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FACULTY OF SPORT, EDUCATION & SOCIAL SCIENCES

Physiological Responses to Load Carriage by Backpack

By

Sam David Blacker

Thesis for the Doctor of Philosophy

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ABSTRACT

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Load carriage (19.3 km, 280 min, 31 kg load) in the field elicited a cardiovascular strain of 72 ± 5 %HRmax and caused neuromuscular impairment (7 ± 8% decrease in jump height (P<0.001) (study 1).

In the laboratory, the reliability of muscle function tests were compared to baseline values at 2, 24, 48 and 72 hours in studies 2 and 3. The most reliable parameters were voluntary isokinetic peak torque of the knee, trunk and shoulder extensors and flexors and isometric knee extension maximal force, and electrically stimulated voluntary activation, doublet contraction and relaxation times and 20:50 Hz ratio.

In study 4, $\dot{V}O_2$ increased by 3.9 ± 2.3 $mL \cdot kg^{-1} \cdot min^{-1}$ over 120 minutes of level walking carrying a 25 kg backpack [LWLC] (P<0.05) which was less when walking unloaded [LW] (1.6 ± 0.6 $mL \cdot kg^{-1} \cdot min^{-1}$, P<0.05) but there was no difference compared to walking downhill carrying a 25 kg backpack [DWLC] (4.3 ± 2.5 $mL \cdot kg^{-1} \cdot min^{-1}$, P>0.05). The differences between conditions appeared to relate to changes in substrate oxidation, neuromuscular impairment and reduced mechanical efficiency.

Neuromuscular function was measured in study 5 at 0, 24, 48 and 72 hours after the treadmill walking conditions described in study 4. LW caused no changes in neuromuscular function. Isometric knee extension force decreased immediately after LWLC (15 ± 11 %, P<0.05) and DWLC (16 ± 17 %, P<0.05), recovering by 72 hours for DWLC only. VA decreased after LWLC only, doublet half relaxation time and 20:50Hz decreased after LWLC and DWLC (P<0.05). LWLC and DWLC were associated with decreases in isokinetic peak torque of knee, trunk extensors and flexors and shoulder flexors (P<0.05) with complete recovery by 72 hours.

Regression models developed in study 6 indicated that participants with the most efficient metabolic and neuromuscular performance during 120 minutes of load carriage (25 kg backpack) on a level gradient had high body mass and high absolute $\dot{V}O_2$max with strong trunk, shoulder and knee flexors.

To examine nutritional interventions to reduce the metabolic cost during, and neuromuscular impairment following, load carriage, participants consumed Placebo [PLA], Carbohydrate [CHO] or Whey Protein [PRO] beverages during 120 minutes of load carriage and for three days of recovery.

During load carriage (study 7), there were no differences in $\dot{V}O_2$, RER, or EMG RMS between conditions at minute 5 (P>0.05). The increase in $\dot{V}O_2$ between 5 and 120 minutes was less during CHO (8 ± 5 %) than PLA (14 ± 6 %, P<0.05) or PRO (17 ± 4 %, P<0.05). RER decreased between minutes 5 and 120 during PLA and PRO only. Peak RMS did not change over time in *m. rectus femoris, m. vastus lateralis, m. semitendinosus,* and *m. biceps femoris*. Attenuation in $\dot{V}O_2$drift during CHO could not entirely be accounted for by higher carbohydrate oxidation rates.

During recovery, neuromuscular function was measured 0, 24, 48 and 72 hours after load carriage (study 8). There was no difference between PLA, CHO or PRO in the decrease in peak torque of knee and trunk extensors and flexors at 0 h. Peak torque of the knee extensors and flexors returned to pre-exercise values at 24 h during PRO followed by CHO at 48 h and PLA at 72 h (P>0.05). Trunk flexors returned to pre exercise value at 24 h for CHO and PRO but 48 h for PLA (P>0.05). Faster recovery of neuromuscular function was probably due to CHO and PRO improving protein balance, thus enhancing repair of muscle tissue damaged during exercise.

In conclusion, load carriage increases $\dot{V}O_2$ and $\dot{V}O_2$drift whilst walking and causes neuromuscular impairment, which can last for up to 72 hours following exercise. Nutritional supplements can reduce $\dot{V}O_2$drift and improve the time course of recovery of neuromuscular function.

List of Publications

The following conference papers were presented at the British Association of Sport and Exercise Sciences (BASES) conference:

Blacker S.D., Fallowfield J.L., Bilzon J.L.J., & Willems M.E.T. (2008). Physiological responses to prolonged load carriage during level and downhill treadmill walking. BASES Conference, University of Brunel, September 3rd 2008

Blacker S.D., Williams N.C., Fallowfield J.L., Bilzon J.L.J., & Willems M.E.T. (2009). Whey protein supplementation improves time course of recovery of muscle function following prolonged load carriage. BASES Conference, Leeds Metropolitan University, September 1st 2009

The following manuscript has been published in a peer reviewed journal:

Blacker S.D., Fallowfield J.L., Bilzon J.L.J., & Willems M.E.T. (2009). Physiological responses to load carriage during level and downhill treadmill walking. Medicina Sportiva 13 (2): 108-124

The following technical report has been published by the Ministry of Defence

Fallowfield J.L., Blacker S.D., Davy T., Delves S., Layden J. & Willems M.E.T. (2008) Physiological responses to load carriage in the field. INM Report No. 2008.048, November 2008

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Authors Declaration

I *Sam Blacker* declare that the thesis entitled *Physiological Responses to Load Carriage by Backpack* and the work presented in the thesis are both my own, and have been generated by me as the result of my own original research. I confirm that:

- This work was done wholly or mainly while in candidature for a research degree at this University;
- Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
- Where I have consulted the published work of others, this is always clearly attributed;
- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
- I have acknowledged all main sources of help;
- Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
- Parts of this work have been published as (please see list of publications)

Signed: S.Blacker.....

Date: 01/11/09.....

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Abbreviations

BCAA	Branched chain amino acid
CHO	Carbohydrate supplement group
CI	Confidence interval
DWLC	Downhill walking (-8 % gradient) with load carriage (25 kg backpack)
EMG	Electromyography
GPS	Global positioning system
HMB	β-Hydroxy-β-Methylbutyrate
HR	Heart rate
HRmax	Maximum heart rate
HR_{rest}	Resting heart rate
HRR	Heart rate reserve
HFF	High frequency fatigue
ICC	Intraclass correlation coefficient
iEMG	Integrated electromyography signal
ITT	Interpolated twitch technique
LFF	Low frequency fatigue
LoA	Limits of agreement
LW	Level walking (0 % gradient)
LWLC	Level walking (0 % gradient) with load carriage (25 kg backpack)
MODREC	Ministry of Defence Research Ethics Committee
MSFT	Multistage fitness test
MVC	Maximal voluntary contraction
%Δ MVC	Percentage change in maximal voluntary contraction force
NS	Non-significant difference
PLA	Placebo supplement group
PRO	Protein supplement group
RER	Respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$)
RMS	Root mean square
RPE	Rating of perceived exertion
VA	Voluntary activation
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_2$drift	Drift in oxygen uptake over time
$\dot{V}O_2$max	Maximum rate of oxygen uptake
$\dot{V}CO_2$	Volume of carbon dioxide expired
20:50 Hz	Ratio of the peak force during electrically stimulated 20 and 50 Hz tetani

Chapter 1. Introduction

Load carriage is the transport of an external mass in a backpack supported on the upper torso by shoulder straps and/or hip belt during human locomotion (Knapik *et al.*, 1996). It is undertaken both as part of recreational pursuits (Lobb, 2004; Machefer *et al.*, 2007) and as an occupational task (e.g. military and emergency services) (Knapik *et al.*, 2004; McLellan and Selkirk, 2004). The physiological responses to load carriage have previously been examined in field and laboratory trials, and the current body of knowledge will be reviewed as part of this thesis, with particular reference to the effect of load carriage on neuromuscular function (Chapter 2). The majority of previous studies have examined the physiological responses during short periods of load carriage (i.e. $\leq$ 15 minutes). However, this time frame does not reflect the duration of most defined load carriage tasks, which have been documented to be from 30 minutes (Richmond *et al.*, 2008) to 10 hours over repeated days (McCaig and Gooderson, 1986).

When the duration of sub-maximal exercise such as load carriage is extended (i.e. > 15 minutes), additional physiological demands are placed upon the load carrier. Three such changes are an increased energy cost of exercise over time, the onset of muscle fatigue and the increased potential of muscle injury. Previous investigations of load carriage of durations greater than 15 minutes have primarily investigated the metabolic and cardiovascular responses during exercise (for example Patton *et al.*, 1991). Since Clarke *et al.* (1955) investigated reductions in muscle strength following load carriage in the field, the effect of load carriage on neuromuscular function (i.e. the force producing capability of a muscle) has received very little attention. Clarke *et al.* (1955) measured changes in strength using cable tension weight stack. However, since this initial study, experimental techniques to examine changes in neuromuscular function have developed significantly. For example; isometric and isokinetic dynamometry (Warren *et al.*, 1999), electrical stimulation (Edwards *et al.*, 1977b), interpolated stimulation during voluntary contraction (Paillard *et al.*, 2005). These procedures have been used to examine the mechanisms responsible for changes in neuromuscular function following running, cycling and ski skating exercise (Millet and Lepers, 2004).

Neuromuscular function is most accurately quantified by the force producing capability of a muscle or muscle group (Warren *et al.*, 1999), a reduction which can last for several days or weeks after the initial exercise bout (Warren *et al.*, 2002). However, changes

in neuromuscular function in the days following a load carriage bout have not been investigated. Reduced force producing capability of a muscle has been shown to impair subsequent exercise and skilled task performance (Byrne *et al.*, 2004) and in animal models to reduce the amount of energy a muscle can absorb, thus increasing susceptibility to muscle strains (Mair *et al.*, 1996). These aspects are of particular importance in occupational settings where further exercise, physical training or skilled tasks are undertaken immediately after and in the days following a load carriage bout. Identifying changes in neuromuscular function and the mechanisms responsible can inform interventions to improve physical performance during and following load carriage. Nutritional supplementation with carbohydrate or whey proteins are two potential interventions to improve neuromuscular function during exercise and recovery (Howatson and van Someren, 2008). However, the effects of carbohydrate and whey protein supplements on neuromuscular function during load carriage and subsequent recovery have not been investigated.

