THE INFLUENCE OF CYCLIC LOADING ON THE EXTENSIBILITY OF HUMAN HAMSTRING MUSCLE-TENDON UNITS *IN VIVO*

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A thesis submitted in partial fulfilment for the degree of

Master of Health Science, Auckland University of Technology

2005

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CERTIFICATE OF AUTHORSHIP

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institute of higher learning, except where due acknowledgement is made in the acknowledgements.

ACKNOWLEDGEMENTS

Many people have contributed to this thesis, and it is a pleasure to acknowledge them here. In particular I would like to thank my family; especially Sylvie, my wife, best friend, and love, and Ella and Grace my daughters, all who have been amazing with their unconditional support, love and patience over these past years, without whom this thesis could not be been completed.

I thank my mum, Diana for her support and interest in the English grammar and context of this thesis. I acknowledge my dad, Ed and my father in law, John Raven who died during the writing of this thesis; I miss them dearly.

I wish to thank my supervisor Dr. Peter McNair for his ideas, guidance, high standards, and feedback over the period taken to complete this work.

A special acknowledgment goes to Dr. Marion Jones, who has been wonderfully supportive during my period of personal grief, thank you.

My colleagues have been an amazing source of support throughout and I thank you all. A special mention must go to (in no particular order) Verna Stavric, Jill Caldwell, Grant Mawston, Wayne Hing, John Molloy, Mark Boocock, Diana Horner, Liz Smyth, Cheryl Keals-Smith, and Jane Mason. Without such special people like you, the process would have been that much harder.

Rona Thorne I thank for sorting out the formatting of this thesis, using her amazing computer skills.

Many thanks go to the participants, for coming forward and giving their time freely to this study.

The Auckland University of Technology Ethics Committee (AUTEC) granted ethical approval for this research on 23rd of June 2004. Approval number 04/125 (refer to Appendix A).

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ABBREVIATIONS

AROM	Active range of motion
BMI	Body mass index
ECM	Extracellular Matrix
EMG	Electromyography
ICC	Intraclass Correlation Coefficient
IPR	Initial passive resistance
IMCT	Intramuscular connective tissue
LLPS	Low load prolonged stretch
MPKA	Maximal passive knee angle
MPR	Maximal passive resistance
MPRT	Maximal passive resistance torque
MTU	Muscle-tendon unit
MVC	Maximal voluntary contraction
PARQ	Physical Activity Readiness Questionnaire
PCSA	Physiological cross-sectional area
PROM	Passive range of motion
PKE	Passive knee extension test
PRT	Passive Resistance Torque
RMS	Root mean square
SENIAM	Surface-EMG for the Non-Invasive Assessment of Muscles
SLR	Straight leg raise test
$Stiff_{100}$	Stiffness calculated over the entire (100%) torque-angle curve.
$Stiff_{10}$	Stiffness calculated over the final 10% of the torque-angle curve.

ABSTRACT

Objective: The objective of this study was to investigate the influence of cyclic loading on the extensibility of hamstring muscle-tendon units *in vivo*.

Study Design: A test-retest randomised controlled trial with repeated measures was undertaken.

Background: Stretching has been commonly promoted to increase the passive extensibility of the muscle-tendon units, yet the mechanism behind its proposed effects remains ambiguous. *In vivo* studies of stretching have mostly been limited to the viscoelastic characteristic of stress-relaxation. Few studies have investigated the characteristic of creep. Animal and cadaver *in vitro* creep experiments have consistently shown increases in the length of the soft tissues, with associated changes in their resistance and stiffness. These results however, might not be representative of human muscle-tendon units under *in vivo* conditions. Additionally, those *in vivo* human studies that have investigated creep phenomenon have contrasting results. To date, no known *in vivo* study has examined passive cyclic loading of human hamstrings to a constant load level.

Method: Using a repeated measures design the extensibility of the hamstring muscles were assessed by a passive knee extension test (PKE) to maximal stretch tolerance using a KinCom® dynamometer. Those participants in the intervention group underwent 45 continuous passive cyclic loadings as the KinCom® dynamometer

moved the knee joint into extension until torque reached 85% of maximal passive resistance torque measured in the passive knee extension test. The control group sat quietly relaxed during the intervention period. Measurements of hamstring passive extensibility using the PKE test were repeated at the end of the intervention.

Results: Following the intervention, the PKE test showed for the cyclic loading group there was a significant ($p < 0.05$) increase in both maximal passive resistance torque (mean 23%) and knee joint angle (mean 6.3%). A significant ($p < 0.05$) decrease in passive resistance torque (mean 11.8%) when re-measured at the baseline position of maximal passive knee angle was observed. A significant increase ($p < 0.05$) was found for passive stiffness over the final 10% of the knee torque-angle curve. No significant difference ($p > 0.05$) was found for passive stiffness for the full (100%) of the torque-angle curve. Of the control group, no significant differences ($p > 0.05$) were observed for all variables of the PKE test. Analysis of cycle one compared to forty-five of the cyclic loading intervention procedure showed a significant ($p < 0.05$) increase in both passive knee joint angle (mean 5.2%) and passive stiffness (mean 28.6%) over the final 10% of the knee joint torque-angle. No significant difference ($p > 0.05$) was found for passive stiffness across the full (100%) knee joint torque-angle.

Conclusion: The findings of the current study demonstrated that after cyclic loading the hamstring muscles lengthened and became stiffer over the final gained range of knee joint motion. Although the current study cannot determine the mechanism

behind the changes in the variables of interest, these findings do provide some evidence that most likely a combination of altered stretch tolerance and local mechanical effects within the muscle-tendon unit, i.e. creep lengthening were responsible.

CHAPTER 1 – INTRODUCTION

1.1 The Problem

This study investigated changes in passive extensibility of the hamstring muscles attributable to the effects of repeated cyclic loading of the knee joint into extension in individuals without pathology.

Despite the popularity of stretching regimes in rehabilitation and sports settings, the mechanism behind its proposed effect remains uncertain. As most biological tissues, skeletal muscle-tendon units behave viscoelastically (Best, McElhaney, Garrett, & Myers, 1994; Fung, 1972; Taylor, Dalton, Seaber, & Garrett, 1990). For example, daily activities of the lower extremity such as walking or running involve repetitive movements and are cyclic in nature (Fung, 1979; Magnusson, Hansen, & Kjaer, 2003a; Thornton, Oliynyk, Frank, & Shrive, 1997; Tsuang et al., 1998). Such constant motions of the lower extremities are believed by some authors to subject the hamstrings to *cyclic loading* (Kroll & Raya, 1997; Thornton et al., 1997). The response of the hamstring muscle-tendon units (as most soft tissue structures) is *creep*, a *viscoelastic* characteristic, defined as continuous lengthening while under sustained or cyclic loading (Fung, 1973; Thornton et al., 1997).

When muscle-tendon unit extensibility and joint range of motion has been reduced due to injury or secondary to contracture, passive stretching techniques are commonly used to increase range of motion of joints crossed by the muscles with insufficient length (Liebesman & Cafarelli, 1994; Smith, 1994; Taylor et al., 1990). These techniques are preformed with the belief that an alteration of the muscle-tendon unit's properties

results in increases in range of motion (Best & Garrett, 1993; Kroll & Raya, 1997; Safran, Garrett, Seaber, Glisson, & Ribbeck, 1988; Taylor et al., 1990). Therefore, it has been hypothesised that if muscles and their related connective tissue have sufficient length the incidence of injury recurrence may be reduced (Anderson & Burke, 1991; De Dyne, 2001; Garrett, 1990, 1996; Worrell, 1994). Such gains in range of motion following passive stretching are termed *passive extensibility*, which Gajdosik (2001) defined as the ability of the muscle-tendon unit to lengthen while the muscle is not active.

A common passive stretching technique consists of lengthening the targeted muscle-tendon units to a set length and then held at a constant length for a given period of time. This technique is termed as *sustained* or *static stretching* (now referred as static stretching) (Wilkinson, 1992; Zachazewski, 1989). However, to simulate daily human activities performed in a rhythmic or cyclic motion, stretching techniques are performed in a repeated or cyclic manner (McNair, Hewson, Dombroski, & Stanley, 2002) and are referred to by a variety of names such as *continuous passive motion*, *ballistic*, *cyclic* or *dynamic stretching* (now referred as dynamic stretching) (Bressel & McNair, 2002; Magnusson, Aagaard, Simonsen, & Bojsen-Moller, 1998a; McNair, Dombroski, Hewson, & Stanley, 2000; Starring, Gossman, Nicholson, & Lemons, 1988).

Until recently, to study and measure the effect of dynamic stretching on the passive extensibility of human muscle-tendon units under *in vivo* conditions has been difficult (Magnusson, 1998b). With the development of dynamometers, indirect measurements of load and lengthening deformation of the muscle-tendon unit under *in vivo* conditions are now possible (Gajdosik, 2001; Keating & Matyas, 1996; Magnusson et al., 2003a).

The majority of human *in vivo* studies however, have investigated the viscoelastic property of *stress-relaxation*, that is, the decrease in passive resistance with the tissue under static or dynamic lengthening (Gajdosik et al., 2004; Lamontagne, Malouin, & Richards, 1997; McNair et al., 2000; McNair et al., 2002; Reid & McNair, 2004). The property of creep under *in vivo* conditions interestingly has received limited attention, in spite of the consistent findings of *in vitro* animal and cadaver studies showing that significant increases in passive extensibility occur. At least three main conclusions from *in vitro* animal creep studies can be made; a) tendon has demonstrated immediate lengthening in proportion to the magnitude of the load applied (De Zee, Bojsen-Moller, & Voigt, 2000; Provenzano, Lakes, Keenan, & Vanderby Jr., 2001; Smutz, France, & Bloswick, 1995; Wang & Ker, 1995; Warren, Lehmann, & Koblanski, 1976; Wren, Lindsey, Beaupreacute, & Carter, 2003), b) low levels of loading applied to tendon over longer periods of time result in greater residual lengthenings compared to heavier loadings and shorter holding times (Warren et al., 1976), and c) tendons subjected to cyclic loading result in reduced stiffness (De Zee et al., 2000; Provenzano et al., 2001; Wang & Ker, 1995; Wren et al., 2003).

Tendon however, is only one component and less extensible in relation to the other structures of the muscle-tendon unit (Lieber, 1992). Taylor et al. (1990) investigated lengthening of rabbit muscle-tendon units *in situ* subjected to cyclic loading. Their findings were consistent with studies of tendon, and concluded that creep lengthening occurs in muscle-tendon units of live animals. While these experiments provide useful biomechanical information on isolated tissue structures, they are limited as the majority were performed on tendons and may not be representative of stretching muscle-tendon units under *in vivo* conditions.

Of human *in vivo* stretching studies that have investigated creep, none have investigated cyclic loading and the majority were uncontrolled clinical trials or case reports that investigated chronic (over days, weeks or months) low-load prolonged stretch effects on orthopaedic pathologies affecting either the hip, shoulder, knee, or ankle joint (Bohannon, Gajdosik, & LeVeau, 1985; Kottke, Pauley, & Ptak, 1966; Light, Nuzik, Personius, & Barstrom, 1984; MacKay-Lyons, 1989; Nuismer, Ekes, & Hom, 1997; Richard, 1986; Richard, Jones, Miller, & Finley, 1988; Rizk, Christopher, Pinal, Higgins, & Frix, 1983; Steffen & Mollinger, 1995). Caution needs to be exercised while interpreting the results of these studies as many have fundamental methodological errors and none used true experimental designs. Nonetheless, overall they have reported increased joint range of motion compared to manual high-load short duration stretches; a finding consistent with a classical *in vitro* rat tendon experiment by Warren et al. (1976). Therefore, creep might offer an alterative means to gain passive extensibility of the hamstring muscles, though investigations initially under controlled laboratory conditions are required to address this conjecture.

Under controlled laboratory conditions, two *in vivo* human studies of note have examined creep. Bohannon (1984) investigated stretching of the hamstring muscles over three consecutive days for eight minutes using a straight-leg raise (SLR) technique. The angle of the SLR of the stretching group was compared to a non-stretching control group. His results showed a non-significant change in hamstring passive extensibility at the end of the three days and concluded his findings were most likely attributed to an inadequate loading level and hold time. In contrast, a study by Madding, Wong, Hallum, & Medeiros, (1987), investigated stretching in a single session, using three levels of sustained loading times (15, 45, and 120 seconds) applied to the adductor

muscles of an intervention group compared to a non-stretching control group. Their results showed significant gains in passive extensibility and reduced load-force (a repeated measure at baseline maximal abduction angle) for all three levels of hold times, accompanied by no significant difference across hold times. Madding et al. findings demonstrated sustained loading resulted in increased passive extensibility, a finding consistent with the animal and cadaver creep models. In addition, a 15 second hold was just as effect for increasing extensibility as the longer hold times.

The paucity of human *in vivo* studies investigating stretching using sustained loading and none using cyclic loading, combined with the contrasting findings under controlled laboratory conditions, highlights the need for further investigation into the effects of creep phenomena. To date there are no known human *in vivo* stretching studies that have investigated cyclic loading of the hamstring muscles.

1.2 Purpose of the Study

The purpose of the current study was to investigate the effect of cyclic loading to a constant load level on the passive extensibility of the hamstring muscle-tendon units in healthy participants.

More specifically using a passive knee extension test protocol on a KinCom® dynamometer the extensibility of the hamstring muscles were measured and the following variables examined prior to and after the above mentioned cyclic loading:

- Maximal passive resistance torque,
- Maximal passive knee angle,

- Passive resistance torque at baseline maximal passive knee angle,
- Passive stiffness calculated over the full (100%) range of loading,
- Passive stiffness calculated over the final 10% of the range of loading.

In addition, the following variables were also measured at cycle one and cycle forty-five of the loading intervention:

- Mean maximal passive knee angle
- Mean passive stiffness for full (100%) range of loading
- Mean passive stiffness for the final 10% range of loading

1.3 Significance of the Problem

The phenomenon of creep under *in vivo* conditions has received little attention by researchers. Therefore, the current study will add to the biomechanical literature knowledge concerning the passive mechanical properties of the hamstring muscles. The findings will also have significance for clinical and sports biomechanists, physiotherapists, rehabilitation therapists, athletes, and coaches by providing additional evidence of the effectiveness or otherwise of stretching the hamstring muscles using a constant cyclic loading paradigm.

CHAPTER 2 - REVIEW OF THE LITERATURE

2.1 Introduction

This chapter is divided into six major sections. The first section discusses the search strategy used, search returns, and includes a summary table of the creep studies in this review of literature. The second section is a review of the structural and physiological factors of the muscle-tendon unit, with an emphasis on its connective tissue that contributes to the passive mechanical properties of the whole structure. The third section briefly reviews the gross anatomy and connective tissue structure of the hamstring muscle group. This section is followed by a review of the biomechanical properties and terminology used to describe muscle-tendon units and its viscoelastic behaviour and their characteristics of stress-relaxation and creep. In the fourth section is a review of the literature of both human and animal studies that have investigated soft tissue structures under sustained or cyclic loading. The fifth section discusses stretching and the use of the passive knee extension test on the dynamometer as an assessment tool, and the reliability of the tests and equipment used in the current study. The chapter concludes with a summary.

2.2 Literature Search: Animal & Human Studies

2.2.1 Introduction

Literature pertaining to both animal and human of passive sustained or cyclic loading and those studies that investigated creep as the dependant measure were considered in this review.

2.2.2 Search Strategy

2.2.2.1 Inclusion Criteria

The following criterion was used to determine which studies would be examined in the literature review.

- *In vivo* studies that used healthy participants, that is, with no history of lower extremity injury or neurological impairment were included
- *In vitro* or *in situ* studies using normal healthy specimens of animal or human cadaver tissues of any of the following; the muscle-tendon unit, muscle, tendon or ligament were considered.
- Studies that used the intervention of sustained or cyclic loading were included. Associated studies to those of creep that used static or dynamic stretching were also considered.
- Clinical trials with simple orthopaedic pathology were considered if they met the above conditions. Case reports were excluded due to their limited information and fundamental methodological errors.
- Associated studies of physiology, morphology, structure, ultrastructure, and biomechanics of muscle, tendon, aponeurosis, and ligament were included for background purposes.

2.2.2.2 Search Strategy, Databases & Resources Searched

A search strategy was used to identify both published and unpublished studies and was limited to papers of the English language. Studies were located electronically using the following databases or resources:

- Allied and Complementary Medicine (AMED, 1985+)

- Cochrane Database of Systematic Reviews
- Cumulative Index to Nursing & Allied Health Literature (CINAHL, 1982+)
- Current Contents (1997+)
- EBSCO Health Databases
- Evidence Based Medicine Reviews
- Medline
- PEDro (Physiotherapy Evidence Database)
- Proquest
- Sports Discus
- Web of Science
- E-Journals
- Unpublished thesis held in New Zealand and Australia

In addition the Internet was searched for information on sustained or cyclic loading, creep, muscle-tendon studies, stretching techniques, static or dynamic stretching, hamstring muscles and treatment protocols. Additionally the names of key authors were used to identify their personal web sites.

Reference lists of all included studies and texts were manually searched for further relevant studies that may have been missed using the search criterion.

2.2.2.3 Search Terms Used

For each database, a search strategy was used to identify studies relating to creep, sustained or cyclic loading. The search terms were modified as required for each database. Individual search terms were combined and phrases formed to target the

desired studies and were used in the descriptors, title, abstract, and within body of text as listed in Table 2.1.

Table 2.1: Search Terms: Keywords Used In The Search.

Keywords		
acute	human	repeat (ed)
behaviour	*in situ*	stretch (s, ing)
compliance	*in vitro*	skeletal
creep	*in vivo*	stiff (ness)
cyclic	length (ening)	stress
deformation	hamstring (s)	strain
duration	load (s, ing)	static
dynamometer (y)	motion	tendon (s)
dynamic	muscle (s)	time
elasticity	range	viscoelastic (ity)
elongate (tion)	passive	
flexible (ility)	periodic	
hamstring(s)	relaxation	

2.2.2.4 Search Returns

The search strategy returned 32 studies that initially appeared to meet the inclusion criteria. Five studies were identified as case reports. Four studies that initially appeared to meet the inclusion criteria were not included as they discussed mathematical modelling of creep. The remaining 23 studies on creep (fourteen *in vitro*, six *in vivo* and three clinical trials) were included in the review (Table 2.2, and 2.3).

Table 2. 2: Brief Summary of Reviewed ***In Vitro*** Creep Lengthening Studies.

Author(s)	Year	Species, Type	Study	Tissue, Structure	Passive Load Type	Load Limit	Level No., Load Amount, Time Applied	Rate Level & Speed	Main Reported Findings	Stiffness	Stats
Warren et al.	1976	Animal, Rat	*In vitro*	Tendon, Tail	Sustained	Failure	Two, N/A, 50 min.	Single, 6.67%	Creep ↑ lengthening, in low-load 1.25% < high-load 2.28%	N/A	$p < 0.01$
Taylor et al.	1990	Animal, Rabbit	*In situ*	Leg, Whole Muscle-Tendon Unit	Repeated	Physiological	Single, 78.4 N, 30 sec holds X10	Single, $10^{o}s^{-1}$	Creep 3.5% lengthening,	N/A	$p < 0.05$
Smutz et al.	1995	Human	*In vitro*	Tendon, Finger	Sustained & Cyclic	Physiological	Four 10, 20, 50, 100 N, 30 & 100 min.	Two, 0.25 & 1 Hz	Creep ↑ lengthening, no diff between conditions	N/A	$p < 0.05$
Wang & Ker	1995	Animal, Wallaby	*In vitro*	Tendon, Tail	Sustained & Cyclic	Failure	Multiple, 10-80 MPa, N/A	Single, 1.6 Hz, sec to days.	Creep 6% lengthening; ↑ load to ↑ length	↓ from 4 to 10% as cycles ↑	$p < 0.05$
Adams & Dolan	1996	Human	*In vitro*	Spine	a) Sustained & b) Cyclic	Physiological	Single, N/A, 5 – 60 min.	Three, 1, 3, 10 sec.	Creep response, ↑ load to ↑ flexion, Flexion a) ↑ 12% b) ↑ 4%	N/A	$p < 0.05$
Thornton et al.	1997	Animal, Rabbit	*In vitro*	Ligament, MCL	Sustained & Cyclic	Physiological	Single, 14 MPa, 20 minutes X 30 cycles	Single, 1 Hz	Creep ↑ lengthening	N/A	$p < 0.05$
Dee Zee et al.	2000	Animal, Pig	*In vitro*	Extensor & Flexor Tendon, Foot	a) Sustained & b) Cyclic	Failure	1 level, N/A, 20 min.	Single, 1.4 Hz, 1,600 cycles, ~ 20 min.	Creep ↑, a) 0.12 b) 0.24% for flexor & a) 0.28% b) 0.22% for extensor lengthening	No change	$p < 0.05$

Note. Ordered by year of publication. N/A = Not available, ↑ = Increase, ↓ = Decrease.

Table 2.2 Continued: Brief Summary of Reviewed *In Vitro* Creep Lengthening Studies.

Author(s)	Year	Species, Type	Study	Tissue, Structure	Passive Load Type	Load Limit	Level No., Load Amount, Time Applied	Rate Level & Speed	Main Reported Findings	Stiffness	Stats
Ker et al.	2000	Animal, Wallaby	*In vitro*	Tendon, Tail & lower limb	Sustained	Failure	Multiple, 18-80 MPa, N/A	Single, 1 sec	Creep ↑ lengthening, ↑ load to ↑ length	N/A	$p < 0.05$
Provenzano et al.	2001	Animal, Rat	*In vitro*	Ligament, MCL	Sustained	Physiological	Multiple, 3.7-9.9 MPa, 100 sec.	Single, 0.32 sec	Creep 0.75% lengthening	↑ stiffness with ↑ load	$p < 0.05$
Thornton et al.	2001	Animal, Rabbit	*In vitro*	Ligament, MCL	Sustained & Cyclic	Physiological	Single, 4.1 MPa, 20 min. X 30 cycles	Single, 1 Hz	Creep ↑ lengthening, ↑ hydration to ↓ lengthening	↑ with ↑ hydration	$p < 0.05$
Schechtman & Bader	2002	Human,	*In vitro*	Tendon, Foot	Cyclic	Failure	Single, 20%, N/A	Single, 4 Hz	Creep ↑ lengthening	↓ as cycles ↑	$p < 0.05$
Thornton et al.	2002	Animal, Rabbit	*In vitro*	Ligament, MCL	Sustained & Cyclic	Physiological	Multiple, 4.1-28 MPa, 20 min. & 30 cycles	Single, 1 Hz, 30 cycles	Creep ↑ 1.3-2.1% lengthening, ↑ load to ↑ length	↑ from 1stst to last cycle	$p < 0.05$
Wren et al.	2003	Animal, Rabbit	*In vitro*	Ligament, MCL	a) Sustained & b) Cyclic	Failure	Nine, 35-75 MPa, a) 17 & b) 50 min.	Single 1 Hz	a & b) Creep ↑ 4-12% lengthening, ↑ load to ↑ length	↓ as cycles ↑	$p < 0.05$
Hingorani et al.	2004	Animal, Rabbit	*In vitro*	Ligament, MCL	Sustained	Physiological	Multiple, 3-54.8 MPa, 100 sec.	Single	Creep ↑ lengthening, ↑ load to ↑ length	N/A	$p < 0.05$

Note. Ordered by year of publication. N/A = Not available, ↑ = Increase, ↓ = Decrease.

Table 2 3: Brief Summary of Reviewed *In Vivo* or Clinical Trial Creep Lengthening Studies.

Author(s)	Year	Species, Type	Study	Tissue, Structure	Passive Load Type	Load Limit	Level No., Load Amount, Time Applied	Rate Level & Speed	Main Reported Findings	Stiffness	Stats
Rizk et al.	1983	Human	Clinical Trial	Adhesive Capsulitis	Sustained	Physiological	Multiple, 8.8- 66.8N, 15 min. intervals over 2 hrs daily	N/A	↑ Joint angle compared to another Rx	N/A	$p < 0.05$
Bohannon	1984	Human	*In vivo*	Hamstrings	Sustained	Physiological	N/A	N/A	No change in joint angle	N/A	$p > 0.05$
Madding et al.	1987	Human	*In vivo*	Adductors	Sustained	Physiological	Multiple, mean 22.6 N, N/A	Single, 5º sec	↑ Abduction & ↓ in force	N/A	$p < 0.05$
McGill & Brown	1992	Human	*In vivo*	Spine	Sustained	Physiological	Single,	Single	↑ Flexion	N/A	N/A
Steffen & Mollinger	1995	Human	Clinical Trial	Contracture	Sustained	Physiological	Multiple, Mean 6.1 N-m, Daily	N/A	No change in joint angle	N/A	$p > 0.05$
Nuismer et al.	1997	Human	Clinical Trial	Contracture	Sustained	Physiological	N/A, N/A, Daily	N/A	↑ Joint angle	N/A	$p < 0.05$
Maganaris et al.	2002	Human,	*In vivo*	Tendon-Aponeurosis	***** Sustained, Isometric Contractions	Physiological, Ultrasonography	Single, 80% of MVC X 10, 1 sec rest between	Single 3 sec	Creep ↑ 13.3% lengthening	N/A	N/A
Claude et al.	2003	Animal, Cat	*In vivo*	Ligament, Supraspinal	Cyclic	Physiological	Three, 20, 40, 60 N, 20 min.	Single 0.1 Hz	Creep ↑ lengthening with ↑ load	N/A	$p < 0.05$
Lu et al.	2004	Animal, Cat	*In vivo*	Ligament, Supraspinal	Cyclic	Physiological	Single, 1 N, 20 min.	Two, 0.1 & 0.5 Hz	Creep ↑ lengthening, 38.9% & 89.4%,	N/A	$p < 0.05$

Note. Ordered by year of publication. N/A = Not available, ↑ = Increase, ↓ = Decrease. ***** Active contraction included, only known ultrasonography study of creep.

