

**SKELETAL MUSCLE DYSFUNCTION IN PEOPLE WITH  
COPD AND RECIPIENTS OF LUNG TRANSPLANTS**

by

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## ABSTRACT

Chronic obstructive pulmonary disease (COPD) is a respiratory condition with multisystemic effects resulting in skeletal muscle dysfunction. Lung transplantation is a treatment option for people with COPD resulting in improved lung function, however, skeletal muscle dysfunction persists and may be worsened following transplantation due to a period of bedrest and the myopathic effects of immunosuppressant medications. We hypothesized that impairments in skeletal muscle function of the quadriceps and hamstrings in people with COPD would be related to changes in skeletal muscle structure and these impairments would be accentuated in lung transplant recipients. Two pilot studies (**Studies 1 and 2**) were performed to establish methodology. In **Study 3**, magnetic resonance imaging showed uniform atrophy of the thigh muscles and intramuscular fat infiltration in the quadriceps and hamstrings of people with COPD compared to age-, sex-, and body mass index (BMI)-matched controls. Notably, eccentric torque normalized to muscle volume was greater in people with COPD compared to controls. **Study 4** showed that despite shorter times to task failure for sustained isometric quadriceps contractions, people with COPD showed similar changes in EMG median frequency and amplitude compared to controls. Quantification of cellular features of the vastus lateralis (VL) in **Study 5** showed a greater proportion of abnormal muscle and small, angular fibers in people with COPD compared to controls. A larger proportion of people with COPD showed increased connective tissue in the VL compared to controls. Comparison of people with COPD to lung transplant recipients in **Study 6** showed a similar amount of muscle atrophy, a wide variation in intramuscular fat infiltration, shorter times to task failure for the quadriceps and small, abnormal histological features of the VL. In summary, these findings suggest that although people with COPD

demonstrate impairments in skeletal muscle structure and function (e.g. atrophy, fat infiltration, reduced endurance), they have a preservation of eccentric torque and motor unit firing properties. The variation of findings in transplant recipients, partly attributable to a small and diverse sample, limited our ability to test our hypothesis of whether changes in skeletal muscle structure and function would be accentuated in this group of individuals.

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## LIST OF ABBREVIATIONS

<b>A<sub>A</sub></b>	– area fraction
<b>ANOVA</b>	– analysis of variance
<b>BFL</b>	– biceps femoris, long head
<b>BFS</b>	– biceps femoris, short head
<b>BMI</b>	– body mass index
<b>BOS</b>	– bronchiolitis obliterans syndrome
<b>CF</b>	– cystic fibrosis
<b>COPD</b>	– chronic obstructive pulmonary disease
<b>CSA</b>	– cross-sectional area
<b>DLT</b>	– double lung transplant
<b>ICC</b>	– intraclass correlation coefficient
<b>FEF<sub>25-75</sub></b>	–mid expiratory flow rate
<b>FEV<sub>1</sub></b>	– forced expiratory volume in one second
<b>FVC</b>	– forced vital capacity
<b>GOLD</b>	– Global Initiative for Obstructive Lung Disease
<b>H&amp;E</b>	– hemotoxylin and eosin
<b>HLA</b>	– human leukocyte antigen system
<b>HLT</b>	– heart-lung transplant
<b>IGF-1</b>	– insulin-like growth factor 1
<b>KE</b>	– knee extensors
<b>KF</b>	– knee flexors
<b>LE</b>	– lower extremity
<b>MF</b>	– median frequency

**MRI** – magnetic resonance imaging

**MVC** – maximal voluntary contraction

**MVV** – maximal voluntary ventilation

**PCr** - phosphocreatine

**PFK** - phosphofructokinase

**pH** – power of hydrogen (concentration of hydrogen ions)

**Pi** – inorganic phosphate

**<sup>31</sup>P-MRS** – phosphorus magnetic resonance spectroscopy

**RF** – rectus femoris

**RPE** – rating of perceived exertion

**SD** – standard deviation

**SEM** – standard error of the mean

**SPSS** – Statistical Package for the Social Sciences

**SLT** – single lung transplant

**SM** - semimembranosis

**ST** - semitendonsis

**TNF- $\alpha$**  – tumor necrosis factor-alpha

**V<sub>E</sub>** – minute ventilation

**VL** – vastus lateralis

**VM** – vastus medialis

**VO<sub>2</sub>** - oxygen consumption

**VO<sub>2</sub>peak** - peak oxygen consumption

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*We have a hunger of the mind which asks for knowledge of all around us, and the more we gain, the more is our desire; the more we see, the more we are capable of seeing.*

*~ Maria Mitchell (1818-1889), Professor of Astronomy, Vassar College*

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## CO-AUTHORSHIP STATEMENT

Sections of this thesis have been published or are in preparation for publication in multi-authored papers in refereed journals. Details of the authors' contributions are provided below.

### PUBLISHED PAPERS

**Mathur S.**, Reid W.D., Levy, R.D. Exercise limitation in recipients of lung transplants.

*Physical Therapy*. 2004;84(12):1178-87.

#### **(Presented in Chapter One)**

The thesis author was responsible for developing the research question in conjunction with the co-authors, performing the literature review, writing and preparing the manuscript for publication. Dr. Reid assisted in developing the research question, writing and editing of the manuscript. Dr. Levy assisted in the development of the research question and provided editing of the manuscript.

Warburton, D.E.R., **Mathur, S.** Skeletal muscle training in people with chronic heart failure and people with chronic obstructive pulmonary disease. *Physiotherapy Canada*. 2004;56:143-157.

#### **(Presented in Chapter One)**

The thesis author was responsible for developing the research question in conjunction with the co-author, performing the literature review on people with COPD, writing and preparing the manuscript for publication. Dr. Warburton was responsible for developing the research question, reviewing the literature on people with heart failure, writing and preparing the manuscript for publication.



**Mathur, S.,** Eng, J.J., MacIntyre, D.L. Reliability of surface EMG during sustained contractions of the quadriceps. *Journal of Electromyography and Kinesiology*. 2005;15(1):102-110.

**(Presented in Chapter Two)**

The thesis author was responsible for performing the research (i.e. data collection), data analysis/interpretation, writing and preparing the manuscript for publication. Dr. Eng was responsible for developing the research question and designing the testing protocol, providing laboratory space and technical support, assistance with data interpretation and editing of the manuscript. Dr. MacIntyre was responsible for developing the research question, designing the testing protocol, providing laboratory space, assistance with data interpretation and editing of the manuscript.

**Mathur, S.,** MacIntyre, D.L., Forster, B.B., Reid, W.D. Influence of exercise-induced injury on knee extension torque in the presence of longstanding quadriceps atrophy: A case report. *Physiotherapy Canada*. 2005; 57:1-9.

**(Presented in Chapter Three)**

The thesis author was responsible for developing the research question and testing protocols, performing the research (i.e. data collection), data analysis/interpretation, writing and preparing the manuscript for publication. Dr. MacIntyre provided laboratory space for data collection and editing of the manuscript. Dr. Forster developed the imaging protocol, provided access to the MRI facility, and editing of the manuscript. Dr. Reid was responsible for developing the research question, assisting with data collection and data interpretation and editing the manuscript.

## MANUSCRIPTS IN PREPARATION

**Mathur, S.,** MacIntyre, D.L., Forster, B.B., Road, J.D., Levy, R.D., Reid, W.D. Skeletal muscle atrophy and intramuscular fat infiltration in people with COPD. *In preparation for submission to: American Journal of Respiratory and Critical Care Medicine.*

### **(Presented in Chapter Four)**

The thesis author was responsible for developing the research question and testing protocols, performing the research (i.e. data collection), data analysis/interpretation, writing and preparing the manuscript for publication. Dr. MacIntyre provided laboratory space for data collection, assisted with data interpretation and editing of the manuscript. Dr. Forster developed the imaging protocol, provided access to the MRI facility, and editing of the manuscript. Dr. Road and Dr. Levy facilitated participant recruitment and editing of the manuscript. Dr. Reid was responsible for developing the research question and testing protocols, assisting with data interpretation and editing of the manuscript.

**Mathur, S.,** MacIntyre, D.L., Road, J.D., Levy, R.D., Reid, W.D. Surface EMG of the quadriceps during a fatiguing contraction in people with COPD. *In preparation for submission to: European Respiratory Journal.*

### **(Presented in Chapter Five)**

The thesis author was responsible for developing the research question and testing protocols, performing the research (i.e. data collection), data analysis, writing and preparing the manuscript for publication. Dr. MacIntyre assisted with developing the research question, assisted with data interpretation, provided laboratory space for data collection and editing of the manuscript. Dr.

Road and Dr. Levy facilitated participant recruitment and editing of the manuscript. Dr. Reid assisted with data interpretation and editing of the manuscript.

**Mathur, S.,** Koehle, M.S., Road, J.D., Levy, R.D., Reid, W.D. Cellular features of the vastus lateralis in people with COPD. *In preparation for submission to: Physical Therapy.*

**(Presented in Chapter Six)**

The thesis author was responsible for developing the research question, performing the research (i.e. data collection), data analysis/interpretation, writing and preparing the manuscript for publication. Dr. Koehle was responsible for data collection (taking the muscle biopsies). Dr. Road and Dr. Levy facilitated participant recruitment and editing of the manuscript. Dr. Reid assisted with data analysis and interpretation and was responsible for editing the manuscript.

**Mathur, S.,** Levy, R.D., MacIntyre, D.L., Forster, B.B., Reid, W.D. Skeletal muscle atrophy and weakness in recipients of lung transplants. *In preparation for submission to: The Journal of Heart and Lung Transplantation.*

**(Presented in Chapter Seven)**

The thesis author was responsible for developing the research question, developing the testing protocols, performing the research (i.e. data collection), data analysis/interpretation, writing and preparing the manuscript for publication. Dr. Levy facilitated participant recruitment and editing of the manuscript. Dr. MacIntyre provided laboratory space for data collection and editing of the manuscript. Dr. Forster developed the imaging protocol, provided access to the MRI facility, and editing of the manuscript. Dr. Reid assisted with data interpretation and was responsible for editing the manuscript.

# 1. LITERATURE REVIEW AND INTRODUCTION TO THESIS<sup>a</sup>

## 1.1 STATEMENT OF THE PROBLEM

Chronic obstructive pulmonary disease (COPD) affects 750 000 Canadians (approximately 3% of adults) (1) and results in significant morbidity and mortality (2, 3). In addition to the debilitating effects on the respiratory system, COPD results in systemic complications, specifically, skeletal muscle dysfunction (4). Lung transplant is a viable option for people with end-stage respiratory disease, resulting in major improvements in lung function and quality of life (5). However, skeletal muscle dysfunction persists and may be worsened following transplant and continues to limit functional capacity (6, 7). Although impairments in skeletal muscle structure and function have been described in people with COPD, there are studies to suggest that skeletal muscle function is preserved in some people with COPD (8, 9). Therefore, we hypothesized that impairments in skeletal muscle function of the quadriceps and hamstrings in people with COPD would be related to changes in skeletal muscle structure and these impairments in structure and function would be accentuated in lung transplant recipients.

The theoretical framework for examining skeletal muscle structural and functional changes in people with COPD and recipients of lung transplants is presented in Figure 1-1. This framework provides an overview of the generic factors (i.e. those that affect muscle in all people) and disease-specific factors (i.e. those that are particularly related to people with COPD or lung

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<sup>a</sup> Parts of this chapter have been published in the following two papers:

**Mathur S.,** Reid W.D., Levy, R.D. Exercise limitation in recipients of lung transplants. *Physical Therapy*. 2004;84(12):1178-87.

Warburton, D.E.R., **Mathur, S.** Skeletal muscle training in people with chronic heart failure and people with chronic obstructive pulmonary disease. *Physiotherapy Canada*. 2004;56:143-157.

transplant) that may contribute to skeletal muscle dysfunction. Further, it provides the specific measures of structure and function that are examined in this thesis to determine which characteristics of skeletal muscle are impaired and which are preserved in people with COPD and recipients of lung transplants.

## **1.2 CHRONIC OBSTRUCTIVE PULMONARY DISEASE**

COPD has been defined by the Global Initiative for Chronic Lung Disease (GOLD) as “a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases” (10). The main clinical symptoms of COPD are cough, sputum production and dyspnea on exertion.

Approximately 750, 000 Canadians (3% of adults) are diagnosed with COPD (1) however, this is likely an underestimate since many people do not seek medical assistance until their symptoms are moderate to severe in nature (2). It is estimated that up to 50% of people with COPD are undiagnosed (3). COPD is the fourth leading cause of death in men and the fifth in women (3). COPD also results in significantly morbidity; it is the fourth leading cause of hospitalization for men and sixth leading cause for women in Canada (2). Economic analysis has revealed that COPD results in direct costs of \$2000 (Canadian dollars) per patient annually (primary and secondary care visits, lab tests and treatments) and \$1198.18 in indirect costs (i.e. work lost due to COPD) resulting in a total annual societal cost of \$3195.52 per patient (11).

Smoking is the major cause of COPD accounting for 80-90% of cases (2), however only a minority of smokers develop the disease, therefore environmental and genetic factors also play role in the development of COPD (12). It has been established that the deficiency of alpha-1 antitrypsin ( $\alpha$ 1-AT) leads to the development of an early-onset, genetic form of COPD. This is a result of a deficiency in the elastase inhibitor resulting in overall breakdown in the elastic fibers of the lung (12). The balance of elastase to elastase inhibitors may also predispose certain people who smoke to develop COPD although a genetic linkage has not been established (12).

Diagnosis of COPD is based primarily on spirometry. The GOLD Guidelines provide cut-points for forced expiratory volume in one second ( $FEV_1$ ) and the ratio of  $FEV_1$  to forced vital capacity (FVC) to characterize severity of disease (see Table 1-1). Symptoms are also an important part of diagnosis however there is no clear relationship between the degree of airflow limitation and the severity of symptoms (10).

The Canadian Thoracic Society COPD Guidelines recommend that people with COPD should be encouraged to maintain an active lifestyle and receive education on self management (3). These goals can be met through a comprehensive respiratory rehabilitation program, however less than 2% of Canadians with COPD have access to such programs (13). Respiratory rehabilitation programs are multidisciplinary in nature and include exercise training, education and psychosocial support. In a meta-analysis of randomized controlled trials examining the effectiveness of the rehabilitation programs, Lacasse et al. (2005) (14) reported that respiratory rehabilitation programs that were at least four weeks long resulted in improvements in quality of life (dyspnea, fatigue and sense of control over the disease) and functional exercise capacity,

measured using walk tests. These data support the potential value of widespread implementation of rehabilitation programs for people with COPD.

### **1.3 LUNG TRANSPLANTATION**

Lung transplantation is a viable option for improving survival and quality of life in selected patients with end-stage lung diseases such as chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis, pulmonary hypertension and cystic fibrosis (5). There are a number of factors that determine whether someone is a candidate for lung transplantation and influence the timing and choice of procedure (i.e. single versus double lung transplant). Life expectancy and quality of life issues need to be considered for each potential candidate for transplantation (15). Factors that indicate the need for lung transplantation have been outlined in a consensus statement from the American Thoracic Society in conjunction with the European Respiratory Society and the International Society for Heart and Lung Transplantation (16).

In people with COPD, double lung transplant has been shown to result in greater lung function, exercise tolerance and a trend towards improved survival compared to single lung transplant, however it may be more suitable for younger candidates (people under the age of 55 years) who are at lower risk and will be expecting to return to a more active lifestyle following transplant than older candidates (17). The improvement in function following single lung transplant is also very favorable. In addition, single lung transplant may be preferred as it increases the number of donor lungs available thereby reducing waiting list time (17). Double lung transplant is the method of choice in people with cystic fibrosis due to the presence of chronic pulmonary infection and generalized bronchiectasis.

Data from the British Columbia Transplant Society (BCTS) indicate that nine single lung and one double lung transplant were performed in 2004 (18). The average waiting time for a lung transplant in 2004 was 12.2 months and there are currently 13 people awaiting lung transplant in this province. Survival statistics gathered in British Columbia from 1995 to 2003 provide a 74.4% survival rate at one year and 54.9% survival rate at five years following single lung transplant, and 77.6% and 58.4% survival rates respectively, following double lung transplant.

Organ rejection is the most important factor in long term survival following lung transplantation (5). Chronic rejection results in a small airway disease known as bronchiolitis obliterans (BO). BO appears to be a result of an "injury response" which results in inflammation of the small airway epithelium (19). Detection of BO can be done using transbronchial biopsy to examine histological changes in the airway epithelium, however this method is limited in that only small biopsies can be obtained and it has poor sensitivity (20). Therefore a clinical description of bronchiolitis obliterans syndrome (BOS) based on changes in  $FEV_1$  from a stable post-transplant value has been developed (20). The stable post-transplant  $FEV_1$  is determined and termed BOS stage 0. Progressive declines in  $FEV_1$  from baseline are defined as BOS stages one through three (see Table 1-2) (20). The BOS-p (potential-BOS) is based on a decline in mid-expiratory flow rate ( $FEF_{25-75}$ ) in addition to  $FEV_1$ , is used to alert the physician to the need for close monitoring (20).

Immunosuppression is essential following transplantation to minimize organ rejection. Standard immunosuppressive therapy consists of three classes of drugs: a calcineurin inhibitor (i.e. cyclosporine or tacrolimus), a purine synthesis inhibitor (i.e. azathioprine or mycophenolate mofetil) and a corticosteroid (i.e. prednisone) (5). Cyclosporine and tacrolimus bind to



intracellular binding proteins (immunophilins) in lymphocytes and inhibit the activity of calcineurin. This leads to an inhibition of DNA transcription and blocks the proliferation of T-cells and the release of interleukin-1 (IL-1) and IL-2 (21). Azathioprine and mycophenolate mofetil inhibit purine biosynthesis which then blocks lymphocyte proliferation (5). The immunosuppressive action of prednisone is to block cytokine gene transcription and secretion from mononuclear phagocytes (5). Together these drugs minimize acute and chronic organ rejection in the transplant recipient.

#### **1.4 EXERCISE LIMITATION IN COPD AND LUNG TRANSPLANT**

Patients with COPD exhibit symptoms of breathlessness and fatigue during exercise, leading to exercise intolerance (22, 23). Conventional research and rehabilitation therapy focused on the inability of the lung to extract and deliver oxygen to the working muscle (i.e. central limitation to exercise). However, it is increasingly apparent that primary organ failure is not the sole determinant of exercise intolerance in patients with COPD (24). In fact, indices of lung function, such as FEV<sub>1</sub> in COPD, are poor predictors of exercise capacity in these patients (25).

Recent research has revealed that patients with COPD have pathophysiologic changes in their skeletal muscle that affect their ability to perform physical work and, thus, to complete activities of daily living (25). Using a rating of perceived exertion (RPE) scale, 43% of patients with COPD rated leg fatigue higher than breathlessness (dyspnea) at maximal exercise (22). Leg fatigue was the primary reason to stop exercising, whereas dyspnea was reported as the primary reason for terminating exercise by only 26% of patients. Similarly, in a healthy control group, 36% reported leg fatigue and 22% reported dyspnea as the primary limiting symptom (22).

Mador et al. (2000) (26) found that up to 50% of patients with COPD demonstrated quadriceps fatigue following cycle exercise, suggesting that lower extremity (LE) muscle fatigue may contribute to exercise limitation in some patients with COPD (26, 27). In another study, quadriceps muscle strength was significantly related to maximal exercise capacity ( $r = .58$ ) and the distance covered in a 6-Minute Walk Test ( $r = .63$ ) in patients with COPD (28). Even with optimal bronchodilator therapy, which increased FEV<sub>1</sub> by 11% from baseline, contractile LE fatigue was still present following cycling exercise in a subgroup of patients who reported LE fatigue as the limiting factor to maximal exercise (27).

In contrast, Richardson and colleagues suggested that maximum exercise was not limited by skeletal muscle function in people with COPD, reporting that a skeletal muscle metabolic reserve exists during exercise in these patients (29). In their study, nine people with severe COPD completed two types of exercise: a maximal cycling exercise test, which uses a large muscle mass and is often limited by cardiorespiratory factors, and single leg extensor exercise, which uses a small muscle mass and is free of cardiorespiratory limiting factors. Both exercises were performed under three different conditions: breathing room air (21% O<sub>2</sub>), breathing 100% O<sub>2</sub> to increase O<sub>2</sub> availability to the muscle, and breathing a 79% helium–21% O<sub>2</sub> mixture (HeO<sub>2</sub> is used to relieve the work of breathing). The peak work rate (W<sub>peak</sub>) achieved during cycling increased when the subjects breathed either 100% O<sub>2</sub> or the HeO<sub>2</sub> mixture compared with breathing room air. Both of these conditions would allow greater O<sub>2</sub> availability to the working muscle, thereby increasing the maximal work output. Compared with breathing room air, the peak work rate on the single leg extensor exercise increased with 100% O<sub>2</sub> but not with the HeO<sub>2</sub>

mixture. In this case, cardiorespiratory factors do not limit exercise, so the use of HeO<sub>2</sub> to relieve the work of breathing would not improve oxygen delivery to the working muscle. However, an increased fraction of inspired O<sub>2</sub> would improve O<sub>2</sub> available to the working muscle, allowing a greater W<sub>peak</sub> to be achieved. The findings of Richardson and colleagues provide evidence that the knee extensor muscles may have the potential to utilize more oxygen if the availability of oxygen is increased. Therefore skeletal muscle may not be the sole factor in limiting exercise in some patients with COPD under experimental conditions (29). However, it is important to consider that, in a clinical setting, breathing 100% O<sub>2</sub> or HeO<sub>2</sub> is rarely used as an adjunct to exercise training in patients with COPD.

Following lung transplantation, there are substantial improvements in pulmonary function and a subsequent improvement in exercise capacity; however, peak exercise remains reduced to 40% to 60% of predicted values even up to 2 years after transplantation. Williams et al. (7) tested maximal exercise capacity in recipients of a single-lung transplant (SLT) (n=6) and in recipients of a double-lung transplant (DLT) (n=7) at 3 months and again at 1 to 2 years after transplantation. At 3 months after transplantation, peak oxygen consumption (VO<sub>2peak</sub>) was 46% of predicted values in the SLT group and 50% of predicted values in the DLT group. At 1 to 2 years after transplantation, there was no improvement in maximal oxygen consumption (VO<sub>2max</sub>) or maximal work capacity in either group, despite improvements in lung function and return to regular activities (i.e., school or work) in most of the recipients of transplants. Evans et al. (6) compared whole-body exercise (cycling) in 9 recipients of SLT who were 5 to 38 months after transplantation versus a control group of subjects without known pathology or impairments. Measurements of VO<sub>2peak</sub> taken during cycling were reduced in the SLT group compared with

the control group ( $p<.001$ ) and were only  $36.8\% \pm 3.1\%$  ( $\bar{x} \pm \text{SD}$ ) of predicted values in the SLT group.

A growing body of evidence points to the role of lower-limb skeletal muscle dysfunction following lung transplantation as the major factor in exercise limitation. This evidence is consistent with the observation that the majority of recipients of lung transplants report lower-extremity fatigue rather than dyspnea as the reason for terminating maximal exercise on a cycle ergometer (22). Using phosphorus magnetic resonance spectroscopy ( $^{31}\text{P}$ -NMR), Evans et al. (6) found that recipients of SLT ( $n=9$ ) demonstrated a lower resting pH of the quadriceps femoris muscle and an earlier drop in pH during bilateral knee extension exercise to exhaustion. In addition, the work rate at which pH fell was correlated with whole-body  $\text{VO}_{2\text{peak}}$ . These findings suggest that an intrinsic abnormality of the skeletal muscle may exist in recipients of transplants and may play a role in exercise limitation. Lands et al. (30) reported that in 9 recipients of SLT and 10 recipients of DLT, most of whom were over 18 months after transplantation, maximal work capacity on a cycle ergometer was most strongly correlated with 30-second work capacity during isokinetic cycling ( $r=.84$ ), rather than with pulmonary function variables such as  $\text{FEV}_1$  ( $r=.58$ ) and residual volume/total lung capacity ( $\text{RV}/\text{TLC}$ ) ( $r=-.52$ ).

## **1.5 SKELETAL MUSCLE ABNORMALITIES IN COPD AND LUNG TRANSPLANT**

A theoretical framework for examining structural and functional changes in skeletal muscle is presented in Figure 1-1. This framework provides an overview of the generic factors (i.e. those that affect muscle in all people) and disease-specific factors (i.e. those that are particularly related to people with COPD or lung transplant) that may contribute to skeletal muscle

dysfunction. A further discussion of these factors is presented in Section 1.5. These factors affect both the structure and function of skeletal muscle. A summary of the structural and functional changes in skeletal muscle of people with COPD and lung transplant recipients is discussed in this section.

The reduced exercise capacity and symptoms of patients with COPD are directly associated with alterations in skeletal muscle metabolism, and/or cellular structure (25). The skeletal muscles of patients with COPD demonstrate limitations in delivery and use of oxygen. These limitations likely contribute to the increased dependence on anaerobic metabolism, early onset of lactic acidosis, and reduction in exercise capacity typically seen in these patients (31, 32) and these alterations persist following lung transplantation (6). Table 1-3 provides an outline of skeletal muscle alterations in people with COPD, lung transplant recipients and with disuse. The following section reviews these limitations.

### **1.5.1 Muscle mass**

Reduced muscle mass and muscle fiber atrophy are commonly observed in patients with COPD (33-35). Reduced muscle mass is thought to contribute to exercise intolerance in patients with COPD (33, 36). Bernard and colleagues reported that the reduction in force produced by the quadriceps was proportional to the decrease in the cross-sectional area (CSA) of the muscle, suggesting that the loss of strength was due primarily to the loss in muscle mass and that the contractile function of the muscle was preserved (33). Intrinsic contractile function of the vastus lateralis has also been shown to be preserved in vitro, using bundles of muscle fibres obtained

through open muscle biopsies from patients with COPD (37). Gosker et al. (2002) (38) found a strong correlation between fat free mass measured using bioelectrical impedance and total mean muscle fiber CSA ( $r=0.87$ ,  $p < 0.001$ ). The authors also reported a fiber atrophy of type 2A/X and type 2X fibers compared to controls, whereas the fiber area of type 1, 1/2A and 2A fibers was similar to controls (38).

Apoptosis, which is the process of programmed cell death, has been observed in skeletal muscle and may result in muscle fiber atrophy (39). Skeletal muscle apoptosis has also been detected in patients with COPD using the TdT-mediated X-dUTP nick end-labeling (TUNEL) technique and by detection of proteolytic fragments. Apoptosis was significantly higher in patients with COPD who had a body mass index (BMI) less than  $20 \text{ kg/m}^2$  compared with those with a normal BMI (40).

Muscle mass has not been described extensively following lung transplantation and there have been no studies on people with COPD following lung transplant. In a group of lung transplant recipients with cystic fibrosis, there was a 31% reduction in the CSA of their quadriceps muscle and a 33% reduction in the quadriceps peak torque compared to controls (41). There was a strong, positive correlation between CSA of the quadriceps and peak torque which was similar to controls, indicating that muscle strength per CSA was preserved in this group of patients (41).

### **1.5.2 Cellular changes in structure**

Histologic skeletal muscle abnormalities have been observed in people with COPD, including (1) a reduction in the percentage of type 1 fibers (slow twitch); (2) a shift towards type 2 fibers

(specifically type 2b fibers); (3) a shift from the myosin heavy-chain 1 (fatigue resistant) isoform to myosin heavy-chain 2a and 2b (more fatigable) isoforms; (4) a reduced capillary-to-fiber ratio; and (5) reduced capillary density (35, 42, 43).

Type 1 fiber proportion has also been shown to be reduced in lung transplant recipients compared to age and sex matched controls ( $24.9\% \pm 4.4\%$  [ $x \pm SD$ ] versus  $56.1\% \pm 2.4\%$  [ $x \pm SD$ ]) (44). Furthermore, Morton et al. (45) showed that the reduction in proportion of type 1 muscle fibers and oxidative enzymes of the quadriceps femoris muscle also was present before transplantation in 18 candidates for lung transplant compared with controls. These patients had severe, end-stage lung disease and a primary diagnosis of COPD (n=8), bronchiectasis (n=5), cystic fibrosis (n=3), pulmonary fibrosis (n=1), and Eisenmenger syndrome (n=1). Three months after transplantation, a second muscle biopsy was taken in 13 of these patients and no change was observed in oxidative capacity or proportion of type 1 muscle fibers compared with the pre-transplant condition. The results of this study suggest that although changes in muscle oxidative capacity are seen following lung transplantation, these changes may be a reflection of changes in muscle that occur in the pre-transplant condition.

### **1.5.3 Energy metabolism of muscle**

A series of metabolic changes occur within the skeletal muscle of patients with COPD, which may play a role in exercise limitation. The oxidative capacity of the skeletal muscles in patients with COPD is reduced compared with that of healthy people and may contribute to the early onset of anaerobic metabolism and exercise limitation (46). This may be associated with increased intracellular acidosis and disproportionate lactate production during exercise (31).

Simon et al., (2001) (47) demonstrated a plateau in the  $\text{VO}_2$  of the lower extremities during cycling exercise in a subgroup of patients of COPD. This was partially explained by a limitation in the ability of the lower extremity muscles to extract oxygen. Early reductions in phosphocreatine and pH during exercise conditions have also been observed in the quadriceps muscles of patients with COPD (48-50).

Skeletal muscle metabolism has also been examined in recipients of lung transplants. Using  $^{31}\text{P}$ -MRS, Evans et al.(6) demonstrated that recipients of lung transplants (5-38 months after transplantation) had a greater decline in PCr/Pi, greater increases in lactate concentrations, and lower resting intracellular pH of the quadriceps femoris muscle, which dropped at a lower metabolic rate with incremental bilateral lower-extremity exercise. In a study by Tirdel et al.(51) four recipients of SLT and 2 recipients of DLT (5- to 28 months post-transplant) underwent near-infrared spectroscopy (NIRS) in conjunction with a standard exercise test to examine peripheral oxygen uptake of the quadriceps femoris muscle. Compared to an age- and sex-matched control group, recipients of lung transplants demonstrated less oxygen desaturation at the level of the vastus lateralis muscle during peak cycling exercise compared with controls, indicating an impaired ability of the muscle to uptake and utilize the available oxygen.

Reduced systemic oxygen extraction, measured from arterial and venous oxygen content during incremental exercise, also has been reported in patients with cystic fibrosis and COPD before and after lung transplantation suggesting that the ability of working skeletal muscle to extract oxygen is impaired in the pre-transplant condition and does not improve after transplantation(52, 53).



#### 1.5.4 Biochemical changes

A series of biochemical abnormalities further worsen the oxidative potential of skeletal muscle in patients with COPD. Researchers have observed reductions in  $\beta$ -hydroxyacyl coenzyme A dehydrogenase activity (an enzyme involved in  $\beta$ -oxidation), citrate synthase, and/or succinate dehydrogenase (46). Cytochrome oxidase, the final enzyme of the electron transport chain, is increased in patients with COPD (54). There is little change or perhaps an increase in the concentration of glycolytic enzymes (46, 55). The biochemical and histologic changes associated with COPD indicate that a shift from aerobic to anaerobic metabolism may occur in these patients.

Similar to findings in patients with COPD, the quadriceps femoris muscles of recipients of lung transplants show reduced oxidative capacity. Wang et al.(44) examined biopsies from the vastus lateralis muscle in 7 recipients of lung transplants (2 with SLT, 4 with DLT, and 1 with HLT) 3 to 24 months after transplantation and compared them with 7 control subjects matched for age and sex. The muscle from transplant recipients had a lower oxidative enzyme activity (ie, citrate synthase, 3-hydroxyacyl-coA-dehydrogenase), and higher activity of the glycolytic enzyme, phosphofructokinase, compared with that of matched controls. The findings of reduced muscle oxidative capacity are in line with the consistent observation of a reduced  $\text{VO}_2$  peak and early onset of lactate acidosis observed in recipients of lung transplants (44). The biochemical and histologic changes associated with COPD indicate that a shift from aerobic to anaerobic metabolism may occur in these patients and this may persist following lung transplantation.

## **1.6 FACTORS AFFECTING SKELETAL MUSCLE IN COPD AND LUNG TRANSPLANT**

Many factors have been identified as possible contributors to skeletal muscle dysfunction in patients with COPD (4). Figure 1 summarizes both the generic and disease-specific factors that may affect skeletal muscle function in people with COPD and recipients of lung transplants.

Because of dyspnea, people with COPD often reduce their level of physical activity and adopt sedentary lifestyles, leading to deconditioning of the cardiovascular system and disuse of the peripheral muscles, especially those in the LEs. Furthermore, Pitta et al., (2005) (56) compared the physical activity level in people with COPD compared to sedentary, older adults using a triaxial accelerometer. People with COPD were found to spend less time standing and walking and more time sitting and lying than the controls, and also had a lower intensity of walking (56). This study provides quantitative data showing that people with COPD have lower levels of daily physical activity and this may contribute to disuse of the peripheral muscles.

Humans exposed to spaceflight, cast immobilization, or bedrest experience decreased muscle mass and muscle CSA, atrophy of type 1 and 2 muscle fibres, and a corresponding decline in peak force and power output (57, 58). Oxygen delivery to the muscle and concentration of oxidative enzymes of the quadriceps are reduced in humans following bedrest (57). Atrophy from bedrest or decreased physical activity likely affects the LE muscles more than the UE muscles owing to relative differences in the type and amount of contractions of these muscles during activities of daily living (33, 57, 59).

Similar to the changes experienced with disuse, patients with COPD experience a reduction in the CSA of their thigh muscles (33) and a reduction in the CSA of type 1 and type 2 fibres of the quadriceps (35). However, muscle strength of the biceps is reduced to a degree similar to that of the quadriceps in patients with COPD, (25, 60) suggesting that loss of muscle strength may be due to other systemic factors. Other factors, such as hypoxemia or hypercapnea, poor nutrition, use of corticosteroids, decreased hormone levels, systemic inflammation and oxidative stress may also contribute to the skeletal muscle abnormalities observed in patients with COPD (25). Similarly, these factors may be accentuated following transplantation due to a prolonged period of bedrest post-operatively, reduced activity level and immunosuppressant therapy.

Altered blood gases may also contribute to skeletal muscle abnormalities in people with COPD (61). Hypoxemia has been related to a decrease in the oxidative capacity of muscle in healthy humans exposed to altitude (62) and these adaptations are similar to the changes observed in the quadriceps muscle in patients with COPD (4, 42). Furthermore the presence of hypercapnea may alter the resting pH of muscle and lead to early onset of muscle fatigue by inhibiting the action of the rate limiting enzyme phosphofructokinase (PFK) and the interaction of the contractile elements with calcium (4).

Nutritional depletion has been well documented in patients with COPD and has been reported in 49% of candidates entering pulmonary rehabilitation (63), and 45% of lung transplant candidates (64). Nutritional depletion contributes to a decline in muscle mass, preferential decrease in CSA of type 2 fibers and a concurrent reduction in the peak force output of the muscle (4). Engelen et al. (1994) (65) found isometric handgrip force to be significantly lower in COPD patients with

nutritional depletion (less than 90% of ideal body weight) compared to patients with adequate nutritional status.

The presence of systemic inflammation and oxidative stress in COPD may also contribute to muscle atrophy. With inflammation there is an increase in the production of reactive oxygen species which can contribute to apoptosis of skeletal muscle nuclei resulting in fiber atrophy (66). As patients with COPD are typically older, the ability of the muscle to regenerate is hampered (67) and fiber atrophy from apoptosis can be permanent and result in reduced muscle strength. Reactive oxygen species can damage structural, transport and regulatory proteins of the cell, alter membrane potentials and alter membrane permeability to ions (68). This damage can affect the muscle's ability for excitation-contraction coupling, carbohydrate metabolism and calcium homeostasis (4) and lead to an inability of the muscle to produce and sustain a contraction, decreasing force output and increasing susceptibility to fatigue. In patients with COPD, local submaximal quadriceps exercise (repeated knee extensions at 30% of maximum voluntary contraction) was found to induce an increase in markers of muscle lipid peroxidation and protein oxidation but unlike the control group, did not result in an increase in antioxidant activity (69). The increase in markers of oxidative stress was inversely correlated with quadriceps endurance time ( $r = -0.66$ ;  $p < 0.05$ ). The results indicate that there may be an oxidant/antioxidant imbalance in people with COPD and this may affect muscle endurance (69).

Skeletal muscle myopathy associated with chronic corticosteroid use has been well documented and results in muscle fiber atrophy, affecting type 2 fibers to a greater extent and a downregulation of the action of insulin-like growth factor (IGF-1) (ATS/ERS, 1999). Long-term use of corticosteroids has been associated with proximal limb muscle weakness, selective type 2

fiber atrophy in peripheral muscle and the diaphragm (70, 71). In patients with COPD, a significant reduction in quadriceps force and respiratory muscle force has been observed and correlates to the average daily dose of corticosteroids (72). Observation of steroid-induced myopathy under the microscope reveals increased fiber size variation, presence of angulated fibers, centrally located nuclei and basophilic staining fibers (70). These findings are similar to those of muscles exposed to prolonged overload and injury and may reflect a decreased regenerative capacity of peripheral muscle in these patients and contribute to muscle atrophy. This may indicate that muscles of patients exposed to corticosteroids are more susceptible to muscle injury, which may lead to a loss of muscle strength and increased susceptibility to fatigue.

Reduced levels of anabolic hormones normally seen with aging have also been shown to affect muscle CSA and force production. Both IGF-1 and testosterone decrease in men and women with age. This reduction may be further accentuated in COPD by interaction with chronic hypoxemia, corticosteroid use and chronic illness, contributing to the decline in muscle strength (36). In a recent study by Casaburi et al. (2004) (73), testosterone supplementation with or without resistance training was given to men with COPD who had low testosterone levels. Testosterone supplementation alone led to an increase in lean body mass of 2.3 kg and a 17% increase in LE muscle strength (1-repetition maximum for leg press). Testosterone supplementation in addition to resistance training led to an increase in lean body mass of 3.3kg and a 27% increase in LE muscle strength (73). The findings of this study support the use of testosterone supplementation in improving muscle function in men with COPD.

Preliminary data from Coronell et al., (74) suggest the potential for a genetic link between muscle function in people with COPD. In a study of 80 males with COPD, an association between the expression of HLA class II alleles and quadriceps and respiratory muscle strength and endurance was found, such that those people who expressed the gene had significantly greater muscle strength and endurance (74). This finding may provide some further insight into why some people with COPD have profound muscle weakness and atrophy while others have relatively preserved muscle function (75).

Following lung transplant, there may be a persistence of skeletal muscle abnormalities associated with the pre-existing condition, however factors associated with the transplant procedure and post-operative management may accentuate these abnormalities or result in alternate changes. Following transplant, there is a prolonged period of bedrest and reduced physical activity, which may result in further changes associated with disuse. Immunosuppressant medications such as cyclosporine and corticosteroids also have a profound effect on skeletal muscle function. The immunosuppressant agent cyclosporine has been shown to impair mitochondrial function. Animal studies have shown that cyclosporine in therapeutic doses can decrease the capacity of the electron transport chain (a source of ATP production during oxidative metabolism) by blocking a calcium-dependent pore in the inner mitochondrial membrane, thus affecting calcium efflux out of the mitochondria and impairing mitochondrial respiration (76). This impairment in calcium transport may lead to an inability of working muscle to utilize oxygen and an early shift toward glycolytic metabolism, especially during exercise, resulting in limited exercise capacity (76, 77). Mercier et al. (77) reported that the impairment in mitochondrial respiration was associated with reduced endurance time in treadmill running in rats given cyclosporine. Similarly, tacrolimus, which is also a calcineurin inhibitor and is prescribed instead of

cyclosporine for many recipients of lung transplants, may have similar effects in muscle (5). Cyclosporine also may cause chronic anemia in some recipients of transplants, resulting in reduced oxygen-carrying capacity of blood (78). However, anemia likely has a minimal effect on  $\text{VO}_2\text{peak}$  during exercise because hemoglobin levels are normal or only mildly reduced in most patients, and reduced  $\text{VO}_2\text{peak}$  is seen in recipients of transplants (79, 80).

## **1.7 SUMMARY**

Current evidence implicates skeletal muscle dysfunction as an important factor limiting exercise tolerance in patients with COPD and recipients of lung transplants. There are a number of factors which may contribute to skeletal muscle dysfunction; however, there is a large variation in the degree of muscle dysfunction experienced by people with COPD and lung transplant recipients (8, 9). It remains unclear whether some aspects of skeletal muscle structure and function are maintained and whether there are some factors that may assist in preserving muscle function in these two groups of people. A further examination of the structural and functional aspects of skeletal muscle may provide further insight into the nature of skeletal muscle dysfunction in people with COPD and recipients of lung transplants.

## 1.8 OBJECTIVES AND HYPOTHESES OF THE THESIS

This thesis will address the question: which aspects of skeletal muscle structure and function are impaired and which are preserved in people with COPD and recipients of lung transplants? This thesis examines both the structure and function of skeletal muscle to address this question. As shown in Figure 1, the structural aspects of skeletal muscle examined in this thesis are muscle atrophy, or a reduction in muscle size, and myopathy, which can manifest as an inability of the muscle to generate tension per unit size. Muscle function is examined through the ability to generate torque, or muscle strength, and the reduction in force generating capacity of a muscle due to preceding activity, or muscle fatigue.

We hypothesized that impairments in skeletal muscle function of the quadriceps and hamstrings in people with COPD would be related to changes in skeletal muscle structure and these impairments in structure and function would be accentuated in recipients of lung transplant. The specific objectives and hypotheses that are addressed in this thesis are outlined below.

**Objective 1.** To examine the differences in concentric and eccentric torques of the knee extensors (KEs) and knee flexors (KFs) in people with COPD compared to age, sex and BMI-matched controls.

**Hypothesis 1.** People with COPD will have lower torques of the KEs and KFs compared to matched controls and the extent of the difference between groups will be similar for concentric and eccentric torque.



**Objective 2.** To determine whether muscle volume and intramuscular fat infiltration of the quadriceps and hamstrings are related to reduced concentric and eccentric torque of the KEs and KFs, respectively, in people with COPD.

**Hypothesis 2.** Reduced muscle volume and increased intramuscular fat infiltration will be correlated with reduced concentric and eccentric torque in people with COPD.

**Objective 3.** To compare the time to task failure of the quadriceps muscle and the motor unit firing properties using surface EMG during a fatiguing contraction of the quadriceps in people with COPD to age, sex and BMI-matched controls.

**Hypothesis 3.** It is hypothesized that people with COPD will have shorter time to task failure compared to controls and this will be due to differences in the ability to recruit and fire motor units.

**Objective 4.** To compare cellular features of skeletal muscle injury in the vastus lateralis in people with COPD to age, sex and BMI-matched controls.

**Hypothesis 4.** People with COPD will show more features of skeletal muscle injury than controls.

**Objective 5.** To compare the skeletal muscle structure (atrophy, fat infiltration) and function (torque and fatigue) of lung transplant recipients to people with COPD and controls.

