Skeletal Muscle & Gait Analysis in Chronic Heart Failure: New Insights into Impaired Exercise Capacity

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This thesis is presented for the degree of Doctor of Philosophy at The University of Western Australia

The School of Sport Science, Exercise and Health
July, 2014
Abstract

Chronic heart failure (CHF) is amongst the most profound health burdens in Australia and worldwide. It is characterized by severe disability that impacts tasks of daily living and is associated with extreme fatigue. Indeed, CHF is one of the most common reasons for hospital admission and general practitioner consultations in the elderly and it is estimated that CHF accounts for >$1 Billion in annual health care costs in Australia ($34 Billion annually in the USA).

Low exercise capacity is a strong independent predictor of prognosis in CHF and used clinically to stratify patients for transplantation and ventricular assist device implantation. However, development of optimal exercise-based treatment to improve functional capacity in CHF is hampered by a lack of understanding of the fundamental mechanisms underpinning exercise intolerance. In many cases, the cardiac function is not directly associated with exercise capacity in CHF and growing evidence points to an important role for skeletal muscle in dictating disease severity and the progression. The majority of studies focusing on skeletal muscle in CHF have either addressed gross physiological function (e.g. muscle mass in relation to exercise capacity) or skeletal muscle histopathology. Surprisingly, few studies of CHF have examined in vivo muscle-level morphology and functional properties and their impact on locomotor biomechanics, despite the known association between these variables in other populations. Therefore, the general aims of the present thesis were: 1) to study in vivo morphological and mechanical properties of skeletal muscle in CHF patients and 2) to investigate how these properties impact on exercise and functional walking capacity. The ultimate goal was to enhance the evidence base for rehabilitation of individuals with CHF.
Study 1 used novel 3D ultrasound imaging techniques to examine detailed muscle-level morphology of the calf muscles in CHF. These muscles were the focus of experimentation because they represent the main source of mechanical work during walking and they are amenable to dynamic *in vivo* imaging. This chapter also examined the relationship between muscle morphology, strength and exercise capacity. Despite a similar overall lean lower limb mass compared to age- and exercise level-matched control participants (CON), CHF patients exhibited ~25% lower combined triceps surae volume and physiological cross-sectional area (PCSA). These reductions in triceps surae size were explained predominantly by the marked reductions in the soleus (SOL). Furthermore, SOL volume, unlike the other triceps surae muscles, correlated strongly with peak oxygen uptake (peak) in CHF. This, together with a strong correlation between plantarflexor strength and peak suggests, for the first time, that the SOL may be a sentinel skeletal muscle in terms of identifying the severity of functional impairment in CHF.

Study 2 investigated how the reduction in the size of the SOL (Study 1) impacts active voluntary force and passive force in CHF. This represents the first *in vivo* functional analysis at an individual skeletal muscle level undertaken in CHF. The smaller SOL of CHF patients resulted in lower peak active (voluntary) and peak passive forces compared to CON. Contrary to our hypothesis, this difference was not present when forces were normalized by SOL PCSA. This detailed muscle level analysis, in contrast to some earlier analyses of joint strength, indicates that reduced muscle force production in CHF is strongly associated with muscle size. This analysis also showed that SOL optimal fascicle lengths (length where peak force is achieved) is shorter in CHF, suggesting that a loss of muscle mass may in part be due to the loss of serial sarcomere
numbers. Exercise to restore muscle lengths (e.g. eccentric exercise) may thus be particularly beneficial in CHF.

Study 3 consisted of a detailed biomechanical inverse dynamic analysis of gait to understand how the differences in the skeletal muscle properties reported in the previous two studies impact on functional walking capacity in CHF. Surprisingly, no difference in preferred speed or overall limb kinematics and kinetics between groups were found, possibly explained by an optimization of the mechanical cost of transport (the amount of mechanical work performed per distance traveled; J/kg/m). Nevertheless, when normalized to triceps surae volume, over two times greater ankle plantarflexion work was required in CHF. This, together with a greater reliance on the ankle to power walking in CHF patients compared to CON, may be a primary cause of their earlier onset of fatigue.

Together, these studies offer a novel examination of the mechanisms explaining the reduced exercise and walking capacity in CHF, using novel combinations of ultrasound imaging and biomechanical modeling not previously applied to study CHF. The results suggest that the SOL is a key muscle in CHF that is particularly affected by muscle wasting and strongly linked to peak aerobic capacity. This raises the possibility of using SOL morphology as an estimate of peak in circumstances where peak is not easily measurable due to lack of resources or trained staff. It also points to a causal link between the morphological and functional alterations of the calf muscles and fatigue during walking in CHF. Thus, improving calf muscle condition, specifically muscle size, may prove important in ameliorating the limitations to exercise and walking capacity in CHF. These findings provide a scientific basis for the design of optimized rehabilitation strategies in CHF, with the specific recommendation to include ankle/calf
based training (or offloading) as an important part of whole-body exercise rehabilitation.
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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>°C</td>
<td>Degree celsius</td>
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<tr>
<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>3DUS</td>
<td>Three-dimensional ultrasound</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>CHF</td>
<td>Chronic heart failure</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>cos</td>
<td>Cosine</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross-sectional area</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual energy X-ray absorptiometry</td>
</tr>
<tr>
<td>EF</td>
<td>Ejection fraction</td>
</tr>
<tr>
<td>eGRF</td>
<td>Estimated glomerular filtration rate</td>
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<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>Hz</td>
<td>Hertz</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>l</td>
<td>Liter</td>
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<tr>
<td>LG</td>
<td>Lateral gastrocnemius</td>
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<tr>
<td>m</td>
<td>Meter</td>
</tr>
<tr>
<td>MG</td>
<td>Medial gastrocnemius</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
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<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>mmol</td>
<td>Millimole</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>ms</td>
<td>Millisecond</td>
</tr>
<tr>
<td>N</td>
<td>Newton</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York health association</td>
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<tr>
<td>PCSA</td>
<td>Physiological cross-sectional area</td>
</tr>
<tr>
<td>r</td>
<td>Correlation coefficient</td>
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</table>
S.D. Standard deviation
s Second
SOL Soleus
T Tesla
V Volt
\( \dot{V}O_2 \) peak Peak oxygen uptake
yr Year

### Chapter 4

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>3D</td>
<td>Three-dimensional</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>CHF</td>
<td>Chronic heart failure</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
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<tr>
<td>cos</td>
<td>Cosin</td>
</tr>
<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>ES</td>
<td>Effect size</td>
</tr>
<tr>
<td>( F_a )</td>
<td>Peak voluntary active force</td>
</tr>
<tr>
<td>( F_p )</td>
<td>Passive force</td>
</tr>
<tr>
<td>F-L</td>
<td>Force-length</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>( k_1 )</td>
<td>Stiffness in the first part of the passive force-length curve</td>
</tr>
<tr>
<td>( k_2 )</td>
<td>Stiffness in the second part of the passive force-length curve</td>
</tr>
<tr>
<td>( k_{1\text{norm}} )</td>
<td>Stiffness in the first part of the normalized passive force-length curve</td>
</tr>
<tr>
<td>( k_{2\text{norm}} )</td>
<td>Stiffness in the second part of the normalized passive force-length curve</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>L</td>
<td>Fascicle length</td>
</tr>
<tr>
<td>( L_0 )</td>
<td>Fascicle length during maximal voluntary contraction of plantarflexors</td>
</tr>
<tr>
<td>( L_{\text{max}} )</td>
<td>Fascicle length at maximum length</td>
</tr>
<tr>
<td>( L_{\text{rest}} )</td>
<td>Fascicle length at neutral angle</td>
</tr>
<tr>
<td>( L_{\text{slack}} )</td>
<td>Passive fascicle slack length</td>
</tr>
<tr>
<td>LG</td>
<td>Lateral gastrocnemius</td>
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<td>m</td>
<td>Meter</td>
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</table>
Moment contribution from co-contraction of the dorsiflexors
$M_{dorsi}$ Net passive ankle joint moment
$M_p$ Peak net ankle joint moment
$M_{peak}$ Moment generated by the plantarflexors
$M_{plant}$ Medial gastrocnemius
MG MegaHertz
MHz Milliliter
ml
$M_{V_{pl}}$ Maximal voluntary isometric plantarflexion contractions
$\Delta M_p$ Difference in the estimated passive soleus moment
N Netwon
N Maximal voluntary isometric plantarflexion contractions
PCSA Physiological cross-sectional area
r Achilles moment arm
s Second
SOL Soleus
TA Tibialis anterior
Vol Volume

Chapter 5

<table>
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<td>Analysis of variance</td>
</tr>
<tr>
<td>CHF</td>
<td>Chronic heart failure</td>
</tr>
<tr>
<td>COT</td>
<td>Cost of transport</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
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<td>J</td>
<td>Joule</td>
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<tr>
<td>kg</td>
<td>Kilogram</td>
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<tr>
<td>m</td>
<td>Meter</td>
</tr>
<tr>
<td>$M_{jpt}$</td>
<td>Net joint moment across the individual joint axes</td>
</tr>
<tr>
<td>MANOVA</td>
<td>Multivariate analysis of variance</td>
</tr>
<tr>
<td>MHz</td>
<td>MegaHertz</td>
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<td>min</td>
<td>Minute</td>
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<tr>
<td>Symbol</td>
<td>Definition</td>
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<td>------------</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>$P_j$</td>
<td>Net power across all three planes of the individual joint</td>
</tr>
<tr>
<td>$P_{ji}^+$</td>
<td>Positive power at the $i^{th}$ joint</td>
</tr>
<tr>
<td>$P_{j\text{pl}}$</td>
<td>Instantaneous power in the individual joint planes</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>$t_1$</td>
<td>Instant of the first heel strike</td>
</tr>
<tr>
<td>$t_2$</td>
<td>Instant of the second heel strike (or toe off)</td>
</tr>
<tr>
<td>$\dot{V}O_2$ peak</td>
<td>Peak oxygen uptake</td>
</tr>
<tr>
<td>yr</td>
<td>Year</td>
</tr>
<tr>
<td>$\omega_{j\text{pl}}$</td>
<td>Joint angular velocity across the individual joint axes</td>
</tr>
<tr>
<td>$W_{ji}^+$</td>
<td>Positive work at the $i^{th}$ joint</td>
</tr>
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</table>
Acknowlegments

Adesso che il lavoro è concluso e dopo tutti gli sforzi profusi per arrivare al traguardo, mi guardo indietro e vedo la strada fatta per arrivare ad ultimare questa tesi. E allora penso che sia giusto ringraziare chi mi ha aiutato in questo percorso e farlo con le mie parole.

Il primo grazie va innanzitutto a Jonas che mi ha accompagnato in ogni fase di questo cammino. Ho dei bellissimi ricordi del tempo speso nell'organizzazione degli esperimenti e degli incontri per discutere dei risultati e degli sviluppi futuri. Questo lavoro non sarebbe stato lo stesso senza la tua guida. Grazie anche a David e Danny per la supervisione fornita a questo progetto. Ho imparato molte cose da voi che spero faranno parte del mio bagaglio di ricercatore; grazie per i vostri insegnamenti e spero che potremo collaborare ancora in futuro.

Grazie a tutti i ricercatori e gli studenti che hanno collaborato al progetto, con i quali è stato un piacere lavorare, per il tempo dedicato che hanno dedicato a questo studio. Un sentito grazie anche a tutte le persone che fanno parte della SSEH: il personale tecnico-amministrativo che è sempre stato molto disponibile nei miei confronti e tutti i dottorandi con cui ho passato dei bei momenti durante questa avventura. Un ringraziamento speciale a Luqman e Christian che hanno condiviso questo percorso quotidianamente con me, tra alti e bassi, tra gioie e dolori. In questi anni non sono solo stati colleghi ed amici, ma hanno anche costituito la mia famiglia australiana.

Ringrazio tutti i partecipanti che hanno donato il loro tempo per i miei studi, senza i quali niente di tutto quello che è stato fatto sarebbe stato possibile. Grazie a tutti i miei amici e colleghi di oltreoceano, Nicola, Luca, Giuseppe e Massimo, per l'aiuto e per i continui incoraggiamenti che mi hanno fatto sentire in questi anni.

Da ultimo, un pensiero va alla mia famiglia a cui dedico questo lavoro. Grazie per avermi costantemente supportato e per la vicinanza che mi fate sentire sempre. Spero che recupereremo presto il tempo passato stando lontani.
Declaration

I declare this thesis is my own composition, all sources have been acknowledged and my contribution is clearly identified in the thesis. For any work in the thesis that has been co-published with other authors, I have permission of all co-authors to include this work in my thesis.

Statement of Contribution

This thesis contains published work and/or work prepared for publication, some of which has been co-authored. The bibliographical details of the work and where it appears in the thesis are outlined below. A statement for each publication that clarifies the contribution of the student to the work is provided.

First author papers submitted/published by the candidate based on the contained in this thesis

Study 1 - Chapter 3


Candidate contribution:

Planning. Review of the literature, development of testing protocols, contribution to formulation of hypothesis and study design.

Data collection. Recruitment and pre-screening of control subject; coordination and management of all the testing sessions, conduction of all the testing sections at UWA, assistance of the testing sections at RPH (peak) and at Envision (MRI scans).

Data analysis. Setup of a system for the reconstruction of muscle volume from 2D ultrasound images, digitizing and segmentation of ultrasound images (ImageJ and Stradwin), segmentation of MRI data (Mimics), data analysis and processing, conduction of statistical analysis (SPSS), organization of meeting for discussion and data interpretation.

Manuscript. Preparation of manuscript draft, preparations of tables and figures, assistance with responses to reviewer comments.
Study 2 - Chapter 4


Candidate contribution:

Planning. Review of the literature, development of testing protocols, contribution to formulation of hypothesis and study design.

Data collection. Recruitment and pre-screening of control subject, coordination and management of all the testing sessions, conduction of all the testing sections.

Data analysis. Design and realization of the software used for analysis of active and passive forces (Opensim and Matlab), digitising of ultrasound images (ImageJ), analysis and processing of all the data, conduction of statistical analysis (SPSS), organization of meeting for discussion and data interpretation.

Manuscript. Preparation of manuscript draft, preparations of tables and figures.

Study 3 - Chapter 5


Candidate contribution:

Planning. Review of the literature, development of testing protocols, contribution to formulation of hypothesis and study design.

Data collection. Recruitment and pre-screening of control subject, coordination and management of all the testing sessions, conduction of all the testing sections at UWA, assistance of the testing sections at RPH (peak).

Data analysis. Design and realization of the software used for 3D gait analysis assessment (Opensim and Matlab), analysis and processing of all the data, conduction of statistical analysis (SPSS), organization of meeting for discussion and data interpretation.

Manuscript. Preparation of manuscript draft, preparations of tables and figures.

 Candidate contribution

Planning. Review of the literature, development of testing protocols, contribution to formulation of hypothesis and study design.

Data collection. Recruitment and pre-screening of older subjects, coordination and management of their testing sessions, conduction of all the testing sections involving older subjects at UWA.

Data analysis. Analysis and processing of older subjects data, conduction of statistical analysis (SPSS), organization of meeting for discussion and data interpretation.

Manuscript. Preparation of manuscript draft, preparations of tables and figures, assistance with responses to reviewer comments.

Oral communications


Poster communications

Panizzolo FA, Maiorana AJ, Naylor LH, Dembo L, Lloyd DG, Green DJ, Rubenson J. Mechanical work at the ankle: a limit to walking capacity in chronic heart failure? Presented at the VII World Congress of Biomechanics, 6-11 July 2014, Boston, USA.

Panizzolo FA, Maiorana AJ, Green DJ, Lloyd DG, Rubenson J. Gait analysis in chronic heart failure patients points to the calf as the source of reduced functional capacity. Presented at the XXIV International Society of Biomechanics, 4-9 August 2013, Natal, Brazil.

Saxby DJ*, Panizzolo FA*, Modenese L, Dunne JJ, Rubenson J, Lloyd DG. Effects of different scaling methods on Opensim model fidelity. Presented at the XXIV International Society of Biomechanics, 4-9 August 2013, Natal, Brazil. *Authors contributed equally.

Panizzolo FA, Green DJ, Lloyd DG, Rubenson J. Soleus fascicle strain is matched in young and old adults at the preferred walking speed. Presented at the XXIII International Society of Biomechanics, 3-7 July 2011, Brussels, Belgium.
"Fatti non foste a viver come bruti ma per seguir
virtute e canoscenza"

"Ye were not form’d to live the life of brutes, but
virtue to pursue and knowledge high"

Dante Alighieri
General Introduction
Chronic heart failure (CHF) is a disease characterized by inability of the heart to pump enough blood to fulfill the body's demands. CHF represents a profound health burden; in Australia alone there is an estimate of 300,000 patients affected and up to 30,000 new diagnoses each year (Australian Institute of Health and Welfare, 2003). Mortality rates among diagnosed CHF patients are dramatically high, with 30 to 40% of patients in the USA dying within one year after receiving the diagnosis (McMurray and Pfeffer, 2005). It is one of the most common reasons for hospital admission for the elderly and ranks among the most costly of any disease, with over $1B annual cost in Australia (Clark et al., 2004) and greater than $30B annual cost in the USA (Lloyd-Jones et al., 2010).

The CHF syndrome results from a large variety of causes, such as injury to the myocardium, ischemic heart disease, hypertension, and diabetes (Kemp and Conte, 2012). Two of the most striking characteristics exhibited by CHF patients are a low exercise capacity and extreme fatigue during exercise (Clark et al., 1996). Importantly, aerobic exercise capacity ($\dot{V}_{O2}\text{peak}$) is amongst the best prognostic indices for CHF (Mancini et al., 1991). Therefore, if our goal is to enhance function for patients suffering with CHF, there is pressing need to further investigate the mechanisms responsible for reduced exercise capacity and high fatigability exhibited by subjects with CHF. This knowledge will serve to inform evidence-based exercise rehabilitation management that, together with traditional pharmacological treatment, can optimize CHF care.

Although impaired cardiac output initiates the syndrome of CHF, it is widely accepted that exercise capacity and cardiac function are poorly correlated (McKelvie et al., 1995). Previous studies have suggested that peripheral factors (skeletal muscle), as opposed to cardiac factors, play a key role in dictating the progression and the severity
of the disease and the limits to peak aerobic capacity. Recognition of the importance of peripheral abnormalities in CHF led to the “muscle hypothesis” of CHF (Clark et al., 1996), which proposes that activation of muscle ergoreceptors generates symptoms and contributes, via reflex mechanisms, to further neurohormonal activation, peripheral vasoconstriction, cardiac burden and consequent disease progression.

Previous research investigating skeletal muscle abnormalities in CHF has mainly focused on either integrated gross physiological function (e.g. muscle mass vs. peak) (Fülster et al., 2013; Minotti et al., 1991) or histopathological analysis of muscle (Sullivan et al., 1990), reporting differences in muscle size, metabolism and microstructure and function. Despite this growing evidence of skeletal muscle limitations in CHF, no studies to date have investigated the relevance of in vivo skeletal muscle morphological and mechanical properties in CHF patients, nor the effects that these limitations might have on whole body biomechanical and energetic function.

Recent advances in ultrasound imaging technology and computational musculoskeletal modeling have allowed human in vivo muscle morphology and mechanics to be explored. These technologies include non-invasive experimental measurements of muscle and tendon structure and their mechanics during gait (Cronin and Lichtwark, 2013), as well as model predictions of their function (Delp et al., 2007; Erdemir et al., 2007; Lloyd and Besier, 2003). Studies applying these technologies to investigate musculoskeletal properties have been conducted on healthy (Hodges et al., 2003; Kwah et al., 2013) and clinical populations, including patients affected by cerebral palsy (Barber et al., 2011; Fry et al., 2004), Parkinson (Yucel and Kusbeci, 2010), Duchenne dystrophy (Jansen et al., 2011), chronic obstructive pulmonary disease (Menon et al.,
Chapter 1 - General Introduction

2012). However, no previous studies in humans have applied these techniques to muscle abnormalities and subsequent biomechanical malfunction in CHF.

Therefore, the aim of the first study of this thesis was to characterize both the overall lower limb lean mass and the detailed morphology of the triceps surae and Achilles tendon in CHF, and to establish their relationship with exercise capacity and muscle strength. The triceps surae was chosen because it is amenable to *in vivo* imaging (Herbert et al., 2011; Maganaris et al., 1998) and it represents an important muscle group in the mechanics of locomotion (Hof et al., 2002). Plantarflexors, indeed, offer a highly relevant comparison between synergist muscles known to possess different fiber types (Gollnick et al., 1974) and they constitute the main source of power during walking (McGowan et al., 2009).

**Hypothesis study 1.** It was hypothesized that the different muscles of the triceps surae would be affected by muscle wasting in a non-uniform way, due to differences in their function and fiber type. Also tested was the relative relationship that lower limb lean mass and the size of the specific triceps surae muscles have with peak, as well as the relationship between muscle size and plantarflexion strength.

The results of the first study revealed a marked reduction in soleus (SOL) muscle size, as opposed to total lower limb lean mass or the other triceps surae muscles, and a strong correlation between the volume of this muscle and the peak in CHF. These findings suggest that the SOL may be a sentinel muscle in CHF and a possible proxy for tracking disease progression. Following this rationale, the second study aimed to further analyze the SOL muscle, exploring how its reduced size impacted on peak active voluntary isometric force production and passive force.
**Hypothesis study 2.** It was hypothesized that a reduction in active and passive forces of the SOL muscle would be present in the CHF population and that these reductions would persist after normalizing for the muscle physiological cross-sectional area.

The third study aimed to explore how the findings obtained at a muscle level in studies 1 and 2 translate to whole body mechanics and energetics. This was achieved by performing a detailed three-dimensional inverse kinematic and kinetic biomechanical gait analysis of walking in CHF patients. Walking was chosen because is one of the most ubiquitous task of daily living and because CHF have displayed an altered walking capacity (Forman et al., 2012).

**Hypothesis study 3.** It was hypothesized that a slower walking speed is selected in CHF to reduce total leg mechanical work and, in addition, that a re-distribution of mechanical work from the ankle to the other lower limb joints would be required to account for the reduced muscle size of plantarflexors. This redistribution was hypothesized to increase with walking speed, and thus the % peak aerobic capacity utilized.

Joint-level biomechanics can prove essential for understanding functional limitations to walking in CHF and how to best structure rehabilitation treatments. Further insight into functional limitations can also be gained through analyses of individual muscles mechanics during gait. This thesis includes an initial analysis of the SOL mechanical function during walking between healthy young and old adults that establishes the effect of aging *per se* and the role of muscle mechanics in dictating locomotor behavior (preferred walking speed) (Appendix). This study paves the way for future
analyses aimed at differentiating the effects of age and CHF on muscle mechanics in walking and its role in dictating locomotor function in CHF patients.

Traditional rehabilitative management of CHF has focused on pharmacological approaches, or forms of exercise rehabilitation that have primarily borrowed principles that are effective in healthy populations. Surprisingly, no previous studies have focused on the specific nature of skeletal muscle functional limitation in heart failure, with the aim of informing optimal exercise rehabilitation. The outcomes of the present thesis will provide important novel information that should assist in the future development of evidence-based strategies for rehabilitation and improvement in the health of patients with CHF. This in turn, has the potential to translate into decreased mortality and improved stratification for transplant and implantation of ventricular assist devices.
References


Chapter 1 - General Introduction


2

Literature Review
2.1 What is chronic heart failure?

Chronic heart failure (CHF) is a complex clinical syndrome in which the ability of the left ventricle to fill with or eject blood is impaired (Hunt et al., 2005). Because of its reduced contractile ability the heart is not able to empty completely and, as a consequence of this, there is a high venous filling pressure and a decrease in the effective work done by the heart (Keith, 1956).

Chronic heart failure is a very severe and debilitating disease worldwide. For example, in the US recent statistics show that 5.1 million people are affected by CHF, with an prevalence that rises to 11.5% in the population over 80 years (Go et al., 2014). Also in Australia CHF is a major health burden with 8.2% of the population over 75 affected by this disease (Australian Institute of Health and Welfare, 2008). Today there are still 2 million of new cases worldwide every year, for a total of 22 million people affected by CHF worldwide (Kemp and Conte, 2012).

The mortality rates associated with CHF are very high with a 5-year survival rate below 50% (McKee et al., 1971). A temporal trend in the mortality of CHF was analyzed by Levy et al. (2002) in men and women in the 65-74 year age range and found that the probability of survival decreased markedly over a 10-year period (Fig. 2.1). However, the probability of survival after diagnosis has slightly increased in the 50 years between 1950 and 2000. Nevertheless CHF still remains one of the most important public health concerns (McMurray and Stewart 2002), which is why there is pressing need to study ways to reduce the risk and manage this condition.

Epidemiological studies have provided insight into what are important risk factors for CHF. The first longitudinal study to provide information on the epidemiology of CHF
was the Framingham study (McKee et al., 1971). The study, which began in 1948 and included 5209 adults from the city of Framingham (Massachusetts), is now in its third generation of participants (Mahmood et al., 2014). In the investigation, the lifetime risk of developing CHF was estimated in people whose ages ranged from 40 to 80 years. The highest risk was found at the age of 80 years and was 20.2% for men and 19.3% for women; the lifetime incidence was strictly correlated with concomitant hypertension (Colucci and Braunwald, 2008). Furthermore, for every 10 year age group from 40 to 70 the risk was almost double for people showing high systolic ( > 160 mmHg) or diastolic ( > 100 mmHg) blood pressure. Further studies have elucidated many risk factors for CHF. These have been extensively reviewed (Kenchaiah et al., 2004) and can be grouped into five main categories: clinical, toxic, biochemical, genetic and morphologic (Table 2.1).

![Figure 2.1](image.png)

**Figure 2.1.** Chronic heart failure mortality rates over a 10-year period for (a) men, and (b) women; from Levy et al., 2002.

A number of medical conditions are precursors to CHF, which causes a number of debilitating symptoms. The precursor conditions include ischemic heart disease, diabetes, and especially hypertension, which are present in three quarters of all CHF patients (Kemp and Conte, 2012). The symptoms exhibited in CHF are due to the reduced cardiac output and the lack of efficient venous return (Kemp and Conte, 2012).
### Table 2.1. Major and minor risk factors for CHF, adapted from Kenchaiah et al., 2004; Colucci and Braunwald, 2008.

<table>
<thead>
<tr>
<th>Type</th>
<th>Major</th>
<th>Minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical</td>
<td>Age</td>
<td>Excessive alcohol consumption</td>
</tr>
<tr>
<td></td>
<td>Sex (male)</td>
<td>Cigarette smoking</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
<td>Dyslipidemia</td>
</tr>
<tr>
<td></td>
<td>Electrocardiographic LV hypertrophy</td>
<td>Renal insufficiency</td>
</tr>
<tr>
<td></td>
<td>Myocardial infarction</td>
<td>Sleep-disordered breathing</td>
</tr>
<tr>
<td></td>
<td>Diabetes mellitus</td>
<td>Low physical activity</td>
</tr>
<tr>
<td></td>
<td>Valve disease</td>
<td>Low socioeconomic status</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coffee consumption</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dietary sodium intake</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased heart rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Impaired pulmonary function</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mental stress and depression</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toxic</td>
<td>Chemotherapeutic agents (doxorubicin, daunorubicin, cyclophosphamide, 5-fluorouracil)</td>
<td>Cocaine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biochemical</td>
<td>Albuminuria</td>
<td>Homocysteine</td>
</tr>
<tr>
<td></td>
<td>Natriuretic peptides</td>
<td>Insulin-like growth factor I</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumor necrosis factor α</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interleukin-6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>Morphological</td>
<td>LV dilatation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased LV mass</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Asymptomatic LV systolic dysfunction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LV diastolic filling impairment</td>
<td></td>
</tr>
<tr>
<td>Genetic</td>
<td>Family history of cardiomyopathy</td>
<td>Genetic polymorphisms</td>
</tr>
</tbody>
</table>
The two major symptoms associated with this CHF are breathlessness (dyspnea) and fatigue during exercise (Clark et al., 1996). These occur when the person’s heart is unable to meet metabolic requirements, and diverts the supply of blood toward more vital organs like the brain and the heart. Therefore, some CHF patients also experience a lack of appetite and nausea due to the reduced blood flow to the stomach. Other symptoms like wheezing or cough are not related to reduced blood flow, but rather to elevated pulmonary capillary pressure caused by the inefficiency of the left ventricle. These symptoms enable diagnosis and identification of the stage of CHF severity using the symptoms classified by the New York Heart Association (NYHA) scale (Table 2.2). This classification scale involves four classes and it is based on the physical disabilities experienced by the patients (Dolgin, 1994).

Table 2.2. New York Heart Association (NYHA) classifications of severity of chronic heart failure; from Kemp and Conte, 2012.

<table>
<thead>
<tr>
<th>Class</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No symptoms on ordinary activity.</td>
</tr>
<tr>
<td>II</td>
<td>Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, dyspnea or angina.</td>
</tr>
<tr>
<td>III</td>
<td>Marked limitation of physical activity. Comfortable at rest, but less than ordinary physical activity results in fatigue, palpitation, dyspnea or angina.</td>
</tr>
<tr>
<td>IV</td>
<td>Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency may be present even at rest.</td>
</tr>
</tbody>
</table>

2.2 Pathophysiology of chronic heart failure

The pathophysiology of CHF is very complex and it generates compensatory effects and interactions between different physiological systems of the human body. It affects many of these systems, such as the cardiovascular, respiratory, musculoskeletal, renal, neuroendocrine, haemostatic, immune and inflammatory (Piepoli and Coats, 2013;
Clark et al., 1996). However, major components of the pathophysiology of CHF involve the cardiovascular system and the pulmonary, or respiratory, system (Drexler and Coats, 1996). In addition the cardiovascular and pulmonary systems are responsible for perfusion of the skeletal muscles to enable physical activity and exercise to be undertaken (Magnusson, 1997). So it is the function and interaction in these three systems (the cardiovascular, pulmonary and skeletal muscle systems) that have been implicated in morbidity and mortality in CHF.

2.2.1 Cardiovascular function

Reduced cardiac output is one of the key factor driving the various systemic effects of CHF. Cardiac output is measured in terms of how much blood is pumped by the heart per minute and is calculated as the product of heart rate multiplied by stroke volume. While in a normal healthy adult this value is around 4-8 l/min (Klabunde, 2011), in subjects affected by CHF it decreases to 4.1±2.0 l/min (Agostoni et al., 2000). Stroke volume is defined as the amount of blood ejected by the heart per heart beat and in a healthy individual is around 60-100 cc (Klabunde, 2011) while in CHF it is reduced by ~50% (McConnell et al., 2006). The reduced cardiac output in CHF is due to the dysfunction of the left and/or the right ventricles, which lead to many signs and symptoms (Table 2.3).