2.3 Structural And Physiological Factors Contributing To The Passive Properties Of The Muscle-Tendon Unit

2.3.1 Introduction

The two principal passive structures that transmit forces are tendons and three layers of connective tissue; the endomysium, perimysium, and epimysium, located within the muscle. Together the muscle and tendon form a complex or unit termed the *muscle-tendon unit* and refers to the whole structure, which includes the contractile elements and their supporting connective tissue structure, the tendon and its aponeurosis (Huijing, 1992; Kannus, 2000; Purslow, 2002). When the whole structure is not being referred to as the muscle-tendon unit; then muscle, tendon, or aponeurosis will be considered as individual components and referred by their respective names.

The structure and physiological properties of the muscle-tendon unit is well documented in the physiology literature (Marieb, 2004; Williams et al., 1995); however, the passive components will be the focus of this section. The muscle-tendon unit, as in all living tissues, is a composite material and therefore displays complex mechanical behaviour (Fung, 1973). However, both muscle and tendon are well ordered hierarchical structures, which allow deductions to be made of their biomechanical properties through examination of the individual components that form the whole (Fung, 1973). There is a caveat however, not only do the individual components of the muscle-tendon unit determine its biomechanical properties but the environment in which the muscle-tendon unit exists has influence on its properties in terms of structure and behaviour (Fung, 1984).

2.3.2 Connective Tissue of the Muscle-Tendon Unit

Until recently, the mechanical behaviour of connective tissue of the muscle-tendon unit has been poorly understood; classically thought as a relatively inert or immobile tissue whose primary function was to give support and structure to its contractile component (Butler, Grood, Noyes, & Zernicke, 1978; Maganaris, Narici, Almekinders, & Maffulli, 2004b). However, it now well known that connective tissue is dynamic and capable of adapting and altering its structure in response to external forces. Such adaptation occurs through alteration of its structural proportions that are required to provide the necessary mechanical properties to resist tension, compression, torsion and lengthening (Culav, Clark, & Merrilees, 1999).

The design of connective tissue within the contractile component of the muscle-tendon unit provides a structural framework for force dispersion and transmission, and protection by limiting the extent of elongation. It is also believed to be the main contributor to the muscle-tendon unit's elasticity consisting of series elastic and parallel components (Kubo, Kanehisa, & Fukunaga, 2001a; Purslow, 1989; Purslow & Trotter, 1994; Young, Paul, Rodda, Duxson, & Sheard, 2000). The serial elastic component consists of the connective tissue found in the tendon and aponeurosis and believed to contribute to the mechanical properties of active (contracting) muscle. The parallel elastic component consists of the connective tissue located within the muscle tissue and believed to contribute to the mechanical properties of passive (relaxed) muscle (Purslow, 1989; Roy, Monti, Lai, & Edgerton, 2003). An important factor in understanding these mechanical components of the muscle-tendon unit requires further examination of the connective tissue that constitutes their respective parts.

The extensive networks of connective tissue located in the muscle-tendon unit are comprised largely of *collagen* type I, but also contain some type III, V and VI. Collagen is a fibrous protein that has unique mechanical properties providing a functional role of support and force transmission (Culav et al., 1999; Liu, Yang, Al-Shaikh, & Lane, 1995). Single collagen molecules are composed of a triple helix - three polypeptide chains that have strong hydrogen-bonded 'water' bridges, which maintain its mechanical strength and stability (Culav et al., 1999). All collagen has been shown to have properties to resist tensile loadings efficiently; for example, tendon typically lengthens less than 8% (Harkness, 1980). The proportion of collagen is approximately 20% of the total volume of connective tissue, the remaining 80% being the *extracellular matrix* (ECM) (Kjaer, 2004). Collagen fibrils are embedded into a hydrophilic ECM composed of 70% water, and 30% proteoglycans, glycoproteins, glycosaminoglycans, ground substance, plus a small amount of elastin (Culav et al., 1999; Magnusson et al., 2003a). Proteoglycans have an important mechanical and structural role as they bind most of the water of the ECM, creating a highly structured gel-like material (Culav et al., 1999; Kannus, 2000; Kottke et al., 1966). Studies by Fung (1988) confirmed the important mechanical role of the ECM in maintaining the integrity of connective tissue. Fung's experimental data revealed that collagen and elastin are so well embedded into the ECM that the collagen and elastin fibers cannot move freely. In contrast, researchers Aspden, (1986) and Frost, (1990) believe increases in length of the muscle-tendon unit during passive loading are due to collagen and elastin fiber rearrangement within the extracellular matrix. However, the findings of two recent time-resolved X-ray diffraction studies (Purslow, Wess, & Hukins, 1998; Puxkandl, 2002) investigating bovine intramuscular connective tissue did not show any collagen and elastin fiber rearrangement, a finding supporting Fung's earlier *in vitro* work. Purslow et al. (1998)

as have others (Hooley, McCrum, & Cohen, 1980; Viidik, Danielsen, & Oxlund, 1982), speculated the additional lengthening observed during passive lengthening is possibly due to interfibrillar sliding and shearing of the ground substance of the ECM.

An important fiber within the ECM is elastin, which for example in tendon comprises approximately 1-2%. Elastin plays an important role in energy absorption and mechanical recovery while capable of undergoing extreme lengthening (up to 150%) (Culav et al., 1999). Upon the removal of force elastin fibers recoil and return to their original length without structural damage (Culav et al., 1999; Kjaer, 2004). More recently elastin has been acknowledged as an important component of the muscle-tendon unit's mechanical protection from injury and movement efficiency (Kjaer, 2004; Liu et al., 1995; Magnusson et al., 2003a).

A large portion of the ECM is water (70%); therefore its hydration is believed to play an important spacing and lubricating role that affect the connective tissue's mechanical and viscoelastic behaviour (Culav et al., 1999; Kjaer, 2004). The consequence of hydration has been demonstrated in animal ligament models under passive cyclic loading. Ligaments were soaked in solutions that either hyper or under hydrated them compared to normal ligament hydration levels. Results showed that hyper-hydrated ligament connective tissue was initially stiffer, yet able to lengthen further compared to under-hydrated ligament connective tissue, which was initially more compliant (the ability of the tissues to lengthen) (Chimich, Shrive, Frank, Marchuk, & Bray, 1992; Thornton, Shrive, & Frank, 2001). Alterations of hydration levels have also been studied in human tissues under *in vitro* conditions. Haut and Haut (1997) investigated patella tendon either soaked in a solution to increase or decrease its water content, and then

subjected to passive cyclic loading. Their results were identical to the animal ligament studies of Chimich et al. (1992) and Thornton et al. (2001), confirming hydration levels similarly affect human connective tissues. The volume of hydration loss during cyclic loading has also been investigated. Hannafin & Arnoczky (1994) investigated cyclic loading of canine tendon (30 cycles, 1 per minute, to a load of 100g) over a variety of times ranging from five minutes to 24 hours. Their results showed a significant reduction ($p < 0.05$) in water volume ranging from 2-5%, demonstrating that significant losses occur over a relatively short period. All of these studies demonstrate the important of hydration and its implications on the muscle-tendon units mechanical and behaviour responses.

2.3.2.1 Structure of Tendon & Its Aponeurosis

The tendon portion of the muscle-tendon unit is a highly organised hierarchical structure consisting of closely packed, parallel bundles of collagen fibers that vary in length and thickness (Kannus, 2000). The tendon is surrounded by smooth connective tissue called the *epitenon*, which, on its inner surface connects with the endotenon a layer binding the collagen fibers and fiber bundles together. At rest, tendon fibers exhibit a regular repeating crimp configuration which is believed to have important mechanical properties and associated viscoelastic behaviour during passive lengthening. It has been suggested crimping acts as an initial absorber of forces through compliance during the initial stage of lengthening (Gathercole & Keller, 1991; Paavola et al., 2002). Non-invasive scanning electron micrography has confirmed that collagen fibers do have a 'wavy like' (crimped) appearance when in an unloaded state, that are easily aligned with little or no tensile force, allowing lengthening (Magnusson et al., 2002; Thornton, Shrive, & Frank, 2002). As force increases the collagen fibers begin to elongate and

straighten leading to a loss of their wavy appearance (Fratzl et al., 1997; Thornton et al., 2002). Since collagen fibers vary in length, uncrimping happens at varying points during the lengthening process. Therefore, collagen fibers are stretched progressively; a process termed *fiber recruitment* (Thornton et al., 1997; Viidik, 1972) and is described as *strain-stiffing behaviour* (Lakes & Vanderby, 1999); a process that appears to play an important role in mechanical behaviour of the connective tissue of the muscle-tendon unit during creep lengthening.

The biomechanical strength of tendon to withstand large unidirectional force-loadings relies on its strong cross-links between the collagen molecules combined with its fiber structure that has a staggered parallel array pattern (Culav et al., 1999). This structural arrangement appears to ensure if any individual collagen fibrils are damaged during daily cyclic loading and unloading, the entire integrity of the tendon is not compromised (Kjaer, 2004). Regular damage however, does appear to occur in tendon as demonstrated in an *in vitro* study by Schechtman and Bader (1997). They investigated fatigue of human cadaver tendon placed under a normal *in vivo* physiological cyclic loading profile. Their results surprisingly showed failure would occur at 300,000 cycles, equating to only four months of normal walking. They concluded failure at this low number of cycles must indicate that a continuous process of non-symptomatic damage and repair occurs in healthy tendon subjected to daily physiological load levels in order to maintain homeostasis.

Schechtman and Bader's findings have been supported by an *in vitro* animal ligament study by Thornton et al. (2002) who investigated cyclic loading at very low physiological load levels. At post intervention, using an electron microscope they

observed micro-failure of individual collagen fibers of the ligament. Although the differences of the tissues studied, Thornton et al. results confirmed that micro-failure of collagen fibers could occur at very low-loadings.

Furthermore, the affect of cyclic loading of simulated human running (high loading) has been measured in the Achilles tendon (Puxkandl, 2002). Results of this study have shown lengthening of the overall structure was in excess of the lengthening of its individual fibers. A finding the authors concluded demonstrated that a considerable amount of sliding or deformation must occur between the strongly bonded bundles of fibrils or collagen fibers within the ECM of the tendon.

The amount of lengthening of the connective tissue of tendon and its associated aponeurosis has been measured in animal *in vitro* models. Zuurbier, Everard, van der Wees, & Huijing (1994) using a series of markers in the tissue investigated passive lengthening of rat gastrocnemius aponeurosis. Their finding showed from proximal to distal the aponeurosis lengthened non-uniformly and ranged from 9.8-52.3%, respectively. In another study, Lieber, Leonard, Brown, and Trestik (1991) showed that passive lengthening of frog semitendinosus aponeurosis also lengthened non-uniformly. This study's results however, were in contrast to Zuurbier et al. findings, showing a lengthening of 8% the aponeurosis, and 2% of its associated tendon. Most likely the differences between these studies was due to the location of the proximal–distal measurement point; Zuurbier et al. measured the entire length of the aponeurosis including its intramuscular connection, while Lieber separated these into two distinct parts. Additionally, differences in the architecture of individual animal species tissue structure has been shown to vary widely (Fung, 1973). Furthermore, in a different study

Lieber (1992) found the magnitude of lengthening was determined by the relative length of the tendon to the length of the muscle, that is, muscle-tendon units with a longer tendon resulted in greater gains in length.

2.3.2.2 Ultrasonography studies of Muscle-Tendon Units, In Vivo

Recently, the development of ultrasonography has allowed direct measurements of loading and lengthening of intact human tendon and aponeurosis tissues during stretching under *in vivo* conditions. A limited number of such studies have investigated the lengthening of human muscle-tendon units under passive stretching, while the majority have used stretching with active muscle, that is, induced isometric muscle contraction by either electrical stimulation or voluntary effort. Nevertheless, due to the paucity of *in vivo* studies on human muscle-tendon units, these studies provide important insights into *in vivo* human soft tissue behaviour.

Measurements during passive stretching of muscle-tendon units have been investigated by a few ultrasonography studies of the gastrocnemius muscle under physiological low levels of load (Herbert, Moseley, Butler, & Gandevia, 2002; Muraoka, Muramatsu, Takeshita, Kawakami, & Fukunaga, 2002). The results of these studies both showed the tendon could lengthen (~2 and 9%, respectively) at low levels of loading. These findings are consistent with *in vitro* animal studies (Lieber et al., 1991; Zuurbier et al., 1994), and are supported by *in vitro* scanning electron micrography studies (Magnusson et al., 2002; Thornton et al., 2002) that showed tendon lengthening occurs at low loading levels due to uncrimping of its collagen fibers.

However, another ultrasonography study showed that the passive lengthening of the aponeurosis and tendon of the gastrocnemius muscle does not occur uniformly along its entire length (Kubo, Kanehisa, & Fukunaga, 2005). Results showed lengthening of the muscle fascicles (52.2%), was significantly greater ($p < 0.05$) than the tendon (11.9%), which was significantly greater ($p < 0.05$) than the aponeurosis (5.4%). In addition, these findings demonstrate the relative differences in lengthening between the two different tissue types, muscle and tendon.

In comparison to passive stretching, many ultrasonography studies have used active stretching (isometric muscle contraction) to measure muscle-tendon units. The results of these studies have been quite different. A study by Maganaris and Paul (2000b) investigated multiple loading levels (20, 40, 60, 80, and 100% of maximal voluntary contraction - MVC) by percutaneous electrical muscle stimulation; applied at 100Hz for one second, to the tibialis anterior muscle-tendon units of five participants. Their results showed that overall lengthening occurred as a function of increasing load, with the tendon lengthening (range 0.8-2.5%) being significantly less ($p < 0.01$) than its aponeurosis (range 2.1-7%). These findings are in agreement with the *in vitro* study of Lieber et al. (1991)

In contrast, Muramatsu et al. (2001) had participants generate an isometric MVC to stretch their Achilles tendon. Results showed non-significant ($p > 0.05$) lengthening differences between the tendon (5%) and its associated aponeurosis (5.9%). Magnusson et al. (2003c) used a similar methodology as Muramatsu et al, and found that the Achilles tendon significantly ($p < 0.05$) lengthened (8%), six times more than its associated aponeurosis (1.4%). The findings of these studies suggest under normal

physiological loadings human tendon lengthenings are actually greater than reported under *in vitro* test conditions (range 2-5%). Furthermore, the structure with the greatest length change is inversed with electrically stimulated active isometric contraction compared to participant isometric MVC. Therefore, these factors require consideration when studied under such conditions. Magnusson et al. speculated that the reasons for the dissimilar findings between these ultrasonography studies might be due to differences in measurement technique, lack of accounting for joint movement, and individual differences of the structures investigated. Additionally, Magnusson et al. speculated that differential strain patterns of tendon and aponeurosis tissues may reflect their different functional roles and hence their mechanical behaviour for flexor and extensor muscles. Also of consideration are the comments of Maganaris and Paul (1999) and Hof (1998) who speculated that length changes calculated from *in vivo* studies that used participant isometric MVC neglect antagonistic co-activation, resulting in possible errors.

Therefore, although ultrasonography studies using active loading provide some insight into lengthening of intact whole muscle-tendon units *in vivo*, the results may not be comparable with those performed under passive loading. It is important to note that Magnusson et al. (2003c) commented that the aponeurosis has been shown to have greater loading during passive stretching than during active contraction loading. Lieber and Friden (2000a) and Lieber, Leonard, and Brown-Maupin (2000b) speculated such differences were due to active muscle 'anchoring' the aponeurosis with some degree of lateral force transmission and therefore limiting its lengthening response to longitudinal forces.

It is important to note that if tendon is as compliant, as shown in the Magnusson et al. (2003c) study, then the operating range of the muscle-tendon unit is increased. Such lengthenings could allow shock absorption during high-impact loadings and therefore play an important role in the prevention of injury. The disadvantage of such compliance is a delay in the time before limb movement begins as the muscle fibers shorten to accommodate the passive lengthening of the compliant tendon. It has been speculated that compliance of this magnitude would affect the proprioception of the joint crossed by the muscle-tendon unit and its associated muscle spindles that act as length transducers, thereby diminishing control of joint positioning (Maganaris, 2002; Narici, 1999). These disadvantages would seem counterintuitive and therefore, though speculative, a balance between control and compliancy must occur.

2.3.2.3 The Sarcomere and Its Connective Tissue

The anatomical features of muscle are well documented in the physiology literature (Marieb, 2004; Williams et al., 1995) and are briefly reviewed in this section in context to the current study. Muscle tissue structure is composed of contractile tissue, as well as an intricate network of interconnected connective tissue of seven types of collagen, predominately type I and III; that compartmentalises the contractile muscle fibers into units of which the basic functional unit is the *sarcomere* (Best & Garrett, 1993; Dvir, 2003).

The sarcomere contains the protein filaments *myosin* and *actin*, the key components of the binding site during *cross-bridge formation*. A state of *filament resting tension* has been proposed to exist between these two filaments while the muscle is at rest (Janke, Proske, & Struppler, 1989; Proske & Morgan, 1999). The filaments require force to

detach the bonds between them and it is believed this process contributes to the stiffness of muscle when initially, stretched (Janke et al., 1989; Proske & Morgan, 1999). After detachment the bonds of the cross bridges reform at the new lengthened position. On removal of the lengthening force, the filaments return to their starting length; however, 'slack' develops due to stable bonds still present at the longer length, causing a reduction in the muscle's stiffness. This phenomenon of filament resting tension is termed *thixotropic behaviour*, and is described as a gel-like, altering from a semi-solid state when at rest to a mobile fluid-like state when stretched (Hagbarth, Hagglund, Nordin, & Wallin, 1985; Lakie & Robson, 1988; Proske & Morgan, 1999). Evidence for such thixotropic behaviour has been supported by a study using X-ray diffraction by Takezawa, Sugimoto, and Wakabayashi (1998). The authors proposed that thixotropic behaviour would be seen as spacing changes of the filaments actin and myosin during passive lengthening. Their results confirmed spacing changes did occur and they concluded this altered state accounted for much of the sarcomere compliance.

Other researchers have identified two sarcomere elastic protein filaments *titin* and *desmin* that are thought to contribute to the sarcomere's mechanical behaviour. Titin has been suggested to be the sarcomere's main source of internal resistance and energy recoil (Trombitas et al., 2003; Wang, McCarter, Wright, Beverly, & Ramirez-Mitchell, 1991; Witt et al., 1998). The elastic nature of titin was demonstrated in an animal study where the binding sites of titin in isolated myofibrils returned to their original position after extensive sarcomere lengthening (Maruyama et al., 1984). Further evidence has been provided by Tskhovrebova, Trinick, Sleep, and Simmons (1997) who observed that titin stiffens the more it was lengthened. The authors believed this stiffening response to be a major contributor to the muscle fibers elastic response. An additional

role for titin has been proposed to maintain the central location of myosin during periods of muscle relaxation, which is believed to be an important structural role in maintaining the integrity of the contractile muscle unit (Funatsu, Higuchi, & Ishiwata, 1990; Horowits & Podolsky, 1987).

The second identified protein, desmin, is believed to have an important structural role by connecting the sarcomere transversely and extends longitudinally outside of the sarcomere. This arrangement creates a mechanical structural framework of *serial* and *parallel* mechanical connections, forming a network for passive force transmission (Roy et al., 2003; Wang et al., 1991).

Outside the sarcomere, connective tissue of the muscle is organised into three layers with a fiber orientation (~60º) that has been shown to be important in force transmission (Kjaer, 2004; Purslow, 2002; Purslow & Trotter, 1994). The deepest layer the *endomysium* embeds the individual muscle fibers forming a complex interconnected meshwork of delicate collagen fibers. These resemble dense three-dimensional hollow honeycomb as illustrated in Figure 2.1, and is believed to be important in lateral force transmission (Purslow, 1989, 2002; Purslow & Trotter, 1994; Trotter & Purslow, 1992; Williams et al., 1995).

The endomysium connects to the *perimysium* a multisheet-layer that compartmentalises the muscle tissue into *fascicles* and has a cross-ply arrangement of crimped collagen fibers. The outermost layer, the *epimysium* merges at the ends of a muscle with the connective tissue of its tendon and aponeurosis. Collectively the endomysium and the perimysium layers are referred to as the *intramuscular connective tissue* (IMCT). This

creates a continuous connection of connective tissue around the muscle fibers outwards to the epimysium and is the main contributor to force transmission during passive stretch (Herbert, 1988; Purslow, 1989; Roy et al., 2003).

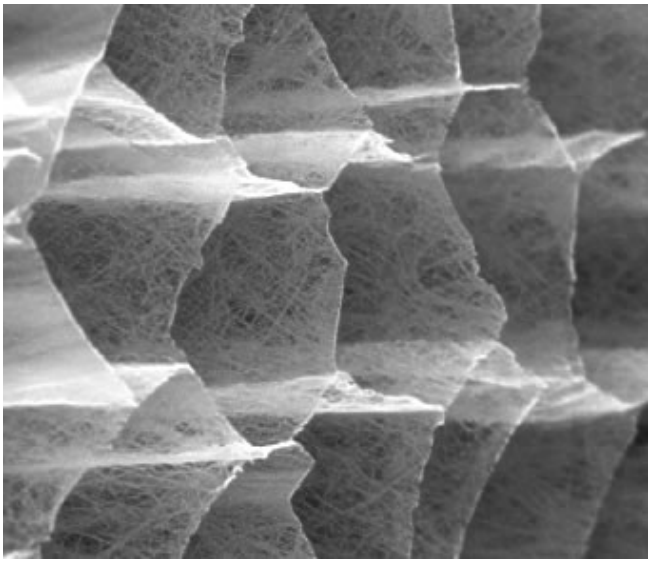

Figure 2.1: Scanning electron-micrograph (magnification X 3200), an oblique view of the endomysium meshwork (part of the IMCT), which separates each muscle fiber.
Note. From Purslow. (2002). *Comparative Biochemistry and Physiology - Part A, 133*, p. 953. Reproduced with permission.

2.3.3 Anatomy and Structure of the Hamstrings

The term *hamstrings* is used in both lay and medical literature and describes collectively the three muscles, *semimembranosus*, *semitendinosus*, and *biceps femoris* (Kroll & Raya, 1997; Palastanga, Field, & Soames, 2002). The hamstring muscle group is frequency subjected to daily repetitive or cyclic loadings during human locomotion. To understand how such loadings might affect the hamstrings its anatomy and muscle architecture (defined by Lieber and Bodine-Fowler (1993) as the geometric arrangement

of the muscle fibers relative to the axis of force production) is of practical importance to provide a basis for its mechanical properties and responses under *in vivo* conditions (Fung, 1984; Lieber & Friden, 2001; Narici, 1999). Although the hamstring muscles are made of similar contractile units (sarcomeres), their individual architectural arrangement varies. The key muscle architecture components are their fiber length, pennation angle (fiber angle relative to the force-generating axis), and their physiological cross-sectional area (PCSA). This is a calculated measurement that estimates the total muscle fiber cross-section area of the muscle (Lieber & Friden, 2000a). The hamstring muscles are, in general, characterised by low pennation angles, long fibers, and having an intermediate PCSA (Table 2.3). Such anatomical characteristics are required to allow lengthening during large excursions of the lower extremities, often performed at high velocity.

Table 2.4: Muscle Architecture of Human Hamstring Muscles
(Data presented as Mean ±SD)

Muscle	**Pennation Angle (º)**	**Fiber Length (mm)**	**PCSA (cm^2)**
Semimembranosus	15 ±3	63 ±5	17 ±2
Semitendinosus	50 ±0	158 ±2	5 ±1
Biceps femoris long head	0 ±0	85 ±5	13 ±3
Biceps femoris short head	23 ±1	139 ±3	N/A

Note. N/A = not available. Adapted from Wickiewicz, Roy, Powell, & Edgerton (1989).

Changes in pennation angle associated with lengthening of the muscle-tendon unit has been demonstrated using ultrasonography studies, though none have reported on the hamstring muscles. Nevertheless, two research groups (Fukunaga, Kawakami, Kuno, Funato, & Fukashiro, 1997; Narici et al., 1996) have investigated the muscle architecture of gastrocnemius and quadriceps muscles, respectively, during passive motion. The findings of these two studies were identical, and revealed that as joint angle changed, muscle fiber length increased up to 18% and pennation angle altered by three degrees from a loaded (passive stretch) to an unloaded state. These findings demonstrate that joint position alters the muscle architecture of the muscle they affect. Such architectural changes have mechanical implications for the muscle-tendon unit and its viscoelastic behaviour during passive stretching.