**Hypothesis 5.** People with lung transplant may have poor skeletal muscle function due to their pre-transplant condition (i.e. COPD) and this is likely worsened by post-operative bedrest and immunosuppressant medications. It is hypothesized that lung transplant recipients will have

greater detrimental changes in their skeletal muscle structure and function compared to people with COPD and controls.

## 1.9 SCOPE OF THE THESIS

This thesis includes seven studies presented in the following seven chapters followed by a discussion chapter which provides an overall summary of the findings and conclusions. Chapters Two and Three report the results of two pilot studies which were used to develop the methodologies employed in the thesis. **Chapter Two** is a pilot study which examines the reliability of surface EMG during muscle fatigue and uses a novel method of examining changes in surface EMG median frequency and amplitude during a sustained muscle contraction.

**Chapter Three** is a case report which was used to pilot the isokinetic strength measurements and to establish the methods for determining muscle volume from multiple slices obtained from magnetic resonance imaging. Chapters Four to Six are individual manuscripts which report the findings from each study on people with COPD. **Chapter Four** examines skeletal muscle atrophy and intramuscular fat infiltration in people with COPD and the relationship of these structural aspects of skeletal muscle to concentric and eccentric torque production in people with COPD. **Chapter Five** evaluates two aspects of quadriceps muscle fatigue in people with COPD: endurance time and motor unit firing properties using surface EMG. **Chapter Six** examines cellular features of muscle injury using needle muscle biopsies of the vastus lateralis muscle of the quadriceps in people with COPD. **Chapter Seven** reports the key findings from the study on recipients of lung transplants and compares the findings to what was found in people with COPD. Finally, **Chapter Eight** provides a summary of the main conclusions from each study, highlights the novel findings from the thesis and outlines directions for future research.

## 1.10 TABLES AND FIGURES

**Table 1-1.** COPD classification from GOLD Guidelines (10)

Stage	Characteristics
0: At Risk	<ul style="list-style-type: none"><li>• normal spirometry</li><li>• chronic symptoms (cough, sputum production)</li></ul>
I: Mild COPD	<ul style="list-style-type: none"><li>• <math>FEV_1/FVC &lt; 70\%</math></li><li>• <math>FEV_1 \geq 80\%</math> predicted</li><li>• with or without chronic symptoms (cough, sputum production)</li></ul>
II: Moderate COPD	<ul style="list-style-type: none"><li>• <math>FEV_1/FVC &lt; 70\%</math></li><li>• <math>50\% \leq FEV_1 &lt; 80\%</math> predicted</li><li>• with or without chronic symptoms (cough, sputum production)</li></ul>
III: Severe COPD	<ul style="list-style-type: none"><li>• <math>FEV_1/FVC &lt; 70\%</math></li><li>• <math>30\% \leq FEV_1 &lt; 50\%</math> predicted</li><li>• with or without chronic symptoms (cough, sputum production)</li></ul>
IV: Very Severe COPD	<ul style="list-style-type: none"><li>• <math>FEV_1/FVC &lt; 70\%</math></li><li>• <math>FEV_1 &lt; 30\%</math> predicted or <math>FEV_1 &lt; 50\%</math> predicted plus chronic respiratory failure</li></ul>

Classification based on post-bronchodilator  $FEV_1$

$FEV_1$ : forced expiratory volume in one second

FVC: forced vital capacity

respiratory failure: arterial partial pressure of oxygen ( $PaO_2$ ) less than 8.0 kPa (60 mm Hg) with or without arterial partial pressure of  $CO_2$  ( $PaCO_2$ ) greater than 6.7 kPa (50 mm Hg) while breathing air at sea level.

**Table 1-2.** Bronchiolitis obliterans syndrome (BOS) classification system (2002) (20)

BOS Stage	Change in forced expiratory volume
BOS 0	$FEV_1 > 90\%$ of baseline and $FEF_{25-75} > 75\%$ of baseline
BOS 0p	$FEV_1$ 81-90% of baseline and/or $FEF_{25-75} \leq 75\%$ of baseline
BOS 1	$FEV_1$ 66-80% of baseline
BOS 2	$FEV_1$ 51-65% of baseline
BOS 3	$FEV_1 \leq 50\%$ of baseline

Abbreviations:

BOS – bronchiolitis obliterans syndrome

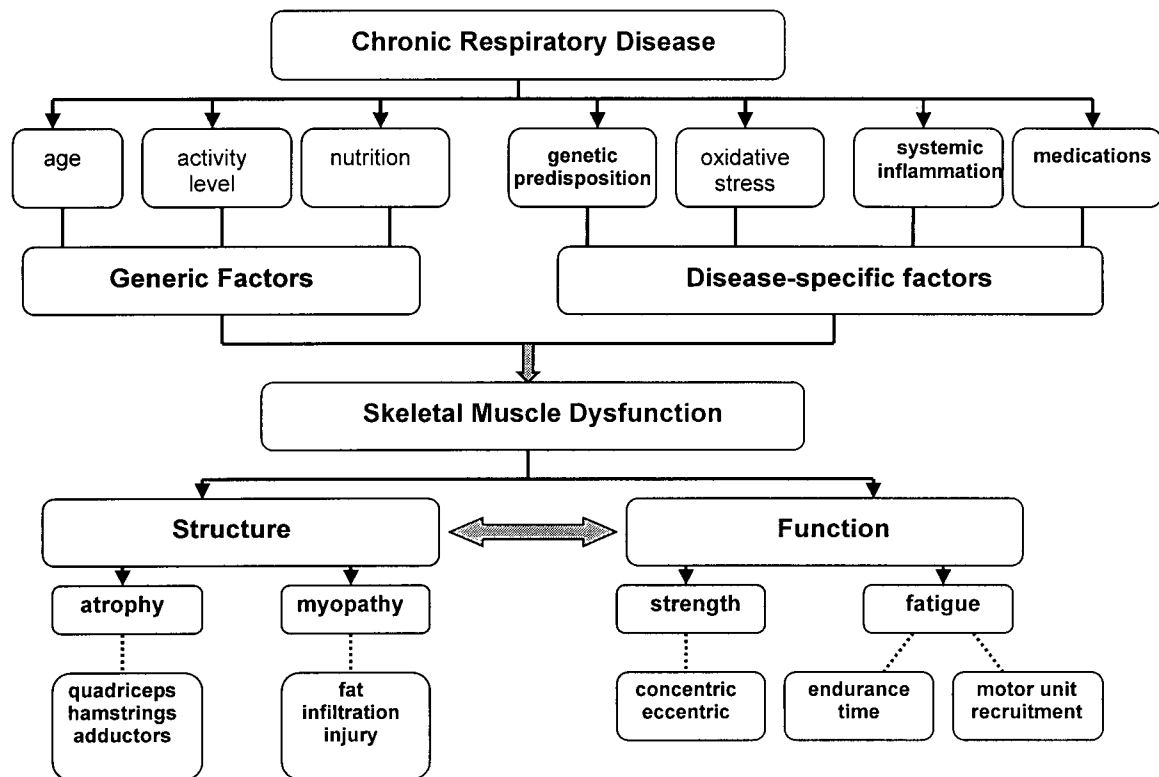
$FEV_1$ – forced expiratory volume in one second

$FEF_{25-75}$  – mid-expiratory flow rate

**Table 1-3.** Comparison of skeletal muscle pathophysiological changes after disuse, in patients with COPD and lung transplant recipients

Skeletal Muscle Changes		COPD	Transplant	Disuse
Functional	↑Muscle Weakness	√(33, 60)	√(41)	√(81, 82)
	↑Muscle Fatigability	√(26, 27, 83)	√(30)	
	Early Onset of Anaerobic Metabolism	√(31, 84)	√(6)	√(85)
Morphologic	Muscle Fiber Atrophy	√(35)		√(57, 86)
	↓Muscle Mass	√(33)	√(41)	√(87-89)
Cellular	↓Percentage of Type 1 Fibers	√(35, 43)	√(44)	√(90)
	↑Percentage of Type 2 Fibers	√(35, 42)	√(44)	√(90)
	↓Capillary to Fiber Ratio	√(35, 42)		√(91)
	↑Apoptosis	√(40)		√(92)
	↑Fatigable Myosin Heavy Chain Isoforms	√(43)		√(93)
Metabolic	↑Intracellular Acidosis	√(49)	√(6)	√(94)
	↑Phosphocreatine Depletion	√(49, 50)	√(6)	√(88, 90)
	↓Reliance on Oxidative Pathways	√(48, 49)	√(6)	√(95, 96)
	↑Reliance on Glycolytic Pathways	√(48, 49)	√(6)	√(96)
Biochemical	↓Oxidative Enzyme Activity	√(35)	√(44)	√(97)
	↑Oxidative Stress	√(69)		√(98)

**Note:** Example references are provided. Adapted from: Warburton and Mathur. (2004) (99)



**Figure 1-1.** Theoretical framework for examining skeletal muscle dysfunction in people with COPD and recipients of lung transplants.

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## 2. RELIABILITY OF SURFACE EMG DURING SUSTAINED CONTRACTIONS OF THE QUADRICEPS<sup>b</sup>

### 2.1 INTRODUCTION

Neuromuscular fatigue has been defined as a reduction in the force generating capacity of a muscle due to previous activity (1). A number of methods exist to quantify fatigue in humans during muscular work. The duration for which a task can be sustained at a given level of maximal voluntary contraction (MVC) has been widely used to quantify fatigue and is termed the endurance time or time to task failure (1). Surface electromyography (EMG) also provides a non-invasive, objective method of measuring the physiological processes occurring during sustained muscular work and is a widely accepted method of quantifying fatigue (2). In addition to objectivity, it can be used to quantify fatigue from different sites on a large muscle group and/or among agonist muscles (3-5). Surface EMG during fatiguing contractions has previously been used to discriminate between people with low back pain from healthy people (6-8) and to assess muscle function following immobilization (9) and exertion-induced muscle injury (10).

Surface EMG is influenced by a number of physiological properties such as motor unit discharge rates and muscle fiber membrane characteristics, as well as non-physiological properties such as electrode size, shape and placement (11). Day to day variation in EMG recording may be associated with differences in electrode re-application such as minor changes in the position of the recording electrodes over the muscle and differences in skin preparation (12). Therefore it is

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important to establish the reliability of measures derived from the EMG signal for both clinical and research settings, especially when used to determine differences in performance over time in the same individual and differences between individuals.

During sustained, submaximal isometric contractions, characteristic changes in EMG occur including an increase in amplitude and a spectral shift towards lower frequency (13). These changes have been observed at contractions levels from 20% MVC up to 100% MVC (14, 15). The increase in amplitude during sustained contractions has been attributed to an increase in neuromuscular activation (rate coding and motor unit recruitment) which is required as the ability of the muscle to generate force decreases with sustained activity (16). The frequency shift has been attributed to peripheral changes, specifically a reduction in muscle fiber action potential conduction velocity and central changes including synchronous firing of motor units and recruitment of new motor units (16, 17). Changes in mean frequency and median frequency are common methods for expressing this frequency shift during sustained contractions (18). Median frequency (MF) is defined as the frequency that splits the power spectrum of the EMG in half and is less susceptible to noise than the mean frequency (19). Previous studies have used the slope of the MF and amplitude to describe the shift in these parameters during fatiguing contractions. However, the slopes of MF and amplitude have previously shown low reliability and are therefore not useful measurements (4, 17, 20). To indicate the presence of muscle fatigue, it is important to determine how the final MF and amplitude of EMG relate to the initial MF and amplitude, respectively. Therefore, we chose to “normalize” the final value to the initial value as a unique measure of fatigue and determine the reliability of “normalized” MF and amplitude compared to the previously used measure of slope.

The quadriceps femoris is made up of four muscles: rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) which lie superficially and the vastus intermedius, which is deep. These muscles work together to generate force, however, previous studies show that differences may exist in the relative activation of these muscles during maximal voluntary contractions and sustained submaximal contractions (21-23). Therefore, differences may also exist in the rate of fatigue and activation among these muscles during sustained submaximal contractions of the quadriceps. The purpose of this study was to examine the test-retest reliability of four measures (initial, final, normalized final and slope) derived from the EMG signal for both MF and amplitude. Three superficial muscles of the quadriceps (RF, VL, and VM) were studied during sustained submaximal contractions at 80% and 20% of MVC. Secondly, differences in MF and amplitude among the three muscle groups were examined.

## **2.2 METHODS**

### **2.2.1 Sample**

Twenty-two subjects (n=11 males and n=11 females) were tested on two occasions, one week apart. Subjects were healthy males and females between the ages of 21 and 34 years of age (age  $27 \pm 4$  years [mean $\pm$ SD], height:  $174.3 \pm 10.5$  cm, weight:  $74.0 \pm 4.6$  kg, BMI:  $24.1 \pm 2.7$  kg/m<sup>2</sup>), recreationally active, with no history of knee pathology, cardiorespiratory or neuromuscular conditions. Subjects were asked to refrain from physical activity on the day prior to and the day of testing to avoid the effects of cumulative muscular fatigue. All subjects signed an informed consent form prior to testing. Ethical approval was granted by the University Clinical Research Ethics Board.

### 2.2.2 Instrumentation

All torque measures were done on the KinCom dynamometer (version 5.30, Chattanooga Group Inc., Hixson TN). The KinCom has previously been shown to be a valid and reliable method for measuring torque produced by a muscle (24, 25).

Surface EMG was collected from three electrode sites on the quadriceps muscle using pairs of self-adhesive, silver, silver-chloride pellet electrodes (7 mm diameter, fixed interelectrode distance of 30 mm, Kendall Meditrace). The raw signal was passed through a differential amplifier, input impedance of 10 Gohm, CMRR of 115dB, and a gain of 1000 (Bortec Electronics, Calgary, Canada). The signal was analogue to digitally converted at 1024 samples per second (AT-MIO-64E-3, National Instruments), and bandpass filtered (second order Butterworth filter) at 10-400 Hz. The frequency content (i.e., power spectrum) was determined for each window using discrete FFT methods, and the statistical definition of 'median' was used to calculate the MF in each window. The first 250 ms segments of each second of EMG were processed on-line using Hanning window processing and Fast Fourier Transform to calculate the MF. Custom off-line processing (MatLab, The MathWorks Inc., Natick MA) was used to detect the point of fatigue and to determine the start and end-points of force and EMG. The end-point of the contraction (i.e. the point of fatigue) was defined as the point where the produced force dropped 20% from the target level. Data collected over a five-second window following the start point and preceding the end point were averaged to obtain a single value for start and final MF and amplitude of EMG.

### **2.2.3 Electrode placement**

EMG recordings were taken from three sites on the quadriceps: rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM). A ground electrode was placed over the patella. Skin was cleaned with alcohol prior to placing the electrodes. Predetermined landmarks were used as a guideline for electrode placement (15 cm from the superior border of the patella for RF, 7 cm superior for VM and 9 cm superior for VL) (26). Subjects were then asked to contract their quadriceps against manual resistance and the belly of the muscle was palpated. The electrodes were placed collar to collar over the largest part of the muscle belly parallel to the orientation of the muscle fibers. The distance of the distal electrode from the patella was recorded to ensure that the same placement could be used during the second testing session.

### **2.2.4 Maximal voluntary contractions**

The subject was seated on the KinCom seat with the axis of rotation of the dynamometer aligned to the knee joint line. The backrest and seat angles were adjusted so that the hip was at approximately 80° of flexion. A strap was placed across the subject's pelvis to minimize hip movement during the tests. The cuff (load cell) of the KinCom was placed at a distance of 50% of the lower leg length (measured from the top of the fibular head to the mid-point of the lateral malleolus). This length was noted to ensure the same placement for the second testing session. The knee joint was placed at 90° of flexion for all testing.



Following a submaximal warm-up contraction, subjects did three isometric maximal voluntary contractions (MVC) of the knee extensors. The highest was used to calculate the target force for the endurance tasks. Subjects were instructed to "push as hard as possible" for a five second period. Visual feedback of the produced force was provided. A one minute break was given between MVC trials. MVCs were done on both legs and on both days of testing, prior to the endurance task. Leg dominance was recorded and was determined by asking each subject over which leg he or she had more control (e.g. which leg they would use to kick a ball).

### **2.2.5 Endurance tasks**

Each subject performed two fatiguing contractions: 80% MVC on one leg and 20% MVC on the other leg. Through random allocation, eleven subjects performed the 20% MVC on their dominant side and eleven performed the 80% MVC on their dominant side. The order of the fatigue tasks was also randomized. Visual feedback of the target and produced force was provided and verbal encouragement was given by the tester to maintain the force at the target level until the target force could no longer be met despite verbal cueing. The endurance time was attained from the software program and was defined as the point where the produced force dropped 20% from the target level.

On the second day of testing, the same endurance task was performed on each leg following the measurement of MVCs. The subjects were not given knowledge of their results until after completion of the second testing session.

### 2.2.6 Statistical analysis

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) (version 10, SPSS Inc., Chicago IL). Descriptive statistics for sample characteristics and for MF and amplitude at each level were expressed using mean  $\pm$  standard deviation. Slope of MF and amplitude was calculated from the linear line of best fit through the set of data points from the start to end value. Normalized final MF and amplitude was calculated as a ratio of the final value to the initial value (e.g. final value/initial value). Scatterplots of the data were inspected to ensure that no outliers existed in the data set. Intraclass correlation coefficient (ICC) (2,1) was used to express relative reliability of the measures (27). ICC expresses the ratio of between-subject variance to within-subject variance and is a unitless value (27). Munro's descriptors for reliability coefficients were used to describe the degree of reliability: 0.00 to 0.25 – little, if any correlation; 0.26 to 0.49 – low correlation; 0.50 to 0.69 – moderate correlation; 0.70 to 0.89 – high correlation and 0.90 to 1.00 – very high correlation (28). Standard error of the measurement (SEM) was used to express absolute reliability of the measure (29). SEM is calculated from the square root of the error variance (i.e. mean of standard deviations from day 1 and day 2) and has the same unit as the tested variable. Smaller values of SEM reflect more reliable measures (29). The SEM was also expressed as a percent of the mean value for the measure to allow for comparison of absolute reliability between measurements (SEM/mean  $\times$  100%). Comparisons among muscles were done using initial, final and normalized final values for MF and amplitude using repeated measures ANOVA (blocked for subject) and post-hoc Tukey's tests.

## **2.3 RESULTS**

### **2.3.1 Torque measures and endurance time**

All twenty-two subjects completed both test sessions. Peak torque on the dominant leg was  $137 \pm 62$  Nm and on the non-dominant leg was  $141 \pm 58$  Nm ( $p=0.790$ ). Start torque for the 80% contraction demonstrated very high reliability:  $103.4 \pm 50.5$  Nm on day 1 and  $100.0 \pm 42.1$  on day 2 (ICC=0.95, SEM 54.7 Nm). For the 20% contraction, start torque was also highly reliable:  $26.6 \pm 13.3$  Nm on day 1 and  $27.2 \pm 12.8$  Nm (ICC=0.97, SEM=10.8). Endurance time for both contraction levels showed high reliability. For the 80% contraction, the mean endurance time was  $59 \pm 33$  s on day 1 and  $57 \pm 22$  s on day 2 (ICC=0.85, SEM=10s). For the 20% contraction, endurance time was  $379 \pm 138$  s on day 1 and  $400 \pm 198$  s on day 2 (ICC =0.96, SEM = 58s).

### **2.3.2 Median frequency**

Figure 2-1 shows the initial and final MF for Day 2; slope and normalized MF for both Days 1 and 2 are shown in Table 2-1. ICCs for test-rest reliability are shown in Table 2-2. Moderate to high relative reliability was found for initial and final MF for both the 80% and 20% MVC for all three muscle groups (ICC ranging from 0.59 to 0.88). SEM ranged from 5% to 11% of the mean (i.e. 68% of the time, the actual measure would be expected to fall in this range). Rectus femoris showed the highest relative and absolute reliability compared to VL and VM for both contraction levels.

Normalized final MF showed moderate to high reliability for all three muscle groups for both contraction levels (see Table 2-2). Slope of MF for the 80% contraction showed low reliability for all three muscle groups with ICCs ranging from 0.28 to 0.35 and was associated with large variability (see Table 2-2). For the 20% contraction, ICCs for slope demonstrated high reliability for RF and moderate reliability for VL and VM. Absolute reliability for slope at 20% however was poor with SEMs being close to twice as large as the mean values (see Table 2-2).

### **2.3.3 Amplitude**

Figure 2-2 shows the initial and final amplitude for Day 2; initial and final amplitude expressed as a percentage of MVC and slope and normalized amplitude are shown in Table 2-3. Reliability coefficients are shown in Table 2-2. For the 80% contraction, SEMs ranged from 22% to 36% of the mean for initial and final amplitude, however relative reliability was moderate to high (ICC=0.58 to 0.84; Table 2-2). The slope of amplitude showed particularly low ICCs for RF and VM. This was likely due to a single subject who had particularly high values for slope of amplitude for all three muscle groups on the first day of testing but was within two standard deviations of the mean on the second day. Normalized final amplitude showed moderate to high relative reliability (see Table 2-2).

For the 20% contraction, all three muscles demonstrated high to very high reliability for all measures with the exception of normalized final amplitude for VL which showed moderate reliability (see Table 2-2). Absolute reliability ranged from 15% to 22% of the mean for initial, final and normalized amplitude. A large degree of variability was found for slope of amplitude at

20%. Absolute reliability was low with SEMs ranging from 35% to 58% of the mean for this measure.

#### **2.3.4 Differences among muscles of the quadriceps**

Initial, final and normalized final values for MF and amplitude from the second day of testing were used for further comparison between muscle groups. Slopes of MF and amplitude were excluded from between-muscle comparisons, as they were associated with large variability. Rectus femoris demonstrated a higher initial MF and lower normalized final MF compared to VL and VM for the 80% MVC ( $p < 0.001$ , Table 2-1). Normalized end amplitude was lower in RF compared to VL and VM for the 80% MVC ( $p < 0.001$ , Table 2-2). For the 20% MVC, no significant differences were found among muscle groups for MF or amplitude (see Table 2-1).

### **2.4 DISCUSSION**

Our study used a unique measure of fatigue, the normalized final MF and amplitude. These measures were found to be more reliable than slope, which is a more commonly used measure of fatigue. We also used both high and low level contractions and found that the reliability of EMG varied depending on the level of the contraction. In addition, differences were found among the muscles of the superficial quadriceps in MF and amplitude of EMG during sustained submaximal contractions at a high contraction level.

### **2.4.1 Reliability of median frequency of EMG**

In our study, moderate to high reliability was found for initial and final MF at 80% and 20% MVC for all three muscle groups. This is in accordance with previous findings of reliability of initial MF with sustained submaximal contractions of the quadriceps (30), elbow extensors (19) and trunk extensors (5, 20). It has previously been shown that the variability associated with repeated contractions differs among the superficial muscles of the quadriceps (4, 23, 30). In our study, RF showed the greatest relative and absolute reliability of initial and final MF compared to VL and VM for the 80% and 20% MVC. Similarly, Kollmitzer et al. (4) found RF to have greater reliability than the vasti during a sustained contraction at 50% MVC. The position of the thigh may account for the greater variability of the EMG recording in VL and VM during knee extension. A slight internal or external rotation at the hip can change the extent to which each of these muscles is recruited thereby increasing between-day variance (4, 10).

Low ICCs were found for slope of MF for the 80% MVC, however the ICCs for the 20% MVC was moderate to high. The associated variability was high for both contraction levels with SEM being four to ten times higher than the mean value. High variability for slope of MF has been documented in a number of muscle groups at varying levels of MVC (5, 17, 20). A regression-based calculation was used to estimate the line of best fit to determine the slope of MF over the duration of the contraction. This may introduce a greater degree of variability than regression-free indices of fatigue such as the normalized final MF. Although variability was high, ICCs for slope of MF during the 20% MVC were high in contrast to the 80% MVC. The ICC considers both the within-subject variability, in addition to the between-subject variability; the greater range of data for the 20% (therefore greater between-subject variability) compared to the 80%

MVC likely resulted in higher ICCs. In addition, contraction time for 20% MVC also showed a better relative reliability ( $ICC = 0.96$  compared to  $0.85$ ) and this may partially account for the higher reliability in the slope measurement. In our study, normalized MF was calculated as an alternate measure to express the change in MF over time. Normalized MF was found to be a more reliable measure compared to slope for both contraction levels and would be preferred as an index of fatigue in making further comparisons.

#### **2.4.2 Reliability of amplitude of EMG**

In our study, reliability of amplitude was moderate to high for all measures except for slope during the 80% MVC and was comparable to the reliability found for MF indicating that both measures are useful in the assessment of muscle fatigue. Previous studies have also reported good reliability for amplitude measures during sustained contractions of the quadriceps (23, 30). Similar to the findings with MF, normalized final amplitude had better absolute and relative reliability than slope and would therefore be a preferred index of fatigue than slope.

For 20% MVC, all measures of EMG amplitude showed high to very high reliability and the reliability was generally higher than that found for the 80% MVC. As the amplitude of the EMG signal is related to the force (2, 31), this may reflect the differences in the ability to hold the force steady at low versus high forces (32). An inverse relationship exists between force production and force variability (i.e. steadiness of force); with higher forces showing greater force variations and hence, an increase in within-subject variability (33, 34). This may have contributed to a greater degree of variability in the amplitude of EMG between-days and

accounted for the lower ICCs for the 80% MVC. It may be useful to quantify the steadiness (e.g. standard deviation, coefficient of variation) of the force in future investigations.

### **2.4.3 Differences among muscles of the quadriceps**

The results of this study suggest that not all muscles of the quadriceps are recruited in a similar manner or contribute to knee extensor force production equally, particularly with submaximal voluntary contractions. The RF showed a higher initial MF and lower normalized MF compared to the vasti during the 80% MVC but not the 20% MVC. These differences may reflect morphological variations among the superficial muscles of the quadriceps. Early anatomical studies have reported a greater proportion of type 1 fibers (~50%) in VM compared to VL (~30%) (35) and a significantly larger type 2 fiber diameter in the RF (74  $\mu\text{m}$ ) compared to VM and VL (65 and 63  $\mu\text{m}$ , respectively) (36). As initial MF has been correlated to the proportion of type 2 fibers in a muscle(4, 37), the difference in fiber type proportion among the quadriceps may account for the significantly higher initial MF found with the 80% MVC. In the 20% MVC this difference was not seen and may be due to the low force requirement for this task where type 2 fibers would not be initially recruited. Future studies should examine fiber type proportions in addition to muscle fatigue to determine whether differences in EMG parameters are related to the cellular composition of the muscle.

The rate of fatigue was also greater in RF, as indicated by the lower normalized final MF, compared to the vasti for the 80% MVC but not the 20% MVC. During the 80% MVC there is greater recruitment of type 2 fibers (38), which likely results in greater recruitment of the RF muscle compared to the vasti. RF has previously been shown to be the most fatigable muscle of



the quadriceps with sustained contractions (22, 30) and to have the lowest threshold for fatigue with cycle ergometry (21). In addition to differences in fiber proportions, RF is a biarticular muscle while the vasti are monoarticular. Ebenbichler et al. (39) who also reported greater fatigue of RF compared to VL and VM (with the hip at a fixed angle) attributed it to differences in electrophysiological behavior between monoarticular and biarticular muscles.

No differences were found among the muscles with the 20% MVC. This is likely due to the lower force requirement for this task. In a 20% sustained contraction, recruitment of type 1 fibers is adequate for the maintenance of initial force (38), therefore all three muscles were likely recruited in a similar manner and fatigued at a uniform rate. Similar to findings by Pincivero et al. (23), no differences were seen between the vasti muscles and both showed a parallel decline in MF and parallel increase in amplitude for both contraction levels.

## **2.5 CONCLUSION**

Although a number of factors can affect the between-day reliability of EMG such as electrode placement, skin preparation, position of the limb and subject performance, the results of this study show that MF and amplitude of surface EMG can be reliably measured across days during sustained fatiguing contractions of the quadriceps. Normalized final values of MF and amplitude are associated with smaller variability compared to slope and are therefore preferred as a measure of fatigue. The differences among the superficial muscles of the quadriceps in MF and amplitude during a high but not a low level contraction may provide some insight into functional differences among these muscles. Future studies should examine the ability of EMG to detect

changes in fatigability of muscles following muscle atrophy or exercise training and its relationship to muscle fiber type proportions.

## **2.6 BRIDGING SUMMARY**

The study presented in this Chapter was used to develop the methodology for examining motor unit firing properties during a sustained contraction of the quadriceps, for the studies described in Chapters Five and Seven of this thesis. This study examines the reliability of endurance time (or time to task failure) and two measures from surface EMG, MF and amplitude. It also introduces the measure of normalized MF and amplitude, which is a novel measure for quantifying muscle fatigue. The normalized MF and amplitude were found to be more reliable than the slope of MF and amplitude which was previously described in the literature, so normalized MF and amplitude were used for further studies in the thesis. The task using 80% of MVC was used for studies in people with COPD and recipients of lung transplants as it resulted in a shorter endurance time and would be better tolerated by these people. This task was also more feasible to conduct during a single test session.

**Table 2-1.** EMG median frequency parameters for 80% and 20% contractions (mean  $\pm$  standard deviation)

Variable	Rectus Femoris		Vastus Medialis		Vastus Lateralis	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
<b>80% contraction</b>						
Initial (Hz)	89.2 $\pm$ 14.8	95.3 $\pm$ 13.8*†	82.8 $\pm$ 10.7	82.2 $\pm$ 12.8	74.6 $\pm$ 13.5	76.2 $\pm$ 12.6
Final (Hz)	70.2 $\pm$ 13.4	74.6 $\pm$ 13.1	72.3 $\pm$ 14.3	72.7 $\pm$ 12.7	63.9 $\pm$ 12.3	68.1 $\pm$ 14.7
Normalized (Hz)	0.78 $\pm$ 0.01	0.78 $\pm$ 0.01*†	0.87 $\pm$ 0.12	0.89 $\pm$ 0.11	0.86 $\pm$ 0.01	0.89 $\pm$ 0.01
Slope (Hz/s)	-0.39 $\pm$ 0.21	-0.35 $\pm$ 0.20	-0.22 $\pm$ 0.24	-0.17 $\pm$ 0.24	-0.19 $\pm$ 0.18	-0.11 $\pm$ 0.15
<b>20% contraction</b>						
Initial (Hz)	90.7 $\pm$ 12.9	91.8 $\pm$ 12.4	80.6 $\pm$ 10.3	83.9 $\pm$ 8.9	81.7 $\pm$ 12.1	81.3 $\pm$ 7.3
Final (Hz)	81.6 $\pm$ 13.6	83.1 $\pm$ 14.1	73.8 $\pm$ 10.2	76.8 $\pm$ 8.0	70.2 $\pm$ 12.7	72.3 $\pm$ 8.4
Normalized (Hz)	0.90 $\pm$ 0.13	0.90 $\pm$ 0.0075	0.92 $\pm$ 0.01	0.92 $\pm$ 0.01	0.86 $\pm$ 0.12	0.89 $\pm$ 0.11
Slope (Hz/s)	-0.0023 $\pm$ 0.0029	-0.0019 $\pm$ 0.0024	-0.0014 $\pm$ 0.00184	-0.0011 $\pm$ 0.0024	-0.0031 $\pm$ 0.0034	-0.0021 $\pm$ 0.0028

Normalized MF = final MF/initial MF

\* significantly different from VM,  $p < 0.001$ † significantly different from VL,  $p < 0.001$

**Table 2-2.** Reliability of median frequency and amplitude measures for 80% and 20% contractions.

	Rectus Femoris		Vastus Medialis		Vastus Lateralis	
<b>MEDIAN FREQUENCY</b>						
<b>80% contraction</b>	<b>ICC</b>	<b>SEM (%)</b>	<b>ICC</b>	<b>SEM (%)</b>	<b>ICC</b>	<b>SEM (%)</b>
Initial (Hz)	0.88	4.96 (5.4%)	0.62	7.25 (8.8%)	0.62	8.06 (10.7%)
Final (Hz)	0.87	4.76 (6.6%)	0.88	4.69 (6.5%)	0.68	7.59 (11.5%)
Normalized (Hz)	0.61	0.054 (6.9%)	0.73	0.058 (6.6%)	0.81	0.038 (4.3%)
Slope (Hz/s)	0.35	0.16 (43.2%)	0.28	0.21 (>100%)	0.31	0.14 (86.1%)
<b>20% contraction</b>						
Initial (Hz)	0.84	5.05 (5.5%)	0.59	6.17 (7.5%)	0.80	4.38 (5.4%)
Final (Hz)	0.87	4.96 (6.0%)	0.70	4.98 (6.6%)	0.67	6.09 (8.5%)
Normalized (Hz)	0.66	0.058 (6.5%)	0.68	0.043 (4.7%)	0.66	0.065 (7.5%)
Slope (Hz/s)	0.89	0.026 (>100%)	0.50	0.015 (>100%)	0.85	0.012 (>100%)
<b>AMPLITUDE</b>						
<b>80% contraction</b>	<b>ICC</b>	<b>SEM (%)</b>	<b>ICC</b>	<b>SEM (%)</b>	<b>ICC</b>	<b>SEM (%)</b>
Initial (μV)	0.65	57.71 (31.8%)	0.74	81.03 (31.9%)	0.84	55.09 (23.4%)
Final (μV)	0.71	52.03 (31.6%)	0.58	102.84 (36.7%)	0.80	65.73 (26.0%)
Normalized (μV)	0.66	0.16 (17.2%)	0.83	0.15 (12.6%)	0.84	0.11 (9.8%)
Slope (μV/s)	0.30	1.30 (>100%)	0.21	2.34 (>100%)	0.76	0.81 (>100%)
<b>20% contraction</b>						
Initial (μV)	0.87	7.41 (20.2%)	0.92	10.82 (17.6%)	0.85	12.58 (21.8%)
Final (μV)	0.92	22.08 (26.5%)	0.97	21.22 (14.1%)	0.83	34.59 (27.7%)
Normalized (μV)	0.91	0.42 (17.5%)	0.88	0.36 (14.8%)	0.68	0.48 (22.0%)
Slope (μV/s)	0.85	0.076 (63.3%)	0.90	0.094 (36.1%)	0.87	0.060 (32.4%)

SEM is given in units provided and expressed as a % of mean of Days 1 and 2.

Normalized MF = final MF/initial MF; normalized amplitude = final amplitude/initial amplitude

**Table 2-3.** EMG amplitude parameters for 80% and 20% contractions (mean  $\pm$  standard deviation)

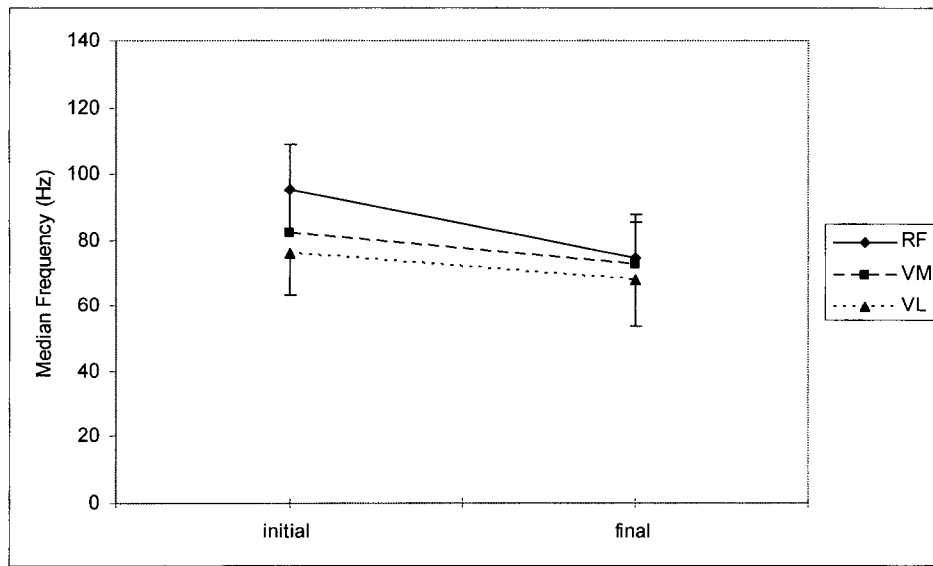
Variable	Rectus Femoris		Vastus Medialis		Vastus Lateralis	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
<b>80% contraction</b>						
Initial ( $\mu$ V)	191.4 $\pm$ 131.5	171.9 $\pm$ 64.8	253.9 $\pm$ 167.1	253.8 $\pm$ 152.0	256.2 $\pm$ 158.4	213.7 $\pm$ 119.8
Final ( $\mu$ V)	182.8 $\pm$ 128.6	145.9 $\pm$ 64.1	287.8 $\pm$ 146.4	272.6 $\pm$ 171.1	280.0 $\pm$ 148.5	225.6 $\pm$ 147.3
Initial (% MVC)	91.4 $\pm$ 18.3%	90.6 $\pm$ 28.4%	87.3 $\pm$ 22.4%	84.0 $\pm$ 20.5%	95.7 $\pm$ 21.8%	91.6 $\pm$ 26.5%
Final (% MVC)	90.5 $\pm$ 24.8%	81.2 $\pm$ 38.4%	107.2 $\pm$ 34.1%	101.2 $\pm$ 47.6%	110.3 $\pm$ 30.6%	97.3 $\pm$ 30.9%
Normalized( $\mu$ V)	1.00 $\pm$ 0.29	0.86 $\pm$ 0.24*	1.23 $\pm$ 0.35	1.15 $\pm$ 0.39	1.17 $\pm$ 0.29	1.06 $\pm$ 0.27
Slope ( $\mu$ V/s)	-0.195 $\pm$ 1.97	-0.704 $\pm$ 1.13	0.683 $\pm$ 2.87	-0.009 $\pm$ 2.384	0.324 $\pm$ 1.86	0.0003 $\pm$ 1.40
<b>20% contraction</b>						
Initial ( $\mu$ V)	37.6 $\pm$ 24.6	35.6 $\pm$ 16.7	61.8 $\pm$ 40.8	61.4 $\pm$ 38.2	56.1 $\pm$ 30.7	59.1 $\pm$ 34.5
Final ( $\mu$ V)	86.7 $\pm$ 89.9	79.9 $\pm$ 68.2	148.0 $\pm$ 123.6	152.1 $\pm$ 119.8	120.1 $\pm$ 79.5	129.6 $\pm$ 87.6
Initial (% MVC)	15.8 $\pm$ 5.9%	16.2 $\pm$ 7.1%	19.5 $\pm$ 7.0%	21.8 $\pm$ 10.4%	21.4 $\pm$ 9.0%	24.0 $\pm$ 10.5%
Final (% MVC)	33.3 $\pm$ 15.4%	36.3 $\pm$ 17.2%	45.1 $\pm$ 18.8%	51.7 $\pm$ 25.7%	45.1 $\pm$ 24.1%	54.8 $\pm$ 26.9%
Normalized( $\mu$ V)	2.45 $\pm$ 1.54	2.35 $\pm$ 1.28	2.45 $\pm$ 1.12	2.43 $\pm$ 0.96	2.20 $\pm$ 0.98	2.16 $\pm$ 0.72
Slope ( $\mu$ V/s)	0.13 $\pm$ 0.25	0.11 $\pm$ 0.13	0.24 $\pm$ 0.32	0.28 $\pm$ 0.29	0.17 $\pm$ 0.14	0.20 $\pm$ 0.19

Initial and final amplitudes are expressed as absolute values ( $\mu$ V) and as percentage of amplitude achieved during MVC

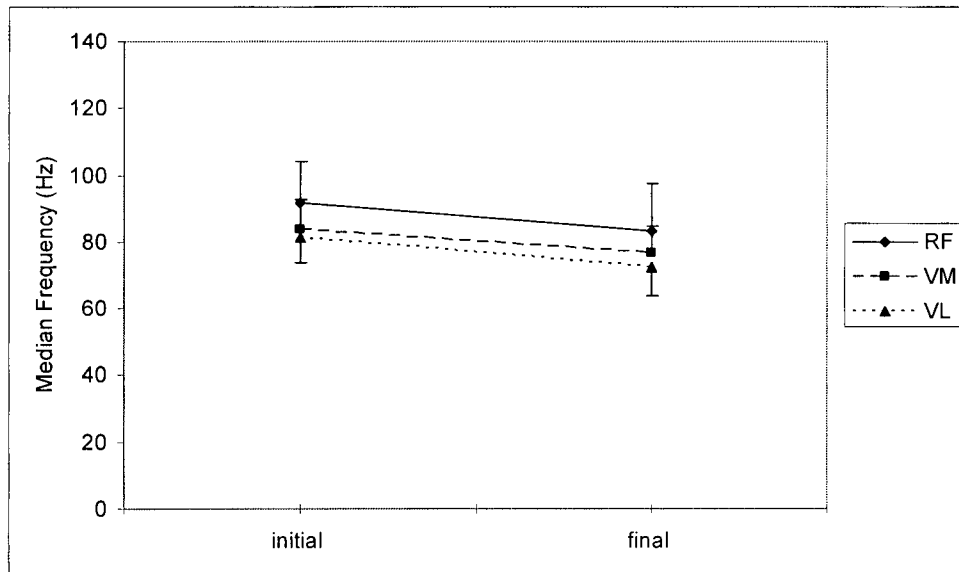
Normalized amplitude = final amplitude/initial amplitude

\* significantly different from VM,  $p < 0.001$

† significantly different from VL,  $p < 0.001$

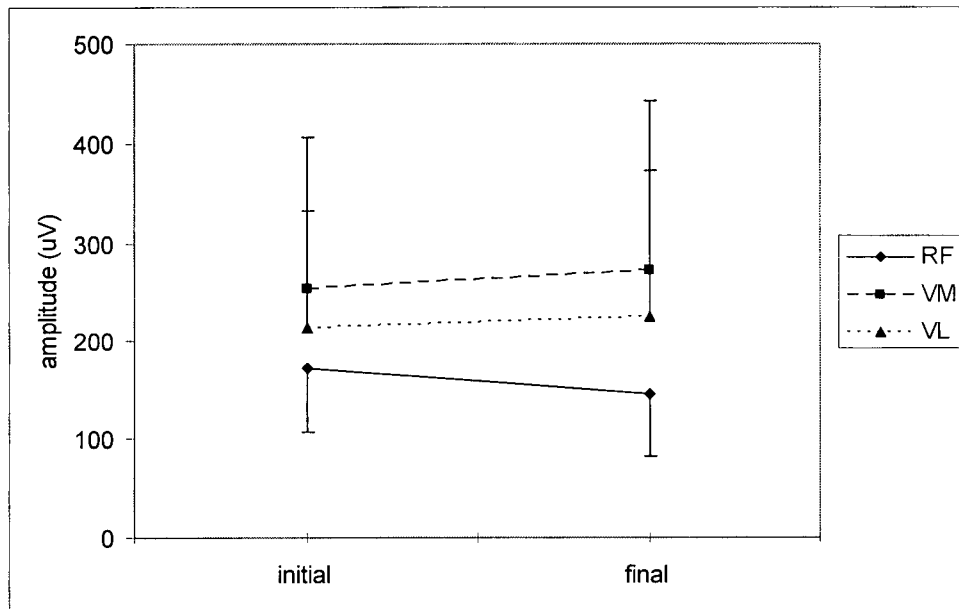


A)

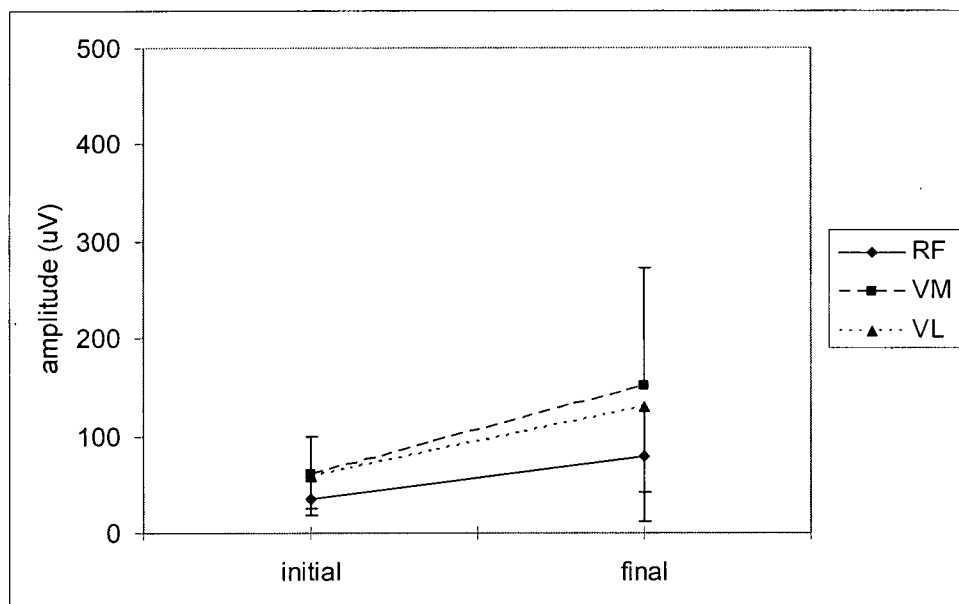


B)

**Figure 2-1.** Initial and final median frequency (MF) on Day 2 for A) 80% contraction and B) 20% contraction (mean  $\pm$  standard deviation). Note: Between-subject SDs are presented however within-subject SDs are used to calculate reliability coefficients.