Left ventricular dysfunction can occur in systole and/or diastole. In systole, the impairment occurs during contraction and ejection, while in diastole there is a dysfunction in the phase of relaxation and ventricular filling. Although around 70% of patients exhibit systolic dysfunction, with most of these patients also presenting with diastolic dysfunction. An ejection fraction (volumetric fraction of blood pumped out the ventricle) of less than 40% is usually used as a parameter to diagnose the presence of a
systolic dysfunction, whilst a value higher than 50% is reported in a healthy heart (Paulus and van Ballegoij, 2010). The resulting reduced cardiac output causes a global reduction in blood perfusion (i.e. hypoperfusion) of various body tissues and organs, which is characteristic of the left ventricular dysfunction (Kemp and Conte, 2012). Left ventricular dysfunction causes also an augmented pressure of the capillaries in the lungs, generated by an augmented left atrial pressure. This elevated pressure forces fluids out of the pulmonary capillaries leading to pulmonary congestion. As a consequence of this, one of the striking symptoms of left ventricular dysfunction is dyspnea [i.e. shortness of breath (Myers, 1996)], among a suite of other signs (Table 2.3).

<table>
<thead>
<tr>
<th>Dysfunction</th>
<th>Symptoms</th>
<th>Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left ventricle</td>
<td>Dyspnea on exertion</td>
<td>Basilar rales</td>
</tr>
<tr>
<td></td>
<td>Paroxysmal nocturnal dyspnea</td>
<td>Pulmonary edema</td>
</tr>
<tr>
<td></td>
<td>Tachycardia</td>
<td>S3 gallop</td>
</tr>
<tr>
<td></td>
<td>Hemoptysis</td>
<td>Pleural effusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cheyne-Stokes respiration</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>Abdominal pain</td>
<td>Peripheral edema</td>
</tr>
<tr>
<td></td>
<td>Anorexia</td>
<td>Jugular venous distension</td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
<td>Abdominal-jugular reflex</td>
</tr>
<tr>
<td></td>
<td>Bloating</td>
<td>Hepatomegaly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nocturia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swelling</td>
</tr>
</tbody>
</table>

Right ventricular dysfunction is often associated with, and caused by, left ventricular failure. The reduced contractility of the right ventricle leads to an increase in the pressure of the vena caval system. The main problem associated with right ventricular
failure is the congestion of systemic capillaries. Congestion of capillaries causes an excessive retention of the fluids in the body, impairing venous drainage (Kemp and Conte, 2012). This leads to the many symptoms that are typical of right ventricular dysfunction (Table 2.3).

CHF is characterized by a reduced mean atrial pressure as a consequence of the reduced cardiac output. Mean atrial pressure is the product of cardiac output multiplied by total peripheral resistance. The reduced cardiac output and increased total peripheral resistance results in hypoperfusion of different body tissues and organs. This is therefore detrimental to the health and normal function of these tissues and organs.

The human body has different mechanisms to compensate for the reduced amount of blood and nutrients reaching the tissues. Although the mechanisms are beneficial in the short term, they rapidly start to be detrimental and exacerbate the severity of the CHF. Distinct compensatory mechanisms are part of the pathophysiology of CHF, among these are neurohormonal adaptations, ventricular remodeling and impairment of the Frank-Starling mechanism (Kemp and Conte, 2012).

Neurohormonal adaptations in the presence of CHF act to increase the main arterial pressure by increasing the total peripheral resistance. The adaptations work by first sensing a reduction in the main arterial pressure that leads to the activation of the sympathetic nervous system. This causes the release of catecholamines, such as norepinephrine and epinephrine, which have direct effects both on the heart and on the peripheries (Triposkiadis et al., 2009). As a consequence of this, the heart rate and its contractility are augmented and the lumina of the peripheral blood vessels’ are reduced, the latter called vasoconstriction. These neurohormonal activation mechanisms present
in CHF are the same as those generated by the body during intense exercise or hemorrhage (Piepoli and Coats, 2013). However, in healthy individuals these mechanisms are turned on only in these circumstances, while in CHF these mechanisms are always active and exacerbate the hemodynamic abnormalities characteristic of this disease (Kemp and Conte, 2012).

Another compensatory mechanism in CHF is ventricular remodeling. This mechanism involves not only morphological changes such as shape, structure and size, but also functional alterations (Curry et al., 2000). These adaptive changes are a consequence of the increased load on the ventricle's walls due to the reduced ejection fraction. Although initially these modifications improve the stroke volume, as the ventricle undergoes this process they start to become counter-productive. In fact, there is an increased wall tension and fibrosis, which in turn reduce the contractility and the potential of increasing myocardial apoptosis. The morphological changes could also produce long term dyssynchrony in the heart activity that in turn might reduce the overall pumping action (Kemp and Conte, 2012).

Lastly, impairment of the Frank-Starling mechanism has a negative impact on the heart’s function in CHF patients (Guyton and Hall, 2010). The Frank-Starling mechanism, first formulated by the Italian physiologist Maestrini as “the heart's law”, describes the relationship between the right atrial pressure and the cardiac output. Briefly, in a healthy individual, this law states that the stroke volume depends on the left ventricular pressure, with the volume increasing with increasing pressure (Fig. 2.2). In other words, the more the heart walls are stretched, the higher is the strength of the heart’s contraction during the systolic phase. Importantly, this relationship is independent of other external factors. In CHF patients their heart attempts to increase
the cardiac output by increasing the atrial pressure, but, as the flattened shape of the
curve shows in Fig. 2.2, at a certain point an increase in pressure does not correspond to
a proportional increase in the contractile strength. Eventually, with the progression of
the disease, the impairment of the Frank-Starling mechanism becomes more pronounced
and the pressure keeps increasing without any change in the heart’s contractile strength.

![Figure 2.2. The Frank-Starling mechanism which shows the response of heart stroke
volume with left ventricular end-diastolic pressure; from Kemp and Conte, 2012.](image)

**2.2.2 Pulmonary function and exercise capacity**

There is a strong association between CHF and pulmonary dysfunction (Mancini, 1995).
CHF affects the pulmonary function via several mechanisms; ventilation and gas
exchange, the focus of this section, but also by providing the energy demands of the
skeletal muscles (see section 2.3). The combined effect of these mechanisms result in
one of the hallmarks of CHF disease severity (Björnstad et al., 2001), namely low levels
of exercise capacity reflected in dyspnea or breathlessness (Myers, 1996).
A number of tests and metrics are commonly used to describe the exercise capacity. These are peak rate of oxygen consumption ($\dot{V}O_2$ peak), ventilatory efficiency and respiratory ratio (RER). Ventilatory efficiency is the slope of the linear relationship between ventilation ($\dot{V}e$) (i.e. the rate of total volume of gas entering the lungs), and the rate of carbon dioxide production ($\dot{V}CO_2$), while RER is the ratio of carbon dioxide production to oxygen consumption (Guyton and Hall, 2010). $\dot{V}O_2$ peak and ventilatory efficiency have been shown to be very good prognostic indicators of morbidity and mortality in CHF (Arena et al., 2008; Piepoli, 1999). Importantly, these metrics also reflect the mechanisms by which CHF patients experience exercise intolerance (Arena et al., 2008; Mancini, 1995).

$\dot{V}O_2$ peak is one of the best prognostic metrics and indicators of exercise intolerance in CHF (Piepoli, 1999). It is well established that patients affected by CHF show remarkably reduced $\dot{V}O_2$ peak values compared to healthy individuals. In fact, while reference values for a normal untrained adult is between 30-40 ml/kg/min, some CHF patients cannot exceed 10 ml/kg/min (Cohen-Solal et al., 1999). Reduced $\dot{V}O_2$ peak in CHF has been reported when this parameter has been assessed both with a walking protocol (Toth et al., 1997) and on a stationary bike (Esposito et al., 2010). Importantly, Mancini et al. (1991) reported that CHF patients with a $\dot{V}O_2$ peak of 14 ml/kg/min have a one-year survival rate similar to that of patients receiving heart transplantation. Furthermore, $\dot{V}O_2$ peak is used to classify the severity of CHF. A value less than 15 corresponds to a severe CHF, a value between 15 and 20 corresponds to moderate CHF, while a value greater than 25 is associated with mild CHF (Harrington et al. 1997; Buller et al., 1991). Therefore, $\dot{V}O_2$ peak and measures of exercise capacity are used to
guide transplant candidacy and listing (Mancini et al., 1991; Myers et al., 1998; Stevenson et al., 1995).

Ventilatory efficiency has also been used as an important prognostic marker for CHF and exercise intolerance (Kleber et al., 2000). In CHF, as in healthy subjects, the relationship between $\dot{V}e$ and $\dot{V}CO_2$ is still linear, but the slope is steeper (Witte and Clark, 2002). In fact, while a slope for a normal subject is around 20.8, in CHF it doubles to 40.7 (Witte and Clark, 2007), which means that greater ventilation is required for a fixed carbon dioxide production (Buller and Poole-Wilson, 1990).

Ventilatory efficiency has been suggested as an alternate prognostic marker of morbidity and mortality to $\dot{VO}_2$ peak. Selig et al. (2010) commented that $\dot{VO}_2$ peak and ventilatory efficiency provide independent and complementary information for the study of intervention in CHF, so stressing the importance of both these indexes to better characterize the disease.

A maximal exercise protocol must be used to obtain $\dot{VO}_2$ peak and ventilatory efficiency. Bard et al. (2006), after an investigation involving more than 350 CHF patients, reported a slope of 37.0 ± 9.0 at peak exercise versus a reduced slope of 33.0 ± 8.9 at submaximal exercise. Importantly, they showed that the best predictor of CHF mortality was the ventilatory efficiency calculated at peak exercise, rather than submaximal exercise. Although, Arena et al. (2008) recommended that ventilatory efficiency should be calculated using all the exercise data collected from initiation to the maximal exertion, attaining maximal exertion was crucial for obtaining the best measures.
Chapter 2 - Literature Review

RER is an important measure to determine whether a maximal exercise state has been reached (Witte and Clark, 2007), to obtain VO\textsubscript{2} peak and maximal ventilatory efficiency. RER reflects the type of metabolism being used: values around 0.7 characterizes lipid metabolism while value of 1.0 and above indicates glucose metabolism (Dunford and Doyle, 2011). An exercise test is normally considered maximal when RER reaches 1.1, while in the CHF population a value of 1.0 or higher is normally used (Witte and Clark, 2007). Basing the tests on higher RER levels improves the prognostic metrics of the exercise capacity in CHF (Mezzani et al., 2003).

A high slope of the ventilatory efficiency relationship during exercise is a consequence of the hyperventilation and dyspnea of CHF patients (Sullivan et al., 1988; Roubin et al., 1990; Fink et al., 1986; Weber et al., 1982). Hyperventilation in CHF arises because of an increase in intrapulmonary pressures, which are related to interstitial fluid accumulation, decreased lung compliance (Ingram and McFadden, 1976; Parker and Gorlin, 1969) and stimulation of pulmonary juxtacapillary receptors (J-receptors) in the lungs (Reed et al. 1978; Parker and Gorlin, 1969; Ingram and McFadden, 1976). These receptors, stimulated by the distension of the pulmonary vessels, are the primary cause of dyspnea. Nevertheless, a direct link between elevated intrapulmonary pressure and dyspnea is still missing (Fink et al. 1986; Szlachcic et al., 1985; Wilson and Ferraro, 1983), and other mechanisms explaining the higher ventilation in CHF have been proposed. These hypotheses include a poor ventilation perfusion ratio (Myers et al., 1992; Roubin et al., 1990; Sullivan et al., 1988), a large fraction of dead space, or an early onset of metabolic acidosis during physical activity (Roubin et al., 1990; Reddy et al., 1988; Weber et al., 1982; Myers et al., 1992).
Hyperventilation in CHF can be explained by a low ratio of ventilation to blood perfusion in the alveoli. In a normal healthy individual a ventilation-perfusion ratio (Ve/Q) close to 1 indicates that all of the oxygen in the ventilated air is used to perfuse the blood via the alveoli. A Ve/Q < 1 indicates that the alveoli have circulating blood that are unventilated (i.e. shunt), while a Ve/Q > 1 indicates the numbers of alveoli that are ventilated but have no blood arriving there (i.e. dead space). Dead space could have an anatomical cause, in the case of those area of the lungs that have to be ventilated but do not contribute to the gas exchange, or a physiological cause. The ventilatory efficiency is related to ventilation-perfusion through the following equation:

\[
\frac{\dot{V}_e}{V_{CO_2}} = \frac{863}{P_{aCO_2} \cdot (1 - \frac{V_d}{V_t})}
\]  

(2.1)

where \(P_{aCO_2}\) is the partial pressure of arterial carbon dioxide, \(V_d\) the volume of dead space and \(V_t\) the tidal volume. It has been suggested that \(P_{aCO_2}\) is within normal values or even reduced in CHF during exercise (Weber et al., 1982; Wilson and Ferraro, 1983; Clark et al., 1997; Franciosa et al., 1984). So the increase in the slope of the ventilatory efficiency has to be caused by augmented dead space. Assuming that anatomical dead space is almost constant among adults, because it is linked to the length of the conducting airways (Myers, 1996), the majority of the increase in \(\dot{V}_e/V_{CO_2}\) slope has to be explained by the difference in physiological dead space.

Physiological dead space is a larger than normal volume of dead space present in CHF possibly due to the inability of the heart to perfuse the top of the lungs (Witte and Clark, 2007). Physiological dead space is also usually manifested by a reduction in diffusing capacity of the lung, which varies with the severity of CHF (Johnson, 2000). On this
point, (Puri et al., 1994) showed that CHF patients have impaired transalveolar diffusion, measured as transcapillary carbon monoxide diffusion. The larger than normal dead space also leads to hyperventilation and abnormal shallow breathing patterns in CHF, measured as a shallow tidal volume.

Even though the mechanism(s) of shallow tidal volumes in CHF is not completely understood, there have been a number of proposed causes. The first, as alluded to above, is a compensation for the large amount of physiological dead space through an increase in respiratory rate while retaining a shallow depth of breath (Myers, 1996). This compensatory mechanism, evident at low intensity exercise, worsens with higher levels of exercise. Therefore, an augmented respiratory rate has to take place to compensate for the reduced tidal volume and the increased physiological dead space (Myers, 1996).

A second explanation for shallow breathing could be the reduced mechanical work of breathing associated with a faster but less pronounced respiration (Ingram and McFadden, 1976). This happens because pulmonary hemodynamics are abnormal, and with the lungs being less compliant the work of breathing increases. Therefore, a strategy adopted by the CHF population is to try to reduce the work of breathing with a more rapid but shallow respiration (Ingram and McFadden, 1976).

A final possible cause of shallow breathing is the functional impairment of the diaphragm (Hammond and Sharp, 1990; Evans et al., 1995; Chua et al., 1995; Mancini et al., 1992; McParland et al., 1992; Ambrosino et al., 1994). The CHF syndrome has been associated with an alteration in the histology (Lindsay et al., 1996), structure (Stassijns et al., 1999) and strength (Meyer et al., 2001) of the respiratory muscle. These
abnormalities could be the cause of the diaphragm’s functional impairment and contribute to the shallow breathing and pulmonary limitations evidenced in this group.

Returning to hyperventilation, as suggested above another possible cause of this may be metabolic acidosis. This is when people shift from an aerobic to anaerobic metabolism in skeletal muscles. Early onset of metabolic acidosis was reported by Weber et al. (1982) and others using the measurements of gas exchange and air flow. Depending on the severity of the CHF patients, the authors reported an anaerobic thresholds occurring at a $\dot{V}_{O_2}$ between 17.0 ± 0.3 and 7.1 ± 1.5 ml/min/kg (Weber et al, 1982). In a normal healthy population, although highly dependent on fitness level, the anaerobic threshold occurred at a marked higher $\dot{V}_{O_2}$, 29.7 ± 5.3 ml/min/kg (Palka and Rogozinski, 1986). The early onset anaerobic metabolism in CHF patients may explain the fatigue they experience at low levels of exercise and hyperventilation. Importantly, these results implicate skeletal muscles as being involved in the exercise intolerance in CHF.

2.3 Pathophysiology and skeletal muscle function in chronic heart failure

Although impaired cardiac output initiates the syndrome of CHF, it is widely accepted that exercise capacity and cardiac function are poorly correlated (McKelvie et al., 1995). This point is supported by a study of Cohn et al. (1993) on a large cohort of CHF patients (n > 500) that showed a low, non-significant correlation ($r = 0.16$) between $\dot{V}_{O_2}$ peak and cardiac output (ejection fraction). This reinforces the view that even if the heart acts as a "primum movens" of the CHF syndrome, adaptive changes involving other systems produce the symptoms of CHF (Piepoli and Coats, 2013). Furthermore, it is believed that impaired peripheral blood flow (Kubo et al., 1991) and skeletal muscle dysfunction (Drexler et al., 1992) are fundamental to the exercise intolerance and,
importantly, disease progression in CHF. Recognition of the importance of these peripheral abnormalities, and the underlying peripheral mechanisms in CHF, has led to the “muscle hypothesis”, as described by Clark et al. (1996),

The “muscle hypothesis” proposes that peripheral abnormalities are centered on pathophysiological mechanisms in the skeletal muscle. Reduced peripheral blood flow and increased vascular resistance are present in the arms (Zelis and Flaim, 1982) and lower limbs (Sullivan et al., 1989; Wilson et al., 1984), both at rest and during exercise, thus affecting the muscle function. Moreover, reduced blood flow in the lower limbs has been shown to be related to a lower \( \dot{V}O_2 \) peak (Sullivan et al., 1989; Wilson et al., 1984, 1983) and to an earlier onset of an anaerobic metabolism in skeletal muscle (Mancini et al., 1990). The anaerobic metabolism leads to stimulation of the muscle metaboreceptors that generate the symptoms of CHF and contribute, via reflex mechanisms, to further neurohormonal activation, peripheral vasoconstriction and consequent disease progression.

Skeletal muscles are centrally important to the progression of the CHF by causing an increase in the ventilatory response to exercise and early symptoms of fatigue (Clark et al., 1996) (Fig. 2.3). As a consequence, there is an activation of the sympathetic nervous system that reduces cardiac function by means of increased vascular resistance and reduced peripheral blood flow. Moreover, a catabolic state is present, perhaps related to cytokine activation (Testa et al., 1996) and insulin resistance (Doehner et al., 2005) in the muscles, causing further muscle wasting. Skeletal muscles therefore have a key role in dictating the advancement of the severity of the disease (Fig. 2.3).
Several abnormalities that are characteristic of the CHF syndrome are observed in the skeletal muscles (Gosker et al., 2000; Rehn et al., 2012; Clark et al. 1996, Minotti et al., 1992a). Five distinct abnormalities of skeletal muscle have been identified: muscle wasting, structure, metabolism, gross function/strength and fiber/cross-bridge mechanics. Because these abnormalities are centrally important to the present thesis they are described in following separate sections.

Figure 2.3. Muscle skeletal hypothesis in chronic heart failure; from Clark et al., 1996.

2.3.1 Muscle wasting

It has long been recognized that significant weight loss and muscle wasting (i.e. cachexia) are major features of advanced CHF and have been reported extensively (Akashi et al., 2005; Katz and Katz, 1962; Mancini et al., 1992; Volterrani et al., 1994; Minotti et al., 1993). Cachexia is broadly defined as the state of muscle wasting and weight loss that occurs in several different chronic disorders (Yeh and Schuster, 1999), although a clear definition of this term is not present in the literature (Anker and Sharma, 2002). Some groups defined CHF patients as ‘cachectic’ when the body fat content was < 29% (females) or < 27% (males) (McMurray et al., 1991), or when the ideal body weight was < 85% (Levine et al., 1990) or even < 80% (Otaki, 1994).
Therefore, in discussing muscle wasting in CHF a distinction between cachetic and non-cachetic patients has to be made for a better interpretation of the results.

Assessing differences in muscle mass has commonly used dual-energy X-ray absorptiometry (DXA) (Cicoira et al., 2001; Williams et al., 2004; Fülster et al., 2013; Toth et al. 1997, 2006, 2010; Lang et al., 1997; Haykowsky et al., 2013; von Haehling et al., 2012; Anker et al., 1999, 2001). DXA allows the investigators to assess subject’s lean body mass, which has good correlation with measured skeletal muscle mass across different healthy and clinical populations (Kim et al., 2002). Nevertheless, the results of the DXA studies have been inconclusive in regards to muscle loss in CHF. A recent study using this technique on a large cohort of CHF patients (Fülster et al., 2013), reported statistical differences in the total, as well as in the upper and lower limb lean body mass between cachetic and non-cachetic patients. Similar findings were also reported by other authors (Anker et al., 2001; Toth et al., 1997). In contrast, other studies using DXA (Toth et al., 2006, 2010) found no differences between CHF subjects and healthy matched controls. However, they did not specify if cachectic or non-cachetic patients were enrolled, possibly leading to misinterpretations of findings.

A limitation in the use of DXA to evaluate muscle wasting is that it only assesses the lean mass from the total body or specific anthropometric segments. DXA is unable to assess synergist muscle groups or individual muscles. Furthermore, in muscle physiology research, characterization of muscles is performed by not only regarding its mass, or volume, but also by other parameters such as muscle cross-sectional area (CSA) or physiological cross-sectional area (PCSA). PCSA is a muscle’s CSA with pennation angle taken into account and is a relevant functional parameter because of its strong correlation with the working capacity of a muscle (Lieber, 2009). The inability of
 DXA to assess all these parameters makes it unsuitable for a precise description of muscle wasting.

**Table 2.4.** A summary of the studies investigating muscle wasting of single muscle compartments.

<table>
<thead>
<tr>
<th>Study</th>
<th>Compartment/muscle investigated</th>
<th>Parameter evaluated</th>
<th>Technique Used</th>
<th>Main findings</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minotti et al. 1993</td>
<td>Total thigh, thigh muscles, knee extensors</td>
<td>Max CSA</td>
<td>MRI</td>
<td>Reduction</td>
<td></td>
</tr>
<tr>
<td>Harrington et al. 1997</td>
<td>Total thigh, Quadriceps</td>
<td>CSA</td>
<td>CT</td>
<td>Thigh no differences Quadriceps reduced</td>
<td></td>
</tr>
<tr>
<td>Williams et al. 2004</td>
<td>Total thigh</td>
<td>Mass</td>
<td>DXA</td>
<td>No differences</td>
<td></td>
</tr>
<tr>
<td>Haykowsky et al. 2014</td>
<td>Total thigh</td>
<td>CSA</td>
<td>MRI</td>
<td>No differences</td>
<td></td>
</tr>
<tr>
<td>Anker et al. 1997</td>
<td>Total thigh, quadriceps</td>
<td>CSA</td>
<td>CT</td>
<td>Reduction</td>
<td>Cachetic vs non cachetic patients</td>
</tr>
<tr>
<td>Magnusson et al. 1994</td>
<td>Quadriceps</td>
<td>Mass</td>
<td>MRI</td>
<td>Reduction</td>
<td></td>
</tr>
<tr>
<td>Magnusson 1997</td>
<td>Quadriceps</td>
<td>Mass</td>
<td>MRI</td>
<td>Reduction</td>
<td></td>
</tr>
<tr>
<td>Gielen et al. 2012</td>
<td>Quadriceps</td>
<td>CSA</td>
<td>CT</td>
<td>Reduction</td>
<td></td>
</tr>
<tr>
<td>Mancini et al. 1992</td>
<td>Calf</td>
<td>Volume</td>
<td>MRI</td>
<td>Reduction</td>
<td></td>
</tr>
</tbody>
</table>
To overcome the limitations of DXA some investigations have used magnetic resonance imaging (MRI) or computed tomography (CT). However, to date, very few studies have used these methods to investigate muscle wasting of specific lower limb muscles and/or muscle compartments in CHF (Table 2.4). In these only Magnusson (1997) has analyzed individual muscles, and this was in the thigh.

### 2.3.2 Structure within muscles

Differences in the skeletal muscle structure associated with the CHF have been extensively reported (Clark et al., 1996; Rehn et al., 2012). Most of the histologic information was obtained by studies investigating biopsies of the vastus lateralis, because of its accessibility, except for one (Mancini et al., 1989) in which the gastrocnemius was examined. The main consistent finding of these investigations is a reduction in the percentage distribution of type I fibers and an increase in the percentage distribution of type II fibers (Schaufelberger et al., 1997; Sullivan et al., 1990; Drexler et al., 1992; Massie et al., 1996).

Reduced fiber CSA has also been reported in several studies investigating fiber structure in CHF (Mancini et al., 1989; Williams et al., 2004; Sullivan et al., 1990; Massie et al., 1996), with an obvious implication for force production. Despite this, is it still not clear which fiber types suffer more atrophy in CHF. The literature tends to consider type II fibers as being more affected by atrophy than type I fibers, as shown in studies that have reported a reduction in type II (or subtypes IIa and IIc) but an unaltered CSA of type I (Mancini et al., 1989; Massie et al., 1996). In contrast, one study has reported that type I fibers have a greater atrophy than type II fibers (Williams et al., 2004).
Another important parameter in the characterization of muscle structure is capillary density. The abnormalities of peripheral vasculature and skeletal muscle in CHF has been extensively reviewed (Duscha et al., 2008), with the results being inconsistent and depending on the method of assessment. When capillary density is defined as the ratio of capillaries per muscle fiber, several studies (Drexler et al., 1992; Duscha et al., 1999; Magnusson et al., 1996; Schaufelberger et al., 1995; Williams et al., 2004) found a 17-32% reduction in CHF compared to control groups (Duscha et al., 2008). Moreover, Duscha et al. (1999) found a correlation between capillary density and VO$_2$ peak. In contrast, Sullivan et al. (1990) and Massie et al. (1996) did not find a significant reduction in capillary density in the CHF group, when it was instead defined as the ratio of capillaries per area of muscle (in mm$^2$). Only Magnusson et al. (1996) showed reduced capillary density in CHF measured this way, contradicting the other mentioned studies. Moreover, using this definition, Mancini et al. (1989) showed an increase in the capillary density of the CHF. However, if the fibers have reduced CSA then there would be more fibers per area of muscle, probably causing the above inconsistencies. The reduced number of capillaries per fiber might possibly indicate the lack of an adequate oxygen supply required for the normal function of the muscle.

2.3.3 Metabolism

Abnormalities in the metabolic function of skeletal muscle have also been extensively reported in studies on humans with CHF (Drexler et al., 1992; Ralston et al., 1991; Broqvist et al., 1992; Sullivan et al., 1990; Schaufelberger et al., 1997; Abozguia et al., 2008; Minotti et al. 1992; Massie et al., 1987a, 1987b) and in animal models of the disease (Arnolda et al., 1991; Simonini et al. 1996, 1999; De Sousa et al., 2002). This literature is almost unanimous in affirming reduced oxidative capacity of skeletal
muscle in CHF, that in turn leads to an early onset of anaerobic metabolism during exercise (Rehn et al., 2012; Clark et al., 1996; Piepoli and Coats, 2013).

Other studies have evaluated oxidative metabolic activity by assessing the levels of phosphocreatine (PCr) and inorganic phosphorus (Pi) in the skeletal muscle, using 31 P-nuclear magnetic resonance (Mancini et al., 1989; Mancini et al., 1992; Massie et al., 1987b; Sullivan et al., 1991; Wilson et al., 1985). The ratio between Pi and PCr is an estimate of ADP concentration. Thus, a high Pi/PCr ratio provides an index of mitochondrial respiration (Mancini et al., 1992). The altered metabolism characteristic of CHF that has been established in these studies may be linked to variations in mitochondrial function or substrate utilization (Wiener et al., 1986).

Abnormalities have been found in the enzymes that regulate aerobic metabolism of CHF (Gosker et al., 2000). Typical enzymes involved in the oxidative process are citrate synthase, succinate dehydrogenase and β-hydroxyacyl, while enzymes responsible for glycolytic metabolism are hexokinase, phosphofructokinase and lactate dehydrogenase (Gosker et al., 2000). No differences in the activity of enzymes involved in the anaerobic metabolism were reported (Mancini et al., 1989; Sullivan et al., 1990) while a decreased activity was reported by Sylven et al. (1991). However, substantially decreased activity of enzymes regulating the aerobic metabolism was instead found in many studies (Mancini et al., 1989; Naveri et al., 1997; Sullivan et al., 1990, 1991). This suggests greater relative dependence on anaerobic metabolism.

There are several other indicators of anaerobic metabolism in CHF. One is a reduction in intracellular pH at peak exercise which has be interpreted as an index of an earlier use of anaerobic metabolism in CHF (Naveri et al., 1997; Massie et al., 1987b; Wilson et
al., 1985; Mancini et al., 1992). Also indicative of anaerobic metabolism is a reported reduction in the density of mitochondria, which produce the decreased oxidative capacity of the muscle itself (Esposito et al. 2010; Gosker et al. 2000; Hambrecht et al. 1997; Drexler et al. 1992). Taken together, these metabolic alterations occurring in the skeletal muscle lead to an earlier anaerobic threshold, which are indicators of the metabolic cause of fatigue in CHF.

2.3.4 Gross muscle function and strength
Numerous studies reported a reduction in skeletal muscle strength in CHF (Harrington et al., 1997; Magnusson et al., 1994; Sunnerhagen et al. 1998; Minotti et al., 1993; Lipkin et al., 1988; Toth et al. 2006, 2010). These studies compared the strength (maximum isometric knee extension torque) of the quadriceps in CHF with respect to age-matched controls.

This literature is almost unequivocal in reporting a marked reduction of quadriceps strength in CHF (Harrington et al., 1997; Magnusson et al., 1994; Sunnerhagen et al., 1998; Toth et al., 2006, 2010). The only two studies that did not report a statistical difference in strength, could have been affected by the small number of CHF participants involved (n= 6) (Harridge et al., 1996), or by having subjects with similar muscle size between the groups (Minotti et al., 1991). This begs the question: is the reduction in strength in CHF muscle specific?

Only a few studies have investigated the strength of muscle groups in CHF other than the knee extensors. Interestingly, while there are consistent findings of reduced strength of quadriceps, the results from other muscle groups are less clear and sometimes contradictory. Sunnerhagen et al. (1998) found reduced strength of the plantarflexors
and dorsiflexors, while Minotti et al. (1992b) found no difference in the dorsiflexors and Carrington et al. (2001) no differences in the plantarflexors. Also Harridge et al. (1996) did not find any difference in the strength of elbow flexors and extensors, as well as in the plantarflexors. Lastly, the adductor pollicis, was investigated by Buller et al. (1991), who did not find any reduction in isometric force production of this muscle in CHF patients compared to controls.