The experimental studies reported in this thesis have investigated the physiological responses to load carriage, with particular reference to neuromuscular performance. Cardiovascular and neuromuscular responses of Royal Marines during a load carriage task in the field were examined to quantify the physiological responses to load carriage in a relevant occupational setting (Chapter 3). Due to restrictions relating to the control of participants and the detail of measures that could be taken in an occupational setting, all subsequent investigations were undertaken in a laboratory. The reliability of a battery of laboratory-based tests (voluntary and electrically stimulated contractions) were established to measure changes in neuromuscular function in the days following load carriage (Chapters 4 and 5). Metabolic and cardiovascular responses were assessed during a two hour bout of load carriage on level and downhill gradients in the laboratory (Chapter 6). The battery of voluntary and electrically stimulated contractions (Chapters 4 and 5) were applied to investigate the effect of a two hour load carriage bout on level and downhill gradients on neuromuscular function 0, 24, 48 and 72 hours after exercise (Chapter 7). The physiological determinants of participants' metabolic and neuromuscular responses to two hours of load carriage were identified to examine how individual physical characteristics influence the physiological responses to load carrying exercise (Chapter 8). Finally, the results of the preceding studies were used to identify and evaluate the effect of nutritional interventions on the metabolic, cardiovascular and neuromuscular responses during load carriage (Chapter 9) and neuromuscular function 0, 24,

48 and 72 hours after exercise (Chapter 10). In Chapter 11, the results from the experimental studies were summarised, potential applications were discussed and suggestions for future research are presented.

Chapter 2. Review of the Literature

2.1. Load Carriage Tasks

Load carriage is undertaken as a recreational pastime and occupational task. Recreational walkers have been reported to typically carry backpack loads ranging from 8 ± 6 to 20 ± 10 kg for durations of several hours and for periods up to and beyond durations of 8 days (Lobb, 2004). Members of emergency services carry breathing apparatus supported by shoulder straps during fire fighting and search and rescue tasks, with load mass ranges between 24 ± 1 and 33 ± 1 kg (Richmond *et al.*, 2008). Duration of load carriage varies depending on the task requirements, which can be as short as 30 minutes (Richmond *et al.*, 2008) and in extreme cases can involve repetitive periods of load carriage in a an 8 to 14 hour shift (Aisbett *et al.*, 2007). Military personnel frequently carry backpack loads to transport equipment during training and operational deployments, with the load carried and duration differing depending on the purpose of the load carriage task. A typical example of a military load carriage task is the final assessment during British Army training, which requires recruits to walk 12.8 km in two hours carrying either a 15, 20 or 25 kg backpack depending on specific job role (Rayson *et al.*, 2000). During operational deployments, repeated bouts of load carriage lasting up to 10 hours over several days with loads of up to 63 kg have been reported (McCaig and Gooderson, 1986).

2.2. Variables Effecting the Physiological Responses to Load Carriage

2.2.1. Addition of Load Mass

Goldman & Iampietro (1962) first showed a positive relationship between load mass (10, 20, 30 kg) and energy expenditure. These findings were extended to develop equations to predict the energy cost of load carriage with different load mass in the field (Epstein *et al.*, 1988; Pandolf *et al.*, 1977; Soule *et al.*, 1978). Borghols *et al.* (1978) investigated the cardiovascular and respiratory responses when carrying 10, 20 and 30 kg loads at rest and walking at 25, 50 and 70 % $\dot{V}O_2$max. As load increased, no differences were observed when standing, but during exercise between 25 and 50 % $\dot{V}O_2$max, regression analysis indicated that each kilogram of weight increased oxygen uptake by 33.5 $mL \cdot min^{-1}$, heart rate by 1

beat·min^{-1} and pulmonary ventilation by 0.6 L·min^{-1}. Further non-linear increases in these parameters were observed with increasing load at 70 % $\dot{V}$ O_2max. Similar increases in cardio-respiratory responses during exercise as load mass is increased have been observed in subsequent studies (Christie and Scott, 2005; Patton *et al.*, 1991; Sudan Pal *et al.*, 2009).

The addition of load mass increases postural sway whilst standing (Heller *et al.*, 2009), and during locomotion causes changes of posture, movement and gait (Attwells *et al.*, 2006). Also, during locomotion, there is greater forward lean of the trunk to support the load of the backpack (Attwells *et al.*, 2006) and an the head position moves further forward (Fiolkowski *et al.*, 2006) resulting in increased forces on the lumbosacral (L5/S1) region of the spine (Goh *et al.*, 1998). In the lower body, Quesada *et al.* (2000) observed increased peaks in internal moments during hip extension, knee extension, and ankle planter flexion with increasing backpack loads equivalent to 0, 15 and 30 % of a participant's body mass. These data suggest a greater energy contribution from these muscle groups with increases in load mass. The addition of carrying a backpack load and resultant changes in gait and posture cause increases in the vertical and antiposterior ground reaction forces and forces necessary for balance (Birrell *et al.*, 2007; Tilbury-Davis and Hooper, 1999). The additional support required to maintain balance during load carriage has been suggested as the reason for the greater musculoskeletal stiffness observed when carrying backpack (40 % body mass) compared to unloaded walking (Holt *et al.*, 2003). These findings support the view load carriage results in greater recruitment of muscle fibres and additional recruitment of other muscle groups compared to unloaded walking, which is related to load mass.

Electromyography (EMG) has been used to quantify different patterns of muscle recruitment with increasing load mass. Pressure on the shoulders increases with load mass (Vacheron *et al.*, 1999), increasing the EMG signal recorded in the *m. trapezius* (Holewijn, 1990). Contrary to these findings, Harman *et al.* (1992) showed no changes in average EMG signal of the *m. trapezius* with increase in backpack load (6, 20, 33, and 47 kg) and Devroey *et al.* (2007) observed no changes in *m. trapezius* activity with increases in load (0-15 % body mass). However, the duration of load carriage in the latter studies was 1 minute (Harman *et al.*, 1992) and 5 minutes (Devroey *et al.*, 2007) and the former study was 20 minutes (Holewijn, 1990). Therefore, changes in muscle activity may be time dependent. Studies examining trunk muscle activity with load carriage have reported mixed findings. Cook &

Neumann (1987) observed increases in *m. erector spinae* muscle activity with increases in load (0-20 % body mass). However, more recently, decreases in *m. erector spinae* muscle activity and increases in abdominal muscle activation have been shown with increases in load (0–20 % body mass), probably due to the additional forward lean observed during load carriage (Al-Khabbaz *et al.*, 2008; Devroey *et al.*, 2007). In the lower body, increases in load mass cause more prolonged activation of the *m. vastus lateralis* (i.e. parts of quadriceps), but not *m. semimembranosus* or *m. semtendinosus* (i.e. parts of hamstrings) (Ghori and Luckwill, 1985).

2.2.2. Absolute and Relative Loads

The addition of carrying an absolute load mass during walking or running causes a greater increase in $\dot{V}O_2$ (relative to body mass) for lighter individuals. For example, in a sample of 12 military personnel, Bilzon *et al.* (2001) showed no relationship between body mass and $\dot{V}O_2$ relative to body mass ($mL \cdot kg^{-1} \cdot min^{-1}$) during treadmill running at 9.5 $km \cdot h^{-1}$ ($r=-0.47$, $P>0.05$). However, with the addition of an 18 kg backpack there was a inverse linear relationship between body mass and relative $\dot{V}O_2$ ($r=-0.87$, $P<0.01$). Due to this relationship between body mass and load mass, and the oxygen cost of load carriage, many researchers use loads relative to a participant's body mass in their investigations. However, the relationship between load mass and its effect on physiological responses other than the oxygen cost have not been investigated (e.g. muscle recruitment and neuromuscular impairment).

The change in metabolic demand with changes in load mass are best illustrated using a theoretical comparison of carrying absolute or relative loads using previously validated predictive equations (Pandolf *et al.*, 1977) (Figure 2.1). The following theoretical example is based on a 25 kg absolute load and a mean participant body mass of 70 ± 10 kg to represent a typical load carriage task in an occupational population (Rayson *et al.*, 2000). From the data it is evident that for the 'average' 70 kg individual carrying an absolute (25 kg) or relative load (36 % body mass), the relative metabolic demand is the same (24.3 $mL \cdot kg^{-1} \cdot min^{-1}$). In the relative load condition, due to the scaling of load to body mass, for any change in body mass the relative metabolic demand remained constant. In the absolute load condition: a change in body mass of ± 10 kg from average, to an 80 or 60 kg individual, causes a change in $\dot{V}O_2$ from the relative load of -4 % (0.9 $mL \cdot kg^{-1} \cdot min^{-1}$) or +5 % (1.2 $mL \cdot kg^{-1} \cdot min^{-1}$), respectively. A

change in body mass of ± 20 kg from average, to an 90 or 50 kg individual, causes a change in $\dot{V}O_2$ from the relative load of -6 % (1.5 $mL \cdot kg^{-1} \cdot min^{-1}$) or +13 % (3.1 $mL \cdot kg^{-1} \cdot min^{-1}$), respectively. These data illustrate that the difference between carrying an absolute or relative load on the metabolic demand is minimal for the majority of an average occupational population. However, the difference in metabolic demand when carrying absolute or relative loads is greater for those with an extremely high or low body mass and is more pronounced for lighter individuals.

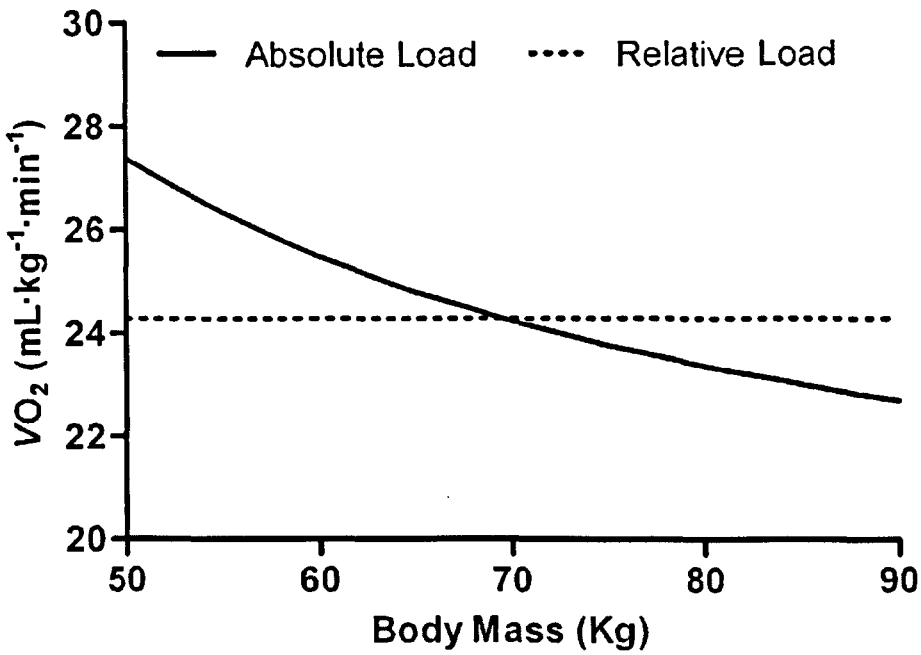


Figure 2.1 – The metabolic demand (relative $\dot{V}O_2$) for individuals (body mass range from 50 to 90 kg) carrying an absolute load of 25 kg and relative load of 36 % body mass. $\dot{V}O_2$ values calculated using equations presented by Pandolf *et al.* (1977).

