $R^2 = 0.75$, $p < 0.001$) (76), and a structured, supervised endurance training program has been shown to positively influence FEV_1 in adolescents and adults with CF (103). Skeletal muscle strength, specifically quadriceps strength, has been

shown to be unrelated to disease severity in adults with CF (76), however hand grip strength has been shown to have a weak but statistically significant relationship with FEV_1 ($r = 0.25$, $p = 0.05$) (76). A supervised, structured strength training program however has been shown to positively influence FEV_1 in adults with CF (103).

2.4.6 Measurement of physical activity

Physical activity can be measured in a number of different ways, including both subjective and objective methods. Common subjective methods include self-report questionnaires such as the Habitual Activity Estimation Scale (104, 105), the Physical Activity self-administered Questionnaire (106), and the International Physical Activity Questionnaire (107), amongst others. Subjective measures are often favoured during clinical research due to their ease of application (time efficient, minimal cost, nil equipment required) (108). However self-report questionnaires are a measure of an individual's *perception* of their physical activity, not an objective measure of physical activity itself nor a measure of the ability of an individual to be physically active (109). Although subjective measures such as the Habitual Activity Estimation Scale and the 7-Day Physical Activity Recall questionnaire are validated for use in CF (110), questionnaires that ask individuals to subjectively report physical activity levels are limited in that participants may over-estimate their activity levels in comparison to physical activity that is objectively measured (109, 110), due to poor memory and biased self-reporting (109, 111).

Physical activity can be objectively measured using a motion sensor such as an accelerometer or a pedometer. Pedometers are easy to use and inexpensive, but lack the

sensitivity of activity monitors that incorporate accelerometry (111). In systematic reviews examining the measurement of physical activity in both healthy populations and amongst adults with CF the use of activity monitors, namely the Sensewear armband, is recommended as a valid and informed choice of measurement (109, 112).

In this research program physical activity was measured objectively using the Bodymedia Sensewear armband (Model MF; Pennsylvania, USA) (Figure 2.3). The device includes a tri-axial accelerometer, a galvanic skin response sensor, a skin temperature sensor, a heat-flux sensor and a near-body ambient temperature sensor. Together with participant characteristics (namely sex, height, weight, hand dominance and BMI) energy expenditure is estimated according to the manufacturer's software equations. As a result the Sensewear armband not only provides an estimate of activity via step count it also provides energy expenditure estimations (113) expressed as mean energy expenditure (kcal/min) or metabolic equivalents (METs). The armband (specifically Model MF) is worn on the rear left arm, over the triceps (mid-humerus) (Figure 2.4).

Figure 2.3 Bodymedia Sensewear armband (Model MF) (114)

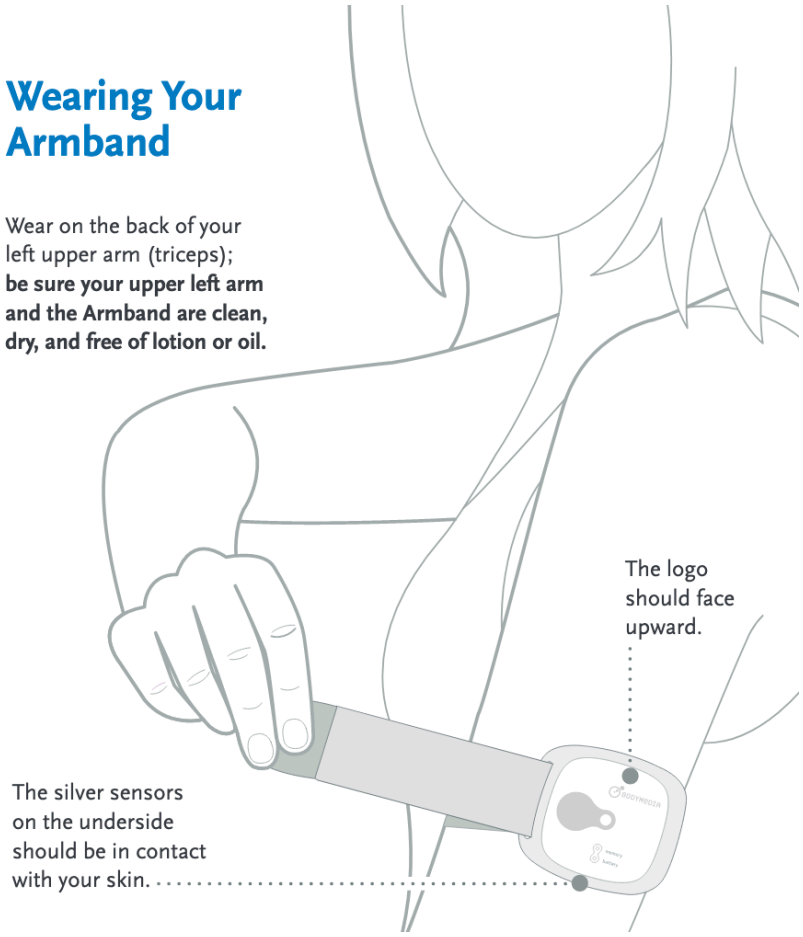

Figure 2.4 Wear position of the Bodymedia Sensewear armband (Model MF) (114)

One MET is equal to the amount of energy the body expends sitting quietly for one hour. Levels of physical activity can be classified in terms of metabolic equivalent as per Table 2.1 (115).

Table 2.2 illustrates this further by listing approximations of METs for specific physical activities (116). A threshold of what is considered mild physical activity intensity ($\geq$ 3 METs) equates to at least normal walking pace, but also encompasses activities such as jogging, cycling, swimming, racquet sports and running (116). This cut-point of METs $\geq$ 3.0 is in accordance with other studies within the adult CF population (96, 117).

Table 2.1 Classification of physical activity in terms of exercise intensity (115)

Level of physical activity	METs
SEDENTARY	<1.6
LOW intensity	1.6 – 3.0
MODERATE intensity	3.0 – 6.0
VIGOROUS intensity	6.0 – 9.0
HIGH intensity	>9.0

Definition of abbreviations: METs = metabolic equivalents

Table 2.2 Common activities and their metabolic equivalents (116)

Physical activity	METs
Normal pace walking (3 – 4.5 km/hr)	3
Brisk pace walking (4.5 – 6km/hr)	4
Fast walking (6+ km/hr)	4.5
Jogging (slower than 10 km/hr)	7
Running (faster than 10 km/hr)	12
Cycling	7
Tennis/squash	7
Swimming	7
Stepper/elliptical	6
Yoga/pilates/stretching	4

Definition of abbreviations: km/hr = kilometre per hour; METs = metabolic equivalents

In the healthy adult population the Sensewear armband has been validated and has demonstrated accurate estimates of energy expenditure both at rest and during physical

activity of low to moderate intensity when compared to the gold standard doubly labelled water method, or indirect calorimetry (118). As a caution, for physical activity of low intensity the Sensewear armband may overestimate energy expenditure (13 - 27%) (119, 120), and for vigorous and high intensity it may underestimate energy expenditure (22%) (119, 120). Accelerometers in general demonstrate a strong positive relationship ($r > 0.80$) with step count for adults with chronic disease (112). Compliance for wearing the Sensewear armband for daily physical activity measurement has been found to be good (>90%) in both the healthy population and amongst adults with COPD (121).

The Sensewear armband has proven to be a valid measure of physical activity in adults with CF (122, 123). To validate this piece of equipment Cox et al. subjected 26 adults with CF to a series of seven physical activity tasks with simultaneous assessment of energy expenditure using both the Sensewear armband and indirect calorimetry (122). The tasks included both static tasks (supine lying, unsupported sitting and upright standing) and active tasks (stationary cycling, walking, a functional upper-limb test and stair-climbing) (122). Good agreement was found between the two measures ($p = 0.03$), with a mean difference of -0.02 METs (95% CI -1.1 to 1.1) (122).

Step count, as measured by the Sensewear armband, has been found to have an error rate of -4% to 3% at both slow (0.89 ± 0.5 m.s^{-1}) and fast (1.97 ± 0.2 m.s^{-1}) gait speeds as compared to manual step counting in the adult CF population (76). This is comparable to that of other commercially available accelerometer devices (namely Fitbit and Garmin Forerunner) (124, 125). It is worth noting that this error rate analysis was conducted on a pilot basis with only eight participants (76).

2.4.7 Measurement of exercise tolerance

Measurement of exercise tolerance will be discussed in terms of measurements of aerobic exercise tolerance and measurements of strength-based exercise tolerance, both measures of interest in this research program.

2.4.7.1 Measurement of aerobic exercise tolerance

Clinical measures of aerobic exercise tolerance recommended for use in CF include the 6MWT, the MST and full cardiopulmonary exercise tolerance testing protocols (70). These measures are explained in detail below.

Six minute walk test

The 6MWT is a standardised self-paced test that is conducted worldwide according to the revised American Thoracic Society guidelines (126), and provides an output of six minute walk distance (6MWD). The test involves having the participant walk laps of a flat 30m course for 6 minutes with instructions to walk as far as possible with scripted encouragement. Heart rate, oxygen saturations and perceived breathlessness (modified Borg scale) are recorded every minute, and a total distance walked is recorded in metres.

The 6MWT is a valid and reliable measure of exercise capacity amongst individuals with chronic lung disease, including adults with CF (126). The 6MWT has been shown to be repeatable in adults with CF (aged 15 - 49) across the spectrum of disease severity (FEV_1 15 - 119% predicted), with a co-efficient of variation of 4.3% (127, 128). Martin et. al. (2013) found that in a population (n=286) of adults with CF a 6MWD of <475m was an independent predictor of transplant or death within 10 years, regardless of disease severity (hazard ratio 2.09 (95% CI 1.13 - 3.90; p = 0.02)) (129).

Modified shuttle test

The MST-25 is conducted according to the standardised guidelines stipulated in the recording used for the test (130). The 25-level MST Is an adaptation of the original modified shuttle test (MST). The MST is a 15 level shuttle test (as outlined below) which was in turn adapted from the incremental shuttle walk test specifically for people with chronic respiratory disease (131). Prior to this, use of the incremental shuttle test was deemed insufficient in eliciting a maximal exercise tolerance response in individuals with chronic respiratory disease (including adults with CF) (132).

The MST and MST-25 require individuals to walk/run at increasing speeds back and forth on a 10m course (131). At the end of each level standardised encouragement is given, and a prompt on the recording indicates that a new level has commenced. Test subjects continue with the test until they either (a) fail to maintain the set pace, or (b) opt to cease testing due to reasons of shortness of breath or fatigue. The MST-25 comprises 25 levels; incorporating the entire 15 levels of the MST, with the addition of extra levels for younger adults (e.g. those with CF) allowing participants to run during the higher shuttle levels. The MST (and the first 15 levels of the MST-25) have a strong relationship with FEV_1 % predicted for adults with CF with moderate to severe lung disease (133). A threshold effect has been noted for those adults with CF mild to moderate lung disease ($FEV_1 \geq 67\%$ predicted) (133). The MST-25 was created specifically to address this problem (134).

The MST has been found to be a valid measure of exercise tolerance within the adult CF population compared to formal metabolic testing. Distance covered during testing strongly correlates with VO_{2peak} ($r = 0.95$, $P < 0.00$) across the spectrum of disease severity (FEV_1 17 – 96% predicted) (132). The MST-25 has also been demonstrated as a reliable measure of

exercise tolerance (test-retest reliability r = 0.99 for distance covered) (134). As per recommendations for use in CF populations, both the 6MWT and the MST-25 were measured in this research program (70).

Cardiopulmonary exercise testing

Cardiopulmonary exercise tolerance protocols measure peak aerobic capacity (VO_{2peak}) using a metabolic cart during a maximal incremental work test (either treadmill or cycle ergometry) (135, 136), and are considered to be the gold standard for measuring aerobic fitness (110). In the healthy population, VO_{2peak} is known to decline by an average of 10% each decade over 30 years of age due to decreasing maximum heart rate and decreased stroke volume associated with age (137). However, this method of testing is time consuming (110) and requires specialised laboratory equipment that is expensive and not widely available to clinicians working in CF centres (130), and as such was not used in this research program.

2.4.7.2 Measurement of strength-based exercise tolerance

The primary measure of non-aerobic exercise tolerance throughout the literature concerning adults with CF is the quadriceps strength test (using handheld dynamometry). Measurement of quadriceps strength as a standardised testing procedure is explained in detail below.

Quadriceps strength was included in this research program to more specifically assess individuals who utilise strength training as a means of routine exercise, so as not to show bias towards participants whose primary form of exercise is aerobic. Quadriceps strength measured isometrically by means of maximum voluntary contraction has been found to be

both a valid and reliable measure within the adult CF population (93, 138). The gold standard measurement of maximum voluntary contraction is via either isometric or isokinetic measurement using a dynamometer (139). The use of a hand held dynamometer has been shown to be a valid and reliable estimate of quadriceps muscle strength (140).

2.5 Inflammation, physical activity and exercise

This subchapter will outline the relationship between inflammation, physical activity and exercise in healthy adults, and the specific role of inflammatory markers IL-6, IL-8 and TNF-α in response to exercise (as investigated in this research program). The relationships between inflammation, physical activity and exercise in chronic disease, children with CF and adults with CF will also be explored, including both aerobic and resistance exercise.

In healthy adults without CF an acute session of exercise is known to produce oxidative stress and a short term pro-inflammatory response (19), most significantly an increase in systemic IL-6 (141). Whereas regular exercise training (i.e. long-distance running training over a 9 month period) appears to be associated with a sustained anti-inflammatory effect, namely a reduction in C-reactive protein levels (20, 21).

The role of IL-6 during exercise is to increase lipolysis of adipose tissue and increase glucose production via the liver (both pro-inflammatory actions) (142), and to enhance insulin sensitivity (also pro-inflammatory) (143). In addition to this IL-6 appears to have a regulatory effect on TNF-α during exercise (anti-inflammatory) (144) and also stimulates the production of IL-10 (anti-inflammatory) (145). The mass result is that following exercise there is a marked increase in systemic IL-6 (146), the main source of this being the

contracting skeletal muscle during exercise (142).

Systemic concentrations of IL-8 are elevated following strenuous exercise that involves eccentric muscle activity (i.e. running) (147), however this increase is not seen following concentric exercise of moderate intensity (i.e. cycle ergometry) (148). The function of IL-8 during exercise is unknown, but it is thought that this chemokine stimulates angiogenesis (142).

Routine exercise, be it an acute bout or steady-state, does not appear to alter systemic TNF-α (142), however prolonged, strenuous exercise (i.e. marathon running) results in a small increase in plasma TNF-α in healthy individuals (149).

2.5.1 Inflammation, physical activity and exercise in chronic inflammatory disease

In adults with mild chronic inflammatory disease similar to that of CF such as multiple sclerosis, chronic heart failure, etc., it has been demonstrated that regular exercise can have an anti-inflammatory effect (8). A 2009 systematic review examining the effects of exercise on inflammatory markers in both adult and paediatric populations with chronic inflammatory disease analysed 19 relevant studies. Twelve of the included studies explored exercise and inflammation in adult cohorts (8), with only one which examined this amongst adults with CF (9). This study of 12 adults with CF with moderate lung disease (aged 23-32, FEV_1 44-66% predicted) found that structured, constant rate exercise (box stepping) led to a greater increase in blood plasma IL-6 and TNF- α compared with healthy adults (9). IL-6 and

TNF-α were both raised pre-exercise (compared with healthy adults), and IL-6 also remained significantly elevated in the adults with CF even after a 120-minute recovery period (9).

In adults with COPD contrasting findings regarding the effect on IL-6 and TNF-α following exercise have been observed. Three studies within the Ploeger et al. systematic review (8) included adults with COPD. An increase in blood plasma TNF-α was demonstrated following both short burst and endurance cycling compared with healthy controls, however no difference in IL-6 was observed between the two groups (150). Whereas in a second study submaximal graded cycling demonstrated an increase in blood plasma leukocytes and IL-6 in adults with COPD compared to healthy controls (151). In contrast, maximal exercise elicited an increase in both leukocytes and IL-6 in both groups, with no difference demonstrated between the two groups (151). Participants with COPD were stratified into groups based on fat-free muscle mass being either normal or decreased (151). Post hoc analysis revealed increased systemic IL-6 following exercise in the COPD group with muscle wasting ($p = 0.05$) when compared with both healthy subjects and individuals with COPD without muscle wasting (151). A third study showed that lymphocyte levels in blood plasma following exercise were greater in healthy controls than individuals with COPD, but the opposite was true of circulating monocytes (152). Unfortunately, these studies have used different systemic inflammatory markers, making it difficult to draw assumptions regarding the inflammatory response to exercise in adults with chronic respiratory disease. Further investigation is needed into the inflammatory response to exercise and physical activity for adults with chronic respiratory disease to be able to draw adequate parallels for adults with CF.

The relationship between inflammation and exercise has also been explored in other

chronic inflammatory diseases. In adults with multiple sclerosis a significant decrease in blood plasma TNF-α was seen two and three hours after a 30 minute bout of cycling at 60% of peak oxygen uptake (VO_{2peak}) (153, 154). In adults with chronic heart failure, significant decreases in blood plasma TNF-α and IL-6 were also seen following a 12-week physical training program consisting of cycling 30 minutes a day at 50 revolutions per minute (155). While in theory the results from these other chronic inflammatory disease groups can be extrapolated to adults with CF, due to the variable nature of different inflammatory profiles within differing disease processes caution is required when interpreting these results and attempting to generalise them to all individuals with chronic disease (8).

The optimal level of physical activity and the impact on the underlying inflammatory process associated with CF remains unknown. More information is needed to better define the relationship between physical activity, exercise and inflammation in individuals with chronic inflammatory disease to be able to understand how physical activity can benefit health without exacerbating the inflammation that is inherent to the underlying disease pathology.

Whilst the Ploeger at al. systematic review is now 10 years old, no additional randomised controlled trials were found examining the relationship between physical activity, exercise and the inflammatory response in adults with chronic inflammatory disease. As such the findings of this systematic review remain pertinent as there are gaps in the current understanding of the relationship between physical activity, exercise tolerance and inflammation amongst adults with CF. Due to the limited findings regarding physical activity, exercise and inflammation in adults with CF, the evidence within the paediatric CF population is presented below.

2.5.2 Inflammation, physical activity and exercise in paediatric CF

Based on the perceived effects of exercise upon inflammation amongst healthy individuals, current recommendations for people with CF support physical activity as an effective anti-inflammatory therapy (8). However, to date the evidence regarding the relationship between inflammation, physical activity and exercise amongst individuals with CF appears to lack consistency. Minimal evidence for the relationship between inflammation, physical activity and exercise in the paediatric CF population currently exists. Of the two relevant studies that were found, differing markers of inflammation were used, making comparison and generalisability therefore difficult. Both these studies are presented below.

The first paediatric study demonstrated that children with CF with normal to mild lung disease (FEV_1 62- 113% predicted) have increased levels of systemic TNF-α and IL-6 following a cycling protocol of a series of 10 x 2 minute bouts (1 minute rest between each bout) at 50% VO_{2peak} (n = 14, mean age 10.5 years) (13). In the second study of a large number (n= 149) of adolescents with CF at a children's hospital in the Netherlands, individuals with decreased exercise tolerance were found to have increased serum immunoglobulin-G levels, however this relationship did not achieve significance (31). Subjects ranged in age from 12 - 18 years (57% male) and had categorically mild CF-related lung disease (mean FEV_1 of 83.23 $\pm$ 18.04 % predicted). Participants were subjected to a maximal cycle ergometry exercise tolerance testing protocol (31). Immunoglobulin-G is, however, not a marker of inflammation in itself (156, 157). It is an antibody produced by the body as an immune response to potential infection (156, 157), hence why it was not explored within this research program. The result of this second paediatric study may have been more indicative of lower levels of inherent colonisation and infection and therefore a

lowered immune response, as opposed to directly relating to inflammation (31).

From this limited evidence base it appears that children and adolescents with CF have raised systemic inflammatory markers following structured exercise. Whether these results can be generalised to the adult CF population with greater disease severity, and the role that habitual physical activity plays in this relationship is unknown. There is minimal evidence regarding the relationship between inflammation, physical activity and exercise amongst adults with CF. This is explored in detail regarding the specificities of physical activity, aerobic exercise tolerance and strength-based exercise tolerance over the next three sections.

2.5.3 Inflammation and physical activity in adults with CF

Minimal evidence currently exists regarding the relationship between inflammation and physical activity in adults with CF. One singular study has examined 13 adults with CF (mean age 29 $\pm$ 8.6 years; 69% male; mean FEV_1 54 $\pm$ 18% predicted) who were admitted to hospital for IV antibiotic therapy for an acute exacerbation (138). Physical activity levels were measured via both the Sensewear armband and the ear-worn activity monitor during admission, and again for one week at a point of time three weeks following discharge from hospital (138). No relationship was found between step count and C-reactive protein. The major focus of this study was the effect of an acute exacerbation on quadriceps strength and physical activity (comparing admission values to discharge values), and as such no values were reported for correlations between inflammatory markers and physical activity.

Adults with CF appear to be as physically active as healthy individuals (75-77), however it is an obvious gap in the literature and our understanding regarding the relationship between how much physical activity an adult with CF participates in habitually *and* how this may relate to their underlying level of inflammation. Further investigation is needed into this area to better understand how the physical activity being recommended to an individual with CF is affecting the underlying inflammation related to their disease pathology.

2.5.4 Inflammation and aerobic exercise in adults with CF

There has been some investigation of the relationship between inflammation and aerobic exercise tolerance in adults with CF to date, and consistency is lacking between the choice of exercise tolerance test and systemic inflammatory marker measured. These studies are outlined in detail in this section, indicating a potential trend for increased levels of systemic inflammation in those with reduced exercise tolerance.

In 2006 a CF centre in Cardiff, Wales examined inflammatory markers in blood plasma of 12 adults with CF who completed a box-stepping exercise to the point of exhaustion (or to a maximum of 20 minutes) at a metronome-set pace of 15 steps/minute (9). Systemic IL-6 and TNF-α were increased at rest and further increased after exercise of approximately five minutes at an intensity of ~3.1 METS (9). This suggests that despite systemic inflammation being inherently increased prior to exercise, systemic inflammation increased further following a short duration high intensity exercise task. Pulmonary inflammatory markers were however not examined. This study also showed that adults with CF aged 23 - 32 years, with an FEV_1 44 - 66% predicted (moderate disease severity) demonstrate increased

systemic inflammation following an externally paced, low to moderate intensity box-stepping exercise (9). These findings provide no indication of the relationship between exercise and inflammation in *older* adults with CF, or those with severe respiratory disease.

More recently it has been observed that levels of blood plasma C-reactive protein are related to aerobic exercise tolerance in adults with CF (79). In a French observational cohort study 102 adults, aged 17 – 67 years, were stratified into two groups dependent on disease severity (severe lung disease group mean FEV_1 35 $\pm$ 9% predicted; mild-moderate lung disease group mean FEV_1 82 $\pm$ 18% predicted) (79). Each participant completed cardiopulmonary exercise testing via an incremental ergometric cycling protocol during an outpatient clinic attendance (79). Systemic C-reactive protein was shown to have a weak but significant negative correlation with VO_{2peak} ($r = -0.34$, $p = 0.006$) (79), indicating that those with higher levels of systemic inflammation have poorer exercise tolerance. No between group differences were observed (79), indicating that inflammation in relation to aerobic exercise tolerance is potentially unrelated to disease severity.