It is unclear if the reduced strength in patients affected by CHF is due to the loss of muscle mass and/or if the contractile properties of the skeletal muscles are compromised. Magnusson et al. (1994) reported that when normalized by CSA the strength per area of muscle (muscle specific tension) is not different in CHF compared to controls. In contrast, studies from Toth et al. (2006, 2010) showed that strength is reduced in CHF when values were adjusted for leg fat-free mass. Also Harrington et al. (1997) found a reduced specific tension in the CHF, even though the patients involved in this study did not present a reduced CSA compared to the controls. More recently, Hopkinson et al. (2013) found only a positive trend in the baseline quadriceps strength between CHF and controls ($p = 0.09$), but no difference in maximal quadriceps force ($p = 0.26$) in response to supramaximal magnetic femoral nerve stimulation. However, twitch response does not necessarily reflect muscle specific tension, but rather impaired central or peripheral motor drive (Herbert and Gandevia, 1999), and thus there is greater evidence for lower muscle specific tension in CHF.

Another well established finding on muscle function is the early fatigability in CHF (Clark et al., 1996; Rehn et al., 2012). There is a rapid decay in the production of isometric and isokinetic torque of knee extensors in the CHF population compared with healthy matched controls (Harridge et al., 1996; Harrington et al., 1997; Toth et al.)
2010; Magnusson et al., 1994; Minotti et al., 1991; Minotti et al., 1993). Similar results were also found in the dorsiflexors by Minotti et al. (1992b) and Harridge et al. (1996), using superimposed twitch interpolations and concluded that the reductions in force production were not caused by impaired central motor drive or by an abnormality in the neuromuscular junction transmission, but rather by an abnormality in the muscle itself (Minotti et al., 1992b).

### 2.3.5 Fiber and cross-bridge muscle mechanics

Previous studies still have not clarified if CHF patients reduced in muscle strength is due to altered muscle’s contractile properties (Toth et al., 2010). Although skeletal muscle contraction is regulated by several physiological systems, it is ultimately governed by the functional properties of myofilament proteins (Toth et al., 2012). The characteristics of these structures regulate the most relevant mechanical properties of the muscle: its force-length (F-L) and its force-velocity relationships (F-V) (Woledge et al., 1985).

Alterations in the proteins responsible for muscle contraction have been reported for both non-human animals (Coirault et al., 2007; van Hees et al., 2007, 2010) and humans with CHF (Miller et al., 2010, 2009; Toth et al., 2005). Van Hees et al. (2007) evaluated the properties of the diaphragm muscle in rats with CHF induced by left artery coronary ligation. They found a reduced maximal force and specific tension generation of type I fibers caused by reduced myosin content, which was associated with increased proteolytic activities of caspase-3 and the proteasome. Moreover, they also reported a reduction in Ca$^{2+}$ sensitivity and a slower cross-bridge cycling kinetics in every fiber type. In a similar study, van Hees and colleagues (2010) also found that passive tension upon stretch was significantly reduced ( > 35%) in the diaphragm fibers of CHF rats but
not in the soleus fibers. They did not report differences in titin size but rather a 25% reduced titin content in the diaphragm compared to controls. Another study on CHF rats (Coirault et al., 2007) revealed a 16-20% reduction of myosin sliding velocity of diaphragm and soleus muscles. Szentesi et al. (2005), also attributed the reduced cross-bridge kinetics and the reduction in force to the diminished ATP consumption in CHF.

The altered cross-bridge kinetics has been confirmed in humans with CHF by two studies investigating single fibers in vitro (Miller et al., 2009, 2010). In the vastus lateralis there were compromised cross-bridge kinetics of both type I and IIa fibers, expressed, in part, by longer attachment time of the myosin. Interestingly, however, even though there was a loss of myosin heavy chain (MHC) content in CHF, because the longer attachment times permitted an increase in the number of myosin heads bound to actin, the CHF specific tension was found to be similar to that of a control population (Miller et al., 2010). The discrepancy in specific tension of type I fibers, compared to the earlier study (Miller et al., 2009), was attributed to the temperature used in the muscle preparations. A decreased Ca\textsuperscript{2+} sensitivity in the MHC of type IIa fibers and alterations in the viscoelastic properties of the lattice structure of MHC type I and IIa fibers were also reported (Miller et al., 2010). Although previous research does not firmly establish the fiber specificity of altered cross bridge kinetics, a loss in myosin of skeletal muscle has been confirmed by several in vitro investigations (Miller et al., 2009; Toth et al., 2005; van Hees et al., 2007) contributing to generally reduced muscle function in CHF (Callahan and Toth, 2013).

The compromised skeletal muscle function in CHF can be partially restored using high intensity resistance training (Toth et al., 2012). The 18 weeks of training caused alterations at cellular and molecular levels in CHF. Interestingly, the training increased
the strength of the subjects not by augmenting muscle size or single fiber CSA but by modifying the contractile properties of the proteins. Surprisingly, they found that training reduced the fractional myofibril area of muscle fibers, thereby reducing the number of bound actin-myosin cross-bridges. When fiber tension was corrected for the reduction of the myofibril fractional area patients exhibited an augmented specific tension. Training also corrects the cross bridge kinetics alterations mentioned before, so that the myosin attachment time was reduced to values characteristic of a healthy population.

Although, as discussed above, several studies reported alterations in the proteins regulating the contractile mechanism, others have shown no differences in humans. An example is the study by Okada et al. (2008) who evaluated the contractile protein function of the vastus lateralis muscle in a group of CHF and controls. In vitro analyses of fibers did not show any difference between groups with respect to unloaded shortening velocity or maximal force. Mixed findings were reported by Toth et al. (2005), where MHC and actin did not change in CHF, despite an inverse correlation being found between the content of MHC and the severity of CHF. The authors also reported a correlation between C-reactive protein and MHC protein synthesis, together with a significantly reduced relative amount of MHC type I fibers and only a trend for MHC type IIx.

This section highlights the abnormalities characteristic of the CHF syndrome previously reported in the skeletal muscles, underlining the paucity of the literature on some specific muscle properties. The "muscle hypothesis", which constitutes the rationale for the present thesis, proposed that alterations of skeletal muscle properties in turn leads to
the reduced exercise and functional capacity displayed by patients affected by this pathology.

### 2.4 Relationship between skeletal muscle properties and peak in chronic heart failure

Skeletal muscle abnormalities have been shown to be strongly related with exercise capacity, and VO$_2$ peak especially, in the CHF population (Rehn et al. 2012; Clark et al. 1996; Witte and Clark, 2007). Therefore, the aim of this section is to investigate the link between muscle properties and exercise capacity in patients affected by this disease. The selected properties discussed are muscle size, strength and endurance because of their well-documented association with a reduced VO$_2$ peak.

Muscle size has been related to VO$_2$ peak. Cicoira et al. (2001) studying a large cohort of non-cachetic CHF patients found a significant correlation between lean mass and absolute VO$_2$ peak ($r = 0.70$; $p < 0.0001$). Fülster et al. (2013) also detected significant relationships between the appendicular skeletal mass (sum of arms and legs mass) and absolute VO$_2$ peak ($r = 0.57$, $p < 0.0001$) in patients without muscle wasting, with a similar association between skeletal muscle mass and VO$_2$ peak was reported also by (Toth et al., 1997). Lastly, Schaufelberger et al. (2001) evaluated functional and morphological parameters in ten NYHA III-IV class CHF patients before and after heart transplantation. They found that maximal work rate was related to their thigh CSA and to the strength of knee extensors ($r = 0.77$, $p = 0.025$). Intriguingly, these relationships persisted also after transplantation (exercise capacity and strength $r = 0.64$, $p = 0.01$).
As mentioned above, muscle strength has also been shown to correlate to exercise capacity in CHF. The largest study was performed by Harrington et al. (1997) who examined 100 CHF patients, equally distributed between the four NYHA classes and an age-matched healthy population. They measured the strength of the quadriceps by means of three isometric contractions with superimposed twitch interpolation of 1ms at 1Hz. Quadriceps strength was found to correlate with VO₂ peak both in CHF and in controls. In the study of Buller et al. (1991) multiple isometric contractions of the quadriceps were performed by ten CHF patients with different severity of this disease and again reported a strong correlation between VO₂ peak and maximal isometric force (r = 0.86).

Other studies showed a correlation between muscle endurance and VO₂ peak. Minotti et al. (1991) recruited 16 CHF patients (58 ± 11yo; mean ± S.D.) and 8 age-matched controls to assess strength and endurance of the knee extensors using a series of isokinetic and isometric contractions. Dynamic endurance was defined as the ratio of the mean peak torque in the last three isokinetic contractions as compared to the first three contractions, after a protocol involving a total of 15 repeated contractions. Their results reported a correlation of r = 0.66 between dynamic endurance and VO₂ peak in the CHF groups at 180°/s that increase to r = 0.9 at 90°/s. Interestingly, no significant correlation was found in the control group. Moreover, dynamic endurance was also the only parameter that correlated with VO₂ peak. Lastly, Gielen et al. (2012) enrolled 60 CHF patients and 60 healthy age-matched controls and randomly divided them in two separate groups by age ( > 65 yo and < 55 yo). Half of the people in each group underwent four weeks of aerobic exercise training while the other half served as benchmark data as they underwent their usual medical care. Both the control and the CHF groups that took part in the exercise program showed a correlation between VO₂
peak and endurance of the quadriceps (CHF, r = 0.51, control r = 0.49) after the four weeks, where endurance was assessed as the period of time the participant could maintain 50% of the maximal isometric force.

In addition to muscle size, strength and endurance other altered musculoskeletal properties have been shown to be related with $\dot{V}O_2$ peak and exercise capacity. These alterations, detailed in a review written by Middlekauff (2010), included a shift from type I fibers to type IIb being correlated with diminished exercise capacity (Mancini et al., 1989). Massie et al. (1987a) reported that CHF patients with the most impaired exercise ability also exhibited the lowest phosphocreatinine and pH levels, implying that an early onset of anaerobic metabolic activity limited their exercise capacity. This was also supported by Massie et al. (1996) in a study involving CHF patients with 2.6 ± 0.2 NYHA classification who found that abnormalities in mitochondrial density and activity were strongly related to decreased peak oxygen consumption.

Whilst the clear association between the aforementioned measures of peripheral muscle function and exercise capacity is strongly suggestive of a causal relationship this may not necessarily be true in all cases. It remains possible that peripheral abnormalities and reduction in exercise capacity occur independently in CHF, although to what extent is difficult to discern, especially given the known strong link between skeletal muscle function and exercise capacity.

2.5 Normal locomotor function and assessment

The previous section highlighted how skeletal muscle abnormalities are linked to the reduced exercise capacity in CHF. But how these abnormalities impact on the whole body ability to perform daily task activities has not been addressed. This section focuses
on basic concepts of locomotor function in healthy adults in order to provide an adequate background for the study of the mechanisms regulating walking in CHF.

Walking is a distinctive pattern of terrestrial locomotion in humans (Sentija et al., 2012). A simple description of walking can be obtained using the spatio-temporal parameters of walking speed, stride length and stride frequency (Zijlstra and Hof, 2003). Because of the interdependency of these parameters, when two of them are known the third one can be calculated. Reduced spatio-temporal parameters indicate that a person's gait is being affected by any number of different pathologies and conditions, such as osteoarthritis (Sturnieks et al., 2008), cerebral palsy (Brégou Bourgeois et al., 2014) or anterior cruciate deficiency (Knoll et al., 2004). Subsequently, these parameters alone are not sufficient to explore why the mechanics of walking have been compromised by a pathology.

Walking mechanics is indeed a multifaceted form of locomotion and the task of regulating balance and posture is an extremely complex motor control problem (MacKinnon and Winter, 1993). Historically an inverted pendulum is the established biomechanical model used to describe the mechanism necessary to stabilize and propel the center of mass during walking (Margaria, 1976; Cavagna et al., 1976). In this model, the potential and kinetic energy associated with the center of mass continuously exchange to minimize the net energy needed to drive the moving system (Saibene and Minetti, 2003).

Metabolic energetics also plays an important in characterizing the mechanics of walking (Saibene and Minetti, 2003). Humans prefer to walk at the particular combination of step length, frequency, and even step width that is energetically optimal (Kuo et al.,
As a consequence, normal healthy adults have been shown to adopt a walking speed that allows them to maximize the economy of walking, where walking economy is often measured as the cost of transport (COT; the amount of energy required to travel a given distance) (Saibene and Minetti, 2003).

Technological development has helped the adoption and application of 3D gait analysis techniques allowing investigation of more complex biomechanical aspects of gait. Gait analysis in three planes of motion is necessary to accurately describe gait mechanics (Eng and Winter, 1995) and the detailed characterization of individual joint kinetics and kinematics. 3D gait analysis, coupled with new musculoskeletal modeling techniques (Delp et al., 2007; Lloyd and Besier, 2003), have also provided important insight into the muscle behavior responsible for specific joint kinetics and kinematics patterns, and characteristics of conditions or pathologies (Modenese and Phillips, 2011; Steele et al., 2010). Importantly, joint level biomechanical analyses have helped to identify mechanisms responsible for generation of normal gait (Winter, 1987).

Among the lower limb joints, particular importance is focused on the kinetics of the ankle joint. Muscles crossing the ankle joint are fundamental to provide propulsion and support during gait (McGowan et al., 2009; Neptune et al., 2001; Winter, 1983). The plantarflexors are also the locus of an important energy saving mechanism that takes place during walking that comprises an interaction between the Achilles tendon and the triceps surae (i.e. the medial and lateral gastrocnemii and the soleus). Fukunaga et al (2001) showed that the isometric behavior of the medial gastrocnemius during walking may help to reduce energy expenditure required to produce the contractile forces to support and displace the body forward. Isometric muscle function is possible because the Achilles tendon acts in a spring-like manner by storing and then releasing elastic-
strain energy (Fukunaga et al., 2001), thus providing the power at the ankle rather than the muscle fibers themselves. Furthermore, Achilles tendon stiffness has been found to be optimal to achieve high efficiency not only in walking but also in running (Lichtwark and Wilson, 2007).

2.5.1 Locomotor adaptations due to aging

Importantly, alterations in gait function and energetics have been reported in aging, and in several musculoskeletal diseases and conditions (DeLuca et al., 1997; Nadeau et al., 1999; Roiz et al., 2010). Of particular importance for CHF is the alteration of gait mechanics associated with aging (Mian et al., 2006; Schmitz et al., 2009), as their age group experiences normal healthy changes to their gait compared to younger people. The gait of CHF therefore has to be seen in reference to age-matched and younger cohorts. Compared to younger adults, healthy older adults present a slower walking speed (Himann et al., 1988; Oberg et al., 1993) and an elevated metabolic cost of walking (Mian et al., 2006). A redistribution of joint torque and power among the lower limb joints is also associated with aging, with a shift from the work produced at the ankle joint to the hip joint (DeVita and Hortobagyi, 2000). This altered joint level mechanics is a possible cause of the compromised metabolic cost of walking.

This redistribution of work from the ankle to the hip joint is probably the consequence of an altered muscle function of the plantarflexors. Although little research has addressed this issue, abnormalities in the in vivo skeletal muscle function of muscles crossing the ankle during walking have been reported in the gastrocnemius (Mian et al., 2007) and soleus (Panizzolo et al., 2013) in an aged population. The research on soleus function in walking was done as part of this thesis (Panizzolo et al., 2013) (Appendix)
to establish the baseline of healthy ankle joint and soleus muscle function from which to compare CHF patients in future studies.

2.6 Locomotor function in chronic heart failure

Very little research has been conducted to analyze walking function in CHF. To this extent, the assessment of locomotor function in patients affected by CHF (Adsett et al., 2011; Cahalin et al., 1996; Roul et al., 1998) has been conducted mainly using the six minute walking test (6MWT) (Adsett et al., 2011; Cahalin et al., 1996; Roul et al., 1998), to establish the basic spatio-temporal parameters of walking. During this simple test the patients are asked to walk the longest distance possible through a corridor (preferably 30 m long), for a total duration of six minutes, adopting their own walking speed (Faggiano et al., 2004). Using this simple protocol researchers were able to assess the severity of the CHF (based on the NHYA classification) simply by examining the total distance covered during the 6MWT (Guyatt et al., 1985; Lipkin et al., 1986).

Consistent with other pathologies and conditions (Sturnieks et al., 2008; Brégou Bourgeois et al., 2014; Knoll et al., 2004), a reduced gait speed and stride length have been found in those with CHF (Pepera et al., 2012). In this work the authors reported an average step length of 0.69 m and an average speed of 1.19 m/s in CHF; compared to 0.71 m and 1.22 m/s in healthy, age-matched controls. Another study by Davies et al. (1992) conducted on CHF patients (class II and III NHYA) supports these findings, showing a significant reduced speed/stature index and stride/stature index compared to controls during a free walking. A reduced stride/stature index was also found in NYHA class III patients compared to age-matched healthy controls when walking on a treadmill with a set speed of 0.5 m/s. But what drives these reductions in walking speed and stride length?
Reductions in speed and alterations in spatio-temporal gait parameters may reflect a biomechanical and/or physiological optimization strategy. Interestingly, a different strategy to optimize walking has been reported for CHF patients compared to a healthy population. Figueiredo et al. (2013) showed that CHF walked at a slower speed than age-matched control participants (0.75 ± 0.14 m/s and 0.98 ± 0.30 m/s, respectively) possibly in order to minimize their ventilatory efficiency rather than COT.

Walking performance and economy can be improved in CHF with training. Beneke and Meyer (1997) showed that CHF patients involved in three weeks of exercise training not only had an increase in their 6MWT and $\dot{V}O_2$ peak, but also decreased their COT. These patients showed an increase in their preferred walking speed (from 0.68 ± 0.33 m/s to 1.16 ± 0.30 m/s), which had a positive effect on their walking economy.

Studies on gait function in CHF have assessed either basic spatio-temporal parameters or whole body COT. There is a lack in the literature on studies investigating joint level function in gait, which are important to identify joint and muscle function that are responsible for compromised economy of walking and exercise intolerance in CHF. This raises the obvious question: are there different relative roles of joints between CHF and healthy individuals? A 3D gait analysis of walking in CHF will permit one to elucidate the link between musculoskeletal function and energetics with the goal of investigating the mechanisms responsible for an early onset of fatigue that is characteristic of CHF.

2.7 Statement of the problem

The literature review above points to the abnormalities present in the skeletal muscle of patients affected by CHF, providing the evidence for possible mechanisms responsible
for reduced exercise capacity and fatigue. Although muscle wasting is associated with CHF, previous literature generally described it as a reduction of muscle size, usually assessed as whole body or limb lean mass. No studies to date have investigated the presence of a possible muscle specificity of muscle wasting. Nor has previous research examined the effects of muscle wasting on specific morphological muscle parameters responsible for force production such as muscle volume, PCSA, fiber lengths and pennation angles, or tendon properties. Furthermore, no research has previously assessed the link among these parameters and exercise capacity and strength.

A clear link between muscle morphological and functional properties in CHF is also missing. Although extensive research has been conducted on muscle strength, this is mainly based on the evaluation of net joint moment measurements. These may miss factors that lead to a dissociation between net joint moment and the actual underlying individual muscle force capacities. Currently, no studies have been conducted on humans with CHF that examine individual muscles and their in vivo active and passive components of force production.

Lastly, how these aforementioned musculoskeletal abnormalities impact on gait function is unknown. Information on CHF walking mainly involves simple walking endurance tests, spatio-temporal data and metabolic energetics at a whole body level. A joint level gait analysis with the purpose of understanding the gait mechanics in CHF has never been performed. Such analysis could provide important information on the causal link between the altered skeletal muscle properties, joint function and VO₂ peak, helping to reveal the basis for functional limitations in CHF and foster evidence-based rehabilitation approaches aimed at restoring walking capacity.
In conclusion, the overall aim of this thesis was to characterize the *in vivo* morphological and mechanical properties of the skeletal muscle (triceps surae) in CHF patients in comparison to healthy age- and exercise-matched control participants, and to investigate how these properties impact on gait mechanics. This was achieved by bringing together novel biomechanical and physiological approaches that have not previously been applied in the context of CHF syndrome. In particular, the outcomes of this thesis should improve the knowledge of triceps surae biomechanical properties and their link with strength and exercise capacity in patients with CHF, as well as the functional adaptations in the joint and lower limb mechanics as a result of CHF. The findings of the present work will provide new information and insights for clinicians and practitioners to design better strategies for disease treatment and rehabilitation that could lead to improvements in CHF patient quality of life and life expectancy.

Each of the experimental chapters focus on specific outcomes, which aims and hypotheses are detailed in Chapter 1.


Chapter 2 - Literature Review


Chapter 2 - Literature Review


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Chapter 2 - Literature Review


Chapter 2 - Literature Review


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Is the soleus a sentinel muscle for impaired aerobic capacity in heart failure?

ABSTRACT

Skeletal muscle wasting is well documented in chronic heart failure (CHF). This paper provides a more detailed understanding of the morphology behind this muscle wasting and the relationship between muscle morphology, strength and exercise capacity in CHF. We investigated the impact of CHF on lower limb lean mass, detailed muscle-tendon architecture of the individual triceps surae muscles (soleus, SOL; medial gastrocnemius, MG and lateral gastrocnemius, LG) and how these parameters relate to exercise capacity and strength. 11 CHF patients and 15 age-matched controls were recruited. Lower limb lean mass was assessed by dual energy X-ray absorptiometry (DXA) and the architecture of skeletal muscle and tendon properties by ultrasound. Plantarflexor strength was assessed by dynamometry. Patients with CHF exhibited ~25% lower combined triceps surae volume and physiological cross-sectional area (PCSA) compared to control subjects (p < 0.05), driven in large part by reductions in the SOL. Only the SOL volume and the SOL and MG PCSA were statistically different between groups after normalizing to lean body mass and body surface area, respectively. Total lower limb lean mass did not differ between CHF and control subjects, further highlighting the SOL specificity of muscle wasting in CHF. Moreover, the volume of the SOL and plantarflexor strength correlated strongly with peak oxygen uptake ($\dot{V}O_2$ peak) in patients with CHF. These findings suggest that the SOL may be a sentinel skeletal muscle in CHF and provide a rationale for including plantarflexor muscle training in CHF care.
3.1 Introduction

Impaired exercise capacity is a hallmark symptom of chronic heart failure (CHF) (Clark et al., 1996; Cohn et al., 1993). The important contribution of peripheral, as against cardiac, factors in the functional limitation which characterizes CHF has long been recognized and is reinforced by the finding that ejection fraction is poorly correlated with exercise capacity (Cohn et al., 1993). Moreover, it appears that dysfunctional skeletal muscles have a direct influence on the reduced exercise capacity evident in patients with CHF (Clark et al., 1996), and may contribute to a progressive deterioration in clinical status. These findings resulted in the ‘muscle hypothesis’ of CHF (Clark et al., 1996), which proposes that skeletal muscle dysfunction is a key factor responsible for reduced exercise capacity in this group.

Consistent with the ‘muscle hypothesis’, muscle wasting is common in patients with CHF (Clark et al., 1996; Mancini et al., 1992), especially in advanced disease. It has been found that limb muscle mass is correlated with reduced exercise capacity (peak oxygen uptake; $\dot{V}_2\text{peak}$) (Cicoira et al., 2001). Indeed, a recent analysis of 200 CHF patients (Fülster et al., 2013) revealed that reduced muscle mass is independently associated with lower absolute $\dot{V}_2\text{peak}$ (ml min$^{-1}$) when age, sex, ejection fraction and co-morbidities are accounted for, and that muscle mass is directly linked to disease progression.

Skeletal muscle wasting is therefore highly relevant to the progression and treatment of CHF. However, whilst an overall reduction in skeletal muscle mass in CHF is evident, a more detailed understanding of the morphology behind muscle wasting and the relationship between muscle morphology, muscle functional capacity (strength) and exercise capacity in CHF remains unexplored. For example, it is not known whether
loss of mass occurs evenly across lower limb muscles, or if certain muscles exhibit proportionately more/less muscle wasting. It is conceivable that predominantly slow-twitch muscles may be more markedly affected, given the reported increase in the proportion of type II fibers (Clark et al., 1996), the reduced blood flow and vascular transport capacity in CHF (Musch and Terrell, 1992) and reports that type I fiber atrophy is related to severity of the disease (Delp et al., 1997). It is also possible that muscle wasting is more prominent in the distal leg muscles, given their greater functional role in generating the mechanical work and power for walking and for the maintenance of posture (McGowan et al., 2009). The “architectural” changes that underlie the loss of muscle mass are also poorly understood. Loss of muscle mass may occur through decreased fiber length, with physiological cross-sectional area (PCSA) of the muscle remaining unaffected, or through loss of PCSA with fiber length remaining unaffected, or a combination of these. The distinction is important, given that whole-muscle architecture is a strong predictor of skeletal muscle mechanical capacity (e.g. strength) (Lieber and Fridén, 2000) and may also impact muscle energetics (Rubenson et al., 2006). Finally, the mechanical function of a muscle is also dependent on in-series tendon properties. Tendons function by storing and releasing elastic energy during locomotion, thereby influencing the contractile behavior of muscle, including work and power production and efficiency (Lichtwark and Wilson, 2007). Tendon mechanics are known to be closely associated with muscle wasting in ageing and chronic unloading (Narici and de Boer, 2011; Stenroth et al., 2012). To the best of our knowledge, a detailed structural analysis combining muscle volume, fiber length and pennation angle along with tendon properties has not previously been undertaken in human CHF, nor have they been analyzed in relation to muscle strength or exercise capacity in this group.
The aims of the present study were therefore: 1) To assess whether muscle wasting in CHF is constant across different muscles of the lower limb, in particular across synergist muscles known to differ in fiber composition and function; 2) To assess the detailed architecture of synergist skeletal muscle and tendon properties in patients with CHF, compared to healthy age-matched control participants (with similar adiposity and exercise activity); 3) To assess how skeletal muscle architecture, muscle strength and exercise capacity are related among CHF patients and control participants. To address these questions we analyzed lower limb lean mass and performed a detailed architectural assessment of the triceps surae (calf muscles) and Achilles tendon, along with functional strength measurements of this muscle group. The triceps surae [soleus (SOL), medial gastrocnemius (MG) and lateral gastrocnemius (LG)] were chosen because they offer a highly relevant comparison between synergist muscles known to possess different fiber types (predominantly slow twitch in SOL vs. mixed fibers in gastrocnemius) (Gollnick et al., 1974). Furthermore, the triceps surae muscles are functionally important, representing the main source of power during walking (McGowan et al., 2009) and have been shown to be a major locus of gait impairment in ageing (DeVita and Hortobagyi, 2000; Panizzolo et al., 2013) (Appendix).

3.2 Materials and methods

3.2.1 Subjects

We recruited 11 subjects with CHF (7 men, 4 women; NYHA class II-IV; EF = 30 ± 10 %). Criteria for exclusion included severe renal (creatinine>250mmol/l or eGFR < 30ml/min/1.73m²) or hepatic (bilirubin > 50mmol/l) dysfunction, unexplained anemia (hemoglobin < 100g/l) or thrombocytopenia (platelets < 100 * 10⁹/l), unstable angina or exercise-induced ischemia at low exercise levels, severe aortic stenosis, severe mitral or aortic regurgitation, or hypertrophic cardiomyopathy. All CHF subjects were medically
stable at the time of testing (no cardiac related admissions to the hospital in the three months prior to testing) and their medications (Table 3.1) were clinically optimized by medical staff (LD).

**Table 3.1.** Medications taken by the chronic heart failure participants involved in the present study.

<table>
<thead>
<tr>
<th>Category</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta blocker</td>
<td>Bisoprolol (Bicor)</td>
</tr>
<tr>
<td></td>
<td>Carvedilol</td>
</tr>
<tr>
<td></td>
<td>Nebivolol (plus nitrate)</td>
</tr>
<tr>
<td></td>
<td>Toprol XL</td>
</tr>
<tr>
<td>Diuretic (water pill)</td>
<td>Bumetanide</td>
</tr>
<tr>
<td></td>
<td>Eplerenone</td>
</tr>
<tr>
<td></td>
<td>Frusemide</td>
</tr>
<tr>
<td></td>
<td>Spironolactone</td>
</tr>
<tr>
<td>Other</td>
<td>Allopurinol (gout)</td>
</tr>
<tr>
<td></td>
<td>Amiodarone (antiarrhythmic)</td>
</tr>
<tr>
<td></td>
<td>Candesartan (ARB)</td>
</tr>
<tr>
<td></td>
<td>Clopidiogrel (anticoagulant)</td>
</tr>
<tr>
<td></td>
<td>Digoxin (glycoside)</td>
</tr>
<tr>
<td></td>
<td>GTN (vasodilator)</td>
</tr>
<tr>
<td></td>
<td>Pantoprazole (reflux)</td>
</tr>
<tr>
<td></td>
<td>Pravastatin (cholesterol)</td>
</tr>
<tr>
<td></td>
<td>Ramipril (ACE inhibitor)</td>
</tr>
<tr>
<td></td>
<td>Risedronate (osteoporosis)</td>
</tr>
<tr>
<td></td>
<td>Warfarin (anticoagulant)</td>
</tr>
</tbody>
</table>

The CHF group was attending supervised exercise training 2-3 times per week for ~ 1 hour per session (treadmill walking, cycle ergometry and resistance training) as part of their multidisciplinary care. The control group consisted of 15 healthy subjects recruited from the local community (9 men, 6 women). Eleven of these control subjects were recruited because they matched the CHF group for age, sex and adiposity and had
similar levels of exercise to those of the CHF patients, and were used in comparative statistics. The additional 4 controls were recruited specifically to validate the ultrasound measurement of muscle volume (a total of 6 participants were used for ultrasound validation). Subjects anthropometric characteristics are presented in Table 3.2. All subjects provided written informed consent prior to participating in the study. All procedures were approved by the Human Research Ethics Committee at The University of Western Australia (approval ID: RA/4/1/2533) and Royal Perth Hospital (approval ID: 2011/019).