The three hamstring muscles have distinctly different structural characteristics. Semimembranosus lies on the posteromedial aspect of the thigh, deep to the other two muscles, and has a large membranous tendon that extends approximately 78% of the muscle length. The distal portion of its tendon has a myotendinous junction extending approximately 52% of its length (Garrett, Rich, Nikolaou, & Vogler, 1989; Williams et al., 1995). An interesting finding is Garrett et al. using magnetic resonance imaging reported the muscle belly of semimembranosus contains a proximal or distal myotendinous junction running essentially through the whole muscle.

Semitendinosus has a myotendinous junction extending through 31% of the muscle belly. Additionally there is a mid-line raphe (a seam of connective tissue) that extends over 49% of its entire length (Garrett et al., 1989). The distal portion of the muscle has a slender cord-like tendon that makes-up approximately 56% of the semitendinosus

length. Distally it blends, sharing a common attachment with semimembranosus (Kroll & Raya, 1997; Palastanga et al., 2002; Williams et al., 1995).

Biceps femoris is divided into long and short head, which blend and form one common tendon extending approximately 66% of the muscle's length (Garrett et al., 1989; Palastanga et al., 2002). The literature reports that the long head of biceps femoris it the most frequently injured (76.5%) of the group (Woodley & Mercer, 2004), which has the least pennation angle and a short fiber length. Speculatively, such muscle architecture may not allow for sufficient elongation during long excursions in positions of passive insufficiency, which could lead to injury.

2.4 Biomechanical Properties, Behaviour Of Muscle-Tendon Units: *In Vitro & In Vivo*

2.4.1 Introduction

The preceding sections have reviewed the individual components of passive elements of the muscle-tendon unit and the gross anatomy of the hamstring muscle group. The aim of this section is to examine the complete muscle-tendon unit and to describe its key biomechanical and passive properties, and the muscle-tendon unit's mechanical response to passive stretching and loading that are of interest in the current study.

2.4.2 The Muscle-Tendon Unit and Its Viscoelasticity

When the muscle-tendon unit is subjected to load and under goes subsequent lengthening, it exhibits viscoelastic behaviour that is considered to have both viscous and elastic properties (Fung, 1972; Taylor et al., 1990). Viscous properties are likened

to the actions of a hydraulic piston (in biomechanical terminology known as a *dashpot*) and characterised by time- and rate-dependence behaviour. The hydraulic piston represents the mechanical response of the muscle-tendon unit as it resists force during passive lengthening, exhibited by an increase in its resistance simultaneously as the rate of lengthening increases. The elastic properties of the muscle-tendon unit have been likened to the actions of a simple spring and is characterised by a linear relationship between lengthening and the applied force, described by Hooke's law. However, the muscle-tendon unit is a composite structure and displays much more complex behaviour than that described by a simple spring or hydraulic piston model. Consequently, when the muscle-tendon unit is under load and lengthens, it responds what has been described as *pseudo-elastic* behaviour (Fung, 1979, 1984). If measurements of force and length are plotted on a graph, a non-linear or curvilinear line is apparent. This is described as the *stress-strain curve* or alternately *load-lengthening* curve as illustrated in Figure 2.2 (Fung, 1972; Provenzano et al., 2001; Taylor et al., 1990).

2.4.3 Stress-Relaxation and Creep Lengthening of the Muscle-Tendon Unit

Two characteristic properties of viscoelastic structures are *stress-relaxation* and *creep*. Stress-relaxation is defined as to the lengthening of a muscle-tendon unit and then held at a constant length for a period of time, the measured force or load at that length reduces gradually and demonstrates the muscle-tendon unit's viscous and elastic behaviour (Fung, 1972; Taylor et al., 1990).

The other property, creep is when the muscle-tendon unit continuously lengthens under a constant load over time with a sustained load. Lengthening of the muscle-tendon unit by a constant loading is based upon the viscoelastic components of the muscle-tendon

unit (Fung, 1972; Taylor et al., 1990). Commonly creep is referred to as *static creep* or *sustained loading* (De Zee et al., 2000; Evans & Wilshire, 1993; Thornton et al., 2002). These names are used to distinguish this form of creep from *cyclic loading* (also referred to as *dynamic* or *cyclic creep*), where the load is altered continuously, from a minimum (often near zero) to a constant maximal load value. As such, an increase in muscle-tendon unit length occurs from each sequential cycle load (De Zee et al., 2000; Thornton et al., 2002; Wang & Ker, 1995). For clarity with this thesis, creep has been referred to as *sustained loading* for static creep and *cyclic loading* for dynamic creep.

When creep is plotted, a length-time graph relationship is represented as a curve as illustrated in Figure 2.2 (De Zee et al., 2000; Fung, 1973; Ker, 2002; Magnusson et al., 2003a). To identify where important changes occur the length-time curve has been divided into regions. The first region is known as the *primary* region and represents normal physiological lengthening at a decelerating rate, followed by the *secondary* region where the rate of lengthening slows and eventually reaches asymptote (where a normal curve approaches a horizontal line) as illustrated in Figure 2.2 (Kannus, 2000; Ker, 2002; Wang & Ker, 1995).

These fundamental mechanical characteristics, stress-relaxation and creep of the viscoelastic properties of the muscle-tendon unit are of interest to researchers, and provide information about its behaviour during passive stretching and loading, respectively (Fung, 1967).

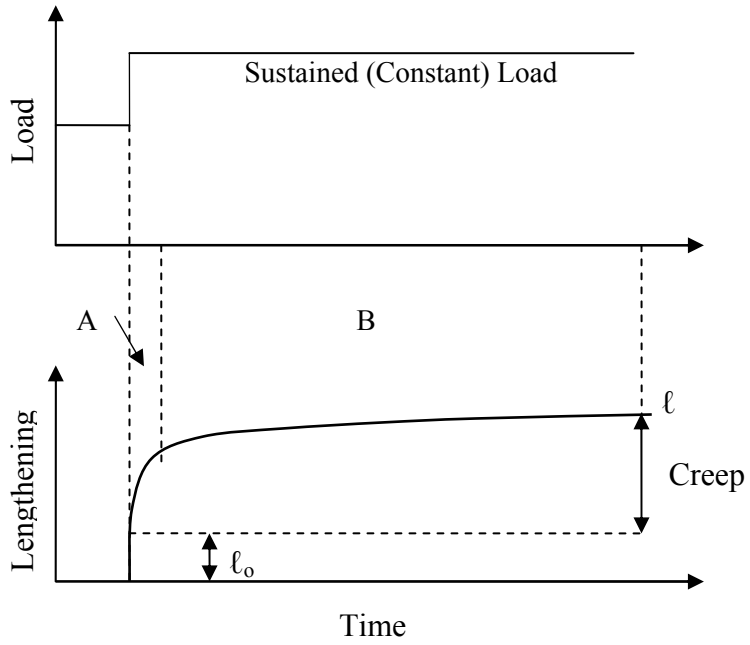

Figure 2.2 Schematic diagram of creep lengthening. ℓ_o= original length, ℓ= final length. A= Primary Region, B= Secondary Region. $\ell - \ell_o$ = Creep Lengthening.

2.4.4 Points of the Stress-Strain Curve Relationship

Particular points of the stress-strain curve relationship are of interest to researchers. When passive lengthening of the muscle-tendon unit occurs under i*n vitro* conditions involving animals or cadavers, initially there is very low almost undetectable resistance. However, when lengthening reaches a point of measurable detection of resistance, this point is termed *initial passive resistance* (IPR) as illustrated in Figure 2.3. With further lengthening of the muscle-tendon unit, ever-increasing resistance occurs until a point of *maximal passive resistance* (MPR) is reached as illustrated in Figure 2.3. It is from this point onward that the connective tissue begins to fail and if lengthening continues, the tissues end in complete failure (Gajdosik, 2001). The location of failure within the muscle-tendon unit has been well documented in animal *in vitro* studies as being in

close proximity to the myotendinous junction (Garrett, Nikolaou, Ribbeck, Glisson, & Seaber, 1988; Garrett, Safran, Seaber, Glisson, & Ribbeck, 1987).

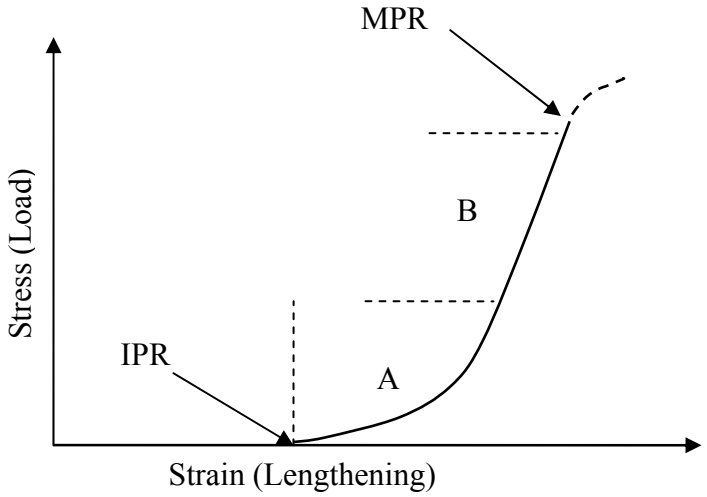

Figure 2.3: Schematic diagram of a typical stress-strain curve for muscle-tendon unit. IPR = Initial Passive Resistance, MPR = Maximal Passive Resistance. A = Toe Region; B = Linear Region.

To monitor changes of the muscle-tendon unit during animal or cadaver *in vitro* studies direct measurements are used. Human studies of *intact* muscle-tendon units performed under *in vivo* conditions however cannot directly measure change and therefore use an indirect measurement technique. Consequently, a different definition of maximal passive resistance is required. The initial point of resistance of the muscle-tendon unit is still termed the IPR However, the process of achieving maximal passive resistance has been referred to as *maximal stretch tolerance* and is defined as the point where maximal discomfort prevents any further stretching (Reid & McNair, 2004; Starring et al., 1988). Changes observed at the maximal stretch tolerance point after stretching interventions has been theorised by number of *in vivo* studies to be due to an immediate increase in the participant's maximal stretch tolerance, rather than a biomechanical

change within the muscle-tendon unit (Halbertsma & Goeken, 1994; Halbertsma, Mulder, Goeken, & Eisma, 1999; Halbertsma, Van Bolhuis, & Goeken, 1996; Magnusson, Aagaard, & Nielson, 2000a; Magnusson et al., 1997; Magnusson et al., 1996b; Magnusson, Simonsen, Aagaard, Sorensen, & Kjaer, 1996a). However, it must be noted that such speculation cannot be determined from the methodology used in these studies and therefore remains conjecture.

2.4.5 Stiffness of the Muscle-Tendon Unit

The stiffness the muscle-tendon unit has been of considerable interest to many researchers. Stiffness measures incorporate both force and motion variables (Fung, 1984; Ker, 2002). *In vitro* studies calculate stiffness as a ratio change in resistive force to changes in length of the muscle-tendon unit ($N\ mm^{-1}$) (Magnusson et al., 2003a; Taylor et al., 1990) or alternatively as Young's modulus (MPa); calculated by multiplying the stiffness value to the tissue's cross-sectional area (Maganaris, 2002; Maganaris & Paul, 1999). Indirect *in vivo* measurements express stiffness as either a change in force to a change in joint angle ($N\ º^{-1}$) (Chesworth & Vandervoort, 1995; Gajdosik et al., 2004; McNair et al., 2002) or as a change in the joint moment (torque) to a change in the joint angle ($N{\cdot}m\ º^{-1}$) (Farley, Houdijk, Van Strien, & Louie, 1998).

Stiffness is associated with greater resistance to motion therefore; the plotted stress-strain curve becomes steeper as illustrated in Figure 2.4 (Liebesman & Cafarelli, 1994). Muscle-tendon units under *in vivo* conditions with increased passive extensibility, that is, greater lengthening, allow greater range of motion of the joint or joints they cross; therefore demonstrate compliance (the reciprocal of stiffness) and display stress-strain curves that are less steep as illustrated in Figure 2.4.

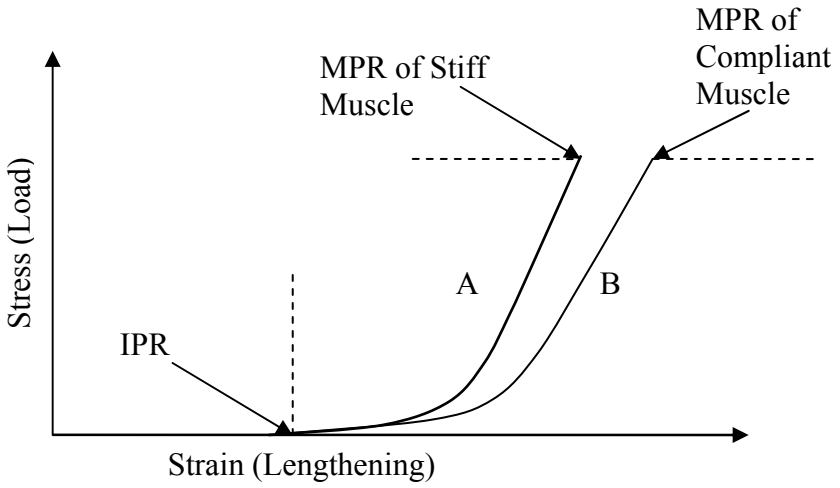

Figure 2.4: Schematic diagram showing plotted curve response of a stiff muscle-tendon unit (A) and a compliant (reduced stiffness) muscle-tendon unit (B). IPR = Initial Passive Resistance, MPR = Maximal Passive Resistance.

It is important for correctness and clarification to note that stiffness as defined here is based upon linear theory (Hooke's Law), and therefore a number of incorrect assumptions are made for complex behaviour observed in composite materials such as the muscle-tendon unit (Fung, 1972; Latash & Zatsiorsky, 1993). For stiffness modelling to be truly accurate, all components that contribute to and influence stiffness must be accounted for. However, such correctness leads to a very complex and impractical mathematical equation (Butler, Crowell III, & Davis, 2003). Therefore, for practical purposes a simple stiffness model is commonly used, a model which more correctly measures *quasi-stiffness* (Butler et al., 2003; Fung, 1972; Latash & Zatsiorsky, 1993). Quasi-stiffness has been accepted as a practical working solution and therefore is used throughout the stretching literature (Best et al., 1994; Hingorani, Provenzano,

Lakes, Escarcega, & Vanderby Jr., 2004; Jensen, Dwyer, Lakes, & Vanderby Jr., 2004; Pradas & Calleja, 1990).

2.4.6 In Vitro & In Vivo Loading Studies Measuring Stiffness

This section reviews studies that have investigated stiffness of the soft tissue structures subjected to sustained and/or cyclic loading, and is divided into two parts. The first part reviews animal and cadaver studies under *in vitro* conditions. The second part reviews studies of animal and human under *in vivo* conditions and includes studies that have used ultrasonography to measure *intact* muscle-tendon units under *in vivo* conditions of humans.

2.4.6.1 Stiffness: In Vitro Loading Studies

In a classical paper, Wang and Ker (1995) investigated stiffness of wallaby tendons under cyclic loading at a frequency of 1.6 Hz. Stiffness was defined as the tangent of the stress-strain curve at the linear region of the curve. Their results showed that cyclic loading influenced the stiffness of the tendon; with each successive cyclic load the tendon became progressively less stiff, as reported the first six cycles reduced by 4, 5, 7, 8, 9, and 10%, respectively. Wang and Ker theorised that the resulting progressive decrease in tendon stiffness was due to the collagen fibers reaching their limit of elongation, at which the tensile force were transferred laterally causing longitudinal shear and sliding within the extracellular matrix. This mechanical process would reduce individual fiber load and the tendon's effective cross-sectional area, and focus the force within the extra-cellular matrix; eventually resulting in failure. Wang and Ker's theory

has recently been supported by a X-ray diffraction study of *in vitro* tissues (Puxkandl, 2002), which confirmed such a process does occur within the tendon structure.

More recently Wren et al. (2003) investigated stiffness of human Achilles tendon using a cyclic loading methodology very similar to Wang and Ker (1995). Loading was applied at a frequency of 1 Hz over 50 minutes. Stiffness was defined as the secant modulus of the stress-strain curve of a line passing through the lowest and highest stress points. Wren et al. reported a mean modulus of 816-822 MPa and significant reduction of stiffness with progressive cyclic loadings, a finding they reported as being consistent with Wang and Ker's study of animal tendon.

However, De Zee et al. (2000) believed studying tendon during cyclic loading using a single loading profile as too simplistic compared to normal physiological loading profiles of tendon. On this premise, they investigated pig tendons under cyclic loading based upon a scenario to simulate the natural physiological loading profile of human Achilles tendon (a loading profile obtained from previous data of running). The tendon was subjected to a cycle frequency of 1.37 Hz, 1,600 cycles, for ~20 minutes. Stiffness was defined as the slope of the linear region (between 50 and 95% of the final portion) of the curve. Their results showed no change in stiffness during the cyclic loading, a finding, which they concluded, showed tendon subjected to a natural physiological loading profile induced creep in the primary region of the deformation-load curve and no mechanical change occurred.

Stiffness however, does not always decrease or remain constant across cyclic loading of soft tissue structures *in vitro*. Thornton et al. (2002) investigated stiffness of rabbit

ligaments (medical collateral) under cyclic loading (30 cycles at a frequency of 1 Hz). Stiffness was defined as the tangent modulus over the final 80% of the stress-strain curve. Their findings showed a significant increase ($p < 0.001$) in stiffness from the first to last cycle. Although Thornton et al. did not discuss a rationale for these differences, a possible reason might be that the tissue's mechanical properties of ligaments were dissimilar to those in tendon.

2.4.6.2 Stiffness: In Vivo Loading Studies

An *in vivo* study by McFaull and Lamontagne (1998) analysed how positioning of the human knee joint affected stiffness of its associated periarticular soft tissue structures. Their results showed that variability in the measurements of stiffness increased as the maximal range of motion of the knee joint was approached, and inter-participant differences in the tissue's stiffness became apparent. Their rationale for such variability of stiffness was that anthropometry and the mechanical properties of a soft tissue's composite mixture when measured at their limits become very sensitive to small changes in knee angle, and therefore need to be taken into consideration.

Individual components of the muscle-tendon unit have recently been shown to differ in stiffness. A study by Kubo et al. (2001a) using ultrasonography, investigated stiffness of the human Achilles tendon, soleus and gastrocnemius muscles under *in vivo* conditions. The tissue structures were passively stretched by a dynamometer from 90 degrees (anatomical position) to 65 degrees of ankle joint dorsiflexion at a rate of five degrees per second Stiffness was defined as the linear portion of the torque-angle curve. The authors found no correlation ($p > 0.05$) between passive stiffness of the muscle-tendon unit and the stiffness measured for the Achilles tendon, while the

tendon's stiffness was found to be negatively correlated to relative increases of resistance. Kubo et al. concluded that the muscle-tendon unit's passive stiffness was not due to the tendon, but rather elements within the muscle.

Stiffness of the muscle-tendon unit has also been investigated under active (muscle contraction) lengthening *in vivo*. A study using ultrasonography by Magnusson et al. (2003c) investigated stiffness of the human Achilles tendon, its associated aponeurosis and the medial gastrocnemius muscle under isometric maximal voluntary contraction. Stiffness was calculated over the tendon's the final 10% of the range of loading. They reported a stiffness mean of 788 MPa, a load value that was comparable to the *in vitro* study by Wren, Yerby, Beaupre, and Carter (2001) of human cadaver Achilles tendon (range 816-822 MPa), a finding they concluded demonstrated that similar mechanical properties and behaviours occurred *in vivo.*

2.4.7 In Vitro, In Situ, & In Vivo Studies Measuring Creep Lengthening

2.4.7.1 Introduction

This section reviews studies that have investigated the viscoelastic characteristic creep of the soft tissue structures when subjected to sustained and/or cyclic loading, and is divided into three parts. The first part reviews animal and cadaver studies under *in vitro* or *in situ* conditions. The second part reviews studies of the spine in animal and human under *in vivo* conditions. The final part reviews human studies (clinical or controlled trials) of soft tissues *intact* under *in vivo* conditions of the upper or lower extremities.

Traditionally *in vitro* studies have used a similar methodology, using clamps to fix the isolated tissue structure in a testing device and applying load, resulting in tissue

lengthening. As the tissue is subjected to tensile force, its viscoelastic behaviour can be directly observed and measured while simultaneously recording its passive resistance and lengthening. Direct measurements use different unit values to those *in vivo* experiments and conversions often cannot be made, leading to difficulty in comparing *in vitro* to *in vivo* studies. However, qualitative comparisons of trends, similarities, and appreciation of the viscoelastic behaviour responses can be formed.

2.4.7.2 Creep Lengthening: In Vitro Animal Studies

A classic paper is that of Taylor et al. (1990) who investigated the viscoelastic behaviour of *in situ* muscle-tendon units of rabbits (extensor digitorum longus) and subjected them to ten repeated cyclic stretches to a sustained load of 78.4 N (calculated to approximate 65% of load to failure). Each cycle was held for 30 seconds before reducing back to an initial load of 1.96 N. Their results demonstrated a significant ($p < 0.05$) mean length increase of 3.5%. Taylor et al. estimated 80% of the length increase took place within the first four repetitions (Taylor et al., p.304, Fig. 6), indicating that the majority of length increases were obtained in the initial cyclic loadings typical of strain-stiffing behaviour (Lakes & Vanderby, 1999), and was a finding consistent with fiber recruitment theory (Thornton et al., 1997; Viidik, 1972).

De Zee et al.(2000) argued normal physiological loading profiles are far more complex for longer periods of time than an idealised single level controlled profile used in studies such as Taylor et al. (1990), and therefore the tissue's mechanical properties might not remain constant during longer cyclic loading. On this basis they investigated pig flexor and extensor tendons under two loading conditions (sustained and cyclic) based upon a scenario modelled on normal human Achilles tendon loading obtained during running

(cycle frequency of 1.37 Hz, 1,600 cycles, ~20 minutes), to simulate a natural physiological loading profile. Their results showed a significant difference ($p < 0.05$) with mean length increases of 0.12% for flexor and 0.28% for extensor tendon during sustained loading and 0.24% for flexor and 0.22% for extensor tendons during cyclic loading. Furthermore, the tendon's lengthening profile displayed a typical creep deformation curve (Dee Zee et al., p. 1353, Fig.1), a finding consistent with Taylor et al. loading of *in situ* muscle-tendon units where greatest lengthening occurred over the initial cyclic loadings.

Although the studies of Taylor et al. (1990) and De Zee et al.(2000) reveal responses of the tissue structures under load; they remain limited to single load levels over a single time. In the classic study by Warren et al. (1976), investigated rat-tail tendons lengthened to failure under two different levels of load and time. These were a *high-load, short duration* condition, that is, a load level termed *full load*, predetermined to be a load level just above normal physiological but below the first yield point of the tendon, and was sustained for five minutes. The second condition was *low-load long duration*, that is, one quarter of the tendon's failure point sustained over 50 minutes. Their findings showed significant differences ($p < 0.01$) in lengthening between the two load/duration levels. The high-load short duration condition responded with rapid lengthening (2.28%), and on release was short-lived leaving a residual length gain of 0.4%. The low-load long duration condition lengthened much more slowly and responded with lengthening of 1.25% on when release a residual length of 0.5% was retained. Warren et al. concluded between the two conditions rat tendon under high-load short duration tendon lengthens some 1.8 times more; however, proportionally rat

tendon under low-load long duration retains a greater residual length, which they attributed to mechanical changes in the tendon's non-recoverable viscous elements.

While Warren et al. (1976) study provided some insight in how soft tissue structures respond to different load levels, it was limited to only two. Wang and Ker (1995) studied wallaby tendons subjected to a wide range levels of loading (range 10-80 MPa) to failure, under two loading conditions, sustained and cyclic (frequency of 1.6 Hz.). Although their study focused on lengthening to failure, nevertheless, their results for both conditions were consistent with the single level loading studies of Taylor et al. (1990) and De Zee et al.(2000), and the two loading levels of Warren et al., that is, creep lengthening occurred across all levels of loading. Furthermore, as the loading level increased during both conditions creep lengthening increased (range 3.8-5.2%), with the exception of the lowest sustained loading level (10 MPa), which after 15 days there was no sign of failure and little lengthening. In contrast, the highest loading level (80MPa) produced a mean length increase of 6% at the point of failure. These findings Wang and Ker concluded, indicated that the 10 MPa load level was such as to produce creep lengthening within the normal physiological loading level (estimated from previous studies to be 13.5 MPa) and was non-damaging in wallaby tendon. Recently, studies using scanning X-ray micrography of tendon (Puxkandl, 2002; Schechtman & Bader, 1997) have confirmed Wang and Ker conclusions that low loading levels are non-damaging, though considerable sliding or deformation within the collagen fibers of the tendon under conditions of normal physiological loading does occur.