Scott & Ramabhai (2000) investigated the effects of carrying absolute loads and loads relative to body mass at 4 $km \cdot h^{-1}$ over a 12 km course. The authors concluded that the relative load decreased the inter participant variation of metabolic ($\dot{V}O_2$) and cardiovascular (heart rate) responses in the group. However, in a recreational or occupational setting, load mass is likely to be set depending on the equipment required for the load carriage task and unlikely to

be distributed between members of a group depending on individual body mass. Therefore, absolute loads are more representative of recreational and occupational requirements.

2.2.3. Load Positioning

To achieve the lowest metabolic cost during load carriage the centre of mass of the load should be located as close to the body and as high in the backpack as possible (Obusek *et al.*, 1997). Stuempfle *et al.* (2004) showed that during 10 minutes of treadmill walking positioning the load centre of mass (25 % body mass) in a high compared to a low position in a backpack resulted in lower $\dot{V}O_2$ (18.6 ± 2.3 vs. 22.2 ± 3.0 $mL \cdot kg^{-1} \cdot min^{-1}$) and ratings of perceived exertion (2.8 ± 0.8 vs. 3.7 ± 1.0) but no differences in heart rate (130 ± 17 vs. 136 ± 25 $beats \cdot min^{-1}$).

Placement of the centre of mass of the load at the top of a backpack results in higher EMG levels of *m. erector spinae* and *m. trapezius*, indicating greater muscle activation, due to differences in posture with the varying load positions (Bobet and Norman, 1984). However, a variety of muscles support the backpack during load carriage (Bobet and Norman, 1982), therefore increases in activity of one muscle group is likely to decrease the contribution from another (Bobet and Norman, 1984). Devroey *et al.* (2007) showed no differences in EMG activity of the shoulders (*m. trapezius par decendens*), trunk (*m. erector spinae longissimus, m. rectus abdominis, m. obliquus externus abdominis*) or lower limbs (*m. rectus femoris*) with thoracic and lumber placement of load in a backpack (0-15 % body mass).

The above data suggest that the optimal load position is close to the body and high in the backpack. Load distribution can be controlled in scientific studies and to a degree by recreational walkers but emergency service and military personnel are restricted by equipment design and the need for easy access to certain items of equipment. Therefore, in an occupational setting the ability to achieve an optimal load distribution is not always possible.

2.2.4. Speed of Movement

There is a positive curvilinear relationship between speed of movement and energy cost during load carriage (Pandolf *et al.*, 1977). Christie & Scott (2005) showed that when walking carrying a 20 kg load, $\dot{V}O_2$ increased from 13.4 ± 1.2 to 26.8 ± 3.0 $mL \cdot kg^{-1} \cdot min^{-1}$ at treadmill speeds of 3.5 and 6.5 $km \cdot h^{-1}$, respectively (i.e. 4.5 $mL \cdot kg^{-1} \cdot min^{-1} \cdot km \cdot h^{-1}$). However,

carrying a heavier load of 65 kg caused a greater increase in $\dot{V}O_2$ from 23.7 ± 3.7 to 42.8 ± 5.6 mL·kg^{-1}·min^{-1} at treadmill speeds of 3.5 and 6.5 km·h^{-1}, respectively (i.e. 6.3 mL·kg^{-1}·min^{-1}·km·h^{-1}). Patton *et al.* (1991) supports these findings, when carrying heavy loads exacerbated the effect of increasing speed of movement on $\dot{V}O_2$. Keren, *et al.* (1981) also showed a positive relationship between $\dot{V}O_2$ and treadmill speed (6.4, 7.2, 8.0, 9.6 and 11.2 km·h^{-1}) whilst carrying a 20 kg backpack. The speed for the transition point between walking and running was approximately 8.2 km·h^{-1} and the rate of rise of $\dot{V}O_2$ with speed was greater when walking (0.6 L·min^{-1}·km·h^{-1}) than when running (0.3 L·min^{-1}·km·h^{-1}).

Kinematic analysis of movement during load carriage on a treadmill has shown that increases in speed (2.2 to 5.8 km·h^{-1}) cause greater musculoskeletal stiffness of the lower limb to maintain stability (Holt *et al.*, 2003) and increases in stride frequency and a shortening of stride length (LaFiandra *et al.*, 2003). These findings suggest greater muscular work at higher load carriage speeds. However, biomechanical changes at speeds above 5.8 km·h^{-1} and muscle activity at any increasing speeds have not been investigated.

2.2.5. Gradient

Load carriage in the field typically involves walking over both positive and negative gradients (Ainslie *et al.*, 2005). Legg *et al.* (1992), showed during treadmill walking at 4.8 km·h^{-1} carrying a 26 kg backpack, that increases in gradient (+0, +2.5 and +5 %) caused rises in $\dot{V}O_2$ (20.9 ± 2.5, 25.3 ± 2.9, 30.7 ± 2.4 mL·kg^{-1}·min^{-1}) and heart rate (122 ± 10, 135 ± 10, 155 ± 10 beats·min^{-1}), respectively. This is supported by the findings of Lloyd and Cooke (2000) which showed increases in $\dot{V}O_2$ and heart rate up to gradients of +20 % when carrying 25.6 kg at 3 km·h^{-1}.

Pimental & Pandolf (1979) first demonstrated (using a statistical model) that the energy cost of carrying a 40 kg load at 3.24 km·h^{-1} on a -10 % gradient was greater than on a level (0 %) gradient. This is in agreement with studies of unloaded walking which have observed a V-shaped relationship between $\dot{V}O_2$ and gradient (Nagle *et al.*, 1990). During unloaded walking $\dot{V}O_2$ initially decreases with gradient but between -6 and -15 % further decreases in gradient cause $\dot{V}O_2$ to increase, the turning point varies between individuals (Wanta *et al.*, 1993). Santee *et al.* (2001), showed during 20 minutes of treadmill walking

carrying 9.1 and 18.1 kg backpacks the mean optimum gradient for lowest $\dot{V}O_2$ during load carriage to be -8 %, further decreases in gradient caused $\dot{V}O_2$ to increase (up to -12 % was investigated). Lloyd & Cooke (2000) observed an initial decrease in $\dot{V}O_2$ from level walking at -5 % gradient, but $\dot{V}O_2$ increased at -12 % gradient and remained constant up to -27 %. Unlike the theoretical data of Pimental & Pandolf (1979), Lloyd & Cooke (2000) and Santee *et al.* (2001) did not observe an increase in $\dot{V}O_2$ above that measured on a level gradient.

2.3. Physiological Responses during Prolonged Load Carriage (≥ 30 minutes)

For the purpose of this thesis the duration of prolonged exercise is defined as ≥ 30 minutes. The majority of studies examining the physiological responses to load carriage have been undertaken over a duration ≤ 15 minutes. However, in most recreational and occupational settings load carriage durations range from 30 minutes to several hours (Section 2.1). Extending the duration of load carriage (i.e. ≥ 30 minutes) causes additional important physiological responses in comparison to shorter bouts (i.e. < 30 minutes).

2.3.1. Metabolic and Cardiovascular Responses

During prolonged sub-maximal exercise gradual increases over time occur in oxygen consumption (i.e. $\dot{V}O_2$drift, Gaesser and Poole, 1996) and heart rate (i.e. cardiovascular drift, Coyle and Gonzalez-Alonso, 2001). $\dot{V}O_2$ and cardiovascular drift have been observed during prolonged load carriage whilst participants walk at fixed pace carrying a constant load (Patton *et al.*, 1991).

Epstein *et al.* (1988) reported walking at 4.5 $km \cdot h^{-1}$ on a +5 % gradient carrying a 25 kg load elicited an initial exercise intensity of 45.5 ± 0.6 % $\dot{V}O_2$max at minute 20 which did not change over the 120 minutes of exercise. However, carrying a 40 kg load resulted in an initial intensity at 20 minutes of 52.1 ± 0.6 % $\dot{V}O_2$max, which steadily increased to 56.2 ± 0.6 % $\dot{V}O_2$max at 120 minutes. The authors concluded that exercise intensities greater than 50 % $\dot{V}O_2$max caused an increase in physical fatigue, leading to an increase in energy cost.

However, Patton *et al.* (1991) showed individuals working at an initial intensity as low as 26.6 ± 0.8 % $\dot{V}O_2$max experienced an increase in energy cost to 29.7 ± 0.9 % $\dot{V}O_2$max

over 120 min walking at 4 km·h^{-1} on 0 % gradient carrying a 49.4 kg load. Patton *et al.* (1991) tested the same participants carrying 5.2, 31.5 or 49.4 kg at speeds of 4, 4.7, 5.7 km·h^{-1} and observed greater $\dot{V}O_2$drift as speed or load increased (Table 2.1). Warber *et al.* (2000) supported these findings, showing that during a 240 minute walk at 5.6 km h^{-1} on +3 % gradient carrying 34.1 kg, exercise intensity increased from 36.5 ± 1.9 to 40.6 ± 2.4 % $\dot{V}O_2$max. $\dot{V}O_2$drift has also been observed when carrying 45.5 kg loads at 4 km·h^{-1} for 230 minutes (Reading *et al.*, 1996) and 225 minutes (Daley *et al.*, 1996). However, Sagiv *et al.* (1994) observed no increase in $\dot{V}O_2$ from an initial exercise intensity of 29 %VO_2max [1] whilst walking for 240 minutes carrying a 50 kg load at 4.5 km h^{-1} on 0 % gradient.

Table 2.1 – Changes in $\dot{V}O_2$ during 12 km of load carriage at speeds of 4.0, 4.7 and 5.7 km·h^{-1} carrying loads of 5.2, 31.5 and 49.4 kg (reproduced from Patton *et al.*, 1991). * indicates a significant change in % $\dot{V}O_2$max over time (P<0.05).