A)



B)

**Figure 2-2.** Initial and final amplitude on Day 2 for A) 80% contraction and B) 20% contraction (mean  $\pm$  standard deviation). Note: Between-subject SDs are presented however within-subject SDs are used to calculate reliability coefficients.

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### 3. INFLUENCE OF EXERCISE-INDUCED INJURY ON KNEE EXTENSION TORQUE IN THE PRESENCE OF LONGSTANDING QUADRICEPS ATROPHY: A CASE REPORT<sup>c</sup>

#### 3.1 INTRODUCTION

Immobilization or non-weight bearing as a result of injury or disease results in numerous adaptive changes in skeletal muscle with atrophy being one of the most recognized consequences of disuse (1). Previous studies have confirmed that generalized thigh muscle atrophy occurs after serious knee injury, ligament injury, tibial plateau fracture, and patellar fracture (1-6). In addition to changes in muscle “quantity” (i.e. cross-sectional area), changes in muscle “quality” (i.e. presence of intramuscular fat) may contribute to loss of muscle function from inactivity or disuse. Several human studies have shown 12 to 30% loss of cross-sectional area following 4 to 17 weeks of disuse. The loss of muscle strength during this time frame is even more pronounced (1), with reported rates of 1 to 6% strength loss per day during the first week (7). Significant deficits in quadriceps muscle strength and postural stability were still present in subjects with a variety of lower-limb injuries, even 40 years after injury (3). Decrements in absolute torque during eccentric activity in these subjects tended to be worse than decrements of concentric or isometric torque (3). However, the extent of torque deficit that can be attributed to muscle atrophy versus other potential factors has not yet been determined.

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<sup>c</sup> A version of this chapter has been accepted for publication: **Mathur, S., MacIntyre, D.L., Forster, B.B., Reid, W.D.** Influence of exercise-induced injury on knee extension torque in the presence of longstanding quadriceps atrophy: A case report. *Physiotherapy Canada*. 2005; 57:1-9.

A well-established technique for the study of gross structure of muscle, magnetic resonance imaging (MRI) distinguishes muscle from fat and connective tissue and can be used to normalize muscle force per unit cross sectional area or unit volume. Used to visualize edema-like abnormalities, patchy fibrosis and fatty infiltration in inflammatory muscle diseases (8, 9), MRI resolution also allows for discrimination among different groups of muscles, enabling examination of atrophy of individual muscles. By normalizing measures of muscle strength to muscle volume, the extent to which muscle atrophy contributes to strength deficit following knee injury can be determined.

Repeated maximal eccentric muscle contraction results in muscle damage, such as sarcomere disruption, inflammation, edema, muscle soreness and loss of muscle force generating capacity (10). In a sedentary 23-year-old woman, isometric torque of the quadriceps decreased 52% immediately after four bouts of 15 maximal isokinetic eccentric contractions and recovered on the fifth day (11). In a study of older individuals who may be more susceptible to muscle damage due to age-related changes, eight sets of 7 to 10 eccentric quadriceps contractions at 75% of eccentric maximum were used to induce muscle damage (12). Isometric quadriceps torque was still reduced 10 days following the exercise stimulus. We speculated that atrophy and weakness of the quadriceps muscle secondary to knee injury might also result in increased susceptibility to exercise-induced muscle damage. However, the effect of eccentric exercise on quadriceps torque in an individual with muscle atrophy following knee injury has not previously been reported.

The purposes of this case report were (1) to describe the muscle volume and torque-generating capacity of the quadriceps in a 46 year old woman, 16 years after an intra-articular tibial plateau fracture and (2) to compare the decline and recovery of quadriceps concentric, eccentric and

isometric torque after exercise-induced muscle damage (eccentric exercise) between the injured lower extremity (LE) and unaffected LE. We hypothesized that the quadriceps muscle of the injured LE would show muscle atrophy and a deficit in torque generating capacity compared to the unaffected LE. Also, the decline in torque following muscle damage would be greater and the recovery of quadriceps torque would be slower in the injured LE compared to the unaffected LE.

### **3.2 CASE DESCRIPTION**

#### **3.2.1 History**

The subject was a 46-year-old woman who sustained a left tibial plateau fracture in a skiing accident 16 years prior to the study. The fracture involved her dominant LE. During open reduction-internal fixation at the time of injury, it was confirmed that a large fragment of the lateral tibial plateau was depressed inferiorly approximately 1 cm. A satisfactory reduction was achieved after the surgery and the subject was then immobilized in a straight leg splint for two months (non-weight bearing). The subject was then touch-weight bearing for one month, and subsequently underwent physical therapy for 1.5 years. Extension and flexion of the knee improved during the initial two years after the fracture but continued to be limited. During the last period of intermittent rehabilitation (1996-2003), the subject performed unilateral weight lifting, stair master and cycling to improve knee function.

The subject provided written informed consent prior to participation in the study. The study protocol was approved by the University of British Columbia's Clinical Research Ethics Board.

### **3.2.2 Examination**

Physical examination at the time of the study showed a range of movement of the left knee of 10-140°. Valgus mobility of the left medial collateral ligament was slightly increased. Tests for anterior and posterior cruciate ligaments and lateral collateral ligament and Apley's meniscus were negative. Patello-femoral joint mobility was normal. No knee joint pain or effusion was elicited by the physical examination or by any of the tests throughout the study. The unaffected knee was normal to testing.

## **3.3 METHODS**

### **3.3.1 Procedure**

Repeated measures of concentric, eccentric and isometric quadriceps torque and delayed onset muscle soreness (DOMS) were acquired on four different days to establish a baseline. Following the baseline, the subject performed a bout of eccentric-exercise on each LE to induce muscle damage. Quadriceps torque and DOMS were measured at 2 hours and at 1, 2, 11, 12, and 13 days after the eccentric-exercise stimulus.

### **3.3.2 Magnetic resonance imaging (MRI)**

MRI was used to determine quadriceps muscle volume. A 1.5 Tesla MRI scanner (1.5T Horizon Echospeed Scanner, General Electric, Milwaukee WI) was used to acquire 100, 5-mm axial

contiguous slices from the femoral-tibial joint line to the anterior superior iliac spine on both thighs. Images were T1-weighted (TE = 8 ms; TR = 650 ms) with a 40 cm<sup>2</sup> field of view and a 512 x 384 pixel matrix (in-plane spatial resolution = 0.78 x 1.04 mm). A representative axial image is shown in Figure 3-1.

NIH Image, version 1.31 ([http://rsb.info.nih.gov/ij/Java 1.3.1\\_03](http://rsb.info.nih.gov/ij/Java 1.3.1_03)) was used to manually outline the rectus femoris (RF) and the vasti (vastus lateralis, vastus intermedius and vastus medialis) on 17 slices starting at the first slice in which the rectus femoris muscle was present: 2 cm below the anterior inferior iliac spine (proximal slice), to the superior aspect of the patella (distal slice). The distance between the proximal and distal slices was divided equally into 16 slices. The gap between measured slices was 2.0 cm or 2.5 cm. The total volume of RF and the vasti (cm<sup>3</sup>) was calculated by summing the product of measured muscle cross-sectional area by the slice thickness of all 17 sections, and the volume of the gaps between slices, estimated using the truncated cone formula (13):

$$\text{volume of gap} = 1/3 * [\text{CSA1} + \text{CSA2} + \text{square root}(\text{CSA1} * \text{CSA2})] * \text{gap thickness}$$

CSA1=cross-sectional area of slice immediately above gap

CSA2=cross-sectional area of slice immediately below gap

This method has previously been validated for estimation of muscle volume (14). Inter-rater reliability of CSA measurement as established on 65 regions of interest by two raters showed an average percentage error between the two raters of 0.4%, with an *r*-value of 0.99.



Muscle volume of the RF and vasti was summed to obtain total quadriceps volume for each LE. These values were used to normalize the isokinetic and isometric torque measurements on each LE by dividing the torque measure by the total quadriceps muscle volume on each LE ( $\text{Nm}/\text{cm}^3$ ).

### **3.3.3 Quadriceps strength**

Maximal voluntary isokinetic (concentric and eccentric) and isometric torque of the quadriceps were measured on the KinCom isokinetic dynamometer (version 5.30, Chattanooga Group Inc., Hixson TN). The KinCom dynamometer has been shown to be reliable and valid for measuring torque produced by a muscle (15, 16). The subject was seated on the KinCom with straps placed across the hips and shoulders. Her knee was carefully aligned with the axis of rotation of the KinCom. The shin pad was placed at 75% of the distance from the head of the fibula to the distal edge of the lateral malleolus.

For isokinetic testing, the subject performed 5 sub-maximal warm-up contractions followed by 3 to 4 maximal voluntary concentric and eccentric contractions at  $30^\circ/\text{s}$  through a range of motion from  $100^\circ$  of flexion to full available extension. For measures of isometric torque, the subject performed one sub-maximal warm-up contraction followed by 3 to 4 maximal voluntary isometric contractions at 70, 90 and 100 degrees of knee flexion. Each contraction was maintained for a minimum of 3 seconds.

For both isokinetic and isometric testing, a 1-minute rest was provided between trials. The subject was instructed to “push as hard as possible” and received visual and verbal feedback during each contraction. Averages of the three highest concentric contractions, three highest

eccentric contractions and three highest isometric contractions at each angle were recorded. Torque values were corrected for gravity and normalized to quadriceps muscle volume ( $\text{Nm}/\text{cm}^3$ ).

#### **3.3.4 Delayed onset muscle soreness (DOMS)**

The amount and location of DOMS was measured on all testing days. At the beginning of each test session (before measures of torque were performed), the subject was asked to sit in a chair and rest for 5 minutes. She was then asked to indicate the amount and location of DOMS when moving from sitting to standing and from standing to sitting in a chair, without pushing on the armrests of the chair. The amount of DOMS was indicated on a 10 cm visual analogue scale (VAS) anchored by the phrase “no soreness” at one end and by “worst soreness ever felt” at the other end. The VAS has previously been used to assess DOMS (17) and shown to be reliable for assessment of pain (18). The location of DOMS was shaded on a body diagram.

#### **3.3.5 Eccentric-exercise stimulus**

The subject performed 60 eccentric contractions (6 sets of 10 repetitions) on each LE using the KinCom dynamometer. The contractions were at 75% of the eccentric maximal force at  $30^\circ/\text{s}$ . The range of movement was set at  $110^\circ$  to  $10^\circ$  knee flexion. A 90-second rest was given between sets. A similar amount of eccentric exercise has been shown to induce muscle damage in older adults who showed muscle atrophy as compared to younger adults (12).

### **3.4 RESULTS**

#### **3.4.1 Muscle volume**

Atrophy of the affected quadriceps was evident in each measured MRI slice throughout the length of the quadriceps, especially in the vasti (Figures 3-1 and 3-2). The atrophy of the vasti was fairly consistent, ranging from 70% to 76% of the right side volume from slice 4 (29 cm above the patella) to slice 14 (5 cm above the patella). The muscle volumes of RF, vasti and the four quadriceps muscles combined of the affected side were 83%, 72%, and 74% of the unaffected quadriceps, respectively.

#### **3.4.2 Delayed onset muscle soreness (DOMS)**

DOMS peaked at 24 hours after exercise-induced muscle damage but was mild even at its peak, i.e. 2.5 cm on a 10 cm VAS (see Figure 3-3). DOMS was slightly greater in the unaffected (right) quadriceps compared to the affected (left). Moving from standing to sitting (i.e. eccentric contraction of the quadriceps) elicited more DOMS than moving from sitting to standing (concentric contraction) on both the right and left quadriceps at 24 hours. DOMS was minimal at 2 hours and 2 days following the eccentric exercise stimulus (Figure 3-3). The location of DOMS was primarily over the muscle belly of the vastus lateralis. There was no muscle soreness reported during the four baseline test sessions or at 11, 12 and 13 days after the eccentric-exercise stimulus.

### 3.4.3 Quadriceps strength

At baseline, concentric, eccentric and isometric torque deficits at 70, 90, 100 degrees of flexion of the affected quadriceps were between 27 and 35% compared to the unaffected side (Table 3-1). After normalizing torque to muscle volume, the mean concentric, eccentric and isometric torque deficits of the affected quadriceps compared to the unaffected side ranged from 1 to 12% (Table 3-1).

The coefficient of variation (standard deviation/mean) for the baseline torque measures ranged from 3 to 13% on the affected LE and 4 to 6% on the unaffected LE (Figures 3-4 and 3-5). Following the eccentric-exercise stimulus, all torque measures for both LEs decreased compared to the baseline measures (see Figures 3-4 and 3-5). On the unaffected LE, both isometric and isokinetic torques improved at two days following the exercise stimulus and remained stable or continued to improve until day 13.

On the affected LE, the isometric torque at 70 and 90 degrees began to improve by day one following the exercise stimulus, whereas isometric torque at 100 degrees did not improve until day two. Isometric torque at 90 and 100 degrees remained stable from day two to day 13. Isometric torque at 70 degrees showed a peak at day two, declined at day 11 and improved between days 11 and 13. Eccentric torque on the affected side remained stable from two hours post-exercise until day 13 and was lower than the baseline measures. Concentric torque showed an improvement at two days post-exercise and then remained somewhat stable and similar to the baseline measures.

### 3.5 DISCUSSION

Our case report showed profound atrophy in the quadriceps muscle long after a tibial plateau fracture in a 46 year old woman, in spite of attempts to specifically train the quadriceps. As in other studies, disuse and immobilization resulted in loss of muscle volume of quadriceps of the affected thigh, i.e. 26% in our study. The degree of atrophy was greater than previously reported (19-21) and was observed in the vasti throughout the length of the muscle, with a total reduction in muscle volume of 28%. Several human studies have also shown reduction in thigh girth and quadriceps cross-sectional area following knee injury and immobilization (6, 19, 21, 22), although atrophy of the specific muscles of the quadriceps has not previously been reported. Muscle biopsy analysis has also demonstrated a decrease in fiber size in both fast- and slow-twitch fibers following knee immobilization (5, 21).

The finding of a torque deficit on the affected quadriceps after tibial plateau fracture is in accordance with previous studies in people with knee injury. Maenpaa et al. (2) reported that isokinetic deficits were prominent in the quadriceps after long-term recovery from patellar dislocation, as would be expected since the patella is part of the extensor mechanism together with the quadriceps muscle, retinacular and ligament elements. Honkonen et al. (4) tested 37 people with tibial plateau fractures 3 to 13 years post-fracture and found that the mean concentric torque deficit of the quadriceps was  $15 \pm 13\%$  in the injured LE compared to the uninjured side. The deficit in torque tended to decrease with time of follow up, indicating that some recovery in strength occurred with time. The follow-up time in our study was 16 years and our subject still had a torque deficit of 27 to 35% despite training post-fracture, suggesting that some changes in the ability of the quadriceps to generate torque are not reversible.

Although no differences in quadriceps muscle strength have been reported between the dominant and non-dominant LE of healthy people (23), the pattern of torque deficit has been shown to differ when the dominant LE is injured compared to when the non-dominant LE is injured (23). In one study (23), the deficit in eccentric torque of the quadriceps was greater than that observed in concentric and isometric torque when the dominant LE was injured, whereas no differences among eccentric, concentric and isometric torque deficit were seen with a non-dominant LE injury. In our subject, although the dominant LE was injured, a similar torque deficit was observed in eccentric, concentric and isometric torque so it did not appear that LE dominance affected the results of this study.

After normalizing torque to muscle volume, it appeared that the reduction in strength associated with disuse following tibial plateau fracture was due largely to muscle atrophy, rather than contractile dysfunction, as the normalized torque ( $\text{Nm}/\text{cm}^3$ ) was similar between the affected and unaffected quadriceps. This contrasts with results of previous studies in which a larger change in muscle strength relative to muscle cross-sectional area were reported with disuse. In addition to muscle atrophy, decrease in neural drive and reduced electromechanical efficiency of skeletal muscle are thought to be responsible for a decrease in voluntary strength (1, 20, 24).

The conflicting results of previous reports regarding whether muscle size or neurological adaptation account primarily for reduced voluntary force are not easily explained, but may be related partly to differences in follow-up time and torque normalization methods in these studies (19-21). Some previous studies were conducted in the acute phase following immobilization. Cruz Martinez et al. (24) found that 6 weeks following immobilization, muscle fiber conduction

velocity had returned to normal although muscle atrophy was still present. Therefore 16 years following fracture, our subject likely regained her ability to voluntarily recruit her quadriceps muscle and atrophy likely accounted for the strength deficit on the affected LE.

The method of torque normalization in the present study used muscle volume rather than anatomical cross-sectional area (ACSA); the latter was used more commonly in other studies. As a measure of muscle size, muscle volume is preferable because it is a closer approximation of a physiological cross-sectional area (PCSA) which takes into account the orientation of the muscle fibers to the longitudinal axis of the muscle (14). Therefore, the normalization method used in our study may better reflect the number of sarcomeres in a muscle and may relate more directly to the force generating capacity of the muscle.

Our subject experienced mild muscle soreness of the quadriceps following exercise-induced muscle damage with the highest rating occurring 24 hours following muscle damage. This pattern is typical of DOMS associated with exercise-induced muscle damage and has been postulated to reflect intramuscular swelling or an inflammatory response within the muscle (10, 25, 26). The intensity of DOMS may also reflect the extent of muscle damage (10).

Surprisingly, the degree of muscle torque loss following muscle damage was similar in the affected and unaffected LEs, although the subject experienced slightly more DOMS in the unaffected LE. This may have resulted from using a relative load for the muscle damage stimulus that applied the contractions at 75% of the eccentric maximum for each LE. Therefore, because the maximum eccentric torque on the affected LE was lower than that of the unaffected LE, less absolute work was performed on the affected LE than the unaffected LE during the

muscle damage stimulus. This may have resulted in a similar degree of muscle damage on both sides. An exercise-induced muscle damage stimulus that applies the same absolute load to both LEs may have induced more muscle damage in the affected quadriceps.

Compared to previous studies, the degree of recovery appeared to be reduced in our subject as torque measures on both LEs did not return to baseline values even 13 days after the initial eccentric exercise stimulus. Serrao et al. (11) demonstrated that the maximal isometric torque of the quadriceps, injured by 60 maximal eccentric contractions, quickly started to recover and continually improved until reaching its pre-exercise level between the fourth and fifth day. Similarly, Stupka et al. (27) found that concentric and eccentric torque returned to baseline values by one week following eccentric exercise. A quicker recovery time may be attributed to the younger age of the subjects in these studies.

In our subject, eccentric torque showed the least amount of recovery in both LEs even though muscle soreness had resolved. This may be due to specific damage caused by eccentric exercise that is detected most by eccentric strength testing rather than concentric and isometric testing. During an eccentric contraction, the muscle is forcibly lengthened and sarcomeres, particularly those on the descending limb of the torque-angle curve, are thought to be ruptured (26, 28). When repeating this movement during testing, these sarcomeres may be put under strain and unable to generate adequate force, particularly under lengthening conditions. In line with this hypothesis, isometric force at 100 degrees also showed delayed recovery in both LEs compared to concentric and isometric force at 90 and 70 degrees. At 100 degrees, the quadriceps muscle is also in a lengthened position, so the sarcomeres damaged by eccentric exercise may not be able to generate adequate force in this position.



### **3.5.1 Clinical implications**

Muscle that has undergone atrophy may have increased susceptibility to damage from eccentric loading. However a program of progressive eccentric exercise may be beneficial in reducing susceptibility to exercise-induced muscle damage (29, 30). Exercise prescription for atrophied muscle may include a regular, progressive program of eccentric exercise and exercise at long muscle lengths (29). However further research needs to be conducted to develop more specific exercise prescription guidelines that incorporate eccentric exercise training. When progressing eccentric exercise programs, physical therapists should take into account DOMS, changes in torque, and prolonged recovery time of atrophied muscles.

This case report has limited generalizability because it is based on the response of a single individual to an intervention. Replication of a structured case report across several subjects would increase the generalizability and credibility of our findings (31).

### **3.6 CONCLUSION**

In spite of rehabilitation, atrophy of quadriceps can be profound long after tibial plateau fracture. An exercise stimulus that leads to muscle damage can result in a longstanding torque deficit even after 13 days and when muscle soreness is absent. Considering the atrophy, low torque and long recovery time in the affected quadriceps, exercise prescription for muscle following immobilization and atrophy should be judicious to avoid overuse.

### **3.7 BRIDGING SUMMARY**

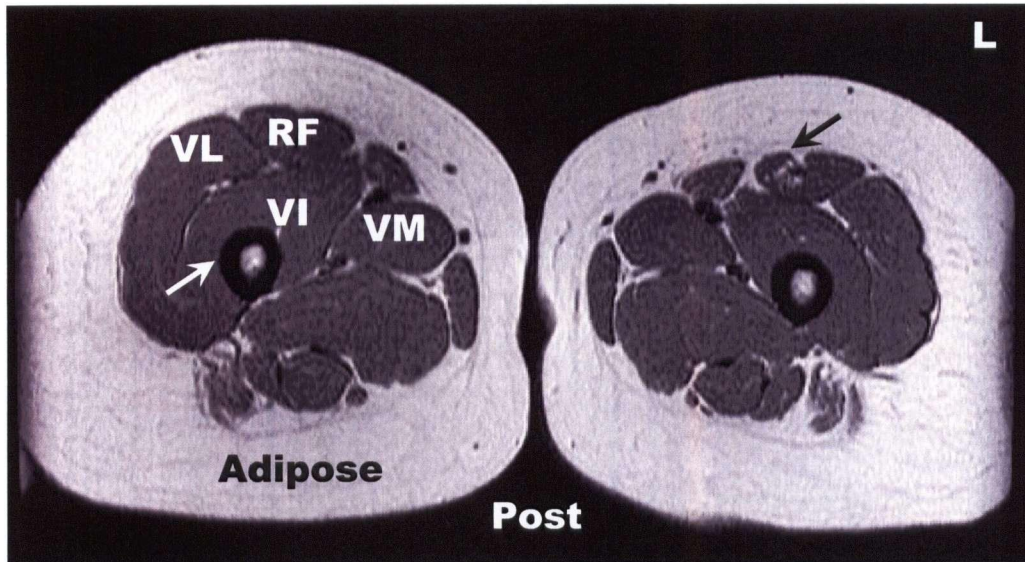
The case report presented in this Chapter was used to develop the methods for estimating muscle volume from multiple axial slices of the thigh obtained from MRI and to pilot the protocol for measuring isokinetic concentric and eccentric torque on the KinCom dynamometer. These methods were further employed in this thesis on people with COPD (Chapter Four) and lung transplant recipients (Chapter Seven). A unique finding in this case report was the presence of unilateral, intramuscular fat infiltration in the rectus femoris muscle of the affected leg. This led to an investigation of intramuscular fat infiltration of the quadriceps and hamstrings and its relationship to muscle function in people with COPD and recipients of lung transplants, presented in Chapters Four and Seven, respectively.

### 3.8 TABLES AND FIGURES

**Table 3-1.** Average baseline torque measures

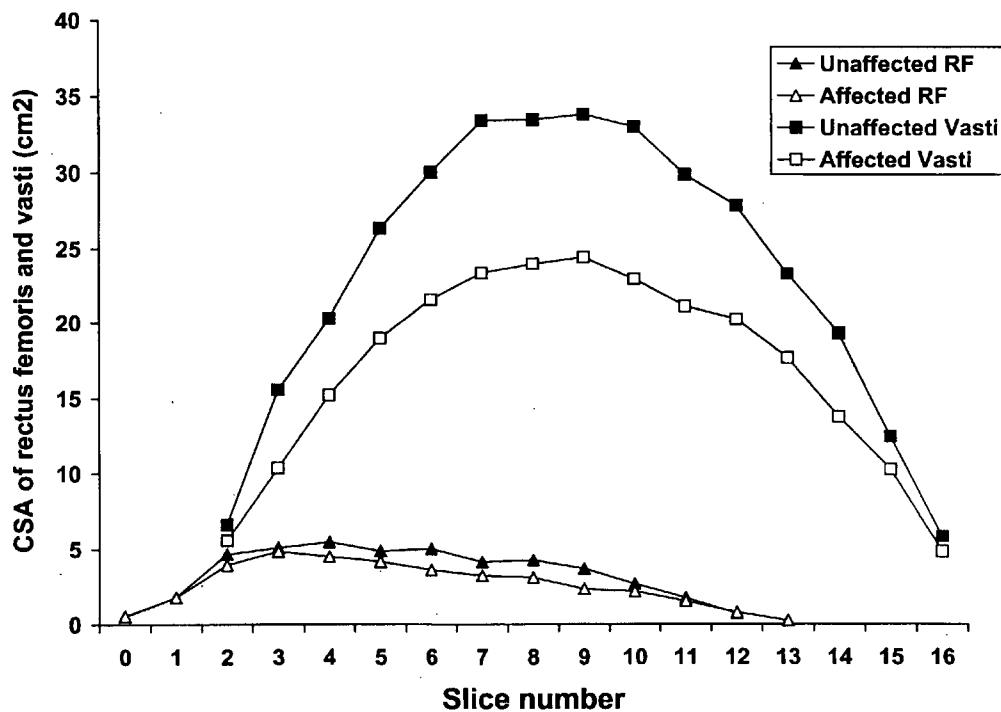
	Right	Left	Left / Right (%)	% Deficit*
<b>Absolute torque (Nm)</b>				
Concentric torque	90	58	65%	35%
Eccentric torque	118	82	70%	30%
Isometric torque at 70°	114	80	70%	30%
at 90°	128	93	73%	27%
at 100°	117	77	65%	35%
<b>Torque normalized to muscle volume (Nm/cm<sup>3</sup>)</b>				
Concentric torque	0.0796	0.07	88%	12%
Eccentric torque	0.1048	0.0992	95%	5%
Isometric torque at 70°	0.1011	0.0962	95%	5%
at 90°	0.1134	0.1125	99%	1%
at 100°	0.1041	0.0925	89%	11%

\* % deficit = (right – left)/right x 100

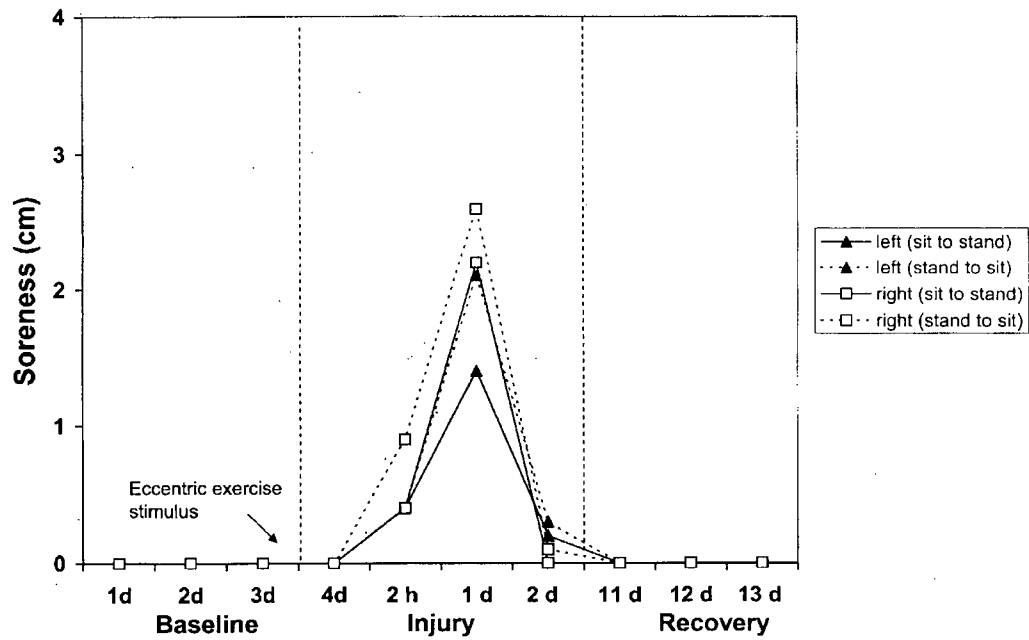


**Figure 3-1.** Axial T-1 weighted image obtained at midhigh (midway between the anterior superior iliac spine and the patella). Visible atrophy of the quadriceps muscle is noted on the left side compared to the right side. Intramuscular fatty infiltration (white pixels indicated by the black arrow) can be observed in the rectus femoris muscle on the affected side.

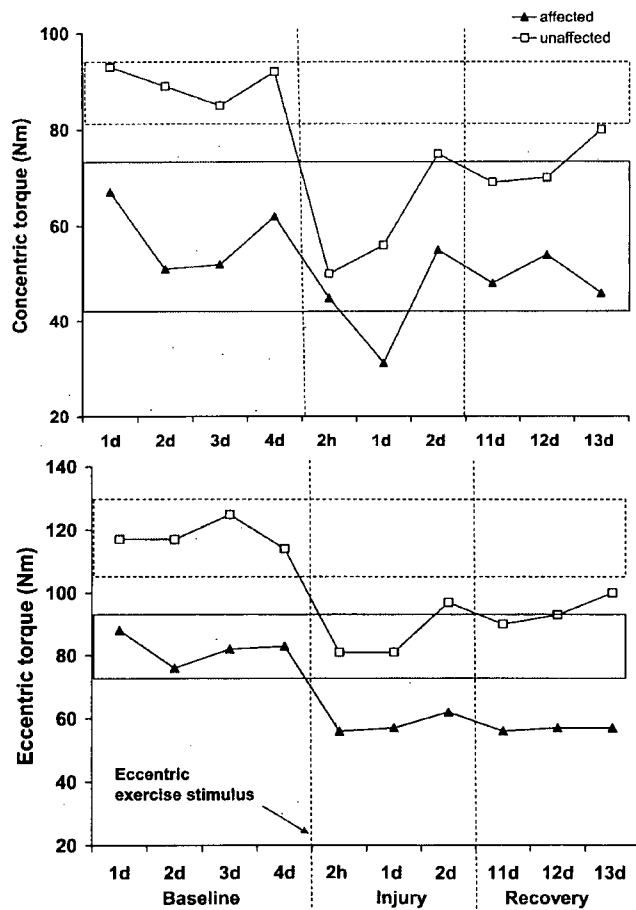
Abbreviations and symbols: Post – posterior, RF – rectus femoris, VI - vastus intermedius, VL – vastus lateralis, VM – vastus medialis; white arrow indicates the femur.



**Figure 3-2.** Cross-sectional area of the rectus femoris (RF) and vasti in the affected and unaffected lower extremities. Slice 0 is the first slice where RF is apparent (2 cm below the anterior inferior iliac spine) and slice 16 includes the superior aspect of the patella. Slices 1 to 15 are located at approximately equal intervals between the two endpoint slices with a gap between consecutive slices of 2.0 cm or 2.5 cm.



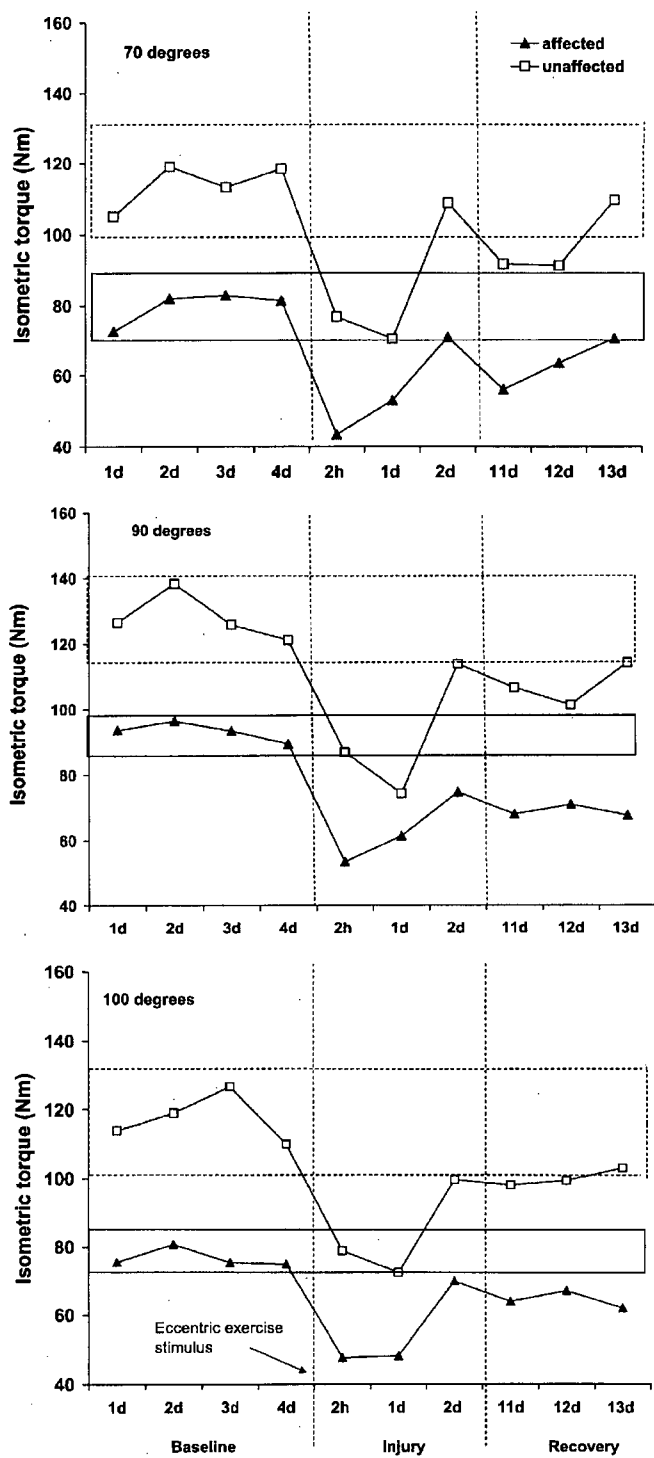
**Figure 3-3.** Delayed onset muscle soreness measured using visual analogue scale during the baseline phase (days 1 to 4) and following eccentric exercise. The first post-exercise measurement was taken 2 hours after exercise and then on days 1, 2, 11, 12 and 13 after exercise.



**Figure 3-4.** Concentric (top panel) and eccentric torque (bottom panel) during the baseline phase (days 1 to 4) and following eccentric exercise for the affected and unaffected lower extremities. The first post-exercise measurement was taken 2 hours after exercise and then on days 1, 2, 11, 12 and 13 after exercise.

**Figure 3-5.** Isometric torque at 70 (top panel), 90 (middle panel) and 100 degrees (bottom panel) of knee flexion during the baseline phase (days 1 to 4) and following eccentric exercise for the affected and unaffected lower extremities. The first post-exercise measurement was taken 2 hours after exercise and then on days 1, 2, 11, 12 and 13 after exercise.





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## 4. SKELETAL MUSCLE ATROPHY AND INTRAMUSCULAR FAT INFILTRATION IN PEOPLE WITH COPD<sup>d</sup>

### 4.1 INTRODUCTION

Skeletal muscle weakness has previously been reported in people with chronic obstructive pulmonary disease (COPD) (1, 2). There are a number of factors that may contribute to muscle weakness in this population such as low levels of physical activity, poor arterial blood gases (hypoxia, hypercapnea), malnutrition, systemic inflammation, oxidative stress and low testosterone levels (3, 4). There is a wide variation in the degree of skeletal muscle weakness observed among people with COPD (5), therefore each individual with COPD likely has a different, relative contribution of factors contributing to skeletal muscle dysfunction. Muscle quantity (i.e. muscle size or mass) is one factor that may contribute to skeletal muscle weakness in people with COPD however the quality of the muscle tissue is another important aspect that must be considered when examining the relationship between muscle mass and strength. Muscle quality is related to the contractility and composition of the muscle tissue (6).

Although quadriceps muscle atrophy has been observed in people with COPD (1), muscle quality has not been examined in this population. Muscle atrophy may be associated with a change in muscle composition, specifically, intramuscular fat infiltration which is the deposition of fat within the epimysium of a muscle. Although the mechanism of intramuscular fat deposition is not well understood, it has been observed in men and women over the age of 70 years (6), people

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<sup>d</sup> A version of this Chapter is in preparation: **Mathur, S.**, MacIntyre, D.L., Forster, B.B., Road, J.D., Levy, R.D., Reid, W.D. Skeletal muscle atrophy and intramuscular fat infiltration in people with COPD. *Thorax*.

with neuromuscular diseases (7), congenital myopathies (8-10), muscular dystrophy (11), chronic denervation and tendon tears (12). In these conditions there may be an abnormal replacement of muscle with fat tissue which may lead to reduced force output. Lastly, fat infiltrated muscle has been observed in obese individuals and correlated to insulin resistance in obese individuals and people with type 2 diabetes (13). Therefore the deposition of fat within a muscle has important implications for both function and metabolism of muscle. Both the size of the muscle and the quality of the muscle may be important factors in the ability of the muscle to generate tension and need to be examined further in people with COPD.

The majority of studies examining muscle strength in people with COPD have measured the force or torque that the muscle can produce at a fixed joint position (i.e. isometric contraction) (2) or while the muscle is actively shortening (i.e. concentric muscle contraction) (1). Eccentric muscle contractions occur when an active muscle is forcibly lengthened by an external load (14) and have not previously been studied in people with COPD. Both concentric and eccentric muscle contractions occur during daily activities: concentric contractions of the knee and hip extensors occur while walking upstairs or rising from a chair whereas eccentric contractions occur to control movements such as the action of the hip and knee extensors when moving from standing to sitting, or walking downstairs. Eccentric contractions are unique in their ability to generate very high forces with lower metabolic requirements compared to concentric contractions (15). Furthermore, it has been found that in older adults there appears to be a relative preservation in eccentric torque, while there is a decline in concentric torque compared to younger adults (16). A detailed examination of both concentric and eccentric muscle contractions in people with COPD may provide further insight into skeletal muscle function in the performance of daily activities in this population.

The purpose of this study was to compare concentric and eccentric muscle torque of the knee extensors (KEs) and knee flexors (KFs), muscle volume and intramuscular fat infiltration of the quadriceps and hamstrings in people with COPD to a control group matched for age, sex and body mass index (BMI). Secondly, we examined the relationships between muscle strength, muscle volume and intramuscular fat infiltration in both groups. We hypothesized that people with COPD would have lower torques of the KEs and KFs compared to matched controls and the extent of the difference between groups will be similar for concentric and eccentric torque. Secondly, we hypothesized that reduced muscle volume and increased intramuscular fat infiltration would be correlated with reduced concentric and eccentric torque in people with COPD and controls.

## **4.2 METHODS**

### **4.2.1 Sample and recruitment**

Twenty people with COPD and 20 controls participated in the study. A sample of twenty subjects based on an expected difference in quadriceps concentric torque between people with COPD and controls of 30% (1), resulted in a power of 0.95. People with COPD had moderate to severe (Stage II to III) COPD based on the GOLD guidelines ( $FEV_1 < 80\%$  of predicted and  $FEV_1/FVC < 70\%$ ) (17), had no acute exacerbations of COPD and had not taken oral corticosteroids (i.e. prednisone) in the six months prior to inclusion in the study. Participants had not been in any formal exercise rehabilitation program for at least one year prior to the study.



The control group was recruited from the general population and matched for sex, age and body mass index to the people with COPD and were free of respiratory disease.

All participants were currently not smoking. Those with co-morbid cardiovascular disease (e.g. heart failure, previous myocardial infarction or cardiovascular surgery), neurological conditions (e.g. stroke, Parkinson's) and lower extremity musculoskeletal problems (knee or hip injury or arthritis) were excluded from the study. The study was approved by the University Clinical Research Ethics Board and each participant provided written, informed consent prior to participation (see Appendix A for informed consent forms).

Height and weight were measured with shoes off and light clothing. Each participant also completed the Physical Activity Scale for the Elderly (PASE) (18). The PASE is a 12-item, self-administered questionnaire which asks about household, leisure time and work-related physical activity over the past seven day period. The PASE has been found to be reliable (18) and valid (19) in older adults and has previously been used in people with COPD (20). Participants underwent spirometry according to the standards described by the American Thoracic Society (21) to measure FEV<sub>1</sub> and FVC to define the presence and severity of COPD.

#### **4.2.2 Muscle volume**

MRI was used to estimate quadriceps, hamstrings and adductor muscle volume. A 1.5 Tesla MRI scanner (1.5T Horizon Echospeed Scanner, General Electric, Milwaukee, WI), was used to acquire 5-mm axial contiguous slices from the femoral-tibial joint line to the anterior superior

iliac spine on both thighs. Images were T1-weighted (TE = 8 ms; TR = 650 ms) with a 40 cm<sup>2</sup> field of view and a 512 x 224 pixel matrix (in-plane spatial resolution = 0.78 x 1.78 mm).

NIH Image, version 1.31 ([http://rsb.info.nih.gov/ij/Java 1.3.1\\_03](http://rsb.info.nih.gov/ij/Java 1.3.1_03)) was used to manually outline the quadriceps [rectus femoris (RF), the vasti (vastus lateralis, vastus intermedius and vastus medialis)], hamstrings (semitendinosis, semimembranosis, biceps femoris long head and short head) and the adductors (adductor brevis, longus and magnus). Individual muscles are shown in Figure 4-1.