Another study (Provenzano et al., 2001) investigated sustained loading (100 seconds) applied at multiple load levels (range 3.7-9.9 MPa) to the medial collateral ligament of

rats. Provenzano et al. estimated from earlier work these loading levels would induce lengthening below a level considered to cause damage. Their findings showed a mean increase of 0.75% in length (significance was unknown as no statistical analysis was reported). Furthermore, findings revealed at low loading levels creep was nonlinear and the rate of lengthening was strongly dependant upon the loading level applied (p = 0.0078), ranging from 0.058 (3.7 MPa) to 0.007 (9.9 MPa). These findings indicated at each level of loading, lengthening occurs with time and as the level of loading increases there is a subsequent reduction in the rate of creep, a findings consistent with the animal tendon study by Wang and Ker (1995) and strain-stiffing behaviour (Lakes & Vanderby, 1999).

Hingorani et al. (2004) using a very similar methodology as Provenzano et al. (2001) investigated the medical collateral ligaments of rabbits. The ligaments were subjected to five levels of sustained loading load (3.0, 8.1, 15.8, 34.9, and 54.8 MPa), held over 100 seconds. Their results were consistent with Provenzano et al. however, they noted creep in the rabbit ligament displayed a greater non-linearity than observed in rat ligament, and believed differences across species might reflect their differing composite tissues components and therefore need to be accounted for when making inferences or comparisons (Fung, 1973).

Thornton et al. (2002) preformed a further investigation of multiple load levels on the medical collateral ligament of rabbits. Using methodology similar to Provenzano et al. (2001), four levels of load (4.1, 7.1, 14, 28 MPa) were applied as either sustained loading for 20 minutes or cyclic loading for 30 cycles at 1 Hz. Only the highest load level (28 MPa) was considered greater than a normal physiological load. Thornton et al.

findings showed at load levels of 4.1 MPa and 7.1 MPa, there was no significant change ($p > 0.05$) in length, while at 14 MPa a significant ($p < 0.05$) length increase (1.3%) did occurred. The highest load level (28 MPa) showed significantly ($p < 0.05$) greater lengthening (2.1%) than the 14 MPa load level. The authors concluded that creep behaviour exhibited in rabbit ligaments had little sensitivity to load levels within the toe-region of the stress-strain curve. However, creep lengthening did increase significantly in the linear region, though at a slower rate. Thornton et al. suggested collagen was well designed to minimise creep in the toe-region, that is, crimp of collagen fibers allows the tissues to be compliant (Latash & Zatsiorsky, 1993; Magnusson et al., 2002); a finding consistent with studies of *in situ* muscle-tendon units (Taylor et al., 1990), tendon (De Zee et al., 2000; Wang & Ker, 1995) and ligament (Hingorani et al., 2004; Provenzano et al., 2001). Furthermore, as load increases, the rate of creep decreases, typical of strain-stiffing behaviour (Lakes & Vanderby, 1999), and is consistent with fiber recruitment theory (Thornton et al., 1997; Viidik, 1972).

Many studies have investigated the viscoelastic property of stress-relaxation based on non-linear theory developed by (Fung, 1972). Thornton et al. (1997) questioned whether creep phenomena required investigating if inferences about its behaviour could be made from past documented stress-relaxation studies. They hypothesised that creep behaviour was inversely proportional to stress-relaxation and therefore could be predicted from published data. Their study investigated this hypothesis by comparing dynamic stretching to a constant length (stress-relaxation) to cyclic loading to constant load (creep) using rabbit ligaments (medial collateral ligament). The ligaments were subjected to 30 cycles at 1 Hz for 20 minutes to a sustained loading level of 14 MPa. Their results showed significant lengthening differences ($p < 0.05$) existed between the

two cyclic techniques. Creep lengthening was significantly less approximately 1.9 times than predicted by inverse stress-relaxation calculations and indicated that inferences cannot be made from previous published data. Thornton et al. concluded that creep and stress-relaxation are two different mechanical phenomena, agreeing with former theory published by Fung (1984), that creep lengthening is more non-linear than stress-relaxation and was most likely due to the different mechanical processes taking place within the micro-structure of the tissues during loading. Additional support for these deductions about creep versus stress-relaxation has been provided by Provenzano et al. (2001), who used rat rather than rabbit ligaments and replicated the methodology used by Thornton et al. and found their results were consistent with Thornton et al.

2.4.7.3 Creep Lengthening: In Vitro Studies of Human Soft Tissues

This section considers those studies involving cadaver soft tissue structures; this includes the spine due to the paucity of sustained and cyclic loading studies of human muscle-tendon unit.

Smutz et al. (1995) investigated sustained loading of human tendons (flexor digitorum) under four levels of loading (10, 20, 50, 100N) considered to cause normal physiological levels of strain. There were three test conditions (one sustained loading, held for 30 minutes; and two cyclic loading frequencies, 0.25 and 1 Hz, held for 100 minutes). Their findings at the four loading levels over all three conditions showed mean length increases of 0.6, 0.9, 1.8, and 2.3%, respectively. There were no significant differences ($p > 0.05$) for lengthening between the three conditions at any of the four load levels. However, significant lengthening differences ($p < 0.05$) were found between sustained loading and 1 Hz cyclic loading at the 10, 20, and 50 N load

levels, and significant lengthening differences ($p < 0.05$) were found between sustained loading and 0.25 Hz cyclic loading at the 20 and 50 N load levels. The only significant lengthening difference ($p < 0.05$) between the 1 Hz and 0.25 Hz cyclic loadings was at the 50 N load level. Although, Smutz et al. did not provide any rational for this behaviour in their discussion, this finding is interesting as soft tissues are thought to be rate dependent (Evans & Wilshire, 1993; Fung, 1972; Taylor et al., 1990) therefore; one would have expected differences to occur at all four load levels between the two cyclic conditions. Significant differences ($p < 0.05$) were found between lengthening for the four load levels of all three conditions. As a result a consistent trend of the four load levels for the three conditions was exhibited as the load level increased a corresponding increase in creep lengthening occurred. All creep curves (Smutz et al., p. 70, Fig.5, 6, 7) for all three conditions were reported as being similar, though the rate and amount of lengthening was greater for sustained loading compared to either cyclic loading condition. Smutz et al. findings are consistent with animal *in vitro* studies (Hingorani et al., 2004; Provenzano et al., 2001; Taylor et al., 1990; Thornton et al., 2002; Wang & Ker, 1995) and indicate that in many ways human tendons behave like animal tendons.

Wren et al. (2003) investigated sustained loading and cyclic loading of human Achilles tendon to failure, using a methodology very similar to Wang and Ker (1995). Nine levels of sustained loading load increased by 5 MPa intervals (range 35-75 MPa) were applied for 17 minutes. The cyclic loading condition used the same load levels, applied at a frequency of 1 Hz over 50 minutes. Furthermore, four markers were used to measure lengthening in sub-regions of the tendon. Their results for both sustain and cyclic loadings showed lengthening of the tendon increased (range 4-12%) as the load level increased a finding similar to animal tendon (Wang & Ker, 1995) and human

cadaver tendon studies (Schechtman & Bader, 1997). Additionally Wren et al. observed lengthening was not uniform across the tendon, with some regions lengthening more than other regions, a finding consistent with animal *in vitro* studies (Lieber et al., 1991; Zuurbier et al., 1994).

Adams and Dolan (1996) investigated sustained loading and cyclic loadings of human cadaver lumbar spinal segments. Using a preconditioning load of 300 N for 15 minutes to reduce post-mortem changes, four test conditions were applied while flexing the spine to approximately 70% of its flexion limit. The first condition examined the rate of loading into flexion under three rate levels (1, 3, and 10 seconds) with the spinal segment being flexed four times. The second condition examined cyclic loading (100 cycles) into flexion using a three second loading/unloading time, over a five minute time period. The third condition examined five minutes of sustained loading in flexion using a one second load/unloading time, while the fourth condition replicated the second but increased the sustained loading time to 60 minutes. The results for the first condition showed a significant ($p < 0.05$) increase in the tissues' resistance, compared to the ten seconds rate, the three seconds rate increased the bending moment by 9%, and the one second by 13%. A finding in support of soft tissue's rate dependence of loading (Evans & Wilshire, 1993; Fung, 1972; Taylor et al., 1990). The results for the three remaining conditions all reported significantly ($p < 0.05$) reduced flexion resistance to bending by 17, 42, and 67%, respectively. Lumbar flexion range of motion increased by 4% for cyclic loading over five minutes and 12 % for sustained loading for 60 minutes (no data was reported for sustained loading for five minutes). Additionally the fourth condition, the interspinal ligament resistance to its bending moment had reached asymptote at approximately 500 seconds. Adams and Dolan concluded from their

findings the time-dependant nature of the human spine and that rapid cyclic loading was resisted by the spinal tissue's structure. Albeit the tissues of this study are rather different from those of the muscle-tendon unit; nevertheless, these findings confirm the implications of sustained loading and cyclic loadings on the mechanical properties of soft tissue structures in humans.

2.4.8 Limitations of Animal and Cadaver Tissue Testing In Vitro

The studies reviewed have shown some consistent findings and trends in whole muscle-tendon units, tendon, ligament, and spinal soft tissue structures under sustained loading and cyclic loading conditions. Caution, however, must be exercised as results that are extrapolated directly from *in vitro* experiments to interpret *in vivo* physiological phenomenon for the following reasons; slippage of the tissue at the clamp site, concentration of forces in the material adjacent to the clamp, use of preserved or thawed tissues may have altered physical properties, and forces of daily physiological tissue loading under *in vivo* conditions may differ to those used in animal or cadaver experiments (Fung, 1973; Maganaris, 2002, 2004a). Some of these limitations have been addressed using *in situ* experiments (Taylor et al., 1990) where part of an isolated tissue structure is excised and placed in a clamp set-up (similar to that used with *in vitro* studies) while preserving its blood and nerve supply to a live animal (Maganaris, 2004a).

2.4.9 Creep Lengthening: Animal & Human In Vivo Loading Studies

2.4.9.1 Introduction

The previous *in vitro* studies show the effect of either sustained or cyclic loading on soft tissue structures in isolation result in similar outcomes. The mechanical properties and physiological composition of composite tissue structures that are *intact* and under *in vivo* conditions might be quite different from isolated single tissue structures under *in vitro* conditions (Fung, 1984). The following section considers studies of creep phenomenon *in vivo* and examines whether their findings are similar to *in vitro* study results.

2.4.9.2 Creep Lengthening: In Vivo Studies of Human Tissues using Ultrasonography

Only one ultrasonography study (Maganaris, 2002) has investigated creep phenomenon using cyclic loading of intact human muscle-tendon units *in vivo*. Maganaris subjected the gastrocnemius muscle-tendon units of six participants to five maximal isometric contractions elicited one second apart, by percutaneous electrical stimulation (100Hz), each for a two second duration. Creep lengthening was determined by measuring lengthening from the myotendinous junction to the tendon's osseous insertion point, induced by all five contractions compared to the length movement of the joint moment at the fifth contraction. Results showed a significant increase ($p < 0.05$) in length from the first (4.7%) to the fifth (6%) contraction and when plotted produced a typical curvilinear curve (Maganaris et al., p. 1025, Fig. 8). Maganaris concluded that this provided evidence that creep lengthening did occur in human muscle-tendon units under *in vivo* conditions. These findings have clinical implications; for example, if the operating range of the muscle-tendon unit is increased, shock absorption during high-impact loadings could occur and therefore play an important role in the prevention of

injury. The disadvantage of muscle-tendon unit creep lengthening might be if a delay occurs in the time taken before limb movement begins, caused by the muscle fibers having to accommodate the increase in length of the tissue structures (Magnusson et al., 2003c).

2.4.9.3 Creep Lengthening: In Vivo Animal and Human Spinal Studies

Few studies have investigated creep under *in vivo* conditions, therefore animal and human spinal creep studies have been included in this section to appreciate their mechanical response.

Claude, Solomonow, Zhou, Baratta, and Zhu (2003) investigated cyclic loading of the supraspinal ligament (level L4–L5) of cats at multiple loading levels under *in vivo* conditions. Three levels of load (20, 40 and 60 N) applied at a frequency of 0.1 Hz over 20 minutes were used. Claude et al. results showed the same trend for all three load levels, that is, the greatest lengthening occurred on the first cycle, followed by a decrease in lengthening with each successive cycle. When the lengthening were plotted a typical creep curve was displayed (Claude et al., 354, Fig. 5). The final creep lengthenings were; 7.0, 11, and 26% for each load level, respectively. Their results are consistent with *in vitro* studies that tested soft tissue structures at multiple loading levels, that lengthening behaviour was dependant on the magnitude of the load applied (Hingorani et al., 2004; Provenzano et al., 2001; Smutz et al., 1995; Thornton et al., 2002; Wang & Ker, 1995; Wren et al., 2003). Furthermore, Claude et al. found the ligament increased its resistance to further lengthening as the number of cycles increased, exhibiting typical strain-stiffing behaviour (Lakes & Vanderby, 1999). In addition, this finding is consistent with *in vitro* studies of whole muscle-tendon units

(Taylor et al., 1990), tendon (De Zee et al., 2000; Wang & Ker, 1995) and ligament (Hingorani et al., 2004; Provenzano et al., 2001), and consistent with fiber recruitment theory (Thornton et al., 1997; Viidik, 1972).

Viscoelastic structures under *in vitro* conditions have shown to be rate-dependant (Adams & Dolan, 1996). Lu, Solomonow, Zhou, Baratta, and Li (2004) hypothesised that dissimilar cyclic loading rates would result in differences of final length under *in vivo* conditions. Using a similar animal model to Claude et al. (2003), the authors investigated cyclic loading comparing two frequencies of 0.1 and 0.5Hz, using a cyclic loading level of one Newton in a 20-minute session. Their findings showed significant ($p < 0.05$) lengthening differences with the higher frequency (0.1Hz) producing greater lengthening (89.4%) compared to the 0.5Hz frequency (38.9%) and displayed typical creep curves (Lu et al., p. 850, Fig.3). These findings confirmed the authors' hypothesis and were consistent with the findings of Adams and Dolan (1996) *in vitro* study.

Animal *in vivo* spinal models have provided some insight into creep phenomenon. McGill and Brown (1992) wondered if creep could be observed in the *in vivo* human lumbar spine. They investigated prolonged positions of spinal flexion (a position commonly adopted by occupations such as bricklaying). Participants wearing a non-invasive electromagnetic device that measured lumbar spine motion (expressed as angle change) maintained a sustained sitting flexion position for 20 minutes. Their results showed a mean increase of 5.5 degrees and the plotted measurements showed the typical creep curve (McGill and Brown, p. 45, Fig. 3a, b). McGill and Brown concluded that the human spine responded with creep lengthening, a finding consistent with the cadaver *in vitro* study by Adams and Dolan (1996) and the *in vivo* studies

which tested creep loading of the feline spine (Claude et al., 2003; Lu et al., 2004). The results of this study therefore show that the mechanical behaviour of *intact* human soft tissues *in vivo* under load are similar to *in vitro* animal or human soft tissue structures.

2.4.10 Creep Lengthening: Clinical Trials of Human Tissues

2.4.10.1 Introduction

All of the studies reviewed so far have been performed on normal (nonpathological) tissue structures under acute conditions and likewise the current study investigated normal tissue structures under a single session (acute response) subjected to cyclic loading. However, because so few human *in vivo* studies have been performed, the following section reviews clinical trials of soft tissues with pathology and have been included in the review to appreciate the mechanical response of muscle-tendon units of *chronic conditions*, that is, associated pathology over prolonged periods of time, ranging from days, weeks to months.

2.4.10.2 Creep Lengthening: Clinical Trial of Low-Load Prolonged Stretch

The use of a sustained loading, termed *low-load prolonged stretch* (LLPS) under *in vivo* conditions, has been reported in the literature in a limited number of clinical trials. The technique LLPS is the *in vivo* equivalent of Warren et al. (1976) low-load long duration procedure. Furthermore, these clinical trials (MacKay-Lyons, 1989; Rizk et al., 1983; Steffen & Mollinger, 1995) have investigated participants with pathogenic soft tissue contractures of the hip, shoulder, knee, and ankle joint. For instance, Rizk et al. (1983) investigated the passive extensibility of 56 shoulders diagnosed with *adhesive capsulitis* using intermittent LLPS. Fifty participants (32 male, 18 female), age range 40-70 years,

were placed into two groups. The first group was treated with conservative physiotherapy consisting of heat, exercises and manual therapy. The second group was treated with intermittent LLPS and transcutaneous electrical stimulation (TENS), a pain-relieving device. Each intermittent LLPS treatment session consisted of 15 minutes loading, five minutes recovery and was repeated over a total treatment session time of two hours for eight weeks. Both groups attended treatment sessions four times a week for the first four weeks. The remaining four weeks, treatment sessions were reduced to three times a week, resulting in a total loading time of 56 hours. The treatment sustained loading level was gradually increased as tolerated over the eight weeks (range 8.8 to 66.7 N). Results showed although both groups showed increases in passive extensibility, and a significant difference ($p < 0.05$) occurred between the groups. The intermittent LLPS group's mean increase was 33.9 degrees compared to 8 degrees for the conservative treatment group. However, caution needs to be exercised due to a number of fundamental methodological errors, which could potentially bias the results. These included no randomisation or blinding of group allocation, no control group, and no blinding of the investigators to assessment measurements. Nevertheless, intermittent LLPS group did show a increase in passive extensibility of the shoulder joint, a response most likely due to mechanical changes in the soft tissue structures, that is, creep lengthening occurred, a finding consistent with the low-load prolonged duration *in vitro* animal model of Warren et al. (1976).

Another clinical trial was performed by Steffan and Mollinger (1995) who investigated the effectiveness of an orthotic spring-loaded device that allowed LLPS to be applied to 28 nursing-home residents (six male, 22 female) with contractures of the knee joint. Sustained loading was applied using a torque-load setting adjusted to each participant's

maximal stretch tolerance (range 0.0-6.1 N·m). All participants had bilateral knee contracture of the soft tissues and acted as self-controls, with LLPS applied to one knee only. The mean wearing time was three hours a day, five days a week. Four baseline measures of passive extensibility were taken in the first month using the passive knee extension test, measured using a standard universal goniometer blinded to the assessor until after the measurement was fixed. The best of three repeated measurements was recorded. In addition, a hand-held dynamometer measured torque (mean 9.7 N·m) at maximal passive extensibility. In the second month, the LLPS intervention was begun lasting a total of six months with monthly measurements recorded. The results of this study showed no significant change ($p > 0.05$) in passive extensibility compared to the control knee. The investigators concluded the applied mean torque-load setting (6.1 N·m) compared to the mean torque-load (9.7 N·m) measured at the point of maximal passive knee joint extension was insufficient to cause a creep effect. This conclusion has implications for investigators using maximal stretch tolerance as the setting of the baseline loading level. It would appear from Steffan and Mollinger's results that the baseline loading level should be set rather by the mean loading force measured at the point of maximal passive extensibility.

In contrast to Steffan and Mollinger (1995) findings, another clinical trial (Nuismer et al., 1997) examined the use of LLPS for contractures of 18 different joints (2 wrist, 12 elbows, and 4 knees) using a variety of similar orthotic spring-loaded devices applied for a daily sustained loading time of seven hours. The reported sustained loading settings for each orthotic device were of a unique scale only known to the manufactures; therefore, the true values used remain unknown as a standardised international unit value and therefore have not been reported here. Nevertheless, the results of this study

after six-months of LLPS application showed a significant ($p < 0.01$) though small increase in the mean passive range of motion (3.3 degrees) compared to the control knee. Similar to Rizk et al. (1983) intermittent LLPS clinical trial, this study demonstrated passive extensibility of pathological joint soft tissue structures also occurs with LLPS. However, caution needs to be exercised when evaluating Steffan and Mollinger findings as their trial had a number of fundamental methodological errors similar to the Rizk et al. clinical trial, which could bias these findings. These included no randomisation of participants entering into the study and no blinding of the investigators during assessment measurements.

2.4.10.3 Creep Lengthening: Randomised Controlled Trials of Human Tissues

This section reviews randomised controlled trials of studies that have investigated creep lengthening. Randomised controlled trials are considered the 'gold standard' for rigour inquiry and methodology (Sackett, Rosenberg, Gray, Haynes, & Richardson, 1996), however, only two known studies, Bohannon (1984) and Madding et al. (1987) have investigated creep under controlled laboratory conditions.

Bohannon (1984) investigated maximal sustained loading applied to the hamstring muscles of ten healthy participants for eight minutes on three consecutive days in an attempt to improve range of motion of the hip joint using a hamstring muscle stretching technique termed *straight-leg-raise* (SLR). The participants were randomised into either an intervention or control group. Using a pre-test trial, mean baseline loading levels were determined by the participant's maximal stretch tolerance, (mean ±SD) 61.8 ±12.7 N for the intervention group and 64.7 ±15.7 N for the control group during a SLR stretch. During the intervention procedure, an auditory electromyographic (EMG)

feedback device was used to alert the participants if any excessive muscle activity from the hamstring muscles occurred; though levels of EMG were not recorded. Bohannon's results showed a non-significant ($p > 0.05$) mean increase in passive range of motion of 4.4 ±5.6 degrees compared to controls 0.6 degrees (no standard deviation for controls were reported). Nevertheless, interim measurements after 15 seconds of sustained loading did show greater passive extensibility compared to controls however, these gains were not retained. The findings of this study indicate that creep lengthening of muscle-tendon units due to sustained loading under *in vivo* conditions applied for short durations might be transient. This result is consistent with the study by Warren et al. (1976), which also reported transient length gains using a high-load short duration protocol. A point of consideration is Bohannon use of an eight minute hold time he believed was clinically relevant, yet he reported, "As the angle of SLR had not plateaued within the eight minute period of loading used in this study, daily increases may have been greater if the loading time had been prolonged to 20 or more minutes as suggested by previous clinical reports" (Bohannon, 1984, p. 495). Therefore, it would appear that this omission had major implications for the outcome of his study, suggesting a significant increase in SLR perhaps would have been achievable. Another factor that potentially affected Bohannon's results was the lack of data monitoring of the EMG feedback device. If the device was set at, a feedback level that allowed muscle activity, the participants would have been able to resist the sustained loading, resulting in reduced passive extensibility.

The other controlled study is by Madding et al. (1987) which investigated three levels of hold times (15, 45, and 120 seconds) of sustained loading applied to the hip joint adductor muscles of 72 healthy participants. The participants were randomised into

either a treatment or control group. Baseline abduction range of motion was determined by the best of three trials, where each participant actively abducted their leg as far as possible, and the range of motion recorded. Returning the abducted leg to neutral, the baseline level of sustained loading was determined upon the investigator passively manually abducting the leg to the baseline abduction range of motion. At this position, a hand-held dynamometer was placed on the medial side of the leg and the force reading recorded as the baseline sustained loading level. During intervention, the investigators manually applied a rate of stretch was five degrees per second and using the hand-held dynamometer maintained sustained loading by manually adjusting the lower extremity to the hand-held dynamometer's force reading. The results for all three hold times showed a significant increase ($p < 0.05$) in passive extensibility (reported as range of motion in degrees; mean ±SD), 7.0 ±2.0, 5.4 ±2.7, 7.2 ±3.0 degrees, respectively, when compared to the control group. In addition, the force loading measured at the baseline range of motion, showed a significant decrease ($p < 0.05$) in force of 4.9 ±2.0, 4.9 ±2.9, 4.9 ±2.9 N, respectively, when compared to the control group. However, the statistical analyses revealed no significant difference ($p > 0.05$) between the three stretch groups. Madding et al. concluded that a 15-second hold was just as effective as longer holds to increase passive extensibility and decrease resistive force. Although their study did not measure range of motion increases simultaneously during the hold times, increases in range of motion would most likely be due to creep lengthening, a phenomena that has consistently been exhibited using sustained loading in both *in vitro* and *in vivo* soft tissue studies. Some caution must be exercised about the accuracy of this study, during hold times, loading levels were applied manually (monitored using a hand-held dynamometer). Furthermore, no measures of reliability for the methodology were

provided and no EMG monitoring was performed to verify that the changes observed were due to passive lengthening rather than active muscle involvement.

2.5 Passive Stretching and Its Assessment Using the Passive Knee Extension Test and Dynamometry

2.5.1 Introduction

This section briefly discusses the rationale for the use of stretching in the clinical setting. Types of stretching techniques are described and common used names are clarified. In addition, the use of dynamometry and the passive knee extension test to assess changes in the properties of muscle-tendon units is discussed. Finally, the section finishes with a review of reliability studies of the modified passive knee extension test and dynamometry.