Speed (km h^{-1})	Load (kg)	Initial % $\dot{V}O_2$max	Final % $\dot{V}O_2$max
4.0	5.2	20.1 ± 0.7	20.1 ± 0.6
	31.5	25.0 ± 0.9	25.5 ± 1.0
	49.4	26.6 ± 0.8	29.7 ± 0.9 *
4.7	5.2	24.0 ± 0.7	24.2 ± 0.8
	31.5	29.3 ± 1.0	32.3 ± 1.2 *
	49.4	34.0 ± 1.0	39.0 ± 1.3 *
5.7	5.2	29.5 ± 1.9	29.6 ± 4.0
	31.5	36.6 ± 1.5	40.4 ± 2.0 *
	49.4	41.7 ± 1.1	50.1 ± 1.7 *

[1] Value calculated from the participants mean $\dot{V}O_2$max (mL·kg^{-1}·min^{-1}) and mean exercise $\dot{V}O_2$ (mL·kg^{-1} min^{-1}) at the start and end of exercise.

Similar trends to the $\dot{V}O_2$drift over time have also been observed with heart rate (Daley *et al.*, 1996; Patton *et al.*, 1991; Reading *et al.*, 1996; Sagiv *et al.*, 1994; Warber *et al.*, 2000), indicating the presence of cardiovascular drift (Coyle and Gonzalez-Alonso, 2001). These findings are unsurprising due to the relationship between the respiratory and cardiovascular system and the changes that occur during exercise (Åstrand, 1956). Warber *et al.* (2000) showed an increase of heart rate from 120 ± 10 to 136 ± 15 beats·min^{-1} over 240 min walking at 5.6 km h^{-1} on +3 % gradient carrying 34.1 kg. Increases in load mass and walking speed cause higher initial exercise heart rates and increases in the rate of cardiovascular drift (Patton *et al.*, 1991). Similarly to changes in $\dot{V}O_2$, Sagiv *et al.* (1994) showed no cardiovascular drift when participants carried 38 kg and 50 kg during 240 minutes of treadmill walking at 4.5 km·h^{-1}.

There are several potential explanations for the differences in $\dot{V}O_2$ and cardiovascular drift between studies. Sagiv *et al.* (1994) participants had relatively high maximum oxygen uptake values ($\dot{V}O_2$max 65.2 ± 5.0 mL·kg^{-1}·min^{-1}) compared to participants in other studies ($\dot{V}O_2$max 52.9 ± 1.9 to 60.3 ± 2.3 mL·kg^{-1}·min^{-1}). Although participants may be working at a similar aerobic intensity (% $\dot{V}O_2$max) less highly trained individuals are likely to fatigue more rapidly potentially contributing to the increased $\dot{V}O_2$ and cardiovascular drift. Sagiv *et al.* (1994) suggest their findings may differ from others due to the utilisation of a new backpack with hip and chest belts which distribute the load more evenly to the large muscle groups of the hips and legs. In the other studies, participants carried the load in a backpack and vest close to the body (Patton *et al.*, 1991) or in backpacks only (Daley *et al.*, 1996; Epstein *et al.*, 1988; Reading *et al.*, 1996; Warber *et al.*, 2000). It is plausible that the load carriage system could cause the observed differences, because the metabolic and muscular efficiency (i.e. oxygen cost and muscle activation per unit of load mass) of carrying loads varies depending on the location and distribution of the load centre of mass (Legg, 1985; Legg and Mahanty, 1985; Obusek *et al.*, 1997). Over prolonged periods these differences will become more pronounced as they may cause increased local fatigue and discomfort (Legg *et al.*, 1997) potentially altering gait resulting in a reduction in economy and increasing $\dot{V}O_2$ and heart rate. As discussed in section 2.2.2, body mass is inversely related to the metabolic cost of load carriage. However, the body mass of the participants in Sagiv *et al.* (1994) study (74 ± 8 kg) was lighter than the participants in four of the five other studies [77 ± 3 kg (Patton *et al.*,

1991), 82 ± 10 kg (Daley *et al.*, 1996), 82 ± 10 kg (Reading *et al.*, 1996), 83 ± 3 kg (Warber *et al.*, 2000)].

The physiological mechanisms responsible for $\dot{V}O_2$ and cardiovascular drift during prolonged exercise are discussed in detail in Chapter 6.

2.3.2. Thermal Responses

After the onset of exercise, body temperature begins to rise, During prolonged exercise, heat is lost if environmental temperature is lower than body temperature, but if environmental temperature exceeds body temperature there is a net heat gain and core body temperature rises (Wendt *et al.*, 2007). Shoenfeld *et al.* (1978) observed increases of core body temperature of 1.5 ± 0.7 °C and heart rate 61 ± 17 beats·min^{-1} during a 12 km walk (6 km·h^{-1}) wearing military clothing carrying 30 kg load in 21-24°C ambient temperature and 45 % relative humidity. However, only data at start and end of load carriage are presented (i.e. no data points in between), therefore it is unclear if the rate of rise of core temperature is steady or plateaus and if there is a relationship with heart rate. Byrne *et al.* (2005) presented more detailed thermal and cardiovascular responses in 35 °C ambient temperature and 55 % relative humidity during three 60 minute load carriage bouts (14 kg, 4.4 km·h^{-1}, 5 % gradient) separated by 15 minutes seated rest. Core body temperature and heart rate increased from 38.1 ± 0.1 to 39.1 ± 0.1 °C and 158 ± 5 to 162 ± 5 beats·min^{-1} between minutes 30 and 150, respectively. The figures in their manuscript show that after an initial rise in core body temperature and heart rate in the first 45 minutes both parameters show gradual increase in the following 135 minutes of load carriage. Byrne *et al.* (2005) also observed a 0.4 ± 0.3 % decrease in body mass following load carriage. However, sweat loss not been examined during load carriage in ambient conditions.

2.3.3. Neuromuscular Responses

During 120 minutes of load carriage on a treadmill (20, 25 and 32 kg backpack) Bobet & Norman (1982) used electromyography (EMG) to examine changes in muscle activation (*m. tibialis anterior, m. vastus lateralis, m. biceps femoris, m. erector spinae* and *m. trapezius*). There were no changes in average EMG signal of any muscles over time in any condition. However, the participant numbers in the study were low (n=4) and inter and intra participant variability of muscle activity was very high. The authors concluded 'a larger sample would be required to draw conclusive statements about muscle activity in load carriage *per se*'.

Therefore, this study may not accurately reflect changes in muscle activity during a prolonged duration of load carriage. However, the force producing capability of a muscle or muscle groups following exercise is considered the most accurate measure of neuromuscular function and a force decrement indicates neuromuscular impairment (Warren *et al.*, 1999) (discussed in detail in section 2.4).

The findings from research examining neuromuscular function following load carriage in field and laboratory based studies are mixed. No changes in vertical jump height (as a measure of explosive leg power) were observed following a best effort 20 km road march carrying 46 kg (mean time 314 ± 70 minutes) (Knapik *et al.*, 1991). Ainslie *et al.* (2003) also observed no change in vertical jump performance following a self paced 21 km hill walk carrying 9.5 kg (mean duration 448 minutes). However, the physical strain on the participants in Ainslie *et al.* (2003) study was relatively low; participants took 448 minutes to walk 21 km and were only carrying a 9.5 kg load resulting in a mean heart rate of 132 ± 21 beats·min^{-1}. Also, Knapik *et al.* (1991) did not describe if participants were adequately familiarised with the vertical jump technique, therefore a learning effect potentially could have occurred between pre and post testing.

In contrast, Warber *et al.* (2000) showed that following 240 minutes of load carriage on a treadmill at 5.6 km·h^{-1} and 3 % gradient, the maximal number of squat lifts completed by participants (45.5 kg barbell at a metronome-cued rate of 25 reps·min^{-1}) decreased from 52 ± 28 to 27 ± 10 reps.

In addition, Clarke *et al.* (1955) measured decreases in the force producing capability of the ankle planter flexors, pectorals, shoulder elevators, knee, hip and trunk extensors and flexors following a series of load carriage bouts. Load carriage consisted of a 12.1 km field based walk at approximately 4.0 km·h^{-1} carrying no load and loads 13, 18 and 27 kg in two different types of backpack either mounted high or low on the shoulders with or without rifles. Strength assessment was conducted using a cable-tension weight stack (described in a previous study Clarke *et al.*, 1954). Results were expressed as 'strength decrement index (SDI)' (Equation 2.1). The magnitude and statistical significance of the decrements in strength due to the march were measured by the t-ratio (the t-value used for a t-test look up table) (Equation 2.2)

Equation 2.1

$$SDI = [(Pre\ Strength - Post\ Strength) / Pre\ Strength)] \times 100$$

Equation 2.2

$$t\text{-}ratio = (Mean_1 - Mean_2) / (\sqrt{Stdev_1^2/n_1 + Stdev_2^2/n_2})$$

Only significant differences in pre and post values (scores with a t-ratio greater than 1.50) were reported in the results of the first study (Figure 2.2), a larger t-ratio value indicates a greater difference between pre and post scores. The t-ratio is a more accurate indicator of actual differences between pre and post scores than the mean SDI's. The mean SDI's are affected by extreme scores (and errors) which increase the means disproportionately to the importance of single cases. However, the t-ratio is not affected, as the extreme scores also produce greater standard deviations which in turn are reflected in the larger standard errors for these measures.

Clarke *et al.* (1955) acknowledge that there was uncontrolled variation which caused large standard deviations, reducing the ability to draw clear conclusions from the results. This may have originated from lack of control in the field based measurements or the equipment used to measure the changes in strength (i.e. cable tension weight stack measuring on a discrete scale). Despite these short falls, strength decrements were apparent in at least two of load carriage conditions in all the muscle groups measured (Figure 2.2). Across the load carriage conditions, decrements were greatest in the trunk and knee extensors and flexors.