When looking towards research with a comparable participant population and environment to that of this research program, Bradley et al. found a strong inverse relationship between inpatient hospital antibiotic treatment-induced change in C-reactive protein over the course of an admission and exercise tolerance as measured by MST amongst adults with CF (n = 20, aged 23 $\pm$ 5 years, FEV_1 60 $\pm$ 25% predicted) (158). However, the relationship between C-reactive protein and exercise tolerance at a particular time point - either pre-IV antibiotics, or post-14 days of IV antibiotics - was not found to be significant. This suggests that the *change* in systemic inflammation in response to hospital treatment as opposed to individual inflammation levels at any one time point has a direct relationship with exercise tolerance.

2.5.5 Inflammation and strength-based exercise in adults with CF

To date, limited studies have explored the relationship between inflammation and skeletal muscle strength in adults with CF. It has been demonstrated that increased muscle mass in adults with CF has an inverse relationship with systemic IL-6 levels ($r = -0.34$), but not with TNF-α (159), however the relationship of muscle mass to strength or exercise tolerance was not explored in this study. Three other studies have examined the relationship between C-reactive protein (93, 138, 160), IL-6, IL-8 and TNF-α (160), and quadriceps strength in adults with CF and found no significant relationship between inflammation and muscle strength (93, 138, 160).

Further investigation into the relationship between skeletal muscle strength and inflammation is needed for adults of all ages with CF, across the disease spectrum. This will provide insight into the benefits and consequences of resistance training for this cohort and enable tailoring of physical activity programs to an individual's inflammatory profile.

Interestingly, the relationships between inflammation and disease severity or between inflammation and age have not been explored in adults with CF. A clear gap in the literature is the relationship between inflammation, physical activity and exercise tolerance in the older adult (i.e. >35 years of age) with CF. In particular, what happens following discharge from hospital in regard to inflammation and habitual physical activity in these individuals remains unknown. Studies measuring an individual's systemic and pulmonary inflammation (across a broad scope of disease severity and age of patients) that are sufficiently powered are needed to enhance our understanding of whether a proposed physical activity program may trigger an unwanted pro-inflammatory response in adults with CF. Further investigation

is warranted in the immediate post-discharge and longer-term periods for adults with CF following an acute exacerbation.

In summary, this research program aims to investigate the relationships between both systemic and pulmonary inflammatory markers, physical activity levels (as measured by Sensewear armband) and exercise tolerance (as measured by 6MWT, MST-25 and quadriceps strength) in adults with CF following hospitalisation for an acute exacerbation. Chapter 3 outlines the methods by which these aims were carried out. Understanding the relationship between physical activity, exercise tolerance and inflammation for adults with CF regardless of age or disease severity and establishing proof of concept is essential to provide an evidence-based approach to exercise prescription for adults with CF.

CHAPTER 3 METHODS

This chapter outlines the methods used in the study included in this thesis. One study was conducted which generated two reports, so the methods of both reports are presented together in this chapter. Study design, study setting, participant requirements, ethical approvals, study procedures, outcome measures and statistical analyses used are outlined.

3.1 Study design

A single-centre prospective cohort study was conducted using a convenience sample of patients admitted to the Adult Cystic Fibrosis Centre at The Prince Charles Hospital. Concurrent assessments were conducted on participants within 48 hours prior to discharge using six different assessment tools (outlined below).

3.2 Setting

The Prince Charles Hospital is a 630-bed tertiary referral hospital located in suburban Brisbane. The hospital forms part of the Metro North Hospital and Health Service of Queensland Health and is the major cardiothoracic hospital for the state of Queensland. The Adult CF Centre, located within the Prince Charles Hospital, is the primary adult CF service in the state of Queensland and provides outreach services to Cairns, Townsville and Mackay, in addition to providing a service to individuals in south east Queensland, northern New South

Wales and the Northern Territory. At present, the Adult CF Centre cares for 300 adult patients, including up to 25 inpatients at any one time. The hospital is also home to the only heart and lung transplantation service in the state of Queensland.

3.3 Participants

Adults diagnosed with CF confirmed by sweat testing or genotype analysis were included in the study if they were admitted to the Thoracic Medicine unit at The Prince Charles Hospital to be managed following a pulmonary exacerbation of CF requiring intravenous (IV) antibiotics, were medically stable and were aged ≥ 18 years. Patients were excluded if they were discharged with home-based IV antibiotic treatment; were pregnant; or if they had any co-morbidities that prevented exercise testing (i.e. severe musculoskeletal or neurological impairments).

Participants were recruited consecutively over a period of 12 months and stratified into three groups based on recognised groupings of disease severity (Mild: FEV_1 ≥ 70% predicted; Moderate: FEV_1 40 - 70% predicted; and Severe: FEV_1 ≤ 40% predicted) (38). The aim was to recruit relatively equal numbers per group if possible, of approximately 10 - 15 per group. Once the quota (n = 15) for each individual group was filled, recruitment to that particular group ceased, but continued consecutively in the other groups until recruitment was finished. This approach was adopted to avoid bias in any one particular severity group, and to enable analysis of each sub-group's inflammatory and physical activity statuses.

A sample size of 28 participants was required to conservatively evaluate a moderate relationship (r = 0.25) between inflammatory markers and physical activity with 75% power and 95% level of significance, as estimated with the Clinical and Translational Science Institute

sample size calculator (161, 162). These values were based on a comparable study which looked at the relationship between lung function, inflammation, exercise tolerance and quality of life in 20 adults with CF, where the standardised response mean was defined at 0.2, 0.5 and $\geq$ 0.8 as small, moderate and high levels of correlation (158).

3.4 Ethics and consent

The study was approved by the Metro North Human Research Ethics Committee (HREC/12/QPCH/289). Site-Specific Assessment approval was granted for this study by the Prince Charles Hospital Research, Ethics & Governance office in 2013 (SSA AU/3/8011116) (Appendix 1).

Note that prior ethics approval (external research) from Griffith University's Human Research Ethics Committee was deemed to be not required for this study, as data collection occurred prior to enrolment in the Master of Philosophy at Griffith University. Email correspondence confirming this is provided in Appendix 2.

Participants were invited to take part in this study via explanatory letters disseminated outlining the study aims of examining a potential relationship between inflammatory markers in blood and sputum, exercise tolerance and physical activity. Signed informed consent was gained from participants prior to data collection.

3.5 Health and safety

All physical activity and exercise tolerance assessments were considered routine practice for physiotherapy and were deemed as causing no potential harm to participants beyond that included during routine physiotherapy practice. Blood samples were collected by the CF registrar, and sputum samples were collected by the CF physiotherapist as per routine care (adhering to local infection control procedures).

The physical resources required for sample processing and storage were provided by The Queensland Lung Transplant Service laboratory, The Prince Charles Hospital. This included: freezer space for sample storage, laboratory space to conduct sample processing, and laboratory consumables to conduct sample processing and storage. There was no cost for these services.

Data were securely stored on a password-protected computer in a locked office in the thoracic physiotherapy office within The Prince Charles Hospital. In compliance with Queensland Health regulations all records are to be kept for seven years following completion of the research. Back-up storage has occurred via the secure Griffith University Research Storage Service.

Guidelines for the Protection of Privacy in the conduct of medical research have been complied with as per recommendations of the Commonwealth Privacy Commissioner under Section 95A of the Privacy Act 1988 and the Privacy Amendment (Private Sector) Act 2000. No details of participants have been nor will be published that may lead to the recognition of the participant.

3.6 Procedures

Potential participants were approached to volunteer for this study following inpatient treatment for a pulmonary exacerbation. Study information was provided to potential participants by the candidate and written consent was obtained for all 32 participants prior to data collection. This time-point was chosen on the basis of patients most likely being in their best state of health, having completed 10-14 days of intravenous antibiotics and relatively intense physical therapies.

Once recruited to this research, participant clinical and demographic information were recorded (lung function, height, weight), from medical chart lung function results. Sputum and blood samples were collected from each participant within 48 hours prior to discharge from hospital (prior to the commencement of exercise tolerance testing).

Within 48 hours prior to discharge measures of respiratory function and exercise tolerance were collected. Disease severity was determined using spirometry (Jaegar Vyntus® Pneumo, made in Germany) to measure FEV_1, collected by an independent assessor according to the European Respiratory Society guidelines (163).

Exercise tolerance was determined using a 6MWT (126), an MST-25, (130), and isometric strength testing of the quadriceps muscle bilaterally (139) (see Section 3.7.2 for further explanation of outcome measures). A short demonstration was performed by the physiotherapist prior to each test, and standardised instructions and encouragement were given. A CF physiotherapist experienced in the management of CF patients conducted all exercise tolerance assessments. No assessor blinding occurred as measures were collected as a part of routine care.

Physical activity was estimated using a multisensory armband device (Bodymedia® Sensewear armband, model MF-SW, Pittsburgh, PA, USA). On the day of discharge, each participant was fitted with a Sensewear armband by the research candidate. Participants were instructed to wear the armband for 5-7 days and then return it to the research candidate at The Prince Charles Hospital via a reply-paid envelope.

Discharge from hospital was determined by self-reported improvement in the patient's symptoms and objective evidence of a reduction in C-reactive protein levels and improvement in lung parameters (FEV_1). Additional data collection regarding anti-inflammatory therapies was not considered feasible, due to the additional burden imposed upon participants and staff.

Participants were followed up for twelve months following discharge from The Prince Charles Hospital to monitor time to next pulmonary exacerbation (days). This was defined as a clinical worsening in a participant's pulmonary status, resulting in any or all of the following symptoms which led to the commencement of intravenous antibiotic therapy: increased cough, shortness of breath, chest pain, loss of appetite, loss of weight, or a decline in lung function tests (34).

3.7 Outcome measures

Primary outcome measures were inflammatory markers in blood and sputum, exercise tolerance (6MWD, MST-25 distance, quadriceps strength adjusted for bodyweight) and physical activity levels during the first week following discharge from hospital for an acute

infective exacerbation of CF. Secondary measures included time to next pulmonary exacerbation.

3.7.1 Inflammatory markers

The three inflammatory markers measured in this research program were IL-6, IL-8 and TNF-α. Other cytokines (for example IL-1β) were not measured as a component of this research due to a lack of specific antigen availability with The Prince Charles Hospital laboratory. Measures were taken via blood and sputum samples (as a measure of both pulmonary and systemic inflammation) within 8 hours of each other.

Blood and sputum sampling protocols

Venous blood samples were collected by a CF registrar within 48-hours prior to discharge and prior to exercise tolerance testing. 5ml of blood was taken from each participant and placed into a Vacutainer™ Serum Separator II Advance tubes (SST™). Blood samples were immediately centrifuged by the research candidate in the on-site laboratory following venepuncture. Samples were centrifuged for 10 minutes at 1000 x gravity at room temperature to separate plasma from haematocrit.

Sputum samples were subsequently spontaneously expectorated and collected in a sterile container by the treating CF physiotherapist. These samples were refrigerated at 4°C for up to 7 days, at which time point the research candidate prepared them for processing. Samples were homogenised by mixing with a phosphate-buffered saline solution at a ratio of 5:1 and heated in a water bath to 37°C for 30 minutes with regular agitation. Sputum was then

centrifuged at 1800 RPM at 4°C for ten minutes to separate supernant from cell pellet (cell pellet was discarded). All samples were stored at -20°C for 24 hours, and then at -80°C in the UQ Transplant and Vascular Disease research laboratory at The Prince Charles Hospital for later analysis.

Blood and sputum measurements

Both blood plasma and sputum IL-6, IL-8 and TNF-α levels were measured by previously optimised in-house ELISA in duplicate and analysed via the Fluostar Omega, using local laboratory protocols derived from international reference standards (164).

ELISA is the most widely used method for single cytokine measurement (165). ELISA is based on the immunology concept of antigen and antibody binding, allowing the detection of small quantities of antigens such as cytokines with high sensitivity (166). ELISA has been demonstrated as a reliable and valid method of measuring IL-6 (167), IL-8 (168, 169) and TNF-α (170, 171) in human biological samples.

3.7.2 Exercise tolerance measures

Exercise tolerance was determined using three measures; 6MWD, MST-25 and quadriceps muscle strength. The measures were conducted in this order for all participants, with a minimum of 60 minutes rest between each test.

Distance walked in 6 minutes

The 6MWT was conducted within 48 hours prior to discharge according to the American Thoracic Society guidelines (172), following collection of blood and sputum samples. The test occurred in an unobstructed corridor with a 30m track marked with cones at each end. Participants were instructed to walk as far as possible for six minutes, back and forth along the track and pivot around the cones at each end, with standardised encouragement provided after each minute. Rests were permitted as necessary; however, participants were encouraged to resume walking as soon as possible. Only one 6MWT was conducted due to time constraints in the 48-hour period prior to discharge from hospital.

Heart rate and oxygen saturation (by pulse oximetry, SaO_2) were measured before and after the 6MWT using a finger probe (Datex, Ohmeda TuffSat, Madison, WI, USA). Prior to the test commencing, participants using a Modified Borg Scale (173) were asked to indicate their current perceived level of shortness of breath on a scale of 0 (none at all) to 10 (very, very severe shortness of breath). Participants were asked again to rate their perceived level of breathlessness on completion of the test. After the test, participants were asked to give a rating of their perceived level of exertion during the test, using a scale from 6 (no exertion at all) to 20 (maximal exertion) (174). A minimum of 60 minutes rest occurred between the 6MWT and other measurements of exercise tolerance.

Modified shuttle test 25

The MST-25 was conducted within 48 hours prior to discharge according to the standardised guidelines stipulated in the recording used for the test (130). The 25-level MST required participants to walk/run at increasing speeds back and forth on a 10m course to the pace of

a recording. Each level in the test lasts for one minute, with the speed of the test increasing by 0.61 km/h each minute. There are a maximum of 25 levels. At the end of each level standardised encouragement was provided by the research candidate, and the participant was reminded that their speed needed to increase to match that of the increasing pace of the recording. Participants continued with the test until they either (a) failed to maintain the set pace on two consecutive beeps, or (b) opted to cease testing due to subjective reasons of shortness of breath or fatigue.

Heart rate and oxygen saturation (by pulse oximetry, SaO_2) were measured before and after the MST-25 using a finger probe (Datex, Ohmeda TuffSat, Madison, WI, USA). Participants were also asked to give a rating of their perceived exertion (174) and a rating of perceived breathlessness (Modified Borg Scale) (173), as stipulated above. A minimum of 60 minutes rest occurred between the MST-25 and other measurements of exercise tolerance.

Quadriceps isometric strength testing

Quadriceps strength was tested bilaterally within 48 hours prior to discharge using hand-held dynamometry (Lafayette Manual Muscle Test System). Testing procedures were undertaken based on the published protocol that exists for individuals with COPD (139) and the Wieboldt study (138) with the addition of a secured inextensible strap (to ensure an isometric contraction). Seated on a plinth at a height where feet were elevated from the floor, with hips flexed at 90° and knees flexed at 90° (refer to Figure 3.1), each participant performed three maximal voluntary knee extension efforts for three seconds on each leg with standardised encouragement, with a resting period of at least 30 seconds between efforts. Strength in kilograms was standardised and expressed as a percentage of each participant's body weight (kg/BW) (175). For analysis, the mean of the two greatest peak force values was calculated.

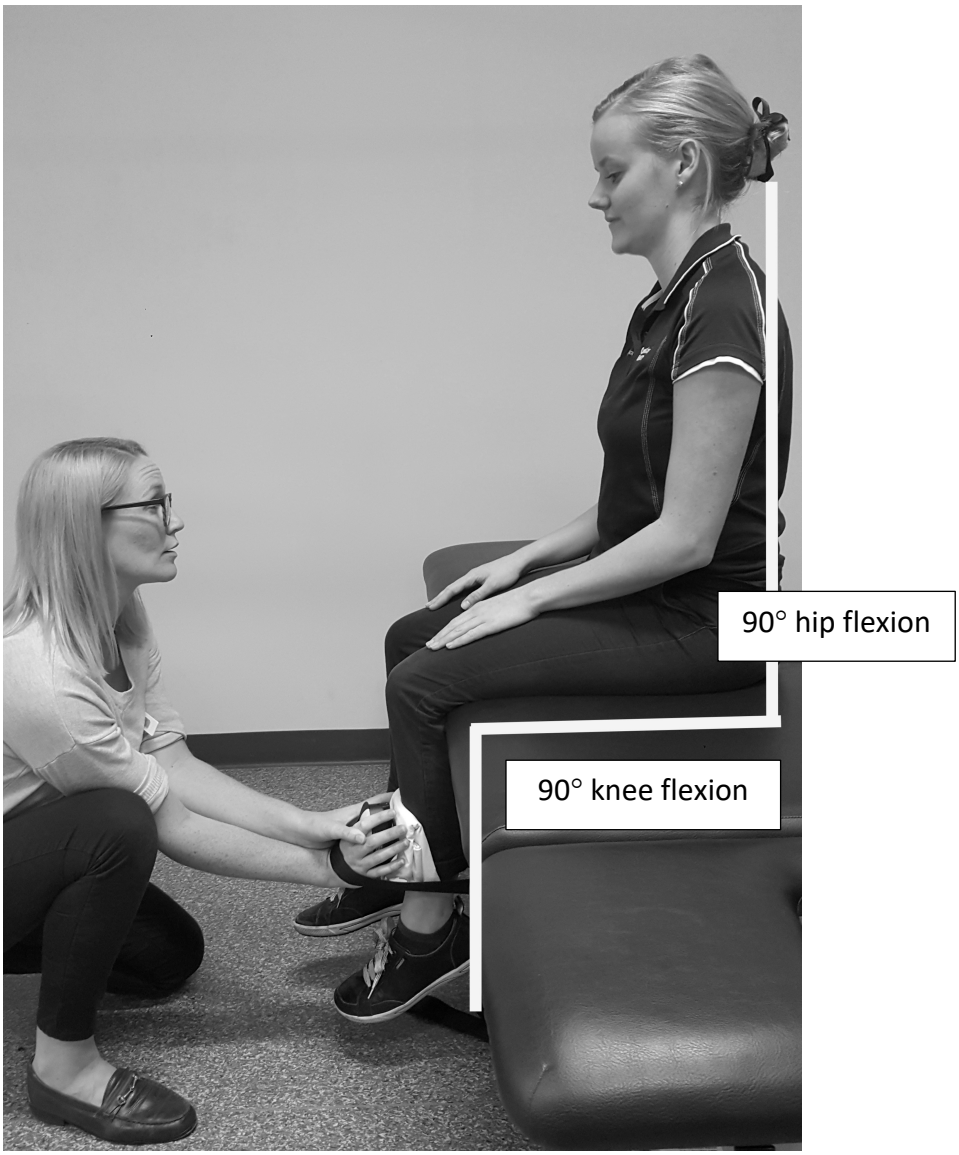

Figure 3.1 Quadriceps isometric strength testing via handheld dynamometer

3.7.3 Physical activity level measures

Physical activity levels were measured using the Bodymedia Sensewear armband (Model MF). Following exercise tolerance testing and prior to discharge home each participant was fitted with a Sensewear armband. Participants were instructed to wear the armband on the

left upper arm as per the manufacturer's recommendations for 5-7 days following discharge and was only removed for bathing and swimming. Participants were instructed to wear the armband overnight. Minimum wear-time of 5-7 days was based on recommendations for chronic lung disease (176) and rehabilitation (177). Participants were provided with written instructions and were provided with the research candidate's contact details in the event of a problem. Participants returned the Sensewear armband to the research candidate at the hospital via a reply-paid envelope. Data are reported as the average of five full days of wear. Outcome measures obtained from the armband included METs and steps. For the purposes of this research METs ≥ 3.0 was used as the cut-off for mild intensity physical activity, as has been used in other studies of adult CF populations (96, 117). This is also in line with the findings of Ionescu et al., who demonstrated increased levels of systemic IL-6 and TNF-α after five minutes of physical activity at an intensity of ~3.1 METs (9).

3.7.4 Measurement of time to next pulmonary exacerbation

All participants were followed up for twelve months following discharge from hospital to monitor time to next pulmonary exacerbation (days). Pulmonary exacerbation was defined as a clinical worsening of respiratory status which required the commencement of inpatient intravenous antibiotic therapy. Symptoms recognised as indicative of worsening respiratory status (34) which were used in this research program include:

- Increased cough
- Increased sputum production
- Shortness of breath

- Chest pain
- Loss of appetite
- Loss of weight
- Decline in pulmonary function tests

All participants who experienced a pulmonary exacerbation and required antibiotic therapy were reviewed at CF outpatient clinics; making it feasible that all participants who clinically deteriorated were identified. Pulmonary exacerbation was confirmed by treating consultant.

If a participant received a lung transplantation or was deceased at the end of the 12-month follow-up period, this was also recorded. If participants did not present to CF outpatient clinics or inpatient wards during the 12-month period, pulmonary exacerbation was deemed not to have occurred.

3.8 Statistical analysis

All statistical analyses were performed with SPSS for Mac v 23.0 (2015, IBM Corp., Armonk, NY, USA). Descriptive analysis was conducted for all measures. Shapiro-Wilk's test and Q-Q plots were used to determine whether continuous data were normally distributed. Physical activity level (METs and steps) over the total time the armband was worn (hours) was determined. Pearson's correlational analyses were conducted to examine the relationships between both plasma and sputum inflammatory markers (IL-6, IL-8 and TNF-α), measures of physical activity in the first week following discharge (METs, steps/day), and exercise

tolerance measures (6MWD, MST-25 and quadriceps strength [kg/m^2]). Correlations were classified as low (r = -.30 to -.50; or .30 to .50), moderate (r = -.50 to -.70, or .50 to .70) or high (r = -.70 to -.90 or .70 to .90) (178).

To investigate any potential relationship between disease severity and inflammation, physical activity and/or exercise tolerance, data were categorised into three groups of disease severity based on the framework of the Australian CF Data Registry's published grading of obstructive lung physiology in CF (44); that is, mild (FEV_1 > 70% predicted), moderate (FEV_1 40 - 70% predicted), and severe (FEV_1 < 40% predicted) lung disease, respectively.

The occurrence and timing of a pulmonary exacerbation in the 12 months following discharge from hospital was determined. Time to next pulmonary exacerbation was recorded in days, with individuals who did not experience a pulmonary exacerbation in this period recorded arbitrarily as 366 days. Participants who required a bilateral single sequential lung transplant within the 12-month follow-up period also experienced a pulmonary exacerbation in the lead-up to requiring this and were coded accordingly. No participants died in the 12-month follow up period.

Multivariate analyses (one-way MANOVA) were conducted to examine the relationships between inflammatory markers and time to next exacerbation, between physical activity and time to next exacerbation, and between exercise tolerance and time to next exacerbation. Non-normally distributed data were natural log transformed prior to performing correlation testing. A p-value of < 0.05 was considered statistically significant.

The relationship between inflammatory markers and physical activity levels following an inpatient treatment for an acute exacerbation was further examined by using independent t-

tests and Mann-Whitney U tests (depending on normality of distribution) to explore whether there was a statistically significant difference for those participants who experienced a pulmonary re-exacerbation within 6 and/or 12 months, and those who did not. Time to next pulmonary exacerbation (days) was used as a covariate.

Correlational analyses were also conducted to examine relationships between measures of exercise tolerance, and between age, sex, BMI and disease severity with all other outcomes.