To estimate muscle volume, the muscles were outlined on 17 slices starting at the first slice in which the RF muscle was present: approximately 2 cm below the anterior inferior iliac spine (proximal slice), to the superior aspect of the patella (distal slice). The distance between the proximal and distal slices was divided equally into 16 slices. The gap between measured slices was between 2.0 and 3.0 cm. The total volume (cm<sup>3</sup>) of each muscle was calculated by summing the product of measured muscle cross-sectional area by the slice thickness of all 17 sections, and the volume of the gaps between slices, estimated using the truncated cone formula as described by Ross et al. (22):

volume of gap =  $\frac{1}{3} * [CSA1 + CSA2 + \text{square root } (CSA1 * CSA2)] * \text{gap thickness}$

CSA1=cross-sectional area of slice immediately above gap

CSA2=cross-sectional area of slice immediately below gap

This method has previously been validated for estimation of muscle volume (23). Inter-rater reliability of CSA measurement as established on 65 regions of interest by two raters showed an average percentage error between the two raters of 0.4%, with an *r*-value of 0.99.

Muscle volumes of the RF and vasti were summed to obtain total quadriceps volume; volumes of semitendinosis, semimembranosis, biceps femoris long head and short head were summed to obtain total hamstrings volume. Adductors were circled as a single muscle group. Further details on estimating muscle mass in people with COPD are provided in Appendix B.

#### **4.2.3 Intramuscular fat infiltration**

Muscle quality was assessed by determining the degree of intramuscular fat infiltration in the quadriceps and hamstrings. Muscles were outlined on three slices along the length of the thigh: upper thigh (40% of distance from the proximal slice to the distal slice), midthigh (50%) and lower thigh (80%). The upper thigh slice was the most proximal slice where the rectus femoris, vasti and semitendinosis muscles had sufficiently large CSAs to generate frequency distributions of signal intensity (i.e. greater than 200 pixels per muscle). The midthigh slice had the largest CSA for the quadriceps muscles. The lower thigh slice captured the largest CSA of the vastus medialis, vastus lateralis, semimembranosis and biceps femoris muscles.

Image J was used to generate frequency distributions of signal intensity for each muscle on each of the three slices (Figure 4-2). The interquartile range (the number of pixels in the 25<sup>th</sup> to 75<sup>th</sup> percentile of signal intensity) was calculated for each muscle on the three slices. This provided an indication of the width of the distribution of signal intensity from each muscle. A greater

interquartile range indicated greater fat infiltration. As a second method of examining the frequency distributions of signal intensity for a given muscle, the coefficient of skewness was calculated for each frequency distribution. The coefficient of skewness is a measure of the asymmetry of a distribution. The normal distribution is symmetric, and has a skewness value of zero whereas a distribution with a significant positive skewness has a long right tail (24) indicative of pixels with higher signal intensity. A description of the development of the methods for quantifying intramuscular fat infiltration from MRI is provided in Appendix C.

#### **4.2.4 Peak torque of the knee extensors and flexors**

Concentric and eccentric torque measures of the knee extensors and flexors were performed on the KinCom dynamometer (version 5.30, Chattanooga Group Inc., Hixson, TN). The KinCom has previously been shown to be a valid and reliable method for measuring torque produced by a muscle (25, 26). A slow angular velocity of 30 degrees per second was chosen for testing as it has been shown to be more reliable than faster movement velocities in people with COPD (27).

The participant was seated on the KinCom seat with the axis of rotation of the dynamometer aligned to the knee joint line. The backrest and seat angles were adjusted so that the hip was at approximately 80° of flexion. A strap was placed across the participant's pelvis to minimize hip movement during the tests. The cuff (load cell) of the KinCom was placed at a distance of 75% of the lower leg length (measured from the top of the fibular head to the mid-point of the lateral malleolus).

Each participant performed measurements of knee extension followed by knee flexion. All testing was done on the dominant leg which was determined by asking each participant over which leg he or she had more control (e.g. which leg they would use to kick a ball). Five submaximum warm-up contractions in both the concentric and eccentric directions were done to familiarize the participant with the movements. The warm-up was followed by three to five maximum voluntary concentric and eccentric contractions to ensure that there were three measurements within 5% of each other. The range of motion for knee extension was set from 100 degrees of knee flexion to full extension and for knee flexion was 10 degrees to 100 degrees of knee flexion. The subject was instructed to “push as hard as possible” and received visual and verbal feedback during each contraction. A one minute rest was provided between trials and a three minute rest was provided between knee extension and knee flexion measurements. The highest of the three concentric contractions and the highest of the three eccentric contractions (i.e. peak torque) was used for analysis. Peak torque values were corrected for gravity.

Peak torque was normalized to muscle volume and expressed in  $\text{Nm}/\text{cm}^3$ . KF torque was normalized to the hamstrings muscle volume (semimembranosis, semitendinosis and biceps femoris) and KE torque was related to the quadriceps muscle volume (rectus, femoris, vastis medialis, lateralis and intermedius) as these are the primary movers for the actions of knee flexion and extension, respectively (28).

#### **4.2.5 Statistical analysis**

Statistical analysis was done using Statistical Package for the Social Science (SPSS) software (version 13.0, SPSS Inc., Chicago, IL). Descriptive statistics (mean and standard deviation) were

computed for sample characteristics, torque, muscle volume and normalized torque measures. Independent samples t-tests were used to compare sample characteristics. One-way ANOVA was used to compare differences between groups for muscle volume, absolute and normalized torque and fat infiltration (interquartile range and coefficient of skewness). The 95% confidence intervals for the mean difference between groups were also calculated. Significance level was set at  $p < 0.05$  and adjusted for multiple comparisons using the Bonferroni correction.

Pearson product moment correlations were used to quantify the relationship between muscle torque and the following variables: age, height, weight, physical activity level (PASE score), quadriceps and hamstrings muscle volume and fat infiltration. These variables were normally distributed (Kolmogorov-Smirnov test of normality). Significance of dichotomous variables (i.e. sex and presence of absence or respiratory disease) was tested using independent samples t-test. Those variables that were significantly correlated to muscle torque or were significantly different, were entered into a multiple, stepwise regression model to predict muscle torque. Variables were entered at  $p < 0.05$  and removed at  $p > 0.10$ .

## **4.3 RESULTS**

### **4.3.1 Sample characteristics**

Sample characteristics are summarized in Table 4-1. Age, height, weight and body mass index (BMI) were similar between groups. People with COPD had lower values for lung function ( $FEV_1$ , FVC expressed as percent of predicted and  $FEV_1/FVC$  ratio) than healthy controls ( $p < 0.001$ ) which was consistent with the GOLD criteria for moderate to severe COPD (17). There

was a trend for physical activity level to be lower in people with COPD compared to the control group ( $p = 0.058$ , Table 4-1).

#### **4.3.2 Muscle volume**

Total quadriceps, hamstrings and adductor muscle volumes were 37.9%, 34.1% and 38.9% lower respectively, in people with COPD compared to the control group ( $p \leq 0.001$ , Table 4-2). The percentage difference in muscle volume between people with COPD and controls was consistent across individual muscles of the quadriceps and hamstrings, ranging from 31.4% to 38.5% (see Table 4-2).

#### **4.3.3 Intramuscular fat infiltration**

Intramuscular fat infiltration as measured using the interquartile range was greater in the people with COPD compared to controls for all muscles across all levels (Table 4-3;  $p < 0.002$ ). The coefficient of skewness, was higher in people with COPD compared to controls in all muscles except for rectus femoris at the 40% level (close to the hip) and semimembranosis at the 80% level i.e. close to the knee (Table 4-4;  $p < 0.002$ ).

#### **4.3.4 Absolute and normalized torque**

Concentric peak torque was 29% lower in people with COPD compared to controls for both KEs ( $p = 0.001$ ) and KFs ( $p = 0.002$ ; Table 4-5). Eccentric peak torque was 20% lower for the KEs ( $p = 0.016$ ) and 14% lower for the KFs ( $p = 0.036$ ) in people with COPD compared to controls

(Table 4-5). There was no significant gender by group interaction; males had higher torques than females in both groups.

Torque was normalized per unit muscle volume and expressed in  $\text{Nm}/\text{cm}^3$  (Table 4-5).

Normalized concentric torque for the knee extensors and flexors was not different between groups. Normalized eccentric knee extensor and flexor torques were higher in people with COPD compared to controls ( $p = 0.013$  and  $0.044$ , respectively).

#### **4.3.5 Regression analysis**

Activity level (PASE score) was not correlated with muscle torque for either the knee extensors or flexors so it was excluded from the regression model ( $r$ -values ranged from  $0.13$  to  $0.21$ ).

Correlations were made between the measures of fat infiltration (IQR and coefficient of skewness) of the quadriceps muscles (rectus femoris and vasti) to KE concentric and eccentric torque and for fat infiltration of the hamstrings (semimembranosis, semitendinosis and biceps femoris) to KF concentric and eccentric torque. These correlations were not significant and ranged from  $-0.06$  to  $0.04$  for the quadriceps and  $-0.2$  to  $0.01$  in the hamstrings.

The correlations between muscle torque and other variables are shown in Table 4-6. Age was correlated to knee extensor concentric and eccentric torque but not with knee flexor torque.

Weight was correlated to all torque measures except for knee extensor concentric torque.

Concentric and eccentric muscle torque for both the knee extensors and flexors were significantly different for sex ( $p = 0.001$  to  $0.019$ ) and presence of respiratory disease ( $p = 0.001$  to  $0.036$ ) so these variables were also included in the regression model.



Regression analysis (Table 4-7) revealed that muscle volume of the quadriceps and hamstrings accounted for largest proportion of the variance in KE and KF torques, respectively. Only in the model for KF eccentric torque, was another variable (sex) included in the model. For all other models, sex, presence of respiratory disease, height, weight and age did not account for a significant proportion of the variability to be included in the model to predict muscle torques.

#### **4.4 DISCUSSION**

This study is the first to show that people with COPD show uniform muscle atrophy of the quadriceps, hamstrings and adductors and in addition have significant intramuscular fat infiltration. We also found that people with COPD had a higher normalized eccentric torque of the KEs and KFs compared to controls and normalized concentric torque was similar to controls. The findings on normalized muscle torque suggest that although there are some factors that may lead to reduced muscle torque production in people with COPD such as muscle atrophy, there may be factors that contribute to the maintenance or improvement of torque generating capacity of muscle, particularly of eccentric torque.

Fat infiltration has been observed in people with neurological and myopathic conditions (7, 11) and has been associated with advanced age (6) and obesity (13). In our study, people with COPD were matched to controls for age, sex and body mass index but still showed greater fat infiltration. Therefore, factors, besides those that were matched or controlled for, related to the multisystemic sequelae of COPD, may contribute to the deposition of fat within muscle. The presence of fat within muscle in people with COPD may have implications for muscle

metabolism. The deposition of fat within a muscle appears to be related to insulin sensitivity which is a risk factor for metabolic disease such as type 2 diabetes (13). The utilization of intracellular fat may be mediated by mitochondrial function and the oxidative capacity of the muscle cell (29). People with COPD have low concentrations of oxidative enzymes (e.g. citrate synthase) (30) and a lower proportion of type I fibers (31) in their quadriceps muscle, which may limit their ability to use intramuscular fat depots and/or affect the insulin sensitivity of the muscle however this relationship has not been examined in people with COPD. Although the mechanism of fat infiltration in muscle is not clear, a study comparing aged mice to younger control mice, found that muscle satellite cells from older mice demonstrated greater gene expression of transcription factors for adipose (32). Further research is required to determine whether this contributes to greater fat deposition in muscle in people with COPD.

Intramuscular fat infiltration may also be related to systemic inflammation. Markers of systemic inflammation such as TNF-alpha and nitric oxide synthases have been shown to be higher in the vastus lateralis of people with COPD than controls (33). In other conditions, such as muscular dystrophy, inflammatory changes in the skeletal muscle appear to be a precursor to intramuscular fat infiltration (11). The relationship between fat infiltration and inflammation in the skeletal muscle of people with COPD may provide insight into the mechanism of intramuscular fat infiltration and requires further examination.

In our study, we did not see a relationship between intramuscular fat infiltration and muscle strength of the KEs and KFs. This is in contrast to the findings of Goodpaster et al. (6), who reported that older people with greater fat infiltration of their thigh muscles, shown by lower muscle attenuation values on computed tomography, had lower isokinetic strength of the KEs.

Muscle fat infiltration has also been associated with mobility limitation in older adults (34). Therefore, in people with COPD it does not appear that intramuscular fat infiltration affects the contractility of skeletal muscle or there may not be a sufficient volume of fat to disrupt torque output. Alternatively, there may be other factors that preserve the contractility of the muscle despite increased fat infiltration such as the ability to recruit and fire motor units, changes in the cellular structure of skeletal muscle or improved efficiency of movement.

People with COPD demonstrated a 34 to 39% reduction in muscle volume across the three major muscle groups of the thigh. Furthermore, there was a uniform reduction in muscle volume of the individual muscles of the quadriceps and hamstrings (i.e. uni- and bi-articular muscles). In one study using computed tomography (CT), a mean reduction in thigh muscle CSA of approximately 24% was found in people with COPD, however the extent of atrophy of individual muscle groups were not identified (1).

Other studies examining muscle mass on people with COPD have not specifically examined individual muscles of thigh. Studies using dual energy x-ray absorptiometry (DEXA) and bioelectrical impedance (35, 36) have shown a reduction in fat free mass in people with COPD compared to controls, however, the measures of fat free mass used in these studies are for the whole body rather than a specific muscle group. To determine the amount of force that is generated per unit volume of muscle, more specific measures of muscle size are required. CT and MRI can provide specific measures of muscle CSA (37). These tools also have the ability to discriminate muscle tissue from fat, bone and connective tissue, thereby providing accurate measures of muscle mass (37). MRI has the additional benefit of not emitting any ionizing

radiation, therefore multiple slices can be acquired through a longer scanning time, without risk to the subject (7).

People with COPD had a reduction in both concentric and eccentric KE and KF torque compared to controls, however eccentric torque was affected to a lesser extent than concentric. This is similar to older adults who show a relative preservation of eccentric compared to concentric torque for the muscles surrounding the ankle (38), knee (39), and elbow (16). Although the mechanism of this difference cannot be determined from this study, previous literature may provide some insights into the relative preservation of eccentric torque in people with COPD.

Eccentric muscle actions require less ATP and less muscle activation to generate force than concentric muscle actions since some of the actin-myosin cross-bridges are broken by the external load rather than by ATP hydrolysis and there is more force exerted per cross-bridge (15). Because people with COPD have a reduced capacity to generate ATP due to poor oxygen extraction and utilization by their skeletal muscle (40, 41), they may rely more heavily on eccentric contractions in their daily activities, which are less metabolically demanding compared to concentric. The non-contractile components of skeletal muscle tissue (i.e. connective tissue components or cytoskeletal proteins) also contribute to eccentric torque generation whereas they detract from concentric torque. An increase in fibrotic tissue has been observed under the light microscope in muscle biopsies taken from the vastus lateralis muscle of people with COPD compared to age-matched controls (42). This may represent increased connective tissue within skeletal muscle, resulting in a stiffer muscle which makes a greater contribution of passive tension to eccentric torque.

This study showed that muscle volume accounts for between 39 to 53% of the variance in concentric and eccentric torque production of the knee extensors and flexors in people with COPD and older adults. The relationship between muscle volume and torque provides evidence that muscle size is an important contributor to muscle strength however there are other factors that account for the remainder of the variance observed in muscle torque. These may be factors related to muscle activation, muscle mechanics or intrinsic muscle fiber properties (14). Sex, age, height and weight likely contribute to the size of the muscle and therefore did not have any additional contribution to the regression model predicting muscle torque. In addition, people with COPD tend to have low muscle mass therefore the factor of respiratory disease also did not account for additional variance in the regression model once muscle volume was included.

Normalized torque provides an estimate of how much force is being produced per unit volume of muscle and allows for comparisons to be made independently of muscle size. Using normalized torque is particularly important when muscle size differs between groups, such as between young and old adults (43). As seen in this study, people with COPD had muscle atrophy and muscle volume was a major contributor to torque, therefore, normalized torque was a more valid measure than absolute torque to compare strength differences between people with COPD and controls. Although people with COPD had lower absolute torque for knee extension and flexion, their torque normalized to muscle volume was similar to controls providing further evidence that muscle size was a major contributor to strength. This is similar to the finding of Bernard et al.(1), where knee extension force was normalized to mid-thigh cross-sectional area and found to be similar between people with COPD and healthy controls. This is also consistent with the finding of Debigare et al. (44), who examined fiber bundles from vastus lateralis and found in-vitro force production to be preserved in people with COPD (44). KE and KF eccentric

normalized torques were higher in people with COPD indicating that more eccentric torque could be generated per unit volume of muscle compared to controls. Again, the mechanism of this difference is unclear from the present study however an increase in muscle connective tissue or cytoskeletal elements, or altered motor unit recruitment in people with COPD may contribute to this change.

#### **4.4.1 Limitations**

An important methodological consideration in this study is that maximal voluntary contractions were used to examine muscle strength and it was assumed that the participants were fully activating their muscles during the knee extension and flexion contractions. Non-volitional methods of stimulating muscle such as magnetic or electrical stimulation can be used to ensure full muscle activation (45). Magnetic stimulation has previously been used in people with COPD to measure muscle fatigue following exhaustive cycling (46, 47). Furthermore, interpolated twitch techniques may be used to superimpose an electrical twitch stimulation on a voluntary contraction to determine whether a muscle is fully activated during a voluntary contraction (48). These techniques may be useful in future studies examining muscle strength to examine whether voluntary recruitment strategies contribute to differences in torque production by people with COPD and healthy older people.

Muscle volume was estimated from T-1 weighted MR images which did not allow us to remove the volume of intramuscular fat from the muscle, therefore, muscle volume may have been overestimated by a small percentage in people with COPD who had greater fat infiltration. As shown in Appendix C, the percent of voxels binned as fat or mixed muscle/fat based on signal

intensity, was approximately 2% in an individual with visible fat infiltration, therefore the overestimation would likely be very small in the sample of people with COPD. Scanning sequences that suppress the signal from fat e.g. short tau inversion recovery (STIR) (49) may be used in future studies to provide a closer estimation of absolute muscle volume.

Physical activity was measured in this study using a self-report scale and although it was similar between groups, there was a trend for people with COPD to have lower PASE scores than controls. In a recent study using accelerometry, people with COPD were shown to have less standing and walking time and a lower intensity of walking in their daily activities compared to healthy sedentary people (50). This degree of specificity could not be captured using the physical activity scale employed in this study. Therefore the relative contribution of physical activity to muscle atrophy and fat infiltration cannot be determined from this study.

#### **4.5 CONCLUSION**

People with COPD demonstrate uniform muscle atrophy of their thigh muscles and greater intramuscular fat infiltration compared to matched controls. Muscle volume is a major contributor to muscle torque production whereas fat infiltration is not related to torque production. Although absolute torque of the KEs and KFs is lower in people with COPD compared to controls, concentric torque normalized to muscle volume is similar to controls and normalized eccentric torque is actually higher in people with COPD. Further research is required to determine what factors may contribute to the enhancement of eccentric torque in people with COPD.

#### **4.6 BRIDGING SUMMARY**

The findings reported in this Chapter are consistent with the initial hypothesis outlined in Chapter One, that people with COPD would have lower muscle volume than controls and this would contribute to muscle torque. However, intramuscular fat infiltration, although higher in people with COPD, did not correlate to torque. Also, people with COPD had lower absolute concentric and eccentric torque of the KEs and KFs than controls but eccentric torque appeared to be affected to a lesser extent than concentric. Furthermore, normalized eccentric torques of the KEs and KFs were higher in people with COPD than controls. This finding on eccentric torque provides evidence that some aspects of skeletal muscle function are preserved in people with COPD. Chapter Five and Six of the thesis further examine two factors that could account for preserved or impaired skeletal muscle performance in people with COPD: motor unit firing properties and cellular structure of skeletal muscle.



## 4.7 TABLES AND FIGURES

**Table 4-1.** Sample characteristics for people with COPD and controls (mean  $\pm$  standard deviation).

	COPD	Control
n	20	20
age (yrs)	68.2 $\pm$ 10.0	64.4 $\pm$ 8.1
sex	11F, 9M	11F, 9M
height (m)	1.66 $\pm$ 0.09	1.67 $\pm$ 0.13
weight (kg)	72.1 $\pm$ 14.6	69.0 $\pm$ 14.4
BMI (kg/m <sup>2</sup> )	26.6 $\pm$ 4.7	24.3 $\pm$ 2.2
FEV <sub>1</sub> (L)	1.34 $\pm$ 0.41	2.28 $\pm$ 0.72
(% predicted)	(51 $\pm$ 17%)†	(81 $\pm$ 20%)
FVC (L)	2.58 $\pm$ 0.47	3.05 $\pm$ 1.11
(% predicted)	(78 $\pm$ 14%)†	(83 $\pm$ 20%)
FEV <sub>1</sub> /FVC (%)	52 $\pm$ 14%†	77 $\pm$ 9%
Physical activity scale	101 $\pm$ 58	131 $\pm$ 37

† significantly different from control,  $p < 0.001$

**Table 4-2.** Muscle volume (cm<sup>3</sup>) of the quadriceps and hamstrings in people with COPD and controls (mean ± standard deviation). The 95% confidence interval (95% CI) is provided for the mean difference between groups.

Muscle	COPD	Control	percent difference*	95% CI
<b>TOTAL Quadriceps (cm<sup>3</sup>)</b>	1459 ± 388 †	2351 ± 724	37.9%	-1263 to -519
Rectus femoris	195 ± 50 †	316 ± 103	38.3%	-173 to -68
Vasti	1265 ± 342 †	2035 ± 627	37.8%	-1097 to -444
<b>TOTAL Hamstrings (cm<sup>3</sup>)</b>	595 ± 158 †	903 ± 251	34.1%	-442 to -174
Semitendinosus	149 ± 45 †	217 ± 71	31.4%	-106 to -30
Semimembranosus	193 ± 58 †	314 ± 94	38.5%	-171 to -70
Biceps femoris – long head	174 ± 55 †	256 ± 79	32.1%	-126 to -39
Biceps femoris – short head	80 ± 25 †	116 ± 37	31.4%	-57 to -16
<b>Adductors</b>	603 ± 185 †	988 ± 290	38.9%	-540 to -228

\* percent difference = (control – COPD)/control x 100

† significantly different from control,  $p \leq 0.001$

**Table 4-3.** Interquartile range (25<sup>th</sup> percentile to 75<sup>th</sup> percentile) of pixel intensity for the quadriceps and hamstrings at the upper, mid and lower thigh (mean  $\pm$  standard deviation). The 95% confidence interval (95% CI) is provided for the mean difference between groups.

Level and muscle	COPD	Control	95% CI
<b>Upper thigh (40% of total thigh length; close to the hip)</b>			
RF	72 $\pm$ 22†	43 $\pm$ 5	16 to 42
Vasti	64 $\pm$ 16†	43 $\pm$ 4	12 to 30
Semitendinosis	70 $\pm$ 18†	46 $\pm$ 7	13 to 34
<b>Midthigh (50% of total thigh length)</b>			
RF	68 $\pm$ 25†	45 $\pm$ 5	8 to 38
Vasti	63 $\pm$ 14†	46 $\pm$ 5	8 to 26
Semitendinosis	88 $\pm$ 28†	50 $\pm$ 11	22 to 54
Biceps femoris (long head)	86 $\pm$ 27†	48 $\pm$ 6	23 to 53
<b>Lower thigh (80% of total thigh length; close to the knee)</b>			
Vastus medialis	75 $\pm$ 28†	47 $\pm$ 4	14 to 43
Vastus lateralis	101 $\pm$ 34†	49 $\pm$ 7	25 to 78
Semimembranosis	102 $\pm$ 35†	63 $\pm$ 16	20 to 60
Biceps femoris (long head)	80 $\pm$ 21†	50 $\pm$ 6	19 to 42
Biceps femoris (short head)	95 $\pm$ 32†	60 $\pm$ 10	18 to 54

† indicates significantly different from control,  $p < 0.002$

**Table 4-4.** Coefficient of skewness of pixel intensity for the quadriceps and hamstrings at the upper, mid and lower thigh (mean  $\pm$  standard deviation). The 95% confidence interval (95% CI) is provided for the mean difference between groups.

Level and muscle	COPD	Control	95% CI
<b>Upper thigh (40% of total thigh length; close to the hip)</b>			
Rectus femoris	1.85 $\pm$ 0.46	1.25 $\pm$ 1.01	-0.04 to 1.26
Vasti	2.28 $\pm$ 0.58†	1.37 $\pm$ 0.63	0.39 to 1.41
Semitendinosus	1.81 $\pm$ 0.76†	0.71 $\pm$ 0.48	0.59 to 1.61
<b>Midthigh (50% of total thigh length)</b>			
Rectus femoris	1.93 $\pm$ 0.59†	0.38 $\pm$ 0.80	0.97 to 2.13
Vasti	2.01 $\pm$ 0.50†	0.95 $\pm$ 0.82	0.51 to 1.59
Semitendinosus	1.47 $\pm$ 0.36†	0.72 $\pm$ 0.41	0.32 to 1.16
Biceps femoris (long head)	1.69 $\pm$ 0.61†	0.85 $\pm$ 0.52	0.35 to 1.31
<b>Lower thigh (80% of total thigh length; close to the knee)</b>			
Vastus medialis	1.76 $\pm$ 0.58†	1.13 $\pm$ 0.59	0.11 to 1.16
Vastus lateralis	1.71 $\pm$ 0.63†	0.86 $\pm$ 0.54	0.33 to 1.38
Semimembranosus	1.48 $\pm$ 0.54	1.11 $\pm$ 0.60	-0.06 to 0.82
Biceps femoris (long head)	1.53 $\pm$ 0.36†	0.63 $\pm$ 0.39	0.54 to 1.24
Biceps femoris (short head)	1.62 $\pm$ 0.66†	0.84 $\pm$ 0.40	0.36 to 1.20

† indicates significantly different from control,  $p < 0.002$

**Table 4-5.** Torque (Nm) and normalized torque (Nm/cm<sup>3</sup>) of the quadriceps and hamstrings in people with COPD and controls (mean  $\pm$  standard deviation). The 95% confidence interval (95% CI) is provided for the mean difference between groups.

Variable	COPD	Control	percent difference*	95% CI
<b>Absolute Torque (Nm)</b>				
Quadriceps concentric	61 $\pm$ 18†	85 $\pm$ 24	28.0%	-36 to -13
Quadriceps eccentric	109 $\pm$ 26‡	139 $\pm$ 45	21.3%	-52 to -10
Hamstrings concentric	24 $\pm$ 8†	34 $\pm$ 11	28.7%	-16 to -5
Hamstrings eccentric	38 $\pm$ 14 ‡	48 $\pm$ 18	22.4%	-19 to -3
<b>Normalized torque (Nm/cm<sup>3</sup>)</b>				
Quadriceps concentric	0.044 $\pm$ 0.016	0.037 $\pm$ 0.009	-17.0%	-0.002 to 0.014
Quadriceps eccentric	0.076 $\pm$ 0.016†	0.062 $\pm$ 0.019	-23.5%	0.003 to 0.02
Hamstrings concentric	0.042 $\pm$ 0.014	0.039 $\pm$ 0.011	-9.1%	-0.004 to -0.012
Hamstrings eccentric	0.063 $\pm$ 0.014†	0.054 $\pm$ 0.013	-17.0%	0.002 to 0.018

\* percent difference = (control – COPD)/control x 100

† significantly different from control,  $p < 0.05$

‡ significantly different from control,  $p < 0.01$

**Table 4-6.** Correlations between KE and KF torque and explanatory variables

<b>Variables</b>	<b>KE concentric</b>	<b>KE eccentric</b>	<b>KF concentric</b>	<b>KF eccentric</b>
<b>Age</b>	-0.38†	-0.47†	0.081	0.16
<b>Height</b>	0.38†	0.42†	0.48†	0.60‡
<b>Weight</b>	0.16	0.37†	0.36†	0.53‡
<b>Quad muscle volume</b>	0.67‡	0.63‡	N/A	N/A
<b>Hams muscle volume</b>	N/A	N/A	0.66‡	0.73‡

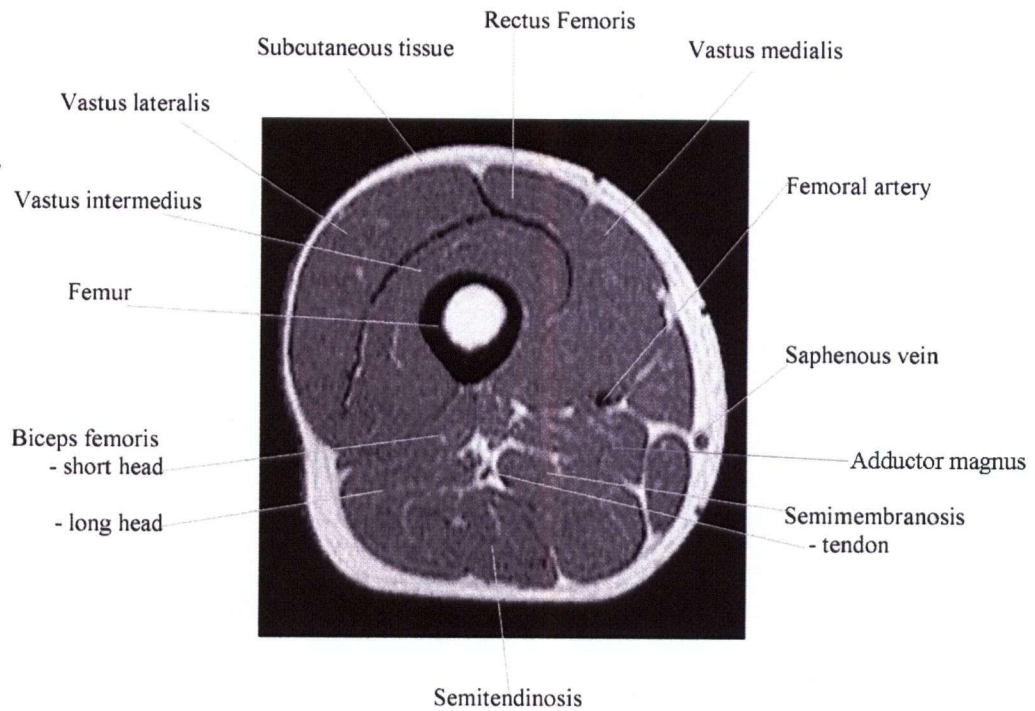
† significant at  $p < 0.05$

‡ significant at  $p < 0.01$

**Table 4-7.** Multiple regression analysis for predicting muscle torque using muscle volume\*

	<b>Predictors</b>	<b>R-squared</b>	<b><math>\beta</math></b>	<b>p-value</b>
<b>KE concentric torque</b>	quadriceps volume	0.454	0.674	< 0.001
<b>KE eccentric torque</b>	quadriceps volume	0.393	0.627	< 0.001
<b>KF concentric torque</b>	hamstrings volume	0.434	0.659	< 0.001
<b>KF eccentric torque</b>	hamstrings volume	0.527	0.588	< 0.001
	gender	0.569	-0.247	0.065

\* excluded variables – PASE score, interquartile range and coefficient of skewness for fat infiltration



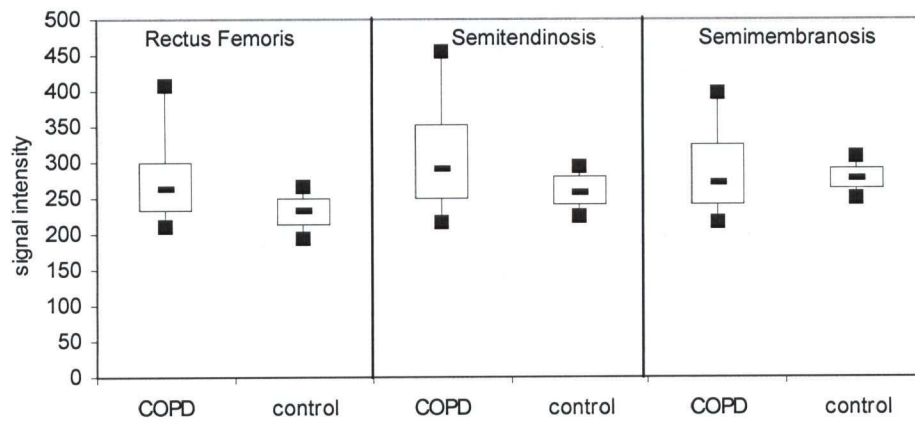
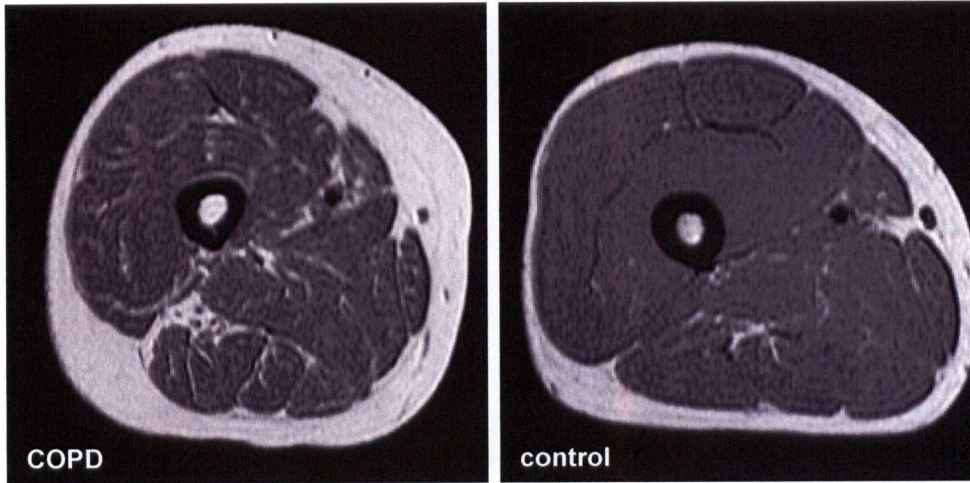
**Figure 4-1.** Axial MRI of a control subject, taken at 70% of total length from the anterior inferior iliac spine to the superior border of the patella (1.5 Tesla, T-1 weighted image). Major anatomical structures are labeled.



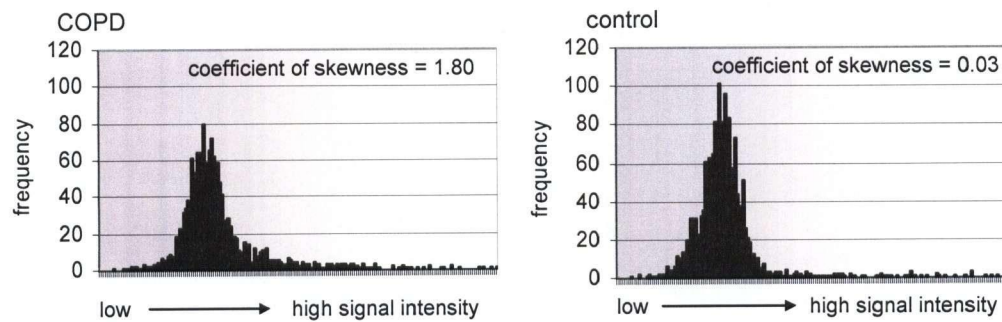
**Figure 4-2.** Examples of axial MR images at midthigh and respective boxplots depicting interquartile range and frequency distributions of signal intensity of muscle from T-1 weighted images of person with COPD (male, 53 years) and a control subject (male, 55 yrs)

**A)** Boxplots of signal intensity for three muscles (rectus femoris, semitendinosis and semimembranosis). The box represents the interquartile range (25<sup>th</sup> to 75<sup>th</sup> percentile), the black bar represents the median and the whiskers represent the 10<sup>th</sup> and 90<sup>th</sup> percentile of signal intensity. Note that the interquartile range is wider for all three muscles in the person with COPD indicating a greater distribution of signal intensity.

**B)** Frequency distribution of signal intensity for the rectus femoris muscle. Note that the coefficient of skewness for the frequency distribution of the control subject (right panel) who has homogenous muscle, is close to zero indicating a normal distribution. The coefficient of skewness for the person with COPD (left panel) who has visible fat infiltration in the rectus femoris is positive, indicating a right skewed distribution.



a)



b)

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## 5. SURFACE EMG OF THE QUADRICEPS DURING A FATIGUING CONTRACTION IN PEOPLE WITH COPD<sup>c</sup>

### 5.1 INTRODUCTION

People with COPD appear to have poor skeletal muscle function that results in reduced exercise tolerance. Leg fatigue has been shown to be a major factor limiting exercise in people with COPD with 43% of people reporting leg fatigue compared to 26% reporting breathlessness as the primary factor limiting maximal exercise (1). In people with COPD compared to healthy controls, the cross-sectional area of the quadriceps muscle is reduced (2), there is a lower concentration of oxidative enzymes (3) and a reduced proportion and size of type I muscle fibers (4). Furthermore, studies using magnetic spectroscopy show lower levels of phosphocreatine and lower pH following quadriceps exercise in people with COPD compared to controls (5, 6). These findings suggest that there is an increased reliance on glycolytic metabolism during muscular work and this may manifest itself as greater fatigability of skeletal muscle during exercise.

Previous studies using twitch interpolation have shown that quadriceps fatigue is present in over 50% of people with COPD following a cycle ergometer test to exhaustion (7, 8) however studies examining the endurance of the quadriceps during local exercise (i.e. repeated knee extensions) show mixed results. Differences in these findings may be a result of differing methodology such as intensity of contraction (percent of maximum voluntary contraction), type of contraction (static or dynamic) or the type of task (whole-body exercise such as cycling or a localized muscle

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contraction such as knee extension). The majority of studies examining localized muscle contractions have used low intensity contractions (10 to 40% of MVC) that primarily task the oxidative pathway (9-11). A higher intensity contraction which tasks anaerobic metabolism may provide insight into a different aspect of muscle metabolism in people with COPD.

Surface EMG can be used to examine the motor unit properties of muscle and is an accepted method for objectively quantifying muscle fatigue (12). Previous studies that have examined surface EMG in people with COPD have only recorded EMG from the vastus lateralis muscle (9, 11, 13, 14) however, the quadriceps muscle is made up of four muscles, rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) which lie superficially and the vastus intermedius, which is deep. Differences exist in the contribution of the quadriceps to knee extension, and a previous study demonstrated that the RF showed greater fatigue than the VL and VM during a sustained contraction of the quadriceps (see Chapter Two). Therefore, it is important for EMG to be recorded from multiple sites on the quadriceps (15, 16).

The purpose of this study was to examine the time to task failure of a high intensity quadriceps contraction to examine changes in surface EMG from three sites on the quadriceps muscle, in people with COPD. Time to task failure and surface EMG parameters were compared between people with COPD and an age-, sex- and BMI-matched control group. The study examined a select group of people with COPD who were limited by leg fatigue rather than ventilation on an incremental cycle ergometer test. We hypothesized that people with COPD would have a shorter time to task failure compared to controls and this would be due to differences in the median frequency (MF) and amplitude of surface EMG.

## 5.2 METHODS

### 5.2.1 Participants

Twenty people with COPD and 20 controls participated in the study. People with COPD had moderate to severe (Stage II to III) COPD based on the GOLD guidelines ( $FEV_1 < 80\%$  of predicted and  $FEV_1/FVC < 70\%$ ), (17) had no acute exacerbations of COPD and had not taken oral corticosteroids (i.e. Prednisone) in the six months prior to inclusion in the study. Participants had not been in any formal exercise rehabilitation program for at least one year prior to the study. People with COPD were excluded from the study if they were limited primarily by perception of shortness of breath and/or ventilatory parameters rather than perception of leg fatigue on an incremental exercise test. Further details of the exercise test are provided in the Methods section. The control group was recruited from the general population and matched for sex, age and body mass index to the people with COPD.

All participants were currently not smoking. Those who had co-morbid conditions such as cardiovascular disease (e.g. heart failure, previous myocardial infarction or cardiovascular surgery), neurological conditions (e.g. stroke, Parkinson's) and lower extremity musculoskeletal problems (knee or hip injury or arthritis) were excluded from the study. The study was approved by the University Clinical Research Ethics Board and each participant provided written, informed consent prior to participation.

Height and weight were measured with shoes off and light clothing. Each participant also completed the Physical Activity Scale for the Elderly (PASE) (18). The PASE is a 12-item, self-

administered questionnaire which asks about household, leisure time and work-related physical activity over the past seven day period. The PASE has been found to be reliable (18) and valid (19) for older adults and has previously been used in people with COPD (20).

### **5.2.2 Spirometry and exercise testing**

Participants underwent spirometry according to the standards described by the American Thoracic Society (21) to measure FEV<sub>1</sub> and FVC to characterize lung function. Maximal voluntary ventilation (MVV) was estimated by multiplying FEV<sub>1</sub> by 35 (22) and expressed as a percent of predicted (23).

Participants underwent an incremental exercise test on an electrically-braked cycle ergometer. Participants were seated on the cycle and connected to a metabolic cart through a mouthpiece (VMax 229, Sensormedics, Yorba Linda, CA). The metabolic cart consisted of a pneumotachograph, O<sub>2</sub> and CO<sub>2</sub> analysers and a mixing chamber. Breath-by-breath analysis was used to obtain minute ventilation (V<sub>E</sub>), oxygen uptake (VO<sub>2</sub>) and carbon dioxide production (VCO<sub>2</sub>). Heart rate was monitored using 12-lead ECG (Quinton Eclipse Premier, Bothell WA) and oxygen saturation (% SpO<sub>2</sub>) was monitored using a pulse oximeter (SiMed S103, Miami FL) connected via a finger probe. Blood pressure was measured by a physician at rest and one minute intervals throughout the test using a manual sphygmomanometer. Participants were asked to use the Rating of Perceived Exertion (RPE) category-ratio scale (24) to rate shortness of breath and leg fatigue separately at the end of each exercise stage throughout the test.

After two minutes of rest, participants began pedaling against no resistance (free wheeling) for one minute as a warm-up. The warm-up was followed by an incremental exercise test to symptom-limited maximum ( $\text{VO}_{2\text{peak}}$ ) where the resistance was increased at one-minute intervals by 10 watts per minute for people with COPD and 20 watts per minute for controls. The goal was a total exercise time of 8-12 minutes.

Peak  $\text{VO}_2$  was expressed relative to body weight ( $\text{mL/kg/min}$ ) and as a percent of predicted  $\text{VO}_{2\text{max}}$  based on the equations derived by Jones et al. (25) for cycle ergometry. At the end of the test, participants were asked to indicate which symptom stopped them from exercising (i.e. shortness of breath, leg fatigue or another symptom). Also,  $V_E$  at end-exercise was compared to estimated MVV to determine whether ventilatory limitation was present at end-exercise (26) and  $V_E$  greater than 80% MVV was defined as ventilatory limitation (27).