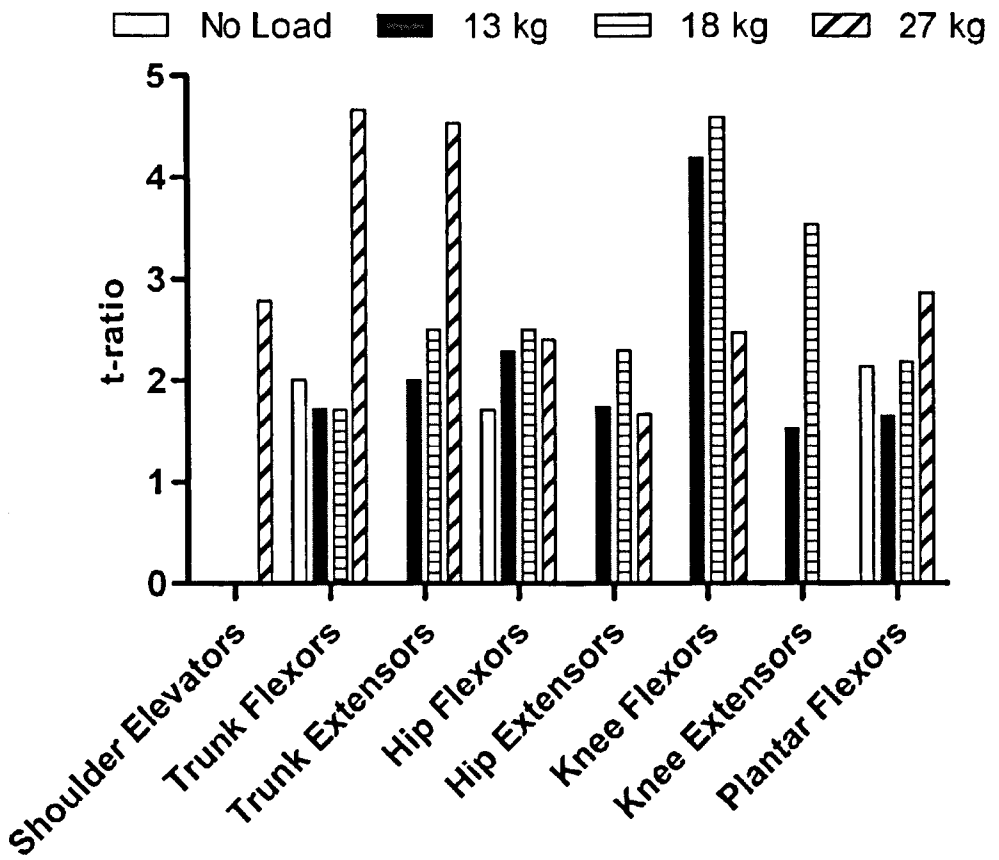


Figure 2.2 – Changes in the force producing capability of muscle groups immediately after a 12.1 km walk at approximately 4.0 $km \cdot h^{-1}$ carrying either no load or backpack loads of 13, 18 and 27 kg. Data are presented as t-ratio. Only statistically significant decreases are shown (reproduced from the data of Clarke *et al.*, 1955).

Clarke *et al.* (1955) noticed that the pre march measures gradually increased on subsequent marches and attributed this to an increase in physical fitness (military fitness test scores). This increase could also be due to a learning effect of the strength tests as no familiarisation procedures were described. The test were re-run over the same distance at the same speed with three different backpacks carrying 18 kg in each in a randomised experimental design with different participants to attempt to reduce these sources of error. The only change in the strength tests was to remove ankle planter flexion and add neck extensor assessment. Once again the results were variable between backpacks and the force producing

capability of all muscle groups measured decreased in at least one load carriage condition (Figure 2.3). The force producing capability decreased in all three load carriage conditions for the hip flexors and trunk extensors only. The greatest decrements again were for the trunk extensors and flexors and the knee extensors. However, the decrements in knee flexors force producing capability were not as high as in the initial load carriage trials.

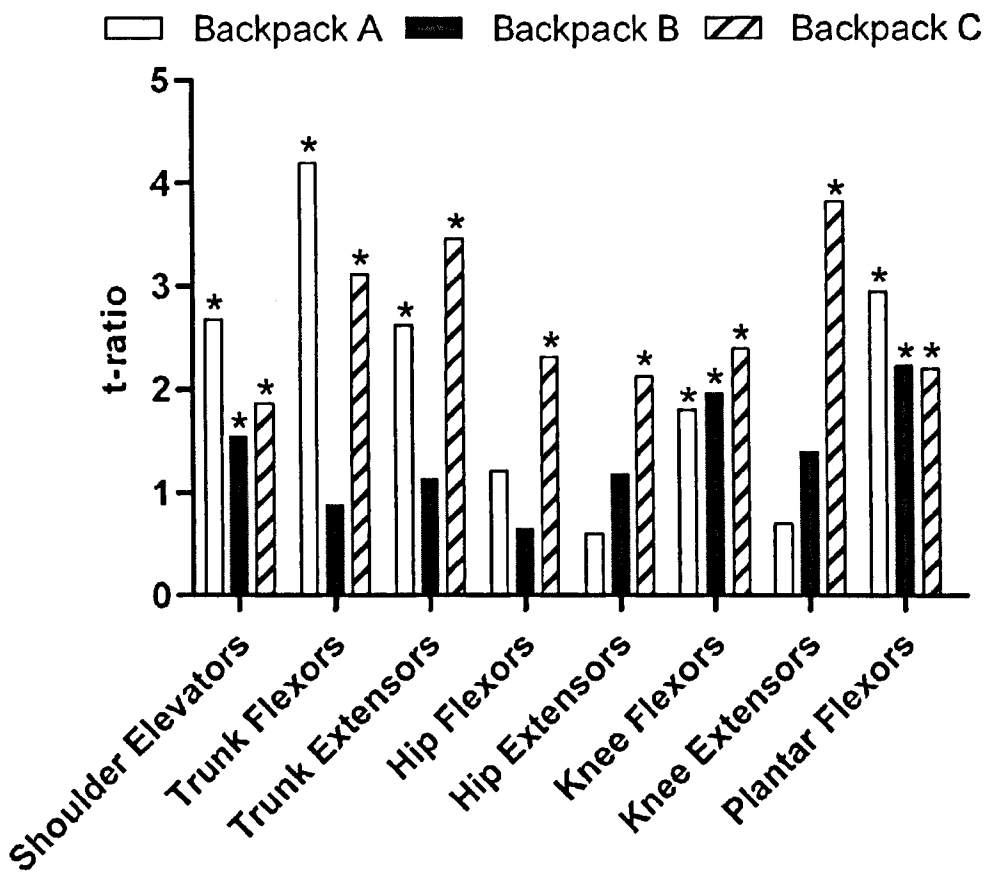


Figure 2.3 – Changes in the force producing capability of muscle groups immediately after a 12.1 km walk at approximately 4.0 $km \cdot h^{-1}$ carrying three different 18 kg backpacks. Data are presented as t-ratio. * Significant difference between pre and post strength scores (reproduced from the data of Clarke *et al.*, 1955).

Frykman *et al.* (1994) observed decreases in trunk, hip and knee angle whilst walking following a 20 km best effort load carriage event carrying 47 ± 11 kg. Similarly, Quesada *et al.* (2000) observed decreases in knee extension moment peaks following 40 minutes of

treadmill walking at 6 $km \cdot h^{-1}$ carrying backpacks equivalent to 15 and 30 % of body mass. These findings support the contentions that load carriages causes neuromuscular impairment of the trunk, hip and knee extensor and flexor muscle groups during load carriage.

Previous research has focused on changes in force producing capacity of the muscles immediately after load carriage. However, the decreases in force producing capability have been shown to last for several days following a damaging exercise bout (Warren *et al.*, 2002). The methods used to assess the decreases in neuromuscular function following load carriage have been basic with little or no attention paid to the reliability and familiarisation of participants. More sophisticated methods have been used to investigate the severity and mechanisms involved in the changes in neuromuscular function following endurance exercise (discussed in section 2.4). Reductions in the force producing capability of a muscle has negative neuromuscular and metabolic consequences (Byrne *et al.*, 2004; Tee *et al.*, 2007). Therefore, if neuromuscular function is compromised in the days following load carriage an individual's physical performance is likely to be impaired. This is particularly important in the occupational setting as following load carriage participants are often required to undertake additional physically demanding and skilled tasks such as setting up and use of equipment and military specific tasks (Knapik *et al.*, 2004), or additional bouts of load carriage (McCaig and Gooderson, 1986).

2.4. Neuromuscular Impairment

Following an exercise protocol or athletic event, reductions in neuromuscular function (i.e. force producing capability) are often referred to as neuromuscular fatigue (Andersson *et al.*, 2008; Lepers *et al.*, 2002; Strojnik and Komi, 1998) or exercise induced muscle damage (Byrne *et al.*, 2004; Howatson and van Someren, 2008). Both terms refer to a neuromuscular impairment caused by the activity and characterised by a decrease in the force producing capability of the muscle (Appell *et al.*, 1992; Edwards, 1981; Tee *et al.*, 2007; Vøllestad, 1997). Reference to neuromuscular fatigue usually (not always) refers to changes in neuromuscular function immediately after exercise (i.e. characterised by a fast recovery). Where as exercise induced muscle damage refers to the effects in the following hours or days and assumes damage to the muscle tissue (i.e. characterised by a slow recovery). Although

some of the differences between neuromuscular fatigue and damage can be determined experimentally (e.g. presence of blood myofibre proteins such as creatine kinase or histological analysis, Appell *et al.*, 1992). It is difficult to distinguish fatigue related reductions in force from injury related reductions especially immediately after exercise (Warren *et al.*, 1999). Therefore, for the purpose of this thesis, the reduction of force producing capability of a muscle group following exercise will be referred to as neuromuscular impairment.

During human locomotion (e.g. load carriage), the basic muscle function is the stretch shortening cycle, where the preactivated muscle is first stretched (eccentric action) and then followed by the shortening (concentric) action (Nicol *et al.*, 2006). Neuromuscular impairment is greatest following eccentric contractions (Newham *et al.*, 1983). Therefore, the muscles activated in this phase of the stretch shortening cycle may have the greatest losses in force generating capacity. During downhill walking greater emphasis is placed on the eccentric component of the stretch shortening cycle in the lower limbs, causing greater neuromuscular impairment in these muscle groups (e.g. *m. quadriceps femoris*) (Clarkson and Hubal, 2002). However, this has not been investigated during downhill walking with load carriage.

The pattern of neuromuscular impairment following exercise has been suggested to be bimodal (Dousset *et al.*, 2007). Typically, there is an immediate reduction in the force production of a muscle, with a small recovery within 1-2 hours. This is followed by a secondary reduction, recovery from which can last for between 4 to 8 days depending on the severity of the exercise bout (Dousset *et al.*, 2007; Nicol *et al.*, 2006).

Hargreaves (2008), describes the complex integration of both central and peripheral mechanisms which may be responsible for the losses in a muscles force producing capability following exercise (Figure 2.4). Experimental procedures to identify the precise sites of neuromuscular impairment exist but have mainly been conducted in vitro or in vivo in animal models. However, the work presented in this thesis focuses on whole muscle responses in human participants.