**Table 3.2.** Anthropometric characteristics of chronic heart failure (CHF) and control groups. Data are means ± S.D.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [yr]</td>
<td>60.7±6.2</td>
<td>61.8±10.0</td>
</tr>
<tr>
<td>Height [m]</td>
<td>1.72±0.07</td>
<td>1.68±0.10</td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>69.9±8.6</td>
<td>72.8±18.0</td>
</tr>
<tr>
<td>Body mass index [kg/m²]</td>
<td>23.5±2.5</td>
<td>25.6±4.8</td>
</tr>
<tr>
<td>Body surface area [m²]</td>
<td>1.8±0.1</td>
<td>1.8±0.3</td>
</tr>
<tr>
<td>Leg length [m]</td>
<td>0.40±0.02</td>
<td>0.38±0.04</td>
</tr>
<tr>
<td>Fat body mass [kg]</td>
<td>17.8±7.2</td>
<td>21.3±11.3</td>
</tr>
<tr>
<td>Total lean body mass [kg]</td>
<td>49.2±8.3</td>
<td>49.0±10.6</td>
</tr>
<tr>
<td>Lower limb lean mass [kg]</td>
<td>8.3±1.5</td>
<td>7.6±1.9</td>
</tr>
</tbody>
</table>

### 3.2.2 Body composition measurements

Overall body composition was determined using dual energy X-ray absorptiometry (DXA) (Luna Prodigy, encore 2004, GE Medical Systems, Madison, WI, USA) on each subject including a measurement of total fat-free mass, total fat mass and bone mineral density. Lean mass for the lower limbs was computed separately by selecting a region
of interest from the greater trochanter to the pubic symphysis and included the leg and foot.

### 3.2.3 Triceps surae volume calculation

Due to the presence of internal cardioverter defibrillators in 10 of the 11 CHF subjects, rendering MRI unsuitable, muscle volume was computed using a three-dimensional ultrasound technique (3DUS) based on a combination of B-mode ultrasound imaging and 3D motion data (Barber et al., 2009). Subjects kneeled with their right leg in a custom-designed plastic water bath equipped with an adjustable metal footplate designed to secure the subject’s foot and to set the ankle joint angle to 0° (i.e. foot perpendicular to the tibia), with the knee in 10° flexion (near maximal extension). The water bath was maintained within a temperature range of 24-25°C to ensure that temperature-dependent variations in the speed of sound were minimized.

Ultrasound images (Telemed, Echoblaster128, Lithuania; 7.5 MHz linear array probe) and 3D marker trajectories of a probe-mounted marker cluster that defined the probe position in space (5-camera Vicon MX system, 250 Hz; Oxford Metrics, UK) were used to calculate muscle volume. The ultrasound and motion capture systems were synchronized using a 3V square pulse generated at the beginning of the images collection by the ultrasound device. The subjects’ lower leg was scanned longitudinally from the popliteal cave to the calcaneus. Three sweeps were performed to scan the whole triceps surae, starting at the lateral side and ending medially so that each sweep overlapped by a minimum of 20 mm. A customized MATLAB script (The MathWorks Inc., USA) was then used to combine ultrasound images and 3D position data and to convert them to Stradwin format (Medical Imaging Research Group, Cambridge University Engineering Department, UK) (Barber et al., 2009). Manual segmentation of
the muscles was performed in Stradwin software by a single investigator (FAP) on approximately 30 slices covering the length of the muscle group (inter slice gap ~ 15 mm). Muscle volumes for the three muscles under investigation (SOL, MG and LG) were computed. The segmentation procedure and analysis of muscle volumes was conducted in a blinded manner to avoid operator bias. To account for the possible variability during data collection and manual segmentation, the collection procedure described above was repeated three times for each subject and an average volume for the SOL, MG and LG computed.

We conducted comparisons between our ultrasound volume measurements and those obtained from axial plane MRI scans of the lower leg in a subsample (n = 6) of control subjects who were able to undergo this form of imaging. MRI scans were collected using a Magnetom Espree 1.5T scanner (Siemens, Erlangen, Germany) with the subjects’ leg position matched to that of the 3DUS experiment.

The reliability of muscle volume measurements for the medial gastrocnemius (MG) obtained using the 3D ultrasound technique has previously been validated against MRI (Barber et al., 2009). Nevertheless, no study has reported the accuracy of this measurement for the whole triceps surae muscle group or the lateral gastrocnemius (LG) and soleus (SOL) muscles. We therefore conducted comparisons between our ultrasound volume measurements and those obtained from axial plane MRI scans of the lower leg in a subsample (n = 6) of control subjects that were able to undergo this form of imaging. During the scan, subjects were lying in a supine position with ankle and knee joint at 0° and 10°, respectively, by means of plastic splint so as to match the water bath condition. MRI images were collected using a Magnetom Espree 1.5T scanner (Siemens, Erlangen, Germany) with a turbo spin echo pulse sequence of 697 ms.
repetition time, 11 ms time to echo, 148 Hz receiver bandwidth, 384x288 voxel image matrix and a 5 mm slice thickness. A total of 90-120 slices were taken for each subject depending on leg length to allow full anatomical coverage from just proximal of the patella to the Achilles tendon insertion on the calcaneous. MRI images were subsequently imported and analyzed directly in Mimics 8.11 (Mimics, Materialise, Ann Arbor, MI, USA); the segmentation process was conducted by a single investigator (FAP). The accuracy of the cross sectional area (CSA) of the Achilles tendon was also assessed by comparing ultrasound and MRI-derived images.

3.2.4 Fascicle length, pennation angle and muscle physiological cross-sectional area (PCSA)

The ultrasound probe described above was first placed over the middle of the selected muscle belly (SOL, MG, LG) following the guidelines of Rubenson et al. (2012), with its longitudinal axis aligned with the orientation of the fascicle. Fascicle length and pennation angle at neutral ankle (0°) and 10° knee flexion joint angles [approximating the angle where passive fascicle forces and net joint torque approach zero, (Arnold et al., 2010; Rubenson et al., 2012)] were calculated in ImageJ, as per Rubenson et al. (2012). For a better characterization of the muscle anatomy, fascicle lengths of the SOL and MG muscles were also calculated in the proximal, medial and distal locations of the muscle, with approximately 4 cm spacing between each location.

The assessment of regional muscle lengths was performed only on the MG and SOL in order to shorten the testing time and avoid discomfort of prolonged experimentation in the CHF group.
Chapter 3 - Muscle morphology in chronic heart failure

For each muscle, physiological cross-sectional area (PCSA) was calculated as:

\[
PCSA = \frac{\text{muscle volume} \times \cos \theta}{\text{fascicle length}}
\]  \hspace{1cm} (3.1)

Fascicle length and pennation angles used for the PCSA calculation were obtained at the mid-belly position of the muscle from images collected in the 3DUS water bath experiment. PCSA was expressed in $cm^2$ and subsequently normalized to body surface area (BSA) calculated as per (Mosteller, 1987).

### 3.2.5 Tendon length and cross-sectional area

A scaled subject specific musculoskeletal model in OpenSim 2.0.2 (Delp et al., 2007) was used to estimate the muscle-tendon unit lengths for each subject at $0^\circ$ of plantarflexion and $10^\circ$ knee flexion. A generic model (Arnold et al., 2010) was scaled using an inverse kinematics algorithm based on the position of 22 retroreflective spherical markers placed on anatomical landmarks and on functionally determined of joint centers (Besier et al., 2003). The algorithm minimizes the distance between the computed joint centers and anatomical reference points on the subject and the generic model. This procedure creates a scaled model whereby the dimensions (length, breadth) of each major skeletal element of the model is adjusted to match the participant’s skeletal morphology, thus allowing an estimate of subject-specific muscle-tendon unit lengths. Markers trajectories were collected by means of an 8-camera VICON MX motion capture system (Oxford Metrics, UK; 100 Hz) while subject was standing and while performing dynamic knee and hip motions to compute functional joint centers (Besier et al., 2003). Tendon length, defined as the free tendon plus aponeurosis (series elastic element), was subsequently calculated as the difference between the estimated muscle-tendon unit length of each of the SOL, MG and LG obtained by the scaled
OpenSim model and the estimated muscle length from ultrasound of the respective muscles (fascicle length *cos(0)) at an ankle angle of 0° (Fig. 3.1). An average of the three tendon lengths was obtained with this procedure.

Figure 3.1. Schematic illustration of the procedure for obtaining triceps surae tendon length. (a) Subject marker set used for motion capture of skeletal elements, (b) subject-specific scaled OpenSim model depicting the MG and SOL muscle tendon units, (c) ultrasound measurement of muscle fascicle length and pennation angle, (d) prediction of tendon length.

Using the same setup and settings described for the determination of 3D muscle volume, ultrasound images of Achilles tendon CSA were taken at the level of the medial malleolus to standardize the measurements across participants. To obtain tendon CSA, images were manually digitized in ImageJ.

3.2.6 Strength

Strength measurements were assessed by means of a robotic dynamometer (M3, Biodex, Shirley, NY, USA). Subjects sat with their right foot strapped to a custom built foot plate designed to align the centre of rotation of the ankle with that of the Biodex
and to minimize heel movement during the contraction. Subjects performed three maximum voluntary contractions with the knee extended (10° flexion) and the ankle dorsiflexed to 10°, approximating the angle at which the maximum torque is produced at the ankle joint (Arnold et al., 2010; Rubenson et al., 2012). The participant’s joint angles were obtained using a 3-camera OptiTrack motion capture system (NaturalPoint, Corvallis, OR; 100 Hz). A minimum of two minutes was observed between repetitions. Peak torque was calculated as the difference between the maximum torque during contraction and the torque measured at rest arising primarily from the weight of the rig and foot and small passive muscle forces (Rubenson et al., 2012). Analog data were collected using a CED data acquisition system running Spike2 V7 software (Micro1401-3; Cambridge Electronic Design, Cambridge, UK; 2000 Hz) and processed by means of a custom written program in MATLAB (The Mathworks Inc., Natick, MA). For each subject the trial with the highest peak torque was reported and used for comparisons.

3.2.7 Exercise capacity

Peak oxygen uptake ($\text{O}_2$ peak) was assessed using a purpose-designed incremental walking protocol on a motorized treadmill, starting at a speed 20% slower than each individual’s preferred speed with stepwise increases in speed and grade every 3 min until the participant reached volitional exhaustion. Indirect calorimetry was conducted using a Vmax Encore gas analysis system (Sensormedics, Yorba Linda, California), which enabled the measurement of expired gas concentrations and volumes. Absolute $\text{O}_2$ peak was expressed in ml min$^{-1}$ and normalized to body mass (ml kg$^{-1}$min$^{-1}$). All tests were performed by an exercise physiologist (AJM) experienced with undertaking cardiopulmonary exercise testing in patients with CHF (Maiorana et al., 2000).
3.2.8 Statistics

Differences in muscle volume (absolute and lean body mass-normalized) and PCSA (absolute and BSA-normalized) were analyzed using a two-way between groups ANOVA with group (CHF/control) and muscle synergist (SOL/MG/LG) as fixed factors. Differences in muscle fascicle length were analyzed using a two-way between groups ANOVA with group (CHF/control) and muscle synergist (SOL/MG/LG) as fixed factors for the mid-belly location. If a significant interaction effect between group and muscle was observed (p < 0.05) a one-way ANOVA with group as a fixed factor was performed for each muscle. A one-way ANOVA was used to determine differences in pennation angle, lower limb lean mass, strength and tendon properties between groups. ANOVAs were performed in SPSS using a Bonferroni post hoc analysis (IBM, Statistic 21, USA).

Linear regression was used to determine correlations between \( \frac{2}{2} \) peak and a) lower limb lean mass, b) muscle volume, c) muscle PCSA (total triceps surae as well as single muscles) and d) strength, and between the muscle PCSA and tendon cross-section. A correlation analysis was also performed between 3DUS- and MRI-derived measurements of muscle volume and the agreement between the two techniques was assessed using the limits of agreement method (Bland and Altman, 1986). Correlation coefficients (r) and significance level (p < 0.05) were determined in SPSS. Values presented are mean ± S.D. unless otherwise stated.

3.3 Results

3.3.1 Subject characteristics

No significant differences in age, height or mass or other anthropometric characteristics were observed between control and CHF groups (Table 3.1); likewise, no significant
differences in leg length (p = 0.14), adiposity (fat body mass) (p = 0.38) were found between control and CHF groups (Table 3.2). The peak was 15.5 ± 3.0 ml kg\(^{-1}\)min\(^{-1}\) in CHF and 35.6 ± 8.3 ml kg\(^{-1}\)min\(^{-1}\) in control (p < 0.0001). It should be noted that one CHF patient and two control participants did not undergo strength testing due to discomfort and in one CHF subject image degradation prevented an accurate SOL volume.

3.3.2 Lean mass and triceps surae muscle volume and PCSA

Total body lean mass and lower limb lean mass as assessed by DXA did not significantly differ between groups (p = 0.95 and p = 0.4, respectively) (Table 3.2). The two-way ANOVA determined a main effect of both group and muscle on absolute volume (p = 0.015 and p < 0.0001, respectively) and volume normalized to lean body mass (p = 0.007 and p < 0.0001, respectively). Post hoc analysis found that volumes collapsed over groups were statistically different between all muscles (p < 0.01). The combined muscle volume of the triceps surae was, on average, 27.2% higher in the control group compared to the CHF group (632 ± 169 ml vs. 497 ± 155 ml; Fig. 3.2a). A significant interaction effect between group and muscle was found in the lean mass-normalized volume (p = 0.03). Post hoc analysis revealed a significant difference in the normalized SOL muscle volume between the CHF and control group (20.5% smaller) but not in the MG or LG (Fig. 3.2b). A trend towards an interaction effect was also observed between group and muscle in the absolute muscle volume (p = 0.08) and was largely due to the small difference in LG volume between the CHF and control group (8.7% smaller) compared to the SOL (27.5% smaller) and the MG (20.9% smaller).

From the volume renderings of the triceps surae muscle group (Fig. 3.3a), an average difference of 20 ± 46 ml was observed between 3DUS and MRI techniques for the
combined triceps surae muscles. This difference amounted to \(2.5 \pm 6.3\%\) difference between measurements of the total triceps surae volume between the two techniques. The average difference in volume for each individual muscle was 4.2%. The overall correlation coefficient between 3DUS and MRI was 0.989 (Fig. 3.3b) and the 95% confidence interval ranged between +47 and -33 ml (Fig. 3.3c).

\[
\text{Figure 3.2. Muscle volume and physiological cross-sectional area (PCSA) values for the total triceps surae and individual plantarflexors. (a) Absolute volume, (b) lean body mass-normalized volume, (c) absolute PCSA, and (d) BSA normalized PCSA. Data are means} \pm \text{ S.D. The (\#) indicates a main effect (p < 0.05; ANOVA) of group (CHF/Control; volume and PCSA collapsed over individual triceps surae muscles). The (*) indicates a statistical difference (one-way ANOVA) between group (CHF/Control). A one-way ANOVA was performed only when an interaction effect between group and muscle were present. TS = triceps surae, SOL = soleus, MG = medial gastrocnemius, LG = lateral gastrocnemius, CHF = chronic heart failure).}
\]

No main effect of group on fascicle length or interaction effect between group and muscle were observed in any ANOVAs. However, a main effect of muscle on fascicle length was observed for the comparison in the distal muscle location (\(p = 0.04;\) Table 3.3). A main effect of both group and muscle were found for absolute PCSA (\(p <\)
A main effect of both group and muscle were also found for BSA-normalized PCSA (p < 0.0001 for both). Post hoc analysis found that PCSA collapsed across groups were statistically different between all muscles (p < 0.01). The PCSA of triceps surae in the control group was, on average, 23.7% higher than in the CHF group (Fig. 3.2c). Interaction effects between muscle and group were found in the absolute PCSA (p = 0.042) and in the BSA-normalized PCSA (p = 0.024). Post hoc analysis found significant differences between the SOL and MG absolute PCSA from the CHF and control groups but not for the LG. The average absolute PCSA of the SOL and MG were 22.2% and 25.3% smaller in the CHF group than in the control group, respectively (Fig. 3.2c). Similar results were observed for the BSA-normalized PCSA whereby only the SOL and MG were significantly different between groups (Fig. 3.2d).

### Table 3.3. Muscle fascicle length and pennation angle of the soleus, and medial and lateral gastrocnemius (GM and GL) in chronic heart failure (CHF) and the control groups.

<table>
<thead>
<tr>
<th></th>
<th>Control Soleus</th>
<th>CHF Soleus</th>
<th>Control MG</th>
<th>CHF MG</th>
<th>Control LG</th>
<th>CHF LG</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fascicle Length [mm]</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>43.4±10.6</td>
<td>38.8±14.1</td>
<td>39.8±11.1</td>
<td>42.3±6.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial</td>
<td>41.5±11.7</td>
<td>37.6±14.1</td>
<td>35.2±10.7</td>
<td>37.7±10.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Distal</td>
<td>31.9±9.9</td>
<td>30.8±10.8</td>
<td>34.4±9.5</td>
<td>40.9±8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pennation angle [°]</strong></td>
<td>23.9±4.7</td>
<td>20.8±3.5</td>
<td>28.2±7.8</td>
<td>22.0±6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24.2±9.3</td>
<td>29.5±6.4</td>
<td>24.2±9.3</td>
<td>22.0±6.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± S.D. (*) indicates a main effect of muscle (collapsed over both CHF and control groups) in the distal region of the muscle (p < 0.05).

A significant correlation between total lean lower limb mass and absolute peak 2 (ml min⁻¹; r = 0.82, p = 0.004; Fig. 3.4a) and total triceps surae volume and absolute peak 2 (ml min⁻¹; r = 0.93, p < 0.0001; Fig. 3.4b) was observed in the CHF group. When analyzed on an individual muscle level, only the SOL body-mass normalized volume was found to correlate with the body mass normalized peak 2 (ml kg⁻¹min⁻¹)
in the CHF group ($r = 0.72, p = 0.018$; Fig. 3.4d). The control group exhibited weaker correlations between lean lower limb mass and absolute peak and triceps surae volume and absolute peak ($r = 0.63, p = 0.06$ and $r = 0.58, p = 0.08$, respectively; Fig. 3.4a,b). Unlike the CHF group, body mass normalized data were not correlated in the control group (Fig. 3.4c,f). Muscle PCSA was independent of peak in all muscles in both the CHF and control groups.

Figure 3.3. 3D ultrasound and MRI-derived muscle volume. (a) Example of 3D volume rendering of the triceps surae created with 3D ultrasound (3DUS) and with MRI from the same individual. SOL = soleus, MG = medial gastrocnemius, LG = lateral gastrocnemius. (b) The correlation between volume calculation using 3DUS imaging and MRI (equation $y = 0.998x + 9.365, r = 0.988, p < 0.001$). The solid line represents the line of best fit and the dotted lines represent the 95% prediction interval. Note: data represent three volume measurements per subject (SOL, MG, LG). (c) Bland-Altman plot of the difference between 3DUS and MRI vs. the average of the MRI and 3DUS values. The horizontal lines on the plot represent the mean difference between MRI and 3DUS and the upper and lower 95% limits of agreement.
Figure 3.4. Relationship between \( \dot{V}O_{\text{peak}} \) and leg muscle size. Relationship between absolute \( \dot{V}O_{\text{peak}} \) (ml min\(^{-1}\)) and (a) lower limb lean mass (kg) and (b) triceps surae volume (ml). Relationship between body mass normalized \( \dot{V}O_{\text{peak}} \) and individual muscle volume in (c) the combined triceps surae, (d) soleus, (e) medial gastrocnemius, and (f) lateral gastrocnemius. Chronic heart failure subjects are displayed in grey triangles (▲) and control subjects in open circles (○).

3.3.3 Tendon architectural parameters

Achilles tendon CSA was significantly smaller in CHF patients compared to the control group (59.2 ± 16.1 mm\(^2\) vs. 73.4 ± 20.0 mm\(^2\) respectively, \(p = 0.046\)). However the estimate of tendon length was not different between the CHF (27.4 ± 2.7 cm) and
control (28.6 ± 2.3 cm) groups (p = 0.2). A trend towards a positive correlation between tendon CSA and the triceps surae PCSA existed for CHF (r = 0.65, p = 0.06) but not for the control group (r = 0.35, p = 0.3).

![Figure 3.5](image)

**Figure 3.5.** Peak plantarflexor torque values and their relationship with peak. Peak plantarflexor torque normalized to (a) body mass and (b) triceps surae PCSA. Data are means ± S.D. (c) Relationship between body mass-normalized peak and body mass-normalized peak plantarflexor torque. Note one additional subject was added to the CHF group that was tested in a parallel study (the correlation based on 11 participants is r = 0.762, p < 0.01). Chronic heart failure subjects are displayed in grey triangles (▲), and control subjects in open circles (○).

### 3.3.4 Strength measurements

Body-mass normalized peak torque (N m kg⁻¹) was 13.3% greater in the control group vs. the CHF group, although this difference was not statistically different (p = 0.3; Fig. 3.5a.). Peak torque normalized by the triceps surae PCSA (N m cm⁻²) was 3.6% smaller...
(non-significant) in the control group vs. the CHF group (p = 0.8; Fig. 3.5b). A strong correlation between body-mass normalized peak torque and peak was found in the CHF group (r = 0.75; p < 0.01) but there was no such relationship in the control group (r = 0.25; p = 0.5; Fig. 3.5c).

3.4 Discussion

There is growing evidence that skeletal muscle is adversely affected in patients with CHF and that it contributes to the reduced exercise capacity characteristic of this condition. Skeletal muscle wasting, in particular, has been shown to be strongly correlated with exercise capacity and disease progression in CHF (Fülster et al., 2013), yet little data exists on the specifics of wasting between muscles, or the involvement of whole muscle-tendon morphology in muscle wasting. The present study investigated lower limb lean mass and detailed muscle-tendon architecture and strength of the triceps surae muscles (calf muscles), an important functional muscle group for maintaining posture and for locomotion (McGowan et al., 2009). We assessed architectural and strength differences between CHF and control subjects, as well as relationships with exercise capacity. Whilst there were no detectable differences in lower limb lean mass as measured by DXA, a clear reduction in the size of the SOL and to a lesser extent the MG, and a reduction in Achilles tendon CSA, were observed in the CHF compared with control subjects. However, these differences did not translate to statistically significant differences in strength. Furthermore, SOL muscle volume and plantarflexor strength were found to strongly correlate with exercise capacity in the CHF group, but not in the control group. These findings offer the possibility that the distal lower limb muscles, and the SOL in particular, may be key skeletal muscles determining exercise capacity and function in CHF patients.
3.4.1 Is muscle wasting in chronic heart failure muscle-specific?

Whilst several studies have identified generalized muscle wasting in CHF (Fülster et al., 2013; Mancini et al., 1992), little is known about the specificity of muscle loss. Interestingly, our 3DUS data revealed that muscle wasting was not uniform across the three muscles of the triceps surae. Although the overall reductions in triceps surae muscle volume and PCSA observed in this study are comparable to one previous MRI-based report assessing triceps surae volume in CHF (Mancini et al., 1992), our novel observation is that the reductions in these parameters are seen only in the SOL, and (with respect to volume) to a somewhat lesser extent in the MG. The lack of atrophy in the LG might be related to a difference in muscle mechanical function (Héroux et al., 2014), or possibly a consequence of its relatively small size and limited contribution to posture (Héroux et al., 2014). The more pronounced reduction in muscle volume in the SOL compared to the MG in CHF patients (Fig. 3.2; in particular when normalized to lean body mass) could suggest that muscle wasting is sensitive to muscle aerobic capacity and fiber composition, given the known differences in type I vs. type II fiber distribution between these muscles (Gollnick et al., 1974). Greater CHF-induced atrophy in type I compared to type II fibers has been reported for the human vastus lateralis (Williams et al., 2004), although the opposite has been found in other work reporting fiber type-specific fiber atrophy in CHF (Mancini et al., 1989). In a rat model of CHF, type I fiber atrophy has been correlated to the severity of the disease (Delp et al., 1997) and, recently, significant atrophy in the rat soleus (predominantly type I fibers) but not the plantaris has been observed (Moreira et al., 2013). Alternatively, the SOL volume may be more affected in CHF due to reduced blood flow (Musch and Terrell, 1992), or possibly because the SOL has been shown to provide the majority of mechanical work among the lower limb muscles during walking (McGowan et al.,
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2009) and thus the effect of CHF may be augmented in this functionally important muscle.

Whereas ultrasound-based measurements of triceps surae volume revealed marked reduction in muscle size (~ 30%), DXA measurements of total lower limb lean mass were, surprisingly, only slightly lower (non-significant) in CHF patients compared to the control group. The ~ 0.7 kg (7.8%) reduction in total lower limb lean mass is similar to that reported by Toth et al. (2010), who also found non-significant differences in lean mass between CHF and an age-matched control group. When taken together, the high-fidelity 3DUS of the calf muscles and whole-limb DXA recording suggest that the SOL and MG may be particularly prominent, and possibly early sites of muscle loss in CHF. Indeed, after normalizing muscle volume to lower limb lean mass a significant difference in the triceps surae volume remains between the CHF and the control group indicating proportionately greater wasting in these muscles. Fülster et al. (2013) found that 20% of CHF patients (NYHA class II-III) exhibited muscle wasting compared with a healthy reference group. Using their criteria, only 30% of participants in the current study would be diagnosed with generalized lower limb muscle wasting, yet these patients exhibited muscle wasting in the SOL and MG. It seems likely that muscle-specific changes in the triceps surae may have gone undetected in previous analyses of overall lower limb lean mass using DXA. This may relate to the relatively small size of the triceps surae muscles (~500g) compared to the lower limb lean mass (~ 7.5 kg) and the lower sensitivity of DXA (~300g sensitivity in detecting muscle mass; (Levine et al., 2000)] compared to 3DUS (~35 ml sensitivity; Fig. 3.3c).

We are unaware of previous studies describing muscle-specific atrophy in CHF. Our data offer the intriguing possibility that calf muscle volume, and the SOL in particular,
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may be a sentinel muscle for early muscle wasting in this disease. Greater distal muscle atrophy has previously been documented in ageing (Mitchell et al., 2012) and in other conditions of unloading, such as bed rest (LeBlanc et al., 1992; Narici and de Boer, 2011) and space flight (Narici and de Boer, 2011). However, in these studies a more prominent reduction in total lower limb lean mass was observed compared to the present study. We propose that a distal-to-proximal gradation in muscle loss may occur due to the prominent role of the distal muscles in body support and propulsion (McGowan et al., 2009). Muscle wasting may be amplified, especially in the major plantarflexor muscles compared to the other leg muscles, including the LG (Héroux et al., 2014), that have a lower contribution to these functions in activities of daily living, and in particular walking. The SOL and MG play a key role in supporting the trunk during single-leg stance and pre-swing (Neptune et al., 2001) and the SOL is fundamental for the horizontal acceleration in the late stance phase, which is necessary to provide propulsion to the trunk (Neptune et al., 2001). Because of their importance to gait mechanics, it can be argued that a ~25% reduction in normalized muscle volume and PCSA in the SOL will have a negative impact on function. It is reasonable to hypothesize, therefore, that alterations in gait and movement mechanics and fatigue in CHF will result as a consequence of the reduction in size of the triceps surae, and especially the SOL.

3.4.2 The relationship between muscle-tendon architecture and wasting in chronic heart failure

Muscle architecture analyses revealed that the loss of muscle volume is primarily a result of a loss of muscle PCSA, with muscle length and pennation angles remaining relatively unchanged. Age-related muscle wasting, on the other hand, has been reported to result, in part, from a reduction in muscle length (Stenroth et al., 2012), although
recent work suggests that the active muscle lengths in the MG may be unaffected by age
(Barber et al., 2013). Our findings therefore suggest a pattern of CHF muscle atrophy
that is distinct from that associated with aging per se. Given that volume (Josephson,
1999) and PCSA (Lieber and Fridén, 2000) influence a muscle’s mechanical work and
force capacity, respectively, our findings suggest a biomechanical basis for impaired
function of the calf muscles in CHF patients. It was somewhat surprising, therefore, that
a more pronounced reduction in strength was not observed between the CHF and
control groups in the present study. Interestingly, a lack of statistically significant
differences in ankle strength is consistent with previous measurements of voluntary
plantarflexor torque in CHF (Harridge et al., 1996), and possibly reflect variability in
voluntary torque measurements arising from factors such as neural drive, co-
contraction, muscle moment arm lengths (Baxter and Piazza, 2014) and/or fiber
operating lengths (Rubenson et al., 2012). Despite the lack of statistical differences in
absolute strength, it is also worth noting that the differences between groups are
minimized when normalized for PCSA.

Similar to other studies addressing triceps surae muscle loss (LeBlanc et al., 1992), the
current study also showed that the Achilles tendon undergoes a concurrent reduction in
cross-sectional area in CHF patients. Tendon remodeling occurs in response to the
mechanical loading stimulus (Smith et al., 2013) and it follows, therefore, that the
reduced triceps surae muscle size in the CHF patients may be associated with reduced
habitual in vivo muscle forces.
3.4.3 The relationship between muscle architecture, strength and exercise capacity in chronic heart failure

Previous studies have reported significant correlations between estimates of skeletal muscle mass and functional capacity in CHF (Fülster et al., 2013; Mancini et al., 1992). Indeed, several studies have reported correlations between total lower limb lean mass or the size of specific muscle groups and \( \dot{V}_\text{O}_2 \text{peak} \) (typically expressed as absolute \( \dot{V}_\text{O}_2 \text{peak} \text{ ml min}^{-1} \)). Our findings suggest, for the first time, that a principal reason for such leg muscle mass correlations may relate specifically to the relationship between SOL muscle volume and \( \dot{V}_\text{O}_2 \text{peak} \). We observed correlations between SOL volume and \( \dot{V}_\text{O}_2 \text{peak} \) (absolute and body mass normalized) in CHF, but no such correlation between \( \dot{V}_\text{O}_2 \text{peak} \) and the MG or LG. Whilst similar correlations between the combined calf muscle volume and \( \dot{V}_\text{O}_2 \text{peak} \) have been observed (Mancini et al., 1992), our finding reinforces the suggestion that the SOL muscle may be of particular importance as a determinant of functional capacity in CHF patients. This may relate to the SOL being an important determinant of aerobic potential due to its high oxidative capacity (Gollnick et al., 1974), as well as its key functional role in gait (McGowan et al., 2009). Indeed the size of the gastrocnemius, a muscle known to have a lower oxidative capacity (Gollnick et al., 1974), did not correlate with \( \dot{V}_\text{O}_2 \text{peak} \) in our study. It should be noted that similar to the present study, Harrington et al. (1997) reported correlations between both quadriceps and thigh cross sectional area and absolute \( \dot{V}_\text{O}_2 \text{peak} \) in CHF. However, the strong correlations observed in this study were likely due, in part, to the high covariance between body mass and muscle size and absolute \( \dot{V}_\text{O}_2 \text{peak} \). When thigh and quadriceps cross sectional area were correlated to body-mass normalized \( \dot{V}_\text{O}_2 \text{peak} \), relatively weak correlations were observed compared to that of the SOL in the present study. Furthermore, it should be noted that Harrington et al. (1997) assessed quadriceps anatomical cross sectional area from a single axial plane...
image, and thus may not represent the average physiological cross sectional area of the muscle (typically requiring volume and fiber length and pennation angle measurements). Interestingly, our results also suggest that it may be reduced muscle volume *per se*, more so than PCSA that is linked with reduced $\dot{\gamma}$ peak in CHF.