2.5.2 Passive Stretching and Dynamometry

Until recently, clinical investigations of the passive properties of muscle-tendon units during dynamic stretching have been difficult, *in vivo*. Traditionally maximal joint range of motion has been used as the dependent variable (Magnusson, 1998b). With the development of isokinetic dynamometers however, it is now possible for indirect measurements of passive loading, resistance, and angle change of the joint or joints of the targeted muscle-tendon unit to be made (Gajdosik, 2001; Keating & Matyas, 1996; Magnusson et al., 2003a). The advantage of measuring muscle-tendon units dynamically, compared to a single static point, is the quantification of resistance, stiffness and range of motion of the joint affected by the muscle-tendon unit throughout

its entire passive extensibility (Muir, Chesworth, & Vandervoort, 1999; Toft, Espersen, Kalund, Sinkjaer, & Hornemann, 1989).

A common indirect measure of *in vivo* hamstring passive extensibility is the *passive knee extension test* (PKE). Clinically, this test utilises force to extend the knee passively until firm resistance is felt or the participant reaches their maximal stretch tolerance point in the hamstring region. The PKE enables measurements of the participant's force histories. Passive resistance force histories to determine cyclic loading values have been used in creep animal studies; (Hingorani et al., 2004; Provenzano et al., 2001) enabling the calculation of the required loading level to be used in the intervention procedure.

The PKE has been modified and is used in many studies utilising the KinCom® dynamometer to measure continuous and synchronous passive resistance, and EMG, while velocity and knee joint angle is controlled during the stretching of the hamstring muscles *in vivo* (Klinge et al., 1997; Magnusson et al., 2000a; Magnusson, Aagaard, Simonsen, & Bojsen-Moller, 2000b; Magnusson et al., 1996b; Magnusson, Simonsen, Aagaard, Gleim et al., 1995; Magnusson, Simonsen, Aagaard, & Kjaer, 1996c; Magnusson et al., 1996a; Magnusson et al., 1996d; Reid & McNair, 2004). During the stretching procedure, the knee is passively extended to the point of maximal stretch tolerance, and the resistance offered by the hamstrings is measured as passive force (N) by the dynamometer's load cell. Passive torque (N·m) about the knee joint is calculated by multiplying the resistance force by the moment arm distance. During the dynamic phase (on application of stretching) the plotted data forms a torque-angle curve. It is from this torque-angle curve passive stiffness is calculated. The main findings of

studies that have used the PKE with the KinCom® dynamometer to stretch the hamstring muscle group to a set range of motion were: (a) static stretches (range 45-90 seconds) resulted in increased knee range of motion (range 5-10 degrees), increased stiffness (26%) and increased passive torque (range 56-57%), while the hold phase resulted in reduced stress-relaxation (range 16-35%), (b) repeated static stretching (range 5-10 repeats) resulted in a decline in stiffness, but return to baseline within one hour, (c) cyclic stretches result in increased range of motion (range 5-10 degrees), increased stiffness (~15%) and passive torque (34%), (d) EMG activity for all tests (static, repeated, and cyclic) remained less than 1% of MVC.

2.5.2 Reliability of the Passive Knee Extension Test and Dynamometry

The reliability of the PKE has been shown to be high in an early study by Gajdosik (1991), who used the PKE in a side lying position. The study used an overhead camera to record the maximal passive knee angle and a hand held dynamometer to record the maximal resistance to passive stretch during the test. Gajdosik's results for test–retest reliability for the maximum passive knee angle of 24 healthy participants was high with an intraclass correlation coefficient (ICC) of .90, and r = .91.

Magnusson, Simonsen, Aagaard, Moritz, and Kjaer (1995) performed a test-retest reliability study of the KinCom® dynamometer and used the PKE on ten male participants to assess the measurement of the torque at the final stretched position. The participants were positioned in the KinCom® with their trunk upright; the thigh supported and elevated to approximately 30 degrees to ensure participants could not attain full knee extension. This thigh position increased tension of the muscle-tendon units during knee extension and lessened the involvement of the capsular structures at

the knee joint. The lever arm of the KinCom®, was attached to the ankle of the participant and passively extended their knee from 80 degrees of knee flexion until a tolerable stretch was felt in the hamstring muscles. This position was held for ninety seconds, and then the limb returned to the start position. This procedure was repeated five times. Once the final tolerable stretch position had been determined, the same procedure was repeated 10 minutes later. The amount of resistance to the PKE as measured by the load cell of the KinCom® was compared, which Magnusson et al. reported the correlation coefficient varied between 0.94 and 0.99 with coefficient of variations from 6.2-9.1%. Their results showed a strong relationship and a lack of difference, which the authors considered were an acceptable levels of reproducibility. More recently, Reid and McNair (2004) used the PKE on the KinCom® dynamometer and examined force and angle at the final stretched position using similar methodology as Magnusson et al. Their results also showed high reliability could be achieved with a high ICC of 0.97, with a lower confidence interval of 0.93. Additionally, performance characteristics of the KinCom® have been performed on the reliability of force, angle, and velocity over two different days, resulting in ICC for all tests the coefficient of determination was greater than .99 (Mayhew, Rothstein, Finucane, & Lamb, 1994).

2.6 Chapter Summary and Conclusion

This review of the literature has attempted to gain a greater understanding of the effect of sustained and cyclic loading on the extensibility of soft tissue structures and its subsequent responses have highlighted the following key issues.

The muscle-tendon unit's connective tissue has been identified as the primary structure that determines its mechanical properties during passive lengthenings. The connective tissue's hierarchical structure and extensive network of interconnections forms the series elastic and parallel elastic components of the muscle and tendon and it is the parallel elastic component that is believed to be responsible for passive viscoelastic behaviour.

Newly discovered protein filaments titin and desmin have been identified to contribute to the muscle-tendon unit stiffness and elastic recovery at low-load though the theory of thixotropic behaviour cannot be ruled out.

Tendon has been shown to be stiffer than the muscle component of the muscle-tendon unit and behave viscoelastically with an ability to store potential energy when lengthened under load and recoil using the energy during recovery.

Ultrasonography has recently revealed that at low-loads both the muscle and tendon are compliant and that their lengthenings are not homogeneous and furthermore, stiffness has been shown to vary within the tissue structures.

Additionally the underlying muscle architecture and joint position influence the muscle-tendon unit's mechanical behaviour. Scanning electron micrographs have revealed that the connective tissue has a crimped appearance that has mechanical implications on creep behaviour at low-load levels and with increases in loading and lengthening. Furthermore, fiber recruitment theory appears to hold true, scanning electron microscopy has shown collagen fibers become progressively straighter in the toe region

and some interfibrillar sliding and helix space reduction does occur. It is interesting that at loading levels within normal physiological levels, micro-damage of collagen fibers occurs confirming fatigue studies that a continuous cycle of non-symptomatic damage and repair process must occur within the connective tissue structures to maintain homeostasis.

Gross anatomy has shown that the hamstrings have extensive connective tissue connections throughout their entire lengthen, and reflect their functional role to transmit force, absorb impact shock, and recoil using the energy to recovery during functional daily tasks such as walking or running.

The daily activities of life, especially locomotion subject our muscle-tendon units to repetitive or cyclic loadings that lead to a creep response. Review of literature of animal and human tissues *in vitro* and *in vivo* studies under conditions of sustained or cyclic loading has revealed that all soft tissues exhibit creep phenomena and that many consistent trends occur in their viscoelastic responses. The main trends are: a) as the magnitude of load was increased, simultaneously the length of the tissue increased and the rate of creep decreased, b) when the rate of loading was increased, greater resistance to further lengthening occurred, and c) cyclic loading of tendon consistently showed a steady and progressive reduction in stiffness with each subsequent repetition or cycle. In contrast, ligament tissue showed the opposite effect, that is, a steady and progressive increase in stiffness.

Only two *in vivo* controlled studies are known to have investigated sustained loading phenomena in humans. Their findings differed, Bohannon (1984) showed no significant

change; though this finding was most likely due to methodological error, while the study by Madding et al. (1987) showed a significant creep response with an increase in passive extensibility and a subsequent reduction in resistance.

In conclusion, to date there is a paucity of controlled *in vivo* studies of human muscle-tendon units and these show differing results. Therefore, further investigation is required to study the viscoelastic characteristic of creep phenomenon in human hamstring muscle-tendon units under the condition of passive cyclic loading.

CHAPTER 3 - METHODS

3.1 Introduction

This chapter describes the method and procedures of the current study, which investigated cyclic loading on the passive extensibility of hamstring muscle-tendon units of healthy young adult students, *in vivo*. There are five sections to this chapter. The second concerns the design and participants. The third section describes technical details of the instrumentation used. Section four discusses the procedure, while the final section describes the data and statistical analysis used.

3.2 Study Design & Participants

A randomised repeated measures design utilising a control and experimental group was undertaken for this study.

3.2.1 Power & Effect Size

Based on an effect size of 0.5, a power of 0.8, and with an alpha of 0.05 a pilot study (n = 10) was conducted. The dependent variable means: maximal passive resistive torque, maximal passive knee angle, passive stiffness for 100% of the torque-angle curve, and passive stiffness for the final 10% of the torque-angle curve were assessed. The sample size based on these criteria required 30 participants to take part in the current study.

3.2.2 Participants

In accordance with the requirements of the Auckland University of Technology (AUT) Ethics Committee (Appendix A), 36 university students were invited to participate on a voluntary basis by means of posted advertisements (Appendix B). Written and verbal explanations of all experimental procedures were provided (Appendix C).

3.2.3 Inclusion Criteria

In respect to inclusion criteria, participants had to be aged 20–29 years, be fit and healthy.

3.2.4 Exclusion criteria

Exclusion criteria included history of orthopaedic or neurological disorders, lower back, and any injury to the lower limb that may have precluded them from performing a hamstring stretch, and any cardiovascular condition that may have stopped them from performing a maximal voluntary contraction (MVC).

Thirty-four participants met the eligibility criteria and completed a *Physical Activity Readiness Questionnaire* (PARQ) (Appendix D) and a *Screening Questionnaire* (Appendix E) followed by signing a *Participant Informed Consent* form (Appendix F) prior to commencement of testing.

3.3 Instrumentation

A KinCom® 500H dynamometer (Kinetic Communicator, Software Version 5.30 Chattex Corp., Chattanooga Group, Inc., TN, USA) was used for all testing procedures. Force and angle data were recorded simultaneously from the KinCom® at a sampling frequency of 500 Hz, and relayed via an analogue/digital board (InstruNet, Model iNet-100B, InstruNet, USA) to a computerised data acquisition system (SuperScope II, Version 3.0, GW Instruments, MA, USA) for storage and subsequent processing. Before each testing session the KinCom® load cell was calibrated using a 98.1 N load and torque readings on the computerised data acquisition system (SuperScope II) were checked with the calculated value to ensure accuracy to within one Newton. The experimental set-up and computerised data collection system is shown in Figure 3.1.

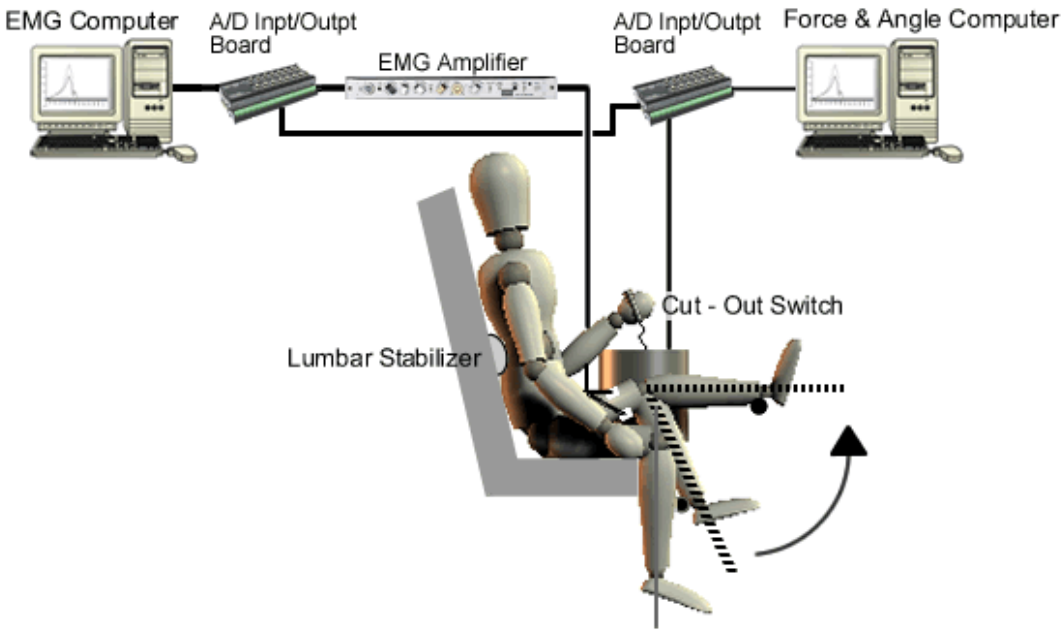

Figure 3.1: Schematic diagram of experimental set-up.

Electromyographic activity (EMG) was recorded using bipolar surface electrodes adhered by self-adhesive disposable pads (Norotrode 30™, Myotronics-Normed, Inc.,

WA, USA). A reference (ground) surface electrode (3M Health Care Products, London, Ontario, Canada) was placed over the bony prominence of the tibia shaft. Data were sampled at 1000Hz, amplified 1000 times by a Grass P5 Series A.C Amplifier (Grass-Telefactor, Model No. P511, Astro-Med, Inc., West Warwick, RI, USA) using a bandwidth of 10 Hz to 2 kHz. The common mode rejection ratio was 90 dB at 60 Hz and the input impedance was greater than 25 MΩ. EMG data signals were relayed to a computer based data acquisition and analysis system for subsequent processing (LabView, Version 6.0, USA).

3.4 Procedure

Each participant was tested in a single session in a temperature-controlled laboratory. Before commencement of testing, the participants performed no warm-up or stretching exercises. Participants sat briefly in the KinCom® to allow adjustment of the seat and lever arm to each individual's lower limb dimensions followed by skin preparation for surface electrode EMG application.

3.4.1 Passive Knee Extension Test

A passive knee extension test similar to that used by other studies was used (Klinge et al., 1997; Magnusson et al., 2000a; Magnusson et al., 2000b; Magnusson et al., 1996b; Magnusson, Simonsen, Aagaard, Moritz et al., 1995; Magnusson et al., 1996a; McHugh, Kremenic, Fox, & Gleim, 1998; Reid & McNair, 2004). Participants were seated in the KinCom® with the trunk 80 degrees to the horizontal seat. A firm lumbar roll was placed behind the lumbar spine (L3-L4 level) to maintain a lordosis and to

avoid posterior pelvic rotation during the stretching procedure. The left thigh of participants was placed on the KinCom® universal stabiliser pad elevating it to an approximate 45 degree angle to the horizontal. The height of the universal stabiliser pad was adjusted to ensure the participant's lower leg during the stretch procedure was unable to reach full knee extension (Magnusson, Simonsen, Aagaard, Moritz et al., 1995). The left thigh was secured with a Velcro® strap onto the universal stabiliser pad. Participants were secured with a Velcro® strap across the chest and a seat belt over the anterior pelvis. The right thigh was supported by a seatbelt looped around participant's lumbar-sacral area and the right knee at the level of the tibial plateau (refer to Figure 3.2). Additionally the right thigh was secured onto the KinCom® seat by another seatbelt and a platform placed under the right foot to maintain stability. The left knee joint's axis of rotation was aligned with the axis of the KinCom® lever arm. The lever arm length was adjusted to place its support attachment just proximal to the lateral malleolus of the left ankle. When participants were secure in position, the limb to be tested was moved to the start angle of 70 degrees of knee flexion below the horizontal. Prior to commencement, computerised EMG oscilloscope tracings were shown to the participants, as a form of feedback, teaching recognition of EMG activity and silence to help ensure muscle activation were minimal during the stretching procedure. Participants were given a hand-held *cut-out* switch and instructed to depress the switch to instantly stop the motion of the KinCom® at the point which they *perceived* maximal stretch tolerance. It is recognised however, by the current study and by Gajdosik et al. (2004) that this end point of stretch is based upon psychophysiology phenomena and may not necessarily represent the true biomechanical endpoint of the hamstring muscle group length. Participants were instructed to close their eyes, assume a relaxed posture and completely relax their muscles about the left knee joint, that is, neither assist or

resist the test and in addition to concentrate on the sensation of stretch at the back of their knee and posterior thigh. On commencement of the test the lower leg of participants were passively extended at a rate of 10 degrees per second by the Kin-Com® dynamometer until perceived maximal tolerance to stretch was reached, at which point, as per verbal instructions, participants activated the hand-held cut-out switch instantaneously arresting any further movement of the KinCom® lever arm and consequentially allowing it to return slowly back to the start position (refer to Figure 3.3). A total of four trials were undertaken. The first trial was used to familiarise participants with the procedure and was discarded. A mean value was calculated from the remaining three trials and considered as the baseline measurement for maximal passive resistive torque and knee angle. The reliability of this procedure has been assessed by Reid and McNair (2004) who showed that the variables force and angle had high intraclass correlation coefficients: .97, with a lower confidence interval of .93.

Electrode sites were prepared using a standardised protocol as published by Surface-EMG for the Non Invasive Assessment of Muscles (SENIAM); i.e. by shaving, rubbing the area with a cleaning solution (Omni Prep® paste, D.O. Weaver & Co., CO, USA), followed by further cleaning with alcohol. Electrodes were placed with respect to the quadriceps vastus lateralis muscle, over the middle third of the muscle belly (refer to Figure 3.4).

With respect to hamstring biceps femoris muscle, the electrodes were placed posteriorly midway between the ischial tuberosity and the lateral epicondyle of the tibia (refer to Figure 3.5). All electrodes were sited and orientated approximately parallel to the direction of the fibres of the muscles of interest as recommended by SENIAM.

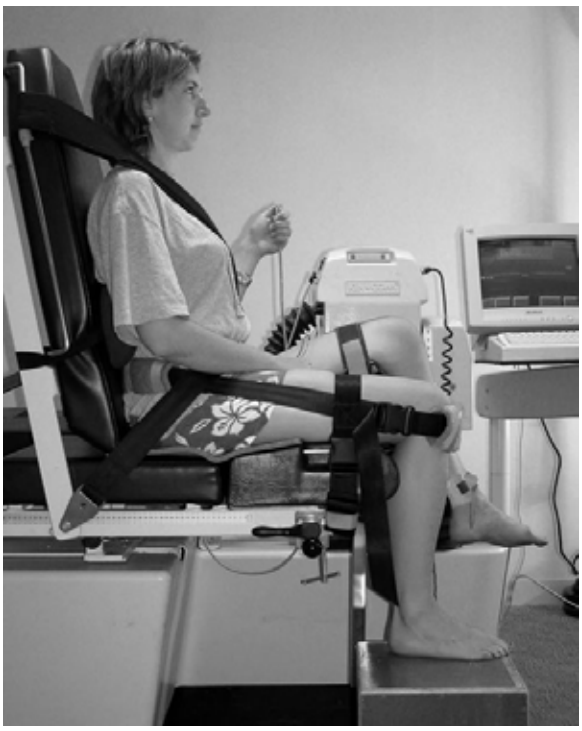

Figure 3.2: The start position for the participant seated in the KinCom® dynamometer for the passive knee extension test.

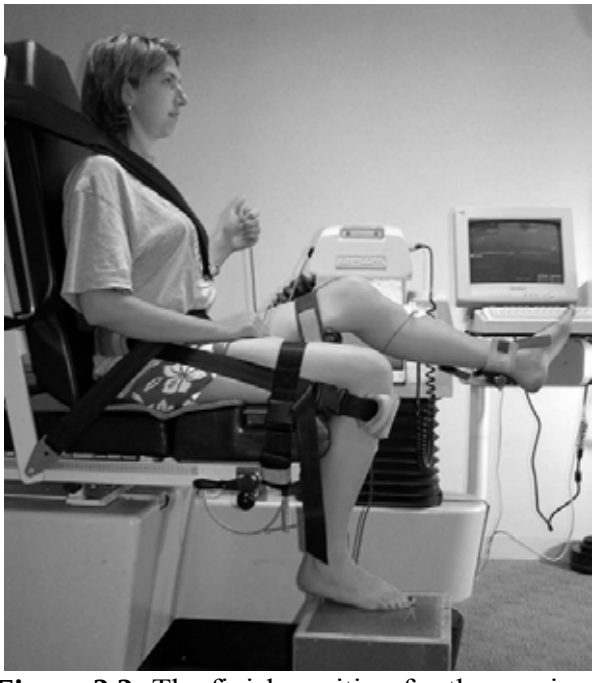

Figure 3.3: The finish position for the passive knee extension test, where the participant voluntarily depressed the 'cut-out' switch, at the point of 'perceived' maximal stretch tolerance.

Figure 3.4: Typical placement (marked by the X) of the vastus lateralis muscle electrode on a participant. The round markers are reference landmarks.

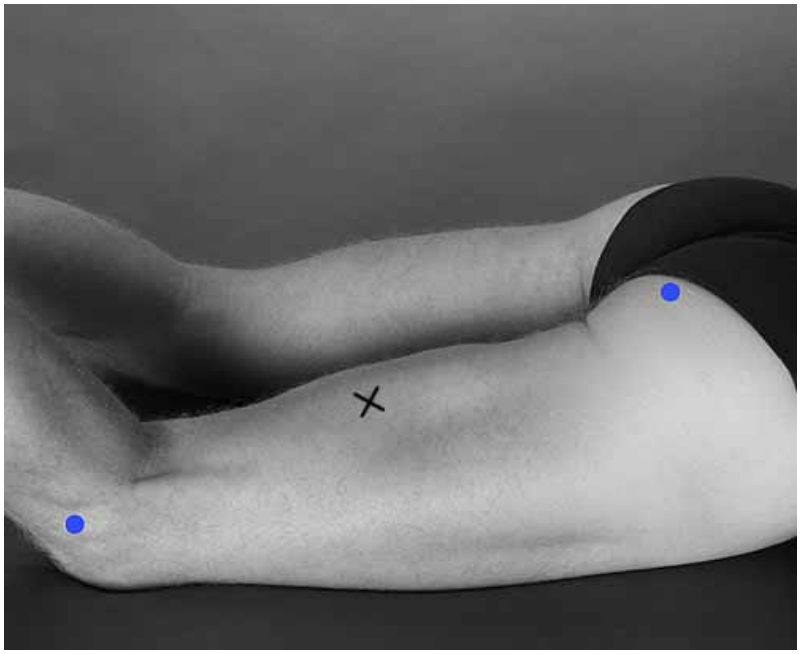

Figure 3.5: Typical placement (marked by the X) of the biceps femoris muscle electrode. The round markers are reference landmarks.

All EMG data were normalised to that collected during an isometric maximum voluntary contraction (MVC) performed at the end of all testing procedures. Root mean square (RMS) values for two-second epochs were calculated for each muscle during these maximal muscle tests (De Luca, 1997). Trials in which the participant's normalised EMG was less than two percent of MVC during the passive knee extension test were considered passive, that is, during the stretching of the hamstring muscle-tendon units the response measured was a passive property, rather than a change in the contractile elements. The data of participants with normalised EMG greater than two percent of their MVC were excluded from data analysis. Normalisation to an MVC is a widely used procedure and remains the method chosen most often by researchers who are seeking information concerning muscle activation levels (Potvin, Norman, & McGill, 1996; Sihvonen, Partanen, Hanninen, & Soimakallio, 1991). EMG data were collected continuously and simultaneously during the passive knee extension procedure performed by the KinCom® dynamometer. EMG data were collected on one computer, while simultaneously angle data were collected on another. Force data were sampled by both machines allowing for synchronisation of the EMG data with the angle data during subsequent analysis.

Participants performed resisted knee extension and knee flexion for MVC with the left lower limb strapped in the KinCom® and with the knee in 40 degrees of flexion. Manual resistance was applied and combined with verbal encouragement from the investigator while participants were asked to perform two maximal efforts for a five to seven seconds period.

The mass of the participant's limb was weighed for gravity correction factoring (determined by cosine of the angle) at 60 degrees to the horizontal to minimise passive tension of the posterior structures, while the participant sat relaxed in the KinCom® dynamometer (Keating & Matyas, 1996; Magnusson et al., 2000b).

The above procedures were undertaken prior to and immediately after the intervention.

3.4.2 Intervention

Participants were randomly assigned by a computerised randomisation programme (Haahr, 2002) to either a control or an intervention group. All data collection was performed at the Physical Rehabilitation Research Centre of the Auckland University of Technology by the investigator.

3.4.2.1 Cyclic Loading Group

After a 10 minute rest interval, the intervention group underwent passive stretching to a predetermined load level, that is, 85% of the mean value of their three maximal passive resistive torque values recorded during the passive knee extension test. Forty-five complete cycles of continuous knee extension and flexion were undertaken. During the cyclic loading procedure, participants remained seated in the KinCom® and continued to assume a relaxed posture. As previously described the same start angle and angular velocity were used. During knee extension, when the KinCom® reached the predetermined 85% force level, the direction of the motion was reversed. At the end of the intervention, participants repeated the procedure of passive knee extension test as previously described. However, this time only two trials were performed. The first trial

again was used to familiarise the participant with the procedure and discarded, while the second trial was used for the post-test measurement and data analyses.