CHAPTER 4 STUDY REPORT 1

Increased Physical Activity Post-Exacerbation is Associated with Decreased Systemic Inflammation in Cystic Fibrosis - An Observational Study.

The following chapter is based on a peer-reviewed submission published in Physiotherapy Theory and Practice. The bibliographic details are:

Burton K, Morris NR, Reid D, Smith D & Kuys S (2019). Increased physical activity post-exacerbation is associated with decreased systemic inflammation in cystic fibrosis – An observational study. Accepted for publication in *Physiotherapy Theory and Practice*, DOI: 10.1080/09593985.2019.1566942

Declaration of candidate contribution

For this co-authored manuscript, the candidate was involved in the experimental design, performed data acquisition, analysed the data, interpreted results, and drafted and critically reviewed the manuscript.

_____ 18/07/2019

Kate Burton / *Candidate and corresponding author* Date

_____ 18/07/2019

Prof Norm Morris / *Principal supervisor and co-author* Date

4.1 Abstract

Background & objective

Cystic Fibrosis is a progressive genetic disease in which systemic inflammation occurs in response to chronic infection in the airways. Maintaining physical activity is fundamental to the management of CF, and improvements in physical activity have been shown to be associated with improvements in lung function and exercise tolerance. At present there is minimal evidence regarding the relationship between physical activity and inflammation in adults with CF.

This study assessed whether physical activity in adults with CF following in-hospital treatment for an acute exacerbation was related to levels of systemic and airway inflammation, and whether physical activity post-discharge predicted for time to next pulmonary exacerbation. Disease severity, age, sex and BMI were also explored as covariates.

Methods

Adults with CF were included following hospitalisation for a pulmonary exacerbation and were followed for 12 months. Inflammatory markers and physical activity were measured immediately post discharge via plasma and sputum concentrations of IL-6, IL-8 and TNF-α. Physical activity was monitored for 7 days via a Sensewear armband. Statistical analyses included Shapiro-Wilk's test and Q-Q plots to determine normal distribution, T-tests, Pearson's correlational analyses and one-way MANOVAs.

Results

Thirty-one adults with CF (13 (42%) female, age 29 $\pm$ 8.7 years, FEV_1 59.1 $\pm$ 23.3% predicted) were prospectively recruited. Physical activity negatively correlated with plasma

inflammation ($r = -0.48$, $p < 0.01$), and positively with disease severity (FEV_1 % predicted) ($r = 0.45$, $p < 0.05$) and body mass index ($r = 0.39$, $p < 0.05$). Daily average steps positively correlated with sputum inflammation ($r = 0.36$, $p = 0.05$). There was no significant relationship between time to re-exacerbation, age, sex and any inflammatory markers or measurement of physical activity (all $p > 0.05$).

Conclusion

Increased physical activity following exacerbation in CF is associated with lower levels of systemic inflammation. Time to re-exacerbation is not related to post-discharge inflammation or physical activity levels.

Key words

Adults, Cystic Fibrosis, Cytokines, Inflammation, Physical Activity.

4.2 Introduction

Cystic fibrosis lung disease is typified by a vicious cycle of airway infection and an exuberant, but ineffective local and systemic inflammatory response (10). Even during periods of clinical stability, elevated concentrations of pro-inflammatory cytokines such as IL-6, IL-8 and TNF-α are detectable in the systemic circulation and in the airways (8-10). The deleterious effects of inflammation may extend beyond local lung destruction and, amongst other effects, systemic inflammation is thought to contribute to muscle wasting (10), which may further impair physical activity (90). Regular physical activity is a critical component of the management of CF, with demonstrated benefits including enhanced sputum clearance, skeletal muscle strength, preservation of bone mineral density, and increased insulin sensitivity, as well as improvements in lung function (28-30).

In healthy adults, the relationship between physical activity and inflammation has been studied (16, 17), with main findings demonstrating that different levels of physical activity may be associated with changes in inflammatory responses. In people with type 2 diabetes (24), COPD (23), human immunodeficiency virus and acquired immunodeficiency syndrome (26), and metabolic syndrome (25), reduced levels of physical activity were associated with higher levels of systemic inflammation. The relationship between regular physical activity and inflammation in CF is not well characterised, and to date there is limited evidence of the association of physical activity with inflammation in adults with CF.

In this study, the overarching aim was to examine the relationship between systemic and pulmonary inflammation and physical activity in adults with CF following hospitalisation for an acute pulmonary exacerbation. It was hypothesised that adults with CF with increased

levels of inflammatory markers would demonstrate reduced physical activity levels post-discharge and that increased systemic and/or pulmonary inflammatory markers and reduced physical activity would predict for time to next pulmonary exacerbation (independent of lung disease severity).

The research questions were:

1. Is there an association between reduced levels of physical activity and increased levels of inflammation in adults with CF following treatment for an acute pulmonary exacerbation? and

2. Do levels of physical activity or inflammation predict time to next pulmonary exacerbation?

4.3 Methods

Design

This was a single-centre prospective cohort observational study with Human Research Ethics approval (HREC/12/QPCH/289 and SSA AU/3/8011116). All participants gave written informed consent prior to participating.

Participants

Participants were recruited consecutively over a 12-month period. To be eligible for inclusion participants needed to be adults with a formal diagnosis of CF (aged 18 and above) who were admitted for inpatient hospital treatment of an acute pulmonary exacerbation. Participants were excluded from the study if they were aged less than 18 years at the time of enrolment,

were discharged with home-based intravenous antibiotic treatment, or were pregnant.

The definition of an acute pulmonary exacerbation was based on international consensus criteria as the presence of any or all of the following symptoms: increased cough, increased sputum production, shortness of breath, chest pain, loss of appetite, loss of weight, and/or a measured decline in pulmonary function (34).

The candidate was responsible for study recruitment, collection of outcome measures (assisted by the Adult Cystic Fibrosis physiotherapy team), sample processing, and data analysis. The CF registrars collected all blood samples.

Procedure

Adults with a diagnosis of CF were recruited at the end of an inpatient admission at The Prince Charles Hospital, Brisbane. Discharge from hospital was determined by self-reported improvement in the patient's symptoms and objective evidence of a reduction in C-reactive protein levels and improvement in lung parameters (FEV_1). Sputum and blood samples were collected within 48-hours of planned discharge from hospital.

To measure the level of physical activity post-discharge, participants were fitted with a Bodymedia® Sensewear armband (Model MF-SW), which was worn for 5-7 consecutive days.

To monitor time to next pulmonary exacerbation (days) all participants were followed up for twelve months. Monitoring of pulmonary exacerbation was made through attendance at regular outpatient clinics and hospital admissions.

Outcome measures

Demographic information was recorded, and respiratory function measured within 48-hours prior to discharge. Lung function was measured using standard spirometry (Jaegar Vyntus® Pneumo, Germany) to measure FEV_1, and collected by an independent assessor according to the European Respiratory Society guidelines (163).

Following venesection, plasma was separated from blood by centrifugation (10 minutes, 1000 x gravity at room temperature) and immediately stored at -80°C for later batch analysis. Spontaneously expectorated sputum samples were collected in a sterile container by the treating CF physiotherapist and processed without delay. Sputum was homogenised by mixing with phosphate-buffered saline in a 5:1 ratio and heating in a water bath to 37°C for 30 minutes, with regular agitation. Homogenised sputum samples were centrifuged at 1800 RPM at 4°C for ten minutes to separate supernatant from cellular component. Supernatant samples were stored frozen to -80°C for later analysis. Cell pellet was discarded. Sputum supernatant and plasma concentration of IL-6, IL-8 and TNF-α were determined using previously optimised in-house ELISA, performed in duplicate and analysed using the Fluostar Omega.

Physical activity levels were measured using the Sensewear armband device; a device which has been validated to estimate physical activity amongst adults with CF (122). Participants wore the Sensewear armband on the left upper arm for 5-7 days following discharge. Participants were instructed to wear the armband overnight. Reported outcome measures were daily average metabolic equivalents (METs; 1 MET = 3.5 ml/kg/min), which are an estimate of energy expenditure per day (122, 179), duration of physical activity at a mild level

(daily average ≥ 3 METs), and daily average steps. BMI was also reported as a component of the Sensewear armband measurements.

Time to next pulmonary exacerbation (days) was defined as the number of days following discharge until the patient required re-admission to hospital for treatment with IV antibiotics for a deterioration in their pulmonary status (158). Pulmonary exacerbation was confirmed by the treating consultant in either Outpatient CF Clinic or in the Emergency Department. Two time-points were examined in this study; participants who experienced a pulmonary exacerbation within the first 6 months (182 days) post discharge, and those who experienced a pulmonary exacerbation within 12 months (365 days) post discharge. If a participant did not experience a pulmonary exacerbation requiring IV antibiotics within 12 months of completing the study this was recorded arbitrarily as 366 days.

Statistical analysis

Statistical analysis was performed with the SPSS statistical package v 22.0 (2015, IBM Corp., Armonk, NY, USA). Data are presented as mean and standard deviation, unless specified otherwise. Physical activity levels (daily average METs), duration of physical activity of at least a mild level (daily average ≥ 3 METs), daily average steps and BMI were determined for each participant (all participants wore the Sensewear armband for > 18 hours/day). Shapiro-Wilk's test and Q-Q plots were used to determine whether continuous data were normally distributed. T-tests and Mann-Whitney U test were used to examine differences in data dependent on normality of distribution. Pearson's correlational analyses were conducted to examine the relationships between both sputum and plasma inflammatory markers (IL-6, IL-8 and TNF-α), measures of physical activity (METs, steps/day), and BMI in the first week following discharge. These correlations were classified as low (r = -.30 to -.50), moderate (r =

-.50 to -.70) or high (r = -.70 to -.90) (178). Multivariate analyses (one-way MANOVA) were conducted to examine the relationships between inflammatory markers and time to next exacerbation, and between physical activity and time to next exacerbation. Non-normally distributed data were natural log transformed prior to performing correlation testing. A p-value of < 0.05 was considered as statistically significant.

4.4 Results

Participant characteristics

Thirty-two adults aged ≥ 18 years with CF were recruited to the study. All participants screened and identified to participate agreed to participate. One participant did not comply with activity monitoring, so data were available for 31 individuals over the study period (18 (58%) male, age 29 $\pm$ 8.7 years, FEV_1 59.1 $\pm$ 23.3% predicted). All participants had a Class I-III mutation (severe) (180), 96% of which were F508del (50% of these were homozygous). In terms of disease severity, 11 participants had mild CF, 12 participants had moderate CF, and 9 participants had severe CF. Demographics, physical activity levels and inflammatory cytokine results in sputum and plasma are provided in Table 4.1. All participants were followed-up for 12 months.

Table 4.1 Participant characteristics on the basis of exacerbation status at 6 and 12 months

Characteristic	All[+] (n=31[*])	Pulmonary exacerbation at 6 months			Pulmonary exacerbation at 12 months		
		Yes (n=16)	No (n=15)	p value	Yes (n=23)	No (n=8)	p value
Age (years)[#]	29 (18-62)	27.8 (18-38)	30.3 (20-62)	0.87	29.4 (18-62)	27.9 (21-38)	0.95
Male	18 (58%)	10 (63%)	8 (53%)	0.58	13 (57%)	5 (63%)	0.47
FEV_1 (% predicted)^	59.1 (23.3)	55.6 (20.6)	62.7 (26.1)	0.44	57.2 (24.3)	64.5 (20.5)	0.48
BMI (kg/m^2)^	21.3 (2.5)	21.3 (2.7)	21.4 (2.6)	0.52	21.1 (2.3)	22.0 (3.2)	0.84
Daily average METs^	1.7 (0.2)	1.8 (0.2)	1.7 (0.3)	0.96	1.7 (0.3)	1.7 (0.2)	0.70
Duration daily average ≥ 3 METs (Hrs:Mins) ^	4:03 (1:20)	4:15 (1:21)	3:51 (1:20)	0.86	4:11 (01:24)	3:41 (1:04)	0.67
Daily average steps^	6351 (2765)	6454 (3562)	6242 (1662)	0.96	6298 (3085)	6503 (1680)	0.75
Plasma IL-6 (pg/ml) [#]	30.9 (14.4-101.4)	26.1 (17.4-38.6)	29.3 (14.4-101.4)	0.10	35.5 (17.4-101.4)	27.1 (14.4-40.0)	0.96
Plasma IL-8 (pg/ml) [#]	36.8 (14.7-145.1)	23.8 (14.7-36.8)	32.9 (18.4-145.1)	0.52	34.2 (14.7-145.1)	26.9 (18.4-105.9)	0.76
Plasma TNF-α (pg/ml) [#]	27.0 (13.6-73.5)	22.9 (15.8-44.3)	28.0 (13.6-73.5)	0.65	27.1 (15.8-73.5)	28.5 (13.6-39.6)	0.55
Sputum IL-6 (pg/ml) [#]	31.9 (16.8-66.75)	32.8 (20.4-66.8)	28.7 (16.8-38.3)	0.25	32.5 (16.8-66.8)	29.0 (20.7-38.3)	0.37
Sputum IL-8 (pg/ml) [#]	125.5 (25.4-533.6)	68.2 (29.0-313.7)	40.8 (25.4-533.6)	0.74	68.2 (25.4-533.6)	37.3 (26.9-279.2)	0.20
Sputum TNF-α (pg/ml) [#]	26.3 (19.2-35.0)	27.2 (20.0-31.3)	26.2 (19.2-35.0)	0.71	26.5 (20.0-31.6)	26.9 (19.2-35.0)	0.91

[#] Median (range), ^Mean (standard deviation), [+]Senseware data unavailable for one subject, *n = 31

Definition of abbreviations: BMI = body mass index; FEV_1 = forced expiratory volume in one second; IL-6 = interleukin-6; IL-8 = interleukin-8; METs = metabolic equivalents; TNF-α = tumour necrosis factor

Relationship between inflammatory markers and physical activity

Plasma concentrations of IL-6, IL-8 and TNF-α demonstrated a low to moderate negative correlation with daily average METs (Figure 4.1, Figure 4.2, Figure 4.3) and a low negative correlation with daily average ≥ 3 METs. There was no association between plasma cytokines and daily average steps (Table 4.2). With the exception of a low positive correlation between sputum TNF-α and daily average steps, there were no significant associations between sputum cytokines and measures of physical activity (Table 4.2).

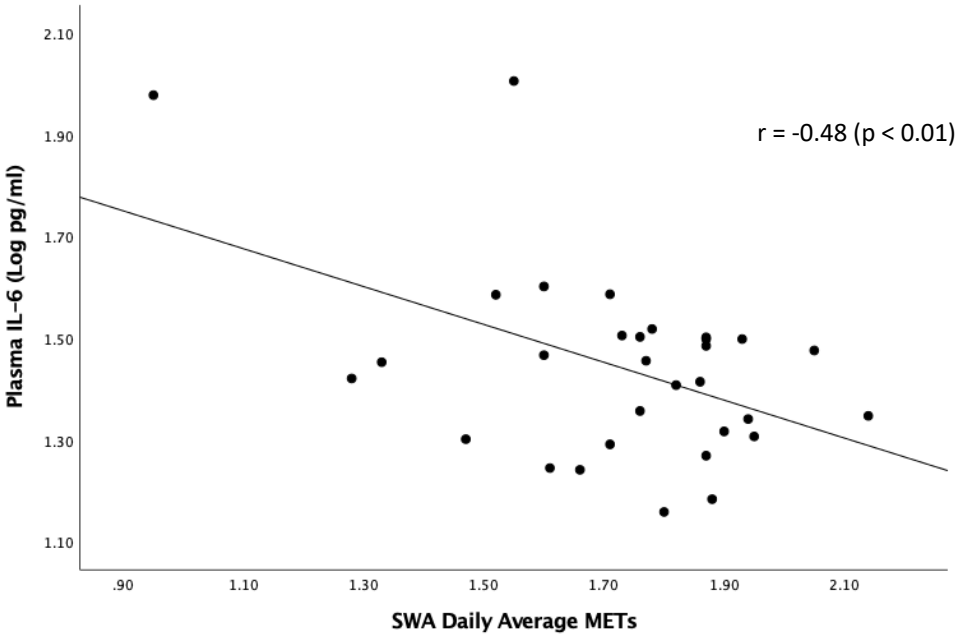

Figure 4.1 Relationship between plasma IL-6 and physical activity

Definition of abbreviations: IL-6 = interleukin-6; METs = metabolic equivalents

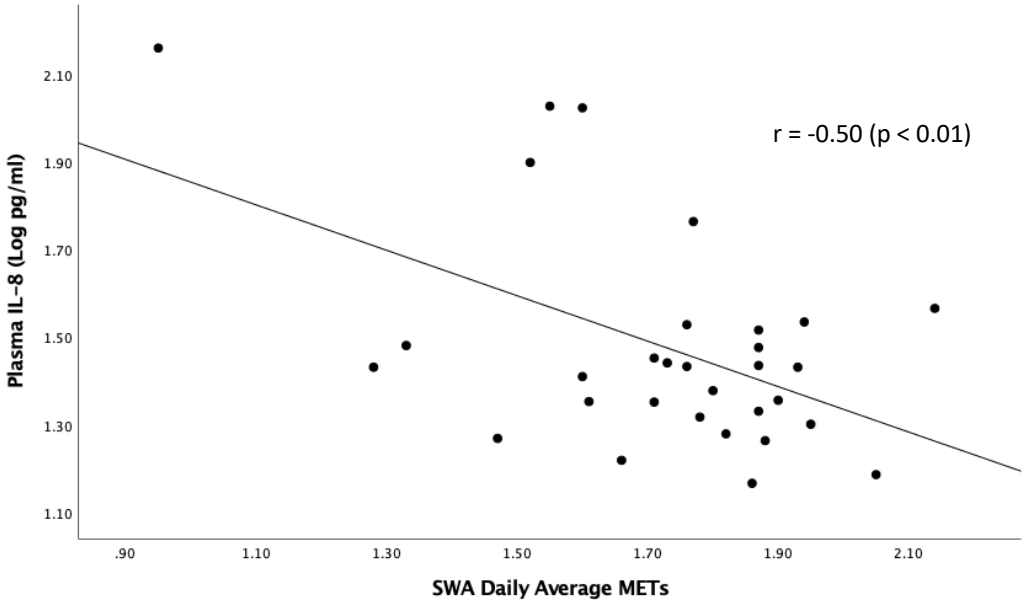

Figure 4.2 Relationship between plasma IL-8 and physical activity

Definition of abbreviations: IL-8 = interleukin-8; METs = metabolic equivalents

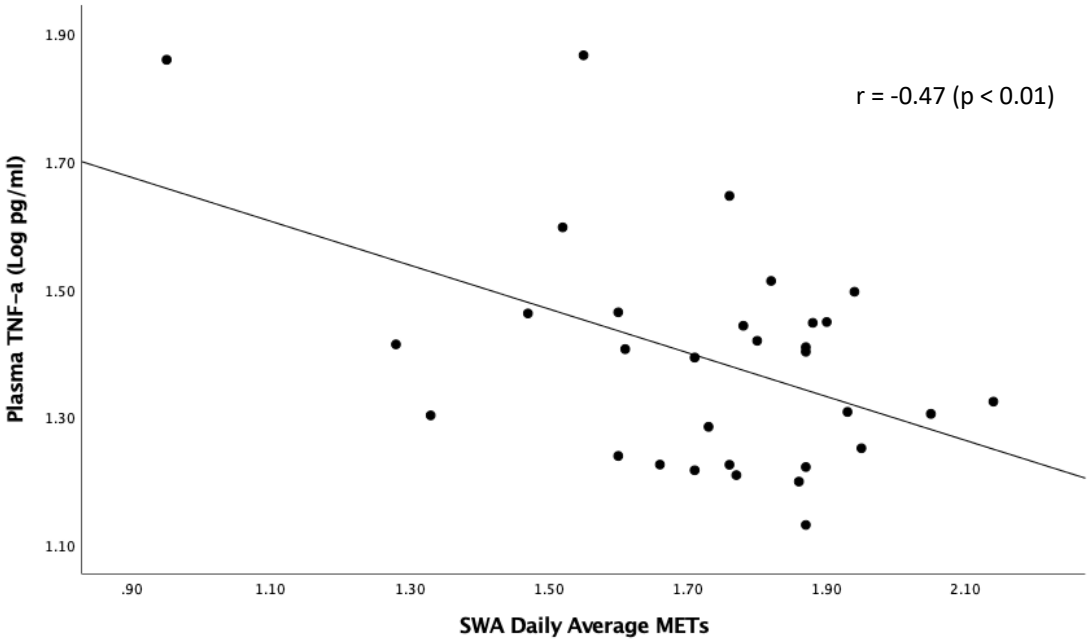

Figure 4.3 Relationship between plasma TNF-α and physical activity

Definition of abbreviations: METs = metabolic equivalents; SWA = Sensewear armband; TNF-α = tumour necrosis factor α.

Relationship between physical activity and disease severity

Daily average steps were found to have a low positive correlation with FEV_1 % predicted (r = 0.45, p < 0.05). No other correlations were found between disease severity and either inflammation of physical activity.

Sex-related differences

Male participants were older (median age 31.9 ± 10.0 years *versus* 24.9 ± 4.9 years, p = 0.01) and had lower lung function than females (mean FEV_1 49.2 ± 19.6% predicted *versus* 72.5 ± 21.7% predicted, p < 0.01) (Table 4.3), predicted values based on Australian and New Zealand adult populations (181). There were no other sex-related differences in either cytokine concentrations or other physical activity parameters (Table 4.3).

Table 4.2 Correlation co-efficients of plasma and sputum cytokines with Sensewear armband data

	Daily average METs	Daily average ≥ 3 METs	Daily average steps
Plasma [pg/ml]			
Log IL-6	**r = -0.48**	**r = -0.40**	r = -0.22
	p < 0.01	**p = 0.02**	p = 0.23
Log IL-8	**r = -0.50**	**r = -0.37**	r = -0.01
	p < 0.01	**p = 0.04**	p = 0.95
Log TNF-α	**r = -0.47**	**r = -0.40**	r = -0.04
	p < 0.01	**p = 0.03**	p = 0.82
Sputum [pg/ml]			
Log IL-6	r = 0.30	r = 0.22	r = -0.02
	p = 0.10	p = 0.23	p = 0.93
Log IL-8	r = -0.04	r = -0.04	r = -0.13
	p = 0.84	p = 0.85	p = 0.50
Log TNF-α	r = 0.18	r = 0.10	**r = 0.36**
	p = 0.34	p = 0.60	**p = 0.05**

Definition of abbreviations: IL-6 = interleukin-6; IL-8 = interleukin-8; METs = metabolic equivalents; TNF-α = tumour necrosis factor α.

BMI-related differences

BMI had a low positive correlation with daily average steps ($r = 0.39$, $p < 0.05$) (Figure 4.4), whereas a moderate negative correlation was seen between BMI and sputum IL-6 ($r = -0.51$, $p < 0.01$) (Figure 4.5). No significant relationship was seen between BMI and IL-8 or TNF-a (either in plasma or sputum).