The link between the SOL function and $\dot{\gamma}$ peak in CHF is also supported by the strong correlation between plantarflexor torque, for which the SOL is the major contributor, and $\dot{\gamma}$ peak in these patients. We are unaware of previous studies correlating plantarflexor strength and $\dot{\gamma}$ peak in CHF, although the strength of quadriceps has been previously shown to correlate with $\dot{\gamma}$ peak in CHF (Minotti et al., 1991). That both the SOL muscle size and plantarflexor strength are correlated to $\dot{\gamma}$ peak only in the CHF group supports the notion that peripheral determinants of $\dot{\gamma}$ peak may be present to a greater extent in CHF patients compared to healthy adults (Clark et al., 1996).

### 3.4.4 Limitations

The present study compared CHF patients to control subjects matched for age and with similar adiposity. We cannot rule out the possibility that reduced activity levels in the CHF patients contributed, at least in part, to the reduced muscle and tendon size and the relationship between muscle volume, strength and exercise capacity observed in this study. There are several factors, however, that lead us to conclude that any influence of activity levels on our findings is likely to have been minimal. Firstly, all CHF patients were engaged in an exercise rehabilitation program and were undertaking supervised exercise 2-3 times weekly, similar to the activity of the control participants. Secondly, our finding that the reduction in muscle volume and PCSA as well as the relationship between muscle volume and $\dot{\gamma}$ peak is highly muscle specific would not be expected
as a result of disuse alone, which should logically be expressed generically. For example, while disuse studies have found muscle loss in distal leg muscles (LeBlanc et al., 1992), they also reported loss in the total lower limb lean mass (LeBlanc et al., 1992). In contrast the present study only found a clear loss of muscle in the SOL, with no differences in total lower limb lean mass from DXA. Thirdly, the SOL has been reported to have a smaller reduction in size (or no reduction) compared to the MG and LG as a result of ageing/disuse (Barber et al., 2013; Morse et al., 2005), whereas in the present study on CHF the SOL presents the largest reduction in size.

3.4.5 Conclusion

The SOL is a key muscle involved in postural control and locomotion. It is classically considered to possess a higher proportion of type I muscle fibers than other muscles of the triceps surae and lower limb. Our findings indicate that SOL wasting is particularly marked in CHF, even when compared to other lower limb muscles in these individuals. Furthermore, the SOL volume correlated strongly with exercise capacity, whereas other individual leg muscle volumes did not, and is largely responsible for the correlation between plantarflexor strength and peak. For these reasons we propose that the SOL is a key muscle reflecting loss of function and exercise capacity in CHF, and may thus be a sentinel skeletal muscle in CHF patients. Finally, our results offer an evidence-base for including calf muscle-specific exercise training, as an important component of whole body training, to help restore functional capacity in CHF.
References


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Chapter 3 - Muscle morphology in chronic heart failure


Voluntary active and passive human soleus muscle forces are dictated by muscle size in chronic heart failure

This chapter is based on the paper: Panizzolo FA, et al. (2014). Voluntary active and passive human soleus muscle forces are dictated by muscle size in chronic heart failure. In review in: Medicine & Science in Sports & Exercise.
Chapter 4 - Active and passive forces in chronic heart failure

ABSTRACT

Reduced skeletal muscle strength has been linked to the compromised exercise capacity characterizing chronic heart failure (CHF). However, it is not clear if the reduction in peak voluntary strength in CHF is associated primarily with reduced muscle size. This shortcoming might stem from the reliance on net joint moment-based measurements that reflect activity in all synergist and antagonist muscles crossing a joint. Therefore, the aim of this study was to investigate the active voluntary specific force (force per muscle physiological cross sectional area, PCSA) and the specific passive force in a single muscle, the soleus (SOL), of CHF patients and age- and physical activity-matched control participants. The SOL was selected as it is a key muscle for postural control and locomotion, and its muscle architecture is affected by CHF. Voluntary active and passive SOL forces and PCSA were obtained by means of a novel approach combining experimental data (dynamometry, electromyography, muscle imaging) with a musculoskeletal model. We found reduced absolute peak voluntary SOL forces (~25%) as well as reduced passive forces (~30%) (at equivalent levels of muscle stretch) in CHF vs. healthy individuals. These differences were eliminated when force was normalized by PCSA, indicating that reduced force output may be strongly associated with muscle size. Nevertheless, the fascicle lengths at which the peak active forces were generated ($L_0$) as well as the passive fascicle lengths were shorter in CHF vs. controls, possibly leading to altered performance of the SOL in functional tasks such as gait. These findings raise the importance of exercise rehabilitation targeting an increase in muscle hypertrophy, and for the calf muscles, exercise that promotes muscle lengthening.
4.1 Introduction

Growing evidence suggests that skeletal muscle contributes to the limited functional capacity that characterizes chronic heart failure (CHF) and to the progression of the disease. Several studies of patients with CHF have reported a reduction in voluntary strength (net joint moments) in the upper and lower limbs (Harrington et al., 1997; Lipkin et al., 1988; Magnusson et al., 1994; Minotti et al., 1993; Sunnerhagen et al., 1998; Toth et al., 2010, 2006), compared to healthy age-matched individuals. These abnormalities have been reported to be closely related to the reduced aerobic exercise capacity exhibited in CHF (Harrington et al., 1997; Panizzolo et al., 2014b; Volterrani et al., 1994), and may, in part, determine the severity of the CHF syndrome.

Importantly, however, it is still not clear if the reduction in voluntary strength is associated primarily with the reduced muscle size that is known to occur in CHF (Fülster et al., 2013; Mancini et al., 1992; Panizzolo et al., 2014b). Several studies that have measured both voluntary strength and muscle size indicate that muscle size alone cannot account for the loss of strength in the quadriceps (Harrington et al., 1997; Toth et al., 2010, 2006). These findings are consistent with reports of lower muscle fiber specific tension (single muscle fiber force per cross sectional area) in diaphragm muscle of CHF-affected rats (Van Hees et al., 2007) and type I fibers from the vastus lateralis of CHF patients (Miller et al., 2009), reflecting a loss of fiber myosin content. However, specific tension in type I vastus lateralis fibers in CHF patients has been reported to be unaffected when a more realistic in vivo temperature was used in the muscle preparations (Miller et al., 2010). In line with this latter isolated fiber study, other functional studies in patients with CHF (Magnusson et al., 1994) have concluded that reductions in muscular strength are commensurate with the loss of muscle size, suggesting that atrophy is indeed the principal factor leading to the functional deficits.
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The lack of clarity surrounding the determinants of voluntary skeletal muscle strength in CHF may stem, in part, from the methods employed in the studies. The aforementioned investigations relied on isometric net joint moments generated under the action of synergist and antagonist muscle groups, which represents a global measurement of gross joint function. However, it is well known that several factors can lead to dissociation between net joint moment and the actual underlying muscle strength (force capacity) (Pinniger et al., 2003; Rubenson et al., 2012). Among these are force sharing between synergist muscles (Jamison and Caldwell, 1993), co-contraction of antagonist muscles (Park et al., 1999), muscle moment arms lengths (Baxter and Piazza, 2014), muscle fiber orientation (pennation angles) and muscle fiber operating lengths, the latter affecting force capacity via force-length characteristics (Rubenson et al., 2012). To the best of our knowledge, there have been no assessments of voluntary strength production in CHF at a single muscle level that account for these factors. As such, a detailed understanding of how voluntary muscular strength is affected in CHF is lacking.

Further insight into the determinants of skeletal muscle force in CHF and whether muscle size dictates reductions in force production can come from analyses of passive muscle forces. Passive forces in cardiac muscle are altered in CHF (van der Velden, 2011), as well as in diaphragm skeletal muscle (van Hees et al., 2010). The differences in passive forces from a healthy population are thought to result from alterations in the intrinsic properties of the muscle that go beyond the overall size of the muscle, most notably in the titin molecule content and structure (Hein et al., 1994; Wu et al. 2002). Surprisingly, as far as we are aware, only one study conducted in a mouse model of CHF (van Hees et al., 2010) has investigated passive forces in appendicular skeletal muscle in CHF. This study reported unaltered passive forces in the soleus (SOL) muscle.
Coupling *in vivo* measurements of both active and passive forces at a muscle level in humans can provide important information for understanding the mechanisms behind the alterations in voluntary skeletal muscle strength associated with CHF. The aim of this study was to investigate the peak voluntary active specific force (force per muscle physiological cross sectional area, PCSA) and specific passive force in the SOL muscle of CHF patients and age- and physical activity-matched control participants. The SOL was selected because it permits an estimation of force production in a single muscle, thus accounting for musculoskeletal morphology and architecture and muscle force-length properties, and minimizing the need to assess co-contraction (Rubenson et al., 2012). Furthermore, SOL has been identified as a primary muscle in which muscle loss occurs in CHF (Panizzolo et al., 2014b) and its size is strongly correlated with the reduced exercise capacity present in CHF (Panizzolo et al., 2014b). We hypothesized that there would be a reduction in the peak active voluntary force and in passive force in CHF patients, compared to a healthy population. We further hypothesized that active and passive force reductions would persist after normalizing for the muscle PCSA (specific force), commensurate with reports of alterations in intrinsic muscle factors, in particular the reduced myosin content in type I fibers (Van Hees et al., 2007; Miller et al., 2009; Toth et al., 2005), and taking into consideration the high percentage of type I fibers in the SOL.
4.2 Materials and methods

4.2.1 Subjects

Patients with CHF and age- and physical activity-matched control participants who were free from other musculoskeletal disorders and lower limb musculoskeletal injuries were recruited for this study. The CHF group included 12 participants (7 men, 5 women) in the class II-IV of the New York Heart Association (NYHA) classification with an ejection fraction of $30.5 \pm 9.6\%$. Criteria for exclusion were described in Chapter 3. The control group was composed of 12 healthy participants recruited from the local community (8 men, 4 women). Ten CHF and all the 12 control participants were also involved in the study described in Chapter 3. The CHF group underwent regular exercise activity 2-3 times per week for $\sim 1$ hour per session (treadmill walking and resistance weight training) as part of their standard patient care. The control participants underwent similar levels of weekly exercise. Because voluntary active strength is known to be affected by age and gender (Lindle et al., 1997) we also performed an analysis aimed to minimize variability arising from age and gender heterogeneity between groups (performed after the initial statistical analysis). To allow for an optimized match between CHF and control participants we paired participants of the same sex, excluding participants who could not be paired within 7 years of age and ensuring that for any age difference greater than 3 years, the older participant was the control subject. This resulted in an average age difference of $2.8 \pm 5.9$ yrs (median difference 2.0) and excluded the largest gender-specific age discrepancies (average $4.8 \pm 11.9$ yrs, median difference 8.3). A complete overview of the anthropometric characteristic of the two groups is reported in Table 4.1. All participants read and signed an informed consent prior to participating in the study and all of the procedures were approved by the Human Research Ethics Committee at The University of Western
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Australia (approval ID: RA/4/1/2533) and Royal Perth Hospital (approval ID: 2011/019).

Table 4.1. Subject characteristics. Full list of participants involved in the study (a) and participants (n = 6) involved in the pair-wise comparison for the calculation of peak voluntary active force (b). Data are means ± S.D.

(a)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age [yr]</th>
<th>Height [m]</th>
<th>Weight [kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>62.7±5.6</td>
<td>1.73±0.06</td>
<td>69.7±8.5</td>
</tr>
<tr>
<td>CHF</td>
<td>63.5±10.9</td>
<td>1.68±0.10</td>
<td>67.9±14.8</td>
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</table>

(b)

<table>
<thead>
<tr>
<th>Group</th>
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<th>Height [m]</th>
<th>Weight [kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>61.2±5.3</td>
<td>1.74±0.06</td>
<td>70.8.7±9.9</td>
</tr>
<tr>
<td>CHF</td>
<td>58.3±8.1</td>
<td>1.65±0.12</td>
<td>72.6±14.8</td>
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</table>

4.2.2 Passive force estimates

The procedures used to estimate passive and active SOL forces were similar to those adopted previously (Rubenson et al., 2012), but with an additional method to assess the synergist muscle force contributions for each active trial. Passive moments were recorded with the participants sitting upright with their right foot and ankle positioned in a dynamometer (Biodex M3, Shirley, NY, USA) and with the knee positioned at 120° of flexion to mitigate the force contribution of the gastrocnemius muscles (Maganaris, 2001). The net passive ankle joint moment ($M_P$) was computed by subtracting the moment generated by the Biodex rig and the weight of the foot, expressed as a percentage of body mass following (de Leva, 1996).
In passive trials surface electromyography (EMG) from the tibialis anterior (TA), the medial and lateral gastrocnemius muscles (MG, LG, respectively) and the SOL were recorded (Noraxon wireless system, Scottsdale, AZ, USA; 2000 Hz) to ensure the muscle crossing the ankle remained inactive. For this purpose, disposable 40 mm Ag/AgCl electrodes (Cleartrace, Conmed, Utica, NY) with an inter electrode distance of 30 mm, were placed on the muscle belly of the muscles above mentioned. For each trial, real-time root-mean-square (RMS) waves of the muscles' activity were computed from the EMG signals (incorporating DC offset; Spike2 V7 software; Cambridge Electronic Design, Cambridge, UK) (Rubenson et al., 2012). Soleus fascicle lengths and pennation angle were recorded using dynamic B-mode ultrasound (Telemed, EchoBlaster128, Lithuania; 25 Hz capture rate; 7.5 MHz 60 mm linear array probe) following the placement and image analysis procedures outlined previously (Panizzolo et al., 2013; Rubenson et al., 2012). Simultaneous measurements of ankle joint flexion/extension angles were made using a portable 3D motion capture system (Optitrack, Corvallis, Oregon, US; 100 Hz). The net joint moment, EMG, ultrasound images and joint angles were recorded synchronously (Micro1401-3; Cambridge Electronic Design, Cambridge, UK; 2000 Hz) by means of a 5 volt TTL-pulse as the ankle was cycled through its full range of motion [the most dorsiflexed and most plantarflexed position tolerated by the participant, corresponding to the maximal fascicle length ($L_{max}$)] at a constant speed of 5°/s over three consecutive cycles. Three initial warm-up cycles were performed prior the recording of any measurements.

Moment data recorded by the Biodex were filtered using 4th-order zero-lag 2 Hz low-pass Butterworth filter (MATLAB, The MathWorks Inc., USA). We estimated that the passive fascicle force reached zero when the $M_p$ over the ankle joint’s range of motion passed through zero (Silder et al., 2007) (Fig. 4.1). To detect the inflexion point...
in $M_p$ where net dorsiflexion and plantarflexion moment converge on zero we first fitted the joint angle vs. $M_p$ data with a 5th-order polynomial based on visual inspection of the data and subsequently computed the first order derivative of this function (MATLAB, The MathWorks Inc., USA) (Fig. 4.1). The corresponding filtered muscle fascicle length at the $M_p$ inflexion angle (Fig. 4.1) was defined as the length where passive SOL forces are first generated ($L_{slack}$). In some instances the inflexion point was slightly above or below zero moment (< 1.5 Nm or ~7% of the peak passive moment). This can occur if the weight of the leg transmits a small moment about the Biodex axis (i.e. small misalignment of ankle center of rotation) or if the moment predicted from weight of the foot has small errors. In these cases the passive moment data was corrected for the offset.

To calculate the passive force ($F_p$) the $M_p$ was downsampled to match the ultrasound data capture rate and divided by each participant's joint angle-specific Achilles moment arm $r$ (see below) and by the cosine of the pennation angle $\theta$ of the corresponding frame:

$$F_p = \frac{M_p}{r \cdot \cos \theta}$$  \hspace{1cm} (4.1)
Figure 4.1. Typical joint angle vs. passive ankle joint moment ($M_p$) curve from one participant. Measured valued (black), 5th-order polynomial fit (red) and inflexion point (blue).

These data were used to construct absolute and normalized passive SOL force-length (F-L) curves for each subject. Absolute passive F-L curves used the measured $F_p$ in Newtons and fascicle lengths ($L$) in mm. Normalized passive F-L curves were created by dividing each participant $F_p$ by his SOL PCSA (Equation 4.5) and normalizing $L$ to $L_{stack}$, [i.e. $(L - L_{stack}) / L_{stack}$]. To enable comparison of absolute $F_p$ between groups, $F_p$ was determined at percent fascicle lengths of 20%, 40%, 60%, 80% and 100% of the maximum fascicle stretch ($L_{max}$), where percent fascicle lengths were defined as $[(L - L_{stack}) / (L_{max} - L_{stack})] * 100$. Passive fascicle stiffness was computed for each participant as the slope of the absolute F-L curves between $L_{stack}$ and 40% stretch ($k_1$) and between 60% - 100% stretch ($k_2$) and between the strain values (0-40% and 60-100%) on the normalized passive F-L curves ($k_{1norm}$ and $k_{2norm}$).
4.2.3 Achilles tendon moment arm

Participant-specific Achilles moment arm ($r$) data were established experimentally on a separate testing day, following the method outlined by (Manal et al., 2010). In this method, B-mode ultrasound (Telemed, EchoBlaster128, Lithuania; 25 Hz) was used to capture Achilles tendon images in the sagittal plane from the participants while their foot was cycled passively across its range of motion in a Biodex dynamometer (M3, Biodex, Shirley, NY, USA). The ultrasound probe (7.5 MHz, 60 mm field of view, linear array probe) was placed longitudinally above the Achilles tendon using a stand-off gel pad (Aquaflex, Parker, NJ, USA). Simultaneously, the trajectories of two retro-reflective markers mounted on the ultrasound probe were recorded by means of a 3D motion capture system (Optitrack, Corvallis, Oregon, US; 100 Hz). Additional anatomical landmarks (first metatarsal, calcaneus, medial malleoli and knee medial condyle) were tracked to calculate the ankle flexion/extension joint angle. A 2D customized graphical interface was developed in Matlab to display both the ultrasound images and the ultrasound probe and the medial malleoli markers in the same coordinate system. The line of action of the Achilles tendon was digitized in this common coordinate system and the moment arm was computed as the perpendicular distance between the tendon line of action and the medial malleoli, which was used as an estimate of the ankle joint center. This procedure was performed at 10 ankle joint angles that spanned the joint’s range of motion. A 10-point moment arm - joint angle curve was obtained for each participant by fitting the moment arm - joint angle data with a 5th-order polynomial, which provided the best fit between the moment arm vs. joint angle relationship based on visual inspection.
4.2.4 Active voluntary force estimates

In the same testing session and with the same experimental set-up as the passive forces estimates, the participants performed maximal voluntary isometric plantarflexion contractions ($MVC_{pt}$) to assess the peak voluntary force capacity of the SOL. The $MVC_{pt}$ were performed initially at $10^\circ$ dorsiflexion because this has been shown to be the angle where the maximal isometric SOL force occurs in young adults (Rubenson et al., 2012) as well as the optimal angle for plantarflexion moment in older adults (Hasson et al., 2011). The measured muscle fascicle length at $10^\circ$ dorsiflexion was designated $L_0$. To assess whether this joint posture resulted in optimal voluntary force in the present study the participants also performed $MVC_{pt}$ at additional joint angles eliciting fascicle lengths that were both longer and shorter than those at $10^\circ$ dorsiflexion. These additional trials were performed only by the participants that were able to tolerate a prolonged protocol ($n = 7$ and $n = 8$, for control and CHF participants, respectively).

A combination of experimental net moment measurements from dynamometry, ultrasound fascicle imaging, electromyography and a scaled participant-specific musculoskeletal model in OpenSim 2.0.2 (Delp et al., 2007) were used to obtain predictions of moments generated by the SOL, as well as the moments generated by synergist muscles and by the co-contraction of plantarflexor muscles. First, a generic lower-limb model (Arnold et al., 2010) was scaled using each patient’s joint axes and centers determined via motion capture data (8-camera VICON MX motion capture system, Oxford Metrics, UK; 100 Hz) from participants in a standing posture as well as dynamic joint motions (Besier et al., 2003). From these trials, an inverse kinematics algorithm was run on the position of 26 retroreflective spherical markers placed on anatomical landmarks and on functionally determined of joint centers (Besier et al.,
that minimized the distance between the OpenSim model markers and the
retroreflective and functionally determined markers. The moment generated by the
plantarflexors ($M_{plant}$) during the maximal voluntary isometric plantarflexion
contractions ($MVC_{pl}$) was calculated as:

$$M_{plant} = M_{peak} - \Delta M_p + M_{dorsi} \tag{4.2}$$

where $M_{peak}$ represents the peak net ankle joint moment calculated as the difference
between the peak recorded Biodex moment during $MVC_{pl}$ and the mean resting Biodex
moment prior to the contraction, $\Delta M_p$ represents the difference in the estimated passive
SOL moment during the $MVC_{pl}$ and the passive SOL moment at rest prior to the
contraction [due to fascicle shortening resulting from tendon stretch; (MacIntosh and
MacNaughton, 2005; Zajac, 1989)], and $M_{dorsi}$ is the moment generated by the co-
contraction of the dorsiflexor muscles. $\Delta M_p$ was calculated as:

$$\Delta M_p = (F_p^{contr} \cos \theta^{contr} \gamma^{contr}) - (F_p^{rest} \cos \theta^{rest} \gamma^{rest}) \tag{4.3}$$

where $F_p$ were obtained for both the fascicle length at the $MVC_{pl}$ and the fascicle length
during the rest period just prior to contraction using a linear interpolation of the passive
F-L relationship ($rest$ and $contr$ superscripts designate rest or $MVC_{pl}$, respectively). The
Achilles moment arm during contraction ($\gamma^{contr}$) were estimated by increasing the
value predicted from the experimental Achilles moment arm - joint angle equation by
the 20% to take in account the increase in moment arm distance reported during
$MVC_{pl}$ with respect to the length at rest (Maganaris et al., 1998).
The $M_{dorsi}$ was predicted by the participant-specific OpenSim model. First, the OpenSim maximal isometric forces of all the dorsiflexors (tibialis anterior, extensor digitorum longus, extensor hallucis longus, peroneus tertius) were adjusted by the same percentage increase or decrease so that the predicted model’s peak isometric dorsiflexion moment at 100% activation matched that of the participant’s experimental maximum net dorsiflexion moment ($MVC_{dorsi}$) recorded in the Biodex dynamometer at 10° plantarflexion, the angle that corresponds approximately to optimal dorsiflexion moments (Arnold et al., 2010). The MVCs were performed only at this joint angle to reduce the total numbers of contractions performed and time spent in the experimental protocol by each participant. This was an important consideration because of the general high fatigability of CHF patients. In this procedure, the OpenSim model was positioned to match the participant’s recorded ankle and knee joint posture. In subsequent measurements of $MVC_{plant}$ the $M_{dorsi}$ was predicted by the OpenSim model by prescribing an activation to all of the dorsiflexors equal to the ratio of the TA’s peak EMG (linear envelope) during the $MVC_{plant}$ to its peak EMG (linear envelope) from the $MVC_{dorsi}$ trial (i.e. this assumed the same activation level for all dorsiflexors).

To take into account the contribution of synergist muscles we predicted the relative percentage contribution of each plantarflexor muscle to the total plantarflexors moment in OpenSim by prescribing the recorded ankle and knee angles and 100% activation of all plantarflexor muscles (peroneus longus, peroneus brevis, flexor hallucis, tibialis posterior, flexor digitorum, MG, LG and SOL). The percent contribution of the OpenSim SOL to the total predicted moment was applied to the experimental $MVC_{plant}$ to define the moment generated by the participant’s S L ($M_d$). Lastly, voluntary SOL active force ($F_a$) was calculated as:

$$118$$
Similar to the procedure used to calculate normalized passive force, voluntary specific active force was obtained by dividing $F_a$ by the participants' SOL PCSA.

The PCSA of the SOL was computed from its volume ($Vol$), resting fascicle length ($L_{rest}$) and pennation angle ($\cos \theta$) at rest (Equation 4.5). These architectural parameters were measured with participants at rest and with their ankle in a neutral plantar flexion angle (0°). These measurements were performed using our 2D and 3D ultrasound procedures as described previously (Panizzolo et al., 2014b) (Chapter 3).

$$PCSA = \frac{Vol \cdot \cos \theta}{L_{rest}}$$ (4.5)

All the calculations for the passive and active force components were performed using custom-written software in Matlab (The MathWorks Inc., Natick, MA, USA).

### 4.2.5 Statistical analysis

Differences in the absolute and normalized passive F-L curves were assessed by testing if $F_p$, or normalized $F_p$, were different between groups (CHF and control) and/or passive fascicle lengths (20%, 40%, 60%, 80% and 100% of stretch) using a two-way repeated measures ANOVA, with Bonferroni post hoc tests. A two-tailed unpaired Student’s t-test with significance level of $p < 0.05$ was used to determine significant differences in the $L_{slack}$ and in the $L_{max}$ as well as in the passive fascicle stiffness ($k_1$ and $k_2$) between the groups. A two-tailed unpaired Student’s t-test ($p < 0.05$) was also used to determine differences in the voluntary active strength between groups. The voluntary active
strength parameters investigated included the peak moment produced by the plantarflexors and by the dorsiflexors, the $F_a$, the specific voluntary active force (N cm$^2$) and the muscle fascicle length at $MVC_{pt}$ ($L_0$). A pair-wise comparison analysis was also run on the $F_a$ on the subset of six age and sex-matched participants. Statistical analysis was performed in SPSS using (IBM, Statistic 21, USA).

4.3 Results

4.3.1 Passive force estimates

A main effect of group on $F_p$ was found ($p = 0.027$) with lower $F_p$ in the CHF group compared to the control group at equivalent levels of fascicle stretch although no interaction effect was found ($p = 0.11$). No differences were found in $k_1$ and $k_2$ between the groups ($p = 0.32$ and $p = 0.85$) (Fig. 4.2a). The $L_{\text{max}}$ was significantly shorter in the CHF group compared to the control group ($p = 0.046$), although no differences were found in $L_{\text{slack}}$ ($p = 0.11$) and in the overall fascicle stretch ($p = 0.34$) (Table 4.2).

Table 4.2. Active and passive fascicle length. Data are means ± S.D.

<table>
<thead>
<tr>
<th></th>
<th>CHF</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{\text{slack}}$ [mm]</td>
<td>30.0±10.6</td>
<td>37.3±8.5</td>
</tr>
<tr>
<td>$L_{\text{max}}$ [mm]</td>
<td>41.9±11.8*</td>
<td>50.9±7.3</td>
</tr>
<tr>
<td>$L_0$ [mm]</td>
<td>26.4±6.4*</td>
<td>33.7±8.4</td>
</tr>
</tbody>
</table>

*indicates a significant difference ($p < 0.05$)

No main or interaction effects were found in the PCSA-normalized $F_p$ between the CHF and control groups ($p = 0.76$ and $p = 0.80$, respectively). Likewise, when the passive F-L curves were normalized by $L_{\text{slack}}$ no significant differences were found in the $L_{\text{max}}$ ($p = 0.70$). Normalized passive fascicle stiffness ($k_{1\text{norm}}$ and $k_{2\text{norm}}$) were not
significantly different between the groups (p = 0.38 and p = 0.46) (Fig. 4.2b). Results were similar when comparisons were performed on the pair-wise analysis.

4.3.2 Active voluntary force estimates

The moment generated by the plantarflexors was ~20% lower in the CHF group compared to the control group (51.8 ± 26.0 Nm and 65.2 ± 20.8 Nm, respectively). Although this difference was not significant (p = 0.18) there was a moderate effect size (ES = 0.57). A non-significant difference (p = 0.45) was found in the moment generated by the dorsiflexors between the groups (26.0 ± 15.4 Nm and 21.7±10.7 Nm, for CHF and control group, respectively). The pair-wise comparison found a reduced plantarflexor moment (p = 0.01; ES = 0.51) in the CHF group compared to the controls (69.5 ± 26.8 Nm and 55.4 ± 27 Nm, respectively) and a non-significant difference (p = 0.3) in the moment generated by the dorsiflexors between the groups (21.3 ± 9.4 Nm and 26.0 ± 16.8 Nm, for the CHF and control group, respectively).

The $F_a$ was ~14% lower in the CHF compared to the control group (non-significant; p = 0.41) (Fig. 4.3a). The $F_a$ normalized by body mass was ~16% lower in the CHF compared to the control group (non-significant; p = 0.25). The pair-wise comparison found a significant ~25% lower $F_a$ in CHF vs. the control group (p = 0.025; ES = 0.76) (Fig. 4.3c). $L_0$ was shorter (~22%) in the CHF group compared to the control group (p = 0.039) (Table 4.1). Likewise, $L_0$ in the pair-wise comparison was significantly shorter (~22%) in the CHF compared to the controls (p = 0.008). No significant differences were found in specific active voluntary force between groups (p = 0.99) (Fig. 4.3b) nor in the pair-wise subset (p = 0.33).
Figure 4.2. Passive force-length (F-L) relationship (a) and passive F-L relationship normalized by individual physiological cross-sectional area and fascicle slack length ($L_{slack}$) (b). Chronic heart failure (CHF) group is displayed in grey triangles (▲), and control group in black circles (●). Average curves are displayed ± S.D. * designates statistically different between groups (p < 0.05; CHF vs. control) on passive force. # designates a significant difference between groups (p < 0.05; CHF vs. control) in maximal passive fascicle length.

The forces derived from the additional joint angles contractions, and therefore with different fascicle lengths, show that the optimal $F_a$ corresponded to the 10° dorsiflexion angle (Fig. 4.4). This occurred for both CHF and control groups, which confirmed the designation of $L_0$ at 10° dorsiflexion.
Figure 4.3. Active force (a, c) and specific active force (c). Results are presented for the whole groups (a, b) and for the pair-wise comparison (c). Chronic heart failure (CHF) group is displayed in grey and control group in black. Data are means ± S.D; * designates a significant difference (p < 0.05) between CHF and the control group.