### **5.2.3 Quadriceps fatigue task**

Each participant performed a fatiguing contraction at an intensity of 80% of their maximal voluntary contraction (MVC). Both the MVC and the endurance task were conducted on the KinCom dynamometer (version 5.30, Chattanooga Group Inc., Hixson TN) which has previously been shown to be a valid and reliable method for measuring torque produced by a muscle (28, 29). All testing was done on the dominant leg which was determined by asking each participant over which leg he or she had more control (e.g. which leg they would use to kick a ball).

The participant was seated on the KinCom seat with the axis of rotation of the dynamometer aligned to the knee joint line. The backrest and seat angles were adjusted so that the hip was at approximately 80° of flexion. A strap was placed across the participant's pelvis to minimize hip movement during the tests. The cuff (load cell) of the KinCom was placed at a distance of 75% of the lower leg length (measured from the top of the fibular head to the mid-point of the lateral malleolus). The knee joint was placed at 90° of flexion for all testing.

To determine the MVC, participants did two to three submaximal warm-up contractions, followed by three isometric maximal voluntary contractions (MVC) of the knee extensors. Participants were instructed to "push as hard as possible" for a five second period. Visual feedback of the produced torque was provided. A one minute break was given between MVC trials. The highest was used to calculate the target torque for the fatigue task (80% MVC).

Following a five minute rest, the fatigue task was conducted. During this task, visual feedback of the target and produced torque was provided and verbal encouragement was given by the tester to maintain the force at the target level until the target torque could no longer be met despite verbal cueing. The time to task failure was defined as the point where the produced torque dropped 20% from the target level and was calculated from the software program. The participant was asked to rate their leg fatigue at the beginning and end of the contraction using the RPE scale.

Surface EMG was collected from three electrode sites on the quadriceps muscle: rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM). The specifics of electrode placement and EMG processing are described in detail elsewhere (see Chapter Two). The reliability of time to

task failure and EMG parameters for an 80% contraction has been reported previously (see Chapter Two). Moderate to high intraclass correlation coefficients (ICCs) were found for time to task failure (ICC = 0.85) and EMG MF (ICC = 0.61 to 0.88) and amplitude (ICC = 0.58 to 0.84) from surface EMG of RF, VL and VM.

#### **5.2.4 Statistical analysis**

Statistical analysis was done using SPSS version 13 (SPSS Inc., Chicago IL). Descriptive statistics for sample characteristics and for MF and amplitude were expressed using mean  $\pm$  standard deviation. Normalized final MF and amplitude were calculated as ratios of the final value to the initial value (e.g. final value/initial value). Independent samples t-tests were used to compare sample characteristics at baseline and were also used to compare initial torque, time to task failure and RPE between groups. A two-way ANOVA with the main factors of group (COPD, control) and muscle (RF, VL, VM) was used to examine between-group and between-muscle differences. Pearson's correlation coefficient was used to examine the relationship between initial torque and time to task failure. Significance level was set at  $p < 0.05$  and adjusted for multiple comparisons.

### **5.3 RESULTS**

#### **5.3.1 Sample characteristics**

Characteristics of the study participants are summarized in Table 5-1. Age, height, weight and body mass index (BMI) were similar between groups. There was a trend for physical activity level to be lower in people with COPD compared to the control group ( $p = 0.058$ , Table 5-1).

### **5.3.2 Spirometry and exercise testing**

Lung function values (absolute and percent of predicted) obtained from spirometry are shown in Table 5-1. People with COPD had lower values for lung function ( $FEV_1$ , FVC expressed as percent of predicted and  $FEV_1/FVC$  ratio) than healthy controls ( $p < 0.001$ ) which was consistent with the GOLD criteria for moderate to severe COPD (17).

At peak exercise, people with COPD achieved a lower peak oxygen uptake ( $VO_{2peak}$ ), expressed in both absolute and as a percent of predicted, compared to the control group (see Table 5-1). Peak workload achieved at the end of the exercise test, both absolute and as a percent of predicted, was also lower in people with COPD compared to controls (Table 5-1). Minute ventilation expressed as a percent of MVV reached  $76.9 \pm 4\%$  of MVV in the COPD group and  $75.9 \pm 14\%$  in the controls ( $p = 0.678$ ). RPE for both leg fatigue and shortness of breath were similar between groups at peak exercise (see Table 5-1); all participants complained of leg fatigue more than shortness of breath at the end of exercise.

### **5.2.3 Endurance task and surface EMG**

The target torques and times to task failure for both groups are shown in Figure 5-1. People with COPD had lower MVC of the knee extensors than controls resulting in a lower target torque for the endurance task ( $69.6 \pm 18.3$  Nm in people with COPD compared to  $92.2 \pm 38.5$  Nm in controls;  $p = 0.023$ ). People with COPD had a 32% shorter time to task failure than controls ( $28 \pm 12$  s compared to  $41 \pm 18$  s;  $p = 0.001$ ). The RPE for leg fatigue at the beginning of the contraction was



zero for both groups and was similar between groups at the end of the quadriceps contraction ( $6 \pm 2$  for COPD and  $7 \pm 1$  for controls,  $p = 0.203$ ). No correlation was found between initial torque (80% of MVC) and time to task failure in people with COPD was  $r = -0.15$  ( $p = 0.52$ ) and in the controls was  $r = -0.11$  ( $p = 0.62$ ).

MF and amplitude for each muscle is provided in Table 5-2. There was no significant group by muscle interaction for any of the EMG measures ( $p = 0.393-0.965$ ). No differences were found between groups for the start, end or normalized EMG amplitude or MF. This was consistent for all three quadriceps muscles.

## **5.4 DISCUSSION**

The results of this study show that people with COPD who were limited by leg fatigue on a cycle ergometer test also had a 32% shorter time to task failure for a high intensity quadriceps contraction than a matched control group. However the changes in the surface EMG from their quadriceps muscle were similar to those seen in the control group. Both groups demonstrated characteristic changes in the EMG amplitude and frequency that are typically observed with sustained muscular contractions held to task failure; specifically an increase in EMG amplitude and a decrease in MF (30). Muscle fatigue of the quadriceps was demonstrated by the change in normalized EMG (final/initial value) to values less than one for MF and greater than one for amplitude, and was similar to what was found in a previous study of young, healthy people using the same protocol (see Chapter Two). The increase in amplitude during sustained contractions has been attributed to an increase in neuromuscular activation (discharge rate and motor unit recruitment) (31). The frequency shift has been attributed a reduction in muscle fiber action

potential conduction velocity and central changes including synchronous firing of motor units and recruitment of new motor units (31, 32). This study shows that people with COPD demonstrate similar changes in motor unit recruitment, firing rate and muscle fiber conduction velocity, to controls, even though the time to task failure was shorter.

Disuse is a factor that has been postulated to contribute to skeletal muscle dysfunction in people with COPD (2, 33). Following a period of disuse (i.e. immobilization), there appears to be an impairment in neuromuscular recruitment patterns resulting in observable changes in the pattern of EMG (34), lower EMG amplitude during maximal contractions (35) and a smaller increase in EMG amplitude with sustained submaximal muscle contractions (34). In this study there were no differences in the initial, final or normalized EMG amplitude between people with COPD and controls, despite a trend for lower physical activity levels in people with COPD as measured by the PASE. Also, both groups reached EMG amplitudes at the end of the task which were higher than what was achieved during the pre-fatigue MVC for all three quadriceps muscles. High EMG amplitudes at the end of the contraction presents further evidence that people with COPD were able to sufficiently recruit their motor neuron pool and neuromuscular propagation was intact (36). The similar EMG MF and amplitudes between the COPD and control groups provides some evidence contrary to the disuse hypothesis in people with COPD; it appears that neuromuscular recruitment is maintained during sustained contractions. However, it is important to consider that immobilization is a very specific model of disuse which results in a dramatic decrease in muscle activity and may not reflect the same changes that occur in skeletal muscle with a gradual reduction in physical activity as experienced by people with COPD.

There is some evidence to suggest that the shift in MF during fatigue may correlate to the proportion of type 1 fibers ( $R^2 = -0.37$ ) and type 2 fibers ( $R^2 = 0.48$ ) (37). Although people with COPD have previously been shown to have a lower proportion of type 1 fibers (38), there was no difference in the normalized MF between people with COPD and controls. Similar to our data, Allaire et al. (13) reported a similar slope of MF during a 60% MVC held to task failure between people with COPD and controls.

A shorter time to task failure in people with COPD despite similar neuromuscular patterns may be attributed to a number of other skeletal muscle properties such as muscle mass and strength, metabolic capacity or contractile function. People with COPD demonstrate muscle atrophy of the quadriceps which contributes to muscle weakness (2) (see Chapter Four). In studies comparing muscle fatigue in groups of people who have differing muscle strength (i.e. males compared to females or younger compared to older adults), people with lower strength (i.e. females, older adults) tend to have longer endurance times (39, 40), however when matched for initial strength, the difference in fatigue tends to diminish (41). One hypothesis to explain this relative fatigue resistance is that lower muscle force results in lower intramuscular pressure being generated, therefore leading to less impediment of blood flow to the working muscle (42). In this study, people with COPD had lower initial torque, but their time to task failure was also lower than the control group and there was no relationship between initial torque and time to task failure in either group. This may have been due to the high contraction intensity of 80% MVC which tends to rely primarily on anaerobic metabolism (43) and therefore is not as highly dependent on blood flow to the muscle. Similarly, the sex difference in muscle fatigue tends to be smaller with higher intensity contractions and under ischemic conditions (44).

A shorter time to task failure in people with COPD may be a reflection of metabolic properties of skeletal muscle in these people. The high intensity contraction used in this study is primarily dependent on anaerobic metabolism (43). In people with COPD, there is a relative increase in the proportion of type 2 muscle fibers (with the reciprocal reduction in the proportion of type 1 fibers) compared to controls, (4) and the concentration of glycolytic enzymes is similar to controls (45, 46). These findings would suggest that the ability to do an anaerobic task would be maintained in people with COPD. However, these people also demonstrate alterations in muscle metabolism which may impair their ability to perform such tasks. At rest, people with COPD have a lower intramuscular pH and ATP concentrations and higher resting muscle lactate (47). People with COPD also show an early drop in muscle pH and steeper rise in the inorganic phosphate to phosphocreatine (PCr) ratio with exercise compared to controls (5, 48). Increases in the concentrations of inorganic phosphate and hydrogen ion within the muscle cell may impair excitation-contraction coupling and/or calcium handling resulting in an earlier onset of fatigue in people with COPD (49). Further studies are required to determine whether these mechanisms of skeletal muscle fatigue are evident in people with COPD.

## **5.5 CONCLUSION**

People with COPD demonstrate a shorter endurance time for a high intensity contraction of the quadriceps however they show similar changes in objective measures of muscle fatigue using surface EMG (MF and amplitude). Therefore, the difference in muscle endurance does not appear to be due to impairments in neuromuscular recruitment but rather other factors such as alterations in muscle metabolism, excitation-contraction coupling and calcium handling may play a role in muscle endurance in people with COPD.

## **5.6 BRIDGING SUMMARY**

The findings in this Chapter are consistent with the initial hypothesis that people with COPD have a shorter time to task failure than controls; however people with COPD did not show any differences in their motor unit recruitment and firing properties as measured with surface EMG, compared to controls. The ability to recruit and fire motor units may be one factor that assists in the preservation of torque per unit volume of muscle that was reported in Chapter Four. One limitation that must be considered is that motor unit properties were examined during an isometric contraction in this Chapter in order to obtain reliable and valid EMG recordings. Therefore, the findings may not be generalizable to concentric and eccentric contractions which were reported in Chapter Four. A discussion of how this may be addressed in future research is provided in Chapter Eight: General Discussion and Conclusions.

## 5.7 TABLES AND FIGURE

**Table 5-1.** Sample characteristics for people with COPD and controls (mean  $\pm$  SD). Results of spirometry and exercise testing are provided as absolute values and as percent of predicted.

	COPD	Control
n	20	20
age (yrs)	68.2 $\pm$ 10.0	64.4 $\pm$ 8.1
sex	11F, 9M	11F, 9M
height (m)	1.66 $\pm$ 0.09	1.67 $\pm$ 0.13
weight (kg)	72.1 $\pm$ 14.6	69.0 $\pm$ 14.4
BMI (kg/m <sup>2</sup> )	26.6 $\pm$ 4.7	24.3 $\pm$ 2.2
Physical activity scale	101 $\pm$ 58	131 $\pm$ 37
<b>Spirometry</b>		
FEV <sub>1</sub> (L)	1.34 $\pm$ 0.41	2.28 $\pm$ 0.72
(% predicted)	(51 $\pm$ 17%) <sup>‡</sup>	(81 $\pm$ 20%)
FVC (L)	2.58 $\pm$ 0.47	3.05 $\pm$ 1.11
(% predicted)	(78 $\pm$ 14%) <sup>‡</sup>	(83 $\pm$ 20%)
FEV <sub>1</sub> /FVC (%)	52 $\pm$ 14% <sup>‡</sup>	77 $\pm$ 9%
<b>Exercise Test</b>		
VO <sub>2</sub> (ml/kg/min)	12.4 $\pm$ 2.8 <sup>‡</sup>	20.8 $\pm$ 5.1
(% predicted)	(57 $\pm$ 19%) <sup>†</sup>	(87 $\pm$ 23%)
Peak workload (W)	70 $\pm$ 18 <sup>†</sup>	145 $\pm$ 49
(% predicted)	(69 $\pm$ 20%) <sup>‡</sup>	(125 $\pm$ 38%)
V <sub>E</sub> (L/min)	38.5 $\pm$ 2.2 <sup>†</sup>	58.8 $\pm$ 18
(% of MVV)	(76.9% $\pm$ 4%)	(75.9% $\pm$ 14%)
RPE (shortness of breath)	6 $\pm$ 1	6 $\pm$ 1
RPE (leg fatigue)	7 $\pm$ 1	8 $\pm$ 1

<sup>†</sup> significantly different from control,  $p < 0.01$

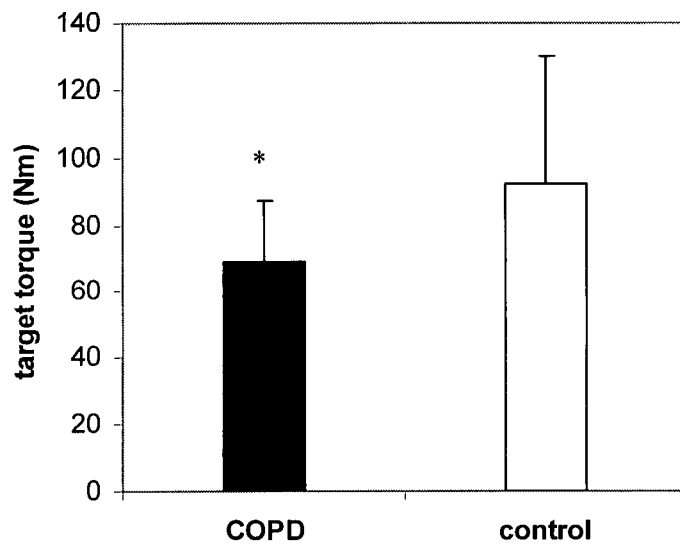
<sup>‡</sup> significantly different from control,  $p < 0.001$

**Table 5-2.** EMG median frequency and amplitude for the rectus femoris, vastus medialis and vastus lateralis in people with COPD and controls (mean  $\pm$  standard deviation). Amplitude is expressed as a percent of the amplitude obtained during a maximal voluntary contraction (%MVC).

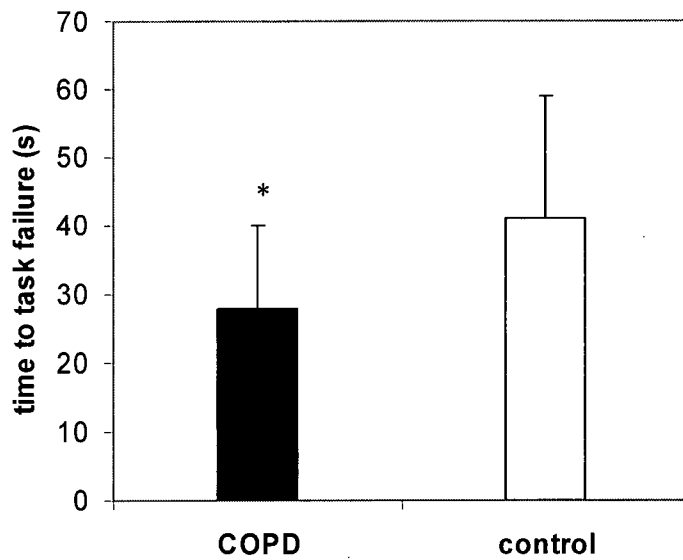
	<b>COPD</b> n = 20	<b>Control</b> n = 20	<b>p-value</b>
<b>Rectus Femoris</b>			
Start frequency (Hz)	79.9 $\pm$ 12.8	78.5 $\pm$ 12.2	0.718
End frequency (Hz)	70.2 $\pm$ 10.6	66.1 $\pm$ 13.0	0.276
Normalized end frequency	0.863 $\pm$ 0.119	0.843 $\pm$ 0.110	0.201
Start amplitude (%MVC)	93.7 $\pm$ 21%	85.6 $\pm$ 24%	0.272
End amplitude (%MVC)	115 $\pm$ 54%	111 $\pm$ 50%	0.791
Normalized end amplitude	1.28 $\pm$ 0.661	1.29 $\pm$ 0.422	0.347
<b>Vastus Medialis</b>			
Start frequency (Hz)	82.4 $\pm$ 11.4	81.7 $\pm$ 13.4	0.854
End frequency (Hz)	74.4 $\pm$ 10.5	69.3 $\pm$ 11.6	0.154
Normalized end frequency	0.895 $\pm$ 0.116	0.854 $\pm$ 0.116	0.122
Start amplitude (%MVC)	95.3 $\pm$ 21%	83.8 $\pm$ 14%	0.050
End amplitude (%MVC)	106 $\pm$ 27%	105 $\pm$ 34%	0.949
Normalized end amplitude	1.14 $\pm$ 0.259	1.26 $\pm$ 0.381	0.235
<b>Vastus Lateralis</b>			
Start frequency (Hz)	81.4 $\pm$ 14.2	79.2 $\pm$ 14.0	0.632
End frequency (Hz)	75.3 $\pm$ 13.9	68.2 $\pm$ 13.6	0.111
Normalized end frequency	0.926 $\pm$ 0.068	0.865 $\pm$ 0.124	0.063
Start amplitude (%MVC)	99 $\pm$ 21%	87 $\pm$ 14%	0.047
End amplitude (%MVC)	108 $\pm$ 24%	116 $\pm$ 38%	0.465
Normalized end amplitude	1.12 $\pm$ 0.232	1.32 $\pm$ 0.376	0.046

Normalized end frequency = end frequency /start frequency

Normalized end amplitude = end amplitude/start amplitude



A)



B)

**Figure 5-1.** Comparison of COPD and control groups for A) target torque and B) time to task failure during a sustained contraction of the quadriceps at 80% of MVC. Bars represent the mean  $\pm$  SD. \* indicates significant difference between groups,  $p < 0.05$ .



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## 6. CELLULAR FEATURES OF THE VASTUS LATERALIS IN PEOPLE WITH COPD<sup>f</sup>

### 6.1 INTRODUCTION

People with chronic obstructive pulmonary disease (COPD) demonstrate reduced muscle mass compared to healthy people and this contributes to muscle weakness (1) (see Chapter Four). However the reduction in muscle volume does not fully account for the reduction in muscle strength (see Chapter Four). Therefore there may be some changes in the cellular structure of skeletal muscle that impair its ability to generate tension. Exertion-induced muscle injury, which is defined as a loss of the structural integrity of the muscle due to prolonged, intense or unaccustomed exercise (2) may contribute to impaired skeletal muscle contractility in people with COPD. People with COPD may be prone to exertion-induced muscle injury even with normal activity such as walking (3) and low to moderate levels of exercise due to predisposing factors such as low physical activity level, systemic inflammation and increased oxidative stress.

People with COPD experience a lower amount and intensity of physical activity in their daily lives, even compared to their sedentary counterparts (4). Lack of physical activity results in a long-standing disuse of their limb muscles, especially of the lower limbs. Following a period of disuse such as cast immobilization, spaceflight or bedrest, skeletal muscle undergoes structural changes which are similar to those seen with exertion-induced muscle injury such as muscle fiber atrophy, atrophy and damage of actin filaments and a disorganization of the sarcomere (5). The

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adaptations that occur within the muscle due to disuse may also increase the muscle's susceptibility to injury with normal loads such as walking (6) and result in decreased muscle strength.

Exertion-induced muscle injury is associated with an inflammatory response which is responsible for the clearance of damaged tissue and for stimulating repair (7). Activated inflammatory cells also produce reactive oxygen species which may accentuate muscle damage (7). The cycle of degeneration-regeneration seen in healthy individuals following muscle injury may be altered in patients with COPD due to increased systemic inflammation (8, 9) and higher concentrations of reactive oxygen species(10, 11) resulting in an amplification of muscle injury through the inflammatory mechanism.

Muscle injury in people with COPD may result in changes in the muscle cellular structure that can be observed under the light microscope. For example, following repetitive strain injuries to contracting muscles in rats, a greater area of connective tissue within the muscle has been observed (12, 13). Connective tissue area has also been observed in the diaphragm of people with airflow obstruction where this muscle is subjected to chronic overload resulting in injury (14) and shown to be higher post-mortem, compared to people with no significant respiratory disease (15). Skeletal muscle satellite cells also appear to have the capacity to differentiate into adipose cell (16). Intramuscular fat infiltration has been observed in the quadriceps and hamstrings in people with COPD using magnetic resonance imaging (MRI) however, it is not possible to determine whether these cells are within the muscle fiber or between fibers from the MRI (see Chapter Four). Examination of muscle biopsy specimens under the light microscope, using H&E staining, may assist in determining the location of the adipose.



This study examines the cellular features that may be consistent with muscle injury in people with COPD under the light microscope. The primary objective was to quantify the area fractions ( $A_A$ ) of normal and abnormal muscle, connective tissue and adipose of the vastus lateralis muscle in people with COPD and compare it to matched, controls. We hypothesized that people with COPD would show an increased  $A_A$  of abnormal muscle, connective tissue and adipose compared to the control group.

## **6.2 METHODS**

### **6.2.1 Participants**

Twenty people with COPD and 20 controls participated in the study. People with COPD had moderate to severe (Stage II to III) COPD based on the GOLD guidelines ( $FEV_1 < 80\%$  of predicted and  $FEV_1/FVC < 70\%$ ) (17), had no acute exacerbations of COPD and had not taken oral corticosteroids (i.e. prednisone) in the six months prior to inclusion in the study. Participants had not been in any formal exercise rehabilitation program for at least one year prior to the study. The participants were recruited from patient lists and posters at pulmonary function labs. The control group was recruited from the general population using newspaper advertisements and posters at community centres. They were matched for sex, age and body mass index to the people with COPD and were free of respiratory disease.

All participants were currently not smoking. Individuals with co-morbid cardiovascular disease (e.g. heart failure, previous myocardial infarction or cardiovascular surgery), neurological

conditions (e.g. stroke, Parkinson's) and lower extremity musculoskeletal problems (knee or hip injury or arthritis) were excluded from the study. The study was approved by the University Clinical Research Ethics Board and each participant provided written, informed consent prior to participation.

Height and weight were measured with shoes off and light clothing. Participants underwent spirometry according to the standards described by the American Thoracic Society (18), to measure FEV<sub>1</sub> and FVC to characterize lung function.

### **6.2.2 Muscle biopsy procedure**

Muscle biopsies were obtained from the vastus lateralis muscle, approximately 15 cm superior to the patella. Participants rested in the supine position, while 0.5% bupivacaine (local anaesthetic) was infiltrated into the skin, subcutaneous tissue and fascia overlying the muscle belly of vastus lateralis. A small incision was made into the skin and a 5-mm diameter biopsy needle was inserted. Suction was applied to obtain a muscle sample of approximately 80 to 100 mg. The sample was oriented and fixed on a cork with mounting medium. The sample was then quick frozen in isopentane, cooled with liquid nitrogen. The frozen sample was then stored at -70 degrees Celsius.

Frozen biopsies were mounted on the cryostat such that the longitudinal axis of the muscle fibers in the biopsy was perpendicular to the surface of the microtome blade. Transverse cryosections (10 µm thick) were cut and stained with hematoxylin and eosin (H&E). Sections were mounted on glass slides and labeled with a code number.

### **6.2.3 Image capturing**

A SPOT digital camera (Diagnostic Instruments Inc., Michigan) Version 2.2 was used to capture images of the vastus lateralis sections. The camera was interfaced with a computer and a microscope (Nikon Microphot, Japan). Images were viewed using the microscope, captured via the digital camera and saved onto the computer system. All images were captured at 40x magnification. A maximum of 20 fields were captured per section (range eight to 20 fields per section).

### **6.2.4 Quantitative evaluation of H&E stained cross-sections**

The images of the vastus lateralis sections were evaluated to determine the proportion of normal muscle, abnormal muscle and connective tissue. Tissue features were classified into one of nine categories as defined in Table 6-1.

Area fractions ( $A_A$ ) of normal muscle relative to abnormal muscle, connective tissue and adipose were quantified using point counting. The computer program Adobe PhotoShop (version 6.0, Adobe Systems Inc., San Jose CA) was used to superimpose a 63-point grid (7 x 9 points) onto each image. To allocate points to categories, the tissue occupying the smallest discernible region in the top right quadrant of the point-intercept was identified and designated to one of the nine categories. Each point was assigned to a category until all 63 points were completed. The total number of points was confirmed prior to moving onto the next image.

$A_A$  of normal muscle, abnormal muscle, connective tissue and adipose (definitions provided in Table 6-1) were calculated using the following equations:

$$\text{Total count} = \Sigma \text{ counts in categories 1-8}$$

$$\text{Area fraction } (A_A) \text{ of normal muscle} = \frac{\Sigma \text{ counts in category 1}}{\text{Total Count}} \times 100$$

$$A_A \text{ of abnormal muscle} = \frac{\Sigma \text{ counts in categories 2-6}}{\text{Total Count}} \times 100$$

$$A_A \text{ of connective tissue} = \frac{\Sigma \text{ counts in category 7}}{\text{Total Count}} \times 100$$

$$A_A \text{ of adipose} = \frac{\Sigma \text{ counts in category 8}}{\text{Total Count}} \times 100$$

Point counting was done by one assessor who was blinded to the identity of the subject. Inter-rater reliability of classification of points was performed on seven identical fields between the assessor and the primary investigator (SM) and reliability coefficients ranged from  $r = 0.92$  to  $0.96$  for normal muscle, abnormal muscle, connective tissue and adipose.

### 6.2.5 Statistical analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 13.0 (SPSS Inc., Chicago, IL). Descriptive statistics [mean  $\pm$  standard deviation (SD)] were calculated for subject characteristics.  $A_A$  of normal and abnormal muscle, connective tissue and adipose were expressed as mean  $\pm$  standard error of the mean (SEM). Independent samples t-tests were used to test for differences between the COPD group and the control group in age, height, weight, body mass index (BMI) and lung function. One-way ANOVA was used to compare the  $A_A$  of normal and abnormal muscle, connective tissue and adipose between groups. Chi-square statistic was used to compare the proportion of subjects in each group who had

counts of connective tissue greater than 10% and adipose greater than 0% on their biopsy.

Significance was set at  $p < 0.05$  and adjusted for multiple comparisons.

## **6.3 RESULTS**

### **6.3.1 Sample characteristics**

Characteristics of the study participants are summarized in Table 6-2. Age, height, weight and body mass index (BMI) were similar between groups. People with COPD had lower values for FEV<sub>1</sub>, FVC expressed as percent of predicted and FEV<sub>1</sub>/FVC ratio than healthy controls ( $p < 0.05$ ) which was consistent with the GOLD criteria for moderate to severe COPD (17).

### **6.3.2 Tissue features**

The largest A<sub>A</sub> of tissue was normal muscle in both the people with COPD and the control group [ $88.2 \pm 1.0\%$  and  $90.5 \pm 0.5\%$  (mean  $\pm$  SEM), respectively;  $p = 0.053$ ]. The A<sub>A</sub> of abnormal muscle, connective tissue and adipose in people with COPD and controls are shown in Figure 6-1. Examples of abnormal tissue features observed in the cross-sections of the vastus lateralis are shown in Figure 6-2. Thirteen people with COPD had an A<sub>A</sub> of connective tissue that was greater than 10% compared to six controls (65% versus 30%,  $\chi^2 = 4.89$ ,  $p = 0.028$ ). However the A<sub>A</sub> of connective tissue was not significantly different between groups ( $10.5 \pm 3.3\%$  in COPD and  $8.9 \pm 2.9\%$  in controls;  $p = 0.101$ ). Point counts were observed on adipose in the vastus lateralis samples of three people with COPD (0.3, 1.0 and 5.5% adipose) whereas no adipose was

observed in any of the control subjects ( $p = 0.226$ ). There was no significant difference in the proportion of subjects showing adipose counts between groups ( $\chi^2 = 3.16$ ,  $p = 0.075$ ).

People with COPD had a higher  $A_A$  of abnormal muscle than controls ( $0.9 \pm 0.2\%$  in COPD and  $0.3 \pm 0.3\%$  in controls;  $p = 0.011$ ). Each category that comprised abnormal muscle is shown in Figure 6-3. Small, angular fibers was the most common feature of abnormal muscle in people with COPD and was significantly higher than controls ( $0.38 \pm 0.09\%$  versus  $0.09 \pm 0.04\%$  in COPD and controls, respectively;  $p = 0.006$ ). The next most common abnormal feature was abnormal cytoplasm ( $0.29 \pm 0.10\%$  versus  $0.15 \pm 0.06\%$ , respectively;  $p = 0.237$ ), followed by inflammatory cells ( $0.10 \pm 0.03\%$  versus  $0.09 \pm 0.02\%$ , respectively;  $p = 0.907$ ).

#### **6.4 DISCUSSION**

This study is the first to use a quantitative method for describing the proportion of normal muscle fibers, abnormal muscle fibers, connective tissue and adipose in a peripheral muscle of people with COPD. We found that people with COPD had a greater  $A_A$  of abnormal muscle fibers in their vastus lateralis compared to age-, sex- and BMI-matched controls. A greater proportion of people with COPD had  $A_A$  of connective tissue greater than 10% of total  $A_A$  in their vastus lateralis compared to controls and only people with COPD had point counts of adipose; however, the  $A_A$  was not significantly different from controls for either of these categories. Differences in the cellular structure of the vastus lateralis muscle of people with COPD may contribute to functional differences of the knee extensors in these people.

Of the abnormal features observed in the vastus lateralis, small, angular fibers were the most common abnormality observed in people with COPD and comprised a higher  $A_A$  than in controls. Small, angular fibers in the vastus lateralis of people with COPD may be indicative of muscle fibers that have undergone atrophy or degeneration. The presence of small, angular fibers is in line with previous findings in people with COPD which show smaller muscle fiber cross-sectional area in the vastus lateralis (19) and lower muscle volume of quadriceps, hamstrings and adductor muscles of people with COPD (see Chapter Four) compared to controls. Although the mechanism of muscle atrophy in people with COPD is not fully understood, skeletal muscle apoptosis has been proposed as a potential cause of muscle fiber atrophy (20). Factors that may contribute to muscle atrophy and/or increased apoptosis in people with COPD include decreased physical activity levels, malnutrition, steroid-induced atrophy and systemic inflammation and oxidative stress (18, 20).

Connective tissue provides both structural integrity to skeletal muscle and also assists with the transmission of tensile loads (21). Endomysial and perimysial connective tissue account for approximately 3-8% of total muscle area in healthy specimens (22). Using a slightly higher cut-off value of 10%, we found that a greater proportion of people with COPD had a high  $A_A$  of connective tissue than controls. Connective tissue has previously been observed, although not quantified, in the vastus lateralis muscle of people with COPD who were diagnosed with steroid-induced myopathy (23) and increased connective tissue was also described in both the perimysium and endomysium of the muscle fibers in people with COPD (24). In the diaphragm of people with airflow obstruction undergoing thoracotomy surgery, the  $A_A$  of connective tissue was reported to be 16.3%,(14) which was higher than what we observed in the vastus lateralis of people with COPD ( $10.5 \pm 3.3\%$ ). A post-mortem study of people with COPD also found a

greater  $A_A$  of collagen in the diaphragm compared to a limb muscle, psoas major ( $24.2 \pm 1.0\%$  in the diaphragm compared to  $12.3 \pm 0.5\%$  in psoas) (15).

Differing mechanisms of muscle injury may account for the higher proportion of connective tissue observed in the limb muscles compared to the diaphragm. Intramuscular collagen has been observed following chronic strain-induced muscle injury in the rat triceps surae muscle and may be a result of an incomplete healing process (12). The diaphragm in people with airflow obstruction is also subjected to constant strain and overload, therefore the mechanism for connective tissue deposition may be similar as in the animal model. However, differing mechanisms for connective tissue deposition may account for the different proportions of connective tissue observed in a limb versus a respiratory muscle. The muscles of the lower limbs in people with COPD are undergoing gradual disuse, which may result in a relative increase in the proportion of connective tissue compared to muscle secondary to muscle fiber atrophy, or an increase in the absolute volume of connective tissue. Studies examining the rat hindlimb following a period of immobilization (a model of disuse) have demonstrated an increase in the volume of intramuscular connective tissue (21, 25) and a decrease in the extensibility of connective tissue (21). This model may provide more insight into the mechanism of connective tissue deposition observed in the limb muscles of people with COPD.

Increased connective tissue in the vastus lateralis may contribute to strength changes observed in people with COPD. A reduction in concentric strength (force generated as the muscle is actively shortening) (26) of the knee extensors has been reported by previous investigators (1, 27).

Connective tissue may replace muscle fibers during the process of muscle atrophy (24), thereby reducing the number of sarcomeres available for tension generation and contribute to reduce



force output. Eccentric strength (force generated as the muscle is actively lengthened) (26) has been found to be maintained in people with COPD (see Chapter Four). During these lengthening contractions, the passive structures of the muscle which are comprised of connective tissue may contribute to the force produced (28). Increased connective tissue that has been observed in this and other studies (23, 24), may contribute to the maintenance of eccentric strength in people with COPD. Please see Appendix D for a description of the relationship between eccentric torque and  $A_A$  of connective tissue.

Although systemic inflammation has previously been implicated as a contributor to skeletal muscle atrophy in people with COPD (8, 9), a very small  $A_A$  of inflammatory cells and inflamed fibers was observed in the vastus lateralis. Minimal evidence of inflammation in the vastus lateralis of people with COPD is similar to the findings of Gosker et al.(24) who observed very few inflammatory cells in the vastus lateralis and reported no difference in inflammatory cells between people with COPD and controls. A limitation of both studies is that specific immunohistochemical techniques to identify subpopulations of inflammatory cells such as macrophages were not used, therefore some inflammatory cells may not have been identified.

Infiltration of inflammatory cells into skeletal muscle has been observed following acute bouts of eccentric or unaccustomed exercise in healthy people and likely contributes to muscle damage (7). If inflammation is a contributor to muscle dysfunction in people with COPD, it may be due to a low grade, insidious process and possibly less to an acute inflammatory reaction affecting a large area of the muscle. Although chronic inflammation may result in a higher concentration of inflammatory markers which have previously been observed in the vastus lateralis of people with COPD, such as increased levels of TNF- $\alpha$  (8), and markers of oxidative stress such as lipofuscin

(29) and nitric oxide synthases (8), an influx of acute inflammatory cells into skeletal muscle may be less obvious in the vastus lateralis of people with COPD. Chronic, low grade inflammation may affect isolated fibers, located diffusely in the muscle, which are not adequately sampled with a needle biopsy.

Adipose was present in the vastus lateralis muscle of three people with COPD but in none of the control subjects. Gosker et al.(24) observed adipose cells in the vastus lateralis of seven of 15 people with COPD using a semi-quantitative method where the severity of the abnormality was rated on a scale of 0 (not present) to 5 (severe). In their study, six people with COPD had a rating score of 1 and one person had a rating of 2 for adipocytes. In our study, the  $A_A$  of adipose was quantified using point counting, which provides a measure of the  $A_A$  of adipose compared to the total area of tissue rather than a categorical ranking. As seen on H&E stained sections in this study, adipose cells appear to be between individual muscle fibers. This may contribute to reduced strength in people with COPD not only by replacing the  $A_A$  of normal muscle fibers, but also by interfering with lateral force transmission along across adjacent muscle fibers (28). The mechanism by which adipose infiltrates skeletal muscle in people with COPD requires further investigation.

## **6.5 CONCLUSION**

People with COPD demonstrate cellular features in their vastus lateralis that are consistent with muscle injury including connective tissue and adipose within muscle tissue. The mechanism by which connective tissue and adipose are formed within the skeletal muscle of people with COPD requires further study. Small, angular fibers were also found to be more common in people with

COPD compared to controls and may be indicative skeletal muscle fiber atrophy. Further research is required to determine how these cellular changes may contribute to muscle function (i.e. torque production) in people with COPD.

## **6.6 BRIDGING SUMMARY**

This Chapter provides an examination of the cellular features of skeletal muscle structure at the light microscope level and is the first study in people with COPD to quantify cellular abnormalities of skeletal muscle using the point counting technique. This study looked specifically at features of skeletal muscle injury in biopsies of the vastus lateralis, as people with COPD may be susceptible to muscle injury due to longstanding disuse and muscle atrophy. The increased  $A_A$  of small, angular fibers in people with COPD reported in this Chapter was consistent with the finding in Chapter Four that showed skeletal muscle atrophy of the quadriceps. The tendency for a greater  $A_A$  of connective tissue provides an avenue for future research examining the role of passive components of skeletal muscle in the maintenance of eccentric torque in people with COPD, also reported in Chapter Four.