3.4.2.2 Control Group

Participants in the control group remained seated in the KinCom® and continued to assume a relaxed posture for 25 minutes. During this period the participants were instructed to avoid any movement to ensure that the position of the pelvis and body position remained unchanged. At the end of the rest period, the control participants repeated the passive knee extension test as previously described. The first trial, as previously described, was used to familiarise participants with the procedure and discarded. The data of the second trial were used as the post-test measurement for data analyses.

3.5 Data and Statistical Analysis

Statistical analyses were undertaken using the Statistical Package for Social Sciences (SPSS) Version 11.0 (SPSS Inc., Illinois, USA).

Control and intervention groups were analysed for baseline comparisons using an Independent t-test for measurements of height, mass and body mass index.

In respect to comparisons across groups and the pre and post measurements, the dependant variables of interest were:

1. Mean maximal passive resistive torque
2. Mean maximal passive knee angle

3. Passive resistance torque at baseline maximal passive knee angle
4. Mean passive stiffness for full (100%) of the torque-angle curve
5. Mean passive stiffness for the final 10% of the torque-angle curve

With regard to these data, descriptive statistics were analysed to determine the appropriateness of utilising parametric analysis. More specifically, data were checked for univariate outliers Z-scores using a Grubbs test. Measures of skewness and kurtosis were also checked for normality. Thereafter, two-factor analysis of variance (ANOVA) with repeated measures was used. The two-factors were time (pre and post intervention), which was the repeated measure, and group (control or intervention). The Huynh-Fedlt epsilon was used to adjust for any departures from sphericity. T-tests were utilised to examine pre-post differences and a Bonferroni method was used to maintain the alpha level.

In respect to analysing the creep response during the intervention cyclic loading after checking assumptions associated with parametric analysis, paired *t*-tests were used for examining differences between cycle number one and cycle number forty-five. The variables of interest were:

1. Mean maximal passive knee angle
2. Mean passive stiffness for full (100%) of the torque-angle curve
3. Mean passive stiffness for the final 10% of the torque-angle curve

For all statistical analyses, significant differences were accepted at the alpha level of $p < 0.05$.

CHAPTER 4 – RESULTS

4.1 Introduction

This chapter is divided into three main sections. The first section provides the description of the participants. The second section presents the results of measures of maximal passive resistance torque, passive resistance torque at maximal angle recorded at baseline, maximal passive knee angle, and passive elastic stiffness attained during the passive knee extension test. The final section covers the analysis of dependent variables during the cyclic loading procedure.

4.2 Participants

A participant recruitment, data screening, and retention flowchart details the process undertaken during the current study as illustrated in Figure 4.1.

Thirty-four participants undertook the study. Retention details, one participant when contacted, withdrew from participation due to being "too busy, had exams" and a second participant on presenting themselves at the Physical Rehabilitation Research Centre did not met the inclusion criteria due to recent low back pain. Furthermore, analysis of EMG data revealed that three participants had excessive EMG activity throughout the testing procedures, that is, greater than two percent normalised EMG of their MVC and were excluded from analysis. Therefore, the final number of participants whose data were analysed was 29, of which 19 were females and 10 were males. Fourteen were in the cyclic loading group of which ten were female and four

were male. Fifteen were in the control group of which nine were female and six were male.

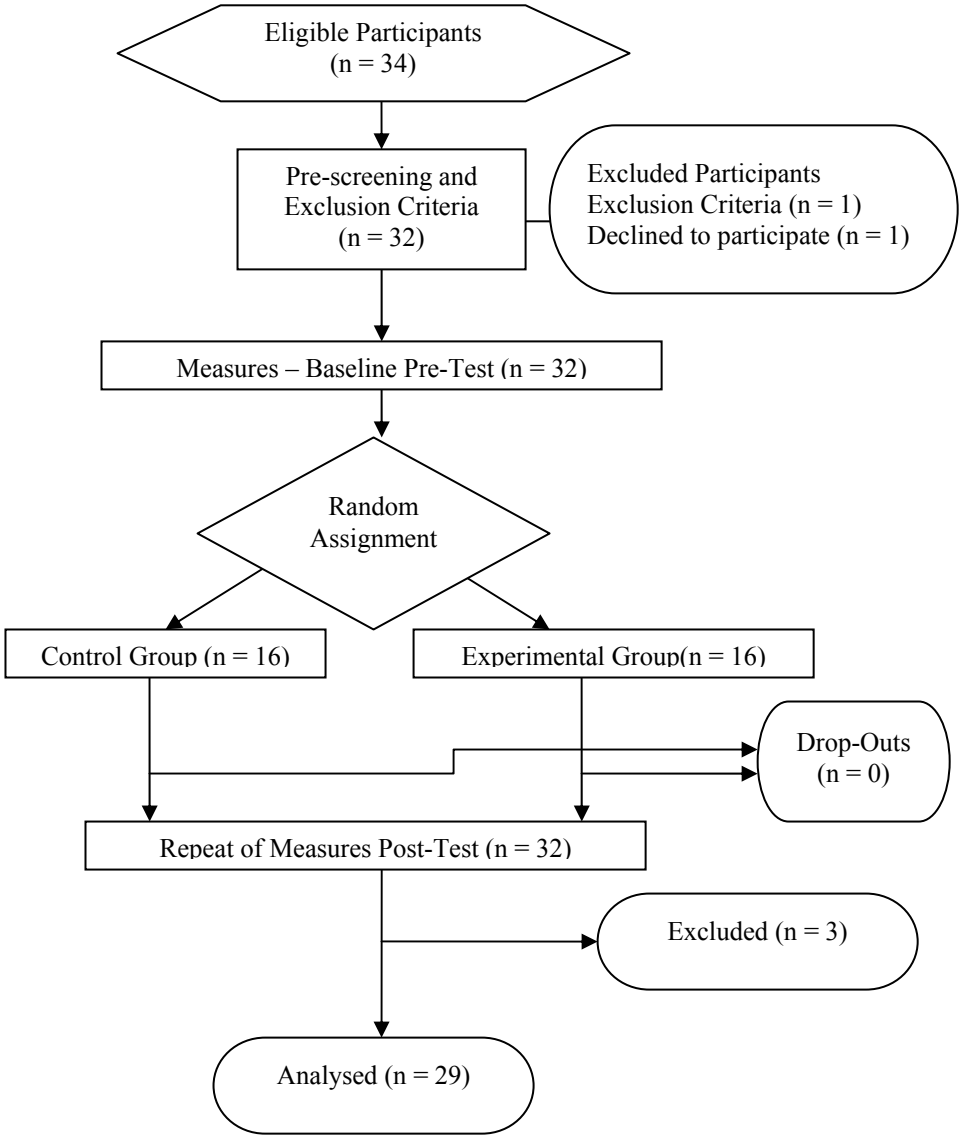

Figure 4.1: Recruitment, Data Screening, and Retention Flowchart of Participants

Descriptive statistics for the participants' age, height, mass and calculated body mass index (BMI) are presented in Table 4.1. For these demographic measurements, independent t-tests examining for differences between the cyclic loading and control groups showed no significant differences ($p > 0.05$).

Table 4.1: Descriptive statistics for age, height, mass, and calculated body mass index (BMI*) for groups control (n = 14) and cyclic loading (n = 15).

	Mean	± SD	Range
Age (yrs)			
Control	22.0	2.8	20.0 – 29.0
Cyclic Loading	22.1	2.7	20.0 – 28.0
Height (cm)			
Control	170.2	6.0	160.0 – 179.0
Cyclic Loading	170.4	8.5	158.5 – 186.0
Body Mass (kg)			
Control	71.6	10.3	56.0 – 94.0
Cyclic Loading	66.7	9.8	50.0 – 85.0
BMI*			
Control	24.7	3.1	20.6 – 29.7
Cyclic Loading	22.9	2.0	19.9 – 26.5

*BMI = mass (kg)/height $(m)^2$

4.3 Passive Knee Extension Test

4.3 1 Maximal Passive Resistance Torque

In respect to maximal passive resistance torque (MPRT), there was a significant main effect for time ($p < 0.05$), no effect for group ($p > 0.05$) and no interaction effect ($p > 0.05$). However, there was a notable trend for an interaction effect (p = 0.07). Figure 4.2 displays the mean (±SD) MPRT values for the cyclic loading and control groups, respectively. Participants in the cyclic loading group produced a mean of 25.7 ±10.3 N·m of torque at baseline and this increased to 31.6 ±13.1 N·m following the cyclic loading intervention (refer to Appendix G, Figure 1 for a typical trace). This corresponds to a 23% increase in MPRT. The control group produced a mean of 25.7 ±13.4 N·m at baseline and 28.1 ±15 N·m after the intervention. This corresponded to a 9% increase in MPRT. Paired t-test examining the

pre to post difference of the cyclic loading and control groups showed a significant effect ($p < 0.05$) for the cyclic loading group but not for the control group.

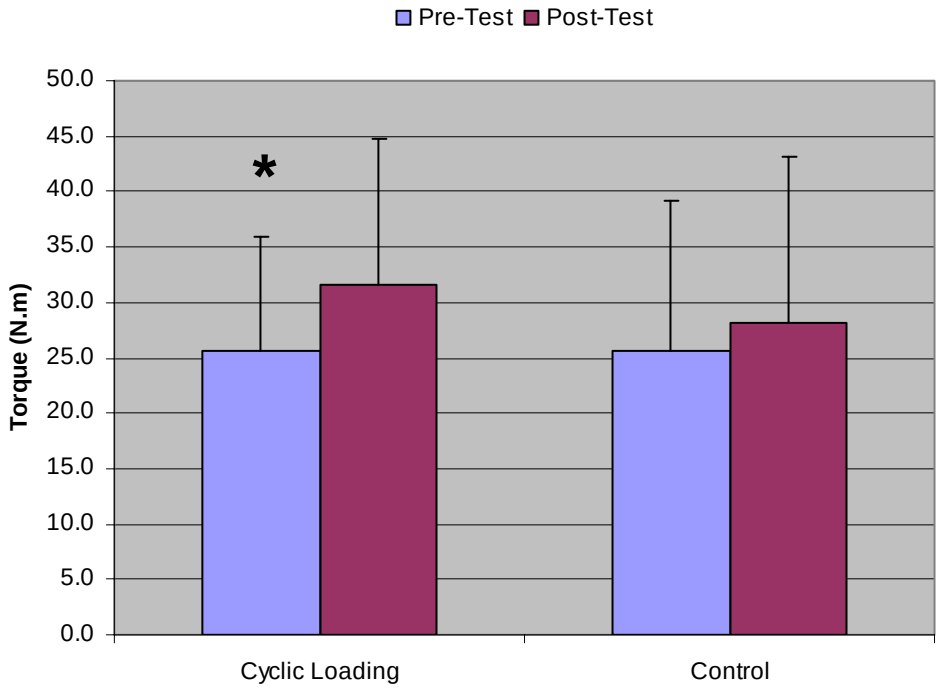

Figure 4.2: Mean (±SD) of MPRT Pre/Post-Test for Cyclic Loading and Control Groups. * denotes significant change ($p < 0.05$).

4.3 2 Passive Resistance Torque at Maximal Angle Recorded at Baseline

In order to indirectly investigate if participants in the current study had altered the physical properties of the muscle as a consequence of the cyclic loading intervention, further analysis of the passive resistance torque (PRT) data was performed. The torque values recorded at the maximal passive knee angle at baseline (MPKA $_{baseline}$) were compared to the torque measurements after the intervention for the same angle. Figure 4.3 displays the mean (±SD) data values for torque at the same angle for participants in the cyclic loading group and the control group, respectively. There was a significant main effect for time and group ($p < 0.05$), and a significant interaction effect ($p < 0.05$). Participants in the cyclic loading group produced a mean of 25.7 ±10.3 N·m of torque at baseline and this decreased to 23.1 ±9.3 N·m following the cyclic loading intervention.

This corresponded to an 11.3% decrease. The control group produced a mean of 25.7 ±13.4 N·m at baseline and 25.6 ±13.0 N·m after the intervention, a change that was not significant.

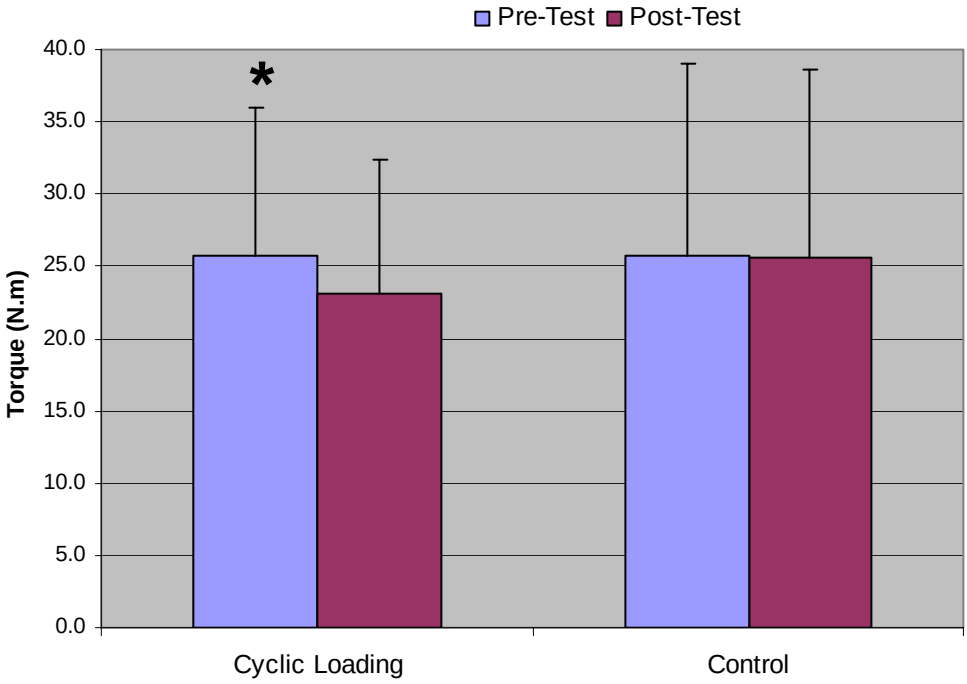

Figure 4.3: Mean (±SD) of PRT at $MPKA_{baseline}$ Pre/Post-Test for Cyclic Loading and Control Groups. ***** denotes significant change ($p < 0.05$).

4.3.3 Maximal Passive Knee Angle

With respect to maximal passive knee angle (MPKA), there was a significant main effect for time and group ($p < 0.05$), and a significant intervention effect ($p < 0.05$). Figure 4.4 displays the mean (±SD) MPKA values for the cyclic loading and control groups, respectively. Participants in the cyclic loading group produced a mean of 96.4 ±9.7 degrees maximal passive knee angle at baseline, and this increased to 102.5 ±10.1 degrees following cyclic loading (refer to Appendix G, Figure 2 for a typical trace). This corresponds to a 6.3% increase in MPKA. The control group

produced a mean of 94.5 ±15.1 degrees at baseline and 96.7 ± 15.2 degrees post-test measurement. This corresponded to a 2.3% increase in MPKA. This change was not significant.

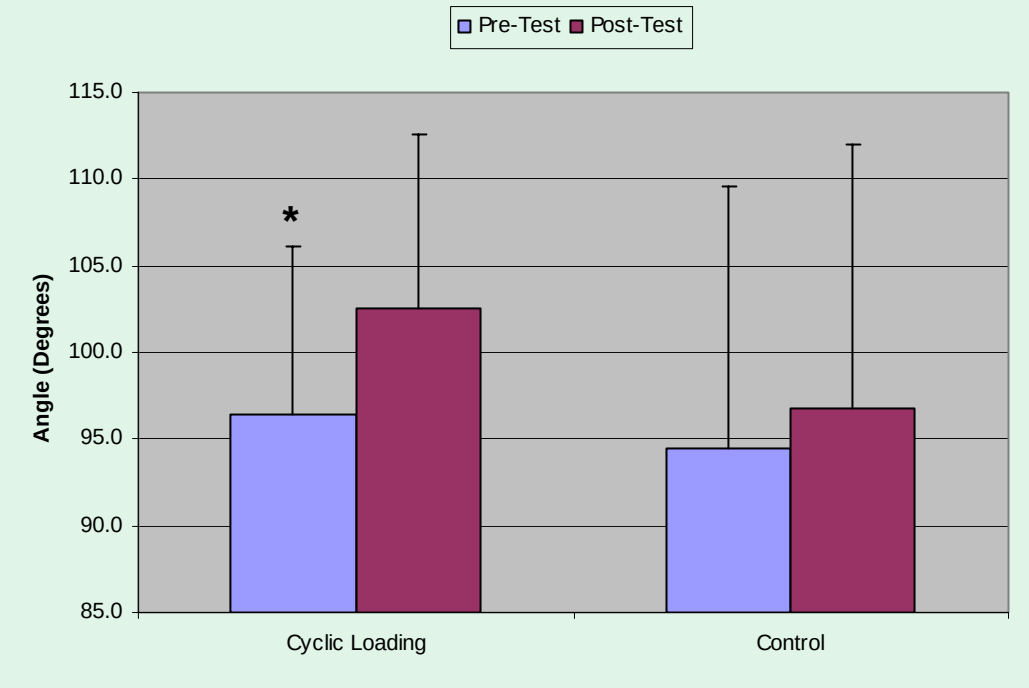

Figure 4.4: Mean (±SD) of MPKA Pre/Post-Test for Cyclic Loading and Control Groups. ***** denotes significant change ($p < 0.05$).

4.3.4 Passive Stiffness over 100 percent of the torque-angle curve

Passive stiffness was calculated as the slope of the torque-angle curve (Δ torque/Δ angle) from initial passive resistance to the maximal passive resistance torque point, that is, 100% of the torque-angle curve ($PS_{100\%}$). There was no significant main effect for time or group ($p > 0.05$) and no interaction effect ($p > 0.05$). Figure 4.5 displays the mean (±SD) data values for the cyclic loading and control groups, respectively. Participants in the cyclic loading group produced a mean of 0.3 ±0.1 Newton-meter per degree at baseline and this increased to 0.4 ±0.2 Newton-meter per degree following the intervention. The control group produced a mean of

0.3 ±0.1 Newton-meter per degree at baseline and 0.3 ±0.1 Newton-meter per degree after the intervention.

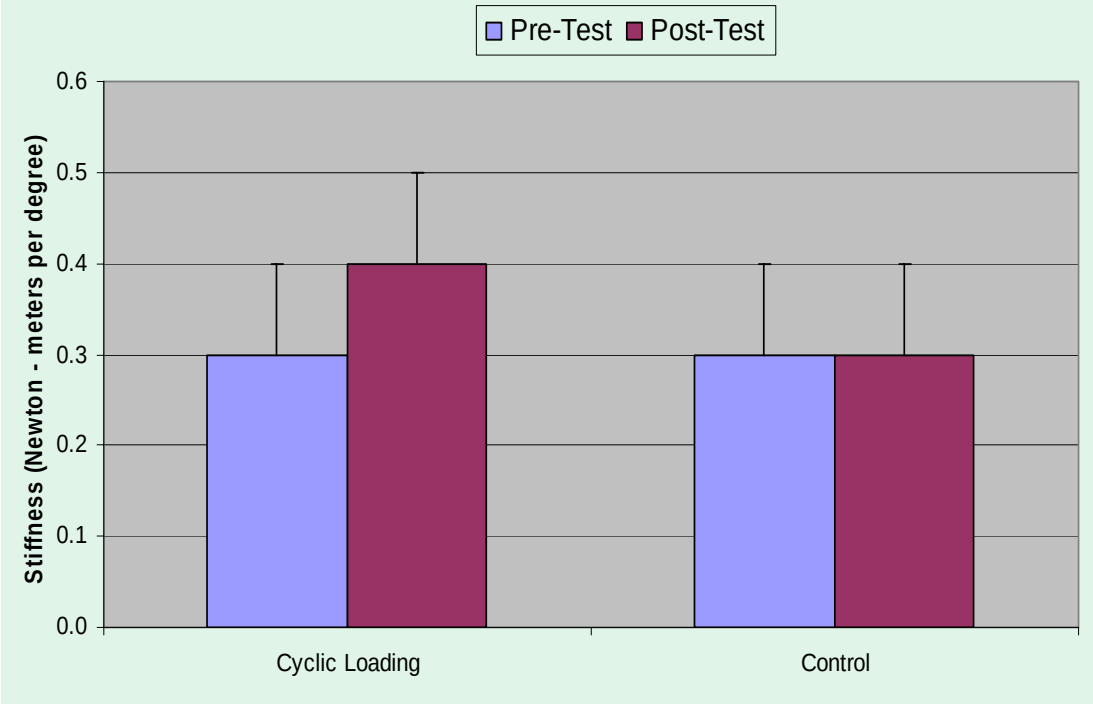

Figure 4.5: Mean (±SD) of $PS_{100\%}$ Pre/Post-Test for Cyclic Loading and Control Groups.

4.3.5 Passive Stiffness for the Final 10 Percent of the Torque-Angle Curve

Passive stiffness was calculated as the slope of the torque-angle curve (Δ torque/Δ angle) over the final 10% of the torque-angle curve ($PS_{10\%}$). There was no significant main effect for time or group ($p > 0.05$) and no interaction effect ($p > 0.05$). However, there was a notable trend for an interaction effect ($p = 0.08$). Figure 4.6 displays the mean (±SD) of values for the cyclic loading and control groups, respectively. Participants in the cyclic loading group produced a mean of 1.0 ±0.1 Newton-meter per degree at baseline and this increased to 1.3 ±0.6 Newton-meter per degree following the intervention. This corresponds to a 30% increase in stiffness of $PS_{10\%}$. The control

group produced a mean of 1.0 ±0.4 Newton-meter per degree at baseline and 1.1 ±0.4 Newton-meter per degree after the intervention. This corresponded to a 10% change in $PS_{10\%}$. Paired t-test examining the pre to post difference of the cyclic loading and control groups showed a significant effect ($p < 0.05$) for the cyclic loading group but not for the control group.

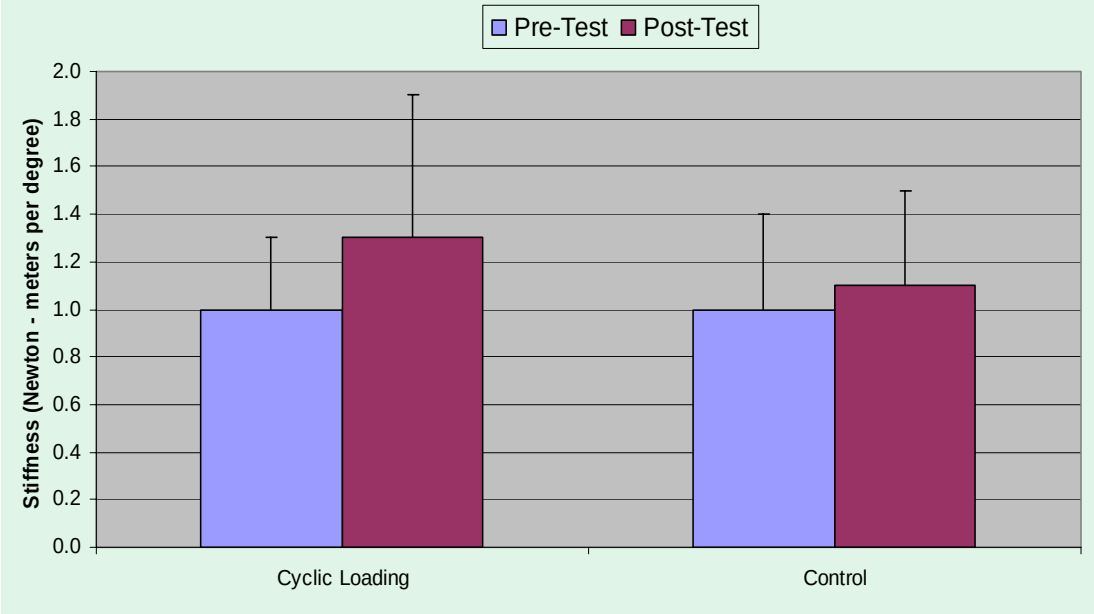

Figure 4.6: Mean (±SD) of $PS_{10\%}$ Pre/Post-Test for Cyclic Loading and Control Groups.

4.4 Cyclic Loading To A Predetermined Load

Analysis of the cyclic loading group's angle data was performed in order to investigate if cyclic loading (creep) had altered from cycle number one to cycle number 45.

4.4 1 Cyclic Loading Knee Joint Angle Change, Cycle No. 1 compared to No. 45

Measurements of maximal passive knee angle ($MPKA_{creep}$) were taken at cycle one and at cycle 45, Figure 4.7 displays the mean (±SD) $MPKA_{creep}$ values for the cyclic

loading intervention group. There was a significant effect for angle change ($p < 0.05$). Participant's cycle number one produced a mean MPKA $_{creep}$ of 89.3 ±9.7 degrees. This measurement increased to 93.9 ±9.2 degrees at cycle 45. This corresponded to an increase of 5.2% in MPKA $_{creep}$.