Figure 4.4. Peak active voluntary force and fascicle length respectively normalized by individual peak voluntary active force ($F_a$) and the muscle fascicle length ($L_0$) measured at 10° dorsiflexion. Data points include measurements taken at joint angles other than 10° dorsiflexion. A data point at 1,1 is included for reference purposes only. Chronic heart failure (CHF) group is displayed in grey triangles (▲), and control group in black circles (●). Average curves are displayed ± S.D.
4.4 Discussion

4.4.1 Peak active voluntary force estimates

The lack of a significant difference in peak voluntary active SOL force between CHF and control participants is surprising considering the substantial (~25%) reduction in plantarflexor muscle size in CHF compared to age-matched healthy individuals observed in the present study and previous studies (Mancini et al., 1992; Panizzolo et al., 2014b). Nevertheless, it should be stressed that this result is in agreement with previous studies investigating plantarflexor strength (net moments) between CHF and healthy individuals (Carrington et al., 2001; Harridge et al., 1996). One factor that might explain the lack of difference in voluntary SOL force in this study, and possibly the low plantarflexor strength in previous studies, are discrepancies in gender-specific ages within the CHF and control groups. Indeed, when we performed a pair-wise sub-analysis comparing six participants paired for age and gender a significant reduction in both $F_a$ (~25%) and plantarflexor moment (~20%) was observed supporting our hypothesis that a reduction in $F_a$ exists in CHF. This finding indicates that a difference was likely obscured in our larger data set due to variability arising from age and gender heterogeneity between groups.

In contrast to our second hypothesis we found no differences in $F_a$ between the groups when normalized by PCSA (voluntary specific force, Fig. 4.3b). This observation was true both for the comparison of the total group and the pair-wise sub-analysis. Our results indicate that the lower peak muscle force in the CHF group might be explained by a reduction in muscle size alone (Panizzolo et al., 2014b). This contradicts some previous investigations (Toth et al., 2010, 2006) in which the authors suggest that the reduced extensor strength (knee joint moments) in CHF is due not only to reduced muscle size but also to contractile dysfunction as well as single muscle fiber studies.
where specific tension of knee extensor muscle has been found to be lower in CHF (Miller et al., 2009). Because these previous studies were performed on the quadriceps, it remains possible that SOL muscle strength is more strongly associated with muscle size per se compared to proximal leg muscles. This interpretation, although speculative, may help explain the previous finding that CHF patients rely less on their hip joint (which depend in part on the quadriceps) and more on their ankle joint, compared to healthy individuals (Panizzolo et al., 2014a). Alternatively, previous work relating net joint moments and muscle size may have misrepresented the actual muscle force capacity owing to musculoskeletal geometry and architecture factors that were not accounted for in joint moment analyses. It should also be noted that at least one study examining isolated muscle fiber specific tension found no differences between CHF patients and control participants despite the lower myosin content in the CHF muscle (Miller et al., 2010). In this study the increased time course for actin-myosin interaction in CHF fibers was understood to compensate for the reduced cross-bridge formation resulting from the lower myosin content. If the same occurs in whole muscles contracting in vivo, this may also help explain the lack of difference in force after normalizing by muscle PCSA.

It is important to acknowledge that, in addition to muscle size and contractile properties, muscle inhibition can also affect peak force production. The inhibition of voluntary muscle activation has been shown to occur in older adults (Leonard et al., 1997; Scaglioni et al., 2002) and thus could have prevented the CHF and control participants from exerting their maximal muscle force capacity during $MVC_{pt}$. Whether muscle inhibition affected the difference in voluntary force remains uncertain. However, previous research has reported similar inhibition levels in the plantarflexors of CHF and healthy individuals, as assessed by a twitch-interpolation protocol (Carrington et al.,
suggesting that inhibition may not greatly contribute to differences in $F_a$. Moreover, if inhibition did contribute to the lower voluntary force capacity of the SOL in CHF it would necessitate a greater absolute specific tension ($N\text{ cm}^{-2}$) for the CHF group in order for the voluntary specific force to remain similar between groups.

4.4.2 Muscle length-dependency of force production

In addition to informing peak voluntary skeletal muscle force capacity in CHF, our work also provides insight into the length-dependency of active force production in CHF (Fig. 4.4). First, it should be stressed that the comparison of $F_a$ between the CHF and the control group was done at a fascicle length that corresponded to the optimal length of the muscle ($L_0$) in both groups, where peak force capacity of the muscle is attained. Therefore, the lower $F_a$ in CHF was not due to a difference in the muscle’s isometric force-length operating range. Moreover, the general shape of the normalized isometric force-length curve (Fig. 4.4) was similar between the CHF and control groups, suggesting that the underlying myofilament mechanics were not altered in CHF SOL, corroborating the interpretation that muscle size by itself is a strong predictor of SOL force capacity in CHF.

While the overall shape of the normalized isometric force-length curve was similar between the groups, a significantly (~20%) shorter $L_0$ was observed in the CHF group (Table 4.2). Although the resting SOL length previously reported (Panizzolo et al., 2014b) was found to be similar between CHF and control groups, this earlier study did not establish a functional optimal fascicle length. A shorter $L_0$ in CHF suggests a loss of sarcomeres in series and likely contributes to the lower muscle volume of the SOL in CHF patients (Panizzolo et al., 2014b). This also raises the question whether muscle
volume is associated differently with \( F_a \) compared to PCSA, however, when \( F_a \) was normalized to SOL volume (N/ml) we likewise observed no differences between the CHF and control groups (p = 0.75 and p = 0.98 in the pair-wise sub-set).

It is unclear how the shorter SOL length in CHF patients impacts the function of the muscle during tasks such as walking. Rubenson et al. (2012) established that the SOL in young adults functions on the ascending limb of the force-length curve during walking. It is possible that the shorter \( L_0 \) in CHF results in the muscle operating at longer lengths across the plateau region, which could optimize force capacity, or possible on the descending limb of the force-length curve where muscle force capacity and stability are compromised (Rassier et al., 1999). This will be an important area of future research in CHF patients.

### 4.4.3 Passive force estimates

The present study provides, to the best of our knowledge, the first estimate of \textit{in vivo} passive human skeletal muscle force-length properties in CHF. As predicted, higher passive SOL force was produced in the control group for a given amount of stretch (Fig. 4.2a). However, in contrast to our hypothesis, passive force is not different after normalizing by muscle PCSA, nor is passive muscle stiffness affected, indicating that muscle size rather than intrinsic muscle properties is a major factor influencing passive force and stiffness in CHF SOL muscle. This finding stands in partial contrast to previous work reporting stiffer cardiac muscle due to alterations in the titin structure (Wu et al., 2002) or decreased passive tension of the diaphragm, due to titin loss (van Hees et al., 2010) in CHF. On the other hand, our results do corroborate data from passive skeletal muscle properties in mice SOL, in which passive forces from CHF-affected animals were likewise not altered after normalizing to muscle cross sectional...
area (van Hees et al., 2010). When considered together, the estimates of voluntary active SOL force and passive SOL force lends credence to the interpretation that muscle size maybe one of the primary sources of altered mechanical function of skeletal muscle in CHF.

It was surprising, however, that for a given absolute muscle length, passive force was significantly higher in CHF SOL compared to the control group. This unexpected finding stems from the fact that over the same ankle range of motion the passive muscle lengths are shorter in CHF patients, in particular at maximal stretch (Fig. 4.2a). The result is that for the same absolute muscle length above $L_{stack}$ the CHF muscle has undergone greater strain, thus generating greater force in titin and other passive load bearing muscle components. The shorter passive muscle lengths in CHF patients corroborate the finding that $L_0$ is shorter in the CHF patients and the possibility that SOL may have undergone a loss of in series sarcomere numbers. Also notable is that the present study is consistent with other experimental studies showing agreement between $L_0$ and the onset of passive force generation (Azizi and Roberts, 2010; Rubenson et al., 2012; Winters et al., 2011). Whereas strain and force for a given absolute muscle length is higher in CHF patients, it should be reiterated that our length- and PCSA-normalized data (Fig. 4.2b) show that passive force for a similar strain is determined primarily by muscle PCSA rather than altered passive intrinsic tissue properties (e.g. titin, collagen).

4.4.4 Functional implications

Considering that smaller muscle size may be a major factor linked to a reduction in force capacity in CHF, exercise that promotes hypertrophy should be a focus for restoring functional capacity in leg muscles. Exercise prescription for CHF is becoming commonplace, but programs that include lower limb resistance training might be
Chapter 4 - Active and passive forces in chronic heart failure especially promising (Maiorana et al., 2000). It is also possible that eccentric muscle training could offer advantages for CHF patients because the high force to metabolic cost ratio (Bigland Ritchie and Woods, 1976) could moderate their hemodynamic and metabolic burden while still delivering a large load stimulus to the muscle. In the case of the SOL, eccentric exercise might also be particularly useful because it has been known to stimulate sarcomerogenesis (Brockett et al., 2001; Butterfield et al., 2005), and thus might restore the muscle fascicle to longer lengths commensurate with healthy age-matched individuals. This would both increase the muscle volume as well as possibly restore muscle force-length-velocity function during locomotion.

Our results also offer insight into the gait mechanics of CHF patients (Panizzolo et al., 2014a). The combination of the shorter SOL muscle fascicles in CHF patients and their greater dorsiflexion during mid-stance of gait (Panizzolo et al., 2014a) may cause significantly greater SOL strain. This might force the muscle on to the descending limb of the F-L curve where large passive forces develop (Rubenson et al., 2012). In this scenario CHF patients would rely more on their passive forces to support the plantarflexion moment during walking, which has the benefit of reducing metabolically expensive active force development. This may help explain why CHF patients rely proportionately more on their ankle for powering walking as speed and metabolic demand increases (Panizzolo et al., 2014a). However, whilst metabolically advantageous, this mechanism might lead to greater lengthening-induced muscle damage (Friden et al., 1983). The muscle’s F-L operating range depends on multiple factors, including tendon stiffness, and a detailed understanding will require further in vivo analyses. Nevertheless, it is intriguing to consider whether specific training that promotes muscle lengthening remodeling would reverse the adaptations in joint mechanics observed in CHF (Panizzolo et al., 2014a).
4.4.5 Limitations

Several limitations of the present study require acknowledgement. These limitations mainly involve the methodology adopted to calculate the $F_a$. Unfortunately, technical difficulties associated with data collection in a clinical population such as CHF prevented us from readily measuring the amount of inhibition during $MVC_{pt}$ during these experiments. Among these, the long duration of the protocol and the difficulty in placing stimulation electrodes on the SOL, in addition to the ultrasound and EMG electrodes, made the assessment of inhibition not feasible in the same testing session. In addition, although the study design strongly relies on experimental data, some parameters (e.g. force sharing between synergist muscles, co-contraction of antagonist muscles) were obtained by means of musculoskeletal modeling. Although we used subject-specific scaled models, they nevertheless rely on assumptions including relative muscle size, tendon stiffness and Hill-type muscle properties, characteristic of these simulations approaches (Seth et al., 2011), although errors resulting from these assumptions are expected to be small [see sensitivity analysis in (Rubenson et al., 2012)].

4.4.6 Conclusion

The present study is the first of its kind to investigate differences in active and passive forces in vivo in humans affected by CHF. The SOL is the major muscle in the plantarflexors group that is involved in postural control and locomotion, and its muscle architecture is strongly altered in CHF (Panizzolo et al., 2014a; 2014b). This work indicates that a primary factor leading to lower voluntary active force production in the SOL is likely a reduction in muscle size. However, shorter muscle fascicles in CHF results in greater passive forces for a given absolute muscle length, and might be linked to changes in CHF gait (Panizzolo et al., 2014a). Exercise that promotes calf muscle
hypertrophy and serial sarcomerogenesis should prove particularly beneficial in CHF patients.
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Gait analysis in chronic heart failure: the calf as a locus of impaired walking capacity

ABSTRACT

Reduced walking capacity, a hallmark of chronic heart failure (CHF), is strongly correlated with hospitalization and morbidity. The aim of this work was to perform a detailed biomechanical gait analysis to better identify mechanisms underlying reduced walking capacity in CHF. Inverse dynamic analyses were conducted in CHF patients and age- and exercise level-matched control subjects on an instrumented treadmill at self-selected walking speeds and at speeds representing +20% and -20% of the subjects’ preferred speed. Surprisingly, no difference in preferred speed was observed between groups, possibly explained by an optimization of the mechanical cost of transport in both groups (the mechanical cost to travel a given distance; J/kg/m). The majority of limb kinematics and kinetics were also similar between groups, with the exception of greater ankle dorsiflexor angles during stance in CHF. Nevertheless, over two times greater ankle plantarflexion work during stance and per distance travelled is required for a given triceps surae muscle volume in CHF patients. This, together with a greater reliance on the ankle compared to the hip to power walking in CHF patients, especially at faster speeds, may contribute to the earlier onset of fatigue in CHF patients. This observation also helps explain the high correlation between triceps surae muscle volume and exercise capacity that has previously been reported in CHF. Considering the key role played by the plantarflexors in powering walking and their association with exercise capacity, our findings strongly suggest that exercise-based rehabilitation in CHF should not omit the ankle muscle group.
5.1 Introduction

Chronic heart failure (CHF) is characterized by a marked reduction in walking capacity that in turn contributes to a reduction in quality of life (Juenger et al., 2002). Indeed, preferred walking speed has been reported to be ~30% lower in CHF compared to healthy age-matched control groups (Beneke and Meyer, 1997; Figueiredo et al., 2013) and walking endurance decreases with increasing severity of the disease (Riley et al., 1992). Notably, walking capacity has also been directly linked with hospitalization and mortality rates in CHF (Forman et al., 2012).

Identifying the mechanisms underlying the reduced walking performance in CHF therefore has important functional and clinical relevance. In many instances, skeletal muscle dysfunction, rather than cardiac function, is fundamental to the exercise intolerance evident in this group (Cicoira et al., 2001; Cohn et al., 1993; Fülster et al., 2013). This underlies the ‘skeletal muscle hypothesis’ of exercise intolerance in CHF (Clark et al., 1996). However, most studies addressing the ‘skeletal muscle hypothesis’ in humans have focused primarily on isolated skeletal muscle, including histology, biochemistry (Sullivan et al., 1990), morphology (Anker et al., 1997; Panizzolo et al., 2014) and strength (Lipkin et al., 1988; Toth et al., 2006). It is likely that these factors all contribute to some extent to reduced functional walking performance in CHF. Yet, unlike other conditions where skeletal muscle dysfunction purportedly leads to impaired walking, no direct study to date has examined the detailed biomechanical response of walking itself in patients with CHF. Such an analysis would provide an effective means to identify the end-effect of skeletal muscle dysfunction on walking mechanics in CHF. This information can help reveal the basis for functional limitations in CHF and foster evidence-based rehabilitation approaches aimed at restoring walking capacity.
The goal of this study was, therefore, to perform a detailed biomechanical gait analysis of walking in CHF patients, compared to healthy age-matched control participants. Recently, we identified that the plantarflexor muscles (the triceps surae) undergo proportionately more muscle wasting in CHF than other lower limb muscles (Panizzolo et al., 2014). Moreover, plantarflexor size, unlike the overall leg lean mass, is strongly correlated with peak aerobic capacity of walking in CHF patients (Panizzolo et al., 2014). These characteristics, together with the finding that the plantarflexors are the main source of work during gait in healthy young and old adults (DeVita and Hortobagyi, 2000; McGowan et al., 2009) and that a reduction in walking speed in older adults is related to the triceps surae function (Panizzolo et al., 2013) (Appendix), suggests that restrictions at the ankle joint might particularly affect the ability of CHF patients to achieve the typical gait speed and mechanics seen in a normal healthy population. Accordingly, we hypothesized: 1) That a slower walking speed is selected in CHF, compared with healthy age-matched individuals, to reduce total leg mechanical work; 2) That the more pronounced wasting reported in plantarflexor muscles in CHF (Panizzolo et al., 2014) requires additional re-distribution of mechanical work during stance from the ankle to the other lower limb joints; 3) That this redistribution would increase with walking speed, and so the % of peak aerobic capacity utilized, because of the association between plantarflexor muscle size and aerobic capacity previously reported in CHF (Panizzolo et al., 2014).

5.2 Methods

5.2.1 Subjects

We recruited 10 subjects (6 men, 4 women) with CHF (NYHA class II-IV; ejection fraction = 30.9 ± 9.7%, mean ± S.D.) and 11 healthy subjects from the local community (8 men, 3 women; see Table 5.1 for subjects characteristics). Ten CHF and all the 11
control subjects were also involved in the study described in Chapter 3. Exclusion criteria for the CHF population are also presented in Chapter 3. All subjects were free from musculoskeletal injury and other musculoskeletal diseases and provided written informed consent prior to participating in the study. All procedures were approved by the Human Research Ethics Committee at The University of Western Australia and Royal Perth Hospital.

Table 5.1 Subject characteristics. Data are means ± S.D.

<table>
<thead>
<tr>
<th>Group</th>
<th>Age [yr]</th>
<th>Height [m]</th>
<th>Weight [kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>63.1±5.6</td>
<td>1.73±0.06</td>
<td>70.1±8.8</td>
</tr>
<tr>
<td>CHF</td>
<td>60.7±9.8</td>
<td>1.67±0.10</td>
<td>73.0±19.0</td>
</tr>
</tbody>
</table>

The chronic heart failure (CHF) group underwent regular exercise activity 2-3 times per week for ~ 1 hour per session (treadmill walking and resistance weight training) as part of their standard patient care. The control subjects underwent similar levels of weekly exercise.

5.2.2 Preferred walking speed

A protocol based on over-ground and treadmill walking trials was used to define each subjects’ preferred treadmill walking speed (Panizzolo et al., 2013) (Appendix). Briefly, subjects' preferred over-ground speed was assessed by timing participants walking along a 20m carpeted surface. A minimum of 5 trials were used to assess mean preferred speeds. For the treadmill trials, preferred walking speed was assessed by permitting subjects to freely self-adjust the treadmill speed, starting at a speed 30% slower than their preferred over-ground speed and incrementing the speed either up or down by 0.01 m/s until they reach their preferred speed. A familiarization treadmill walking session was undertaken prior to assessing preferred walking speeds.
5.2.3 Joint and lower limb mechanical work and cost of transport (COT)

Biomechanical measurements were collected with subjects walking on an instrumented split-belt treadmill measuring 3D ground reaction forces (Bertec, Columbus, OH, USA; 2000 Hz) at three different walking speeds: the subject’s preferred speed, a speed 20% faster than their preferred and a speed 20% slower than their preferred speed.

Three-dimensional (3D) gait analysis was performed on each subject during their treadmill walking trials. The marker set used for 3D motion capture (VICON, Oxford Metrics, UK; 100 Hz) was composed of 26 retro-reflective markers placed on selected bony anatomical landmarks of the shoulders, pelvis and lower limbs. Single markers were placed on the left and right calcanei, left and right head of the first and fifth metatarsals, left and right anterior and posterior superior iliac spines, the sternum, the seventh cervical vertebrae, and the left and right acromion processes. Clusters of three markers were attached to the thighs and shanks of both legs. Functional joint centers for the hip and knee were defined using the procedures of (Besier et al., 2003). All markers and force trajectories were filtered using a zero-lag 4th order low pass Butterworth filter with a 5-7 Hz optimal cut-off frequency that was selected using a custom residual analysis algorithm (MATLAB, The MathWorks Inc., USA). Marker positions collected during a static trial were used to generate a subject-specific musculoskeletal model in OpenSim 2.0.2 (Delp et al., 2007). The generic OpenSim musculoskeletal model (Arnold et al., 2010) was scaled using an inverse kinematics algorithm based on the position of the markers placed on anatomical landmarks and on the functional joint centers previously determined. Joint angles and net moments were computed using inverse kinematics and inverse dynamics performed in the joint coordinate systems of the scaled model for the walking trials. These calculations were made directly in OpenSim by combining 3D markers trajectories and measured ground reaction forces.
Joint power was then computed as:

\[ P_{jt} = M_{jt} \cdot \omega_{jt} \]  (5.1)

Where \( P_{jt} \) is the instantaneous power (in individual joint planes), \( M_{jt} \) is the net joint moment (across the individual joint axes) and \( \omega_{jt} \) is the corresponding joint angular velocity. The instantaneous power in each joint \( P_j \) was subsequently computed as the net power across all three planes of the individual joint. Positive joint work during the stance and across the stride was computed at each joint by integrating the positive values of the instantaneous joint power over the stance and stride times, respectively:

\[ W_{ji}^{+} = \int_{t_2}^{t_1} P_{ji}^{+} \, dt \]  (5.2)

Where \( W_{ji}^{+} \) is the positive work at the \( i^{th} \) joint, \( P_{ji}^{+} \) is the positive power at the \( i^{th} \) joint; \( t_1 \) is the instant of the first heel strike and \( t_2 \) is the instant of the second heel strike (for the calculation of positive work in stance \( t_2 \) is toe off). Heel strike and toe off were automatically determined using the Detect Gait Cycle events plug-in in VICON (Oxford Metrics, Oxford, UK).

The total positive work in the lower limbs was computed both for the stance phase and for the entire stride from the sum of each joint (left and right legs were computed individually and summed) and normalized to the lower limb lean mass (see below). The distribution of total work between individual joints was computed by dividing the total lower limb work by the positive work in the individual joints (sum of left and right joints). The total mechanical cost of transport (COT) J/kg/m was calculated by dividing
the lower limb lean mass-normalized positive work over the stride by the distance traveled over the stride.

The work produced in plantarflexion during stance was computed separately and normalized to the triceps surae volume (see below). Similarly, the specific COT for ankle plantarflexion was computed as the sum of the left and right triceps surae volume-specific positive ankle plantarflexion joint work divided by the distance traveled over one stride. A minimum of five non-consecutive strides per speed were used for generating mean kinematic, kinetic and work data for each individual subject, which were subsequently combined to calculate group mean data.

5.2.4 Lean lower limb mass and plantarflexor volume

Overall body composition was determined using dual energy X-ray absorptiometry (Luna Prodigy, encore 2004, GE Medical Systems, Madison, WI, USA) on each subject. Lean mass for the lower limbs was computed separately by selecting a region of interest from the great trochanter to the pubic symphysis and including the leg and foot.

Plantarflexor volume was computed using a three-dimensional ultrasound technique (3DUS) based on a combination of B-mode ultrasound imaging and 3D motion data, the procedures of which have been described in detail in (Barber et al., 2009; Panizzolo et al., 2014) (Chapter 3).

5.2.5 Peak and sub-maximal oxygen consumption

On a separate testing day, peak oxygen uptake ($\dot{V}_\text{O}_2 \text{ peak}$) was assessed using an incremental walking protocol on a motorized treadmill (Panizzolo et al., 2014). Indirect calorimetry was conducted using a Vmax Encore gas analysis system (Sensormedics,
Yorba Linda, California), which enabled the measurement of expired gas concentrations and volumes. Absolute peak was expressed in ml/min and normalized to body mass (ml/kg/min). Sub-maximal oxygen consumption at the three subject-specific speeds described above were also obtained.

5.2.6 Statistical analysis

A 3x2 mixed model repeated measures ANOVA was performed to evaluate differences between the control and CHF groups across the three testing speeds. The significance level was set at p < 0.05 (SPSS Inc., Statistic 21, USA). ANOVA analyses included joint kinematics and kinetics, the positive work in the combined lower limb (normalized by lower limb lean mass) and the plantarflexor work at the ankle normalized to the triceps surae volume. We analyzed both the mechanical work performed during the stride and stance phase, as well as the mechanical cost of transport (work per distance travelled). Main effects of group and speed and interaction effects were evaluated in these analyses. The distribution of work produced across joints was evaluated with a 3x2 MANOVA (significant level of p < 0.05) including the three lower limb joints. An arcsin conversion was applied to the percentage joint contribution prior to performing this analysis. Joint and speed were set as multivariate factors and group as a univariate factor. Where significant main and/or interaction effects were detected in ANOVA or MANOVA analyses a Bonferroni post hoc test was conducted.

5.3 Results

5.3.1 Preferred walking speed and spatio-temporal parameters

Preferred walking speed was not significantly different between groups. Spatio-temporal gait parameters including stride length, stride frequency, duty factor, stance and swing times were similarly not significantly different between groups (Table 5.2).
Table 5.2. Spatio-temporal parameters at different testing speeds. Data are means ± S.D.

<table>
<thead>
<tr>
<th>Speed</th>
<th>Group</th>
<th>Testing speed</th>
<th>Stance time [s]</th>
<th>Swing time [s]</th>
<th>Duty factor</th>
<th>Stride frequency [Hz]</th>
<th>Stride length [m]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>Control</td>
<td>0.81±0.13</td>
<td>0.77±0.07</td>
<td>0.51±0.06</td>
<td>0.60±0.01</td>
<td>0.79±0.08</td>
<td>1.03±0.14</td>
</tr>
<tr>
<td></td>
<td>CHF</td>
<td>0.79±0.20</td>
<td>0.80±0.12</td>
<td>0.51±0.07</td>
<td>0.61±0.03</td>
<td>0.78±0.10</td>
<td>0.96±0.21</td>
</tr>
<tr>
<td>Preferred</td>
<td>Control</td>
<td>1.00±0.16</td>
<td>0.69±0.06</td>
<td>0.48±0.05</td>
<td>0.59±0.01</td>
<td>0.87±0.08</td>
<td>1.16±0.16</td>
</tr>
<tr>
<td></td>
<td>CHF</td>
<td>0.99±0.26</td>
<td>0.74±0.13</td>
<td>0.48±0.06</td>
<td>0.61±0.02</td>
<td>0.84±0.13</td>
<td>1.11±0.22</td>
</tr>
<tr>
<td>Fast</td>
<td>Control</td>
<td>1.21±0.21</td>
<td>0.64±0.05</td>
<td>0.45±0.04</td>
<td>0.59±0.01</td>
<td>0.93±0.07</td>
<td>1.28±0.18</td>
</tr>
<tr>
<td></td>
<td>CHF</td>
<td>1.19±0.31</td>
<td>0.67±0.12</td>
<td>0.46±0.07</td>
<td>0.59±0.02</td>
<td>0.91±0.14</td>
<td>1.23±0.24</td>
</tr>
</tbody>
</table>

Figure 5.1. Comparison of joint angles, moments and powers (top to bottom) for the preferred walking speed across the gait cycle in chronic heart failure (solid lines) and control subjects (dotted lines); (a) ankle, (b) knee and (c) hip. Data are group means; the shaded regions represent the S.D. of the mean. The vertical lines represent toe off (solid lines represent the CHF group and the dotted line the control group). Positive joint angles represent flexion (dorsiflexion at the ankle) and negative angles represented extension (plantarflexor at the ankle). Positive moments represent net flexion joint moments (dorsiflexion at ankle) and negative moments represent net extension joint moments (plantarflexion at ankle). Positive powers represent instantaneous joint power generation and negative powers represent instantaneous joint power absorption. The * represents statistically different ankle joint dorsiflexion in stance (p < 0.05).
5.3.2 Joint kinematics and kinetics

No differences were found between CHF and control participants in peak joint moments or powers at the ankle, knee or hip across all three speeds. A main effect of group was reported in the kinematics of the ankle. The CHF group exhibited a higher dorsiflexion peak in stance (p = 0.01) but no differences in the total ankle range of motion (p = 0.2). Joint kinematics, kinetics and power traces for the preferred speed are displayed in Fig. 5.1.

5.3.3 Total lower limb lean mass-specific and plantarflexor volume-specific work and COT

Total leg lean mass-specific mechanical work and COT across the stride were on average 10.2% and 15.6% greater in CHF versus control (Fig. 5.2), although no statistical main effect of group was observed in either of these parameters (p = 0.2 and p = 0.1, respectively). A significant main effect of speed was present in both lower limb lean mass-specific total work (p < 0.001) and COT (p < 0.001). Post hoc tests revealed that when collapsed across groups differences were present between all speeds for lower limb lean mass-specific total work. Post hoc tests also revealed that the slowest and preferred speeds did not differ in lower limb lean mass-specific COT, but that each was lower than the COT at the fastest speed. No significant interaction effects for positive leg lean mass-specific work or COT were present. The same statistical findings existed for the total leg work when normalized to body mass.

A main effect of group on the triceps surae volume-specific plantarflexion work and COT in stance was observed (Fig. 5.3); p = 0.024 and p = 0.008, respectively. The amount of work produced by the CHF group was 52.8%, 69.2% and 69.2% higher than the control group (for the three testing speeds); the COT was also 68.4%, 74.0% and
54.4% higher than the control group (for the three testing speeds). A main effect of speed was observed on the triceps surae volume-specific plantarflexion work and COT in stance (p < 0.001 and p = 0.002, respectively). Post hoc tests revealed that when collapsed across groups the triceps surae volume-specific plantarflexion work were different from each other between all speeds and that the COT at the slowest and preferred speeds were not different from each other but both were lower than that at the fastest speed. No interaction effect was observed between group and speed (p = 0.2 and p = 0.3, respectively).

Figure 5.2. Total positive mechanical work (a) and cost of transport (b) produced in the lower limb during the stride normalized by lean leg mass for the three testing speeds (mean ± SE; CHF black bars, control white bars). The * represents a significant difference from the -20% speed collapsed across groups (speed main effect post hoc) and the § represents a significant difference from the preferred speed collapsed across groups (speed main effect post hoc).
5.3.4 Distribution of joint work

Across all the testing speeds the hip produced the highest amount of positive work, while the knee joint produced the least work, in both control and CHF groups. Nevertheless, a different distribution of work during stance was found between CHF and the control group (Fig. 5.4). Main multivariate effects of group and speed on work distribution were not present (p = 0.3 and p = 0.08, respectively) but an interaction effect was found between joint and group (p = 0.01) and between speed and joint (p = 0.005). Post hoc analyses revealed differences in the percent work between groups at the ankle at all speeds (p = 0.011, p = 0.023, and p = 0.001) and at the hip only at the
slowest speed (p = 0.044, p = 0.13 and p = 0.055). These results reflected that the percentage of work produced at the ankle joint was higher in the CHF, while an opposite trend was found for the hip, and that the ankle produced proportionately more work in CHF vs. control participants as speed increased (Fig. 5.4).

**Figure 5.4.** The distribution of positive mechanical work (group mean data) across lower limb joints at the three different testing speeds. (a) Slower than preferred, (b) preferred and (c) faster than preferred speeds. The # represents significant differences (MANOVA post hoc analyses) between groups at the specific joints.
5.3.5 Aerobic capacity

\( \tilde{V}_2 \) peak was significantly higher (p < 0.001) in the control group than in CHF (35.8 ± 7.9 and 15.8 ± 2.8 ml/kg/min, respectively). While no differences were reported in the submaximal oxygen consumption values between groups at any of the testing speeds, a main effect of group (p < 0.001) and a main effect of speed (p = 0.002) were reported when oxygen consumption was expressed as a percentage of maximum aerobic capacity (Table 5.3).

Table 5.3. Oxygen consumption expressed as a percentage of the peak oxygen consumption (\( \tilde{V}_2 \) peak) for the three testing speeds. Data are means ± S.D.