Table 4.3 Participant demographics and outcome measures

Characteristic	All[+](n = 31)	Males (n = 18)	Females (n = 13)
Age (years)[#]	29 (18-62)	31.9 (18-62)	24.9 (20-35)
FEV_1 (% predicted)^	59.1 (23.3)	49.2 (19.6)	72.5 (20.8)
BMI (kg/m^2)^	21.3 (2.5)	21.6 (2.8)	21.5 (4.4)
Daily average METs^	1.7 (0.2)	1.7 (0.2)	1.7 (0.3)
Duration daily average ≥ 3 METs (hours:minutes)^	4:03 (1:20)	4:04 (1:22)	4:02 (1:20)
Daily average steps^	6351 (2765)	6414 (3424)	6265 (1578)
Time to next IV antibiotics (days)^*	186 (128)	178 (138)	197 (117)
Sputum IL-6 (pg/ml)^	31.1 (10.6)	33.9 (13.1)	29.3 (5.0)
Plasma IL-6 (pg/ml)^	30.9 (19.2)	31.2 (18.7)	28.0 (20.7)
Sputum IL-8 (pg/ml)^	64.7 (127.2)	85.8 (84.8)	180.6 (156.9)
Plasma IL-8 (pg/ml)^	36.8 (30.7)	38.1 (29.4)	26.1 (33.6)
Sputum TNF-α (pg/ml)^	26.2 (3.7)	26.5 (3.3)	26.4 (4.4)
Plasma TNF-α (pg/ml)^	27.0 (14.1)	27.2 (13.9)	24.8 (16.1)

[#] Median (range), ^Mean (standard deviation), *n=23 (9 subjects did not require IV antibiotics in the follow-up period), [+]Sensewear armband data unavailable for one subject

Definition of abbreviations: BMI = body mass index; FEV_1 = forced expiratory volume in one second; IL-6 = interleukin-6; IL-8 = interleukin-8; METs = metabolic equivalents; TNF-α = tumour necrosis factor α

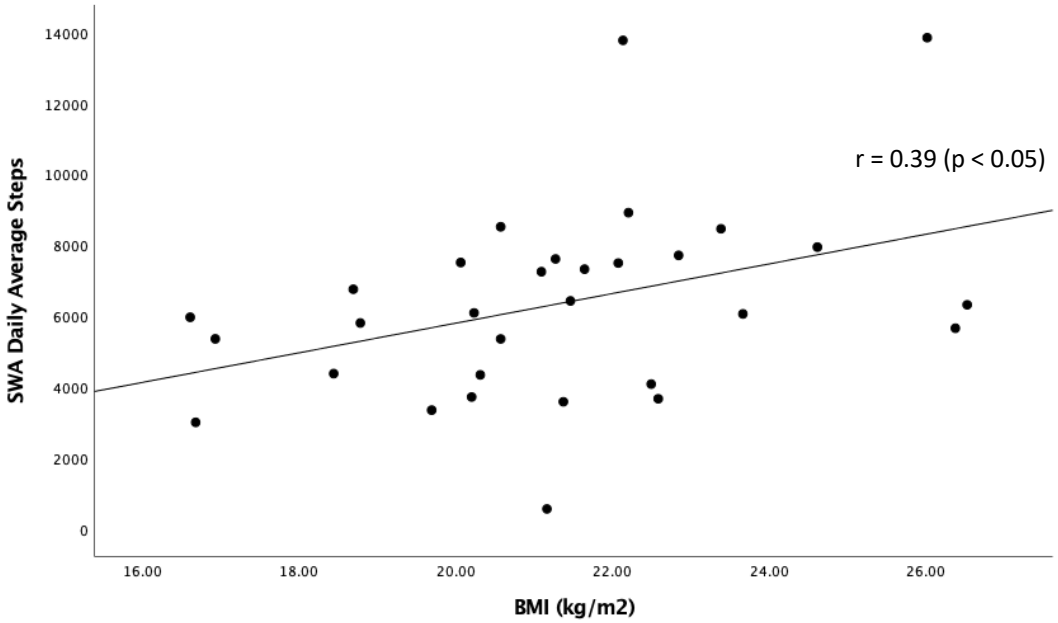

Figure 4.4 Relationship between BMI and physical activity

Definition of abbreviations: BMI = body mass index; SWA = Sensewear armband

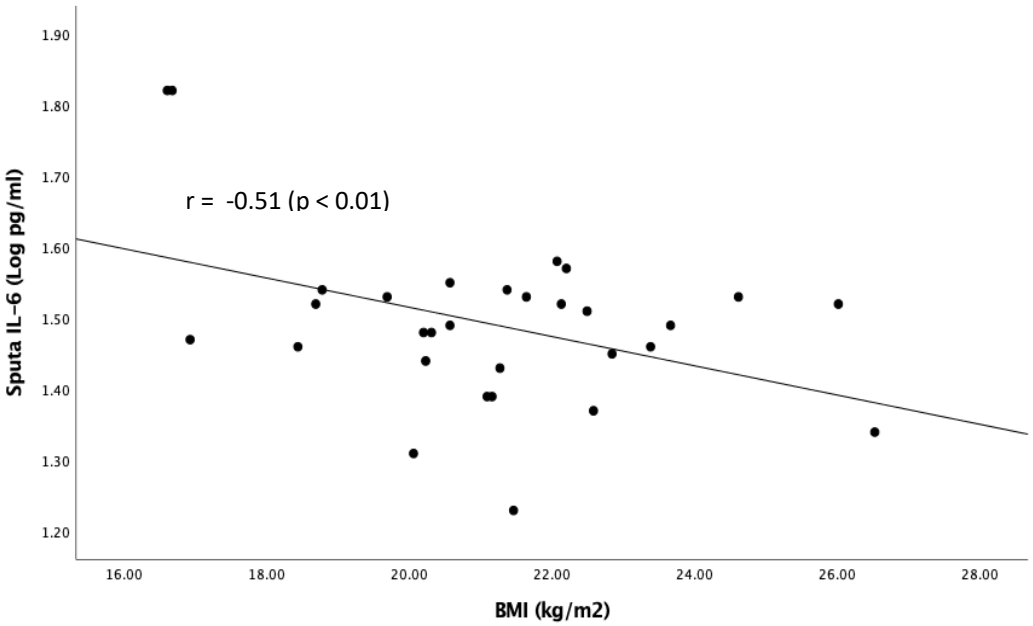

Figure 4.5 Relationship between BMI and pulmonary inflammatory markers

Definition of abbreviations: BMI = body mass index; IL-6 = interleukin-6; IL-8 = interleukin-8.

Differences related to time to next pulmonary exacerbation

Nine of 31 participants did not require further IV antibiotics in the year following enrolment. The median (range) time to next course of IV antibiotics among the 23 subjects who experienced a pulmonary exacerbation was 129 (13 - 366) days. At six months there was no statistically significant difference in time to re-exacerbation based on an individual participant's systemic inflammatory markers at discharge [$F(6, 52) = 0.94$, $p > 0.4$, Wilk's $\Lambda = 0.82$, partial $\eta^2 = 0.1$], sputum inflammatory markers [$F(6, 52) = 1.59$, $p > 0.1$, Wilk's $\Lambda = 0.72$, partial $\eta^2 = 0.16$], or physical activity levels (Daily average METs) [$F(3, 26) = 0.51$, $p > 0.6$, Wilk's $\Lambda = 0.95$, partial $\eta^2 = 0.06$]. Similarly, at 12 months there was no statistically significant difference in time to re-exacerbation based on an individual's systemic inflammatory markers [$F(6, 52) = 0.93$, $p > 0.4$, Wilk's $\Lambda = 0.82$, partial $\eta^2 = 0.1$], sputum inflammatory markers [$F(6, 52) = 1.25$, $p > 0.2$, Wilk's $\Lambda = 0.76$, partial $\eta^2 = 0.13$], or physical activity levels (Daily average METs) [$F(3, 26) = 0.75$, $p > 0.5$, Wilk's $\Lambda = 0.92$, partial $\eta^2 = 0.08$].

There was no significant difference in clinical characteristics, physical activity levels or cytokine levels between participants who did or did not require IV antibiotics in the 12-month follow-up period (Table 4.1).

4.5 Discussion

This is the first study in adults with CF to demonstrate that the level of physical activity following hospitalisation for an acute pulmonary exacerbation is significantly associated with the degree of systemic inflammation. A healthy BMI was also found to be an independent predictor of increased levels of physical activity. However, the hypothesis was not confirmed that levels of inflammation and physical activity following hospitalisation for an acute pulmonary exacerbation would predict time to next exacerbation.

The relationship between systemic inflammation and physical activity in CF has not been studied in detail in adults with severe lung disease. A previous study of a large number of adolescents with CF who were clinically stable found a non-significant negative relationship between exercise tolerance and serum immunoglobulin-G levels (31). The adolescents studied had well-preserved lung function (FEV_1 of 83.2 ± 18.0% predicted) (31), but the suggestion of an inverse relationship between systemic inflammation and exercise tolerance supports these findings in adult patients recovering from a pulmonary exacerbation who in general had moderate-severe CF-lung disease.

Study Report 1 assessed both systemic and airway inflammation concurrently and related these measures to physical activity. Participants were assessed following inpatient treatment for a pulmonary exacerbation, as this was the time-point when patients were

considered most likely to be at their best, having completed 10-14 days of IV antibiotics and relatively intense physical therapies. Study Report 1 was also interested in whether inflammation and physical activity levels following discharge could predict time to next pulmonary exacerbation.

Increased concentrations of systemic (but not airway) cytokines were found to be associated with decreased physical activity levels. Interestingly, no correlation was found between cytokine levels in sputum and plasma, suggesting that these represent distinct "compartments" in terms of the host immune response. These findings are consistent with earlier observations that demonstrated reduced exercise tolerance during pulmonary exacerbations is associated with increased levels of C-reactive protein (138, 158).

The novel finding of Study Report 1 is that systemic inflammation appears to persist even after aggressive in-hospital treatment for a pulmonary exacerbation. Although the reason for this is unclear one possibility is that this may be associated with the level of physical activity. In a previous study amongst healthy adults, a moderate to vigorous level of physical activity (measured via accelerometry) was shown to be associated with reduced C-reactive protein (15). This has also been seen amongst healthy older adults, where replacing a previously sedentary period with 30 minutes a day of moderate to vigorous activity was associated with a significant reduction in C-reactive protein (18). Conversely to this, other studies have shown that bouts of intensive exercise (such as marathon

running or weightlifting) increase levels of systemic inflammation (16, 117), which suggests a balance is important. This may be particularly important in a condition such as CF where a degree of background inflammation is already present (117).

Physical activity intensity needs to be considered as a potential variable in the relationship between physical activity and inflammation. The cut-point for mild physical activity was set at 3.0 METs based on the known relationship between systemic IL-6 and TNF-α and this level of activity in adults with CF (9), as used in other studies amongst adults with CF (76, 94, 96, 117, 182). This study found a low negative correlation between systemic inflammation and daily average ≥ 3 METs. Alternatively, a cut-point of 4.8 METs has also been used when examining physical activity in adults with CF (183). Had the 4.8 METs cut-point been used within this research program, it is possible that this would have influenced the outcome of the relationship between physical activity intensity and systemic inflammation - however this remains unknown as the data collected does not allow for this analysis to be conducted (the Sensewear software arbitrarily counted time spent at METs $\geq$ 3). However, the strongest correlation seen between systemic inflammation and physical activity within this research program was on the basis of *daily average* METs. This relationship which would still stand regardless of what METs cut point was used to establish either mild or moderate physical activity, maintaining the integral message of this research program. The intensity of physical activity *during hospitalisation* for the acute pulmonary exacerbation was not assessed,

but all patients were encouraged to participate in a tailored exercise regimen throughout their hospital stay.

Participants in this study performed on average only 1.7 METs/day following hospital discharge, which is classified as low level physical activity (184). The explanation for this low level of physical activity is likely multifactorial including lung disease severity, nutritional status, level of inflammation, and also psychosocial factors (including motivation, employment status, family commitments). All of these potential confounders need to be considered in future studies of exercise and physical activity in CF.

Interestingly, in this cohort FEV_1 at discharge did not predict physical activity levels (or time to next exacerbation), which suggests that reduction in systemic inflammation is at least as relevant to recovery of physical activity post-exacerbation as improvements in lung function. Although systemic inflammatory cytokine levels at discharge were good predictors of physical activity in the ensuing week, assessments of inflammation at one-month post-discharge were not repeated, which may have been informative. This concept is further explored in chapter six.

Over half of all patients had clinically deteriorated and needed further IV antibiotics by six months post-discharge, and two-thirds had deteriorated by the 12-month time-point, however physical activity was not found to be an independent predictor of time to next exacerbation. This is consistent with the findings of two previous studies in adults with CF that found physical activity levels as measured over 5-7 days in the outpatient

environment were unrelated to exacerbation frequency in either the preceding year (96) or over a three year follow-up period (99).

Further longer-term studies of systemic inflammation and physical activity in adults with CF are needed, which may identify opportunities to intervene with specifically tailored physical activity programs (185) that delay pulmonary deterioration. Body mass index positively correlated with daily average steps, which suggests that a healthy weight in adults with CF is an independent determinant of physical activity which has been demonstrated previously (186). There was a weak relationship between sputum IL-6 levels and BMI, but overall Study Report 1 findings suggest that nutritional status and inflammation in adults with CF are not strongly linked.

A limitation of this study is that physical activity or levels of inflammation were not assessed at the time of admission nor prospectively followed up to observe how these parameters may change with treatment. This would have allowed relative changes in physical activity and inflammation to be more comprehensively characterised and provide further information on causality. As mentioned, re-assessment at one-month post-discharge would also have been helpful, as by this time-point patients should be closer to their usual state of health and well-being (94). Additionally, a control group was not used due to the nature of this clinician-led study within the constraints of the hospital environment. Reference values are well-established in the literature for both measurement of physical activity via the Sensewear armband (122, 123), and for

measurement of systemic inflammatory markers (187). These considerations need to be factored into future studies of physical activity and inflammation in adults with CF.

In summary, Study Report 1 demonstrates that there is an association between reduced levels of physical activity and increased levels of systemic inflammation in adults with severe CF-related lung disease following intravenous antibiotic treatment for a pulmonary exacerbation. Second, neither levels of physical activity nor inflammation predict time to next pulmonary exacerbation in adults with CF following inpatient treatment for an acute exacerbation. These findings also reinforce the importance of maintaining a healthy BMI to allow adults with CF to engage in physical activity. These results could inform on a future randomised controlled trial examining the effect of a post-discharge physical activity program and its effect on systemic inflammation in adults with CF.

CHAPTER 5 STUDY REPORT 2

Increased Exercise Tolerance Post-Exacerbation is Not Associated with Decreased Systemic Inflammation in Cystic Fibrosis – An Observational Study.

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This report has been prepared for publication but has not yet been submitted.

Declaration of candidate contribution

For this co-authored manuscript, the candidate was involved in the experimental design, performed data acquisition, analysed the data, interpreted results, and drafted and critically reviewed the manuscript.

_____ 18/07/2019

Kate Burton / *Candidate and corresponding author* Date

_____ 18/07/2019

Prof Norm Morris / *Principal supervisor and co-author* Date

Study Report 1 (Chapter 4) demonstrated that systemic inflammation has an inverse relationship with physical activity levels in the first week after discharge from hospital following inpatient treatment for a pulmonary exacerbation in adults with CF. Time to next pulmonary exacerbation was not related to inflammatory markers and/or physical activity levels. Physical activity was shown to have a low positive correlation with disease severity; however, no sex or age-related differences were seen in either inflammation or physical activity. BMI was shown to have a positive relationship with physical activity, and an inverse relationship with pulmonary inflammation.

Study Report 2 (Chapter 5) is an extension of the first report which examined the relationship between inflammatory markers and exercise tolerance, both aerobic and strength based. Time to next pulmonary exacerbation and its relationship to inflammatory markers and/or exercise tolerance were also explored, in addition to BMI, age, sex and disease severity as covariates.

5.1 Abstract

Background & objective

Measuring exercise tolerance is integral in the holistic management of adults with CF, however the relationship between exercise tolerance and inflammation in CF is not well documented. This report evaluated the relationship between exercise tolerance and inflammation in adults with CF following a pulmonary exacerbation, including any differences related to sex, age, BMI or disease severity. Time to next pulmonary exacerbation was also investigated as an independent variable.

Methods

Adults with CF were included following hospitalisation for a pulmonary exacerbation and followed up for 12 months. Exercise tolerance was measured within 48 hours prior to discharge via 6MWT, MST-25 and isometric quadriceps strength. Inflammation was measured via plasma and sputum concentrations of cytokines IL-6, IL-8 and TNF-α. The relationship between exercise tolerance and inflammatory markers was evaluated using appropriate parametric and non-parametric statistics.

Results

Participants included 32 adults with CF (18 (56%) male, aged 28.8 ± 8.8 years, FEV_1 59.4 ± 23.0% predicted) at completion of hospitalisation for an acute pulmonary exacerbation. No associations were found between plasma or sputum cytokines and measures of exercise tolerance (6MWD, MST-25, quadriceps strength). Positive correlations were

found for 6MWD and MST-25 with FEV_1 (6MWD: r = 0.48, p = 0.005; MST-25: r = 0.81, p < 0.001) and FEV_1 % predicted (6MWD: r = 0.43, p < 0.05; MST-25: r = 0.64, p < 0.001). Males had greater quadriceps strength than females (*t* (30) = 3.779, p = 0.001). At 12 months there was no statistically significant difference in time to re-exacerbation based on an individual's exercise tolerance [F (3, 26) = 0.52, p < 0.6, Wilk's Λ = 0.95, partial η^2 = 0.05].

Conclusion

These results indicate that the inflammatory status of an adult with CF at time of discharge post-pulmonary exacerbation has limited ability to predict exercise tolerance (aerobic or strength-based). Exercise tolerance did not influence time to re-exacerbation. Disease severity (FEV_1 % predicted) showed a positive correlation with both measures of aerobic exercise tolerance, however MST-25 appears to be a stronger predictor of lung function compared to 6MWD. Sex and age were not found to be related to exercise tolerance.

5.2 Introduction

Adults with CF who experience a pulmonary exacerbation are encouraged to participate in exercise as a component of their inpatient physiotherapy program (28), however little is known on the relationship between exercise tolerance and inflammation in adults with CF. The benefits of exercise in adults with CF are well established, including improved sputum clearance (27), increased muscle hypertrophy (29), improved bone mineral density (30) and enhanced insulin sensitivity (29), accompanied by improvements in lung function (12). In addition, exercise tolerance has been identified as an independent predictor of morbidity and mortality in adults with CF (31).

In children with CF, an acute bout of exercise has been shown to be associated with increases in systemic inflammatory TNF-α and IL-6 (8). As children move into adolescence, those who decrease in exercise tolerance have been shown to experience a higher mortality rate, a steeper decline in pulmonary function and a greater increase in inflammatory markers (31) compared to their peers who maintain a higher level of exercise tolerance.

In healthy adults, *exercise training* has been found to produce a short-term, anti-inflammatory response (19). In the healthy population an *acute bout of exercise* causes liver, fat and muscle metabolism and a subsequent increase in systemic IL-6 and IL-8 (188, 189). These levels of inflammation return to normal within a few hours of ceasing exercise (22, 188).

Both cross-sectional and longitudinal exercise training studies have demonstrated the long-term anti-inflammatory effect of regular exercise (16). The first study report of this thesis (Chapter 4) demonstrated that lower levels of systemic inflammation were associated with greater levels of physical activity after an exacerbation. It is also well-established that improvements in physical activity are associated with improvements in exercise tolerance, in both the healthy population (190) and amongst both children and adults with CF (32, 76, 96).

Currently, the relationship between exercise tolerance and inflammation in adults with CF remains unclear. Preliminary evidence suggests that low levels of inflammatory markers are associated with increased exercise tolerance in adults with CF (i.e. an inverse relationship). Bradley et al. (2001) found a strong inverse relationship between the *change* in C-reactive protein over the course of an admission and MST amongst adults with CF. However, the relationship between C-reactive protein and MST at a particular time point (either pre-IV antibiotics, or post-14 days of IV antibiotics) was not found to be significant. Hence it is unclear if there is a relationship between exercise tolerance and inflammatory markers themselves, or between exercise tolerance and the improvement in an individual's inflammatory status in response to treatment over the course of a hospitalisation.

In the healthy population there is a significant difference in aerobic exercise tolerance between the sexes from as early as adolescence (191, 192), with this disparity continuing through to adulthood (98, 193). Several factors have been identified

including differences in morphology, and differences in hormonal profiles (98). In prepubescent individuals with CF however, no difference exists in aerobic exercise tolerance between males and females (95), but this changes during puberty (95) to align with the findings of the healthy population. Initial evidence appears to suggest this difference in exercise performance between males and females with CF continues into adulthood (96, 194).

In other chronic respiratory disease populations such as COPD, reduced quadriceps strength has been shown to be related to exercise tolerance and physical activity (195, 196). To date, no differences have been found in relation to sex when comparing quadriceps strength in adults with cystic fibrosis (76). Adults with CF have however been found to have significantly weaker skeletal muscle strength than their age-matched controls, due to their reduced lean muscle mass (88).

The aim of this report was to examine the relationship between systemic and pulmonary inflammation and exercise tolerance in adults with CF following hospitalisation for an acute pulmonary exacerbation. It was hypothesised that those adults with CF with increased levels of inflammation would demonstrate reduced exercise tolerance following hospitalisation for an acute pulmonary exacerbation and that inflammation and reduced exercise tolerance would predict the time to next pulmonary exacerbation. Secondary aims were to explore any age, sex, BMI and disease severity-related differences between inflammation and exercise tolerance, and to look

at the interrelationship between different measures of exercise tolerance.

The specific research questions were:

1. Is there an association between reduced exercise tolerance and increased levels of inflammation in adults with CF following hospitalisation for an acute pulmonary exacerbation?

2. Does inflammation or exercise tolerance predict time to next pulmonary exacerbation?
3. Is age, sex, BMI and/or disease severity related to either inflammation or exercise tolerance?

5.3 Methods

Design

This was a single-centre cohort observational study, conducted prospectively (HREC/12/QPCH/289 and SSA AU/3/8011116). Prior to recruitment to this study all participants provided written informed consent.

Participants

Participants were recruited consecutively over 12-months. Eligibility for inclusion was as follows: adults with a formal diagnosis of CF (aged 18 years and above) who were

admitted for inpatient hospital treatment of a pulmonary exacerbation. Exclusion criteria included those who were discharged with home-based IV antibiotic treatment, or those who were pregnant.

Procedure

Participants were included in this study at the completion of an inpatient admission for a pulmonary exacerbation at The Prince Charles Hospital, Brisbane. The definition of a pulmonary exacerbation at presentation was determined by the treating CF consultant, and was based on international consensus criteria as the presence of any of the following symptoms: increased sputum production, increased cough, chest pain, shortness of breath, loss of weight, loss of appetite, and/or a measured decline in pulmonary function (34).

Blood plasma and sputum samples were collected within 48-hours prior to discharge home from hospital. The candidate was responsible for participant recruitment, outcome measure collection (assisted by the CF physiotherapists), processing of samples, and data analysis. Medical registrars for the CF team collected all blood plasma samples.

Spontaneously expectorated sputum samples were collected by the CF physiotherapist and processed immediately. Participant demographic information was recorded, and respiratory function was measured within 48-hours prior to discharge. Forced expiratory volume in one second (FEV_1) was measured using standard spirometry (Jaegar Vyntus® Pneumo, Bonn, Germany), collected by an independent assessor.

Discharge from hospital was determined by self-reported improvement in the patient's symptoms and objective evidence of a reduction in C-reactive protein levels and improvement in lung parameters (FEV_1).