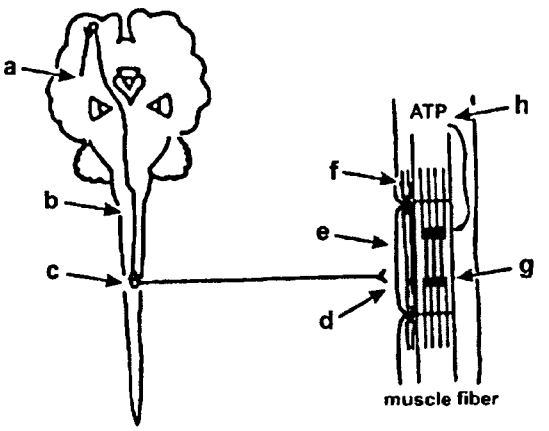


Figure 2.4 – Potential sites of neuromuscular impairment: a, excitatory input from the motor cortex; b, excitatory drive to the lower motoneuron; c, motoneuron excitability; d, neuromuscular transmission; e, sarcolemma excitability; f, excitation-contraction coupling; g, contractile mechanism; h, metabolic supply of energy. (reproduced from Bigland-Ritchie, 1981).

Armstrong *et al.* (1991) suggested the mechanisms responsible for neuromuscular impairment and the recovery of neuromuscular function following exercise can be divided into two distinct stages. First, an initial event that initiates the injury process, and second the loss of intracellular Ca^{2+} homeostasis and initiation of the Ca^{2+} overload phase. Explanation of the initial event causing muscle injury can be further divided into mechanical stress and metabolic stress models. The mechanical stress model is more widely accepted as the cause of muscle injury and implies that the mechanical stress from the forces placed on the cross bridges during the stretch shortening cycle induce failure in some fibres (Tee *et al.*, 2007). Experiments on mice muscles have shown that the degree of muscle damage is related to a combination of speed of lengthening of the muscle, peak forces and exercise duration (Kuipers, 1994). The metabolic stress model proposes that the initial events leading to muscle injury are caused by metabolic deficiencies in the working muscle which could increase the vulnerability of the working muscle fibre to mechanical stress (Tee *et al.*, 2007). The initial mechanical stress to fibres results in damage to the ultra-structure, extracellular matrix and possibly the capillaries, triggering an inflammatory response (Clarkson and Hubal, 2002).

It is assumed that the initial mechanical overload on the muscle fibres induces an increase in intracellular calcium concentration resulting in a loss of cellular Ca^{2+} homeostasis,

which may trigger a chain of events after cessation of exercise (Armstrong, 1990). This may explain the secondary loss of force in the subsequent days after exercise (Nicol *et al.*, 2006). Cytosolic Ca^{2+} levels are closely regulated within muscle cells by a number of buffering and translocation mechanisms, which can become overwhelmed with the rise in intracellular calcium concentration following muscle injury (Tee *et al.*, 2007). This leads to the activation of a number of Ca^{2+} dependent proteolytic and phospholipolytic pathways which degrade the structural and contractile myofibre proteins and well as the myofibre membrane (Armstrong, 1990; Kuipers, 1994).

Duchen *et al.* (1990) showed that a reduction in energy supply to the cells or under hypoxic conditions Ca^{2+} is released from internal stores into the cytoplasm. Therefore, during prolonged exercise when energy stores are reduced the consequences of elevated cytosolic Ca^{2+} may be exacerbated, particularly in the presence of severe glycogen depletion (Tee *et al.*, 2007). This may be limited to individual fibres rather than whole body glycogen depletion, as histological analysis of single fibres from runners following a marathon revealed damage was confined to fibres where there was almost complete glycogen depletion (Warhol *et al.*, 1985).

However, Warren *et al.* (2002) argues that not all of the neuromuscular impairment following exercise can be due to damage to the muscle structure. The authors suggest the loss in force producing capability is primarily due to failure to activate the force-generating structures (i.e. excitation-contraction coupling process) and in the following days there is no secondary loss of force. However, most of the data on which these arguments are justified are based on animal models. Although work is ongoing (e.g. Corona *et al.*, 2008; Ingalls *et al.*, 2004), Warren *et al.* (2002) indicated that it is experimentally problematic to identify all the potential sites of failure in the excitation contraction coupling process that may cause the reduction in force producing capability, which is especially true in human participants.

Neuromuscular impairment following pronged exercise in humans could be due to one or a combination of the mechanisms discussed above.

2.4.1. Measurement of Neuromuscular Impairment

Warren *et al.* (1999) conducted a detailed evaluation of the measurement tools used for the study of neuromuscular impairment following exercise primarily involving eccentric contractions. Walking with a load in the laboratory and field involves repeated cycles of

concentric and eccentric actions (i.e. stretch shortening cycle) (Nicol *et al.*, 2006). The eccentric components are likely to induce greater muscle injury and consequently neuromuscular impairment (Newham *et al.*, 1983).

Warren *et al.* (1999) argue in their review that maximal voluntary contraction torque provided the most accurate measure of changes in neuromuscular function following exercise. Range of motion is also a good indicator of neuromuscular function in a rehabilitation or clinical setting, but has poor inter-rater reliability and is rarely used in muscle injury research. Blood myofibre proteins (e.g. creatine kinase, lactate dehydrogenase and glutamic-oxaloacetic transaminase) correlate poorly with decreases in the force producing capability of a muscle and are therefore considered to be poor measures of neuromuscular impairment. Histology and soreness ratings are also poorly correlated with changes in neuromuscular function. In addition, Clarkson & Hubal (2002) suggested that as histology is usually assessed from a small needle biopsy (i.e. 10 to 50 mg) it only represents a small fraction of the muscle(s) involved. Also, the biopsy procedure has been shown to produce some changes that may be mistaken for muscle tissue damage caused by exercise (Roth *et al.*, 2000).

Measurement of neuromuscular impairment following exercise using maximal voluntary contractions has been undertaken using both isometric (Edwards *et al.*, 1977b; Millet and Lepers, 2004) and isokinetic contractions (Eston *et al.*, 1996; Nottle and Nosaka, 2005; Paddon-Jones *et al.*, 2000). However, although force producing capability is reduced by fatigue or muscle injury it may also be increased by the prior activity through increased muscle temperature and/or potentiation (Sargeant, 1994). Prior activity causes potentiation through phosphorylation of the regulatory light chains of myosin, which causes increased sensitivity of the contractile proteins to Ca^{2+} thereby enhancing the contractile response due to a greater rate of attachment of cross bridges (Rassier and Macintosh, 2000). Other mechanisms suggested for potentiation include, increased recruitment of higher order motor units and a change in the pennation angle (the angle formed by the fascicles and the inner aponeurosis) (Tillin and Bishop, 2009). However, there is less experimental evidence to support these mechanisms.

It is debatable whether a voluntary maximal effort elicits maximal recruitment of all motor units, even in highly motivated individuals (Paillard *et al.*, 2005; Warren *et al.*, 1999).

The level of voluntary activation can be assessed using an interpolated electrical stimulation during a voluntary maximal contraction (Paillard *et al.*, 2005; Shield and Zhou, 2004). Thus, the technique can be used to distinguish between the central or peripheral nature of neuromuscular impairment following exercise (Paillard *et al.*, 2005). The electrical stimulus can be applied directly to the motor nerve or via percutaneous electrical stimulation over the muscle belly, in direct comparison no differences have been observed between techniques (Newman *et al.*, 2003). The assessment of voluntary activation during a maximal contraction using an interpolated twitch was first proposed by Merton (1954). Briefly, participants performed a maximal voluntary isometric contraction and a single stimulus was applied to the muscle during the plateau phase of the contraction and at rest, immediately post contraction. Voluntary activation is then calculated (Equation 2.3). The peak force of a single electrical stimulus is markedly smaller than the force produced during a maximal contraction. Therefore, it has now become more common to use two or more stimuli (2–100 Hz) to assess voluntary activation so that evoked force increments are more readily detected during the maximal contraction (Shield and Zhou, 2004).

Equation 2.3

$$\%VA = 100 - (\text{Superimposed MVC Doublet} / \text{Post MVC Doublet}) \times 100$$

Allen *et al.* (1995b) and Behm *et al.* (1996) both raised concern that activation deficits were not being observed using the interpolated twitch technique when there was a central component to neuromuscular impairment, because the interpolated stimulus was masked by the noise of the MVC. Kooistra *et al.* (2007) also questioned the accuracy of the interpolated twitch technique to assess an exact percentage of voluntary activation. Recently, there has been debate regarding the accuracy and application of the interpolated twitch technique as a valid measure of the voluntary activation of a muscle (de Haan *et al.*, 2009a, 2009b; Horstman *et al.*, 2009; Taylor, 2009a, 2009b). In this debate questions have been raised as to whether the relationship between the superimposed and voluntary force is linear or non-linear ('S' shaped). A non-linear relationship may be due to the shortening of the muscle during contraction and potentiation during the stimulus (de Haan *et al.*, 2009a). The effect this has on the accuracy of the calculation of percentage voluntary activation has been questioned (Equation 2.3) (de

Haan *et al.*, 2009a; Taylor, 2009a). In addition, de Haan *et al.* (2009a) questioned whether measures of voluntary activation at a single joint angle could be extrapolated to other conditions. However, the general consensus of authors of these debates is that although the interpolated twitch technique may not provide an accurate measure of the percentage of voluntary activation it is best measure available to indicate the central or peripheral origin of neuromuscular impairment.

Electrical stimulation, typically a twitch, doublet or tetani, can be used to assess the contractile properties of the muscle as changes in these parameters indicate sites of failure in the excitation-contraction coupling process (Allen *et al.*, 1995a; Jones, 1996). These parameters are important as it has been suggested that approximately 75 % of the strength loss following muscle damaging exercise can be accounted for by failure of the excitation-contraction coupling process (Warren *et al.*, 2002). A decrease in the peak force of a stimulation indicates neuromuscular impairment and may be caused by a combination of damage to the muscle structure, reduced myofibrillar Ca^{2+} sensitivity and reduced Ca^{2+} release (Allen *et al.*, 1995a). Decreases in contraction time and rate of force development of a stimulated contraction indicate impairment of the excitation contraction coupling process, probably reflecting a reduced Ca^{2+} release (Martin *et al.*, 2005). A slowing of relaxation velocity reflected by a prolonged half relaxation time or greater rate of force decrease of a electrically stimulated contraction indicates slower cross bridge detachment, related to the uptake or sensitivity to Ca^{2+} (Allen *et al.*, 1995a).