<table>
<thead>
<tr>
<th>Speed</th>
<th>Group</th>
<th>Relative ( \tilde{V}_2 ) peak [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow</td>
<td>Control</td>
<td>28.0±0.1</td>
</tr>
<tr>
<td></td>
<td>CHF</td>
<td>55.0±0.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30.1±0.1</td>
</tr>
<tr>
<td>Preferred</td>
<td>CHF</td>
<td>64.3±0.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>34.0±0.1</td>
</tr>
<tr>
<td>Fast</td>
<td>CHF</td>
<td>65.7±0.1</td>
</tr>
</tbody>
</table>

5.4 Discussion

5.4.1 Speed and overall gait mechanics

One reason why CHF patients may have previously exhibited reduced walking function, including speed, is to compensate for lower limb muscle mechanical capacities (Clark et al., 1996; Harrington et al., 1997; Toth et al., 2006). Our first hypothesis, that a reduction in preferred speed is present and is associated with equal levels of mechanical work compared to a faster walking healthy control group was, however, not met. Surprisingly, the preferred speed, both overground and on the treadmill, were not different between groups, nor were the spatio-temporal gait parameters. Interestingly, the similarity in gait function between the CHF and control groups extended to joint
Chapter 5 - Gait mechanics in chronic heart failure

kinetics and kinematics, with few clearly discernible differences in either joint angles, net joint torques or power with the exception of a more dorsiflexed ankle joint in CHF patients (Fig. 5.1). One possible explanation for the lack of difference in preferred speed in our study compared to others (Beneke and Meyer, 1997; Figueiredo et al., 2013) might be the training status of our patients. Because our CHF patients underwent moderate levels of treadmill exercise it is possible that their similar walking speed was due to a training effect (Beneke and Meyer, 1997). Thus, despite the CHF patients presenting with severely limited peak, regular training may possibly promote similar sub-maximal walking patterns. Another, associated reason for our lack of difference in walking speed, versus previous studies, is the disease severity. If previous studies included subjects with more severe CHF (e.g. NYHA III-IV), then they may have been less capable of achieving "normal", albeit compensated, walking speeds.

5.4.2 Relative muscular work and distribution of joint work

The similarity in speed and joint mechanics at first hand suggest that there are no major biomechanical gait differences present in CHF patients. However, a second possible strategy to offset the pronounced loss of muscle mass in the triceps surae of the CHF patients (Panizzolo et al., 2014) is to redistribute work from the ankle to other joints (DeVita and Hortobagyi, 2000). Contrary to this hypothesis, we found that there was an increase in the proportion of work performed at the ankle in CHF. Because of the reduced size of the plantarflexor muscles in CHF, this results in muscle volume-specific work that is over twice that in the healthy control group (Fig. 5.3a). Therefore, CHF patients apparently choose not to compensate for their reduced plantarflexor muscle volume, but instead to work at substantial higher relative effort (for their available muscle mass) at the ankle. Considering that the ankle is a key joint in producing the mechanical work of walking (McGowan et al., 2009), the much higher joint work per
unit of muscle volume may influence both the acute effort during the stance phase \( (J/ml\) over the stance, Fig. 5.3a) as well as the effort to travel a given distance \( (J/ml/m\) Fig. 5.3b). These factors may be a primary cause of the earlier symptoms of walking fatigue characteristic of this population (Clark et al., 1996), and may be one important limitation to walking aerobic capacity. It should be stressed that these analyses only assess the net work at each joint and it remains possible that transfer of energy via two-joint muscle function (e.g. gastrocnemius) affect the relative contribution of the various muscle groups. Investigation in to this effect using more sophisticated computational modeling (e.g. static or dynamic optimization) is warranted.

It is intriguing to consider whether the higher dorsiflexion angles during stance in CHF (Fig. 5.1) also lead to altered \textit{in vivo} muscle mechanics. Rubenson et al. (2012) reported operating lengths of the soleus in healthy young adults that are conserved to the ascending limb of the force-length curve during gait. The higher dorsiflexion angles in CHF may lead to longer muscle lengths that generate passive forces, thus partly compensating for the reduced active force capacity of the muscle. At present this mechanisms remains speculative and requires further \textit{in vivo} analyses to test.

Surprisingly, the greater demand on the ankle to produce the limb mechanical work increased, rather than decreased, with speed (Fig. 5.3a, Fig. 5.4), further taxing the small triceps surae muscle mass. Our earlier work on the triceps surae in CHF established a strong link between muscle size and peak (Panizzolo et al., 2014), leading to our prediction that mechanical work would be ‘shunted’ away from the ankle as CHF patients walk closer to their aerobic capacity, thus potentially shifting reliance on to other less aerobically limiting muscle groups. The finding that the opposite occurs may in fact help to explain the strong link between the size of the triceps surae and
peak during walking. Indeed, these muscles were key in powering walking, compared to the control group that relied more on the hip, and were responsible for the majority of mechanical work during fast walking.

5.4.3 What dictates preferred walking speed and joint work modulation in CHF?

Our results offer some intriguing questions about what factors influence the self-selected walking mechanics in CHF. Firstly, why did the CHF patients not walk slower despite their reduction in muscle size and impaired aerobic capacity? A possible explanation may be an optimization of speed relative to the mechanical COT. We found that the mechanical work required to travel a given distance was not reduced when shifting from the preferred speed to a slower walking speed in either group (Fig. 5.2b). On the other hand, the COT was elevated in both groups when walking faster than the preferred speed. In this regard, the self-selected speed may represent the fastest speed possible before increasing the mechanical COT. This is plausible since the cumulative (repetitive) loading affects fatigue and energy use in the musculature. Mechanical gait optimization may represent an alternative, or possibly parallel, determinant of gait speed to the respiratory optimization recently reported in CHF patients (Figueiredo et al., 2013).

Secondly, why do CHF patients rely on the ankle to a greater extent to power walking compared to healthy age-matched adults? It has been established previously that ageing causes an increased reliance on the hip to power walking (DeVita and Hortobagyi, 2000; Silder et al., 2008). Our data on healthy older adults supports this, with over 50% of the work attributed to the hip in this group (Fig. 5.4). It is not entirely clear why, in the CHF group, the contributions of each joint to the total mechanical work resemble those in healthy young adults (Farris and Sawicki, 2012), especially considering the
reduced muscle volume of the ankle plantarflexors. A first possible explanation might be found in the hip flexor function. These muscle groups have been found to be weaker in CHF (Minotti et al., 1993; Toth et al., 2010, 2006), or functionally impaired. On the other hand, previous studies (Carrington et al., 2001; Harridge et al., 1996; Panizzolo et al., 2014), indicate that the strength of ankle plantarflexors, despite their prominent loss of muscle volume, may be less affected in CHF compared to hip muscle strength. Thus, a greater reliance on the ankle may reflect a more pronounced loss of the muscle force capacity at the hip in CHF compared to healthy older adults.

5.4.4. Summary

The preferred speed and overall joint kinematics and kinetics are similar between CHF and age- and exercise level-matched subjects. Nevertheless, a marked increase in the muscle volume-specific plantarflexion work and a greater reliance on the ankle over other joints to power walking in CHF patients may help explain their decreased walking capacity. The present study strengthens the finding that the plantarflexor muscles (triceps surae) are a key muscle group limiting exercise capacity (Panizzolo et al., 2014) and should be included in the design of exercise based rehabilitation specific to this population.
References


Chapter 5 - Gait mechanics in chronic heart failure


General Discussion
Chapter 6 - General Discussion

The overarching aim of this thesis was to study the *in vivo* morphological and mechanical properties of skeletal muscle in chronic heart failure (CHF) patients, and to investigate how these properties impact on functional walking and exercise capacity and on the biomechanics of walking. This thesis developed and integrated new musculoskeletal imaging techniques, in combination with biomechanical experimentation and modelling not previously performed in CHF or healthy older adults. The work undertaken revealed novel structure-function relationships in CHF skeletal muscle and gait. These findings enhance the evidence-base for specialized rehabilitation treatments for individuals with CHF.

6.1 Summary of findings

The aim of Study 1 was to characterize the morphological properties of triceps surae and Achilles tendon in CHF by coupling whole-body and lower limb dual energy X-ray absorptiometry (DXA) imaging with a novel three-dimensional (3D) ultrasound imaging technique, not previously applied in CHF patients. Study 1 also aimed to establish the link between musculoskeletal architecture and the reduced exercise capacity and strength known to affect CHF patients. Despite a similar overall lower limb lean mass compared to age- and exercise level-matched control participants, patients with CHF exhibited ~25% lower combined triceps surae volume and physiological cross-sectional area (PCSA), explained predominantly by the soleus (SOL). The reduction in muscle size of the triceps surae was also accompanied by a reduction in the cross-sectional area of the Achilles tendon, suggesting the presence of an overall adaptation of the muscle-tendon complex in the CHF. Also, the volume of the SOL, unlike the other triceps surae muscles, and the plantarflexor strength correlated strongly with exercise capacity (peak) in CHF, but not in the control group (r = 0.723 and r = 0.746, respectively). These findings suggested, for the first time, that the
SOL muscle may be a sentinel skeletal muscle determining exercise capacity in CHF. These alterations in muscle morphology are predominantly different to those observed due to disuse or ageing, suggesting that adaptations in CHF skeletal muscle are not caused only by disuse but they are also related to the CHF condition per se.

Because of the marked reduction in SOL size in CHF compared to the total lower limb lean mass, and its strong association with exercise capacity (Study 1), the second study explored how the reduction in muscle size of the SOL impacts active voluntary force and passive force in CHF. This work combined ultrasound muscle imaging with biomechanical experimentation and modeling, and represents the first muscle-level mechanical investigation in humans affected by CHF, thus extending previous analyses that focused on global measurements of joint strength. The smaller SOL of CHF patients resulted in lower absolute voluntary active and passive forces at equivalent levels of strain compared to healthy individuals. However, contrary to our hypothesis, this difference was not present when normalized by PCSA or volume. This detailed muscle level analysis indicated that the reduced muscle force output in CHF may be more strongly associated with muscle size than previously thought. Nevertheless, evidence of altered muscle properties was observed in the CHF patients. The CHF group exhibited both a shorter optimal fascicle length (i.e. the length where peak voluntary isometric force was achieved) and passive fascicle lengths, compared to the age-matched control group. The shorter passive fascicle lengths in CHF resulted in higher passive force for a given absolute muscle length. Although it is unclear how this impacts a functional task such as walking, it is possible that the shorter fascicle lengths in CHF results in the muscle operating at longer lengths compared to that of healthy individuals. Conversely, a shorter optimal fascicle length in CHF might accommodate a
more compliant Achilles tendon in CHF; the greater compliance may be the result of the smaller tendon cross sectional area (Study 1).

The third study consisted of a detailed biomechanical inverse dynamic analysis of gait to understand how the differences in the skeletal muscle properties reported in the previous two studies impact on the walking capacity in CHF. Surprisingly, and in contrast with our hypotheses, no difference in preferred speed or overall limb kinematics and kinetics between the CHF and control groups were found, possibly explained by an optimization of the mechanical cost of transport (the amount of mechanical work performed per distance traveled; J/kg/m). Nevertheless, when normalized to triceps surae volume, over two times greater ankle plantarflexion work was required in CHF. This, together with a greater reliance on the ankle to power walking in CHF patients compared to age-matched healthy individuals, may be a primary cause of the earlier onset of fatigue known to affect CHF.

Also included in this thesis is the study of Panizzolo et al. (2013), (Appendix). This work was a component of this thesis but not included as a main chapter because it was not on CHF per se, but assessed the effect on in vivo SOL muscle mechanics in walking due to ageing. This work found that the muscle length pattern of the SOL is altered in older adults walking at speeds matching the preferred speed of young adults. Interestingly however, at the slower preferred walking speed of older adults, the SOL length pattern is similar to that of the young adults at their preferred speed. Therefore, a slower preferred speed in older adults may reflect an optimization of muscle mechanics. Importantly, this study forms the basis for future comparisons of in vivo SOL muscle mechanics during walking in CHF that permit a detailed understanding of age- vs. disease specific alterations.
6.2 Integrated synthesis of results

Taken together, the findings of these studies offer a novel interpretation of the mechanisms explaining the reduced exercise and walking capacity in CHF. This thesis suggests that the triceps surae, and especially the SOL, is a key muscle in CHF that is particularly affected by muscle wasting and strongly linked to peak aerobic capacity. It also points to a causal link between the morphological and functional alterations of the calf muscles and fatigue during walking in CHF.

The morphological and gait analyses were coherent in identifying the plantarflexor group as the principle factor differentiating the CHF and control groups. Why the plantarflexors should be more affected in CHF compared to proximal muscles remains unclear. It is possible that, due to their important role in generating the majority of mechanical work (McGowan et al., 2009; Winter, 1983), more prominent maladaptation (or disuse) is apparent in CHF. Alternatively, the distal plantarflexor group may be more affected by a reduction in blood flow and oxygen supply (Musch and Terrell, 1992).

As well as displaying pronounced muscle wasting, the plantarflexors, and in particular the SOL size, correlated strongly with peak (Study 1). Interestingly, this stands in contrast to the control group. This might be explained if skeletal muscle is the primary limit to aerobic energy expenditure in CHF, compared to the delivery of oxygen in healthy individuals (Sullivan et al., 1989). Gait analysis in CHF (Study 3) offers an alternative (or complementary) explanation for the correlation between the triceps surae size and peak. Study 3 revealed that a larger proportion of the total mechanical work of walking is supplied by plantarflexion in CHF, compared to healthy age- and exercise-matched participants, and that the relative contribution of plantarflexion work
in CHF increases with faster walking speed. Therefore, it is plausible that a greater proportion of the metabolic energy use in CHF is expended by the plantarflexor muscles as the metabolic demand of walking increases with speed. Considering that peak was assessed in an incremental walking protocol, the higher relative demand placed on the plantarflexor muscles in CHF could explain why a stronger correlation exists between these muscles and peak in CHF compared to the control group.

Whereas the higher dependence on the plantarflexors in walking is consistent with the link between muscle size and peak in CHF, it represents a paradox with regard to muscle loading. Indeed, the greater reliance on the small triceps surae muscles in CHF results in a marked (> 2x) relative load in these muscles (J/kg and Nm/PCSA ankle plantarflexion work and peak moments). Why CHF patients use their relatively small triceps surae muscles proportionately more in walking rather than offloading them, either by adopting a slower speed or by relying more on other joints to power walking, was surprising and the explanation for this remains uncertain. One possibility for the greater reliance on the plantarflexors in CHF might be that other joints in CHF are relatively weaker, despite the largest reduction in muscle size occurring in the triceps surae (Study 1). While there is no direct evidence for this, some previous studies on knee and hip strength found that reductions in net joint torque could not be explained by muscle size alone (Harrington et al., 1997; Toth et al., 2010, 2006), unlike the predicted SOL force in the present thesis (Study 2). It may therefore be that knee and hip muscles are in fact relatively weaker in CHF, thus explaining the higher reliance on the smaller plantarflexors. Without undertaking detailed measurements, such as those of Study 2, this remains speculative.
An alternative and intriguing explanation for the greater reliance on the plantarflexors to power walking stems from the findings that muscle fascicles are shorter (Study 2) and that the ankle undergoes greater dorsiflexion during stance (Study 3) in CHF compared to the control group. The combined effect of these two alterations is that CHF SOL muscles may undergo greater lengthening strain during stance, possibly resulting in passive force generation within the muscle fibers. A passive force mechanism in CHF muscle would decrease the reliance on metabolically expensive active force generation (Griffin et al., 2003), an outcome that would be highly advantageous for CHF patients who suffer from a severely compromised metabolic capacity (Study 1). Currently the length operating range of the SOL of the CHF patients during walking remains unknown. However, results from a previous study in healthy young individuals (Rubenson et al., 2012) and an annexed study carried out as part of this thesis on healthy young and older participants [Panizzolo et al., 2013; (Appendix)] suggest that the SOL operates at lengths where passive force may be generated. This finding paves the way for future analyses aimed at differentiating the effects of age and CHF on muscle mechanics in walking and its role in dictating locomotor function in CHF patients.

Although the exact reason for the greater reliance on the comparatively small plantarflexor group during walking in CHF is not known, this finding highlights a novel mechanism that may underscore the low exercise capacity observed during walking in CHF (Toth et al., 1997). The combined data from Studies 1-3 suggest, for the first time, that it is the marked elevation in the relative work in the plantarflexors per se, compared to other muscle groups, that may limit walking capacity and endurance in CHF. Study 3 also suggests that it is cumulative effort (work per distance travelled) that dictates locomotor behavior in CHF as opposed to the acute production of mechanical work or joint moments within a single step. Lower loading in the small triceps surae during
stance could easily be achieved by adopting a slower walking speed (Study 3), thus offloading the high relative work and moments produced in this muscle group. However doing so does not lower the work per distance travelled (cost of transport, Study 3) and thus walking slower offers little advantage if it is the cumulative work to cover a given distance that dictates fatigue in CHF.

Study 1 and 3 provide evidence that the SOL may act as a main determinant of exercise and walking capacity in CHF. Study 2 suggests that the primary factor influencing this relationship is muscle wasting, as opposed to other possible neuromuscular factors such as neural drive (Leonard et al., 1997) or the muscles specific tension (Maganaris et al., 2001), although the contribution of these factors cannot be ruled out completely before further study. Importantly, Study 2 identified that the source of the reduced muscle size (mass) is not only a reduction in muscle cross sectional area, as was concluded in Study 1, but is also a result of reduced fascicle length. The observation that both the optimal fascicle length and the fascicle lengths during passive stretch were shorter in CHF, but that the strain in passively stretched muscle is similar (Fig. 4.1), indicates a loss of serial sarcomere numbers. The discrepancy between Studies 1 and 2 is due to the functional assessment of fascicle length (Study 2) compared to the measurements at a single common joint angle (Study 1). Why CHF patients would experience a loss of serial sarcomeres is not known. Possible reasons might be due to a loss of joint excursion (Koh and Herzog, 2004), although joint excursion during walking are similar, or possibly a remodeling signal specific to CHF.

By combining novel 3D muscle imaging techniques and detailed biomechanical analyses not previously adopted in CHF or healthy older adults, this thesis discovered that the triceps surae, and especially the SOL, is a sentinel muscle group in CHF that
can potentially serve as a secondary indicator for CHF health. The exact causal factors for the maladaptation in the triceps surae remains unknown, but muscle wasting both in muscle PCSA and length appear the major factor leading to loss of strength. Exercise to promote muscle hypertrophy and lengthening could be key for restoring functional capacity in CHF.

6.3 Comparison to other conditions: is CHF a unique musculoskeletal pathology or extreme deconditioning?

Studies of this nature, utilizing novel musculoskeletal imaging technologies and biomechanical methods, have not previously been applied in the context of CHF to assess muscle morphology and function. Despite this, previous work has been undertaken in other populations where muscle dysfunctions arise as a result of conditions where the muscle is not the primum movens. These conditions offer a useful comparison to the musculoskeletal limitations found in the CHF syndrome.

It is logical to compare CHF to ageing, where alterations in musculoskeletal structure and function and modifications in gait mechanics are known to occur. Studies on ageing indicate a global deterioration and loss of function of the skeletal muscle properties compared to younger individuals. Investigations comparing young and old adults report reduction in lower limb lean mass (Janssen et al., 2000), muscles size (PCSA and volume; Narici et al., 2003), and in other muscle parameters linked to the force production such as fascicle length and pennation angle (Narici et al., 2003). These structural alterations are expected to be responsible for the reduction in strength associated with ageing (Narici et al., 2003). However, unlike the effects of ageing, the analyses of CHF in this thesis only exhibited clear reductions in size in the triceps surae,
with minimal differences found in total lower limb lean mass. This suggests that the lower limb muscles in CHF are particularly affected compared to the more general effect of age. It is also interesting to note that, in ageing, the gastrocnemius muscles (lateral and medial) undergo greater muscle wasting (Morse et al., 2005a) than the SOL while the opposite is the case in CHF (Study 1). Furthermore, no differences were found in the pennation angle of the SOL or other triceps surae muscles (gastrocnemius muscles) in CHF patients compared to that found in old vs. young adults.

In older adults muscle force decay is not only due to architectural parameters but is also due to functional aspects. Among these, studies have reported a lower specific force (Morse et al., 2005b), a higher co-activation of antagonist muscles (Klein et al., 2001; Macaluso et al., 2002), and a reduction in motor unit activation capacity (Harridge et al., 1999). Conversely, Study 2 found that the activation of agonist and antagonist muscles and computational predictions of their contribution to net joint moments were not affected by CHF. Although we did not measure specific force or neural inhibition directly using muscle electrical stimulation techniques, our findings also suggest that these parameters were likely unaltered in CHF. That specific force is unaffected by CHF is supported by at least one study investigating individual muscle fiber force in the vastus lateralis from CHF patients and control participants (Miller et al., 2010).

Gait has also shown to be affected in aging, probably in part as a consequence of the muscle abnormalities reported above. Older adults adopt slower preferred walking speeds [(Himann et al. 1988; Panizzolo et al. 2013; (Appendix)], possibly because of adaptive musculoskeletal mechanisms specific of ageing (Mian et al., 2007; Panizzolo et al., 2013). In a paper completed as part of this thesis, Panizzolo et al. (2013) concluded that a reduction in speed may permit muscles to function in a mechanically
similar manner to that of younger adults. At a joint level, among the most important alteration displayed by older adults is the redistribution of mechanical work from the ankle joint to the hip joint (DeVita and Hortobagyi, 2000). Remarkably, however, neither a differences in walking speed nor a larger amount of work produced at the hip joint were found in CHF (Study 3). In contrast to ageing, CHF patients redistributed work from the hip to the ankle.

Comparisons between ageing and CHF point to disease-specific adaptations in muscle structure and locomotor function. Bed rest offers a further comparison in which the disuse and deconditioning remodelling signal is more pronounced. An extensive review by Narici and de Boer (2011) listed the musculoskeletal abnormalities associated with bed rest. A primary alteration is a loss in muscle size; subjects forced to stay in bed reported a reduction of 3% in the volume of the thigh after only 7 days (Ferrando et al., 1995). This reduction has been shown to increase to approximately 30% after 90-120 days, as reported in studies investigating quadriceps and calf muscle (Shackelford et al., 2004). Interestingly, bed rest atrophy has been reported to occur in all lower limb muscles (Narici and de Boer, 2011), similar to ageing, but in contrast to the results in CHF patients in Study 1. There are nevertheless some reports of a more marked reduction in size in distal compared to proximal muscles (Belavý et al., 2009; LeBlanc et al., 1997), akin to the present observations in CHF. Moreover, similar to CHF, studies on the SOL and vastus lateralis indicate that atrophy associated with recumbency affects type I fibers more than type II fibers (Hortobágyi et al., 2000; Rudnick et al., 2004; Trappe et al., 2004). These morphological alterations have an impact on muscle function, with reported reduction of muscle strength of 10%-50% occurring in the plantarflexors and in the knee extensors after 20-120 days (Kubo, 2004; LeBlanc et al., 1992). Disuse atrophy and functional deficits associated with bed rest thus appear to
share some similarities with the modifications observed in CHF, including fiber type specificity of muscle wasting and possibly a distal-proximal gradation of muscle loss. Other alterations associated with inactivity such as reduced oxidative capacity or increased apoptosis have also been observed in CHF (Rehn et al., 2012), thus raising the hypothesis that alterations of skeletal muscle in CHF patients are due, at least in part, to extreme deconditioning (Rehn et al., 2012).

Further support for such a ‘deconditioning’ theory can be taken from findings on chronic obstructive pulmonary disease (COPD). Although, COPD and CHF represent two distinct pathophysiological processes, several similar musculoskeletal alterations have been reported including reduced muscle size [thigh, (Bernard et al., 1998), rectus femoris (Seymour et al., 2009)], proportionately greater loss of type I fibers (Jobin et al., 1998) and reduced endurance (Swallow et al., 2007).

It is still not clear if the majority of the alterations in the skeletal muscle of CHF are driven by extreme conditioning or by a disease-specific myopathy (Rehn et al., 2012). Comparing the findings of the present work with those of other models of secondary muscle dysfunction does provide several examples in support of the theory (Miller et al., 2010; Toth et al., 2011) that CHF is a unique musculoskeletal condition. These include both muscle-level architecture as well as functional gait parameters. It should also be stressed that the CHF patients recruited in these studies underwent regular exercise rehabilitation that was similar to the weekly exercise levels of the control group. Therefore, it is unlikely that the CHF patients in this thesis were subject to much greater muscle deconditioning due to disuse compared to the control group. Therefore, the complex adaptive mechanisms revealed by this thesis may very well be related to the disease per se, and not just to an aging or inactivity phenomenon. This can be the
consequence of elevated oxidative stress (Grieve, 2003), prolonged and occult inflammation (Braunwald, 2008), profound impairment in peripheral oxygenation (Drexler, 1995), or intrinsic muscle disease which is comparable to the myocardial idiopathic dilated cardiomyopathy.

6.4 Future research into the mechanisms of skeletal muscle adaptation in CHF

To further elucidate whether alterations in the skeletal muscle of CHF represent a disease-specific condition or are also associated with unloading and disuse, it will be fundamental to design larger studies that more closely control for these factors. Control populations should be carefully selected so that they closely match not only for anthropometry and body composition but also for activity levels, thus ruling out the possibility that the effect of the disease on the skeletal muscle will be concealed by other factors such as inactivity. To date, few studies control for activity levels between CHF and healthy individuals (Rehn et al., 2012). While the studies conducted in this thesis compared CHF patients undertaking regular exercise (as part of their routine care) with a control participants that engaged in similar overall exercise loads, future research will benefit from research designs where the same exercise is prescribed for all participants.

In addition to highly controlled studies involving a large cohort of participants, further in-depth physiological and biomechanical analyses will also aid in establishing the disease-specific vs. deconditioning nature of the skeletal muscle alterations in CHF. Future work in CHF might consider extending the investigations on single muscle properties to strengthen the relationship between skeletal muscle alterations and their
impact on whole body function, with the ultimate goal to provide subject-specific rehabilitative protocols. As an example, while additional information was offered by this thesis regarding the generation of isometric force in skeletal muscle, it is still not clear if the total amount of mechanical work generated by the muscle fibers is similarly affected in CHF. This is an important consideration since skeletal muscle, including the plantarflexors, function to perform mechanical work during walking in addition to functioning isometrically. Preliminary dynamometer-based (Biodex) isovelocity experiments have been conducted over the course of this thesis exploring the mechanical work capacity of the SOL muscle in CHF and control participants. Initial results seem to indicate that the ability of the SOL muscle to produce mechanical work is likewise compromised in CHF and due to a loss of muscle volume (Fig. 6.1). Further analysis of additional participants will be required to establish accurately the relationship between muscle work capacity and muscle size in CHF.

![Figure 6.1. Preliminary data (n = 4) for work produced by the plantarflexors in isokinetic condition (5°/s). Control in black and chronic heart failure (CHF) in grey. Data are means ± S.D.](image)

Study 2 provided data on both active isometric and passive force-length data of the SOL. However, equally important to the generation of mechanical work is the force-
velocity (F-V) relationship, which has not been investigated yet at single muscle level in the context of CHF. Providing information on the F-V properties of skeletal muscle in CHF will offer a better insight into the mechanisms leading to impaired work capacity and will help determine whether mechanical work is indeed limited by muscle size or muscle mechanics. Protocols involving isokinetic contractions across a range of shortening velocities can be used to elucidate the F-V relationship in humans in vivo.

Investigation into mechanical work capacity could provide further practical importance to indicate which type of exercise is most suitable for restoring the ankle joint function in CHF. A rehabilitative protocol involving isometric-based contraction will act to augment strength at the specific joint angle by means of increased neural adaptations (Kitai and Sale, 1989), whereas a isokinetic-based will restore muscle contractility (Akima et al., 1999).

In addition to isolated muscle force and work experiments, analyses of gait is also an important aspect to understanding the functional differences in skeletal muscle in CHF. Research from our laboratory [(Rubenson et al. 2012; Panizzolo et al. 2013, (Appendix)] showed how the SOL function changes between young and old adults during walking and how its stretch-shorten cycle during walking may influence preferred walking speed in young and old adults. The rationale to conduct a similar analysis at a muscle level in CHF has been provided by the altered gait function (Study 3) and active and passive force production (Study 2). Studies of this nature integrating isolated force-length-velocity data with measurements of in vivo fascicle lengths and velocities during walking will help answer more mechanistic questions concerning the F-L and F-V properties in this population and how they might impact locomotor function.
Lastly, a musculoskeletal modeling approach could be applied to obtain information otherwise not measurable \textit{in vivo}, such as individual muscle force production during gait. Incorporating the rich dataset developed in this thesis with musculoskeletal modeling approaches (e.g. EMG-informed modeling, static or dynamic optimization or computed muscle control) will facilitate unprecedented analyses of individual muscle mechanics during functional movement tasks in CHF. With these studies it will be possible to further target specific muscle parameters for rehabilitation.

\textbf{6.5 An evidence-base for optimizing exercise rehabilitation}

The ultimate goal of this thesis was to provide useful information on CHF skeletal muscle and gait that could assist therapists and clinicians to design optimized rehabilitation, thus restoring function and improving the patients' quality of life. Previous analyses investigating the mechanisms leading to impaired exercise capacity and fatigue in CHF have either addressed gross physiological function (e.g. examining general lower limb lean mass or strength and their relation to peak oxygen uptake) or skeletal muscle histopathology. The present thesis used novel imaging and experimental and modelling biomechanical techniques to drive forward new structure-function analyses at an individual muscle level. These analyses show that the skeletal muscle, and potentially the SOL more than other muscles, is an important factor that determines walking exercise capacity and possibly fatigue in CHF. These findings provide scientific rationale for including calf muscle-specific exercise training, as an important component of whole body training, to help restore the functional capacity in CHF since those strategies focusing on principal muscle limitations are most likely to optimise outcomes and/or to prevent further deterioration.
This begs the question: what form of exercise will optimize the rehabilitation of skeletal muscle, and in particular the SOL in CHF? The general benefits associated with exercise prescription in CHF are well established and they include enhancement of the aerobic capacity, skeletal muscle structure and function, peripheral blood flow, neurohormonal and endothelial function, with some evidence for improvements in central haemodynamics, cardiac volumes and mortality (Selig and Hare, 2007). Several studies (Beckers et al., 2008; Degache et al., 2007; Delagardelle et al., 2002) reported that endurance/strength training is superior to endurance training alone in ameliorating muscle function and peak.