To monitor time to next pulmonary exacerbation (days) participants were followed-up for twelve months after discharge from hospital. Attendance at regular outpatient clinics and/or hospital admissions were monitored to measure time to next pulmonary exacerbation. If a participant did not experience a pulmonary exacerbation requiring IV antibiotics within 12 months of study completion this was recorded arbitrarily as 366 days.

Outcome measures

Plasma and sputum supernant concentration of IL-6, IL-8 and TNF-α were determined using in-house ELISA, performed in duplicate and then analysed (Fluostar Omega). Plasma and sputum samples were prepared for analysis as outlined in Study Report 1 (Chapter 4).

Exercise tolerance was determined using a 6MWT (126), an MST-25 (130), and isometric strength testing of the quadriceps muscle bilaterally (refer to Chapter 3.6.2 for a more detailed explanation) (139). A short demonstration session was performed prior to each test, and standardised instructions and encouragement were given. A physiotherapist experienced in the management of adults with CF conducted all exercise tolerance

testing. No blinding occurred as these measures were collected as a part of routine care. A minimum of 60 minutes rest occurred between each measure of exercise tolerance.

Participants completed the 6MWT within 48 hours prior to discharge according to the American Thoracic Society guidelines (172) (as outlined in Chapter 3.6.2). The test occurred in a 30-metre corridor with standardised encouragement. Heart rate, oxygen saturations, breathlessness (using the Modified BORG scale) and distance achieved were recorded both pre and post testing. Due to time constraints in the 48-hour period leading up to discharge only one 6MWT was conducted.

The MST-25 was conducted within 48 hours prior to discharge for each participant as outlined in Chapter 3.6.2. The test was undertaken in a 10-metre corridor where participants walked and/or ran at increasing speeds to the pace of the recording. At the end of each level standardised encouragement was given, and the participant was reminded that their speed needed to increase to match that of the increasing pace of the recording (131). Participants continued with the test until they either (a) failed to maintain the set pace on two consecutive beeps, or (b) opted to cease testing due to subjective reasons of shortness of breath or fatigue. Results are presented as distance (metres). Heart rate and pulse oximetry were measured before and after the MST using a finger probe (Datex, Ohmeda TuffSat, Madison, WI, USA). Participants were asked to rate their perceived exertion and breathlessness (Modified Borg Scale) both pre-and post-testing.

Quadriceps isometric strength was evaluated within 48 hours prior to discharge using hand-held dynamometry (Lafayette Manual Muscle Test System, Lafayette, IN, USA). This was based on the published protocol that exists for individuals with COPD (139) and the Wieboldt study (138), as outlined in Chapter 3.6.2. Each participant performed three maximal voluntary knee extension efforts for three seconds on each leg with standardised encouragement, with a resting period of at least 30 seconds between efforts. Strength (kilograms) was expressed as a percentage of each participant's body weight in order to standardise this measurement (175). For analysis, the mean of the two greatest peak force values was calculated.

Time to pulmonary re-exacerbation (days) was defined as the number of days following discharge from hospital until the participant experienced another acute pulmonary exacerbation that required inpatient treatment inclusive of IV antibiotics (158). Pulmonary exacerbation was diagnosed by the treating consultant in either the emergency department or outpatient CF clinic. This outcome was measured at the 12-month time point following discharge from hospital.

Statistical analysis

Q-Q plots and Shapiro-Wilk's test were used to determine whether continuous data were normally distributed. T-tests and the Mann-Whitney U test were used to examine the differences in data dependent on normality of distribution. Data are presented as mean and standard deviation, unless specified otherwise. Pearson's correlational

analyses (or Spearman's rho for non-parametric data as required) were conducted to examine the relationships between both sputum and plasma inflammatory markers (IL-6, IL-8 and TNF-α), measures of exercise tolerance (6MWD, MST-25 and quadriceps strength), age, sex, BMI and disease severity. Correlations were classified as low (r = -.30 to -.50), moderate (r = -.50 to -.70) or high (r = -.70 to -.90) (178). One-way multivariate analysis of variance (MANOVA) was conducted to assess the relationship between exercise tolerance and time to next exacerbation. Prior to performing correlation testing all non-normally distributed data was natural log transformed. Statistical analysis was performed using the SPSS statistical package v 22.0 (2015, IBM Corp., Armonk, NY, USA). A p-value of < 0.05 was considered statistically significant.

5.4 Results

Participant characteristics

Participants included thirty-two adults (18 male) aged ≥ 18 years with CF (age 28.8 ± 8.8 years, FEV_1 59.4 ± 23.0% predicted). Disease severity across the spectrum was represented by this population (mild n=11, moderate n=12, severe n=9). All participants screened and identified to participate agreed to participate.

Table 5.1 outlines participant demographics, exercise tolerance and plasma and sputum inflammatory cytokine results. Participants were followed up for 12 months. No associations (r > 0.3) were found between plasma or sputum cytokines and measures of exercise tolerance (6MWD, MST-25, quadriceps strength) (Table 5.2).

Sex-related differences

Participant demographics and outcome measures according to sex are detailed in Table 5.3. Whilst there was no sex-related difference in the 6MWD, male participants with CF had statistically significantly greater quadriceps strength (1.3 ± 0.2 kg/BW) following inpatient treatment for an acute exacerbation compared to adult females with CF (1.0 ± 0.2 kg/BW); (t (30) = 3.779, p = 0.001). Male participants were also older than their female counterparts (median age 31.9 years *versus* 24.9 years, p = 0.013) and had poorer lung function (mean FEV_1 49.2% predicted *versus* 72.5% predicted, p = 0.003) (Table 5.3). There were no other sex-specific differences in either cytokine concentrations or other exercise tolerance parameters (Table 5.4).

Relationship between exercise tolerance and disease severity

A positive correlation was found between 6MWD and both FEV_1 (r = 0.48, p = 0.005) and FEV_1% predicted (r = 0.43, p < 0.05) (Figure 5.1). MST-25 had a strong positive correlation with both FEV_1 (r = 0.81, p < 0.001) and FEV_1% predicted (r = 0.64, p < 0.001) (Figure 5.1). No relationship was found between either quadriceps strength or inflammatory markers and disease severity.

Table 5.1 Characteristics of participants on the basis of exacerbation status at 12 months

Characteristic	All (n=32)	Deterioration at 12 months		p value
		Yes (n=23)	No (n=9)	
Age (years)[#]	28.8 (18-62)	29.2 (18-62)	27.9 (21-38)	0.95
Male	18 (56%)	13 (54%)	5 (63%)	0.47
FEV_1 (% predicted)^	59.4 (23.0)	57.5 (24.5)	64.5 (20.5)	0.48
BMI (kg/m^2)^	21.8 (3.6)	21.7 (3.7)	22.0 (3.2)	0.84
6MWD (m)[#]	634 (450-800)	638 (450-800)	624 (520-750)	0.72
MST-25 (m) [#]	840 (420-1590)	830 (420-1510)	905 (430-1590)	0.44
Quadriceps strength (kg/BW)[#]	1.2 (0.7-1.6)	1.1 (0.8-1.6)	1.2 (0.7-1.5)	0.77
Plasma IL-6 (pg/ml) [#]	28.5 (14.4-145.1)	28.4 (17.4-101.4)	27.1 (14.4-40.0)	0.96
Plasma IL-8 (pg/ml) [#]	27.0 (14.7-145.1)	27.0 (14.7-145.1)	26.9 (18.4-105.9)	0.76
Plasma TNF-α (pg/ml) [#]	25.4 (13.6-73.5)	24.8 (15.8-73.5)	28.5 (13.6-39.6)	0.55
Sputum IL-6 (pg/ml) [#]	31.1 (16.8-66.75)	32.5 (16.8-66.8)	29.0 (20.7-38.3)	0.37
Sputum IL-8 (pg/ml) [#]	64.7 (25.4-533.6)	68.2 (25.4-533.6)	37.3 (26.9-279.2)	0.20
Sputum TNF-α (pg/ml) [#]	26.2 (19.2-35.2)	26.5 (20.0-31.6)	26.9 (19.2-35.2)	0.91

[#] Median (range), ^Mean (standard deviation).

Definition of abbreviations: 6MWD = six minute walk distance; BMI = body mass index; BW = body weight; FEV_1 = forced expiratory volume in one second; IL-6 = interleukin-6; IL-8 = interleukin-8; MST-25 = Modified Shuttle Test (25-level); TNF-α = tumour necrosis factor α

Table 5.2 Correlation co-efficients of plasma and sputum cytokines with measures of exercise tolerance

	6MWD	MST-25	Quadriceps strength
Plasma [pg/ml]			
Log IL-6	r = -0.01	r = -0.08	r = -0.23
	p = 0.97	p = 0.70	p = 0.21
Log IL-8	r = 0.13	r = 0.22	r = -0.05
	p = 0.47	p = 0.22	p = 0.78
Log TNF-α	r = -0.03	r = 0.08	r = -0.19
	p = 0.88	p = 0.67	p = 0.30
Sputum [pg/ml]			
Log IL-6	r = -0.29	r = -0.11	r = 0.29
	p = 0.12	p = 0.57	p = 0.12
Log IL-8	r = -0.11	r = 0.04	r = -0.28
	p = 0.54	p = 0.83	p = 0.13
Log TNF-α	r = 0.13	r = 0.08	r = 0.17
	p = 0.49	p = 0.67	p = 0.37

Definition of abbreviations: 6MWD = Six Minute Walk Distance; IL-6 = interleukin-6; IL-8 = interleukin-8; MST-25 = Modified Shuttle Test (25-level); TNF-α = tumour necrosis factor α.

Interrelationship between measures of exercise tolerance

6MWD positively correlated with MST-25 (r = 0.51, p = 0.002). A low-level positive correlation was seen between quadriceps strength and MST-25 (r = 0.40, p = 0.02). No significant relationship was seen between quadriceps strength and 6MWD (r = 0.22, p > 0.1).

Table 5.3 Participant demographics and outcome measures

Characteristic	Males (n = 18)	Females (n = 14)
Age (years)[#]	31.9 (18-62)	24.9 (20-35)
FEV_1 (% predicted)^	49.2 (19.6)	72.5 (20.8)
BMI (kg/m^2)^	21.6 (2.8)	21.5 (4.4)
6MWD (m)^	623 (91)	648 (86)
MST-25 (m)^	895 (420)	870 (208)
Quadriceps strength (kg/BW)^	1.3 (0.2)	1.0 (0.2)
Time to next IV antibiotics (days)^*	178 (138)	197 (117)
Sputum IL-6 (pg/ml)^	33.9 (13.1)	29.3 (5.0)
Plasma IL-6 (pg/ml)^	31.2 (18.7)	28.0 (20.7)
Sputum IL-8 (pg/ml)^	85.8 (84.8)	180.6 (156.9)
Plasma IL-8 (pg/ml)^	38.1 (29.4)	26.1 (33.6)
Sputum TNF-α (pg/ml)^	26.5 (3.3)	26.4 (4.4)
Plasma TNF-α (pg/ml)^	27.2 (13.9)	24.8 (16.1)

[#] Median (range), ^Mean (standard deviation), *n=23 (9 subjects did not require IV antibiotics in the follow-up period).

Definition of abbreviations: : 6MWD = six minute walk distance; BMI = body mass index; BW = body weight; FEV_1 = forced expiratory volume in one second; IL-6 = interleukin-6; IL-8 = interleukin-8; IV = intravenous; MST-25 = Modified Shuttle Test (25-level); TNF-α = tumour necrosis factor α

Table 5.4 Correlation co-efficients of plasma and sputum cytokines with sex

	Sex
Plasma [pg/ml]	
Log IL-6	r = -0.02 p = 0.93
Log IL-8	r = 0.06 p = 0.73
Log TNF-α	r = -0.01 p = 0.98
Sputum [pg/ml]	
Log IL-6	r = -0.22 p = 0.23
Log IL-8	r = 0.37 p = 0.04
Log TNF-α	r = -0.06 p = 0.74

Definition of abbreviations: IL-6 = interleukin-6; IL-8 = interleukin-8; TNF-α = tumour necrosis factor α.

Differences related to time to next pulmonary exacerbation

In the year following enrolment, nine participants did not require further IV antibiotics. For the 23 subjects that did experience a pulmonary exacerbation within the 12-month follow-up period, the median time to next IV antibiotics required was 129 (range 13-366) days. At 12 months there was no statistically significant difference in time to re-exacerbation based on an individual's exercise tolerance [F (3, 26) = 0.52, p < 0.6, Wilk's

$\Lambda = 0.95$, partial $\eta^2 = 0.05$] (Table 5.1). Age, sex, BMI and disease severity were not related to time to next pulmonary exacerbation ($p > 0.14$).

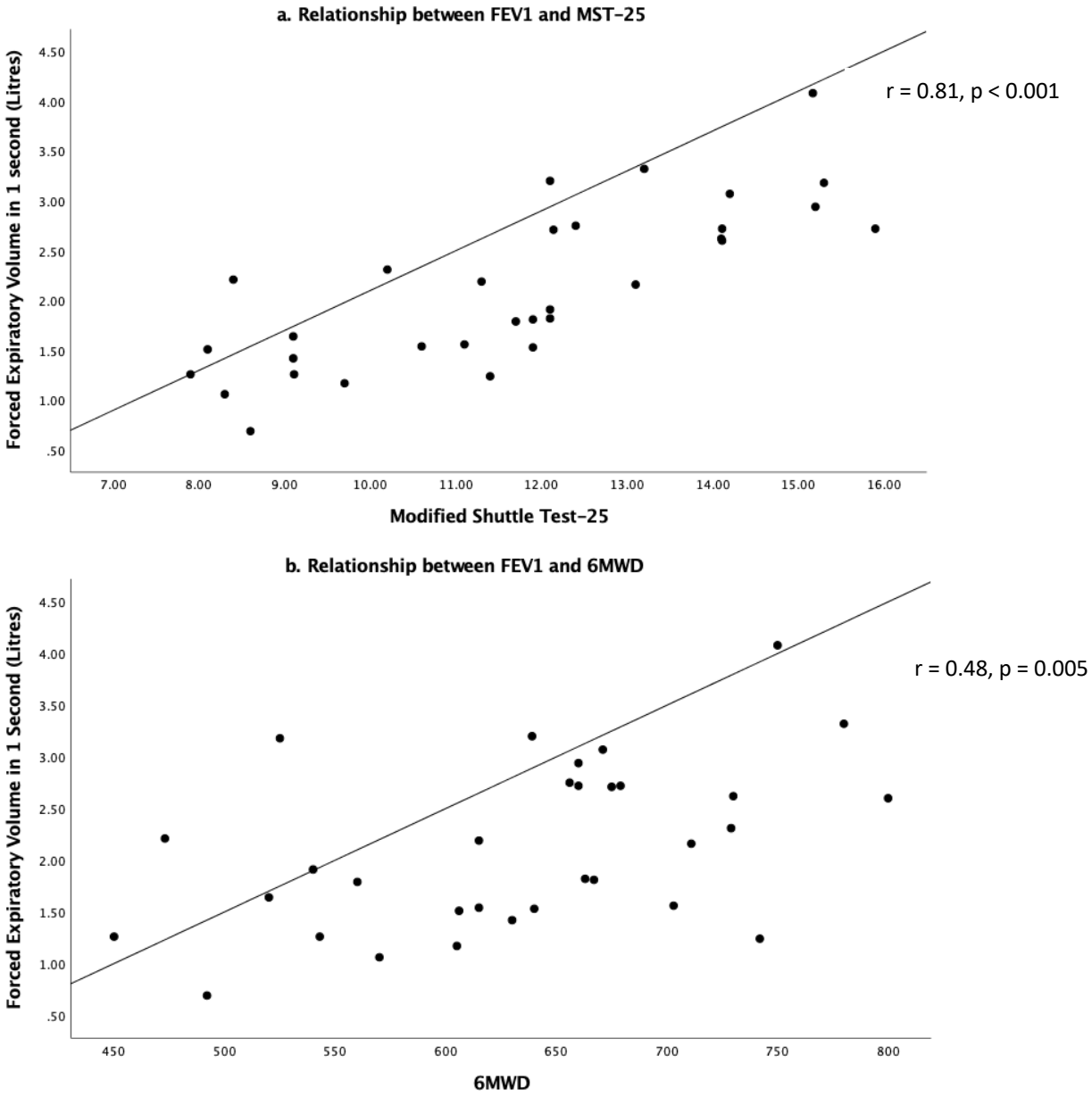

Figure 5.1 Relationship between FEV_1 and exercise tolerance

Definition of abbreviations: 6MWD = Six Minute Walk Distance; FEV_1 = Forced Expiratory Volume in One Second; MST-25 = Modified Shuttle Test 25.

5.5 Discussion

This study found that exercise tolerance as measured using the 6MWT and the MST-25 is not related to markers of inflammation (either systemic or pulmonary) following an inpatient admission for a pulmonary exacerbation in adults with CF. Despite the known involvement of cytokine release precipitating immune function in CF, these results appear to indicate that the inflammatory status of an adult with CF at the time of discharge post exacerbation is not related to exercise tolerance.

Interestingly Bradley et al. (158) found a strong inverse relationship between the treatment induced change in C-reactive protein over the course of an admission and the modified shuttle test. These findings suggest that an individual's exercise tolerance was related to how well their body responds to hospital treatment from a systemic inflammation perspective. Similar to the results of the current study, Bradley et al. reported that the relationship between C-reactive protein and the modified shuttle test at a particular timepoint (either pre-IV antibiotics, or post-14 days of IV antibiotics) was not significant. Bradley et al. (158) (n = 20) studied a smaller sample of participants who had comparable lung function and exercise tolerance as the current study, however were moderately younger (23 ± 5 years vs 28 ± 8 years). Further exploration is needed in an adult CF population to determine whether there is a correlation between treatment induced change in inflammation (specifically IL-6, IL-8 and TNF-α) over the course of an admission and exercise tolerance. It is of note to mention that comparing specific inflammatory markers (IL-6, IL-8 and TNF-α) with C-reactive protein is not ideal due to C-

reactive protein being indicative only of the presence of inflammation, not its source or location (197).

Study Report 1 in this research program demonstrated no statistically significant difference in time to re-exacerbation based on an individual's inflammatory markers (neither systemic nor pulmonary). This current report, Study Report 2, also demonstrated no difference in re-exacerbation rate at 12 months following discharge based on an individual's exercise tolerance in the adult CF population. This is surprising given the positive association found between exercise tolerance and disease severity as measured using FEV_1 and/or FEV_1 % predicted. It is well established in the literature that a decline in FEV_1 is a strong predictor of the need for readmission (198).

FEV_1 was found to be a stronger predictor of MST-25 than 6MWD in this current study. This is comparable to the findings of others in people with both CF (76) and COPD (199), where 6MWD is often poorly associated with lung function when compared to measures of aerobic function. By comparison, aerobic exercise (as induced by MST-25 and comparable exercise with graded increments) is well established in the literature to have a significant correlation with FEV_1 in the adult CF population (102).

Adult males with CF were found to have a significantly greater quadriceps strength (as a percentage of bodyweight) than their female counterparts. Surprisingly, this contrasts to a previous study by Troosters et al. (76) which found no difference in quadriceps strength between male and female adults with CF.

Given the participants in the current study were older (32 ± 8 years vs 26 ± 8 years) and had more severe disease (FEV_1 59.4 ± 23% predicted vs 65 ± 19% predicted) than the Troosters study, it could have been hypothesised that the males and females of the current study would have had similar indices of exercise tolerance. Moreover, despite the known disparity between pubescent males and females in levels of physical activity (95), it was interesting to find no association between sex and aerobic exercise tolerance in this current study. Furthermore, in the Troosters study quadriceps strength was found to be a significant contributor to the variance in the 6MWD (76), whereas in the present study no correlation was found between quadriceps strength and 6MWD. This research program did, however, find a significant but low-level positive correlation between quadriceps strength and MST-25 ($r = 0.40$, $p = 0.02$), suggesting that adults with CF with greater quadriceps strength perform better on this specific form of aerobic exercise testing. This does not translate to 6MWD, possibly due to the known ceiling effect of the 6MWT for adults with CF.

One other potential consideration for the interplay between inflammation and exercise tolerance is possible abnormalities in skeletal muscle oxidative metabolism in individuals with CF. Werkman et al. (81) demonstrated that adolescents (aged 12-18 years) with mild CF who were clinically stable had no intrinsic metabolic constraints or abnormalities in oxygenation in response to exercise, when compared with healthy age-matched control subjects. However, in a separate study it was found that adolescents aged 12-18 ($n = 149$) with CF with a higher immunoglobulin-G level had a significantly lower exercise tolerance (31). To date it is not yet known if this difference continues

amongst adults with an underlying chronic inflammatory state, however the results of this research program suggest that this difference may not extend to adulthood, at least in those recently discharged following a pulmonary exacerbation.

A major limitation of the current study is that neither levels of inflammation nor exercise tolerance were assessed at time of admission and prospectively followed so as to allow a time course evaluation of these parameters over the course of treatment. Such an analysis would have enabled a more comprehensive characterisation of relative changes in inflammation and exercise tolerance and may have provided information on potential causality. A further limitation is that re-assessment of inflammation and exercise tolerance did not reoccur post-discharge, as this would have given an insight into inflammation and exercise tolerance at a time when participants are closer to their "normal" state of well-being (94). These are considerations that should be included in future exploratory studies of exercise and inflammation in adults with CF.

In summary, this study demonstrated that levels of inflammation in adults with CF following treatment for a pulmonary exacerbation do not correlate with measures of exercise tolerance. Furthermore, inflammation, exercise tolerance, age, sex, BMI and disease severity could not predict time to next pulmonary exacerbation. These findings suggest that the commonly used outcome measures of 6MWD and MST-25 for exercise tolerance are not necessarily reliable indicators of the impact of CF and its treatment. Measures of exercise tolerance (6MWD and MST-25) did have a positive correlation with FEV_1, and MST-25 was found to be a stronger predictor of FEV_1 compared to 6MWD. No

relationship was found between BMI and measures of exercise tolerance. Male participants were found to have greater quadriceps strength than their female counterparts, however no other sex-related differences were found between inflammation and exercise tolerance.

CHAPTER 6 GENERAL DISCUSSION

Cystic fibrosis is the most common autosomal recessive genetic disease in Australia (200), characterised by increased infection and inflammation (7), increased production of respiratory secretions, and decreased exercise tolerance (3). For adults with this disease, a high symptom-burden and the need for frequent and lengthy inpatient hospitalisations can markedly impact upon quality of life (3). Incorporating physical activity into daily routine and improving exercise tolerance are fundamental to physiotherapy treatment in CF and have been shown to improve sputum clearance, improve lung function and lead to skeletal muscle hypertrophy (27, 29). Adults with CF are known to have increased levels of circulating inflammatory markers compared to healthy individuals (8-10), however at present little is known about the relationship between physical activity or exercise tolerance and inflammation (either systemic or pulmonary) in adults with CF.

This thesis examined concepts related to inflammation, physical activity and exercise tolerance in a clinical adult CF population and is divided into three stages. The first stage explored the relationship between inflammation and physical activity in adults with CF. Physical activity as a predictor of time to next pulmonary exacerbation was also examined. The second stage of the thesis sought to explore the relationship between inflammation and exercise tolerance in adults with CF. Exercise tolerance as a predictor of time to next pulmonary exacerbation was also examined. The third stage explored

the interrelationship between inflammatory markers, physical activity levels, exercise tolerance, age, sex, BMI and severity of lung disease.