Edwards *et al.* (1977a) first introduced the concept of comparing the responses of human skeletal muscle to electrical stimulation (0.5-2.0 s duration) at low (10-20 Hz) and high (50-100 Hz) frequencies. Expressing the peak force of the two stimulations as a ratio (low frequency force: high frequency force), has been used to assess for the presence of low frequency fatigue (LFF) and high frequency fatigue (HFF) (Jones, 1996). An increase in the 20:50 Hz ratio indicates HFF which is only apparent for a short (60 to 120 s) after high intensity exercise (Strojnik and Komi, 1998; Tomazin *et al.*, 2008) and has been associated with K^+ accumulation in the t-tubules and interfibre spaces of the muscle (Jones, 1996). A decrease in the 20:50 Hz ratio is indicative of LFF, an effect which can last for hours or days after the initial exercise induced muscle injury (Edwards *et al.*, 1977a; Warren *et al.*, 2002). Two processes have been suggested to contribute LFF (Jones, 1996). First, a reduction in the

release of Ca^{2+} from the sarcoplasmic reticulum during the excitation contraction coupling process. Second, damage to the sarcomeres in the middle of the fibre, which are elongated by the stronger sarcomeres at the ends of the fibre during the stretching of the muscle at long lengths. This second process has become known as the 'popping sarcomere' theory (Morgan, 1990). This theory suggests that the elongated sarcomeres in the central portion of the damaged muscle fibre will generate little force while the shortened end regions determine the contractile characteristics of the whole fibre (Jones, 1996).

The reliability of voluntary contractions has previously been investigated (Dervišević *et al.*, 2007; Impellizzeri *et al.*, 2008; Morton *et al.*, 2005). However, this has not typically been conducted over a time scale suitable for assessing recovery of neuromuscular function following exercise (i.e. 0 to 72 h post exercise). Only the reliability of the MVC and %VA with electrically evoked twitch has been investigated over such a timescale (Morton *et al.*, 2005). The reliability of measures to assess recovery of neuromuscular function will be examined and discussed in detail in Chapters 4 and 5.

2.5. Effect of Endurance Exercise on Neuromuscular Function

The measurement techniques described in section 2.4 have been used to examine changes in neuromuscular function following running, cycling and ski events ($\geq$ 20 minutes duration). However, changes following load carriage have not been examined. Previous research has primarily focused on the *m. quadriceps femoris* (knee extensors), due to their primary involvement in the stretch shortening cycle during prolonged exercise.

The most common technique used to examine neuromuscular function following prolonged exercise is the isometric maximal voluntary contraction (MVC). Decreases in MVC force have been observed following running (15 – 63 %), cycling (9 – 18 %) and cross country skiing (8 - 27 %) (Table 2.2). The greater neuromuscular impairment following prolonged running compared to cycling and skiing are probably due to the greater force per cross-bridge during the eccentric phase of the stretch shortening cycle (Nicol *et al.*, 2006). Compared to running, cycling places less emphasise on the eccentric component during the stretch shortening cycle, thus less is a force is transferred through the lower limb (Tee *et al.*, 2007). During cross country skiing, the braking action in the stretch shortening cycle is long and

relatively smooth and the vertical force seldom exceeds 1.5 times body weight (Nicol *et al.*, 2006). However during running the peak impact forces are much higher and the duration of breaking is very short (50-120 ms) (Nicol *et al.*, 2006). This repeated loading will cause the greater neuromuscular impairment following running than skiing based activities (Nicol *et al.*, 2006). During load carriage, the additional weight of a backpack will increase the downward forces during locomotion (Birrell *et al.*, 2007). These forces will be absorbed during the foot strike by the muscles supporting the backpack and involved in locomotion. This may increase the damage to these muscle groups resulting in greater neuromuscular impairment following exercise.

Table 2.2 – Percentage change in force of isometric maximal voluntary extension of the knee extensors from pre value following prolonged exercise. All exercises conducted on treadmill or cycle ergometer unless stated. (NS=non significant change).

Study	Exercise	Time Post Exercise (h)				
		0	6	24	48	120
Skof and Strojnik (2006)	6 km run at anaerobic threshold (20 minutes)	NS				
Martin *et al.* (2004b)	30 minute downhill run	-15				
Millet *et al.* (2003a)	30 km running race (189 ± 27 min)	-24				
Davies and Thompson (1986)	240 min treadmill run at maximum at 65-70 % $\dot{V}$ O_2max	-25				
Nicol *et al.* (1991)	42 km running race	-26				
Petersen *et al.* (2007)	42 km running race (154 ± 4 min)	-23			NS	NS
Place *et al.* (2004)	300 min treadmill run at 55% maximal aerobic velocity	-28				
Gauche *et al.* (2006)	55 km road running race (417 ± 55 min)	-63		-20	-26	
Millet *et al.* (2002)	65 km road running race (duration 511 ± 92min)	-30				
Lepers *et al.* (2001)	30 min cycling at 80% maximal aerobic power	-13				
Bentley *et al.* (2000)	30 min cycling at 80% $\dot{V}$ O_2max	-12	-6			
Millet *et al.* (2003c)	140 km road cycling race (duration 278 ± 25 min)	-9				
Lepers *et al.* (2002)	300 min cycling at 55% maximal aerobic velocity	-18				
Zory *et al.* (2006)	3x1.2 km cross country skiing sprints	-10				
Millet *et al.* (2003b)	42 km ski skating race (160 ± 18 min)	-8				
Takashima *et al.* (2007)	50 km ski skating race (222 ± 42 min)	-27		NS	NS	
Suzuki *et al.* (2006)	Ironman triathlon (3.8 km swim, 180 km cycle, 42.2 km run)	/	/	-30		

Electrically evoked responses of the *m. quadriceps femoris* following endurance exercise are mixed. The stretch shortening cycle during running is most similar to load carriage (compared to cycling and ski skating). Thus the mechanisms which may cause neuromuscular impairment during load carriage are likely to be most similar to those responsible for the neuromuscular impairment observed during running exercise. Therefore, changes following running based events are discussed here in more detail.

Millet *et al.* (2003a) observed a 10 % decrease from pre value in twitch peak torque following a 30 km running race. Skof *et al.* (2006) also observed a decrease of 14 % of twitch peak torque following a 6 km run at anaerobic threshold. However, Davies & Thompson (1986) and Gauche *et al.* (2006) observed no changes in twitch peak torque following a 240 min treadmill run at maximum at 65-70 % $\dot{V}O_2$max and a 55 km road race, respectively. Conversely, increases in twitch peak force have been shown following a 65 km road race (Millet *et al.*, 2002) and a 300 min treadmill run at 55 % maximal aerobic velocity (Place *et al.*, 2004). Millet *et al.* (2002) attributed this increase to potentiation of the muscle caused by the long duration of the exercise. As discussed in section 2.4.1, any change in the peak torque of electrically evoked stimulations depends on the effects of potentiation (i.e. increasing force) and neuromuscular impairment (i.e. decreasing force) (Rassier and Macintosh, 2000). Increases in twitch contraction time and the rate of force development have been shown following a range of prolonged running events (Millet *et al.*, 2002; Millet *et al.*, 2003a; Skof and Strojnik, 2006). Decreases in the half relaxation time and the rate of force relaxation have only been observed after a 65 km running race (Millet *et al.*, 2002).

A decrease in the ratio of low (10-20 Hz) and high (<50 Hz) frequency stimulations indicates the presence of LFF (Jones, 1996). LFF has only been observed following a 30 km running race (Millet *et al.*, 2003a) but was not shown following four other prolonged running events (Davies and Thompson, 1986; Petersen *et al.*, 2007; Place *et al.*, 2004; Skof and Strojnik, 2006). Decreases in voluntary activation of the knee extensors have been shown from the pre event value following a 30 km running race (98 vs. 93 %) (Millet *et al.*, 2003a), 300 min treadmill run at 55 % maximal aerobic velocity (99 vs. 78 %) (Place *et al.*, 2004), 65 km ultramarathon (82 vs. 60 %) (Millet *et al.*, 2002). Decreases in the central activation ratio (95 vs. 93 %) have also been shown immediately after a 55 km road race, however had fully recovered at 24 and 48 h post (Gauche *et al.*, 2006). These findings indicate that both central

and peripheral mechanisms are responsible for the neuromuscular impairment following prolonged running (Paillard *et al.*, 2005).

Following prolonged cycling events the changes in the responses to electrically evoked stimulations are also varied. A 30 min cycle at 80 % $\dot{V}O_2$max has been shown to reduce twitch peak torque, half relaxation time and the rate of force development (Lepers *et al.*, 2001). Also, following 300 min cycling at 55 % maximal aerobic velocity, decreases in twitch peak torque, contraction time and maximal rate of force development have been observed (Lepers *et al.*, 2002). No changes have been observed in the low to high frequency stimulation ratio following prolonged cycling events (Millet *et al.*, 2003c). Voluntary activation has been shown to decrease from the pre value following 30 minutes cycling at 80 % maximal aerobic power (84 vs. 73 %) (Lepers *et al.*, 2001), 30 min cycling at 80 % $\dot{V}O_2$max (95 vs. 91 %) (Bentley *et al.*, 2000) and 300 min cycling at 55 % of maximal aerobic velocity (94 vs. 86 %) (Lepers *et al.*, 2002) but not after a 140 km cycling race (99 vs. 97 %) (Millet *et al.*, 2003c). Millet *et al.* (2003b) showed that following a 42 km ski skating marathon the evoked twitch peak force and rate of force development and increased and contraction time decreased. There was no difference in voluntary activation pre and post race (99 vs. 98 %). But the low to high frequency stimulation ratio increased following exercise.

Although the longer duration running events show the greatest decreases in MVC force (Table 2.2) the changes in electrically evoked muscle characteristics do not appear to follow any trends; the reason for this is unclear. The most prominent changes in the electrically evoked stimulation characteristics were observed following a 65 km ultramarathon (Millet *et al.*, 2002). However, the changes in the second longest event, 55 km road race (Gauche *et al.*, 2006), were less pronounced than some of the shorter and lower intensity events. The differences in electrically evoked stimulations appear to vary between events and may be due to differences in exercise protocols (i.e. combination intensity and duration), participant characteristics (i.e. fitness and familiarity of the exercise mode) or environmental factors (e.g. terrain, temperature, gradient). The effects of load carriage on the voluntary and electrically evoked responses of the muscle have not been investigated.