Interestingly, a simple training program based on regular heel-raise exercises has been shown to target the SOL muscle, increasing its strength and thickness in the elderly (Fujiwara et al., 2010). However, specific training modalities may be particularly beneficial and effective in CHF. For example, an innovative and beneficial training modality for CHF might be the use of eccentric muscle training. Endurance-type eccentric training has been adopted for rehabilitation in other chronic diseases (Roig et al., 2008) and this modality has been found to result in greater strength gains, muscle mass, and fiber hypertrophy (Friden et al., 1983; Hortobágyi et al., 1996; Lastayo et al., 2000) compared to concentric muscle training alone. The rationale for this type of training in CHF is based on several functional and metabolic advantages. First, load intensity is an important stimulus for tissue growth (Enoka, 1988) and eccentric training has an advantage in generating high loads for a remarkably low energetic cost (Bigland Ritchie and Woods, 1976; Lastayo et al., 2000). This characteristic of eccentric training is ideally suited to CHF, permitting a high load stimulus within a group known for their severely restricted metabolic scope (i.e. “more for less” principle). Eccentric training has also been shown to preferentially recruit type II fibers (Friden et al., 1983), thus
providing a more selective stimulus to ameliorate muscle weakness compared to concentric training considering the shift in muscle fiber distribution from type I to type II fibers in CHF (Schaufelberger et al., 1997; Sullivan et al., 1990; Drexler et al., 1992).

Finally, eccentric training is known to increase fiber length via sarcomerogenesis (Butterfield et al., 2005), which might be beneficial in CHF which presents shorter optimal fascicle length at least in the SOL (Study 2).

Although training the plantarflexors in CHF to restore their function seems reasonable, a completely different alternate approach could also be proposed to enhance the performance of this muscle group. Study 3 highlighted how the plantarflexors have a high relative effort due to their small size and because they provide the majority mechanical work of locomotion compared to the other joints. This implies that, simply in walking, CHF patients are already exercising this muscle group to a greater extent than other lower limb muscles. Therefore, whether further taxing this muscle group by implementing dedicated muscle exercise will relieve the symptoms of muscle wasting or exacerbate the condition (possibly through negative adaptive signals) remains an open question. If the latter were true, unloading the plantarflexor group, as opposed to increasing load stimulus, could in turn be beneficial. Following this rationale, targeting other muscle groups during exercise-based rehabilitation might permit an enhancement in the global function and a delayed onset of fatigue. Although thought-provoking, this hypothesis remains speculative and only further studies will be able to elucidate which is the best training program for CHF.

Whilst this thesis advocates for optimal exercise rehabilitation practices for CHF, it recognises that a combination of exercise and more common pharmacological prescription may most benefit CHF patient management. It is important, therefore, that
a broader understanding of the interaction between exercise and pharmacological treatments are generated. Traditional medications for the management of CHF syndrome include angiotensin-converting enzyme (ACE), β-blockers and diuretics as spironolactone (McMurray et al., 2003). Human growth hormone (HGH), insulin-like growth factors (IGF), testosterone and anti-inflammatories could be prescribed to treat the abnormalities reported at muscle level although their action under specific muscle exercise training is currently unknown. Although the main function of statins is to lower cholesterol level, previous investigations have reported a plethora of pleiotropic effects, including reduction of inflammation and improvement of endothelial function associated with this drug in the treatment of CHF (van der Harst et al., 2006). Nevertheless, from a perspective whereby the skeletal muscle regulates the progression of the disease, the use of statins should be considered carefully in CHF. Several side effects of statins on muscle properties have been noted (Parker and Thompson, 2012), and it may be preferable to limit their use in CHF in order to avoid to further reduce performance of the skeletal muscle. Discerning the skeletal muscle abnormalities resulting from statin use vs. the CHF syndrome itself will prove valuable research.

6.6 Conclusions

Using innovative combinations of ultrasound imaging and biomechanical experimentation and modeling, this thesis offers a novel examination of the mechanisms leading to the reduced exercise and walking capacity in CHF. The results suggest that the SOL is a key muscle in CHF, particularly affected by muscle wasting and strongly linked to peak aerobic capacity. This raises the possibility of using SOL volume as an estimate of exercise capacity in circumstances where peak is not easily measurable due to a lack of resources or adequate trained medical staff. Further research to establish whether this highly accessible measurement could possibly be used as a simple
Chapter 6 - General Discussion

prognostic marker is warranted, especially with the clear applicability in less developed
countries where trained staff and resources are scarce. This thesis also proposes a causal
link between the morphological and functional alterations of the calf muscles and
limited exercise capacity and fatigue during walking in CHF. As a consequence, it is
proposed that improving calf muscle condition, and specifically muscle size, may prove
important in ameliorating the limitations to exercise and walking capacity in CHF.
These findings provide a scientific basis for the design of optimized rehabilitation
strategies in CHF. Clinicians and medical practitioners should follow the specific
recommendation to include ankle/calf based training (or offloading) as an important part
of whole-body exercise rehabilitation.
References


Chapter 6 - General Discussion


Chapter 6 - General Discussion


This Appendix is based on a paper published as: Panizzolo FA, et al. (2013). Soleus fascicle length changes are conserved between young and old adults at their preferred walking speed. *Gait & Posture* 38, 764-9.
ABSTRACT

Older adults have been shown to naturally select a walking speed approximately 20% slower than younger adults. We explore the possibility that a reduction in preferred speed in older adults represents a strategy to preserve the mechanical function of the leg muscles. We examined this question in the soleus muscle in eight healthy young (25.8 ± 3.5 years) and eight healthy older adults (66.1 ± 2.3 years) who were paired so that their preferred speed differed by ~20%. Soleus muscle fascicle lengths were recorded dynamically using ultrasound, together with simultaneous measurements of soleus EMG activity and ankle joint kinematics while a) older adults walked on a treadmill at a speed 20% above their preferred speed (speeds matched to the preferred speed of young adults), and b) young and older adults walked their preferred treadmill speeds. Analyses of mean muscle fascicle length changes revealed that, at matched speeds, older adults had a statistically different soleus fascicle length pattern compared to young adults, where the muscle’ stretch-shorten cycle during stance was diminished. However, older adults walking at their preferred speed exhibited a more pronounced stretch-shorten cycle that was not statistically different from young adults. Conserving muscle length patterns through a reduction in speed in older adults may represent a physiologically relevant modulation of muscle function that permits greater force and power production. Our findings offer a novel mechanical explanation for the slower walking speed in older adults, whereby a reduction in speed may permit muscles to function in a mechanically similar manner to that of younger adults.
A.1 Introduction

One of the primary characteristics of gait in older adults (OA) is a reduction in preferred walking speed. Indeed, others have reported around 20% lower habitual walking speeds in healthy community dwelling older adults (1.0-1.3 m/s) compared to young adults (YA) (1.3-1.5 m/s) (Himann et al., 1988; Oberg et al., 1993). This age-related reduction in walking speed reflects a reduced motor capacity that may be linked to a greater incidence of falls (Cesari et al., 2002) and a comparatively high metabolic rate in older adults for a given speed (Martin et al., 1992). Examining the muscular mechanisms linked with a reduced walking speed in older adults may prove important for understanding and treating gait deficits associated with ageing.

The underlying muscular mechanisms responsible for a reduced walking speed in OA compared to YA remain unclear. Previous literature has reported alterations in the structural and functional properties (Christou et al., 2011) of skeletal muscle and tendon linked with aging, such as a loss of muscle mass (sarcopenia), in particular in the lower limbs (Janssen et al., 2000), reduced pennation angle and fascicle lengths (Narici et al., 2003) and lower tendon Young’s modulus and stiffness (Onambele et al., 2006). These changes in muscle-tendon properties have important implications for the force and power capacity and efficiency of muscle during walking (Lichtwark et al., 2005; Zajac, 1989) and it is logical to assume that they contribute to the altered gait mechanics and control in OA (Barrett et al., 2008). Yet, exactly how these muscle-tendon characteristics influence speed selection in OA is not known. Is it possible, for instance, that a slower self-selected walking speed in OA represents a strategy to preserve the muscle’s mechanical milieu at a level similar to those of YA? It has been proposed that humans and other species select speeds that lead to optimal function of skeletal muscles including minimization of force, mechanical power and energy expenditure (Saibene
and Minetti, 2003; Neptune et al., 2008; Rubenson et al., 2004; Watson et al., 2011). A conservation of muscle function at preferred walking speeds across the age span would suggest that the slower speeds selected by OA may be a strategy to maintain optimal muscle mechanical function.

The focus of this study was to explore the aforementioned question by assessing how the soleus muscle functions 1) when OA walk at speeds matched to the faster preferred speed of YA and 2) during walking at preferred speeds in YA and OA. Specifically, we examined the differences in soleus muscle fascicle length changes between YA and OA given that this property is known to be intimately linked to muscle function (Zajac, 1989). We hypothesized that the soleus would lengthen less during the early part of stance (dorsiflexion) and shorten less during the latter part of stance and early swing when the muscle is active (plantar-flexion) in OA compared to YA when walking at matched speeds, similar to that reported for the stretch-shorten cycle of the medial gastrocnemius muscle (Mian et al., 2007), but that these differences would diminish when OA use their preferred walking speed. A corollary hypothesis was, therefore, that the muscle fascicle length change that occurs during stance in OA is greater when walking at their preferred speed compared to a faster-than-preferred speed. The soleus muscle represents a muscle of choice in addressing this question since it is amenable to dynamic in vivo imaging (Cronin et al., 2009; Rubenson et al., 2012), because the plantar flexors, and the soleus in particular, have been identified as the primary source of mechanical work during gait (Neptune et al., 2008; McGowan et al., 2009), and finally due to the finding that the primary locus of gait impairment in OA is at the ankle plantar flexors (Graf et al., 2005).
A.2 Methods

A.2.1 Subjects

Sixteen healthy male subjects free from previous lower-limb injuries and musculo-skeletal disorders were recruited for this study. They were divided in two groups of eight by age: YA 25.8 ± 3.5 years, mean ± S.D.; OA 66.1 ± 2.3, years mean ± S.D. The YA group was composed of the same subjects recruited for a separate parallel study on soleus mechanics during walking and running (Rubenson et al., 2012). Anthropometric characteristics including tibia length, weight and height were not statistically different between groups (0.41± 0.03 m, 1.75 ± 0.06 m and 70.3 ± 9.2 kg, mean ± SD for YA vs. 0.40 ± 0.02 m, 1.74 ± 0.06 m and 72.1 ± 9.4 kg, mean ± S.D. for OA, respectively).

All subjects provided written, informed consent prior to participating in the study and all of the procedures were approved by the Human Research Ethics Committee at The University of Western Australia.

A.2.2 Self-selected walking speed and gait kinematics

Subjects performed over-ground and treadmill walking trials. Their preferred over-ground speed was assessed by timing participants walking 10m along a carpeted surface. A minimum of 5 trials were used to assess mean preferred speeds. For the instrumented treadmill trials (Bertec, Columbus, OH, USA), preferred walking speed was assessed by permitting subjects to freely self-adjust the treadmill speed, starting at a speed 30% slower than their preferred over-ground speed and incrementing speed by 0.01 m/s until they reach their preferred speed. This procedure was repeated five times from which a mean self-selected treadmill speed was determined and used in subsequent tests. All participants attended a familiarization session on the treadmill prior to data collection.
Older adults were paired with younger adults so that the preferred walking speed between each pair differed by 19 ± 2%. Each subject was tested at their preferred treadmill speed, while OA were also tested at a speed 20% greater than their preferred speed, thus matching within 0.16 m/s the preferred speed of their paired YA. This permitted a case-matched comparison of the soleus muscle function 1) between preferred speeds in YA and OA and 2) between preferred speeds in YA and a 20% faster-than-preferred speed in OA that matched the preferred speed of YA (thus controlling for relative speed in OA).

Three-dimensional gait analysis was performed on each person during their treadmill walking trials. To this-end, 22 retro-reflective markers were attached to the subject’s pelvis and lower limb body landmarks and motion was collected using an 8-camera VICON MX motion capture system (Oxford Metrics, UK; 100 Hz). Marker placement and joint modeling were in accordance with the UWA lower body model (Besier et al., 2003). All marker trajectories were filtered using a zero-lag 4th order low pass Butterworth filter with a 6 Hz cut-off frequency, which was defined using a custom residual analysis algorithm (MATLAB, The MathWorks Inc., USA). To determine the stance and swing phases, individual leg ground reaction forces were collected from the treadmill force plates at a frequency of 2000 Hz and synchronized with the motion data. Ankle joint angles were defined following the ISB guidelines (Wu et al., 2002) with negative values representing plantarflexion and positive values representing dorsiflexion (0° represents a neutral angle with the foot perpendicular to the tibia). Knee joint angles were defined with negative values representing extension and positive values flexion (0° knee fully extended).
A.2.3 Muscle length changes and activation

While the subjects walked on the treadmill, dynamic B-mode ultrasound images of the soleus muscle (Telemed, SonoBlaster128, Lithuania; image capture 70 - 80 Hz) were collected using a 7.5 MHz linear array low-profile probe (transducer field width = 60 mm) attached to their right calf using an elastic bandage (Elastoplast Sport, Beiersdorf, Australia). The probe placement was standardized across subjects using a location over the medial gastrocnemius (MG) where the MG muscle-tendon junction was visible in the distal portion of the scan (Rubenson et al., 2012). Soleus muscle fascicle lengths were measured in the mid-region of the ultrasound scan and calculated as the distance between the fascicle endpoints, defined as the intersection of the fascicle with the superficial and deep aponeurosis. Fascicle endpoints were digitized manually using ImageJ software (Abràmoff et al., 2004) by two investigators (one investigator analyzed all OA data). The digitized data were filtered using a 4th order zero-lag 5 Hz low-pass Butterworth filter (MATLAB, The MathWorks, Natick, MA). An analysis of muscle fascicle lengths across the stride on a sub-set of trials from all YA participants indicated minimal and non-significant differences between investigators; cross correlations were 0.97 – 0.99 with zero lag and the average difference in length from all data points was 0.9 mm. A rigid cluster composed of three non-collinear retro-reflective spherical markers was fixed to the ultrasound probe to verify that its movement relative to tibia was minimal during walking. The probe movement relative to the tibia across the stride was within 5.2°, 2.8° and 4.9° for movement in the tibia’s sagittal plane, frontal plane and coronal plane, respectively. Fascicle lengths were normalized by dividing them by the length of the resting fascicle at 8° of dorsiflexion. This joint posture was selected as it represents the mean joint angle corresponding to the soleus muscle slack length (the length above which passive forces first appear) in YA (Rubenson et al., 2012). Resting fascicle lengths were recorded with subjects seated in a dynamometer (Biodex, M3,
Shirley, NY, USA) with the foot rigidly strapped to a customized plate allowing an accurate adjustment of joint angle. Although we did not directly assess muscle slack lengths in OA, previous studies have reported that there are no differences in the ankle angle at which passive force in plantarflexors develop between YA and OA (Gajdosik et al., 1999). Therefore, this method was adopted to represent a functionally relevant assessment of muscle length changes relative to resting muscle lengths.

During the treadmill walking trials surface electromyography (EMG) from the soleus muscle was simultaneously measured (Noraxon, Scotsdale, Arizona, USA or Motion Lab Systems, Baton Rouge, LN, USA; 2000 Hz) with the motion and ultrasound data using the VICON system. EMG signals were high-pass filtered (4th order Butterworth, cutoff 30 Hz), rectified and low-pass filtered (4th order Butterworth, cutoff 7 Hz) to obtain an EMG linear envelope. The EMG linear envelope recorded during rest (non weight bearing) was subtracted from the walking values and the signal was subsequently normalized to the peak value across the stride.

A.2.4 Statistical analysis

A minimum of five non-consecutive gait cycles were used to compute subject mean muscle fascicle length, EMG linear envelope and joint kinematics. Spatial-temporal gait parameters including stride length, stride frequency, duty factor, stance and swing times were calculated (Table A.1). Continuous muscle and joint kinematics in the sagittal plane data were normalized to 101 data points for each of the five strides and used to compute mean subject data, which in turn were used to compute mean group data used in descriptive statistics and group comparisons.
Analysis of the muscle fascicle length pattern was performed using a one-tailed unpaired Student’s t-test to determine significant differences in the amount of active lengthening and shortening, as well as average normalized muscle fascicle lengths across the stride and in stance and swing separately. Muscle lengthening was defined as the increase in length from the time the muscle began to lengthen after heel-strike to the maximum fascicle length in stance; shortening was defined as the decrease in length between the maximum fascicle length in stance to the minimum fascicle length (observed just after toe-off and in conjunction with a return to baseline muscle activity).

To account for the false discovery rate due to multiple comparisons, additional post hoc testing was performed on muscle fascicle length measurements using the Benjamini method (Benjamini and Hochberg, 1995) (see Table A.2). Statistical procedures (paired Student’s t-test) were also used in the same manner described above to determine if significant differences arise due to changing speed among the OA participants (analyses were performed both on the group and on each individual). Secondary to our analysis of muscle length, a two-tailed Student’s unpaired t-test was used to determine significant differences in joint angles and mean EMG linear envelope between the two groups. The significance level was assessed at $p < 0.05$. Effect sizes were calculated using Cohen’s $d$ method. Data are reported as mean ± S.D. unless otherwise stated.

**Table A.1.** Spatio-temporal parameters in young adults (YA) and old adults (OA) for preferred and matched walking speeds.

<table>
<thead>
<tr>
<th></th>
<th>YA</th>
<th>OA (preferred)</th>
<th>OA (matched)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testing speed [m/s]</td>
<td>1.14 ± 0.16</td>
<td>0.96 ± 0.17*</td>
<td>1.15 ± 0.22§</td>
</tr>
<tr>
<td>Stance time [s]</td>
<td>0.71 ± 0.05</td>
<td>0.69 ± 0.05</td>
<td>0.65 ± 0.04§</td>
</tr>
<tr>
<td>Swing time [s]</td>
<td>0.44 ± 0.04</td>
<td>0.49 ± 0.05</td>
<td>0.45 ± 0.04§</td>
</tr>
<tr>
<td>Duty factor</td>
<td>0.62 ± 0.03</td>
<td>0.59 ± 0.02</td>
<td>0.59 ± 0.01</td>
</tr>
<tr>
<td>Stride frequency [Hz]</td>
<td>0.87 ± 0.05</td>
<td>1.18 ± 0.10*</td>
<td>1.09 ± 0.07*§</td>
</tr>
<tr>
<td>Stride length [m]</td>
<td>1.31 ± 0.17</td>
<td>0.82 ± 0.18*</td>
<td>1.06. ± 0.26*§</td>
</tr>
</tbody>
</table>

* Significantly different from YA ($p < 0.05$)

§ Significantly different from OA preferred ($p < 0.05$)
A.3 Results

A.3.1 Comparison of muscle mechanics and joint kinematics at matched walking speeds in YA and OA

In the YA, from early stance, the soleus muscle lengthened by 10.6 ± 8.4% (normalized muscle lengths) and subsequently shortened rapidly during the second half of stance by 23.7 ± 4.5%. In contrast, OA exhibited significantly less lengthening during early/mid-stance (3.7 ± 3.3% normalized muscle lengths; p < 0.05) and less shortening during the second half of stance and early swing (14.9 ± 5.7% normalized muscle lengths; p < 0.05). Significantly shorter mean normalized fascicle length across the total gait cycle (0.86 ± 0.16 YA vs. 0.73 ± 0.09 OA) and both in stance (0.86 ± 0.16 YA vs. 0.74 ± 0.09 OA) and swing phases (0.86 ± 0.16 YA vs. 0.74 ± 0.09 OA) independently were also found in OA compared to YA (Table A.2, Fig. A.1a). A Cohen’s d analysis indicated that the effect of age on the above variables were large (Table A.2).

Absolute resting lengths (mm) were not significantly different between YA (37.7 ± 5.3 mm) and OA (37.9 ± 6.9 mm). Absolute muscle fascicle lengths at heel-strike (33.2 ± 4.9 and 30.7 ± 5.9 mm for YA and OA, respectively) were also not significantly different. No significant differences were present in ankle and knee kinematics between YA and OA walking at matched speeds (Table A.3). However, the mean EMG linear envelope value in OA was significantly higher (p < 0.05) than in YA during the overall gait cycle at walking speeds matched to the YA and showed a large effect size (Table A.3; Fig. A.1c).
Table A.2. P-values relative to the variables analyzed to assess differences in normalized fascicle lengths pattern. Analyses were conducted on the entire stride, as well as the stance and swing phases independently. Significance was initially set at $p < 0.05$ and subsequently adjusted for multiple comparison error using the Benjamini *post hoc* method (Benjamini and Hochberg, 1995) using the 5 muscle fascicle length parameters. Effect sizes calculated with Cohen’s $d$ method are reported in brackets.

<table>
<thead>
<tr>
<th></th>
<th>Stance phase</th>
<th></th>
<th></th>
<th>Swing phase</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean length</td>
<td>Lengthening</td>
<td>Shortening</td>
<td>Mean length</td>
<td>Mean length</td>
</tr>
<tr>
<td>YA - OA (preferred)</td>
<td>0.482</td>
<td>0.217</td>
<td>0.099</td>
<td>0.311</td>
<td>0.396</td>
</tr>
<tr>
<td>YA - OA (matched)</td>
<td>0.034*</td>
<td>0.025* (1.01)</td>
<td>0.002* (1.2)</td>
<td>0.022*</td>
<td>0.026*</td>
</tr>
<tr>
<td>OA (preferred)-OA (matched)</td>
<td>0.001*</td>
<td>0.027* (0.87)</td>
<td>0.117</td>
<td>0.002*</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

* Denotes significant differences after *post hoc* analysis

A.3.2 Effect of speed on muscle fascicle length in OA

Significantly greater lengthening during stance ($7.6 \pm 5.8\%$ normalized muscle lengths) and greater shortening during plantar-flexion in late stance and early swing ($19.0 \pm 8.8\%$ normalized length) were observed when OA walked at their preferred speed (Table A.2). Mean muscle fascicle normalized lengths across the stride ($0.84 \pm 0.10$), stance ($0.86 \pm 0.09$) and swing phases ($0.81 \pm 0.14$) likewise increased significantly between faster than preferred and preferred walking in OA (Table A.2). The effect sizes of the differences in muscle lengths due to speed in OA were large (Table A.2). When assessed on an individual basis, the increase in muscle lengthening and shortening observed in OA during the stance phase between walking at their preferred speed and a 20% faster speed was statistically different in 7 of the 8 OA (based on an average of 5 strides per speed).
Figure A.1. Comparison of matched walking speeds in young adults (YA) and old adults (OA): (a) normalized muscle length, (b) ankle joint angle and (c) normalized EMG linear envelope as a percent of gait cycle (heel-strike to heel-strike). YA dotted lines and OA solid lines respectively. The shaded regions represent the S.D. of the mean and the vertical lines represent toe off.
A.3.3 Comparison of muscle mechanics and joint kinematics at preferred walking speeds in YA and OA

The overall shape of the normalized muscle fascicle length curve both during stance and during the stance and swing phases were not different between YA and OA walking at the matched speed (Table A.2; Fig. A.2a). No significant differences were found in the normalized fascicle lengthening or shortening, nor in the mean fascicle length across the total gait cycle, or the mean lengths in the stance and swing phases between the two groups (Table A.2). As with the normalized length data, the pattern of absolute fascicle lengths in the two groups was not significantly different in any parameter.

No significant differences were found in ankle and knee range of motion or minimum and maximum angles between YA and OA (Table A.3). The average value of normalized EMG linear envelope across the stride was higher in OA than in YA and showed a large effect size (p < 0.05, Table A.3; Fig. A.2c).

<table>
<thead>
<tr>
<th></th>
<th>YA</th>
<th>OA (preferred)</th>
<th>OA (matched)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ankle angle [max]</td>
<td>15.9°± 3.8°</td>
<td>15.8° ± 3.6°</td>
<td>15.2° ± 2.0°</td>
</tr>
<tr>
<td>Ankle angle [min]</td>
<td>-12.9° ± 7.2°</td>
<td>-10.7° ± 5.2°</td>
<td>-13.1° ± 5.9°§ (0.46)</td>
</tr>
<tr>
<td>Knee angle [max]</td>
<td>71.9°± 8.5°</td>
<td>69.0° ± 4.5°</td>
<td>69.9° ± 5.1°</td>
</tr>
<tr>
<td>Knee angle [min]</td>
<td>-0.3° ± 3.6°</td>
<td>2.2° ± 8.2°</td>
<td>1.2° ± 6.4°</td>
</tr>
<tr>
<td>Mean EMG</td>
<td>0.23 ± 0.06</td>
<td>0.32 ± 0.06* (1.9)</td>
<td>0.38 ± 0.11* (1.8)</td>
</tr>
</tbody>
</table>

* Significantly different from YA (p < 0.05)
§ Significantly different from OA preferred (p < 0.05)
Figure A.2. Comparison of preferred walking speeds in young adults (YA) and old adults (OA): (a) normalized muscle length, (b) ankle joint angle and (c) normalized EMG linear envelope as a percent of gait cycle (heel-strike to heel-strike). YA dotted lines and OA solid, lines respectively. The shaded regions represent the S.D. of the mean and the vertical lines represent toe off.
A.4 Discussion

In accordance with our hypothesis, there were marked differences in the soleus fascicle length patterns between the two age groups when OA were asked to match the preferred speed of YA. More specifically, when OA walked 20% faster than their preferred speed there was a lack of a stretch-shorten cycle during the stance-phase, whereby the soleus fascicles remain close to isometric, unlike the stretch-shorten cycle observed in YA. Surprisingly, we also observed a shorter average muscle fascicle length in OA compared to YA at matched walking speeds. Because no significant differences were found in the range of motion of the ankle between groups, this finding is likely explained by a higher stretch of the Achilles tendon in OA, thus mitigating the strain in the muscle fibers themselves. This difference in tendon behavior is supported by the fact that older adults have shown to have a more compliant Achilles tendon than YA (Onambele et al., 2006).

A similar reduction in fascicle stretch-shorten behavior has also been observed previously in the medial gastrocnemius muscle during walking at 1.1 m/s between YA and OA (Mian et al., 2007), and thus appears to be a general characteristic of the triceps surae with aging. The possibility that the gastrocnemius muscle influences the soleus muscle function via its action at the knee has recently been investigated (Maoyi et al., 2012). Gastrocnemius-soleus interaction could have influenced the change in the length pattern observed between preferred and fast walking in OA, however, given that the knee angles between these speeds were not significantly different this effect is likely small.

Also in accordance with our hypothesis, when OA walked at their preferred speed the soleus muscle fascicles exhibited a greater stretch-shorten cycle during the stance phase.
(compared to when they walked at a 20% faster speed) and an overall length pattern that was comparable to that of YA walking at their preferred speed. Interestingly, not only is the pattern of muscle fascicle lengths matched at preferred speeds, but an increase in the average muscle fascicle lengths in OA between fast and preferred speeds resulted in mean muscle fascicle lengths that were also matched. It has previously been argued that YA select walking speeds that result in optimal energetics and muscle mechanics (Saibene and Minetti, 2003; Neptune et al., 2008). Albeit a single muscle analysis, the modulation of active soleus muscle fascicle lengths between fast and preferred walking speeds in OA supports our theory that slower walking in OA may be a strategy adopted to allow their muscles to function mechanically similarly to those of YA.

What could be the possible benefit of maintaining a stretch-shorten cycle of the soleus muscle during walking? It is well accepted that muscles undergoing active stretching produce higher force for a given level of activation (Zajac, 1989). It has also been well documented that eccentric muscle activity is metabolically less expensive than concentric or isometric activity (Bigland Ritchie and Woods, 1976). Maintaining a stretch-shorten-cycle in OA through a reduction in speed may thus lower relative muscle activation and energy use, and in turn reduce fatigue and the energy cost associated with walking. It has also recently been shown that the soleus muscle functions across the ascending limb of its force-length curve during walking in YA (Rubenson et al., 2012). Therefore, an increase in muscle fascicle length during stance increases its length-dependent force capacity as higher forces are required; it has been argued that this can represent a strategy to simplify the control of force regulation during gait (Rubenson et al., 2012). Furthermore, deactivating the soleus muscle as it shortens on the ascending limb of the force-length curve in the second half of stance will result in a more rapid decay of muscle force compared to if the muscle functions
isometrically. A rapid decay of force will increase the rate of elastic energy return in the Achilles tendon and thus the ability to power the ankle joint via the release of stored elastic energy during toe-off. It should also be noted that an increase in mean muscle fascicle length at the preferred speed in OA may improve the overall force capacity of the muscle. We do not know where the soleus functions on its force-length curve in OA, but given the increased compliance of the Achilles tendon reported in OA (Onambele et al., 2006) it is reasonable to assume that the muscle fibers function at shorter lengths and thus possibly also on the ascending limbs of the force-length curve.

Greater relative soleus muscle activation was present in OA compared to YA (Table A.3; Fig. A.1b, A.2b) walking at both matched and preferred speeds, which is consistent with previous studies (Schmitz et al., 2009). A higher relative activation across the stance-phase may be required to achieve the necessary force and moment production due to a loss in force capacity [specific tension; (Morse et al., 2005)], or may represent force-length-velocity affects, although the nature of these remain unclear. The higher average relative muscle activity may also represent a general elevation in co-contraction in OA that is required to maintain stability (Mixco et al., 2012). Older adults likewise have significantly higher activation during the swing phase compared to YA, where the muscle is inactive. Therefore, a co-contraction strategy may also be adopted in OA to increase their limb stability during the swing phase. Interestingly, the higher swing-phase activation, coupled with higher Achilles tendon compliance (Onambele et al., 2006), may help explain the smaller fascicle lengthening during the swing phase in OA.

The present study focused on length changes in a single region of one muscle. The extent to which these findings extend to other muscles and other regions of the soleus muscle are not known, and thus whether they reflect a general mechanism remains
unclear. Furthermore, it remains possible that normalizing muscle fascicle lengths to optimal fascicle lengths in OA could alter the functional assessment of the fascicle length pattern, although the overall direction of the length change will remain unchanged.

Despite these aforementioned limitations, given that the soleus muscle has been reported to be one of the most important muscles responsible for generating the mechanical work of walking (Neptune et al., 2008), and since muscle length patterns are central in dictating the muscle’s mechanical performance (Zajac, 1989), the conservation of muscle fascicle length pattern through a reduction in speed in OA may indeed represent a physiologically relevant modulation of muscle function. While we cannot rule out that changes in muscle fascicle length patterns result from a reduction in speed brought about by other factors, our work on the soleus muscle offers a novel mechanical explanation for the slower walking speed in OA, whereby a reduction in speed permits muscles to function in a similar manner to that of YA.
Appendix

References


Appendix