## 6.7 TABLES AND FIGURES

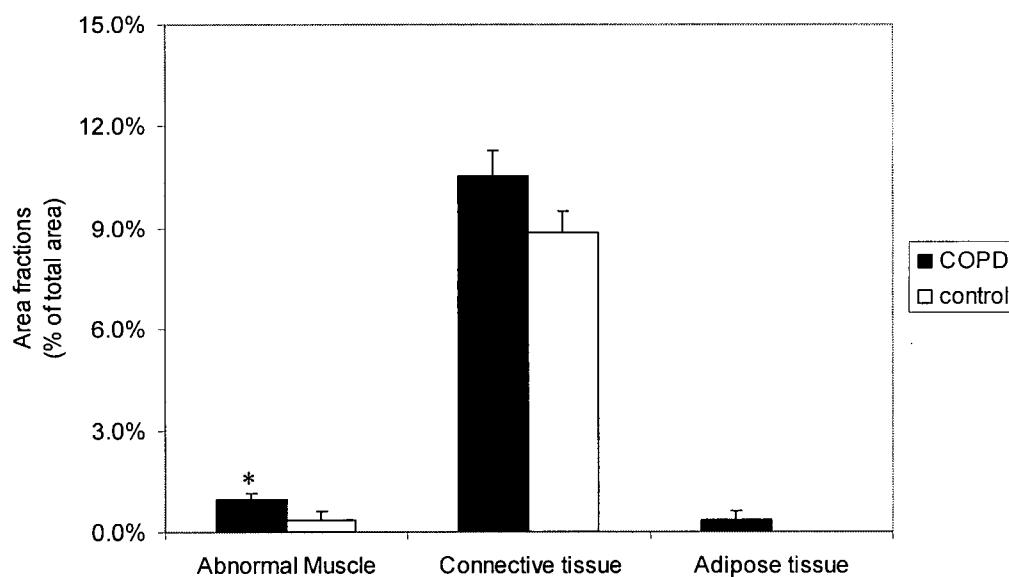
**Table 6-1.** Categories for quantification of tissue features for point counting

#	Category	Definition	
0	no count	space, artifact, epimysial connective tissue, nerve, large blood vessels.	no count
1	normal muscle, capillary	polygonal fiber with acidophilic cytoplasm, plasma membrane and peripheral nuclei or capillary (small blood vessel with endothelium only).	normal
2	internal nuclei	fiber with $\geq 1$ internally located nuclei (sarcoplasm between nucleus and sarcolemma), <i>8 pixels of sarcoplasm between nucleus and sarcolemma</i> .	abnormal
3	small angulated fibre	(a) small fiber ( $\leq 1/3$ the lesser fiber diameter of the five largest fibers in the field) or (b) fibers with "spear-like" extensions or extensions that are less than 45 degrees or (c) fiber with $\geq 2$ acute angles ( $< 90^\circ$ )	abnormal
4	inflamed/necrotic fiber	fiber with $\geq 1$ inflammatory cell or necrotic mass of inflammatory cells and muscle debris without plasma membrane.	abnormal
5	abnormal cytoplasm, lipofuscin	includes: (a) fiber with pale acidophilic peripheral cytoplasm and enlarged peripheral nuclei with or without visible nucleoli, or (b) fiber with pale acidophilic peripheral cytoplasm and deep acidophilic "fuzzy" cytoplasm in the central region, or (c) split or whirled fibers, or (d) vacuoles or (e) uneven cytoplasm staining unrelated to processing or (f) fiber with dull or light gray staining, or (g) cytoplasmic fragmentation or (h) lipofuscin (brown-yellow pigmentation $\geq$ area of a muscle nucleus).	abnormal
6	inflammatory cell	cell in the interstitium that has a round-shaped nucleus consistent with a monocyte, macrophage, or lymphocyte.	abnormal
7	collagen or fibroblast	protein fibrils of endomysial or perimysial connective tissue or a cell located in the interstitium with spindle shaped nucleus that is consistent with a fibroblast.	collagen
8	adipocyte	empty space surrounded by cell membrane consistent with size and shape of adipocyte.	adipose

**Table 6-2.** Sample characteristics for people with COPD and controls (mean  $\pm$  standard deviation)

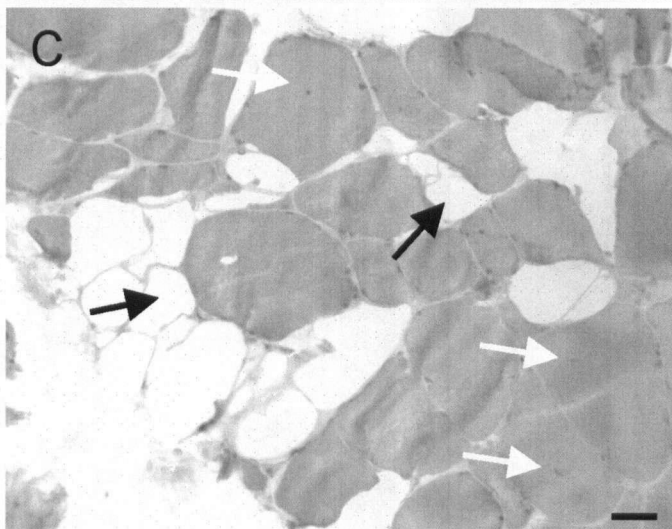
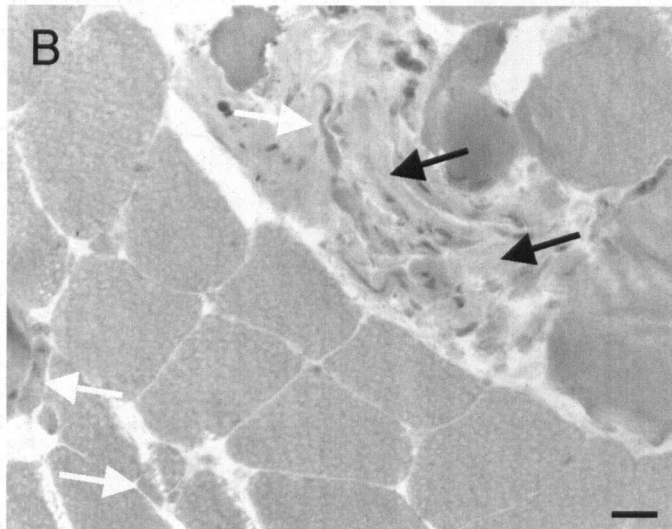
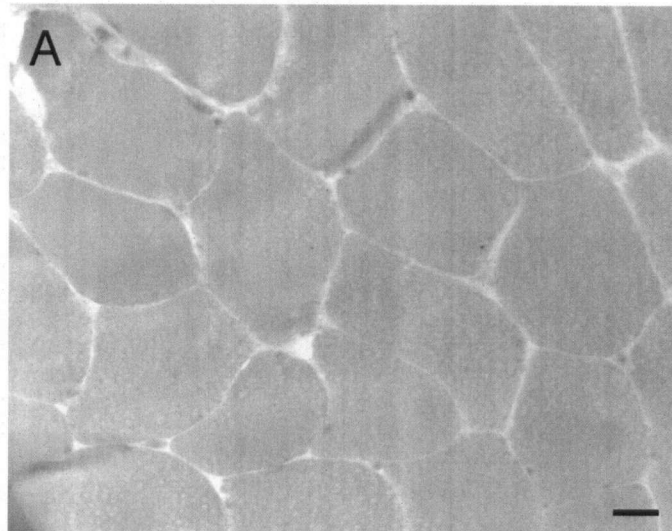
	<b>COPD</b>	<b>Control</b>
n	20	20
age (yrs)	68.2 $\pm$ 10.0	64.4 $\pm$ 8.1
sex	11F, 9M	11F, 9M
height (m)	1.66 $\pm$ 0.09	1.67 $\pm$ 0.13
weight (kg)	72.1 $\pm$ 14.6	69.0 $\pm$ 14.4
BMI (kg/m <sup>2</sup> )	26.6 $\pm$ 4.7	24.3 $\pm$ 2.2
FEV <sub>1</sub> (L)	1.34 $\pm$ 0.41	2.28 $\pm$ 0.72
(% predicted)	(51 $\pm$ 17%)†	(81 $\pm$ 20%)
FVC (L)	2.58 $\pm$ 0.47	3.05 $\pm$ 1.11
(% predicted)	(78 $\pm$ 14%)†	(83 $\pm$ 20%)
FEV <sub>1</sub> /FVC (%)	52 $\pm$ 14%†	77 $\pm$ 9%

† significantly different from control,  $p < 0.001$

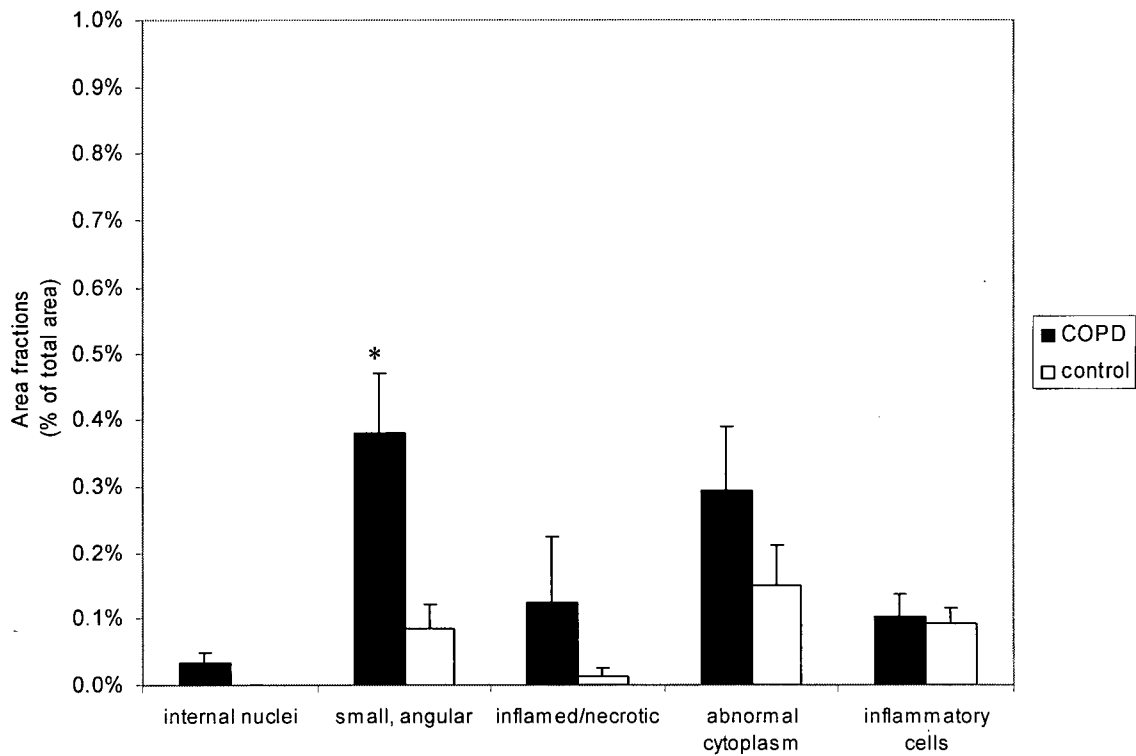


**Figure 6-1.** Area fractions of abnormal muscle, connective tissue and adipose (% total area) of H&E stained cross-sections measured by the point counting technique for the COPD (n=20) and control groups (n=20). \* indicates significantly different area fraction for the COPD group compared to the control group ( $p < 0.05$ ). Bars represent mean  $\pm$  SEM.

**Figure 6-2.** Examples of features of H&E stained cross-sections of the vastus lateralis in people with COPD. **A)** normal muscle; **B)** small, angular fibers (white arrow) and connective tissue (black arrow); and **C)** adipose (black arrow), internal nuclei (white arrow). Scale bars = 10  $\mu\text{m}$







**Figure 6.3.** Area fractions for subcategories of abnormal muscle (% total area) of H&E stained cross-sections measured by the point counting technique for the COPD (n=20) and control groups (n=20).

\* indicates significantly different area fraction for the COPD group compared to the control group ( $p < 0.05$ ). Bars represent mean  $\pm$  SEM.

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## 7. SKELETAL MUSCLE DYSFUNCTION IN RECIPIENTS OF LUNG TRANSPLANTS: A COMPARISON TO PEOPLE WITH COPD<sup>g</sup>

### 7.1 INTRODUCTION

Despite improvements in lung function, recipients of lung transplants still experience a limitation in peak exercise capacity. In the majority of lung transplant recipients, peak exercise capacity reaches only 40-60% of predicted maximum even up to two years following transplantation (1, 2). A growing body of evidence points to the role of lower-limb skeletal muscle dysfunction following lung transplantation as the major factor in exercise limitation. Lung transplant recipients demonstrate an increased reliance on anaerobic metabolism during quadriceps exercise (1) suggesting that an intrinsic abnormality of skeletal muscle may exist in recipients of transplants and may play a role in exercise limitation. This evidence is consistent with the observation that the majority of recipients of lung transplants report lower-extremity fatigue rather than dyspnea as the reason for terminating maximal exercise on a cycle ergometer (2). Furthermore, maximal work capacity on a cycle ergometer is more strongly correlated with 30-second work capacity during isokinetic cycling ( $r=.84$ ), rather than with pulmonary function variables such as forced expiratory volume in 1 second ( $FEV_1$ ) ( $r=.58$ ) and residual volume/total lung capacity ( $RV/TLC$ ) ( $r=-.52$ ) (3).

Pre-transplant condition [i.e. chronic obstructive pulmonary disease (COPD)] is associated with skeletal muscle abnormalities that contribute to exercise limitation, and these changes may

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<sup>g</sup> A version of this Chapter is in preparation: **Mathur, S.**, Levy, R.D., MacIntyre, D.L., Forster, B.B., Reid, W.D. Skeletal muscle atrophy and weakness in recipients of lung transplants. *The Journal of Heart and Lung Transplantation*.

persist following lung transplantation. These changes include reduced muscle mass (4) and muscle fiber atrophy (5), muscle weakness (6), decrease in the proportion of type 1 muscle fibers and oxidative enzymes (5, 7), and increased reliance on anaerobic metabolism (8, 9). Following lung transplant, a prolonged period of bedrest and low activity levels and the use of immunosuppressant medications such as corticosteroids (10) and cyclosporine (11) may lead to further impairments in skeletal muscle structure and function.

Transplant recipients have previously been shown to have a lower proportion of type 1 muscle fibers and lower concentrations of oxidative enzymes than controls (12). Few studies have quantified the functional characteristics of skeletal muscle, beyond work output on a cycle ergometer, and related function to skeletal muscle structure in recipients of lung transplants. In one study, quadriceps strength was lower in recipients of lung transplants with cystic fibrosis (CF) compared to healthy controls and correlated to quadriceps cross-sectional area (CSA) (10). Specific measures of muscle mass, strength and fatigue would provide insight into the relationship between skeletal muscle structure and function in transplant recipients.

Previous studies compare transplant recipients to a healthy control group but comparisons have not been made to people who have a chronic respiratory condition such as COPD, therefore it is not clear whether the changes in skeletal muscle are due to the pre-transplant condition or to factors related to post-transplant care such as a period of prolonged bedrest and the use of immunosuppressant medications.

The purpose of this chapter is to describe the structural and functional characteristics of the quadriceps and hamstrings muscles in recipients of lung transplants and compare them to people

with COPD who have not undergone lung transplantation, and to a control group from the general population.

## **7.2 METHODS**

Recipients of lung transplants were recruited through the Solid Organ Transplant Clinic at Vancouver Hospital. Personal letters were sent to 22 lung transplant recipients. Interested participants (n=15) contacted the investigator and were booked for initial assessment and screening. Three participants were ineligible for participation due to co-morbid conditions limiting their ability to participate [osteoarthritis of the knees (n=1), active infection (n=2)]. Two participants dropped out following the first testing session due to illnesses unrelated to the study procedures. All participants provided written, informed consent (see Appendix A) and the study was granted ethics approval by the University Clinical Research Ethics Board.

Transplant recipients underwent four testing sessions. Procedures for testing and data acquisition are outlined in the preceding chapters of the thesis. During the first testing session, participants completed spirometry and an incremental exercise test on a cycle ergometer (see Chapter Five). Knee extensor (KE) and flexor (KF) torque and quadriceps fatigue were assessed in a second testing session (see Chapter Four). MRI scan was conducted during the third testing session and used to determine muscle volume and intramuscular fat infiltration as described in Chapter Four. A needle biopsy of the vastus lateralis as described in Chapter Six, was conducted at the final testing session.



Statistical analyses comparing transplant recipients to people with COPD and controls was done using one-way ANOVA and Tukey's post-hoc t-tests ( $p < 0.05$ ) using the Statistical Package for the Social Science (SPSS) (version 13.0, SPSS Inc., Chicago, IL).

### **7.3 RESULTS**

The following sections compare and contrast the findings of the transplant group to the major findings in people with COPD and the control group which are outlined in the previous chapters of the thesis. The relevant data from the COPD and control groups have been repeated in this chapter for ease of comparisons.

#### **7.3.1 Sample characteristics of lung transplant group**

Ten recipients of lung transplants participated in the study (see Table 7-1 for a summary of the sample characteristics). Seven were single lung transplant recipients (SLT) and three were double lung transplant (DLT) recipients. All DLT recipients had a pre-transplant diagnosis of cystic fibrosis. Pre-transplant diagnoses for SLT recipients included COPD ( $n=5$ ), alpha-1 antitrypsin deficiency ( $n=1$ ) and sarcoidosis ( $n=1$ ). Time post-transplant ranged from 0.75 years to nine years. All transplant recipients were receiving a regimen of immunosuppressant medications consisting of a calcineurin inhibitor (cyclosporine or tacrolimus), a purine synthesis inhibitor (azathioprine or mycophenolate mofetil) and a corticosteroid (i.e. prednisone) (see Table 7-1). None of the participants were showing signs of organ rejection (bronchitis obliterans syndrome stage 0 or 1, see Table 7-2).

Compared to the sample of people with COPD and the controls, transplant recipients had a younger mean age ( $p=0.001$ ); three of the transplant recipients were under the age of 40 years, whereas all people with COPD and controls were 48 years of age or older. The oldest participant in the transplant group was 68 years old whereas in the COPD and control groups, the oldest participants were 81 years old. Height, weight and BMI were similar between groups.

Lung transplant recipients had higher absolute forced expiratory volume in one second ( $FEV_1$ ) ( $p=0.02$ ) and forced vital capacity (FVC) ( $p=0.014$ ) than people with COPD.  $FEV_1$  as a percent of predicted was similar between groups ( $p=0.079$ ) and FVC as a percent of predicted was higher in the lung transplant group ( $p=0.033$ ).  $FEV_1$  to FVC ratio was similar between groups ( $p=0.709$ ). Although the transplant recipients have improved lung function compared to their pre-transplant condition, only those with DLT approach normal lung function values (see Table 7-2). SLT recipients are still limited by their native lung and therefore do not have normal lung function values. This may account for the similarities between the transplant and COPD groups for lung function.

Lung transplant recipients achieved a higher absolute  $VO_2$  than people with COPD ( $p=0.008$ ) however their  $VO_2$  expressed as a percent of predicted was similar to that of people with COPD ( $p=0.73$ , see Table 7-3). This discrepancy was likely due to the younger participants in the transplant group who had a higher absolute  $VO_{2peak}$  but when expressed as a percent of predicted (which depends on age), their relative  $VO_{2peak}$  was similar to that of the older people with transplant and the people with COPD. Peak work rate achieved was similar between groups ( $p=0.053$ ).

Leg fatigue was the main subjective factor limiting peak exercise on a cycle ergometer in transplant recipients (RPE for leg fatigue  $9 \pm 1$ , shortness of breath was  $6 \pm 2$ , Table 7-3). In seven of the participants, minute ventilation reached 80% or less as a percent of maximum voluntary ventilation (MVV) (see Table 7-3) indicating that ventilation was not likely to limit peak exercise in these subjects.

### **7.3.2 Skeletal muscle atrophy and intramuscular fat infiltration in recipients of lung transplants**

#### *Muscle volume*

Muscle volume of the quadriceps, hamstrings and adductors in lung transplant recipients was similar to that of people with COPD and approximately 30% lower than controls for all muscle groups ( $p < 0.01$ ) (see Table 7-4). Similar to the COPD group, there was a uniform reduction in muscle volume compared to the control group across individual quadriceps and hamstring muscles ranging from 23.4 to 38.6 % (see Table 7-4).

#### *Intramuscular fat infiltration*

Transplant recipients showed a wide range in their degree of intramuscular fat infiltration varying from two young male subjects (27 and 37 years old) who had little to no visible fat infiltration in their quadriceps and hamstrings to a young female subject (36 years old) who had a large degree of fat infiltration in both muscle groups (see Figure 7-1). Although there appeared to be a trend for the transplant recipients to have wider interquartile ranges and higher

coefficients of skewness than the control group (see Tables 7-5 and 7-6), the differences were not significant between transplant and controls, with one exception; the coefficient of skewness for the vasti on the lower thigh reached significance (Table 7-6). There were no differences between the people with COPD and the transplant recipients in interquartile ranges or coefficients of skewness.

#### *Peak torque of the knee extensors and flexors*

Absolute torques of the KEs and flexors KFs were not different in the transplant compared to the COPD or control groups (Figure 7-2A). Torque was normalized to muscle volume to take into account differences in muscle volume between groups. In the transplant group, there were no differences in normalized torques of the KEs and KFs compared to the COPD group or the control group (Figure 7-2B).

Absolute and normalized torque data for individual subjects are shown in Figures 7-3 and 7-4, respectively. Four of the ten transplant recipients (3 females and 1 male) had absolute concentric torques that were below the mean for the COPD group (see Figure 7-3). The other six transplant recipients (all males) had absolute concentric torque values that were higher than the mean of the COPD group. Subjects with concentric KE and KF torques below the mean for the COPD group also had low absolute eccentric torques with one exception; the male subject had a KE eccentric torque that was above the mean for the COPD group (see Figure 7-3). Normalized concentric KE torque showed a similar pattern, with the same four subjects having torques below the mean for the COPD group. However, for the other normalized torques the majority of the transplant recipients had values below the mean for the COPD group (see Figure 7-4). The same four

transplant recipients who had the highest absolute torques, had normalized torque values that were above the mean for the COPD group.

As concentric and eccentric torques are lower in females compared to males in both the COPD and control groups (see Chapter Four), we compared the absolute and normalized torque for the three female transplant recipients, who had the lowest torque values of the group, to a subgroup of only the females from the COPD group. Even compared to the mean value for the females with COPD, these three transplant recipients had lower absolute and normalized torques for both the quadriceps and hamstrings.

#### *Factors contributing to torque production*

Due to the small sample of transplant recipients, multiple regression analyses could not be carried out to determine which factors were the most important in explaining the variability in KE and KF torque.

The degree of intramuscular fat infiltration of the quadriceps and hamstrings was not correlated to concentric or eccentric torque of the KEs and KFs, respectively. Correlations between interquartile range and torque ranged from -0.01 to 0.35 and coefficient of skewness and torque ranged from 0.01 to 0.58. This was similar to the finding in people with COPD.

Correlations between torque and muscle volume, which was the major contributor to torque in people with COPD and controls, were also high in the transplant group with r-values ranging from 0.80 to 0.97 (see Figure 7-5). Correlations were also made between KE and KF torque with

age, height, weight and PASE score in the transplant group and correlation coefficients are shown in Table 7-7. The correlations between height, weight and PASE score were not significant in the transplant group. Age was significantly correlated to concentric but not eccentric torque of the KE and KF.

### **7.3.3 Surface EMG during a fatiguing contraction of the quadriceps in recipients of lung transplants**

Transplant recipients had quadriceps endurance times that ranged from 10 to 39 seconds, which was lower than that found in the COPD ( $p = 0.02$  compared to COPD) and control groups ( $p < 0.001$  compared to controls, Figure 7-6).

Initial and final amplitude and frequency of EMG was similar among the three groups. Although the endurance time was lower than the COPD and control groups, the normalized amplitude and frequency which are indicators of muscle fatigue, were also similar among the all three groups (see Table 7-8). There were no differences between the RF, VL and VM muscles of the quadriceps in the amplitude or frequency of EMG. This was similar to what was seen in the COPD and control groups.

### **7.3.4 Cellular features of the vastus lateralis in recipients of lung transplants**

Transplant recipients had similar area fractions ( $A_A$ ) of normal muscle, abnormal muscle, connective tissue and adipose as people with COPD and controls (see Figure 7-7). Although the difference was not significant, six out of ten transplant recipients had an  $A_A$  of connective tissue

that was greater than 10% which was similar to people with COPD (13 out of 20 subjects) and more than the controls (six out of 20 subjects). The  $A_A$  of adipose was greater than zero in one transplant recipient, three people with COPD and none of the controls.

The subcategories of abnormal cellular features of muscle are shown in Table 7-9. The transplant group did not show any differences from the COPD or control group in these features.

#### **7.4 DISCUSSION**

This study is the first to demonstrate both skeletal muscle atrophy and intramuscular fat infiltration in recipients of lung transplant and to relate muscle atrophy of the quadriceps and hamstrings to concentric and eccentric torque of the KEs and KFs, respectively. The sample of transplant recipients in this study was diverse in age, activity level, pre-transplant diagnosis and number of years post-transplant which likely led to the wide range of skeletal muscle structure and function observed in the sample.

Intramuscular fat infiltration of their quadriceps and hamstrings that was comparable to that seen in people with COPD was observed in all except two lung transplant recipients. Some of the factors resulting in fat infiltration may be similar in people with COPD and transplant recipients, such as muscle atrophy and low physical activity levels. However, transplant recipients are also exposed to corticosteroids for a prolonged period of time, which may also contribute to intramuscular fat infiltration (13). The two subjects who had the least fat infiltration were young males, with CF. One had a very recent transplant (9 months prior to testing) and therefore exposed to immunosuppressant medications for the shortest period of time. The other subject

had his transplant two years prior to testing which was similar to the majority of other participants, however he participated regularly in cycling exercise. Regular cycling may have contributed to the maintenance of his quadriceps and hamstrings structure and function. The subject with the greatest degree of fat infiltration was a young female with CF. She had the longest post-transplant course (9 years post-transplant at the time of testing) and thus the most prolonged course of immunosuppressants, which may have contributed to her increased intramuscular fat infiltration. Although she participated in physical activity, it tended to be of lower intensity, consisting mostly of walking on her job or for exercise.

The muscle atrophy and weakness observed in our study is comparable to previous findings on transplant recipients. Pinet et al. (10) found a 33% reduction in quadriceps torque and 31% reduction in CSA in twelve cystic fibrosis patients who had undergone lung transplantation compared to a matched control group. Also similar to our findings, quadriceps peak torque was correlated to quadriceps CSA in transplant recipients ( $r = 0.77$ ,  $p < 0.001$ ) (10). Pantoja et al. (14) found a reduction in voluntary and electrically stimulated ankle dorsiflexion isometric torque in nine transplant recipients compared to a matched control group. In a large study by Van der Woude et al. (15) on 184 transplant recipients with mixed pre-transplant diagnoses, isometric force of the quadriceps, biceps and triceps was measured using hand-held dynamometry. In this study, people with CF had significantly lower muscle strength than the other transplant recipients whose pre-existing conditions included COPD, alpha-1 anti-trypsin deficiency, pulmonary fibrosis or pulmonary hypertension. This result is contrary to our findings where two of the male subjects with CF had the highest absolute torques out of the sample while the female subject with CF had one of the lowest absolute torque measures. The authors noted that the subjects with CF in their study had lower BMI than the other subjects which may have resulted in lower muscle



force (15). In our study, there was a small range of BMI and people with CF did not have the lowest BMI of the group. The CF subjects in our sample were younger than the other subjects which may explain the higher torque in the two male subjects who were 27 and 37 years old. The female subject was also young (36 years old), however her transplant was nine years ago which was the longest post-transplant duration in our sample. She would therefore be exposed to immunosuppressant medications for the longest period of time which may have led to greater myopathic effects of these medications.

Similar to the findings in people with COPD, there was a strong correlation between torque of the KEs and KFs and muscle volume of the quadriceps and hamstrings, respectively. Although the majority of transplant recipients had absolute muscle torques that were higher than the COPD group, normalized muscle torque tended to be below the mean for the COPD group. This may indicate that transplant recipients, unlike people with COPD, have impairments in muscle contractility. This may be due to factors that are unique to transplant recipients such as long-term exposure to corticosteroids (10). Further investigation of muscle contractile properties using in-vitro techniques are warranted.

A relationship between intramuscular fat infiltration and torque production was not observed and this was likely due to some transplant recipients having high fat infiltration and also having high normalized and absolute torque values compared to others in the sample (e.g. 68 year old male with pre-existing COPD). In the younger transplant recipients, a pattern was observed: the two young, male subjects who had the highest torques also showed little to no intramuscular fat infiltration and the young female with CF who had the lowest torques tended to have the highest fat infiltration of the transplant recipients.

Lung transplant recipients had the shortest endurance time for the sustained quadriceps contraction at 80% of maximum voluntary contraction (MVC) of all three groups of people. However, the median frequency (MF) and amplitude of EMG was similar to that seen in the COPD and control groups. Therefore the difference in endurance time may be explained by differences in muscle metabolism rather than motor unit activation and recruitment.

Transplant recipients show metabolic changes in their skeletal muscle that are similar to people with COPD and may contribute to a shorter endurance time, despite adequate motor unit recruitment and firing. In a study by Evans et al.(1) transplant recipients demonstrated a lower resting pH of the quadriceps muscle and an earlier drop in pH during bilateral knee extension exercise to exhaustion. This suggests that there is an earlier onset of glycolytic metabolism in transplant recipients with exercise. Also, the immunosuppressant drug cyclosporine may impair mitochondrial respiration by blocking calcium efflux from the mitochondria (16). Mitochondrial dysfunction may lead to an inability of working muscle to utilize oxygen and an early shift toward glycolytic metabolism, especially during exercise (16, 17). Similarly, tacrolimus, which is also a calcineurin inhibitor and is prescribed instead of cyclosporine for many recipients of lung transplants, may have similar effects in muscle (18). Calcium uptake and release by the sarcoplasmic reticulum and potassium homeostasis across the sarcolemma are important aspects of skeletal muscle contractility and are linked to muscle fatigue in humans (19). Abnormalities in skeletal muscle calcium and potassium regulation and have been observed in lung transplant recipients and may result in impairments in excitation-contraction coupling and contribute to the early onset of muscle fatigue (19).

In addition to muscle fiber size, the cellular features of skeletal muscle have not previously been quantified in lung transplant recipients and may have an impact on muscle function. Transplant recipients showed a trend towards a greater proportion of connective tissue and small angular fibers compared to the control group and similar to findings in people with COPD, however these differences did not reach significance. Small angular fibers are consistent with muscle atrophy which was observed in the transplant recipients in and people with COPD. Connective tissue has been observed in the diaphragm of people with COPD (20) and may reflect chronic muscle injury. Six of the ten transplant recipients, including one young male subject with CF, had an area fraction of connective tissue of greater than 10% which is higher than expected for normal muscle. This may be a reflection of connective tissue replacement with injury due to muscle atrophy or disuse. Adipose was observed in only one transplant recipient. This subject also had a high degree of intramuscular fat infiltration observed on the MRI.

The results of this study are based on a small and diverse group of lung transplant recipients, which is an important consideration when interpreting the results. The participants ranged in age from 27 years to 68 years old and had a mean age that was younger than both the COPD and the control groups. Therefore changes in skeletal muscle such as atrophy and endurance time, which were comparable between the transplant and COPD group are actually more profound in the transplant group, since it consists of a younger group of people.

The transplant recipients had differing pre-transplant diagnoses, ranged in time since transplant from nine months to nine years and were therefore receiving immunosuppressant therapy for different periods of time, were receiving different immunosuppressant regimens, and had a range

of physical activity levels from one subject who cycled regularly to some subjects who did not participate in any regular physical activity.

## **7.5 CONCLUSION**

This study is the first to show that lung transplant recipients have intramuscular fat infiltration and uniform muscle atrophy of the major muscle groups of thigh. Muscle volume and endurance time of the quadriceps was lower in this group of transplant recipients even compared to an older control group. Although muscle size is correlated to torque production, it appears that muscle torque is not fully explained by muscle size and other factors may be causing impairments in skeletal muscle contractility. The presence of small, angular fibers and increased connective tissue observed under the light microscope are consistent with muscle atrophy. Our study showed that some transplant recipients have muscle structure and function that is well preserved, while others demonstrate profound changes in their skeletal muscle. Further studies should use a larger, more homogenous sample to assist in determining the factors contribute to good or poor skeletal muscle structure and function in recipients of lung transplants.

## **7.6 BRIDGING SUMMARY**

This Chapter provides a detailed examination of skeletal muscle structure and function of the quadriceps and hamstrings in a group of lung transplants recipients and compares them to the findings in people with COPD, presented in Chapters Four to Six of the thesis. The findings of this Chapter did not support our initial hypothesis of the thesis that recipients of lung transplants would show greater changes in skeletal muscle structure and function compared to people with

COPD. However, a limitation of this study was the small and diverse sample of participants. Although some lung transplant recipients did have impaired muscle function compared to people with COPD (i.e. lower concentric and eccentric muscle torque), others did not demonstrate this difference. The findings in this Chapter highlight the diversity and variation in skeletal muscle structure and function found among lung transplant recipients. A number of factors may account for these differences such as pre-transplant diagnosis, type of transplant, time since transplant, dose and type of immunosuppressant medications and level of physical activity. This study only examined a small, diverse group of transplant recipients; therefore the influence of these factors could not be systematically examined.

## 7.7 TABLES AND FIGURES

**Table 7-1.** Sample characteristics of lung transplant recipients (n=10).

Sex	Age	Height	Weight	BMI	Pre-Tx Diagnosis	Type of Tx	Years post-Tx	Medications
M	27	1.83	77.0	23.0	CF	DLT	0.75	Tacro, MMF, Pred
F	36	1.55	58.9	24.5	CF	DLT	9	Tacro, MMF, Pred
M	37	1.69	63.2	22.1	CF	DLT	2	Tacro, MMF, Pred
M	48	1.78	69.4	21.9	alpha-1 antitrypsin	SLT	2	CSA, MMF, Pred
M	53	1.73	90.0	30.1	COPD	SLT	1.5	CSA, MMF, Pred
M	57	1.76	56.2	18.1	sarcoidosis	SLT	1	CSA, MMF, Pred
F	64	1.58	47.0	18.8	COPD	SLT	7	CSA, MMF, Pred
F	65	1.64	68.8	25.6	COPD	SLT	2	Tacro, AZA, Pred
M	67	1.78	58.9	18.6	COPD	SLT	4	Tacro, MMF, Pred
M	68	1.75	89.8	29.3	COPD	SLT	2	Tacro, AZA, Pred
mean ± SD	52	1.72 ± 0.09	70.2±12.9	23.7±4.2			3.1±2.8	

CSA – cyclosporine, Tacro – tacrolimus, AZA – azathioprine, MMF - mycophenolate mofetil, Pred - prednisone

**Table 7-2.** Lung function parameters for lung transplant recipients.

Sex	Age	FEV <sub>1</sub> (L) (% pred)	FVC (L) (% pred)	FEV <sub>1</sub> /FVC	BOS* Stage
M	27	3.80 (83%)	5.10 (91%)	75%	0
F	36	2.57 (96%)	2.96 (97%)	87%	0
M	37	3.34 (86%)	4.12 (89%)	81%	0
M	48	1.16 (28%)	2.51 (48%)	46%	0
M	53	1.45 (39%)	2.04 (43%)	71%	0
M	57	1.91 (50%)	2.49 (52%)	77%	0
F	64	1.59 (72%)	2.19 (79%)	73%	0
F	65	1.22 (51%)	1.80(58%)	68%	0
M	67	0.87 (26%)	2.20 (52%)	40%	1
M	68	2.05 (59%)	3.16 (70%)	65%	1
<b>mean ± SD</b>					
<b>Lung transplant</b>		2.00 ± 0.98 (59 ± 25%)†	2.86 ± 1.04 (68 ± 20%)†	68 ± 15%	N/A
<b>COPD</b>		1.34 ± 0.41 (51 ± 17%)†	2.58 ± 0.47 (78 ± 14%)	52 ± 14%†	
<b>Control</b>		2.28 ± 0.72 (81 ± 20%)	3.05 ± 1.11 (83 ± 20%)	77 ± 9%	

\* BOS – bronchiolitis obliterans stage (ranges from 0 to 3)

† significantly different from controls,  $p < 0.05$

**Table 7-3.** Exercise capacity and physical activity level in lung transplant recipients.

Sex	Age	VO <sub>2</sub> peak (ml/kg/min) (% pred)	Workload (Watts)	V <sub>E</sub> (L) (%MVV)	RPE (legs)	RPE (SOB)	PASE
M	27	33.4 (75%)	181 (71%)	111.6 (83%)	10	8	78
F	36	24.0 (69%)	92 (83%)	64.8 (72%)	9	6	155
M	37	29.2 (74%)	212 (111%)	94.7 (81%)	7	5	88
M	48	14.8 (44%)	90 (44%)	32.5 (80%)	10	9	131
M	53	14.0 (45%)	90 (45%)	40.6 (80%)	10	8	113
M	57	19.1 (67%)	70 (41%)	55.5 (83%)	9	7	172
F	64	17.1 (70%)	70 (108%)	44.0 (79%)	10	5	82
F	65	14.3 (58%)	75 (76%)	33.3 (78%)	9	6	119
M	67	9.9 (42%)	58 (35%)	21.3 (70%)	10	2	59
M	68	15.6 (59%)	113 (62%)	54.5 (76%)	9	7	91
<b>mean ± SD</b>							
<b>Lung transplant</b>		19.1 ± 7.4 (61 ± 13%)†	105 ± 51 (67 ± 27%)†	55.2 ± 27.0 (78 ± 4%)	9 ± 1	6 ± 2	108 ± 36
<b>COPD</b>		12.4 ± 2.8† (57 ± 19%)†	70 ± 18 (69 ± 20%)†	38.5 ± 2.2 (77 ± 4%)	7 ± 1	6 ± 1	101 ± 58
<b>Control</b>		20.8 ± 5.1 (87 ± 23%)	145 ± 49 (125 ± 38%)	58.8 ± 18 (76 ± 14%)	8 ± 1	6 ± 1	131 ± 37

† significantly different from controls, p &lt; 0.05



**Table 7-4.** Muscle volume (cm<sup>3</sup>) of the quadriceps, hamstrings and adductors in transplant recipients and the control group.

Muscle	Lung Transplant	Control	percent difference*
<b>TOTAL Quadriceps</b>	1634.2 ± 476.9 cm <sup>3</sup> †	2350.54 ± 723.7 cm <sup>3</sup>	30.5%
Rectus femoris	205.7 ± 60.9†	315.6 ± 103.0	34.8%
Vasti	1428.5 ± 428.4†	2034.9 ± 627.0	29.8%
<b>TOTAL Hamstrings</b>	635.6 ± 133.6 cm <sup>3</sup> †	903.4 ± 250.8 cm <sup>3</sup>	29.6%
Semitendinosis	166.4 ± 44.2 †	217.1 ± 71.2	23.4%
Semimembranosis	192.7 ± 57.3†	313.8 ± 93.5	38.6%
Biceps femoris – long head	188.5 ± 47.9†	256.1 ± 79.1	26.4%
Biceps femoris – short head	88.0 ± 21.6†	116.2 ± 37.1	24.3%
<b>Adductors</b>	665.2 ± 202.9cm <sup>3</sup> †	987.6 ± 290.2	32.6%

† significantly different from controls, p < 0.001

**Table 7-5.** Interquartile range (25<sup>th</sup> percentile to 75<sup>th</sup> percentile) of signal intensity for the quadriceps and hamstrings at three levels of the thigh (mean  $\pm$  standard deviation).

Level and muscle	Lung transplant	COPD	Control
<b>Upper thigh (close to hip)</b>			
RF	59 $\pm$ 19	72 $\pm$ 22†	43 $\pm$ 5
Vasti	56 $\pm$ 12	64 $\pm$ 16†	43 $\pm$ 4
Semitendinosus	57 $\pm$ 15	70 $\pm$ 18†	46 $\pm$ 7
<b>Mid thigh (midway between hip and knee)</b>			
RF	58 $\pm$ 24	68 $\pm$ 25†	45 $\pm$ 5
Vasti	50 $\pm$ 12	63 $\pm$ 14†	46 $\pm$ 5
Semitendinosus	58 $\pm$ 18	88 $\pm$ 28†	50 $\pm$ 11
Biceps femoris (long)	60 $\pm$ 20	86 $\pm$ 27†	48 $\pm$ 6
<b>Lower thigh (close to knee)</b>			
Vastus medialis	58 $\pm$ 12	75 $\pm$ 28†	47 $\pm$ 4
Vastus lateralis	73 $\pm$ 31	101 $\pm$ 34†	49 $\pm$ 7
Semimembranosis	74 $\pm$ 23	102 $\pm$ 35†	63 $\pm$ 16
Biceps femoris (long)	60 $\pm$ 15	80 $\pm$ 21†	50 $\pm$ 6
Biceps femoris (short)	74 $\pm$ 22	95 $\pm$ 32†	60 $\pm$ 10

† indicates significantly different from control,  $p < 0.002$

**Table 7-6.** Coefficients of skewness of pixel intensity for the quadriceps and hamstrings at three levels of the thigh (mean  $\pm$  standard deviation).

Level and muscle	Lung transplant	COPD	Control
<b>Upper thigh (close to hip)</b>			
RF	2.05 $\pm$ 1.07	1.85 $\pm$ 0.46	1.25 $\pm$ 1.01
Vasti	1.67 $\pm$ 0.88	2.28 $\pm$ 0.58†	1.37 $\pm$ 0.63
Semitendinosus	1.56 $\pm$ 0.77	1.81 $\pm$ 0.76†	0.71 $\pm$ 0.48
<b>Mid thigh (midway between hip and knee)</b>			
RF	1.49 $\pm$ 0.96	1.93 $\pm$ 0.59†	0.38 $\pm$ 0.80
Vasti	1.94 $\pm$ 0.78	2.01 $\pm$ 0.50†	0.95 $\pm$ 0.82
Semitendinosus	1.57 $\pm$ 0.99	1.47 $\pm$ 0.36†	0.72 $\pm$ 0.41
Biceps femoris (long)	1.74 $\pm$ 0.83	1.69 $\pm$ 0.61†	0.85 $\pm$ 0.52
<b>Lower thigh (close to knee)</b>			
Vastus medialis	1.98 $\pm$ 1.01	1.76 $\pm$ 0.58†	1.13 $\pm$ 0.59
Vastus lateralis	2.11 $\pm$ 0.99†	1.71 $\pm$ 0.63†	0.86 $\pm$ 0.54
Semimembranosis	1.54 $\pm$ 0.58	1.48 $\pm$ 0.54	1.11 $\pm$ 0.60
Biceps femoris (long)	1.74 $\pm$ 0.72	1.53 $\pm$ 0.36†	0.63 $\pm$ 0.39
Biceps femoris (short)	1.18 $\pm$ 0.56	1.62 $\pm$ 0.66†	0.84 $\pm$ 0.40

† indicates significantly different from control,  $p < 0.002$

**Table 7-7.** Pearson's correlation coefficients between explanatory variables and muscle torque of the knee extensors (KEs) and flexors (KFs).

variable	KE concentric	KE eccentric	KF concentric	KF eccentric
Age	-0.64†	-0.54	-0.66†	-0.58
Height	0.57	0.57	0.51	0.62
Weight	0.38	0.26	0.31	0.18
PASE score	-0.36	-0.48	-0.35	-0.23

† significant at  $p < 0.05$

**Table 7-8.** Median frequency (MF) and amplitude of surface EMG during a sustained quadriceps contraction.

	<b>Lung Transplant</b>	<b>COPD</b>	<b>Control</b>
<b>Rectus Femoris</b>			
Start frequency (Hz)	93.2±19.2	79.9±12.8	78.5±12.2
End frequency (Hz)	76.2±15.3	70.2±10.6	66.1±13.0
Normalized end frequency	0.832±0.150	0.863±0.119	0.843±0.110
Start amplitude (%MVC)	88.5±31%	93.7±21%	85.6±24%
End amplitude (%MVC)	118±29%	115±54%	111±50%
Normalized end amplitude	1.43±0.42	1.283±0.661	1.289±0.422
<b>Vastus Medialis</b>			
Start frequency (Hz)	92.7±11.0	82.4±11.4	81.7±13.4
End frequency (Hz)	80.1±8.6	74.4±10.5	69.3±11.6
Normalized end frequency	0.872±0.113	0.895±0.116	0.854±0.116
Start amplitude (%MVC)	77.3±22%	95.3±21%	83.8±14%
End amplitude (%MVC)	98.9±21%	106±27%	105±34%
Normalized end amplitude	1.333±0.297	1.138±0.259	1.262±0.381
<b>Vastus Lateralis</b>			
Start frequency (Hz)	81.5±9.0	81.4±14.2	79.2±14.0
End frequency (Hz)	72.7±10.3	75.3±13.9	68.2±13.6
Normalized end frequency	0.897±0.117	0.926±0.068	0.865±0.124
Start amplitude (%MVC)	81.1±28%	99±21%	87±14%
End amplitude (%MVC)	101±18%	108±24%	116±38%
Normalized end amplitude	1.343±0.353	1.117±0.232	1.321±0.376

Normalized end amplitude = end amplitude/start amplitude

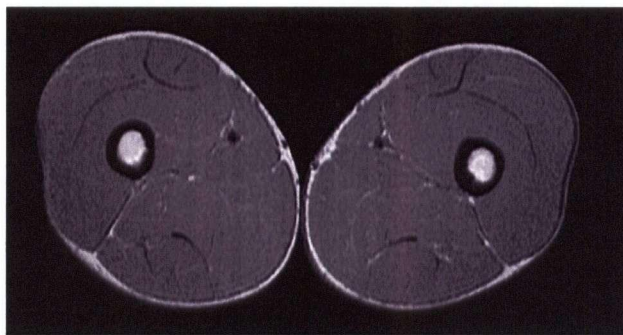
Normalized end frequency = end frequency /start frequency

**Table 7-9.** Area fractions ( $A_A$ ) for subcategories of abnormal muscle (mean  $\pm$  standard deviation).

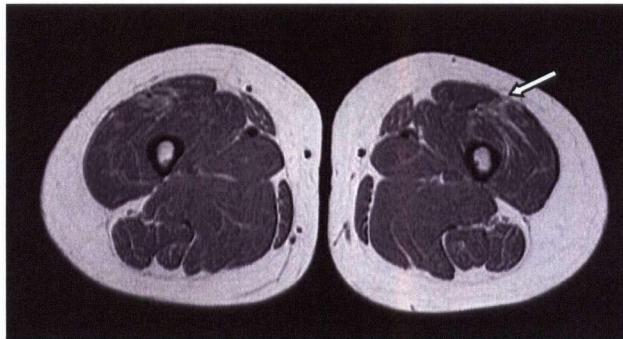
Group	Area Fraction ( $A_A$ ) (% of total area)				
	Internal nuclei	Small, angular fibers	Inflamed, necrotic fibers	Abnormal cytoplasm	Inflammatory cells
<b>Lung transplant</b>	0.00 $\pm$ 0.00%	0.34 $\pm$ 0.11%	0.08 $\pm$ 0.08%	0.11 $\pm$ 0.04%	0.07 $\pm$ 0.03%
<b>COPD</b>	0.03 $\pm$ 0.02	0.38 $\pm$ 0.09	0.12 $\pm$ 0.10	0.29 $\pm$ 0.10	0.10 $\pm$ 0.03
<b>Control</b>	0.00 $\pm$ 0.00	0.09 $\pm$ 0.04	0.01 $\pm$ 0.01	0.15 $\pm$ 0.06	0.09 $\pm$ 0.02



**A)** male, 27 years old

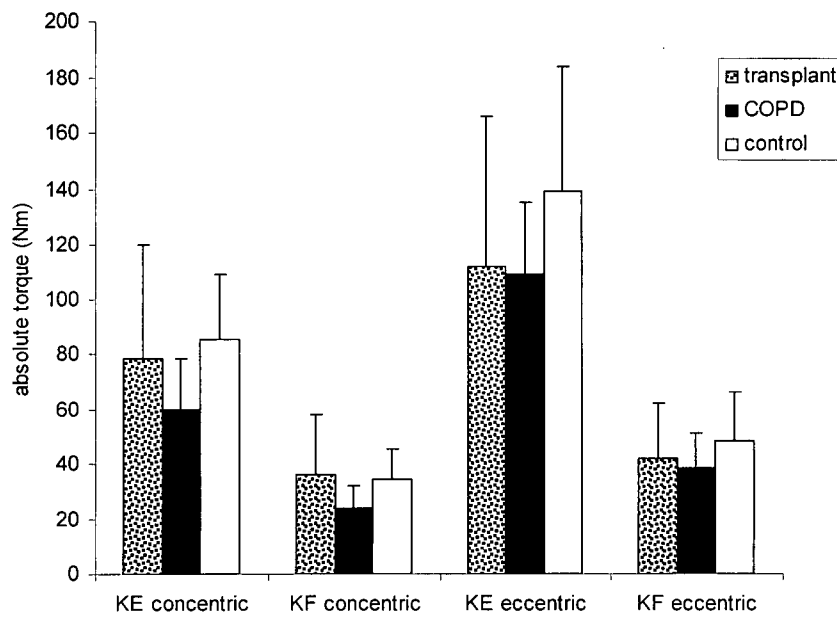


**B)** male, 37 years old

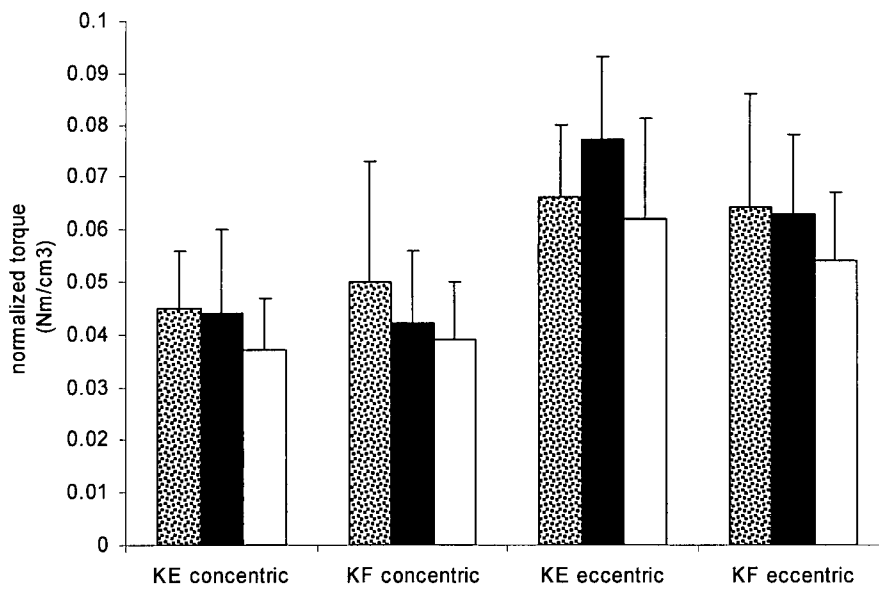


**C)** female, 36 years old

**Figure 7-1.** Axial MRI of the thighs (T-1 weighted, 1.5 Tesla) in three recipients of double lung transplants: **A)** male, 27 years old; **B)** male, 37 years old; and **C)** female, 36 years old. Note very little intramuscular fat infiltration in the male subjects and the higher degree of fat infiltration, particularly in the rectus femoris and vastus lateralis muscles (bilaterally) of the female subject as indicated by the arrow.