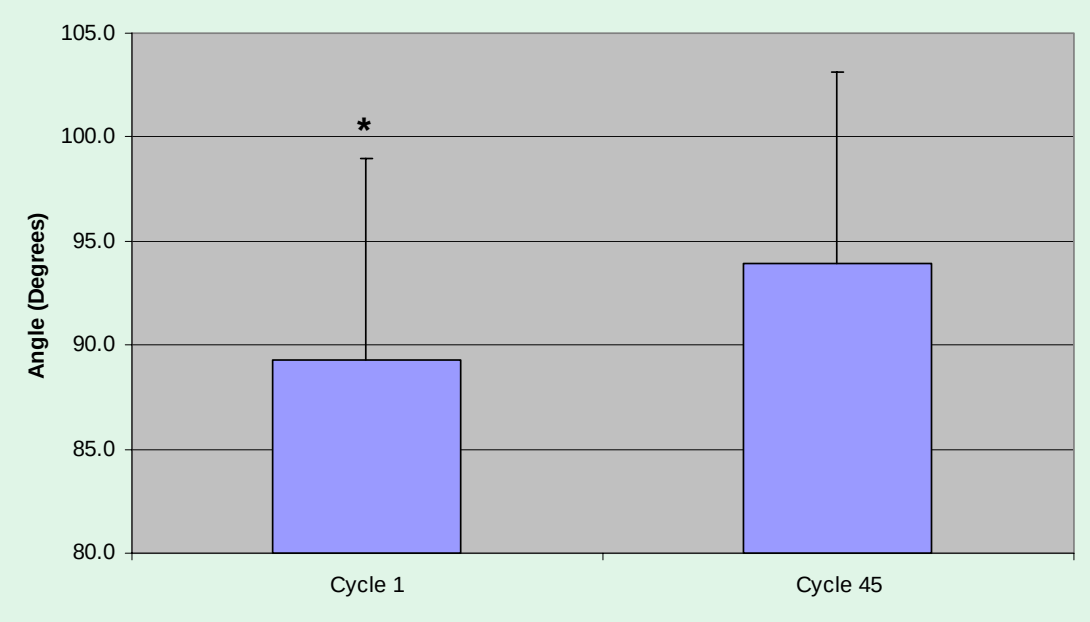

Figure 4.7: Mean (±SD) of cyclic loading MPKA $_{creep}$ Cycle No. 1 compared to Cycle No. 45. * denotes significant change ($p < 0.05$).

In addition, MPKA $_{creep}$ was plotted for each cycle across cycles one to forty-five. The resulting curve (reported as mean angle in degrees and plus or minus the standard error of the mean) demonstrates a typical creep curve form is illustrated in Figure 4.8.

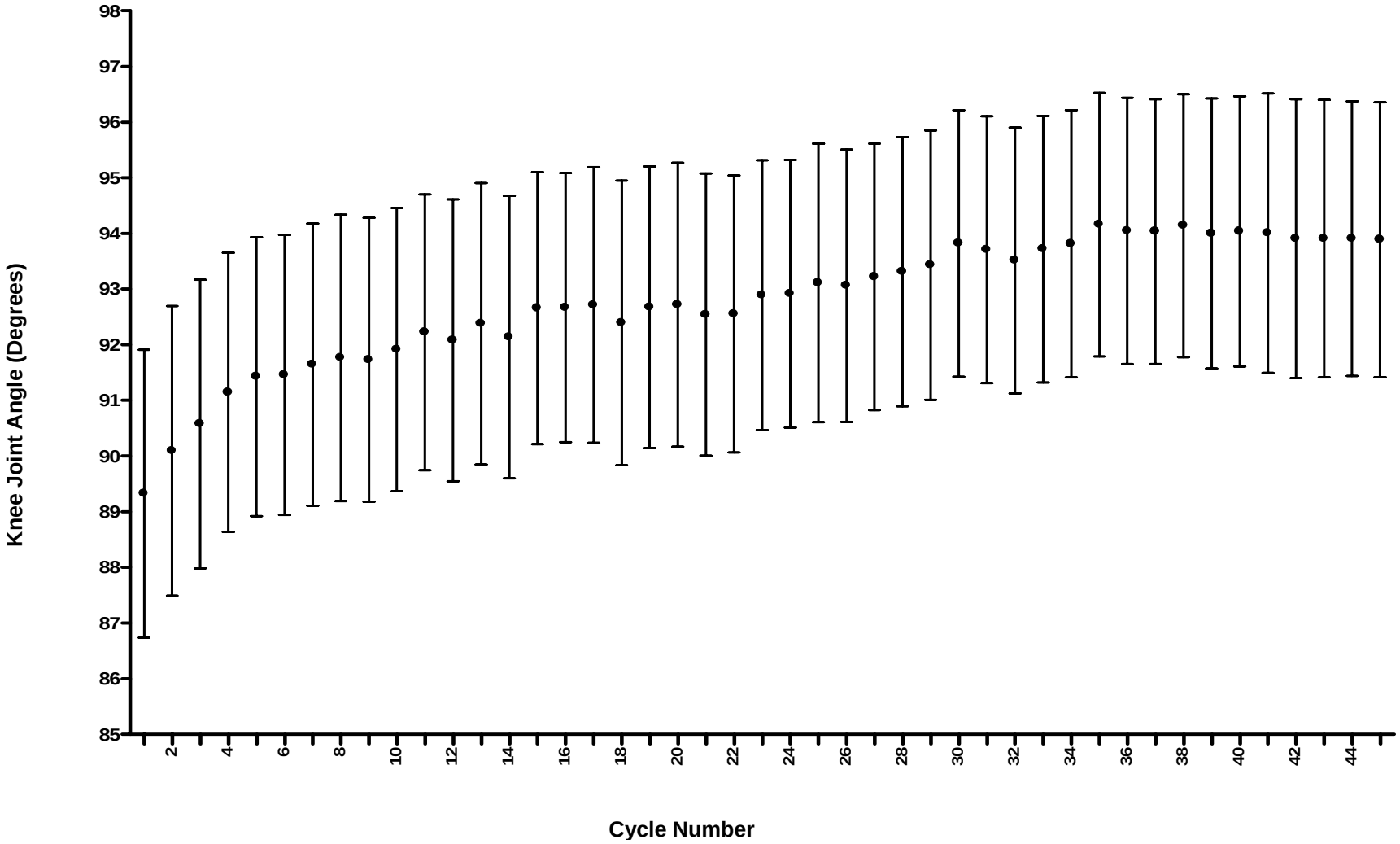

Figure 4.8: MPKA $_{creep}$ for each cycle, reported as mean angle ±SEM.

4.4.2 Cyclic Loading Passive Stiffness for 100 percent of the Torque-Angle Curve, Cycle No. 1 compared to Cycle No. 45

Passive stiffness was calculated as the slope of the torque-angle curve (Δ torque/Δ angle) over 100% of the torque-angle curve ($PS_{creep100\%}$) at cycle one and at cycle 45. Figure 4.9 displays the mean (±SD) of $PS_{creep100\%}$ values for the cyclic loading intervention. Participants in the cyclic loading group produced a mean $PS_{creep100\%}$ value at cycle one of 0.3 ±0.1 Newton-meter per degree. At cycle 45 the measurement had not changed (0.3 ± 0.1 Newton-meter per degree).

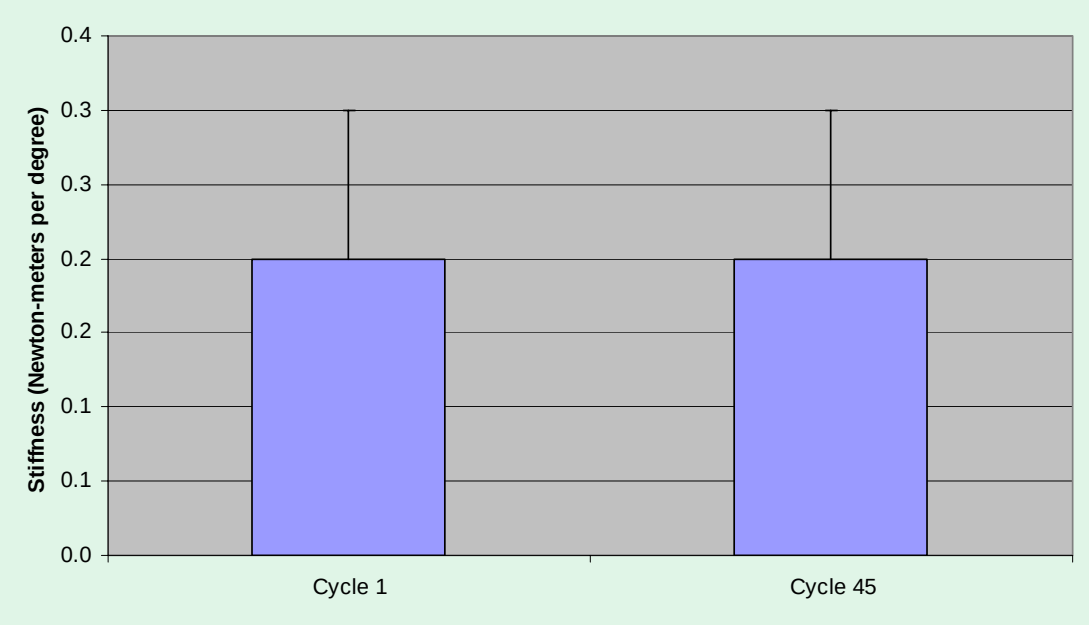

Figure 4.9: Mean (±SD) of cyclic loading $PS_{creep100\%}$ Cycle No. 1 compared to Cycle No. 45.

4.4.3 Passive Stiffness for the final 10 percent of the torque-angle curve, Cycle No. 1 compared to Cycle No. 45

Passive stiffness was calculated as the slope of the torque-angle curve (Δ torque/Δ angle) over the final 10% of the torque-angle curve ($PS_{creep10\%}$) at cycle one

and at cycle 45. Figure 4.10 displays the mean (±SD) of $PS_{creep10\%}$ values for the cyclic loading intervention. There was a significant interaction effect ($p < 0.05$). Participants at cycle one produced a mean $PS_{creep10\%}$ of 0.7 ± 0.3 Newton-meter per degree. This measurement increased to 0.9 ± 0.3 Newton-meter per degree at cycle 45. This corresponded to a 28.6% increase in $PS_{creep10\%}$.

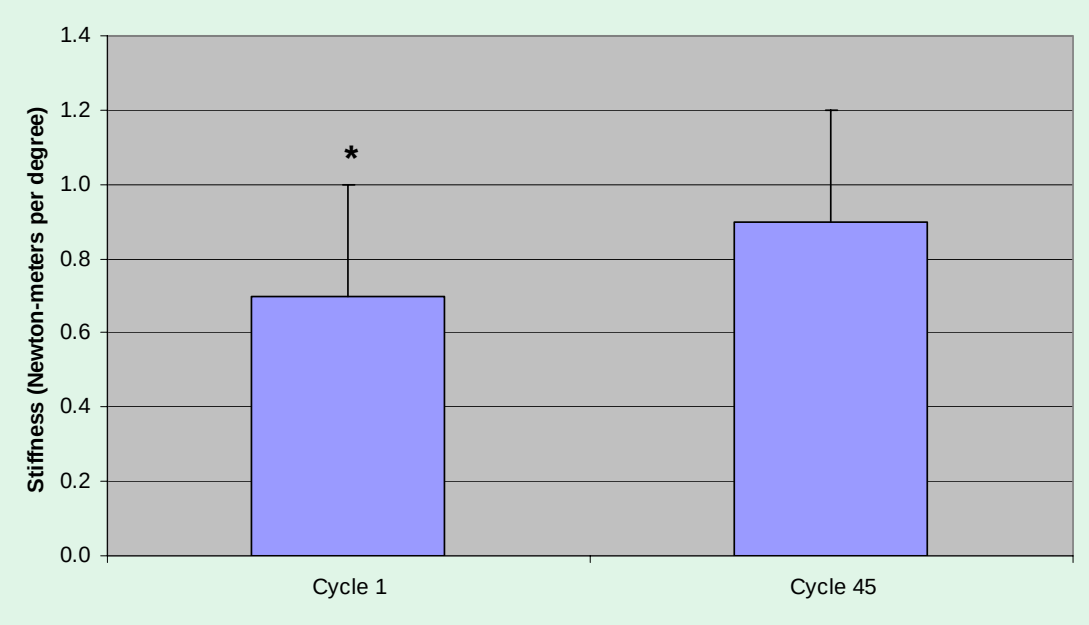

Figure 4.10: Mean (±SD) of cyclic loading stiffness $PS_{creep10\%}$ Cycle No. 1 compared to Cycle No. 45. ***** denotes significant change ($p < 0.05$).

CHAPTER 5 – DISCUSSION

5.1 Introduction

This chapter is divided up into four sections. The first section discusses the current study's results from the repeated passive knee extension test. The second section includes a discussion of the current study's results from the cyclic loading procedure. The third is concerned with the limitations of the study. Finally, the chapter ends with recommendations for future research and a conclusion statement.

5.2 Passive Knee Extension Test

The passive knee extension test was chosen for three reasons: (a) The test enabled measurements of the participant's force histories. Passive resistance force histories have been used to determine cyclic loading values in animal studies investigating creep (Hingorani et al., 2004; Provenzano et al., 2001), and enabled the calculation of the required loading level used in the intervention procedure. (b) The passive knee extension test helps determine an appreciation of the relationship between force or torque and angle through the range of motion and has been shown to be reliable (Magnusson et al., 1998a; Reid & McNair, 2004). (c) Has clinical relevance by simulating stretching techniques where individuals stretch to the point of *maximal stretch tolerance* rather than the more subjective standard of stretching to a sensation of 'tightness' or 'moderate' stretch (Magnusson, 1998b).

5.2.1 Maximal Passive Resistance Torque and Maximal Passive Knee Angle

The results of the current study demonstrated that a single session of cyclic loading to the hamstring muscles resulted in a 23% increase in maximal passive resistance torque with an associated increase in maximal passive knee angle of 6.3%. There are no known observations in the published existing literature that have used a cyclic loading (creep) intervention with the passive knee extension test to compare. Albeit different from cyclic loading, Magnusson et al. (1998a) did report increases in maximal passive resistance torque and maximal passive knee joint angle. Dynamic stretching of the hamstring muscles (to a predetermined joint angle), over 10 consecutive cycles was examined. On completion of the cycles the results of the passive knee extension test showed a significant increase ($p < 0.05$) in maximal passive resistance torque (41.8%) with an associated significant increase ($p < 0.05$) in knee joint range of motion (although no percentage change was reported). The magnitude of the increase in maximal passive resistance torque was, however, approximately two-fold as compared to the current study. These differences are most likely due to a number of factors. Firstly the mechanical properties of the tissue structures measured was different. Magnusson et al. investigated stress-relaxation where as the current study investigated creep. Secondly, tissue lengthening due to stress-relaxation in animal ligament models have been reported to progress at a faster rate than creep (Hingorani et al., 2004; Provenzano et al., 2001; Thornton et al., 1997). Thirdly, Magnusson et al. used a stretching rate twice that used in the current study. As muscle-tendon units are known to be rate-dependent and exhibit greater resistance due to their viscoelasticity (Taylor et al., 1990), this factor would also most likely account for their greater torque result.

The increases of maximal passive resistance torque and maximal passive knee angle have been reported *in vivo* studies that have investigated static stretching of the hamstring muscles over a number of weeks. Reid and McNair (2004) investigated a static stretching protocol over 30 seconds, repeated three times for five consecutive days over a period of six weeks, to the hamstring muscles of healthy individuals. Similar to the current study, the passive knee extension test was used on a KinCom® dynamometer applied at a rate of 5 degrees per second as method to assess their intervention. Their results showed a significant increase ($p < 0.05$) in maximal passive resistance torque (57.4%). Additionally in another study Magnusson et al. (1996a) investigated stretching of the hamstrings twice a day, holding each stretch for 45 seconds over three weeks. Results also showed a significant increase ($p < 0.05$) in maximal passive resistance torque (56.3%). The magnitude of change in both of the studies was greater than those found in the current study. Nevertheless, they provide evidence that similar viscoelastic responses occur in the muscle-tendon units of hamstrings during stretching regimes. The differences between studies (Reid & McNair; Magnusson et al.) and the current study, as previously discussed, are most likely due to the measurement of stress-relaxation versus creep, which are two separate mechanical properties of the tissue structures.

The 6.1 degree increase in maximal knee angle found in the current study was in keeping with other studies of sustained loading. Madding et al. (1987) reported a 6.5 degree increase in sustained loading, while Bohannon (1984) noted a 4.1 degree increase after a daily loading time of 15 seconds. In addition, studies of static stretching of the hamstring muscles have also reported similar increases in maximal knee angle to the current study. Starring et al. (1988) used the passive knee extension test to assess an

Autorange® device that stretched the hamstrings of healthy individuals by straight-leg raise (SLR) to maximal tolerance, for a 15 minute hold time, on five consecutive days. Their results demonstrated a 15.4 degrees increase. These increases were also reported by Reid and McNair (2004) and Magnusson et al. (1996a) when using intervention periods of six and three weeks respectively, increases of 10.1 and 10 degrees were found.

5.2.2 Passive Resistance Torque at Maximal Angle Recorded at Baseline

Passive resistance torque re-measured at the maximal knee angle baseline during the current study, showed a significant decrease in torque of 11.8%. No known *in vivo* studies using ultrasonography or *in vitro* studies have used this methodology. However, this finding is consistent with Madding et al. (1987) who showed a decrease in resistance force of 57.5%, although the magnitude of the decrease was approximately five times greater. These differences are most likely associated with different methodologies used to determine the setting of the baseline loading level. The current study's mean loading level (73.9 N) was determined using the passive knee extension test to stretch the hamstring muscles to maximal stretch tolerance and was applied by the KinCom® dynamometer at a rate of 10 degrees per second. In contrast, Madding et al. used an active method, where participants actively abducted their leg as far as they could under their own effort. A hand-held dynamometer was then used to measure the force reading at this baseline point (mean value was 12 N). The current study's load determination method involved placing the hamstring muscle-tendon units at an initially greater length due to the greater initial loading force. At this point further lengthening would most likely have been limited due the process of fiber recruitment (Thornton et al., 1997; Viidik, 1972). In contrast, Madding et al. protocol of a manually loaded

determination method would have set initial lengthening of the muscle-tendon units at a point where greater lengthening could occur, resulting in a greater magnitude of change, as reported. Furthermore, maximal load levels required to influence lengthening may be specific to different muscle groups, reflecting their passive connective tissue composite differences (Fung, 1984).

Additional support for the current study's findings, from an *in vivo* dynamic stretching study by McNair et al. (2000) who dynamic stretched the triceps surae muscles of young healthy participants on the KinCom® dynamometer, for one minute at a rate of 10 degrees per second. McNair et al. showed a 10.5% reduction in maximal passive resistance force when re-measured at the same angle, which was a similar value found by the current study.

5.2.3 Passive Stiffness for the Final 10 And 100 Percent of the Torque-Angle Curve

The current study found no change in stiffness occurred over 100% of the torque-angle curve demonstrating no change in resistance of the muscle-tendon units to cyclic loading occurred. In contrast, a number of cyclic loading animal *in vitro* tendon studies (Wang & Ker, 1995; Wren et al., 2003) and a human cadaver tendon study (Dee Zee et al., 2000), have all reported decreases in stiffness over either half or the whole of the load-lengthening curve. These studies investigated lengthening of isolated tendon, which is known not to be as compliant as muscle tissue (Kubo et al., 2005), and therefore may not be representative of whole muscle-tendon units *in vivo*.

Stiffness over the final 10% of the torque-angle curve showed a significant increase ($p < 0.05$) of 30%. While there are no known stiffness values reported for human *in*

vivo studies under cyclic loading conditions, the current findings are similar to the dynamic stretching work done by Magnusson et al. (1998a) who reported a significant increase ($p < 0.05$) in stiffness of approximately 22%. Static stretching studies have also reported similar stiffness changes. For instance, Reid and McNair, (2004) reported a 26.3% increase in stiffness of the hamstring muscles over the final 10% of maximal passive knee angle.

Magnusson et al. (1996a) believed that a decrease in torque measured at the same original (baseline point) knee joint angle indicated a change in the tissues' mechanical properties. Such a decrease (11.3%) was observed in the current study.

The possible reasons for the finding of an increase in stiffness could be due to two mechanisms. Firstly, lengthening becomes more and more limited with increasing levels of force as demonstrated in animal models (Fung, 1984). The underlying mechanical process for this phenomenon is *fiber recruitment* and is due to collagen fibers elongating and straightening with increasing force (Fratzl et al., 1997; Thornton et al., 2002). This process of 'uncrimping' happens at varying points as the collagen fibers are stretched progressively until all fibers have been recruited (Thornton et al., 1997; Viidik, 1972). The phenomenon of fiber recruitment results in an exponentially increase in passive resistance to the tissue lengthening (Gajdosik, 2001). Such changes in length of soft tissue structures have been shown to occur under sustained and/or cyclic loading, in animal and cadaver models (De Zee et al., 2000; Huijing, 1992; Lieber, 1992; Smutz et al., 1995; Wang & Ker, 1995; Wren et al., 2003; Zuurbier et al., 1994), in cadaver *in vitro* spinal models (Adams & Dolan, 1996), and in spinal feline ligament *in vivo* models (Claude et al., 2003; Lu et al., 2004). Secondly, a possible

reason is related to hydration of the tissues. Seventy percent of collagen's ECM is water and is believed to play an important spacing and lubricating role that affect the connective tissue's mechanical and viscoelastic behaviour (Culav et al., 1999; Kjaer, 2004). During cyclic loading studies have shown there is a reduction in hydration of the soft tissues as the number of cycles increase resulting in increased resistance to lengthening (Hannafin & Arnoczky, 1994). This finding has been reported in animal ligament models (Chimich et al., 1992; Thornton et al., 2001) and in animal and human tendon models (Hannafin & Arnoczky, 1994; Haut & Haut, 1997).

The current study's findings of increased resistance and joint range of motion could also be attributed to an alteration in *stretch tolerance* rather than a change in the mechanical structure of the muscle-tendon units. This suggestion is supported by a number of *in vivo* studies examining stretching (Halbertsma & Goeken, 1994; Halbertsma et al., 1999; Halbertsma et al., 1996; Magnusson et al., 2000a; Magnusson et al., 1997; Magnusson et al., 1996b; Magnusson et al., 1996a). Stretch tolerance is a phenomenon where the participant's perception of the end point of the stretch is based on discomfort or pain and causes them to stop further stretching (Gajdosik, 2001). Any sensory change, i.e. inhibition, could decrease the participant's perception of pain for that level of stretch and therefore would lead to participants tolerating more tension (greater passive resistive force) as the participant moves further into their range of motion. However, the exact mechanism and structures responsible for stretch tolerance in *in vivo* studies at present remains unknown (Magnusson et al., 1998a).

In summary, the current study showed significant increases in maximal passive resistance torque, with an associated increase in maximal passive knee angle. In

addition, there was a significant decrease in passive resistance torque when measured at the original maximal passive knee angle. Furthermore, stiffness was shown to significantly increase over the final 10% of the torque-angle curve.

5.3 Cyclic Loading To A Predetermined Load

This section discusses the current study's findings during the intervention of cyclic loading applied to a predetermined calculated load of 85% of the participant's passive knee extension test mean maximal stretch tolerance value (as previously described in the Methods section). The variables of maximal passive knee angle and stiffness measured at 10 and 100% of the torque-angle curve are discussed with regard to first cycle as compared to the forty-fifth cycle.

5.3.1 Maximal Passive Knee Angle: Cycle One Compared to Cycle Forty-Five

Maximal passive knee angle increased by 5.2%. There are no known human *in vivo* cyclic loading studies that have investigated this variable. Albeit different, from cyclic loading, the maximal passive knee angle results from a study by Magnusson et al. (1998a) who investigated dynamic stretching of the hamstrings reported a significant increase in maximal passive knee angle across 10 cycles (although no data was reported). This finding is in agreement with the current study. Additionally, findings of an ultrasonography by Maganaris (2002), which investigated cyclic loading of the gastrocnemius muscle using active muscle contractions also supports the findings. The authors reported significant lengthening at the first cycle (4.7%) and at the fifth cycle (6%). The plotted changes of this study displayed a typical creep curve (Maganaris, p.

1025, Fig. 8) which is a similar curve that plotted from the current study (refer to Figure 4.8). The large differences in the magnitudes of change between the Maganaris investigation and current study are most likely attributed to different methodologies used. In addition, lengthening might be specific to different muscle groups, a reflection of their passive connective tissue composite differences (Fung, 1984).