Using the same cohort, one observational study incorporating quantitative design was undertaken in this program of research. Two separate but related reports, based on three stages were produced (as outlined in Chapter 1). The following chapter presents the main findings from these two reports, incorporating synthesis with current literature and the potential implications of the findings for clinical best practice. Limitations of the research program and considerations for future research are also discussed in detail.

6.1 Summary of study findings

6.1.1 Stage 1 findings

The aim of Stage 1 of this research program was to examine the relationship between pulmonary and systemic inflammation and *physical activity* in adults with CF in the first week following discharge from hospital following a pulmonary exacerbation. Plasma concentrations of IL-6, IL-8 and TNF-α were found to correlate negatively with both daily average METs and daily average ≥ 3 METs. Plasma cytokines had no significant relationship with daily average steps; however, a low positive correlation was demonstrated between sputum TNF-α and daily average steps.

The secondary aim of Stage 1 was to establish if inflammation and reduced physical activity would predict time to next pulmonary exacerbation. In the 12-month follow-up period 23 participants (74%) required further IV antibiotics due to subsequent pulmonary exacerbation. There were no differences in clinical characteristics, activity levels or cytokine levels between participants who did or did not require further IV antibiotics in the 12-month follow-up period.

Median time to next IV antibiotics required was 129 days. At both 6 months and 12 months there was no statistically significant difference in time to re-exacerbation based on a participant's systemic inflammatory status at discharge or physical activity levels (daily average METs), demonstrating that neither inflammation nor physical activity predicted time to next pulmonary exacerbation in this cohort of adults with CF following acute inpatient treatment.

6.1.2 Stage 2 findings

The aim of Stage 2 of this research program was to examine the relationship between pulmonary and systemic inflammation and *exercise tolerance* in adults with CF at the end of hospital treatment for a pulmonary exacerbation. It was hypothesised that adults with CF with increased levels of inflammation would demonstrate reduced exercise tolerance following hospital treatment for a pulmonary exacerbation, however this hypothesis proved to be incorrect. No associations were found between plasma cytokines and measures of exercise tolerance (6MWD, MST-25, quadriceps strength).

Further to this, it was hypothesised that inflammation and reduced exercise tolerance would predict time to next pulmonary exacerbation, however again, this hypothesis was proven to be incorrect. At 12 months there was no difference in time to re-exacerbation based on exercise tolerance.

6.1.3 Stage 3 findings

The aim of Stage 3 of this research program was to investigate the interrelationship between inflammatory markers, physical activity levels, exercise tolerance, age, sex, BMI and severity of lung disease (FEV_1 % predicted). In Study Report 1 daily average steps were found to have a positive relationship with disease severity (FEV_1), demonstrating that adults with CF with more severe disease participate in less physical activity than their peers with more well-preserved lung function. No sex-specific differences in either cytokine concentrations or physical activity parameters were found.

Exercise tolerance was found to be related to disease severity, whereby the 6MWD had a moderate positive correlation with both FEV_1 and FEV_1 % predicted and the MST-25 had an even stronger positive correlation with both FEV_1 and FEV_1 % predicted. Not surprisingly, 6MWD had a moderate positive correlation with MST-25. Quadriceps strength had a low positive correlation with the MST, but was not found to be associated with 6MWD, nor with disease severity. This was interpreted as adults with CF with more

severe disease having a lower aerobic exercise tolerance than their peers with more well-preserved lung function, but not necessarily reduced skeletal muscle strength.

In this research program male participants had significantly greater quadriceps strength following inpatient treatment for an acute exacerbation than their female counterparts ($p = 0.001$). Males were also significantly older with lower lung function (mean FEV_1 % predicted), however no other sex-specific differences were found.

Physical activity was found to be related to BMI in adults with CF, with a low positive correlation found between daily average steps and BMI. BMI was also found to be related to pulmonary inflammation, with a moderate negative correlation found between BMI and sputum IL-6, indicating that adults with CF with a lower BMI had higher levels of the inflammatory marker IL-6 in their sputum. BMI was demonstrated to have no relationship with either IL-8 or TNF-a (in both plasma and sputum).

6.2 Clinical implications

From this research program several clinical implications are able to be drawn and will be discussed in later sections. This research program discovered a relationship between inflammation, BMI and physical activity. As such, consideration of both the inflammatory profile and BMI of an adult with CF needs to occur when encouraging physical activity, irrespective of sex. Consideration needs to be paid to the difference between exercise tolerance measures and physical activity behaviours – as what an

adult with CF *can* do under clinical testing conditions appears to be in contrast to the level of physical activity performed outside of the inpatient environment. Finally, the promotion of physical activity programs comprised of both aerobic and strengthening exercises should be considered for adults with severe CF-related disease.

6.2.1 Clinical implication 1 –The inflammatory profile of an adult with CF needs to be considered when introducing physical activity programs

Health professionals should consider the inflammatory profile of an adult with CF when working collaboratively with the individual to enhance their participation in physical activity, both in the inpatient and outpatient hospital environments. This research program found that for adults with CF following an exacerbation, individuals with a greater degree of systemic inflammation were less physically active. Given there was no relationship between the degree of inflammation and exercise capacity measured in the clinical setting, inflammatory markers may be a more sensitive measure of physical activity. Hence in adults with CF the degree of systemic inflammation appears to be related to how much physical activity these individuals actually do on a daily basis, not how much they *can* do under clinical testing conditions. It is possible that the degree of systemic inflammation may play a role in how physically active the individual *actually is* outside the hospital environment. It is also possible that the level of systemic inflammation *itself* may be impacting on the individual's ability to be physically active.

Clinically this means that when the health professional is encouraging adults with CF to increase their habitual physical activity, recognition and consideration of the inflammatory profile of an individual may need to be taken into account.

6.2.2 Clinical implication 2 – Differences exist between physical activity capability (i.e. exercise tolerance) and behaviour (i.e. physical activity) in adults with CF

Physical activity programs are routinely prescribed by health professionals for adults with CF following an inpatient admission based on exercise tolerance at time of discharge – however this research program indicates that there is minimal correlation between the two. It appears that in adults with CF, at the time-point following inpatient hospital treatment for an acute exacerbation, systemic inflammation correlates with physical activity, but does not correlate with exercise tolerance. Measurement of exercise tolerance is not a surrogate measure of physical activity. In other words, just because individuals with CF have a certain exercise tolerance as measured by the 6MWD or the MST-25 – this may not then translate into a higher level of physical activity. This raises the broader question of what drives physical activity behaviours, and where does inflammation fit into this picture?

Prepubescent healthy children are naturally active, often enhanced by physical activity encountered within schooling, group sports and recreational activities (201). As children

reach puberty and enter adolescence physical activity levels begin to decline, and continue to decline throughout the lifespan (202). This pattern is also reflected in the CF population, as detailed in Chapter 2. In healthy adults participation in physical activity has been found to be determined by a complex combination of motivations, abilities and opportunities (203, 204), coupled with self-efficacy and past behaviours (205). While adherence with treatments (including physical activity) has been thoroughly explored in both children and adults with CF (206-208), studies specifically exploring physical activity behaviours in adults with CF are lacking.

Perceptions of physical activity have been explored in adolescents with CF with an overall positive perception of the benefits of physical activity upon their health (209-211). Recurrent themes of worsening illness narrative and lack of parental support, however, influence the perception of physical activity in adolescents with CF (209-211). For adults with CF it could be hypothesised that physical activity behaviour is driven by their intrinsic motivation, ability and opportunity, however the progression of their disease and increasing symptom burden as they age (47) poses additional barriers to being physically active, despite what formal exercise testing suggests they are able to do.

6.2.3 Clinical implication 3 – Maintaining a healthy BMI appears to be important for maintenance of physical activity for adults with CF

When prescribing physical activity health professionals should monitor BMI and be aware of the implications that a low BMI may have on physical activity levels for adults with CF following an acute exacerbation. This research found that individuals with a low BMI had the lowest levels of physical activity and the greatest inflammatory response. These results could be interpreted as maintenance of a healthy BMI appearing to be a 'protective factor'. Typically, a number of deleterious consequences of CF effects BMI as a person with CF ages (2, 212). This includes issues such as pancreatic insufficiency and the consequent malnutrition that may occur, CF-related diabetes, and CF-related liver disease (212). These processes have an impact on the maintenance of an individual's BMI (212-214).

One factor that may contribute to maintaining a healthy BMI is maintenance of lean muscle mass. Maintenance of lean muscle mass, via a combination of both resistance and aerobic exercises (215), and diet (212), may equip an adult with CF to better tolerate the consequences of inherent infection and inflammation (216). This research program found a relationship between a lower BMI and higher levels of pulmonary inflammation, however no relationship between quadriceps strength and either inflammation or BMI, suggesting that inflammation appears to be related to BMI irrespective of lower limb strength. Best practice does however encourage the consideration of body composition in addition to BMI when promoting nutrition within

adults with CF (217). The consequences of obesity in regard to prognosis are, although rare in CF, equally poor to that of malnutrition (217). Therefore dietetic care in CF should aim to maintain or improve muscle mass instead of focusing solely on increasing BMI (217).

This would suggest that the management of CF in all facets should be multidisciplinary. Health professionals should work closely with CF-dietitians, among others, to ensure the BMI of adult with CF is not being impacted negatively by the expectations of the physical activity that is being encouraged. BMI should be factored in as a consideration when prescribing physical activity for adults with CF following an acute exacerbation.

6.2.4 Clinical implication 4 – Sex is not a factor when prescribing physical activity programs for adults with CF – focus instead on individual physical activity behaviours

The research program found that sex was unrelated to physical activity and exercise tolerance in adults with CF. Additionally, while adult males with CF have greater quadriceps strength than their female counterparts, this did not translate to a difference in function (i.e. physical activity levels) nor performance (for example in 6MWD or MST-25). This is surprising as the greater decline in lung function in females with CF with age is well-recognised (218). However, these findings suggest that sex appears unrelated to physical activity or exercise tolerance.

The lack of difference between the sexes in regards to physical activity differs to that of previous studies where adult females with CF were found to be less physically active than their male counterparts (77, 94, 96, 97) (Chapter 2.4.4). The reason for this remains unclear, although it may be a result of how physically active the female participants in this research program were compared to previous studies, despite being of similar age, BMI and disease severity (FEV_1% predicted) (77, 94, 96). Females in this research program were slightly more active than the female participants in the Savi et al. study (mean daily average METs 1.7 ± 0.3 *versus* 1.6 ± 0.2) (96). The other two comparable studies provided overall (male + female) mean time spent performing physical activity, however did not break this down to sex-related means, making direct comparison for female participant cohorts difficult (77, 94). Despite this, total cohort (male + female) mean time spent performing mild to moderate physical activity of participants in this research program was similar to that of the Ward et al. study (94). Cox et al. used a different delineation for physical activity (≥ 4.8 METs), ruling out a direct comparison (77).

Sex differences are known in CF; for example, females decrease physical activity levels during adolescence compared to their male age-matched peers (95), and life expectancy for females with CF is lower than that of their male counterparts (219). Findings from this research program in adults with CF supports the concept that sex is irrelevant when it comes to prescription of and encouragement of physical activity. Instead the focus should be on positively influencing and encouraging healthy physical activity behaviours. When considering behavioural model constructs where motivations, abilities and

opportunities are thought to drive physical activity behaviours (203, 204), it is possible that sex-related differences may exist for adults with CF. Past exposure to different types of physical activity or sport is likely to affect an individual's motivation for participation in their prescribed physical activity program (204). Additionally, roles and responsibilities of an individual within their family unit and varying levels of family support may affect the opportunity for exercise (204). This qualitative research was however, beyond the scope of this thesis, but would be beneficial to be further explored in a future study. CF care teams should be encouraged to thoroughly discuss each individual's motivations, abilities and opportunity for exercise in order to positively influence the behaviour that drives physical activity.

6.2.5 Clinical implication 5 – Physical activity prescription for adults with severe CF-related lung disease should include strength-based training

Adults with CF with severe lung disease (FEV_1 < 40% predicted) do not appear to have the same limitations when it comes to performance of strength-based exercises, as skeletal muscle strength (specifically quadriceps) was not found to be associated with disease severity. This is in contrast with aerobic exercise, where lung disease severity had a negative correlation with exercise tolerance as demonstrated on both the 6WMT ($r = 0.43$, $p < 0.05$) and the MST-25 ($r = 0.64$, $p < 0.001$). These findings suggest that adults with severe CF-related disease could potentially benefit from physical activity programmes prescribed with a specific focus on strength training.

CF care teams (particularly physiotherapists working in this field) should consider *how* to encourage adults with CF to increase their physical activity following an acute exacerbation. The optimal physical activity prescription for people with CF has not been established (28). Traditionally, physiotherapists working with adults with CF prescribe an aerobic-based physical activity program derived from exercise tolerance outcome measures (28). However, the findings of the current study may prompt physiotherapists to consider prescribing physical activity programs that include a strength-based component for adults with severe CF-related lung disease. This is in line with expert consensus recommendations for children and adults with CF which recommend a combination of aerobic and strength-based physical activity on a daily basis (220).

6.3 Strengths and limitations

A number of strengths and limitations must be acknowledged when discussing the merits of this research program. Strengths include the recruitment of a clinically relevant cohort that demonstrates diversity in regard to age and disease severity, and the use of specific inflammatory markers in both blood and sputum. Limitations include the lack of blinding in outcome measurement, the lack of data regarding participant anti-inflammatory therapies, and the lack of potentially confounding data encompassing disease expression and available therapies.

6.3.1 Strength 1 – Inclusion of a diverse clinical population

A major strength of this research program was the recruitment of a diverse clinically relevant *adult* CF cohort. This research program included adults with CF who were older and with more severe lung disease than what has previously been reported (8, 9, 31, 76, 138, 158, 221); an indication of the changing landscape in CF. In Australia adults with CF now have a longer life expectancy with a median age of 35.6 years, an increase of almost 10% from two years prior (44). However with longer life comes the cost more severe co-morbidities including CF-related liver disease, CF-related diabetes and osteoporosis (2, 44).

This change in the co-morbid profile of CF is expected to continue to change over the next decade or more (222), with the advent of CFTR modulators extending life expectancy for individuals with CF (223). It is worth noting that no individuals recruited to this research program received CFTR-modulators during the course of 12-month follow-up, therefore the potential effect of CFTR modulators on systemic inflammation is not a confounding factor for this research program. Consequently, the findings of this research program, due to the diverse range of age and disease severity of participants, are widely applicable to all adult CF cohorts regardless of local area population nuances.

6.3.2 Strength 2 – Investigation of specific inflammatory markers

Another strength of this research program was the investigation of specific inflammatory markers IL-6, IL-8 and TNF-α in both blood and sputum. By measuring IL-6, IL-8 and TNF-α specifically it was possible to more accurately explain not only *how* this inflammation is occurring (i.e. produced by muscle, produced in response to sepsis, etc. as per Chapter 2.3.2), but also *where* in the body the inflammation was taking place; specifically, in the lungs or more broader systemically.

This is in contrast to what is typical of clinical research in CF, where C-reactive protein has been widely used as a measure of systemic inflammation. C-reactive protein is often the inflammatory marker of choice as it is routinely measured during infective exacerbations and reported within routine inpatient pathology (224), and therefore requiring no additional laboratory work. As previously mentioned in Chapter 5.5, the use of C-reactive protein as an inflammatory marker has limitations as it provides no information in regard to how or where the inflammation is occurring within the body (197).

6.3.3 Limitation 1 – Lack of assessment blinding

The first potential limitation is that during exercise tolerance outcome measurement tests assessors were physiotherapists known to participants. It could be suggested that conducting exercise tolerance measures using an assessor unknown to participants may

have provided measures that were more independent. However, the use of a senior physiotherapist from the CF care team may have also influenced participants to work harder during outcome measure testing. This is not necessarily a negative outcome as exercise tolerance measures were aimed at achieving the best performance of participants.

In the clinical environment it was not possible to use an independent assessor of exercise tolerance; as is often the case in 'real world' clinical environments. In the participating facility one senior physiotherapist within the CF team coordinates treatment and discharge planning (including outcome measure assessment) for the inpatient case-list on a daily basis. The CF team in this participating facility also comprises other senior outpatient physiotherapists; however, they do not routinely provide a service to the inpatient case-list. It was not possible to reallocate work distribution to enable independent assessment to occur.

6.3.4 Limitation 2– The lack of data regarding anti-inflammatory therapies

Another potential limitation is the lack of information regarding individuals who were receiving azithromycin anti-inflammatory treatment regularly. Individuals receiving this therapy may have had altered levels of systemic and pulmonary inflammation as a consequence of this medication (225). This may have influenced observed physical activity levels and presented a confounding variable. Due to a large range of clinical data

already being collected from participants in this research program, additional data collection would have imposed further burden on participants and staff which was not considered feasible. This data was not collected contemporaneously and was not able to be gained in retrospect. This may be a contributing factor in terms of inflammation modification during physical activity or effect upon exercise tolerance.

6.3.5 Limitation 3 – The lack of potential confounding variable data related to variance in disease expression

In a patient population such as CF, it would be remiss to not discuss the multitude of variance in disease expression and available therapies (226, 227). The participant population of this research program was standardised, in that all were adults who had been admitted for IV antibiotic therapy following an acute exacerbation. All participants were considered at their "best" health-wise as were being discharged from inpatient hospital stay. However, a number of variables remain amongst the greater population of adults with CF, including those recruited to this research program. Variables may include CF genotype, bacterial (and fungal) colonisation, antibiotic profile, CFRD-status, and CFLD-status (228). While considered valuable, collection of these measures was beyond the scope of this research. These variables need to be considered when appreciating the generalisability of this research to a given cohort adults with CF, and the potential interaction of these factors with an individual's level of inflammation, physical activity and exercise tolerance.

6.3.6 Limitation 4 – *Change* in inflammatory markers, physical activity levels and exercise tolerance was not explored.

This research program did not explore the *change* in inflammatory markers alongside the change in physical activity or exercise capacity during the course of hospitalisation for an exacerbation. Having done so would have provided information regarding how an individual responds to hospital treatment from an inflammatory and physical activity perspective, and would have allowed for a direct comparison with the work of Bradley et al. (158). Bradley used similar measures of exercise tolerance (MST-15 vs MST-25) and also explored correlations with inflammation, however this was done with C-reactive protein (as opposed to the specific measurement of IL-6, IL-8 and TNF-α). As this research program looked at inflammation, physical activity and exercise tolerance *at one time point only,* limited comparisons can be drawn between the pre-existing evidence base and this research program.

6.4 Considerations for future research

This research program forms the basis of further study aimed at exploring the effect of physical activity intensity on systemic inflammation in adults with CF. Investigating the relationship between both the *amount* of physical activity, the *intensity* of physical activity and inflammation in adults with CF needs to be explored on a larger scale to further our understanding of the effect of prescribed physical activity upon

inflammation. In addition to this, exploring the relationship between *type* of habitual physical activity (strength-based or aerobic) and inflammation in adults with CF would enhance this understanding. Exploring inflammatory markers and physical activity levels at one-month post discharge would help us to understand the relationship between the two variables in the "real world" environment; and exploring the potential relationship between sedentary time and inflammation would help shape specific recommendations for physical activity for adults with CF. Lastly, exploring the effect of CFTR modulator therapies upon physical activity and exercise tolerance would be of benefit to today's population of adults with CF.

6.4.1 Relationship between physical activity amount, physical activity intensity and inflammation in adults with CF

The findings of this research program seem to suggest that the number of steps an adult with CF takes on a daily basis relates to their exercise tolerance (6MWD and MST-25), however the metabolic equivalents achieved in a day (relating to physical activity intensity) appears to be unrelated to their exercise tolerance. From this it could be hypothesised that low-intensity steady-state physical activity that occurs regularly (i.e. daily) may be the most beneficial for adults with CF who have a constant underlying state of inflammation. This needs to be further explored in a randomised controlled trial comparing a program of regular low-intensity steady-state physical activity versus regular high-intensity interval training to better understand how much physical activity

and at what intensity is the most beneficial from an inflammatory perspective for adults with CF.

It is known that in healthy adults the immune response to physical activity is a J-curve response – engaging in mild to moderate physical activity is known to enhance immune function, however too much or too little physical activity may actually impair the body's immune response to infection (229, 230). In theory this could be the same with physical activity and the inflammatory response in adults with CF. For clinical populations with a pre-existing state of *chronic* inflammation however, such as people with CF, the inflammatory response to physical activity may not be J-curved. The overarching aim of this future research would be to understand exactly how much physical activity adults with CF need to participate in to have a positive influence on inflammation.

6.4.2 Relationship between type of habitual physical activity (strength-based or aerobic-based) and inflammation in adults with CF

Further research is warranted into the effect of different types of habitual physical activity upon inflammation, namely the differences between habitual aerobic physical activity (i.e. running, jogging, walking) and habitual skeletal muscle strength-based physical activity (i.e. weight training). In this research program there was no significant relationship between quadriceps strength and inflammatory markers, however this was a one-off measurement of strength. Ideally this concept should be explored further via

an intervention trial comparing a program of structured aerobic training versus a program of structured weight training, measuring the effect on specific inflammatory markers.

The aim would be to tailor what *type* of physical activity health professionals prescribe to adults with CF to have a positive influence on the individual's level of inflammation, encompassing the individual's preferences. Understanding what *type* of physical activity positively effects the inflammatory profile of an adult with CF will help health professionals to better prescribe physical activity as a treatment across the spectrum of disease severity to reduce morbidity amongst this patient cohort.

6.4.3 Exploration of physical activity levels and inflammation in the inpatient environment vs at one-month post-discharge

Further research would also ideally explore physical activity levels and inflammation at one-month post-discharge following hospitalisation for an acute pulmonary exacerbation. Inflammation at time of discharge and physical activity levels in the week post-discharge did not inform on risk of re-exacerbation, however inflammatory and physical activity levels on initial discharge from hospital may not be reflective of physical activity levels at one month (94, 138). Ward et al. (94) demonstrated that adults with CF significantly increase their physical activity levels one-month post-discharge following hospitalisation for a pulmonary exacerbation, however the relationship with

inflammation was not explored.

Future research could look to explore physical activity and inflammation levels at time of admission for an acute pulmonary exacerbation, at point of discharge, and again at one-month (or greater) follow-up. This would provide further information on what happens from an inflammatory profile and a physical activity perspective once an adult with CF returns home and is no longer receiving the support and encouragement of their health professional CF team in the inpatient environment. This encouragement of physical activity during hospitalisation for an acute pulmonary exacerbation of CF may itself contribute to a reduction in inflammation. This research program, however, was not designed to address this question, which would need to be answered in an appropriately designed, prospective intervention study comparing an inpatient program of structured physical activity against a control group of adults with CF receiving standard treatment.

6.4.4 Exploration of sedentary behaviours and inflammation in adults with CF

Another direction for future research would include the exploration of sedentary behaviours in adults with CF and the relationship with inflammation or exercise tolerance. It has been established that physical inactivity in adults with CF significantly contributes to reduced exercise tolerance (76). It is known that sedentary behaviour defined as < 1.5 METs in healthy adults is associated with raised inflammatory markers,

namely C-reactive protein and IL-6 (231). Replacing 30 minutes a day of sedentary time with moderate to vigorous intensity physical activity has been shown to significantly lower IL-6 levels in healthy adults (232), suggesting that moderate to vigorous intensity physical activity can modulate the inflammatory profile.