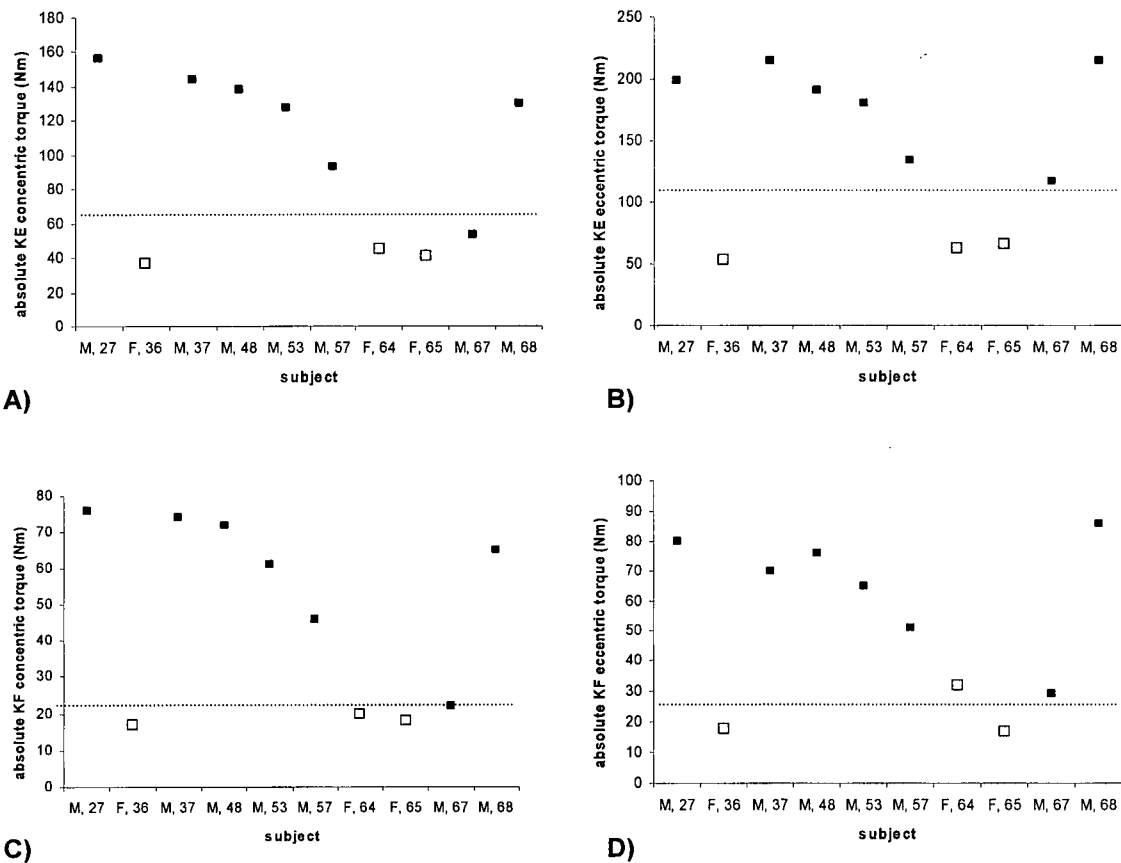
A)



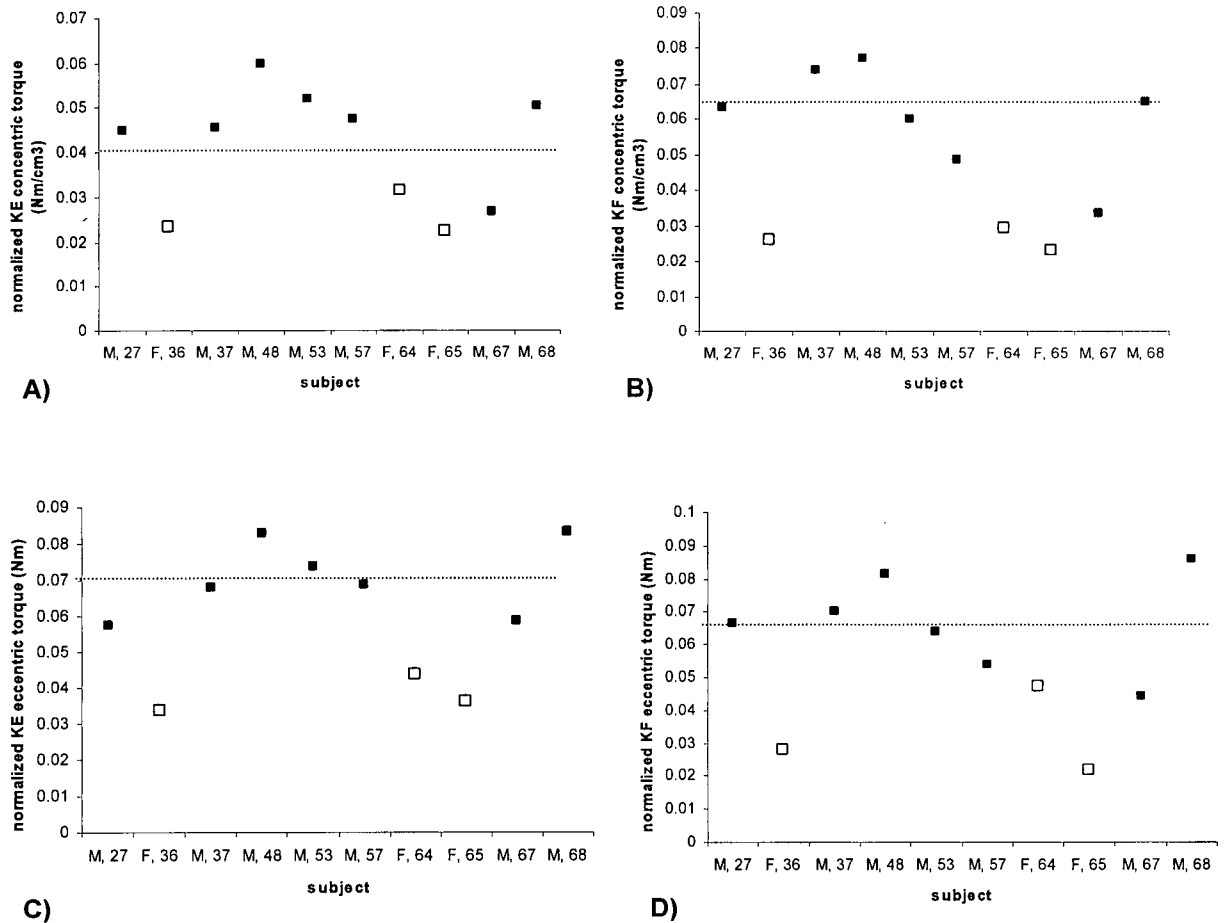
B)

**Figure 7-2.** Knee extensor and flexor torque expressed in A) absolute (Nm) and B) normalized to muscle volume of the quadriceps and hamstrings, respectively (Nm/cm<sup>3</sup>). Bars represent mean  $\pm$  standard deviation.

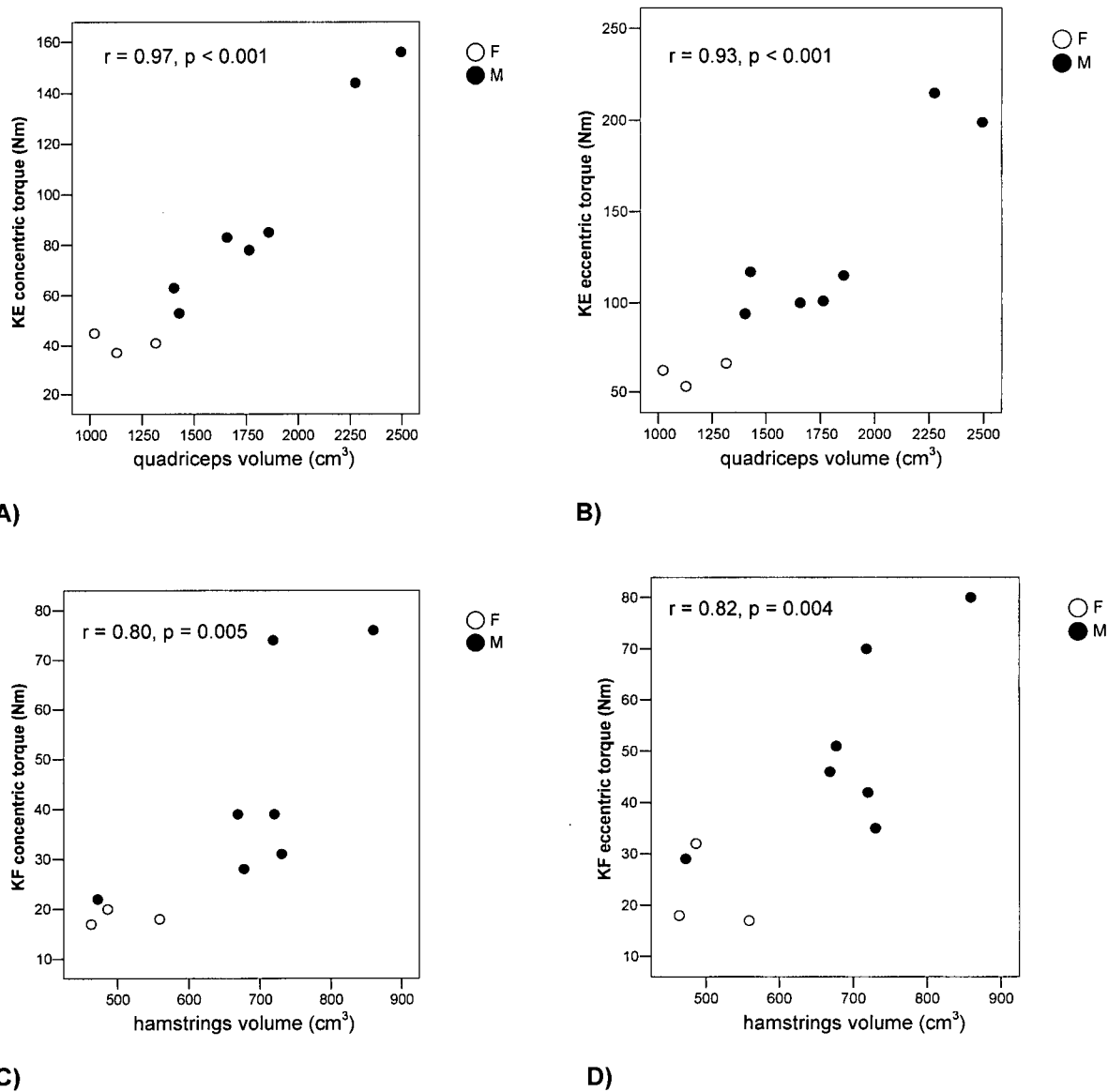




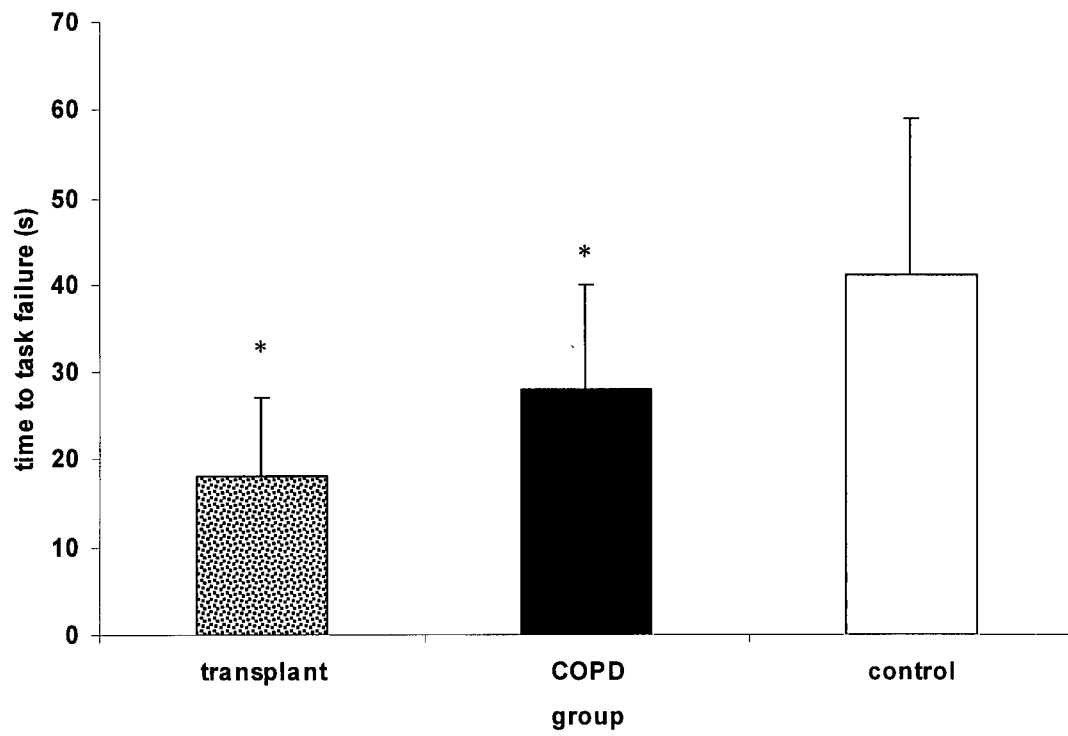
**Figure 7-3.** Absolute torque (Nm) for individual transplant recipients: A) concentric knee extensor (KE) torque; B) eccentric KE torque; C) concentric knee flexor (KF) torque and D) eccentric KF torque. Dashed line represents the group mean for people with COPD. Open squares denote female subjects and closed squares denote male subjects.



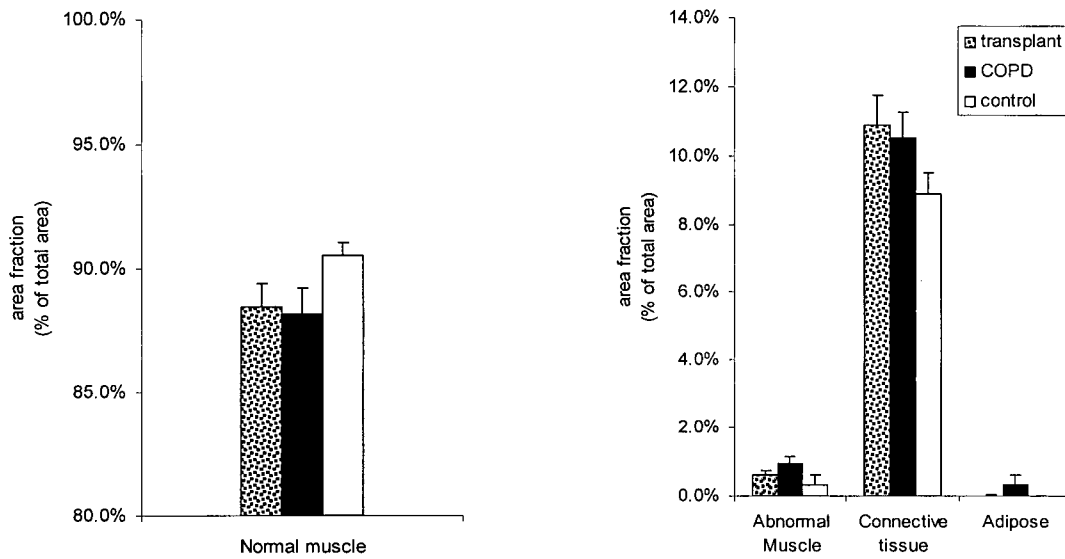
**Figure 7-4.** Torque normalized to muscle volume ( $\text{Nm}/\text{cm}^3$ ) for individual transplant recipients: A) concentric knee extensor (KE) torque per quadriceps volume; B) eccentric KE torque per quadriceps volume; C) concentric knee flexor (KF) torque per hamstrings volume and D) eccentric KF torque per hamstrings volume. Dashed line represents the group mean for people with COPD. Open squares denote female subjects and closed squares denote male subjects.



**Figure 7-5.** Scatterplots depicting the relationship between torque and muscle volume A) Knee extensor (KE) concentric torque to quadriceps volume B) KE eccentric torque to quadriceps volume C) knee flexor (KF) concentric torque to hamstrings volume and D) KF eccentric torque to hamstrings volume.



**Figure 7-6.** Time to task failure for sustained contraction of the quadriceps (80% of MVC) in transplant recipients, people with COPD and a control group. Bars represent mean  $\pm$  standard deviation. \* depicts significantly different from control group at  $p \leq 0.001$ .



**Figure 7-7.** Area fraction ( $A_A$ ) of a) normal muscle and b) abnormal muscle, connective tissue and adipose in transplant recipients, people with COPD and controls (mean  $\pm$  SEM).

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## **8. GENERAL DISCUSSION AND CONCLUSIONS**

### **8.1 GENERAL DISCUSSION**

Skeletal muscle dysfunction is a broad term used to describe the changes observed in the skeletal muscle of people with COPD and lung transplant recipients. Muscle dysfunction may be related to both changes in skeletal muscle structure and/or function. This thesis provided a detailed examination of the structure and function quadriceps and hamstrings muscles in people with COPD and lung transplant recipients. The sample of people with COPD consisted of a select group of individuals who were limited by leg fatigue more than breathlessness at the end of a symptom-limited, incremental exercise test on a cycle ergometer. The sample of lung transplant recipients was composed of a diverse group of individuals differing on a number of pre-transplant and post-transplant factors. Muscle structure was examined through the estimation of muscle atrophy, intramuscular fat infiltration and cellular features of muscle injury. Muscle function was examined through measurement of concentric and eccentric torque, surface EMG and endurance time during a fatiguing contraction. The following sections highlight the main conclusions from each study which comprised the thesis and relate the findings of the studies to each other.

#### **8.1.1 Chapter Two: Reliability of surface EMG during sustained contractions of the quadriceps**

Surface electromyography (EMG) can be used to quantify changes in motor unit firing properties during muscle fatigue. The study presented in Chapter Two examined the reliability of two

measures from surface EMG [median frequency (MF) and amplitude]] and endurance time (the time a muscle contraction is held to a target force level) in healthy adults. These measures were examined during two submaximal contractions; a low level contraction performed at 20% of maximal voluntary contraction (MVC) and a high level contraction performed at 80% of MVC. The study also provided a novel method of quantifying muscle fatigue from surface EMG, normalized final MF and amplitude, and compared its reliability to a previously used method, the slope of MF and amplitude.

The results of the study showed that the new method of quantifying surface EMG during fatiguing contractions, normalized MF and amplitude, was more reliable than the slope of MF and amplitude. The normalized MF and amplitude was therefore chosen for further studies in people with COPD and lung transplant recipients (Chapters Five and Seven). Endurance time for both contraction levels was also highly reliable. Although both contraction levels showed good reliability, the 80% contraction level was chosen for further studies in people with COPD and lung transplant recipients as it results in a shorter endurance time which would be better tolerated for these people. It was also more feasible to obtain an endurance time for the 80% contraction with a single test session.

Although this pilot study was conducted on a group of healthy adults, the results were translated to our two patient groups. The reliability of the fatigue measures may not be the same between healthy adults and people with COPD and transplant recipients, however previous studies on isometric and isokinetic muscle strength testing show similar reliability between healthy people and people with COPD (1). This may provide some evidence that a high level, isometric contraction may also have similar reliability in people with COPD.

### **8.1.2. Chapter Three: Influence of exercise-induced injury on knee extension torque in the presence of longstanding quadriceps atrophy: A case report**

The case report presented in Chapter Three was used to develop the methods for estimating muscle volume from magnetic resonance imaging (MRI) and to pilot the protocol for measuring isokinetic concentric and eccentric torque on the KinCom dynamometer.

MRI was piloted as the method for examining muscle atrophy in this case report. MRI is a non-invasive tool that provides high resolution images and allows for distinctions to be made between muscle, fat and connective tissue (2). It also allows for individual muscles to be visualized so that atrophy of specific muscles can be examined. Another benefit of MRI is that unlike computed tomography, it does not emit any ionizing radiation (2) so that longer scan times can be used to capture multiple images, without putting the participant at increased risk from radiation.

The method for estimating muscle volume from multiple images was developed in this study. Images were selected based on the anatomical origin and insertion points of the muscles and on the level with the largest cross-sectional area (CSA) of the muscle group. An anatomy atlas (3) and a MRI atlas (4) were used to identify individual muscles and clearly define the boundaries of each muscle. Muscle volume was estimated from the CSA measured on 17 images taken at equal intervals along the length of the thigh with gap widths of 2.5 or 3 cm between measured images. This was based on the recommendations from Tracy et al. (5) who described the error associated with different gap intervals between measured slices. The inter-rater reliability for measuring muscle CSA was established between two investigators and found to be very high ( $r$ -

value = 0.99, mean percent error = 0.4%). This methodology was applied to further studies on people with COPD and lung transplant recipients in this thesis.

In addition to describing muscle atrophy, muscle volume was used to express concentric and eccentric torque measures per unit volume of muscle (i.e. normalize the torque measurement to muscle volume). Normalized torque measures were also applied to people with COPD and lung transplant recipients to examine differences in concentric and eccentric torque per unit volume between these groups and matched controls.

This case report also showed the presence of unilateral intramuscular fat infiltration in the rectus femoris muscle of a healthy adult with a knee injury. This led to a further investigation of intramuscular fat infiltration in people with COPD and lung transplant recipients in this thesis.

Although this study was a case report and has limited generalizability, it provided an opportunity to pilot a number of methods used in future studies for this thesis. Most importantly, it revealed the presence of intramuscular fat infiltration which was a unique finding following knee injury, and led to the investigation of fat infiltration of the quadriceps and hamstrings in people with COPD and lung transplant recipients.

### **8.1.3 Chapter Four: Skeletal muscle atrophy and intramuscular fat infiltration in people with COPD**

This study reported four unique findings in people with COPD: (1) intramuscular fat infiltration was found in the quadriceps and hamstrings in people with COPD and was greater than matched

controls; (2) compared to matched controls, absolute eccentric torque was less affected than concentric torque in people with COPD; (3) eccentric torque normalized to muscle volume was higher in people with COPD compared to matched controls and (4) individual quadriceps and hamstring muscles demonstrated uniform muscle atrophy in people with COPD compared to matched controls. Some of these findings were inconsistent with the first and second hypotheses, set forth in Chapter One: concentric and eccentric torques were affected to different extents in people with COPD and intramuscular fat infiltration did not appear to be related to muscle torque production. However, as hypothesized, muscle volume was lower in people with COPD and contributed to reduced torque production.

This study applied a novel imaging method, MRI, to quantify muscle atrophy and also intramuscular fat infiltration in people with COPD. As described previously, MRI is a non-invasive, low risk, imaging method that allows for the differentiation of tissues based on brightness or signal intensity. By using MRI, multiple slices along the length of the thigh could be captured to provide an estimate of muscle volume, rather than cross-sectional area at a single slice. Secondly, intramuscular fat infiltration could be quantified and compared between groups using MRI. No previous study has shown the presence of intramuscular fat infiltration in people with COPD, although this finding has been reported in people with type 2 diabetes, genetic skeletal muscle disorders and older adults (6-9). One limitation of the MR imaging sequence employed in this study was that we were unable to remove the volume of intramuscular fat within the epimysium from the total muscle volume. Therefore, volumes of the quadriceps, hamstrings and adductors included intramuscular fat, although this was likely a small proportion of the total muscle volume. Further studies may employ more advanced imaging sequences

which suppress the signal from fat to provide an estimate of absolute muscle volume and use this value to normalize muscle torque.

Eccentric torque has not previously been reported in people with COPD and provides a unique method of assessing muscle function in people with COPD. The finding that eccentric torque was less affected than concentric torque and that normalized eccentric torque was higher in people with COPD compared to matched controls suggests that there are some factors that help preserve torque in these people whereas other factors contribute to poor muscle function. This thesis further examined two factors that could account for preserved or impaired muscle performance in people with COPD: motor unit firing properties and cellular structure of skeletal muscle.

#### **8.1.4 Chapter Five: Surface EMG of the quadriceps during a fatiguing contraction in people with COPD**

The findings presented in Chapter Five provide support for the maintenance of motor unit firing properties in people with COPD, which may be one factor that contributes to the preservation of torque in these people. This study examined quadriceps muscle fatigue during a high level (80% of maximal voluntary contraction), isometric contraction. Endurance time was defined as the time the contraction could be held to the target level and provided an indication of local muscular endurance of the quadriceps. Surface EMG was used to examine the motor unit firing properties of the quadriceps muscle during a sustained isometric contraction held to fatigue, according to the techniques described in Chapter Two, and was found to be similar between people with COPD and matched controls. Although quadriceps endurance time was shorter in people with

COPD as hypothesized in Chapter One, Hypothesis 3, the ability to recruit and fire motor units appeared to be maintained contrary to the original hypothesis. The maintenance of this functional property of skeletal muscle may contribute to the preservation of torque in people with COPD.

Although this study suggests that motor unit firing properties are maintained in people with COPD during isometric contractions, these findings may not be generalizable to concentric and eccentric contractions. Future studies may employ EMG during concentric and eccentric contractions and compare the amplitude and frequency of the EMG signal in people with COPD compared to controls to determine whether the firing properties are similar. Furthermore, the interpolated twitch technique which stimulates the muscle directly, could be applied to determine whether there is adequate recruitment of motor units during dynamic contractions in people with COPD.

#### **8.1.5 Chapter Six: Cellular features of the vastus lateralis in people with COPD**

This study provided an examination of the cellular features of skeletal muscle structure at the light microscope level and looked specifically at features of skeletal muscle injury in biopsies of vastus lateralis. Skeletal muscle of the lower limbs in people with COPD may be susceptible to injury due to longstanding disuse and atrophy. This was the first study in people with COPD to quantify cellular abnormalities of skeletal muscle using the point counting technique.

The findings of this study are in line with the findings on muscle atrophy in people with COPD reported in Chapter Four. There was a greater area fraction ( $A_A$ ) of small angular fibers in people with COPD compared to matched controls. Although muscle fiber cross-sectional area was not

measured directly in this study, the small angular fibers that were observed are likely indicative of muscle cells that have undergone atrophy.

Another important finding of this study was the observation of increased connective tissue in the vastus lateralis in people with COPD. Although this structural difference in people with COPD may be seen as maladaptive, it may result in increased muscle stiffness and account for the preservation of eccentric torque in people with COPD. Due to methodological limitations in this study, a correlation between eccentric torque and  $A_A$  of connective tissue was not found. Other techniques such as the open muscle biopsy employed by Debigare et al. (10) to isolate muscle fiber bundles from the vastus lateralis in people with COPD may be used in a study of eccentric tension generation and muscle stiffness in-vitro, and provide a further understanding of the relationship between stiffness of the muscle fiber and the preservation of eccentric torque. A non-invasive technique, magnetic resonance elastography, has been applied in human skeletal muscle in-vivo to estimate stiffness of the biceps brachii muscle at rest and during an isometric muscle contraction (11). This may be a useful technique in examining stiffness of skeletal muscle in people with COPD.

#### **8.1.6 Chapter Seven: Skeletal muscle dysfunction in recipients of lung transplants: A comparison to people with COPD**

In this study, the findings on skeletal muscle structure and function in recipients of lung transplants were compared to those in people with COPD reported in the preceding three chapters. Lung transplant recipients undergo a period of low activity level prior to their transplant secondary to their respiratory condition. Immediately following the transplant, there is a period of bedrest and a slow return to physical activity. Furthermore, following the transplant,



patients are put on immunosuppressant drugs to reduce the risk of organ rejection (12). These drugs, particularly corticosteroids and calcineurin inhibitors, are known to have deleterious effects on skeletal muscle (13-16).

Together, these two factors may result in further impairments in skeletal muscle structure and function in transplant recipients compared to people with the pre-transplant condition, such as COPD, who do not undergo transplantation. Alternatively, following transplantation, the improvement in lung function leads to a reduction in symptoms, which may encourage transplant recipients to return to higher levels of physical activity than in their pre-transplant condition. This may counter some of the effects of inactivity and medications, and assist in the preservation of skeletal muscle structure and function.

The results of this study on lung transplant recipients showed a wide variation in the degree of skeletal muscle dysfunction observed however some unique findings are noteworthy. First, all transplant recipients demonstrated muscle atrophy compared to the control group, even though the control group was older than the transplant group. Previous studies have not provided specific measures of muscle size of individual muscles of the quadriceps and hamstrings in transplant recipients. Second, intramuscular fat infiltration was shown for the first time in lung transplant recipients. Third, concentric and eccentric skeletal muscle strength varied among the transplant recipients with some having very high torque and others having very low torques compared to people with COPD and controls. Lastly, although endurance time for the sustained quadriceps contraction was significantly shorter in transplant recipients than people with COPD and controls, amplitude and median frequency of surface EMG were similar among the three groups, indicating that motor unit firing properties appear to be intact in transplant recipients.

The small and diverse sample of lung transplant recipients is a limitation of this study.

Transplant recipients varied in their age, sex, pre-transplant diagnosis, type of transplant (single or double lung), time post-transplant and physical activity level, which are all factors that may have affected skeletal muscle. As the sample size was small, there were not enough subjects to explore the proportional contribution of various factors that may have accounted for differences between subjects. Another limitation of this study was that the control group was not closely matched to the transplant recipients in terms of age (the mean age of the control group was higher than the transplant group) and sex (there was a higher proportion of females in the control group compared to the transplant group). These baseline differences further emphasize the impairments in skeletal muscle function observed in the transplant group; that is, differences observed between the transplant group and a group of people who were older and had a greater proportion of females, are more profound than if there were seen compared to an age- and sex-matched control group. Secondly, not all people with lung transplant in this study had COPD as their pre-transplant condition; therefore comparisons of the pre- and post-transplant states are limited.

The results of this study show that there is a large degree of variation among transplant recipients in skeletal muscle structure and function, therefore the findings were not completely in line with the original hypothesis outlined in Chapter One. Few large studies have been done to examine skeletal muscle in lung transplant recipients however this may be useful in examining subgroups of transplant recipients differing in factors such as pre-transplant diagnoses, type of transplant, time post-transplant, age, sex and activity level, and dosage of immunosuppressant drugs to assist in determining what factors are important in skeletal muscle function post-transplant.

## **8.2 CONTRIBUTIONS TO THE FIELD OF STUDY**

This thesis makes the following novel contributions to the field of research examining skeletal muscle dysfunction in people with COPD and lung transplant recipients:

(1) Intramuscular fat infiltration of the quadriceps and hamstrings is greater in people with COPD compared to age, sex and BMI-matched controls. It is also observed in some recipients of lung transplants however a wide range exists among these people. Intramuscular fat infiltration can be quantified using T1-weighted MRI by measuring the skewness of the histogram of signal intensity generated from a region of interest.

(2) Absolute concentric and eccentric torque of the quadriceps and hamstrings are both lower in people with COPD compared to controls, however when torque is normalized to muscle volume, this difference is no longer observed. Eccentric torque shows a relative preservation compared to concentric torque in people with COPD and normalized eccentric torque is actually greater in people with COPD compared to matched controls. The relative preservation of eccentric torque is not observed in lung transplant recipients.

(3) The major muscle groups of the thigh (i.e. quadriceps, hamstrings and adductors) show uniform atrophy of approximately 35% in people with COPD and of approximately 30% in recipients of lung transplants compared to control. Atrophy was also uniform for individual muscles of the quadriceps and hamstrings and was consistent between biarthrodal muscles such as rectus femoris and uniartrodal muscles such as the vasti.

(4) People with COPD and lung transplant recipients demonstrate similar EMG amplitude and MF during a sustained isometric contraction of the quadriceps held to fatigue, despite a shorter endurance time for this task compared to controls. This provides evidence that the ability to recruit and fire motor units does not contribute to increased fatigability observed in the quadriceps muscle.

(5) Changes observed in the cellular structure of the vastus lateralis are consistent with muscle injury and may affect muscle function in different ways. Small, angular fibers may reflect muscle atrophy and contribute to reduced muscle function (i.e. loss of torque). The tendency for increased connective tissue may contribute to increased passive stiffness and assist in the preservation of eccentric torque.

In summary, this thesis provides evidence that although skeletal muscle dysfunction may be present in people with COPD, there are some factors that result in preserved muscle function in some situations such as in eccentric torque production.

### **8.3 DIRECTIONS FOR FUTURE RESEARCH**

Future research that stems from the findings in this thesis includes two major areas of investigation. First, an in-depth examination of eccentric muscle torque and the mechanisms that contribute to the preservation of eccentric torque compared to concentric torque in people with COPD is warranted. An examination of muscle stiffness including measurements of passive tension, presence of connective tissue and structural proteins within the sarcomere (e.g. titin) and

their relative contributions to eccentric torque production may provide some insights on the preservation of eccentric torque in people with COPD. The lower energy requirement and relative efficiency of eccentric compared to concentric contractions may provide a mechanism by which people with COPD can move more efficiently and also lead to a relative preservation of eccentric torque. Movement efficiency in people with COPD and their potential use of eccentric contractions to perform activities of daily living has not previously been studied and requires further examination.

Second, further studies on intramuscular fat infiltration in people with COPD are warranted. Future research should examine whether skeletal muscle satellite cells in human muscle have the potential to differentiate into adipose cells and what triggers this differentiation process. The volume of intramuscular fat should be measured using more sophisticated MRI protocols to allow for a further exploration of the relationship between fat infiltration and muscle tension generation. Also, studies examining the impact of intramuscular fat on glucose and fatty acid metabolism, mitochondrial function and insulin resistance are required to gain a better understanding of the implications on skeletal muscle metabolism in people with COPD.

#### **8.4 SUMMARY AND CONCLUSIONS**

This thesis examined both the structure and function of the quadriceps and hamstrings to address whether there are aspects of skeletal muscle structure and function that are impaired and whether some aspects are preserved in people with COPD and recipients of lung transplants. The findings of this thesis suggest that although people with COPD demonstrate muscle atrophy, intramuscular fat infiltration, poor muscle endurance and changes in cellular structure indicative

of muscle injury, they have a preservation of eccentric torque and show no impairment in motor unit firing properties during fatigue. Also, cellular changes which are often seen as maladaptive, such as increased connective tissue, may play a role in maintaining torque by contributing to passive tension. Lung transplant recipients show a wide range of changes in skeletal muscle structure and function which may be related to a number of pre-transplant and post-transplant factors.

Directions for future research that arise from this thesis include a further exploration of the mechanisms contributing to the maintenance of eccentric torque and for the deposition of fat within skeletal muscle.

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**Study Procedures:**

You will be required to come on four separate days. The total time required to participate in the study is about 5 hours. On the first day (Visit 1), you will perform a breathing test and an exercise test. For the breathing test, you will be required to take as deep a breath as possible, and then blow forcefully through the mouth into a tube to measure the airflow out of your lungs. For the exercise test, you will be required to pedal on a stationary bike as the resistance is gradually increased for 20 minutes or so, until you can no longer continue exercising at the higher levels of resistance. In order to measure your maximum exercise capacity, you will be encouraged to continue pedaling until shortness of breath or leg fatigue force you to stop pedaling. During the test, you will be required to breathe through a tube attached to a sterile mouthpiece on one end and a gas analyzer on the other. Electrodes will be placed on the skin over your heart to measure your heart rate and rhythm. Visit 1 should take about 80 minutes in total.

On Visit Two, the strength of your thigh muscles (quadriceps) will be tested. You will be seated in a chair with a seat belt. A padded lever will be placed against your shin. You will be asked to straighten your knee as strongly as possible against the lever. The test will take about 30 minutes in total.

On Visit Three, you will undergo a MRI (magnetic resonance imaging) exam of your thigh muscles. The MRI uses a magnet to visualize cross-sections of the body. You will be asked to remove any jewelry (rings, watches etc.) and articles of clothing that contain metal (belt buckles, zippers, metal buttons). If you have a pacemaker you will not be imaged, as the pacemaker cannot function in the magnetic field of the imager. You will be asked to lie on a table, which will then be moved automatically through the imager. You will be asked to lie still for a period of five to ten minutes at a time while the images are being taken. Some people may feel “closed in” during the MRI however there is a constant intercom with the technician and you may get out at any time. Visit 2 should take about 60 minutes in total.

On Visit Four, at least ten days after Visit One, a medical doctor will remove a small muscle sample in order to examine it under a microscope for possible causes of atrophy and weakness. A local anaesthetic will be used to numb a small area of skin and muscle on the side of your thigh muscle, midway between you knee and your hip. A tiny incision will be made and a 5mm sterile needle will be inserted to remove a small muscle sample (about 100mg, which is less than 0.01% of the total mass of the thigh muscle). The skin will be closed with Steri-Strips. You can remove the Steri-Strips seven days following the procedure. Your muscle tissue sample will be frozen and used only for the purposes of this study. If you choose to withdraw from the study, your tissue will be destroyed using standard procedures. Visit 4 should take about 30 minutes in total.

The exercise test and spirometry will be done at Vancouver Hospital. The MRI and muscle biopsy will be done at UBC Hospital. Quadriceps strength and endurance will be measured at G.F. Strong Rehab Research Lab.

**Exclusions:**

If you do not understand sufficient English to comprehend the informed consent form, you will be excluded from the study.

If you suspect that you may be pregnant, have any injury or disease of the knee or leg which may be worsened by exercise, any diseases of the heart or arteries, or clotting disorder, other lung conditions or previous lung surgeries, cancer, or have used steroids within the past 2 months, then you will be excluded from the study.

**Risks:**

There are minimal risks associated with testing procedures outlined in the study. During the exercise test on the cycle, there is a remote possibility (less than 1 in 10,000) of experiencing a heart attack or abnormal heart rhythm. Following the muscle strength test, you may experience some mild fatigue and soreness in the thigh muscle immediately afterwards but this should not last longer than 1 to 2 hours. Immediately following the muscle biopsy, you may feel some slight local discomfort but should not be incapacitated in any way. There is a slight possibility of infection with the muscle biopsy. There is a risk that you may feel "closed in" during the MRI procedure.

**Compensation of Injury**

Signing this consent form in no way limits your legal rights against the sponsor, investigators or anyone else.

**Benefits:**

An honorarium will be paid for your participation in the study. After completion of the study, you will be given access to the information from your tests and the study results if you request.

**Alternative Treatments:**

There are no alternative treatments to this study protocol. You do not have to participate in this study to receive treatment for your condition. Participation is voluntary.

**Confidentiality:**

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by Health Canada and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices.

**Contact:**

If you have any questions or desire further information with respect to this study, or if you experience any adverse effects, you should contact the principal investigator or co-investigators at the numbers listed on page 1 of this consent form. If you have any concerns about your treatments or rights as a research subject you may contact the Research Subject Information Line in the UBC Office of Research Services, 604-822-8598.

**Patient Consent:**

I understand that participation in this study is entirely voluntary and that I may refuse to participate or I may withdraw from the study at any time without any consequences to my continuing medical care.

I have received a copy of this consent form for my own records. I consent to participate in this study.

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Subject Name (Printed)

---

Subject Signature

Date

---

Witness Name (Printed)

---

Witness Signature

Date

---

Investigator's Name (Printed)

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Investigator's Signature

Date

**Study Procedures:**

You will be required to come on three separate days. The total time required to participate in the study is about 3 hours. On the first day (Visit 1), you will perform a breathing test and an exercise test. For the breathing test, you will be required to take as deep a breath as possible, and then blow forcefully through the mouth into a tube to measure the airflow out of your lungs. For the exercise test, you will be required to pedal on a stationary bike as the resistance is gradually increased for 20 minutes or so, until you can no longer continue exercising at the higher levels of resistance. In order to measure your maximum exercise capacity, you will be encouraged to continue pedaling until shortness of breath or leg fatigue force you to stop pedaling. During the test, you will be required to breathe through a tube attached to a sterile mouthpiece on one end and a gas analyzer on the other. Electrodes will be placed on the skin over your heart to measure your heart rate and rhythm. Visit 1 should take about 80 minutes in total.