The current study's cyclic loading results are supported by the findings of creep loading studies of the human spine *in vivo* (Adams & Dolan, 1996) and animal studies that used an *in vivo* feline spinal ligament model (Claude et al., 2003; Lu et al., 2004). All of these studies reported significant differences in tissue lengthening when comparing the first to last cycle. Additionally, the current study's findings of lengthening are consistent with studies of whole muscle-tendon units *in situ* (Taylor et al., 1990), and *in vitro* studies of tendon (De Zee et al., 2000; Wang & Ker, 1995; Warren et al., 1976) and ligament (Hingorani et al., 2004; Provenzano et al., 2001; Thornton et al., 1997; Thornton et al., 2002).

5.3.2 Passive Stiffness: Cycle One Compared to Cycle Forty-Five

Passive stiffness increased by 28.6% over the final 10% of the curve. There are no known human *in vivo* cyclic loading studies with which to compare these results. The current study's findings however, are consistent with Magnusson et al. (1998a), who compared the first cycle to the tenth cycle. They reported a significant increase in stiffness (~15%) over the final 10% of the torque-angle curve. The differences in magnitude of stiffness in this study as compared to the current study are most likely due to the different mechanical mechanisms that were measured. The current study's findings of increased stiffness are also consistent with an *in vitro* study of rabbit

ligaments (Thornton et al., 2002). The authors of this study reported a significant increase ($p < 0.05$) in ligament stiffness from the first to last cycle. However, in contrast, animal tendon models (De Zee et al., 2000; Wang & Ker, 1995; Wren et al., 2003) all consistently reported stiffness decreased with time and number of cycles. De Zee et al. calculated stiffness values for the final 50% of the length-cycle curve and showed a significant decrease ($p < 0.05$) in stiffness from the first 10 cycles to the last 10 cycles. Possible reasons for these reported differences across all of the *in vitro* studies compared to the current study are most likely attributed to measurements of isolated tendon as compared to whole muscle-tendon units.

5.4 Limitations & Delimitations

This section will identify and discuss the limitations of the current study with respect to the age and health of the participants, the chosen muscle group, and methodological procedure used. Finally, the delimitations of the equipment are discussed.

The participants in the current study were young and healthy with an average age of 22 years (range, 20-29 years). Since aging is considered a factor that affects the viscoelastic properties of the muscle-tendon unit (Gajdosik et al., 2004). The findings of the current study might not be directly applicable to other populations, in particular the older adult.

The current study investigated the muscle-tendon units of participants with non-diseased or injured tissue structures. Inferences from these findings might not be applicable to participants whose range of motion is limited by pathological tissues and the associated

physiological and structural changes. Such changes would most likely influence and ultimately alter the tissues mechanical responses to loading and lengthening (Best & Garrett, 1993; Fung, 1973).

In the current study the chosen biarticular muscle, the hamstring group, was investigated for its viscoelastic behaviour. Some similarities in viscoelastic behaviour exist between muscle groups (Fung, 1973). However, findings from the current study might not accurately represent all those characteristics found for all other muscle groups.

A testing protocol of passive knee extension in the seated position was used in the current study. As joint position has been shown to affect the viscoelastic behaviour of the muscle-tendon unit (Fukunaga et al., 1997), the findings in position might not be applicable to other joint positions.

An acute single session was used to investigate viscoelastic behaviour, which limits the findings of the current study to this time domain. Viscoelastic behaviour has been shown to be transient (Bohannon, 1984) and therefore the current study's results might not be applicable to longer time durations.

The current study only investigated cyclic loading using a single level of load and rate on the hamstrings muscle group. The study's findings may not be applicable in other series of differing levels of loading or rate as the viscoelastic behaviour of the muscle-tendon unit to creep has been shown to be load and rate-dependant by *in vitro* studies (Adams & Dolan, 1996; Hingorani et al., 2004; Provenzano et al., 2001; Smutz et al.,

1995; Thornton et al., 2002; Wang & Ker, 1995; Wren et al., 2003) and *in vivo* animal studies (Claude et al., 2003; Lu et al., 2004).

The availability of the software programme (Kinetic Communicator, Software Version 5.30 Chattex Corp., Chattanooga Group, Inc., TN, USA) used may have been a delimitation to this study. Classically a loading rate of five degrees per second has been used to simulate typical dynamic stretching regimes when performed on the KinCom® dynamometer (McNair et al., 2000; McNair et al., 2002). Therefore, to achieve a maximal amount of repetitions the slowest loading rate that could be used to simulate a typical dynamic stretching regime was ten degrees per second. The five degrees per second increase may have influenced the results of passive maximal resistive torque and stiffness.

5.5 Recommendations & Future Research

The current study's findings should contribute to the knowledge and understanding of how cyclic loading influences the viscoelastic properties of the human hamstring muscle group, *in vivo*. However many questions remain. In light of this study's finding and available literature in this area, this section will now discuss five key areas identified for future research.

The investigation of the mechanical properties of muscle-tendon units needs to be undertaken in a wider population. The current study (as have the majority of creep studies within the literature) focused on the viscoelastic response of healthy muscle-tendon units. Future studies need to investigate sustained and cyclic loading over a

wider variety of populations in particular these would include the older adult, and persons with diseased and or injured tissues.

The current study was restricted to the hamstring muscles. Exploration of other biarticular muscle groups to would allow comparisons to be made of their individual mechanical responses to sustained and cyclic loading.

Appropriate and applicable methodological procedure (position of joints during stretching and the session time) used in the stretching of the hamstring muscle-tendon units requires further exploration. In particular, the use of relevant stretching positions and regimes commonly prescribed by sportspersons and rehabilitation therapists. These could include testing other joint positions where the biarticular hamstring group are placed under lengthening in combination with a stretching regime over a number of weeks.

Other methodological procedures that need consideration are the variables of levels of load, rate, and recovery time of sustained or cyclic loading, to simulate more closely human daily activities that subject the muscle-tendon units to complex loading and lengthening profiles.

There is still much research to be done, not only to understand the mechanical properties of the muscle-tendon unit, but also to improve stretching techniques and injury prevention procedures for the hamstring muscle group. A multidisciplinary collaboration between molecular biologists, morphologists, bioengineers, biomechanics, clinicians, and even psychologists will help unite the various disciplines and enhance

the understanding of the muscle-tendon unit. Ultimately, the scientific information obtained from these studies will likely lead to more effective and efficient prevention treatment regimens with improved clinical outcomes.

5.6 Summary and Conclusions

Daily human activities such as walking and running involve repetitive or cyclic motions. These activities subject the hamstring muscles to continuous cyclic lengthening and loading. A significant number of hamstring injuries have been attributed to mechanical factors related to the viscoelasticity of the tissues. Stretching has been proposed as a mechanism to alter viscoelasticity by increasing passive extensibility of the muscle-tendon unit and therefore could reduce the chance of injury. Studies involving stretching of human hamstrings have almost exclusively investigated the viscoelastic property of stress-relaxation, while creep has received little attention. Investigations of creep behaviour have stretched soft tissue structures by passive sustained loading or cyclic loading and have been mostly *in vivo*, *in vitro* animal, and human cadaver studies, plus a more limited number of ultrasonography or clinical trials. All of these studies have reported increases in length of the soft tissue structures and change in their resistance and stiffness following intervention. However, to date no known study had examined passive cyclic loading to a constant load of human hamstrings *in vivo*. Therefore, the purpose of the current study was to investigate the influence of cyclic loading on the extensibility of the human hamstring muscle-tendon units *in vivo*.

The current study used a test-retest randomised controlled trial with repeated measures. The hamstring muscles were assessed by the PKE test to maximal stretch tolerance

using a KinCom® dynamometer. An intervention group underwent 45 continuous cyclic loadings taken to a predetermined load of 85% of their maximal passive resistance torque. The control group did not stretch and at quietly during the intervention procedure. Measurements of the hamstring muscles passive extensibility was repeated using the PKE test at the end of the intervention.

The main findings of the current study were: a) a significant increase in maximal passive resistance torque, b) a significant increase in maximal passive knee angle, c) an associated significant reduction in passive resistance torque, when re-measured at baseline maximal knee angle, and d) a significant increase in stiffness over the final 10% of the torque-angle curve.

In addition, the findings of the intervention of cyclic loading to a predetermined load, when comparing cycle one to cycle forty-five showed: a) a significant increase in maximal passive knee angle, and b) a significant increase in stiffness over the final 10% of the torque-angle curve.

In conclusion, the current study using a constant cyclic loading paradigm could not determine the mechanism behind the changes in the hamstring muscles. However, the changes in the variables of interest provide some evidence that most likely a combination of altered stretch tolerance and local mechanical effects within the muscle-tendon unit, i.e. creep lengthening were responsible for these changes.

APPENDICES

APPENDIX A

MEMORANDUM

Student Services Group - Academic Services

To:	Peter McNair
From:	**Madeline Banda**
Date:	23 June 2004
Subject:	04/125 Influence of age on viscoelastic properties of human hamstring muscle-tendon unit during cyclic loading

Dear Peter

Your application for ethics approval was considered by AUTEC at their meeting on 14/06/04.

Your application has been approved subject to amendment and/or clarification of the following:

1. The Information Sheet should state that research is being done as part of thesis or qualification
2. Compensation clause: "…participants would be covered by ACC…"

Please consider this point/these points and provide a response to me in writing, as soon as possible. Please note that where approval is given subject to specified conditions being met, this does not constitute full approval. The conditions must be met before full approval is granted and research can begin. Please quote the application number and title in all correspondence.

Yours sincerely

Madeline Banda

Executive Secretary

AUTEC

CC: Erik Dombroski

From the desk of ...Private Bag 92006, Auckland 1020 Tel: 64 9 917 9999
Madeline Banda New Zealand ext 8044
Academic Services E-mail: madeline.banda@aut.ac.nz Fax:64 9 917 9812
Student Services Group

APPENDIX B

Participants Required for STRETCHING Study

Are you healthy and mildly active or sedentary – i.e. you do not participate in sporting or regular exercise programmes?

If you fit the above description and are aged 20-29 years, you are requested to participate in a stretching study that will investigate the flexibility of the hamstring muscles.

Participants will sit in a testing machine and undergo an initial maximal stretch test participants will be randomly assigned to either a stretching (intervention) or relaxation (control) group. The stretching group will have one lower limb undergo relaxed dynamic stretching for 15 minutes. The relaxation (control) group will sit quietly for 15 minutes. All participants will then undergo a repeat of the initial maximal stretch test. The entire session will take approximately 60 minutes.

REIMBURSEMENT:

If you are not on campus you will receive a $20 petrol voucher to cover costs of transport to get to the testing session. A free car park will also be supplied.

INTERESTED? FOR MORE INFORMATION:

Contact:
Erik Dombroski
Master student,
AUT Physical Rehabilitation Research Centre,
Akoranga Campus,
Northcote,
North Shore City.
Telephone: (09) 9179624 or 021-455040,
E-Mail: erik.dombroski@aut.ac.nz

APPENDIX C

Participant Information Sheet

Date Information Sheet Produced: 31.05.04

Project Title:
The viscoelastic properties of human hamstring muscle-tendon unit during cyclic loading.

Invitation
You are invited to take part in a research study to examine stretching of hamstring muscles of the leg during relaxed sitting. You have been selected for this study as you meet the entry criteria of being a healthy individual who lives a sedentary or mildly active life and are aged (20-29 years).

What is the purpose of the study?
This study is being done as part of the researcher's Master of Health Science thesis. The aim of this study is to investigate flexibility of the hamstring muscles of young aged persons.

How are people chosen to be asked to be part of the study?
Potential participants are recruited through notices placed on Auckland Student Movement (AuSM) notice boards at the Auckland University of Technology. Additionally public advertisements posted in local and university newspapers are used. Participants are asked to contact the researcher Erik Dombroski (contact details at the end of this sheet) at the Physical Rehabilitation Research Centre for further information. Potential participants are sent or given the information sheet and are contacted in not less than one working week (5 days). At this point the potential participants are given the opportunity to ask any further questions about the study. If the potential participant agrees to take part and fulfils the entry criteria a laboratory testing session will be arranged at a time and date suitable for the participant.

What happens in the study?
The study involves a single testing session at the Physical Rehabilitation Research Centre held at the Akoranga Campus of Auckland University of Technology. All participants will be weighed, their height measured, and their lower leg length measured. Participants are asked to complete an AUT 'Consent Form' and two brief questionnaires asking about their present activity levels, past medical and physical history. Participants will need to wear a pair of shorts and a comfortable fitting top. If not a gown and a pair of shorts will be provided.

Participants will be randomly assigned to either a control group or stretch intervention group. Each participant is seated on the KinCom® dynamometer (a machine for measuring stretch and movement) with firm seating, back support and seatbelts to ensure stability maintenance of position. The participants' ankle is placed upon the KinCom's® lever arm. The participant is provided with a hand-held 'cut-out' switch, asked to close their eyes, and assume a relaxed posture. The participants' lower leg will be passively extended by the Kin-Com® until the participant perceives maximal tolerance to stretch at which point they will activate the 'cut-out switch' which instantly

stops any further movement of the lever arm and immediately returns the leg to the starting position. This procedure will be repeated 4 times.
After a ten minute rest, the stretch intervention group will undergo 15 minutes of passive dynamic stretching to a level set at 85% of the recorded maximal stretch tolerance test. While this is performed the participant remains seated in the KinCom® and assumes a relaxed posture.
The relaxation group will remain seated in the KinCom® and sit quietly relaxed for 15 minutes.

At the conclusion of this procedure both participant groups finish the session with a repeat of the initial four 'maximal stretch tolerance' tests as described already. Total participation time is estimated to take 75 minutes.

What are the discomforts and risks?
There is a risk of a participant having their muscles stretched to a point which may cause a muscle strain.

How will these discomforts and risks be alleviated?
The principle researcher will be with the participants throughout the entire testing procedure. All participants will be fully informed of the proceedings before beginning testing. All participants will hold and control an emergency 'cut-out' switch that they may use at anytime during the testing procedure should they feel they are in physical danger or discomfort. All participants are able to communicate with the researcher throughout the testing procedures and may withdraw at anytime from the study. Additionally there is a back-up manual keyboard 'cut-out' control that the researcher will be within reach of throughout the testing procedure.

What are the benefits?
Participants will learn about the flexibility of their hamstring muscles. Individuals who demonstrate reduced flexibility will receive recommendations for rehabilitation therapy to improve range of motion and function to within limits typically found within their respective age group.

What compensation is available for injury or negligence?
There is a risk of a participant having their muscles stretched to a point which may cause a muscle strain. In the unlikely event of a physical injury as a result of participation in this study, the researcher who is a registered physiotherapist can provide treatment and has access to a first aid kit, ice and bandages. Furthermore participants would be covered by the Accident Compensation Insurance legislation with its limitations.

How will my privacy be protected?
Confidentiality will be maintained throughout the study in the following ways: No material recorded that could identify you, will be used in any reports on this study. Data collected in this study will be kept in a secure cabinet in a locked office. Data collected in paper form will be shredded or if in electronic form erased at the completion of the study.

How do I join the study?
If you are interested following learning of the study through local notices and advertorials, you can contact and discuss your possible participation with the study researcher (refer the name and address at the end of this information sheet). If the potential participant agrees to take part and fulfils the entry criteria a laboratory testing session will be arranged at a time suitable for the participant.

What are the costs of participating in the project? (including time)
Participation in this study will not cost you anything, though if you are not already on campus you will be required to travel to and from the Physical Rehabilitation Research Centre one time. Petrol vouchers of $20 will be provided and a 'free' car park will be made available. The entire duration of the session including pre-screening protocols prior to the hamstring muscle testing will be approximately 60 minutes.

Opportunity to consider invitation
If you wish to participate you will have one week to decide. You will have the right to choose not to participate. If you do agree to take part you are free to withdraw from the study at anytime, without having to provide a reason.

Opportunity to receive feedback on results of research
The results of the study will be published in a scientific journal and presented at scientific conferences. It is usual that a substantial delay, between the end of the data collection and the publication and/or presentation of the results. If the participant wants to discuss the outcomes of the study before this process occurs if they may request time to discuss with the principal researcher.

Participant Concerns

Any concerns regarding the nature of this project should be notified in the first instance to the Project Supervisor. Concerns regarding the conduct of the research should be notified to the Executive Secretary, AUTEC, Madeline Banda, madeline.banda@aut.ac.nz , 917 9999 ext 8044.

Researcher Contact Details: Researcher: Erik Dombroski
Address: AUT, Private Bag 92006, Auckland
Phone: (09) 9179624 or 021-455040

Project Supervisor Contact Details: Dr. Peter McNair
Address: AUT, Private Bag 92006, Auckland
Phone: (09) 9179999 ext 7146

Approved by the Auckland University of Technology Ethics Committee on 30th June 2004, AUTEC **Reference number** 04/125

APPENDIX D

The Viscoelastic Properties Of Human Hamstring Muscle-Tendon Unit During Cyclic Loading.

Date: _____/_____/ 2004

ID No.: _____

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE

1. Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?

☐ Yes ☐ No

2. Do you feel pain in your chest when you do physical activity?

☐ Yes ☐ No

3. In the past month, have you had chest pain when you were not doing physical activity?

☐ Yes ☐ No

4. Do you lose your balance because of dizziness or do you ever lose consciousness?

☐ Yes ☐ No

5. Do you have a bone or joint problem that could be made worse by a change in physical activity?

☐ Yes ☐ No

6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?

☐ Yes ☐ No

If answered YES please list medications:

_____

7. Do you know of any other reason why you should not do physical activity?

☐ Yes ☐ No

If answered YES please state reason:

_____

_____

APPENDIX E

The Viscoelastic Properties Of Human Hamstring Muscle-Tendon Unit During Cyclic Loading.

Date: _____/_____/ 2004
ID No.: _____

SCREENING QUESTIONNAIRE

1. Is your general health good? ☐ Yes ☐ No
   If no, what problems do you have? _____
2. Are you currently taking any medication? ☐ Yes ☐ No
   If yes, please specify: _____
3. Do you have any current conditions that affect your lower legs? ☐ Yes ☐ No
   If yes, please specify (e.g. where?): _____
4. Do you have any current disease process such as cancer, hepatitis or AIDS? ☐ Yes ☐ No
   If yes, please specify: _____
5. Do you have uncontrolled high or low blood pressure? ☐ Yes ☐ No
6. Do you have a heart condition? ☐ Yes ☐ No
7. Have you ever had a stroke? ☐ Yes ☐ No
8. Do you have uncontrolled epilepsy? ☐ Yes ☐ No
9. Do you have any open wounds, skin rashes or conditions? ☐ Yes ☐ No
   If yes, please specify: _____
10. Have you had radiotherapy or chemotherapy in the past 6 weeks? ☐ Yes ☐ No
    If yes, please specify: _____
11. Do you have any bladder or bowel problems? ☐ Yes ☐ No
    If yes, please specify: _____
12. Do you have osteoarthritis in your legs? ☐ Yes ☐ No
    If yes, please specify (e.g. where?): _____
13. Have you had low back pain in the past 6 weeks? ☐ Yes ☐ No
    If yes, please specify: _____
14. Do you suffer from altered sensation in your legs, i.e. 'pins and needles'? ☐ Yes ☐ No
    If yes, please specify (e.g. where?): _____
15. Have you had a muscle or tendon injury in your legs in the past 6 weeks? ☐ Yes ☐ No
    If yes, please specify (e.g. where?): _____
16. Have you had any physiotherapy treatment in the past 3 months? ☐ Yes ☐ No
    If yes, please specify (e.g. what for?): _____

APPENDIX F

Consent to Participation in Research

This form is to be completed in conjunction with, and after reference to, the AUTEC Guidelines (Revised January 2003).

Title of Project: **The Viscoelastic Properties Of Human Hamstring Muscle-Tendon Unit During Cyclic Loading**

Project Supervisor: **Dr. Peter McNair**

Researcher: **Erik Dombroski**

- I have read and understood the information provided about this research project (Information Sheet dated 31.05.04)
- I have had an opportunity to ask questions and to have them answered.
- I understand that I may withdraw myself or any information that I have provided for this project at any time prior to completion of data collection, without being disadvantaged in any way.
- If I withdraw, I understand that all relevant data and transcripts, or parts thereof, will be destroyed.
- I agree to take part in this research.
- I can receive a copy of the report from the research if requested.

Participant Signature: .....……..

Participant Name: ……

Participant ID No.: ……

Participant Contact Details (if you wish to receive a copy of the report):

……..

……..

……..

……..

Date: / / 2004

Approved by the Auckland University of Technology Ethics Committee on 30 June 2004 **AUTEC Reference number** 04/125

Note: The Participant should retain a copy of this form.

APPENDIX G

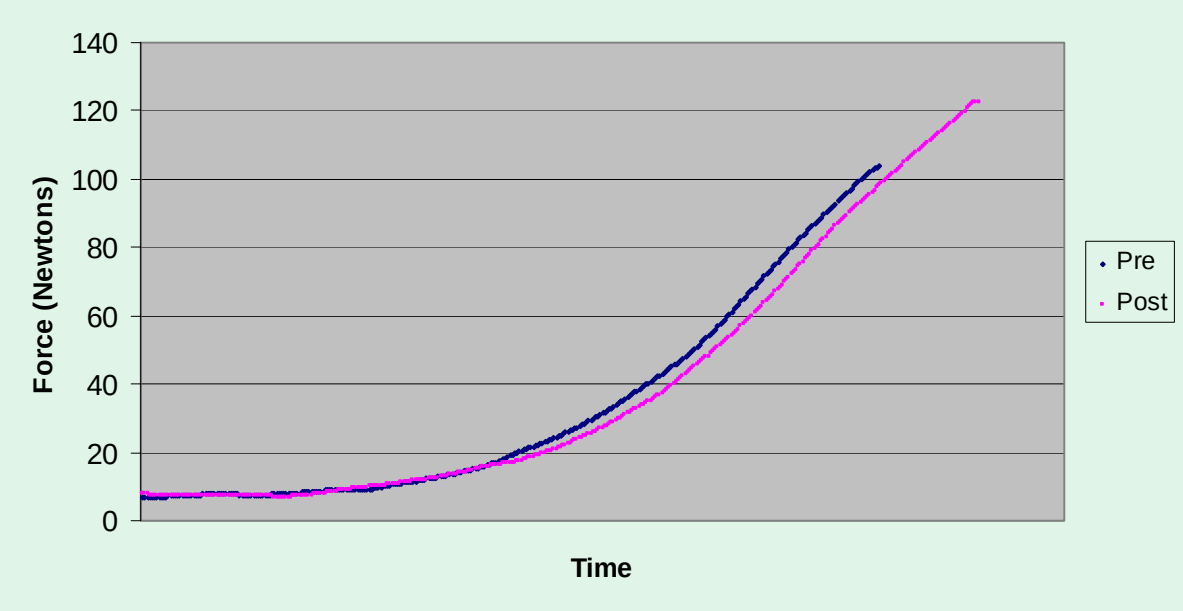

Figure 1: Typical cyclic loading participant pre-post maximal passive resistance traces.

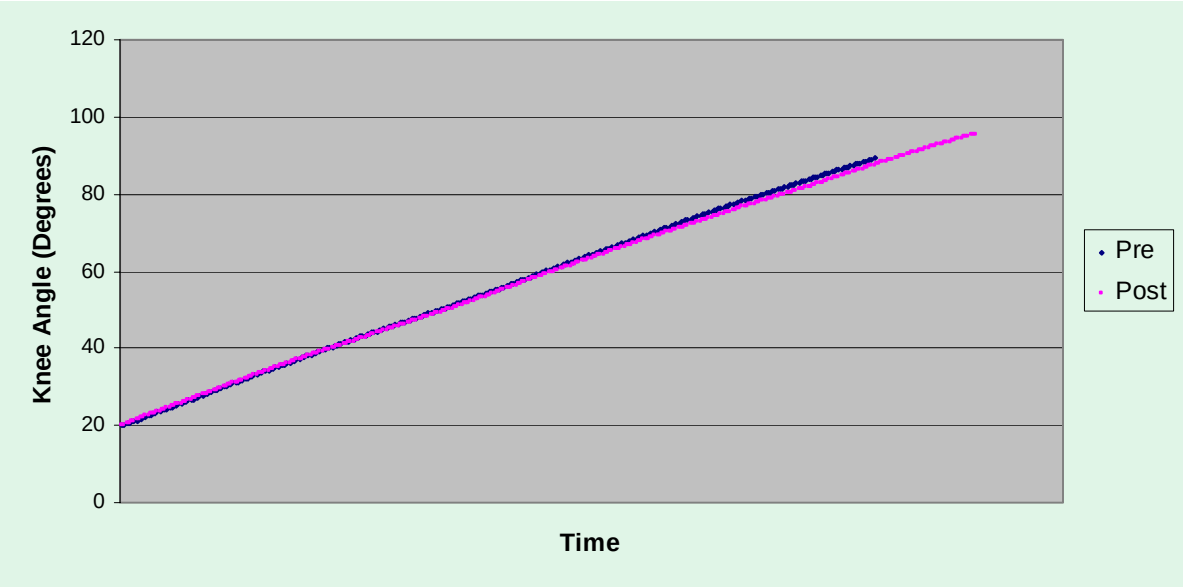

Figure 2: Typical cyclic loading participant pre-post maximal passive knee angle traces.

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