This relationship between sedentary behaviour and inflammation has also been seen in other chronic disease populations such as adults with type II diabetes, where reduced levels of moderate to vigorous intensity physical activity have shown to correlate with increased levels of IL-6 ($\beta = 0.23 \pm 0.07$, $p = 0.002$) (233). Similar to healthy adults, adults with type II diabetes who decreased their sedentary time of one hour a day predicts a 24% (95% CI 1.0, 48.0) reduction in C-reactive protein at 6-month follow-up (234). The specific relationship between sedentary time and inflammation in adults with CF is not yet known. Understanding the consequences of physical inactivity for adults with CF (and, as a health professional, helping patients to understand and act on this), and acknowledging that systemic inflammation has a relationship with physical activity, will help further direct recommendations for adults with CF.

6.4.5 Exploration of the effect of modulator therapies upon physical activity and exercise tolerance

This research program did not include any participants receiving CFTR modulator therapies in conjunction with the study period, including 12-month follow-up. Now that

adults with CF in Australia are able to receive such treatments the relationship between inflammation and physical activity in this patient population should be explored.

Current CFTR modulators include ivacaftor, lumacaftor, tezacaftor and elexacaftor (235, 236). These are a relatively new class of drugs that improve the production and/or function of the defective CFTR protein on the cell surface, improving transport of sodium and chloride ions (235, 236). Systematic reviews examining these modulator therapies have demonstrated improved clinical outcomes for individuals with CF, including improved quality of life, improved respiratory function, and reduced rates of pulmonary exacerbations (235, 236). Future research could explore the relationship between inflammation, physical activity and exercise tolerance in adults with CF receiving CFTR modulator therapies and replicate this research program. This would potentially provide further insight into the relationship between cellular pathophysiology in CF and inflammation and physical activity.

6.5 Conclusion

This thesis examined the relationships between physical activity, exercise tolerance and inflammation in a clinical adult CF population following an acute pulmonary exacerbation. The main findings include:

- Systemic inflammation demonstrated an inverse relationship with physical activity (daily average METs and METs $\geq$ 3). This suggests that adults with CF who are less

physically active following discharge home from hospital have higher levels of blood plasma IL-6 and IL-8.

- Pulmonary inflammation (TNF-α in sputum) is positively related to daily average steps. This suggests that individuals who take more steps on a daily basis have higher levels of TNF-α, however the cause of this remains unknown.
- Time to next pulmonary exacerbation is not related to inflammation, physical activity or exercise tolerance. As CF is a multifactorial and complex disease, it is likely that with a cohort of diverse individuals with CF (n=32) including different genotypes, different antibiotics received during inpatient care, different anti-inflammatory therapies received, differences in CF related diabetes status etc., time to next pulmonary exacerbation was unable to be attributed specifically to inflammation, physical activity or exercise tolerance.
- No relationship was observed between systemic and pulmonary markers of inflammation and exercise tolerance (6MWT, MST-25 and quadriceps strength). This suggests that performance during an exercise tolerance test (either aerobic or strength-based) is unrelated to underlying level of inflammation following hospitalisation for an acute pulmonary exacerbation for adults with CF.

Further investigation within this thesis explored the relationships between inflammation, physical activity, exercise tolerance, age, sex, BMI and disease severity in adults with CF following hospitalisation for an acute pulmonary exacerbation. The main findings include:

- Physical activity has an inverse relationship with disease severity. Adults with CF with a lower FEV_1 % predicted had a lower daily average step count. This suggests that adults with CF with more severe lung disease are less physically active.
- Sex is not related to inflammation, physical activity or aerobic exercise tolerance. Sex does however appear to be related to strength-based exercise tolerance, as adult males with CF demonstrated significantly greater quadriceps strength on testing than their female counterparts, regardless of age or disease severity.
- Aerobic exercise tolerance has an inverse relationship with disease severity. Adults with CF with poorer lung function performed worse on both the 6MWT and the MST-25.
- Strength-based exercise tolerance is not related to disease severity. This is in contrast to the aerobic exercise tolerance measures and their relationship with disease severity. This suggests that adults with severe CF-related disease do not face the same limitations during strength-based exercise as they do during aerobic exercise.
- Physical activity has a positive relationship with BMI. Adults with CF with a lower BMI had a lower daily average step count, demonstrating that nutrition is linked with habitual physical activity.
- Pulmonary inflammation has an inverse relationship with BMI. Adults with CF with a lower BMI had higher levels of IL-6 in their sputum, demonstrating that nutrition is linked with pulmonary inflammation.

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CHAPTER 8 APPENDICES

APPENDIX 1 – Metro North HREC & governance approvals

Queensland Government

Queensland Government

Queensland Health

11 December 2012

Enquiries to: R&ETPCH@health.qld.gov.au
Philip_Lee@health.qld.gov.au
Office Ph: (07) 3139 4198
(07) 3139 4500
Our Ref: PL/JL/Final Approval

Miss Kate Myslinksi
Physiotherapy Department
The Prince Charles Hospital

Human Research Ethics Committee
Metro North Hospital and Health Service
The Prince Charles Hospital
Administration Building, Lower Ground
Rode Road, Chermside QLD 4032

Dear Miss Myslinski,

RE: **HREC/12/QPCH/289: Inflammatory markers and physical activity capacity in the adult cystic fibrosis population following an acute exacerbation requiring hospitalisation.**

This project was considered by Metro North Hospital and Health Service - The Prince Charles Hospital Human Research Ethics Committee (HREC) at its meeting held 6 December 2012.

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007), NHMRC and Universities Australia Australian Code for the Responsible Conduct of Research (2007)* and the *CPMP/ICH Note for Guidance on Good Clinical Practice.*

I am pleased to advise that the Human Research Ethics Committee has granted final approval of this research. The documents reviewed and approved include:

Document	*Version*	*Date*
Patient Information Sheet/Consent Form	1	12 November 2012
Protocol	1	16 November 2012
Application NEAF (AU/1/8110111)		

Please note the following conditions of approval:

1. The Principal Investigator will immediately report anything which might warrant review of ethical approval of the project in the specified format, including:

   a. Unforeseen events that might affect continued ethical acceptability of the project.

   b. Serious Adverse Events that materially impact on the continued ethical acceptability of the project. In addition the Investigator must provide, at least six monthly, a summary

of serious adverse events, in the specified format, including a comment as to suspected causality.

2. Amendments to the research project which may affect the ongoing ethical acceptability of a project must be submitted to the HREC for review. Major amendments should be reflected in a cover letter from the principal investigator, providing a description of the changes, the rationale for the changes, and their implications for the ongoing conduct of the study. Hard copies of the revised amendments, the cover letter and all relevant updated documents with tracked changes must also be submitted to the HREC coordinator as per standard HREC SOP. Further advice on submitting amendments is available from http://www.health.qld.gov.au/ohmr/html/regu/regu_home.asp

3. Amendments to the research project which only affect the ongoing site acceptability of the project are not required to be submitted to the HREC for review. These amendment requests should be submitted directly to the Research Governance Office/r (by-passing the HREC).

4. Proposed amendments to the research project which may affect both the ethical acceptability and site suitability of the project must be submitted firstly the HREC for review and, once HREC approval has been granted, submitted to the RGO.

5. Amendments which do not affect either the ethical acceptability or site acceptability of the project (e.g. typographical errors) should be submitted in hard copy to the HREC coordinator. These should include a cover letter from the principal investigator providing a brief description of the changes and the rationale for the changes, and accompanied by all relevant updated documents with tracked changes.

6. The HREC will be notified, giving reasons, if the project is discontinued at a site before the expected date of completion.

7. The Principal Investigator will provide an annual report to the HREC and at completion of the study in the specified format.

8. The Hospital & Health Service Administration and the Human Research Ethics Committee may inquire into the conduct of any research or purported research, whether approved or not and regardless of the source of funding, being conducted on hospital premises or claiming any association with the Hospital; or which the Committee has approved if conducted outside The Prince Charles Hospital & Health Services.

HREC approval is valid for **3 years** from the date of this letter.

Should you have any queries about the HREC's consideration of your project please contact Philip Lee on the above phone numbers or email addresses. The HREC terms of Reference, Standard Operating Procedures, membership and standard forms are available from http://www.health.qld.gov.au/ohmr/html/regu/regu_home.asp

You are reminded that this letter constitutes ethical approval only. **You must not commence this research project at a site until separate authorisation from the Hospital & Health Services CEO or Delegate of that site has been obtained.**

A copy of this approval must be submitted to the relevant Hospital & Health Services Research Governance Officer/s or Delegated Personnel with a completed Site Specific Assessment (SSA) Form for authorisation from the CEO or Delegate to conduct this research at The Prince Charles Hospital.

Queensland Government

19 March 2013

Enquiries to: RGOTPCH@health.qld.gov.au
Office Ph: (07) 3139 4407
Our Ref: PL/JL/SSA Approval

Miss Kate Myslinski

c/o Physiotherapy Department
The Prince Charles Hospital
Rode Road
Chermside, QLD 4032

Research Governance Office
Metro North Hospital and Health Service
The Prince Charles Hospital
Administration Building, Lower Ground
Rode Road, Chermside QLD 4032

Dear Miss Myslinski

HREC reference number: HREC/12/QPCH/289
SSA reference number: SSA/13/QPCH/82
Project title: Inflammatory markers and physical activity capacity in the adult cystic fibrosis population following an acute exacerbation requiring hospitalisation

Thank you for submitting an application for authorisation of this project. I am pleased to inform you that authorisation has been granted for this study to take place at the following site:

The Prince Charles Hospital

The following conditions apply to this research proposal. These are additional to those conditions imposed by the Human Research Ethics Committee that granted ethical approval.

1. Proposed amendments to the research protocol or conduct of the research which may affect the ethical acceptability of the project are to be submitted to the HREC for review. A copy of the HREC approval/rejection letter must be submitted to the RGO;
2. Proposed amendments to the research protocol or conduct of the research which only affects the ongoing site acceptability of the project, are to be submitted to the research governance officer;
3. Proposed amendments to the research protocol or conduct of the research which may affect both the going ethical acceptability of the project and the site acceptability of the project are to be submitted firstly to the HREC for review and then to the research governance officer after a HREC decision is made.

I am pleased to advise Governance approval of this research project. The documents reviewed and approved include:

Document	*Version*	*Date*
SSA (AU/3/8011116)		

Please complete the Notification of Commencement Form once commencement of this protocol has occurred at this site (http://www.health.qld.gov.au/tpch/documents/form_notification.dot) and return to the office of the Human Research Ethics Committee.

On behalf of the Research, Ethics and Governance Unit, we wish you every success in your research project.

Yours sincerely

[redacted]

Anne Carle
Research Governance Officer
Metro North Hospital and Health Service

Office	Postal	Phone
Research, Ethics & Governance Office The Prince Charles Hospital	Administration Building, Lower Ground Rode Road, Chermside Q 4032	(07) 3139 4407

APPENDIX 2 – Email correspondence – Griffith Ethics

From: Human Research Ethics [mailto:research-ethics@griffith.edu.au] **Sent:** Monday, 19 January 2015 10:20 AM **To:** Kate Burton **Cc:** Rick Williams; Kim Madison **Subject:** Re: Prior Review Application = Outside of Scope?

Dear Kate,
Thank you for your below email, we can confirming that this stage of your research project does not require ethical clearance from Griffith University as the data collection phase was covered by Prince Charles Hospital HREC approval and only the data analysis phase will occur at Griffith.

As you have mentioned, please retain this email and your approval from PCH for your confirmation as proof that Griffith Ethics Approval is not required.

Kind regards,
Marnie Lawson
Human Research Ethics & Integrity
Office for Research
Griffith University

research-ethics@griffith.edu.au
Ph: 5552 9251
Fx: 5552 9058

On 19 January 2015 at 09:43, Kate Burton <Kate.Burton@health.qld.gov.au> wrote:
Hi Marnie,

No – no further engagement with participants will occur, and the protocol is no longer active at TPCH.
I was planning on reviewing re-admission data at 12 month follow-up, but this will occur electronically (as per my Qld Health HREC).

So I guess that means I don't need the Griffith Review?
When it comes time for my confirmation (March 2015), do I just include these emails as proof that a Griffith review was not required in this situation?

Kind regards,

Kate Burton
Senior Physiotherapist
Supportive & Specialist Palliative Care
Gold Coast Hospital & Health Service
Ph: 0402 969 682
Ph: (07) 5626 8440
kate.burton@health.qld.gov.au

From: Human Research Ethics [mailto:research-ethics@griffith.edu.au] **Sent:** Friday, 16 January 2015 10:11 AM **To:** Kate Burton **Subject:** Fwd: Prior Review Application = Outside of Scope?

Hi Kate,
I discussed your application with my Manager to confirm whether it is necessary for you to get ethics approval from Griffith for your research, his response is below. Please let me know whether, based on this you would like the review to go ahead (i.e. you may require further engagement with participants, or the protocol is still active with TPCH)?

Kind regards,
Marnie Lawson
Human Research Ethics & Integrity
Office for Research
Griffith University

research-ethics@griffith.edu.au
Ph: 5552 9251
Fx: 5552 9058

---------- Forwarded message ----------
From: **Rick Williams** <rick.williams@griffith.edu.au>
Date: 15 January 2015 at 16:04
Subject: Re: Prior Review Application = Outside of Scope?
To: Human Research Ethics <research-ethics@griffith.edu.au>
Cc: Kim Madison <k.madison@griffith.edu.au>
Marnie

Provided there is no further engagement with participants (includes follow-up if they are identified or quoted in research outputs) then GU ethics approval is not required. However, it is the project, not the researcher, that has ethics approval so, unless the protocol was completed or withdrawn, the TPCH HREC approval is still operating and the PR can just go ahead.

Regards

On 15 January 2015 at 13:40, Human Research Ethics <research-ethics@griffith.edu.au> wrote:
Rick / Kim,
The below email and attachment (Prior Review Application) relates to a project in which all the data collection was completed while the researcher was employed at Prince Charles (not at Griffith). Kate has now moved to Griffith and is at the analysis / publication stage of the project. This does not require approval from Griffith now as data collection is complete, correct?

Kind regards,
Marnie Lawson
Human Research Ethics & Integrity
Office for Research
Griffith University

research-ethics@griffith.edu.au
Ph: 5552 9251
Fx: 5552 9058

---------- Forwarded message ----------
From: **Kate Burton** <Kate.Burton@health.qld.gov.au>
Date: 14 January 2015 at 12:58
Subject: RE: FW: re: prior ethics approval
To: Human Research Ethics <research-ethics@griffith.edu.au>
Hi Marnie,

Sorry I missed your call. Yes that's correct, all of the data was collected between December

2013 and April 2014 while I was working at the Prince Charles (with their HREC approval).
I enrolled in my M.Phil in February 2014 – as there's a 2/12 crossover do I need to have Griffith ethical clearance?
All I am completing under Griffith is my analysis and publication phases.

PS – email is usually best for me, or alternatively you can reach me on 0402 425 521 after hours.

Kind regards,

Kate Burton
Senior Physiotherapist
Supportive & Specialist Palliative Care
Gold Coast Hospital & Health Service
Ph: 0402 969 682
Ph: (07) 5626 8440
kate.burton@health.qld.gov.au

From: Human Research Ethics [mailto:research-ethics@griffith.edu.au] **Sent:** Wednesday, 14 January 2015 11:29 AM **To:** Kate Burton **Subject:** Re: FW: re: prior ethics approval

Hi Kate,

I have just left a message on your mobile phone to call me.

I wish to clarify the data collection dates for your application, it appears by your recorded dates that data collection has been completed? If this is correct and you are only going to be involved in the analysis and publication phases, no data collection, then ethics clearance is not required.

Kind regards,
Marnie Lawson
Human Research Ethics & Integrity
Office for Research
Griffith University

research-ethics@griffith.edu.au
Ph: 5552 9251
Fx: 5552 9058

On 12 January 2015 at 12:15, Kate Burton <Kate.Burton@health.qld.gov.au> wrote:
Good afternoon,

Please see attached my documentation to apply for Prior Ethics Approval from Griffith for research that was conducted at the Prince Charles Hospital (with Qld Health HREC approval). This research is the topic of my Griffith M.Phil.

Attached are the following documents:
- Prior Ethics Cover sheet
- Qld Health Ethics Submission
- HREC approval letter
- SSA approval letter
- Signed S18

I will also forward you a signed S17 once my Assistance Supervisor Professor Norm Morris (Griffith) has returned from overseas leave.

If there is anything else you require please don't hesitate to ask.

Kind regards,

Kate Burton
Senior Physiotherapist
Supportive & Specialist Palliative Care
Gold Coast Hospital & Health Service
Ph: 0402 969 682
Ph: (07) 5626 8440
kate.burton@health.qld.gov.au

APPENDIX 3 - Published manuscript included in the thesis

PHYSIOTHERAPY THEORY AND PRACTICE
https://doi.org/10.1080/09593985.2019.1566942

Increased physical activity post-exacerbation is associated with decreased systemic inflammation in cystic fibrosis – An observational study

Kate Burton BPhyt [a,b,c], Norman R. Morris PhD, BPhty [a,d], David Reid BSc MB ChB MRCP FRACP MD[b,e], Daniel Smith MBChB, MRCP (UK), FRACP, PhD[b], and Suzanne Kuys PhD, B Phty (Hons), PGDip Pub Hlth[a,f]

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ABSTRACT

Background and Objective: We assessed whether measured physical activity in adults with cystic fibrosis (CF) following in-hospital treatment for an acute exacerbation was impacted by levels of systemic and airway inflammation, and whether physical activity post-discharge predicted for time to next pulmonary exacerbation.

Methods: Adults with CF were included following hospitalization for a pulmonary exacerbation, and were followed for 12 months. Inflammatory markers and physical activity were measured immediately post-discharge via sputum and plasma concentrations of interleukin-6, interleukin-8, and tumor necrosis factor-α. Physical activity was monitored for 7 days via a Sensewear Armband. Statistical analyses included Shapiro-Wilk's test and Q–Q plots to determine normal distribution, t-tests, Pearson's correlational analyses, and one-way MANOVAs.

Results: Thirty-one adults with CF (13 females, 28.8 ± 8.8 years, forced expiratory volume in 1 s (FEV_1) 59.4 ± 23.0% predicted) were prospectively recruited. Physical activity negatively correlated with plasma inflammation ($r = -0.48$, $p < 0.01$), and positively with FEV_1 ($r = 0.45$, $p < 0.05$) and body mass index ($r = 0.39$, $p < 0.05$). There was no significant relationship between time to re-exacerbation and any inflammatory markers or measurement of physical activity (all $p > 0.05$).

Conclusion: Increased physical activity following exacerbation in CF is associated with lower levels of systemic inflammation. Time to re-exacerbation is not related to post-discharge inflammation or physical activity levels.

ARTICLE HISTORY
Received 23 February 2018
Revised 19 October 2018
Accepted 24 November 2018

KEYWORDS
Adults; cystic fibrosis; cytokines; inflammation; physical activity

Introduction

Cystic fibrosis (CF) lung disease is typified by a vicious cycle of airway infection and an exuberant, but ineffective local and systemic inflammatory response (Rottner, Freyssinet, and Martinez, 2009). Even during periods of clinical stability, elevated concentrations of pro-inflammatory cytokines such as interleukin (IL)-6, IL-8, and tumor necrosis factor-α (TNF-α) are detectable in the airway and systemic circulation (Ionescu et al., 2006; Ploeger, Takken, de Greef, and Timmons, 2009; Rottner, Freyssinet, and Martinez, 2009). The deleterious effects of inflammation may extend beyond local lung destruction and, among other effects, systemic inflammation is thought to contribute to muscle wasting (Rottner, Freyssinet, and Martinez, 2009), which may further impair physical activity (Gruet, Troosters, and Verges, 2017). Regular physical activity is a critical component of the management of CF, with demonstrated benefits including enhanced sputum clearance, skeletal muscle strength, preservation of bone mineral density, and increased insulin sensitivity, as well as improvements in lung function (Button et al., 2016; Cooper, 1994; Eliakim, Raisz, Brasel, and Cooper, 1997).

In healthy adults, the relationship between physical activity and inflammation has been studied (Edwards and Loprinzi, 2018; Kasapis and Thompson, 2005; King, Carek, Mainous, and Pearson, 2003; Nilsson, Bergens, and Kadi, 2018), and the main findings demonstrated that different levels of physical activity may be associated with changes in inflammatory responses. In people with type-2 diabetes (Hamer et al., 2014), chronic obstructive pulmonary disease (Loprinzi, Walker, and Hyo, 2014), human immunodeficiency virus and acquired immunodeficiency syndrome (Wirth et al., 2015), and metabolic syndrome (Pitsavos et al., 2005), reduced levels of physical activity were associated with higher

CONTACT Kate Burton Kate.burton@griffithuni.edu.au

levels of systemic inflammation. The relationship between regular physical activity and inflammation in CF is not well characterized, and to date there is limited evidence of the association of physical activity with inflammation in adults with CF.

In this study, our overarching aim was to examine the relationship between pulmonary and systemic inflammation and physical activity in adults with CF at the end of hospital treatment for a pulmonary exacerbation. We hypothesized that patients with increased levels of inflammation would demonstrate reduced physical activity levels post-discharge and that inflammation and reduced physical activity would predict for time to next pulmonary exacerbation. The research questions were: (1) Is there an association between reduced levels of physical activity and increased levels of inflammation in adults with CF following treatment for a pulmonary exacerbation; and (2) Do levels of physical activity levels or inflammation predict time to next pulmonary exacerbation?

Methods

Design

This was a single-center prospective cohort observational study with Human Research Ethics approval (HREC/12/QPCH/289 and SSA AU/3/8011116). All participants gave written informed consent prior to participating.

Participants

Participants were recruited consecutively over a 12-month period. To be eligible for inclusion, participants needed to be adults with a formal diagnosis of CF (aged 18 and above) who were admitted for inpatient hospital treatment of a pulmonary exacerbation. Participants were excluded from the study if they were aged less than 18 years at the time of enrolment, were discharged with home-based intravenous antibiotic treatment, or were pregnant.

A pulmonary exacerbation was defined based on international consensus criteria as the presence of any or all of the following symptoms: increased cough; increased sputum production; shortness of breath; chest pain; loss of appetite; loss of weight; and/or a measured decline in pulmonary function (Bradley, McAlister, and Elborn, 2001).

The chief investigator (KB) was responsible for study recruitment, collection of outcome measures assisted by the Adult CF Physiotherapy team, sample processing, and data analysis. The Adult CF Medical Registrars collected all blood samples.

Procedure

Adults with a diagnosis of CF were enrolled in this study at the end of an inpatient admission at The Prince Charles Hospital, Brisbane. Discharge from hospital was determined by self-reported improvement in the patient's symptoms and objective evidence of a reduction in C-reactive protein (CRP) levels and improvement in lung parameters (forced expiratory volume in 1 s; FEV_1). Sputum and blood samples were collected within 48 h of planned discharge from hospital.

To measure the level of physical activity post-discharge, participants were fitted with a Bodymedia® Sensewear Armband (SWA) (Model MF-SW); Pittsburgh, PA, USA, which was worn for 5–7 consecutive days. To monitor time to next pulmonary exacerbation (days), all participants were followed-up for 12 months. Monitoring of pulmonary exacerbation was made through attendance at regular outpatient clinics and hospital admissions.