On Visit Two, (at least four days after Visit One), the strength of your thigh muscles (quadriceps) will be tested. You will be seated in a chair with a seat belt. A padded lever will be placed against your shin. You will be asked to straighten your knee as strongly as possible against the lever. Following the strength test, you will undergo a MRI (magnetic resonance imaging) exam of your thigh muscles. The MRI uses a magnet to visualize cross-sections of the body. You will be asked to remove any jewelry (rings, watches etc.) and articles of clothing that contain metal (belt buckles, zippers, metal buttons). If you have a pacemaker you will not be imaged, as the pacemaker cannot function in the magnetic field of the imager. You will be asked to lie on a table, which will then be moved automatically through the imager. You will be asked to lie still for a period of five to ten minutes at a time while the images are being taken. Some people may feel "closed in" during the MRI however there is a constant intercom with the technician and you may get out at any time. Visit 2 should take about 80 minutes in total.

On Visit Three, at least ten days after Visit One, a medical doctor will remove a small muscle sample in order to examine it under a microscope for possible causes of atrophy and weakness. A local anaesthetic will be used to numb a small area of skin and muscle on the side of your thigh muscle, midway between your knee and your hip. A tiny incision will be made and a 5mm sterile needle will be inserted to remove a small muscle sample (about 100mg, which is less than 0.01% of the total mass of the thigh muscle). The skin will be closed with Steri-Strips. You can remove the Steri-Strips seven days following the procedure. Your muscle tissue sample will be frozen and used only for the purposes of this study. If you choose to withdraw from the study, your tissue will be destroyed using standard procedures. Visit 3 should take about 20 minutes in total.

The exercise test, spirometry, lung volumes and inspiratory muscle testing, MRI and anthropometry and muscle biopsies will be done at Vancouver Hospital. Quadriceps strength and endurance will be measured at G.F. Strong Research Lab.

**Exclusions:**

If you do not understand sufficient English to comprehend the informed consent form, you will be excluded from the study. If you suspect that you may be pregnant, have any injury or disease of the knee or leg which may be worsened by exercise, any diseases

of the heart or arteries, or clotting disorder, other lung conditions or previous lung surgeries, cancer, or have used steroids within the past 2 months, then you will be excluded from the study.

**Risks:**

There are minimal risks associated with testing procedures outlined in the study. During the exercise test on the cycle, there is a remote possibility (less than 1 in 10,000) of experiencing a heart attack or abnormal heart rhythm. During the exercise test, you may feel "closed in", which could cause some anxiety, or even a panic attack in people who have previously had panic attacks. Following the muscle strength test, you may experience some mild fatigue and soreness in the thigh muscle immediately afterwards but this should not last longer than 1 to 2 hours. Immediately following the muscle biopsy, you may feel some slight local discomfort but should not be incapacitated in any way. There is a slight possibility of infection with the muscle biopsy. There is a risk that you may feel "closed in" during the MRI procedure.

**Compensation of Injury**

Signing this consent form in no way limits your legal rights against the sponsor, investigators or anyone else.

**Benefits:**

An honorarium will be paid for your participation in the study. After completion of the study, you will be given access to the information from your tests and the study results if you request.

**Alternative Treatments:**

There are no alternative treatments to this study protocol. You do not have to participate in this study to receive treatment for your condition. Participation is voluntary.

**Confidentiality:**

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by Health Canada and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices.

**Contact:**

If you have any questions or desire further information with respect to this study, or if you experience any adverse effects, you should contact the principal investigator or co-investigators at the numbers listed on page 1 of this consent form. If you have any concerns about your treatments or rights as a research subject you may contact the Research Subject Information Line in the UBC Office of Research Services, 604-822-8598.

**Patient Consent:**

I understand that participation in this study is entirely voluntary and that I may refuse to participate or I may withdraw from the study at any time without any consequences to my continuing medical care.

I have received a copy of this consent form for my own records. I consent to participate in this study.

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Subject Name (Printed)

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Subject Signature

Date

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Witness Name (Printed)

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Witness Signature

Date

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Investigator's Name (Printed)

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Investigator's Signature

Date

## **APPENDIX B. ASSESSMENT OF MUSCLE MASS IN PEOPLE WITH COPD**

### **B.1 INTRODUCTION**

The assessment of muscle mass in people with COPD is gaining wider use in the clinical and research settings. Reduced fat free mass and this has been shown using dual energy x-ray absorptiometry (DEXA) and bioelectrical impedance in some groups of people with COPD (1, 2) however, others have reported that some subgroups of people with COPD have a normal fat free mass index (3-5). Fat free mass has been correlated to handgrip force (3, 4) and torque of the quadriceps and biceps (3). However, the measures of fat free mass used in these studies are for the whole body rather than a specific muscle group. To determine the amount of force that is generated per unit volume of muscle, more specific measures of muscle size are required. Computed tomography (CT) and magnetic resonance imaging (MRI) can provide specific measures of muscle cross-sectional area (CSA) (6). These tools also have the ability to discriminate muscle tissue from fat, bone and connective tissue, thereby providing accurate measures of muscle mass (6).

As specific measures of muscle mass such as CT and MRI are costly, it is important to determine whether a single slice can provide adequate information on muscle size compared to acquiring of multiple slices to estimate muscle volume. It has been suggested that muscle volume is a better measure of muscle mass for normalizing torque measures than CSA as it provides a better estimation of the physiological CSA of muscle (7). Physiological CSA takes into account the angle of fiber pennation, providing a better estimate of the number of sarcomeres in the muscle, therefore it is preferred to CSA for normalizing torque measures (8). Secondly, anthropometric

measures of limb circumference and skinfold provide a simple, inexpensive method of estimating muscle mass that can be used in the clinical setting (9, 10). This measurement may also be useful in estimating regional muscle mass when more sophisticated technologies are not available, such as in a pulmonary rehabilitation setting.

The relationship between muscle CSA and muscle volume using MRI and muscle CSA estimated using anthropometric measurements and MRI in people with COPD and matched controls, are presented in this Appendix.

## **B.2 METHODS**

### **B.2.1 Sample**

Twenty people with COPD and twenty age-, sex- and BMI-matched controls were included in this analysis. Sample characteristics have been previously described in Chapter Four.

### **B.2.2 Determination of muscle volume and cross-sectional area**

MRI was used to determine quadriceps and hamstrings muscle CSAs and volumes as previously described in Chapter Four. Quadriceps and hamstrings CSAs at the 50% of thigh length and hamstrings CSA at the 70% were determined. These levels were chosen as the 50% or mid-thigh level is commonly used in the literature to quantify thigh muscle CSA. The segment closer the knee (70% of thigh length) was chosen for the hamstrings as this represented the level where the hamstrings had their greatest CSA in our subjects. Muscle volumes of the RF and vasti were



summed to obtain total quadriceps volume; volumes of semitendinosis, semimembranosis, biceps femoris long head and short head were summed to obtain total hamstrings volume.

### **B.2.3 Anthropometric measurements**

Measures of thigh circumference and skinfold were made using the protocol established by Housh et al.(9). These measures were used to estimate the cross-sectional area of the quadriceps and hamstrings at 50% of thigh length.

### **B.2.4 Statistical Analysis**

Muscle CSA was correlated to muscle volume using Pearson's product moment correlation coefficients. The following descriptors were used to assess the strength of the correlations: low - 0.26 to 0.49; moderate - 0.50 to 0.69; high - 0.70 to 0.89 and very high - 0.90 to 1.00 (11). The Bland-Altman procedure was used to compare the anthropometric estimation of quadriceps and hamstring CSA (i.e the alternative measure) to the MRI measurement of CSA (i.e. the criterion measure). The Bland-Altman plot displays the difference between the alternative and criterion measure on the y-axis against the average of the two measures on the x-axis (12). The limits of agreement (i.e. mean  $\pm$  2 standard deviations) are constructed around the mean difference between the two measures. If the mean difference between the two measures is different from zero, there is either underestimation or overestimation of the criterion measure (12).

## **B.3 RESULTS**

### **B.3.1 Muscle cross-sectional area versus volume**

Muscle CSA at 50% of thigh length for the quadriceps had a very high correlation with quadriceps muscle volume ( $r = 0.964$ ,  $p < 0.001$ ), and CSA of the hamstrings at 50% had a high correlation with hamstrings volume ( $r=0.859$ ,  $p < 0.001$ ) (Figure B-1). CSA of the hamstrings at 70% had a comparable correlation to that found for quadriceps at 50%, to their respective muscle volumes ( $r=0.941$ ,  $p < 0.001$ ) (Figure B-1).

### **B.3.2 Muscle cross-sectional area estimated using anthropometric measures**

Using the Bland-Altman procedure, the level of agreement was between the estimation of quadriceps and hamstrings CSA using anthropometric measures and MRI was assessed (Figure B-2). The equation to estimate CSA from anthropometric measures has only been validated in males (9), so only male subjects were included in this analysis ( $n = 18$ ). The mean difference between the measurements as seen on the x-axis (Figure B-2) was negative for both the quadriceps and hamstrings showing that the equation tended to underestimate the actual muscle CSA compared to the MRI measurement. For the quadriceps, there appeared to be a greater underestimation using the equation in men who had large CSAs, however this trend was not observed in the hamstrings.

## **B.4 SUMMARY OF FINDINGS**

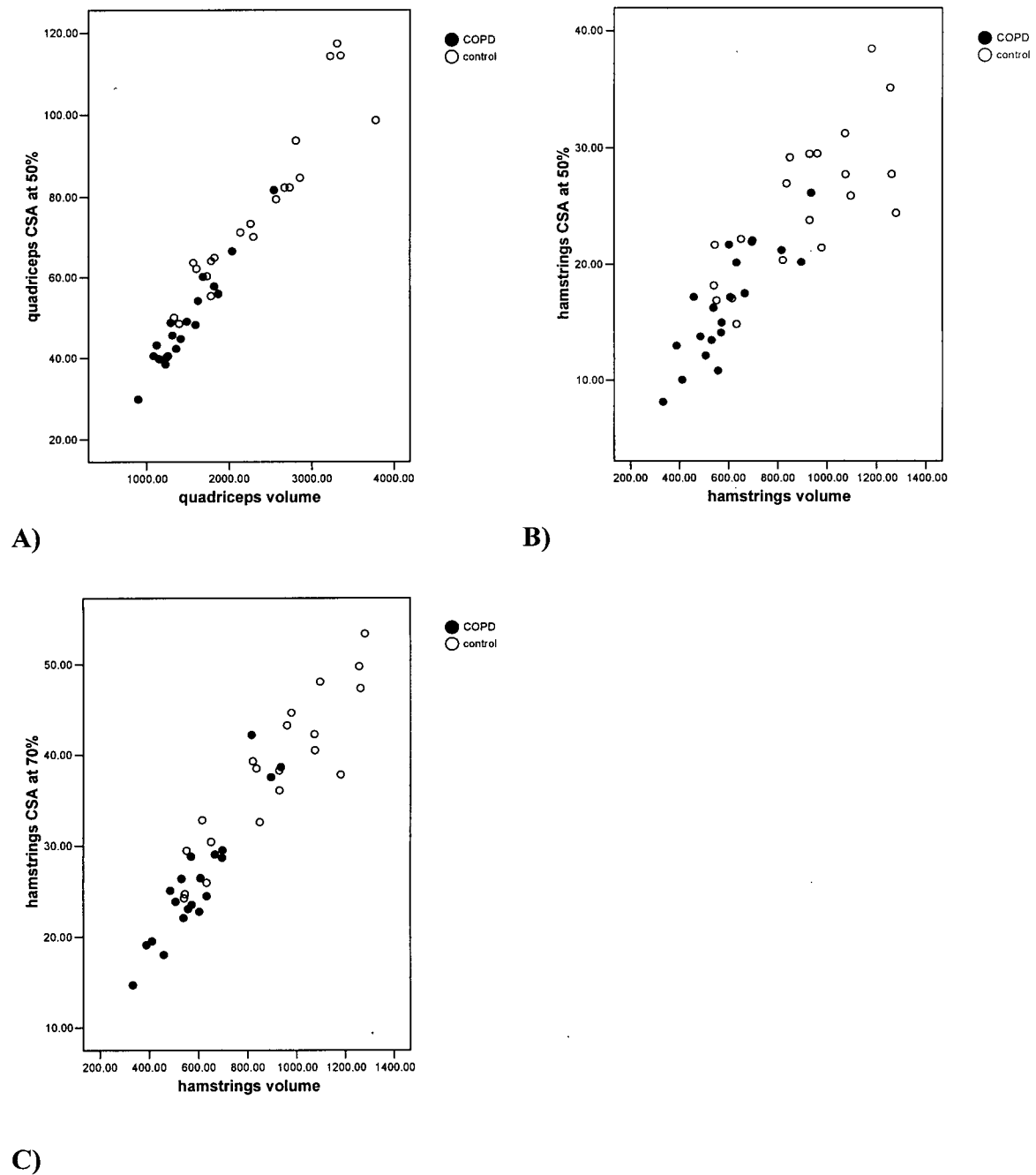
Muscle volume has been proposed to provide a better representation of muscle architecture by taking into account the pennation angle of the muscle fibers, the length and shape of the muscle (8). This may provide a better representation of the number of sarcomeres present in the muscle for tension generation than the CSA at a single slice and therefore be a better correlate to torque. Although muscle volume maybe preferred, multiple images are required to calculate volume and the greater the number of slices, the better the estimation of muscle volume (7). Acquiring more images increases the time and therefore the cost of the MRI and/or CT scan. It will also increase the exposure to radiation in the case of CT which poses greater risk to the participant.

We found that muscle volume was highly correlated to quadriceps and hamstrings CSA at 50% of thigh length however a better correlation was found between hamstring volume and its CSA at 70% of thigh length. At 70% CSA, the hamstrings reach their largest CSA and all four hamstring muscles are present whereas only three are present at the 50% level. Therefore if CSA of a muscle at a single slice is being used as an indicator of muscle size, it is important to determine the level at which the muscle of interest reaches its greatest CSA rather than simply using the 50% or mid-thigh level.

Anthropometric measurement is simple, inexpensive method to estimate muscle CSA of the quadriceps and hamstrings, as validated equations are available (9). However there are a number of assumptions that must be considered when using these measurements and these have been previously described (10, 13). In our study, the estimated CSA tended to underestimate the actual muscle CSA of the quadriceps and hamstrings and the underestimation for the quadriceps

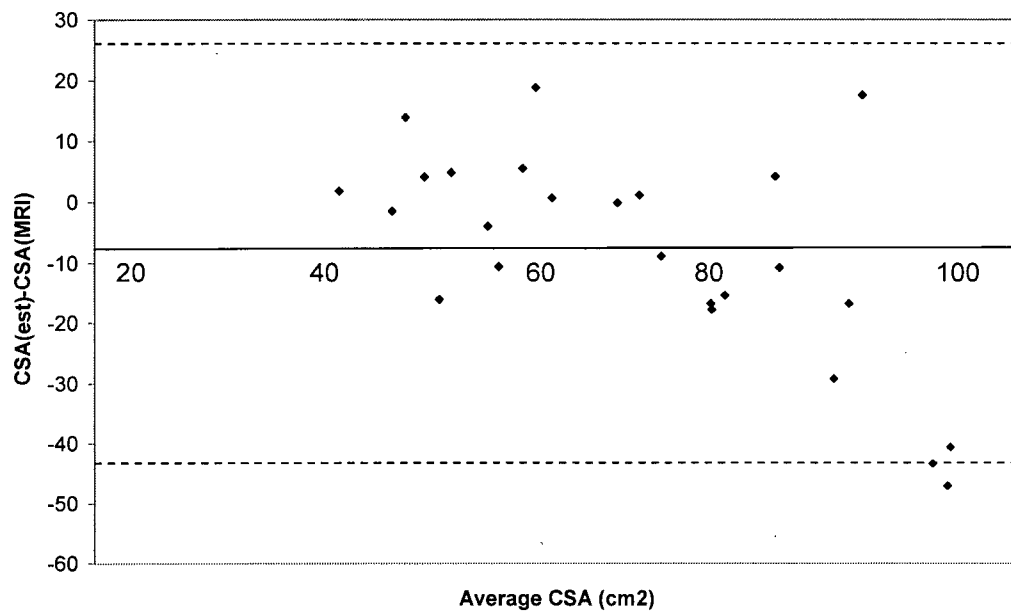
was greater in people with large CSAs. This is similar to the finding of Marquis et al. (14) who reported up to a 30% error in estimation of thigh muscle CSA compared to CT in people with COPD. The use of anthropometric measurements may be useful when more sophisticated techniques are not available, however the limitations of this method must be considered. In older people and in people with COPD, the use of these equations may be further violated as these people tend to have less lean mass and increased inter- and intramuscular fat leading to errors in estimation (15). The usefulness of anthropometric estimations of CSA in measuring change over time has been questioned, as they are not sensitive enough to monitor the improvements in muscle size observed with training (9). Therefore, they are best reserved for single assessments of muscle CSA.

## B.5 FIGURES

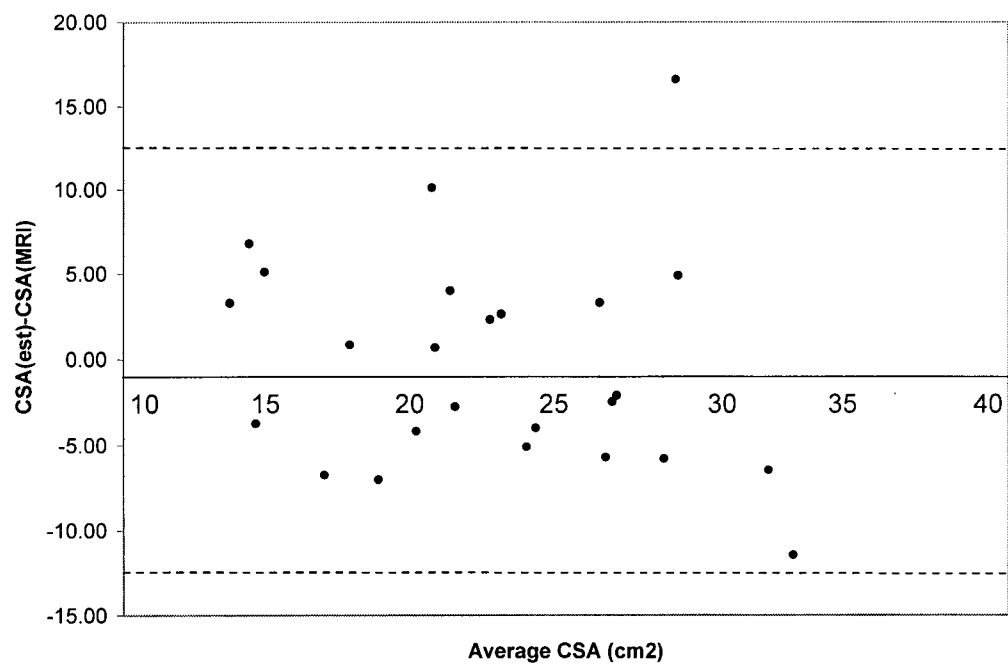


**Figure B-1.** Scatterplots depicting the relationship between muscle volume and cross-sectional area of **A)** the quadriceps at 50% or mid-thigh, **B)** the hamstrings at 50% and **C)** the hamstrings at 70% of total thigh length.

**Figure B-2.** Bland-Altman plots comparing cross-sectional area estimation from anthropometric measures [CSA(est)] to measurement from MRI [CSA(MRI)] of the **A)** quadriceps and **B)** hamstrings. Solid line represents the mean difference between measurements and dashed lines represent the two standard deviations above and below the mean difference.



A)



B)

## B.6 REFERENCES

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## **APPENDIX C. DETERMINATION OF INTRAMUSCULAR FAT INFILTRATION FROM MAGNETIC RESONANCE IMAGING**

### **C-1 INTRODUCTION**

Magnetic resonance imaging (MRI) provides an accurate, non-invasive method of quantifying intramuscular fat (1). It also poses little risk as it does not emit ionizing radiation (2). Unlike computed tomography, MRI does not provide an absolute value for tissue density to quantify muscle composition. However, fat infiltration results in elevated signal intensity in the T1-weighted MR image because fat infiltrated muscles have a shortened T1 relaxation time, resulting in brighter voxels (i.e. three-dimensional pixels)(1, 3). Fat infiltration of skeletal muscle has previously been quantified from simple spin-echo MR images using ranking scales to describe the signal intensity (4, 5), to more complex methods that calculate T1 relaxation times from multiple inversion recovery sequences (2), or scanning techniques that suppress the signal from fat e.g. short tau inversion recovery (STIR) (6, 7).

T1-weighted images have varying degrees of brightness or signal intensity, which can be used to distinguish tissues. Connective tissue such as tendon has very low signal intensity and appears black on MRI whereas fat appears very bright or white and skeletal muscle appears grey (see Figure C-1). For the purpose of this thesis, a method for analysing the signal intensity from the quadriceps and hamstrings muscles was developed to quantify intramuscular fat infiltration and to make comparisons between recipients of lung transplants, people with COPD and control subjects. A description of how the method of analysis was developed is provided in this

Appendix. Analysis was conducted using NIH Image, version 1.31 ([http://rsb.info.nih.gov/ij/Java 1.3.1\\_03](http://rsb.info.nih.gov/ij/Java 1.3.1_03)).

## **C.2 METHOD 1: MEAN SIGNAL INTENSITY FROM A REGION OF INTEREST**

The first method used to quantify intramuscular fat infiltration was to calculate the mean signal intensity for a region of interest (i.e. an individual muscle). As fat appears bright on the MRI and therefore has a high signal intensity, it was hypothesized that the mean signal intensity from a visibly fat infiltrated muscle would be higher than that of a homogenous muscle with little to no visible fat infiltration. The mean signal intensity for two quadriceps and two hamstring muscles at 50% of thigh length were calculated for four subjects and are shown in Table C-1. Two subjects had visible fat infiltration and two had dark grey, homogenous muscle (see Figure C-1). Although the mean signal intensities from the muscles of subjects A and B who had visible fat infiltration were higher than that for subjects C and D who had homogenous muscle, the differences were small (Table C-1). Furthermore, there were other factors that affected the validity of this method.

Absolute signal intensity values from conventional spin-echo MR images depend on a number of factors such as the individual's interaction with the magnetic field, heterogeneity of the magnetic field, coil positioning, tuning and receiver gain (1, 8). Therefore, the absolute signal intensity from a region of interest may differ between people even if the tissue has the same composition. This is further confounded by the heterogeneity of the magnet field which affects the signal intensity of a tissue within the same individual over multiple images.

The heterogeneity of the magnetic field was examined by circling areas of subcutaneous fat of the same size on the medial and lateral aspect of the thigh at 40%, 50%, 70% and 80% of thigh length on two subjects (subjects A and D). As seen in Figure C-2, subcutaneous fat, which was assumed to have the same composition on all aspects of the thigh for the same individual, showed different signal intensities depending on whether it was medial or lateral in the magnetic field. The pattern of where the fat was brighter was different for the two individuals that were examined, with one showing higher signal intensity on the medial aspect and the other showing higher signal intensity on the lateral aspect.

Secondly, there was heterogeneity depending on whether the images were closer or farther away from the centre of the magnetic field. As seen in Figure C-2, the images that were taken closer to the centre of the magnetic field (i.e. slices 2 and 3), were brighter than those taken further away from the centre of the magnetic field. Due to these limitations, the mean of absolute signal intensity from a region of interest could not be used to compare the degree of intramuscular fat infiltration between people.

### **C.3 METHOD 2: BINNING VOXELS BASED ON DISTRIBUTION OF SIGNAL INTENSITY**

The second method used to quantify intramuscular fat infiltration was to determine the range of signal intensities that represented fat, muscle and tendon, then bin the voxels from a region of interest into these categories. Fat, muscle and tendon have distributions of signal intensity that do not overlap, therefore the categories for each tissue are discrete. Regions of subcutaneous fat, homogenous muscle and the quadriceps tendon were circled on an image at 50% of thigh length

for two subjects (subjects A and D) to determine categories for each individual. The categories for each tissue are shown in Table C- 2. Fat has the highest signal intensity and falls within a narrow range, so this category ranged from its minimum signal intensity to its maximum signal intensity. Tendon also has a narrow range and has the lowest signal intensity, so this category ranged from its minimum signal intensity to its maximum signal intensity. Muscle has an intermediate signal intensity so its lower cutoff was set at the mean signal intensity for muscle minus two standard deviations and its upper cutoff was set at the maximum signal intensity for muscle. Lastly, a mixed category was defined for voxels that fell between the upper cutoff for muscle and the lower cutoff of fat and represented voxels that had a combination of muscle and fat.

It was hypothesized that a muscle with more visible fat infiltration would have a greater number of voxels in the category for fat or in the mixed fat/muscle category, than a homogenous muscle. The classification of rectus femoris muscle into categories of fat, tendon, muscle and mixed fat/muscle at 50% of thigh length is provided as an example (see Table C-3).

It was found that even in a muscle that showed lots of visible fat infiltration (i.e. subject D), very few voxels (0.2%) could be categorized as fat based on signal intensity and only 1.8% of voxels fell into the mixed fat/muscle category. In the person with homogenous muscle, no voxels were seen in the fat category and 0.9% of voxels fell in the mixed fat/muscle category. Therefore, this method did not appear to be sensitive enough to detect differences in intramuscular fat infiltration. Another limitation of this method was that categories would have to be determined for each image analysed, therefore would be inefficient for a large sample of subjects.

#### **C.4 METHOD 3: EXAMINING HISTOGRAMS OF VOXEL INTENSITY**

The third method used to describe intramuscular fat infiltration was to describe the distribution of signal intensity from a region of interest. This was done by plotting a frequency distribution of signal intensity for a region of interest and describing the skewness of the distribution in two ways: firstly, by calculating the interquartile range of signal intensity and secondly by calculating the coefficient of skewness. Interquartile range describes the number of voxels in the 25<sup>th</sup> to 75<sup>th</sup> percentile of signal intensity and provides a measure of the spread of the signal intensity (i.e. the range covered by the middle half of the data). Interquartile range can be displayed visually on a boxplot (see Figure C-3). As seen in Figure C-3, wider the interquartile range indicates a greater range of signal intensity from the muscle and reflects a greater degree of fat infiltration.

As a second method of examining the frequency distribution of signal intensity for a given muscle, is to calculate the coefficient of skewness to describe the histogram. The coefficient of skewness is a measure of the asymmetry of a distribution. The normal distribution is symmetric, and has a skewness value of zero whereas a distributions with significant positive or negative coefficients of skewness have a long right or left tail, respectively ((9)). A distribution that is skewed to the right represents a muscle with fat infiltration. frequency distributions of signal intensity and their coefficients of skewness for Subjects A and D are shown in Figure C-3.

Interquartile range and coefficient of skewness gave the best quantitative description of fat infiltrated muscle and also allowed for between-group comparisons to be made. Therefore, this method was chosen for the analysis of intramuscular fat infiltration in Chapters Four and Seven of the thesis.

## C.5 TABLES AND FIGURES

**Table C-1.** Mean signal intensity from individual muscles of four subjects. Subjects A and C and visibly homogenous muscle with little fat infiltration; Subjects B and D had fat infiltrated muscle.

Muscle	Subject A	Subject B	Subject C	Subject D
Rectus femoris	275	310	244	250
Vasti	275	286	256	265
Semitendinosis	274	291	238	254
Biceps femoris	279	278	238	239

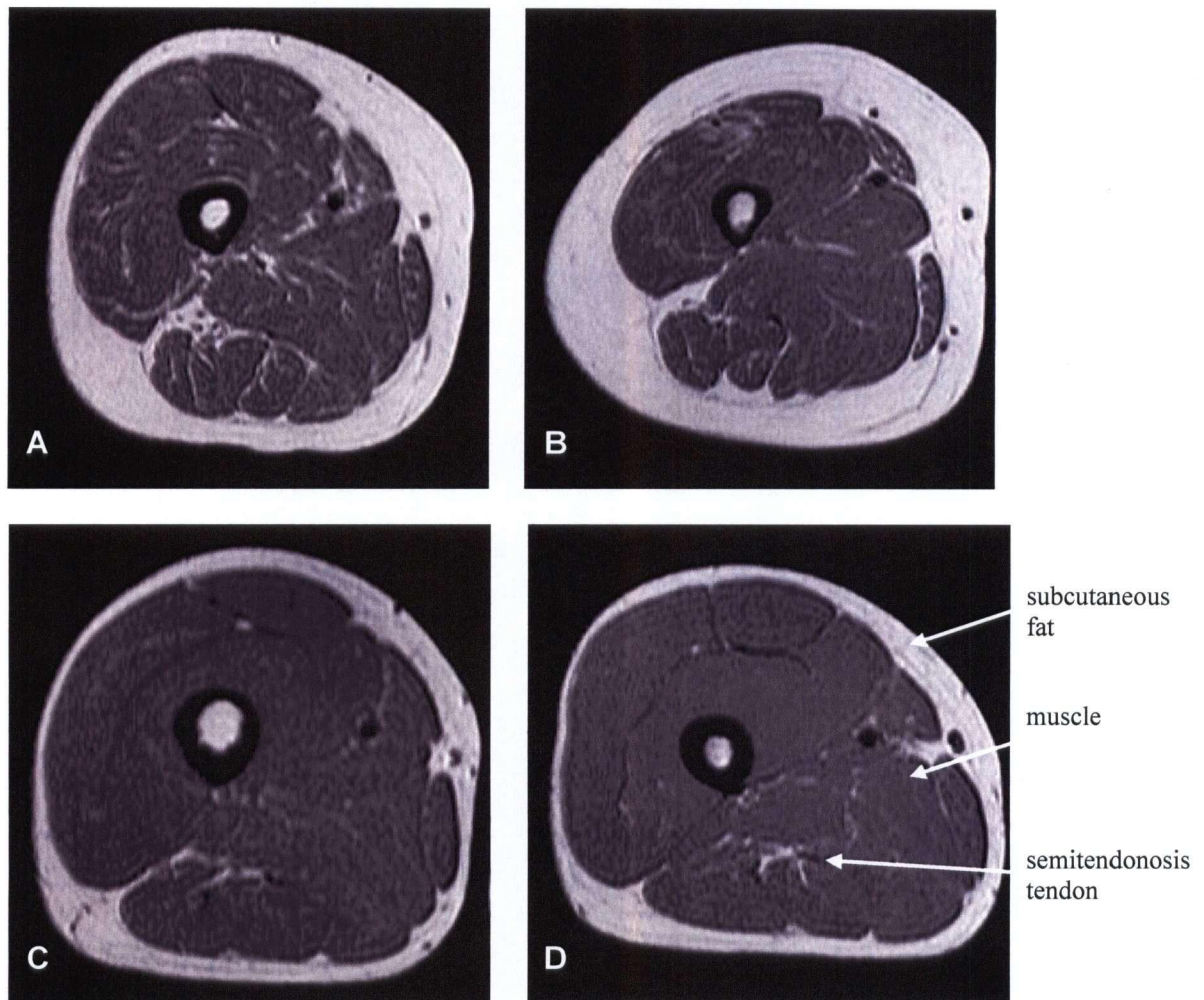
**Table C-2.** Classification of fat, muscle, tendon and mixed (fat/muscle) voxels based on signal intensity.

<b>Tissue</b>	<b>Lower Cutoff of signal intensity</b>	<b>Upper Cutoff of signal intensity</b>	<b>Subject A</b>	<b>Subject D</b>
Subcutaneous fat	minimum	maximum	566-825	579-719
Muscle	mean – 2 SDs	maximum	200-276	117-411
Tendon	minimum	maximum	0-82	0-131
Mixed (fat and muscle)	I > maximum for muscle	I < minimum for fat	277-565	412-578



**Table C-3.** Classification of rectus femoris muscle at 50% of thigh length into categories based on signal intensity.

<b>Subject</b>	<b>% of voxels in fat range</b>	<b>% of voxels in muscle range</b>	<b>% of voxels in tendon range</b>	<b>% of mixed voxels (fat/muscle)</b>
Subject A – visible fat infiltration	0.2%	97.6%	0.4%	1.8%
Subject D – homogenous muscle	0%	98.1%	0.6%	0.9%

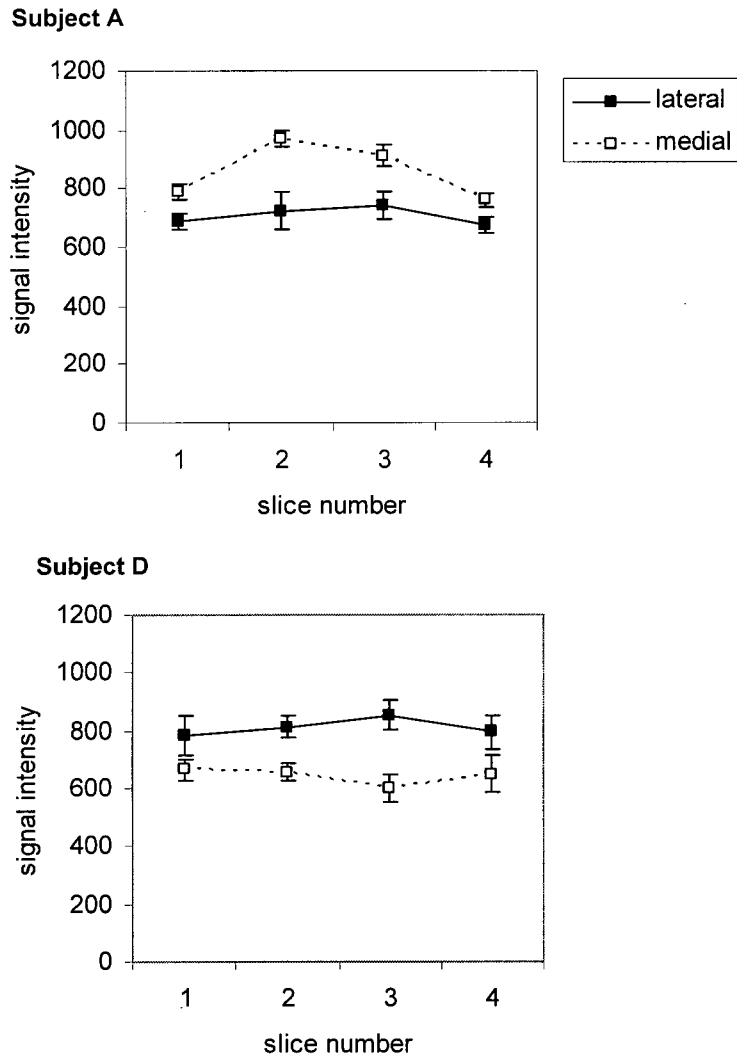


**Figure C-1.** Axial MRI of midthigh in four subjects (T-1 weighted, 1.5 Tesla).

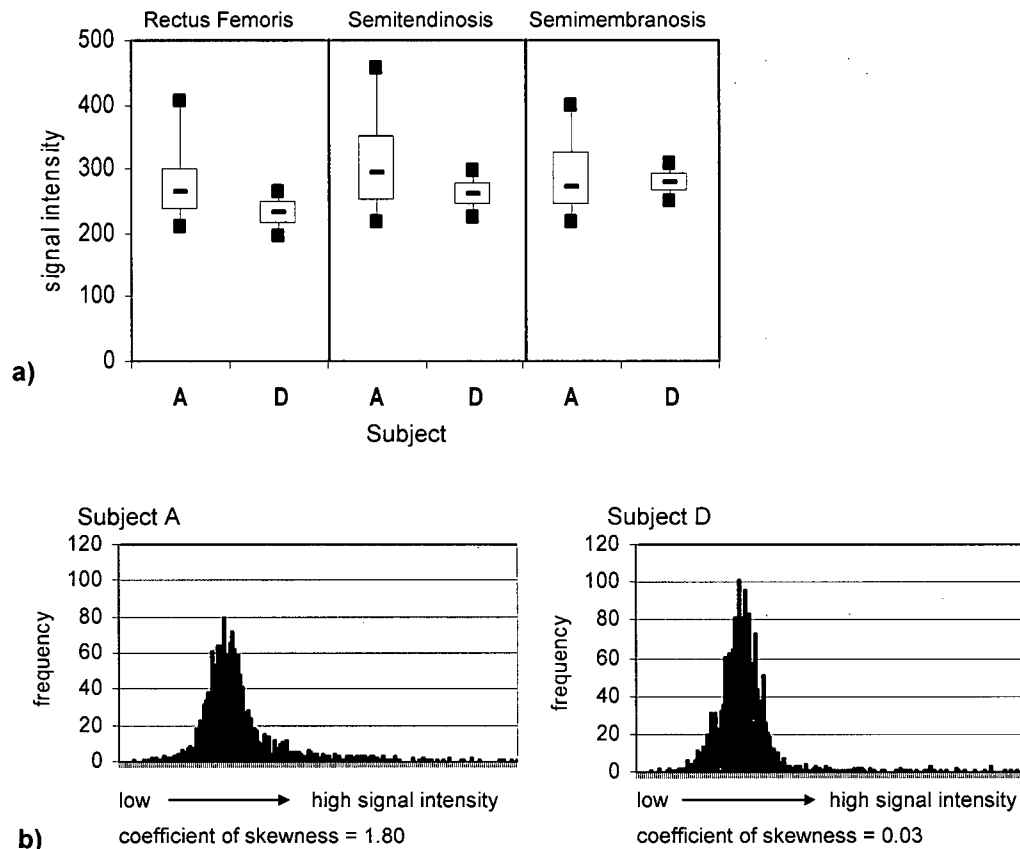
Subjects A and B show intramuscular fat infiltration of the thigh muscles, and

Subjects C and D show homogenous muscle with little to no fat infiltration.

Panel D shows the differing signal intensities for tissues: fat appears white, muscle appears grey and tendon appears black.



**Figure C-2.** Mean signal intensity ( $\pm$  standard deviation) of subcutaneous fat on the medial and lateral aspects of the thigh in Subjects A and D. Measures of signal intensity were taken at four slices along the length of the magnetic field. Slices 2 and 3 are closest to the centre of the magnetic field and Slices 1 and 4 are further from the centre of the magnetic field.



**Figure C-3. a)** Boxplot of signal intensity for three muscles (rectus femoris, semitendinosis and semimembranosis) for Subject A (fat infiltrated muscle) and Subject D (homogenous muscle). The box represents the interquartile range (25<sup>th</sup> to 75<sup>th</sup> percentile), the black bar represents the median and the whiskers represent the 10<sup>th</sup> and 90<sup>th</sup> percentile of signal intensity. Note that the interquartile range is wider for all three muscles in subject A who has fat infiltrated muscle, indicating a wide range of signal intensity. **b)** Frequency distribution of signal intensity for the rectus femoris muscle. Note that the coefficient of skewness for subject A who has visible fat infiltration in the rectus femoris is positive, indicating a right skewed distribution. The coefficient of skewness for the frequency distribution for Subject D who has homogenous muscle, is close to zero indicating a normal distribution.

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## **APPENDIX D: RELATIONSHIP BETWEEN ECCENTRIC TORQUE AND AREA FRACTION OF CONNECTIVE TISSUE IN PEOPLE WITH COPD AND CONTROLS**

### **D.1 DESCRIPTION OF FINDINGS**

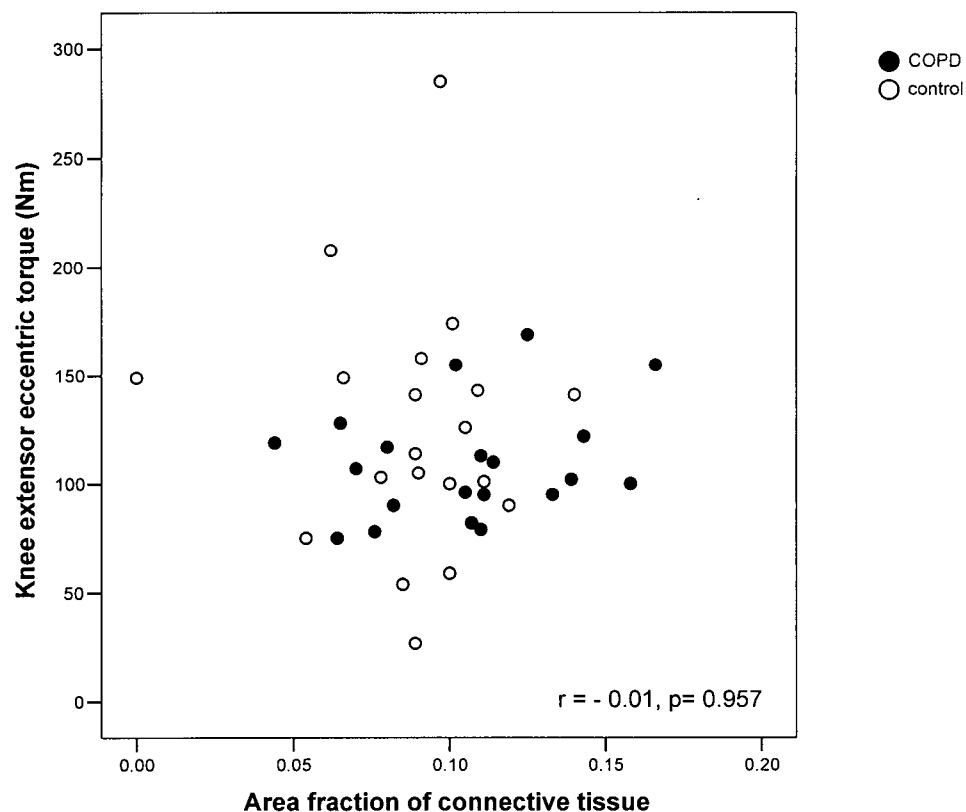
This Appendix describes the relationship between eccentric torque measured on a KinCom dynamometer, described in Chapter Four, and the area fraction ( $A_A$ ) of connective tissue measured from a biopsy of vastus lateralis, described in Chapter Six. Figure D-1 shows a scatterplot of knee extensor torque against area fraction of connective tissue in people with COPD and controls ( $n=40$ ).

A correlation between eccentric torque and  $A_A$  of connective tissue was not found in this study ( $r = -0.01$ ,  $p = 0.957$ ), however this may be due to some limitations in the measurement techniques employed. The needle biopsy provided a very small sample of muscle (less than 100 mg) which is less than 0.01% of the total quadriceps muscle mass. Therefore, it may not be representative of the entire muscle group. Also, once the sample was removed, visible adipose and connective tissue is teased away from the sample prior to mounting and freezing. Therefore some connective tissue on the periphery of the muscle biopsy may have been lost during preparation leading to an underestimation of the  $A_A$  of connective tissue. In regards to the sample preparation, a specific stain for collagen (e.g. picrosirius red) was not used in this study and may have led to an underestimation of the area of connective tissue in the muscle sections. Lastly, the measurement of eccentric torque was done using a voluntary contraction of the knee extensors in an in-vivo setting where other factors (e.g. joint mechanics, velocity of movement,

subject motivation) play a role in the torque output. These factors may have confounded the relationship between the measured eccentric torque and the area fraction of connective tissue.



**D-2. FIGURE**



**Figure D-1.** Scatterplot of knee extensor eccentric and area fraction of connective tissue in the vastus lateralis muscle of people with COPD and matched controls (n=40).