Outcome measures

Demographic information was recorded and respiratory function measured within 48 h prior to discharge. Lung function was measured using standard spirometry (Jaegar Vyntus® Pneumo, Bonn, Germany) to measure FEV_1, and collected by an independent assessor according to the European Respiratory Society guidelines (Miller et al., 2005).

Following venesection, plasma was separated from blood by centrifugation (10 min, 1000 × g at room temperature) and immediately stored at −80°C for later batch analysis. Spontaneously expectorated sputum samples were collected in a sterile container by the treating CF physiotherapist and processed without delay. Sputum was homogenized by mixing with phosphate-buffered saline in a 5:1 ratio and heating in a waterbath to 37°C for 30 min, with regular agitation. Homogenized sputum samples were centrifuged at 1800 RPM at 4°C for 10 min to separate supernatant from cellular component. Supernatants were stored frozen to −80°C for later analysis. Cell pellet was discarded.

Sputum supernatant and plasma concentration of IL-6, IL-8, and TNF-α were determined using previously optimized in-house enzyme-linked immunosorbent assay (ELISA), performed in duplicate and analyzed using the Fluostar Omega.

Physical activity levels were measured using the SWA device, a valid measure of physical activity

among adults with CF (Cox et al., 2014). Participants wore the SWA on the left upper arm for 5–7 days following discharge (only removed for bathing/swimming). Participants were instructed to wear the armband overnight. Reported outcome measures were daily average metabolic equivalents (METs; 1 MET = 3.5 ml/kg/min), which are an estimate of energy expenditure per day (Cox et al., 2014; Kuys et al., 2011), duration of physical activity at a moderate-high level (daily average ≥3 METs), and daily average steps. Body mass index (BMI) was also reported as a component of the SWA measurements.

Time to next pulmonary exacerbation (days) was defined as the number of days following discharge until the patient required re-admission to hospital for treatment with intravenous antibiotics for a deterioration in their pulmonary status (Bradley, McAlister, and Elborn, 2001). Pulmonary exacerbation was confirmed by the treating consultant in either Outpatient CF Clinic or in the Emergency Department. Two time points were examined in this study; participants who experienced a pulmonary exacerbation within the first 6 months (182 days) post-discharge, and those who experienced a pulmonary exacerbation within 12 months (365 days) post-discharge. If a participant did not experience a pulmonary exacerbation requiring intravenous antibiotics within 12 months of completing the study, this was recorded arbitrarily as >365 days.

Analysis

Statistical analysis was performed with the SPSS statistical package v23.0 (2015, IBM Corp., Armonk, NY, USA). Data are presented as mean and standard deviation, unless specified otherwise. Physical activity levels (daily average METs), duration of physical activity at a moderate-high level (daily average ≥3 METs), daily average steps, and BMI were determined for each participant (all participants wore the SWA for >18 h/day). Shapiro-Wilk's test and Q–Q plots were used to determine whether continuous data were normally distributed. t-Tests and Mann–Whitney U test were used to examine differences in data dependent on normality of distribution. Pearson's correlational analyses were conducted to examine the relationships between both sputum and plasma inflammatory markers (IL-6, IL-8, and TNF-α), measures of physical activity (METs, steps/day), and BMI in the first week following discharge. These correlations were classified as low (r = −0.30 to −0.50), moderate (r = −0.50 to −0.70), or high (r = −0.70 to −0.90) (Hinkle, Wiersma, and Jurs, 2003). Multivariate analyses (one-way MANOVA) were conducted to examine the relationships between inflammatory markers and time to next exacerbation, and between physical activity and time to next exacerbation. Non-normally distributed data were natural log transformed prior to performing correlation testing. A p-value of <0.05 was considered as statistically significant.

Results

Thirty-two adults (18 male) aged ≥18 years with CF were recruited to the study. One participant did not comply with activity monitoring, so data were available for 31 individuals over the study period. All participants had a Class I–III mutation (severe) (Castellani et al., 2008), 96% of which were F508del (50% of these were homozygous). Demographics, physical activity levels, and inflammatory cytokine results in sputum and plasma are provided in Table 1. All participants were followed-up for 12 months.

Plasma concentrations of IL-6, IL-8, and TNF-α demonstrated a low-to-moderate negative correlation with daily average METs (Figure 1) and a low negative correlation with daily average ≥ 3 METs. There was no association between plasma cytokines and daily average steps (Table 2). With the exception of a low positive correlation between sputum TNF-α and daily average steps, there were no significant associations between sputum cytokines and measures of physical activity (Table 2). Daily average steps were found to have a low positive correlation with FEV_1 (r = 0.45, p < 0.05).

Male participants were older (median age 31.9 years *versus* 24.9 years, p = 0.01) and had lower lung function than females (mean FEV_1 percentage predicted 49.2% *versus* 72.5%, p < 0.01) (Table 3), predicted values based on Australian and New Zealand adult populations (Brazzale, Hall, and Swanney, 2016). There were no other gender-specific differences in either cytokine concentrations or other physical activity parameters.

BMI had a low positive correlation with daily average steps (r = 0.39, p < 0.05) (Figure 2), whereas a moderate negative correlation was seen between BMI and sputum IL-6 (r = −0.51, p < 0.01) (Figure 2). No significant relationship was seen between BMI and IL-8 or TNF-a either in plasma or sputum.

Eight of 31 participants did not require further intravenous antibiotics in the year following enrolment. The median (range) time to next course of intravenous antibiotics among the 23 subjects who experienced a pulmonary exacerbation was 129 (13–366) days. At 6 months, there was no statistically significant difference in time to re-exacerbation based on an individual participant's systemic inflammatory

Table 1. Characteristics of subjects on the basis of exacerbation status at 6 and 12 months.

		Pulmonary exacerbation at 6 months[d]			Pulmonary exacerbation at 12 months[d]		
Characteristic	All[c] (n = 31[e])	Yes (n = 16)	No (n = 15)	p value	Yes (n = 23)	No (n = 8)	p value
Age (years)[a]	28.8 (18–62)	27.6 (18–38)	30.1 (20–62)	0.87	29.2 (18–62)	27.9 (21–38)	0.95
Male gender	18 (56%)	10 (59%)	8 (53%)	0.58	13 (54%)	5 (63%)	0.47
FEV_1 (% predicted)[b]	59.4 (23.0)	56.1 (20.9)	62.7 (26.1)	0.44	57.5 (24.5)	64.5 (20.5)	0.48
BMI (kg/m^2)[a,b]	21.8 (3.6)	22.1 (4.3)	21.4 (2.6)	0.52	21.7 (3.7)	22.0 (3.2)	0.84
Daily average METs[b]	1.7 (0.2)	1.8 (0.2)	1.7 (0.3)	0.96	1.7 (0.3)	1.7 (0.2)	0.70
Duration daily average ≥ 3 METs (Hrs:Mins) [b]	4:03 (1:20)	4:15 (1:21)	3:51 (1:20)	0.86	4:11 (01:24)	3:41 (1:04)	0.67
Daily average steps[b] [a]	6351 (2765)	6454 (3562)	6242 (1662)	0.96	6298 (3085)	6503 (1680)	0.75
Plasma IL-6 (pg/ml) [a]	28.5 (14.4–145.1)	26.2 (17.4–38.6)	29.3 (14.4–101.4)	0.10	28.4 (17.4–101.4)	27.1 (14.4–40.0)	0.96
Plasma IL-8 (pg/ml) [a]	27.0 (14.7–145.1)	23.9 (14.7–36.8)	32.9 (18.4–145.1)	0.52	27.0 (14.7–145.1)	26.9 (18.4–105.9)	0.76
Plasma TNF-α (pg/ml) [a]	25.4 (13.6–73.5)	22.9 (15.8–44.3)	28.0 (13.6–73.5)	0.65	24.8 (15.8–73.5)	28.5 (13.6–39.6)	0.55
Sputum IL-6 (pg/ml) [a]	31.1 (16.8–66.75)	32.8 (20.4–66.8)	28.7 (16.8–38.3)	0.25	32.5 (16.8–66.8)	29.0 (20.7–38.3)	0.37
Sputum IL-8 (pg/ml) [a]	64.7 (25.4–533.6)	68.2 (29.0–313.7)	40.8 (25.4–533.6)	0.74	68.2 (25.4–533.6)	37.3 (26.9–279.2)	0.20
Sputum TNF-α (pg/ml) [a]	26.2 (19.2–35.0)	27.2 (20.0–31.3)	26.2 (19.2–35.0)	0.71	26.5 (20.0–31.6)	26.9 (19.2–35.0	0.91

[a] Median (range), [b]Mean (standard deviation), [c]Senseware data unavailable for one subject, [d]Time to next IV antibiotics unavailable for one subject, [e]n = 31, one subject was excluded due to insufficient SWA data.
Definition of abbreviations: BMI = body mass index; FEV_1 = forced expiratory volume in 1 s; IL-6 = interleukin-6; IL-8 = interleukin-8; METs = metabolic equivalents; SWA = Sensewear armband; TNF-α = tumor necrosis factor-α.

markers at discharge [F (6, 52) = 0.94, $p > 0.4$, Wilk's Λ = 0.82, partial η^2 = 0.1], sputum inflammatory markers [F (6, 52) = 1.59, $p > 0.1$, Wilk's Λ = 0.72, partial η^2 = 0.16], or physical activity levels (Daily average METs) [F (3, 26) = 0.51, $p > 0.6$, Wilk's Λ = 0.95, partial η^2 = 0.06]. Similarly, at 12 months, there was no statistically significant difference in time to re-exacerbation based on an individual's systemic inflammatory markers [F (6, 52) = 0.93, $p > 0.4$, Wilk's Λ = 0.82, partial η^2 = 0.1], sputum inflammatory markers [F (6, 52) = 1.25, $p > 0.2$, Wilk's Λ = 0.76, partial η^2 = 0.13], or physical activity levels (Daily average METs) [F (3, 26) = 0.75, $p > 0.5$, Wilk's Λ = 0.92, partial η^2 = 0.08]. There was no significant difference in clinical characteristics, activity levels, or cytokine levels between participants who did or did not require intravenous antibiotics in the 12-month follow-up period (Table 1).

Discussion

This is the first study in adults with CF to demonstrate that the level of physical activity following hospital treatment for an acute exacerbation is significantly associated with the degree of systemic inflammation. A healthy BMI was also found to be an independent predictor of increased levels of physical activity. However, we did not confirm our hypothesis that levels of inflammation and physical activity following in-hospital treatment of a pulmonary exacerbation would predict time to next exacerbation.

The relationship between systemic inflammation and physical activity in CF has not been studied in detail in adults with severe lung disease. A previous study of a large number of adolescents with CF who were clinically stable found a non-significant negative relationship between exercise capacity and serum immunoglobulin-G levels (van de Weert-van Leeuwen et al., 2012). The adolescents studied had well-preserved lung function (FEV_1 of 83.2 SD 18.0% predicted) (van de Weert-van Leeuwen et al., 2012), but the suggestion of an inverse relationship between systemic inflammation and exercise capacity supports our findings in adult patients recovering from a pulmonary exacerbation who in general had moderate–severe CF-lung disease.

Our study assessed both systemic and airway inflammation concurrently, and related these measures to physical activity. We chose to assess patients following inpatient treatment for a pulmonary exacerbation, as this was the time point when patients were considered most likely to be at their best, having completed 10–-14 days of intravenous antibiotics and relatively intense

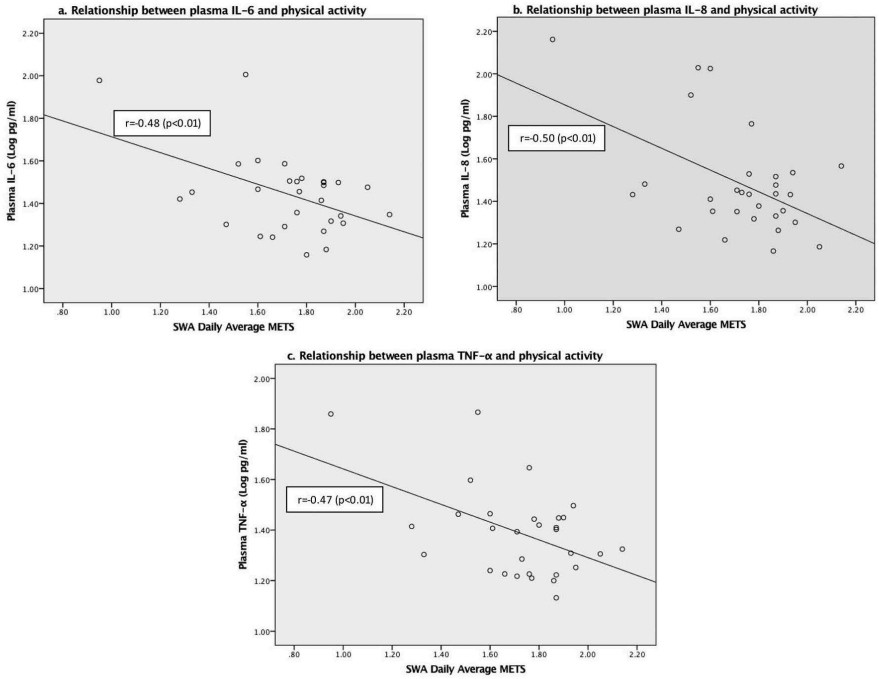

Figure 1. Relationship between plasma inflammatory markers and physical activity.

Table 2. Correlation co-efficients of plasma and sputum cytokines with SWA data.

	Daily average METs	Daily average ≥3 METs	Daily average Steps
Plasma [pg/ml]			
Log IL-6	***R* = −0.48**	***R* = −0.40**	*R* = −0.22
	***p* < 0.01**	***p* = 0.02**	*p* = 0.23
Log IL-8	***R* = −0.50**	***R* = −0.37**	*R* = −0.01
	***p* < 0.01**	***p* = 0.04**	*p* = 0.95
Log TNF-α	***R* = −0.47**	***R* = −0.40**	*R* = −0.04
	***p* < 0.01**	***p* = 0.03**	*p* = 0.82
Sputum [pg/ml]			
Log IL-6	*R* = 0.30	*R* = 0.22	*R* = −0.02
	p = 0.10	*p* = 0.23	*p* = 0.93
Log IL-8	*R* = −0.04	*R* = −0.04	*R* = −0.13
	p = 0.84	*p* = 0.85	*p* = 0.50
Log TNF-α	*R* = 0.18	*R* = 0.10	***R* = 0.36**
	p = 0.34	*p* = 0.60	***p* = 0.05**

Definition of abbreviations: IL-6 = interleukin-6; IL-8 = interleukin-8; METs = metabolic equivalents; SWA = Sensewear armband; TNF-α = tumor necrosis factor-α.

physical therapies. We were also interested in whether inflammation and physical activity levels following discharge could predict time to next pulmonary exacerbation.

We found that increased concentrations of systemic but not airway cytokines were associated with decreased physical activity levels. Interestingly, we found no correlation between cytokine levels in sputum and plasma, suggesting that these represent distinct "compartments" in terms of the host immune response. Our findings are consistent with earlier observations that demonstrated reduced exercise capacity during pulmonary exacerbations is associated with increased levels of CRP (Bradley, McAlister, and Elborn, 2001; Wieboldt et al., 2012).

The novel finding of our study is that systemic inflammation appears to persist even after aggressive in-hospital treatment for a pulmonary exacerbation. Although the reason for this is unclear, one possibility is that this may be associated with the level of physical activity. In a previous study among healthy adults, a moderate-to-vigorous level of physical activity measured via accelerometry was shown to be associated with reduced CRP (Edwards and Loprinzi, 2018). This has also been seen among healthy older adults, where replacing a previously sedentary period with 30 min a day of moderate-to-vigorous activity was associated with a significant reduction in CRP (Nilsson, Bergens, and Kadi, 2018).

Table 3. Participant demographics and outcome measures.

Characteristic	All[d] (n = 31)	Male Gender[e] (n = 18)	Female Gender[e] (n = 13)
Age (years)[a]	28.8 (18–62)	31.9 (18–62)	24.9 (20–35)
FEV_1 (% predicted)[b]	59.4 (23.0)	49.2 (19.6)	72.5 (20.8)
BMI (kg/m^2)[b]	21.8 (3.6)	21.6 (2.8)	21.5 (4.4)
Daily average METs[b]	1.7 (0.2)	1.7 (0.2)	1.7 (0.3)
Duration daily average ≥ 3 METs (hours:minutes)[b]	4:03 (1:20)	4:04 (1:22)	4:02 (1:20)
Daily average steps[b]	6351 (2765)	6414 (3424)	6265 (1578)
Time to next intravenous antibiotics (days)[bc]	186 (128)	178 (138)	197 (117)
Sputum IL-6 (pg/ml)[b]	31.1 (10.6)	33.9 (13.1)	29.3 (5.0)
Plasma IL-6 (pg/ml)[b]	28.5 (18.9)	31.2 (18.7)	28.0 (20.7)
Sputum IL-8 (pg/ml)[b]	64.7 (127.2)	85.8 (84.8)	180.6 (156.9)
Plasma IL-8 (pg/ml)[b]	27.0 (30.3)	38.1 (29.4)	26.1 (33.6)
Sputum TNF-α (pg/ml)[b]	26.2 (3.7)	26.5 (3.3)	26.4 (4.4)
Plasma TNF-α (pg/ml)[b]	25.4 (14.0)	27.2 (13.9)	24.8 (16.1)

[a]Median (range),[b]Mean (standard deviation), [c]n = 23 (9 subjects did not require IV antibiotics in the follow-up period), [d]SWA data unavailable for one subject, [e]Time to next IV antibiotics unavailable for one subject.

Definition of abbreviations: BMI = body mass index; FEV_1 = forced expiratory volume in 1 s; IL-6 = interleukin-6; IL-8 = interleukin-8; METs = metabolic equivalents; SWA = Sensewear armband; TNF-α = tumor necrosis factor-α.

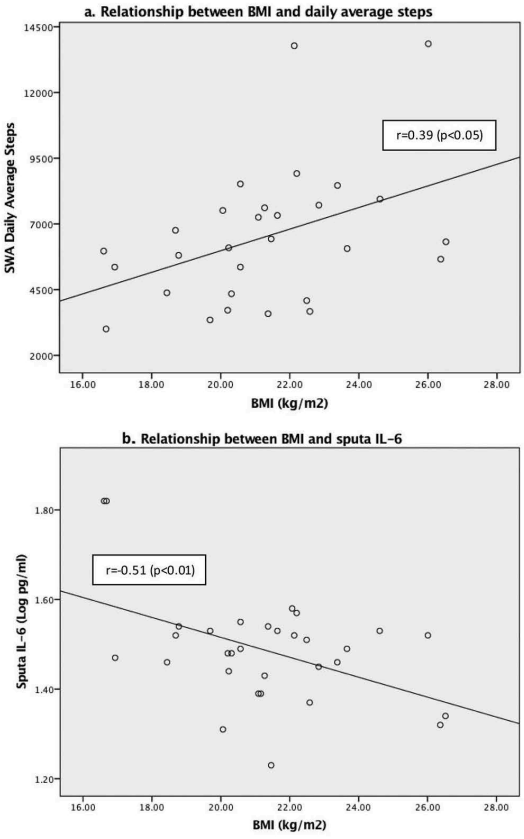

Figure 2. Relationship between BMI and physical activity.

Conversely to this, other studies have shown that bouts of intensive exercise (e.g. marathon running or weightlifting) increase levels of systemic inflammation (Kasapis and Thompson, 2005; van de Weert-van Leeuwen, Arets, van der Ent, and Beekman, 2013), which suggests a balance is important. This may be particularly important in a condition such as CF where a degree of background inflammation is already

present (van de Weert-van Leeuwen, Arets, van der Ent, and Beekman, 2013).

We did not assess intensity of physical activity during treatment of the pulmonary exacerbation, but all patients were encouraged to participate in a tailored exercise regimen throughout their hospitalization. Participants in this study performed on average only 1.7 METs/day following hospital discharge, which is classified as light physical activity (Jette, Sidney, and Blumchen, 1990). The explanation for this low level of activity is likely multifactorial including lung disease severity, nutritional status, level of inflammation, and also psychosocial factors (e.g. motivation, employment status, and family commitments). All of these potential confounders need to be considered in future studies of exercise and physical activity in CF.

Interestingly, in this cohort, FEV_1 at discharge did not predict for physical activity levels or time to next exacerbation, which suggests that reduction in systemic inflammation is at least as relevant to recovery of physical activity post-exacerbation as improvements in lung function. Over half of all patients had clinically deteriorated and needed further intravenous antibiotics by 6 months post-discharge, and two-thirds had deteriorated by the 12-month time point. Although systemic inflammatory cytokine levels at discharge were good predictors of physical activity in the ensuing week, we did not repeat assessments of inflammation at 1-month post-discharge, which may have been informative. Physical activity levels in the week post-discharge did not inform on risk of re-exacerbation, but similarly, physical activity levels on initial discharge from hospital may not be reflective of physical activity levels at 1 month (Wieboldt et al., 2012). Ward et al. (2013) demonstrated that adults with CF significantly increase their physical activity levels 1-month post-discharge following hospitalization for a pulmonary exacerbation, however they did not explore how this may relate to inflammation. The other question raised by our study findings is whether encouraging physical activity during treatment of a pulmonary exacerbation in CF may itself contribute to a reduction in inflammation. However, our study was not designed to address this question, which would need to be answered in an appropriately designed, prospective structured physical activity intervention study.

Further longer term studies of systemic inflammation and physical activity in adults with CF are needed, which may identify opportunities to intervene with specifically tailored physical activity programs (Urquhart and Vendrusculo, 2017) that delay pulmonary deterioration. BMI positively correlated with daily average steps, which suggests that a healthy weight in adults with CF is an independent determinant of physical activity which has been demonstrated previously (Moorcroft, Dodd, and Webb, 1997). There was a weak relationship between sputum IL-6 levels and BMI, but overall our findings suggest that nutritional status and inflammation in adults with CF are not strongly linked.

A limitation of our study is that we did not assess physical activity or levels of inflammation at the time of admission and prospectively follow how these parameters with treatment. This would have allowed relative changes in physical activity and inflammation to be more comprehensively characterized and provided more information on causality. As mentioned, re-assessment at 1-month post-discharge would also have been helpful, as by this time point, patients should be closer to their usual state of health and well-being (Ward et al., 2013). Additionally, we did not use a control group for this study due to the nature of this clinician-led study within the constraints of the hospital environment. Reference values are well-established in the literature for both measurement of physical activity via the SWA (Cox et al., 2014; Dwyer et al., 2009), and for measurement of systemic inflammatory markers (Kleiner et al., 2013). These considerations need to be factored into future studies of exercise and inflammation in adults with CF.

In summary, this study demonstrates that there is an association between reduced levels of physical activity and increased levels of systemic inflammation in adults with severe CF-related lung disease following intravenous antibiotic treatment for a pulmonary exacerbation. Second, neither levels of physical activity nor inflammation predict time to next pulmonary exacerbation in adults with CF following inpatient treatment for an acute exacerbation. Our findings also reinforce the importance of maintaining a healthy BMI to allow adults with CF to engage in physical activity. Our results will inform on a future randomized controlled trial examining the effect of a post-discharge physical activity program and its effect on systemic inflammation in adults with CF.

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