2.6. Consequences of Neuromuscular Impairment

Neuromuscular impairment is likely to result in reductions in performance of exercise and control tasks in the hours and days following an initial exercise bout (Byrne *et al.*, 2004). Sargeant (1994) suggested that the reduction in force producing capability could be viewed as a 'down regulating output to prevent a metabolic crisis' and thus protecting the musculoskeletal structures from further damage. However, this down regulation is likely to have a negative impact on exercise and skilled task performance (Byrne *et al.*, 2004). The consequences of neuromuscular impairment which are particularly important following load carriage as participants may be required to perform further bouts of load carriage (Lobb, 2004; McCaig and Gooderson, 1986; Rayson *et al.*, 2007), continue with physical training programmes (Rayson, 1998; Wilkinson *et al.*, 2008) or undertake skilled tasks (Knapik *et al.*, 2004). Byrne *et al.* (2004) recently reviewed the theoretical and applied implications of neuromuscular impairment following exercise and further research has been conducted since (Chen *et al.*, 2007; Chen *et al.*, 2008; Marcora and Bosio, 2007; Tee *et al.*, 2007; Twist and Eston, 2005). The implications of neuromuscular impairment can be divided into four main categories that are discussed below.

2.6.1. Strength and Power Generating Performance

As discussed previously, neuromuscular impairment is best quantified by the decrease in force producing capacity of a muscle group (section 2.4.1). Therefore, decreases in muscular strength (e.g. ability to lift, carry or move objects) during isometric, concentric and eccentric contractions can last for days following the initial exercise bout (Warren *et al.*, 1999). For example, Byrne & Eston (2002b) showed that following 10 sets of 10 barbell squats, isometric strength was reduced by 35 % 1 hour after exercise and steadily returned towards baseline over the following days, remaining 5 % below baseline at day 7. However, peak power during a 30 second Wingate test was only reduced by 13 % 1 hour after exercise, but decreased by 18 % and 16 % of pre exercise values at 24 and 48 hours, respectively, before beginning to recover. These findings suggest that neuromuscular impairment has a greater effect on power generating performance during the second phase of muscle injury (described previously in section 2.4). In comparison, Sherman *et al.* (1984) also observed similar patterns of recovery of maximal peak torque (isokinetic leg extension 180 $°\cdot s^{-1}$) and work capacity (total work during 50 isokinetic leg extensions 180 $°\cdot s^{-1}$) at 0, 24, 72 120, 168

hours after a 42.2 km running race. The Wingate test on a cycle ergometer (Byrne and Eston, 2002b) utilises a more dynamic movement than the repeated isokinetic contractions (Sherman *et al.*, 1984) resulting in differences in the contractions during stretch shortening cycles of these movements. Therefore, the differences in recovery patterns of force and power between studies are likely to be due to the methodology used to measure power (i.e. cycling vs. dynamometry). In support of the contention that neuromuscular impairment impairs high intensity exercise performance, Twist & Eston (2005) observed decreases in repeated (10x) 10 m running sprint times 0.5, 24 and 48 hours after an eccentric muscle damage protocol (10 x 10 maximal vertical jumps, interspersed 1 minutes rest).

Numerous studies have shown neuromuscular impairment to cause reductions in muscular power during vertical jump tests (for review see; Komi, 2000). Byrne & Eston (2002a) investigated the effect of muscle injury induced by 10 sets of 10 barbell squats (70 % body mass) on 3 different types of vertical jump technique. Muscle injury caused a decrease in isometric strength and the decrease in vertical jump performance was greater for the squat than the countermovement and drop jump techniques. The authors suggested this may be because the countermovement and drop jumps incorporate an active pre-stretch phase which enhances the concentric (propulsive) muscle action, which is not present in the squat jump technique.

2.6.2. Endurance Performance

Tee *et al.* (2007) recently reviewed the metabolic effects of neuromuscular impairment and showed that muscle injury causes decreased insulin sensitivity, greater glycogen depletion and increases in metabolic rate at rest and during exercise. These changes are likely to impair endurance exercise performance.

Following a 65 km ultramarathon, Millet e*t al.* (2000) showed decreases in vertical jump performance (squat and countermovement) and corresponding decreases in RER and increases in ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}O_2$) during 4 minutes of running on an asphalt track (11 $km \cdot h^{-1}$) and cycling on an ergometer (1.5 $W \cdot kg^{-1}$ body mass). However, neuromuscular impairment only impaired economy during cycling ($mLO_2 \cdot kg \cdot min^{-1} \cdot W^{-1}$) but not running ($mLO_2 \cdot kg^{-1} \cdot km^{-1}$). More recently, in a laboratory based study, Chen *et al.* (2007) demonstrated the consequences of neuromuscular impairment during endurance exercise following a 30 minute downhill (-15%) run at 70 % $\dot{V}O_2$peak. Knee extensor MVC decreased

by 21 % following the downhill run with corresponding increases in measurements of running economy during 5 minutes of level running at 85 % $\dot{V}O_2$peak [minute ventilation (+4-9 %), heart rate (+4-7 %) and respiratory exchange ratio (RER) (+4-9 %)]. Similar changes in economy were observed during level running at 65 and 75 % $\dot{V}O_2$peak and were apparent for the 3 days following downhill running as force production of the knee extensors recovered. Similar trends in MVC force and parameters of running economy were observed in a subsequent study using the same downhill running protocol to induce muscle damage (Chen *et al.*, 2008).

Neuromuscular impairment has also been shown to decrease the distance covered during running and cycling time trials. Marcora & Bosio (2007) observed a 21 % decrease in knee extensor MVC force following 10 x 10 drop jumps, interspersed by 1 minute rest, and no change in the control group (no drop jumps). Neuromuscular impairment in the experimental group resulted in a 4 % decrease in distance covered in a 30 minute time trial performance compared to controls. Recently, Twist & Eston (2009) showed that eccentric muscle damage (10 x 10 maximal voluntary vertical jumps) decreased peak isokinetic torque (60 $°\cdot s^{-1}$), increased $\dot{V}_E$ and $\dot{V}_E / \dot{V}O_2$ during 5 minutes cycling at 60 and 80 % of maximum power and impaired 5 minute time trial performance.

Montain *et al.* (2000) investigated the effects of muscle injury induced by eccentric contractions on the thermoregulatory responses to 50 minutes of treadmill walking at 45-50 % $\dot{V}O_2$max in the heat (40 °C, 20 % relative humidity). Compared to the control condition muscle injury (identified by elevated soreness and creatine kinase concentration) resulted in a higher metabolic rate (+6 %) and greater increases in core body temperature (+0.2 °C) 7 hours after the muscle injury protocol. However, these changes are moderate and there were no thermoregulatory responses during treadmill walking 25 and 30 hours after muscle injury protocol. No measurements were made immediately after muscle injury. The effects of neuromuscular impairment on thermal responses (i.e. changes in body temperature at rest or during exercise) whilst measuring changes in the force producing capacity of the muscle or in moderate climatic conditions have not been studied.

2.6.3. Neuromuscular Control

Neuromuscular control and proprioception are also affected by muscle injury, which may effect skilled task performance (Byrne *et al.*, 2004). Deschenes *et al.* (2000) observed a

reduction in isometric MVC force of the knee extensors for 5 days following exercise induced muscle injury protocol (four sets of 25 alternating concentric and eccentric contractions of the knee extensors at 30 $°\cdot s^{-1}$). Accompanying the reduced force producing capability, there was a corresponding reduction neuromuscular efficiency (torque/iEMG) which lasted for 10 days following the initial muscle injury bout, suggesting that greater central activation (nervous stimulation) was required for the achievement of maximal force. Motor control has been also shown to be impaired following eccentric contractions of the elbow flexors during a visual pursuit tracking task (Pearce *et al.*, 1998). In addition, proprioception has been impaired following muscle injury to the elbow flexors, as participants underestimated the attainment of a target force and were unable to match joint angles (Saxton *et al.*, 1995).

2.6.4. Muscle Strains

Mair *et al.* (1996) showed that when rabbit m. *digitorum longus extensors* were fatigued, force producing capability was reduced between 93 to 97 %, resulting in a 70 to 92 % reduction in energy absorbed compared to controls. The authors conclude this is likely to increase the risk of muscle strain in fatigued muscle. Equally, force not absorbed by the muscle is likely to be transferred to the supporting structures (i.e. connective tissue and skeleton). In support of these findings, Gabbett *et al.* (2008) reported the incidence of musculoskeletal injury to be greater during sports training and competition when training loads are high or later in sporting events (i.e. when muscle force producing capability is reduced). Thus neuromuscular impairment may increase the risk of musculoskeletal injury, a problem that is well documented during load carriage (Knapik *et al.*, 1996) and in environments where load carriage is undertaken (i.e. military training) (Knapik *et al.*, 2001).

2.7. Strategies to Reduce Neuromuscular Impairment

In a recent review of the literature, Howatson & van Someren (2008) examined the current strategies used to prevent and treat neuromuscular impairment during and following exercise. The most common approaches identified in the review were nutritional and pharmacological supplementation, electrical therapy, stretching, massage and light exercise. Evidence of the effects of these strategies in reducing neuromuscular impairment is mixed and appears to be dependent on the mode of exercise-induced muscle damage and dose of treatment.

For participants undertaking load carriage in an occupational and recreational setting, the use of electrical therapy and massage is unrealistic due to the specialist equipment and techniques required. Evidence suggests that stretching and subsequent exercise provides an analgesic effect only but does not aid the recovery of neuromuscular function (Howatson and van Someren, 2008). Also, in both occupational and recreational settings the intensity of subsequent exercise is unlikely to be able to be controlled. Nutritional and pharmacological supplements present an attractive option to reduce neuromuscular impairment as they can be used both during and after load carriage. There is greater evidence of the beneficial effects of nutritional supplementation on reducing neuromuscular impairment than pharmacological interventions (Howatson and van Someren, 2008). Also, the use of dietary supplements to aid exercise performance been documented in occupational groups (Arsenault and Kennedy, 1999; Flakoll *et al.*, 2004) and sports performers (Maughan *et al.*, 2004). Two common and commercially available dietary supplements are carbohydrates and proteins and research suggests they may have beneficial effects in preventing or treating neuromuscular impairment as discussed below.

2.7.1. Carbohydrate Supplementation

The effects of carbohydrate supplementation during endurance exercise are well documented and include improved endurance capacity (i.e. time to exhaustion) and prolonged exercise performance (i.e. faster times to complete set distance) (for reviews see Ivy, 1999; Jeukendrup, 2004; Peters, 2003). These effects have been shown even with relatively low rates (16 $g \cdot h^{-1}$) of intake and generally no greater improvements have been observed with higher intake rates (Jeukendrup, 2004). Tsintzas & Williams (1998) conclude in their review that experimental evidence suggests there are two physiological mechanisms responsible for the ergogenic effect carbohydrate ingestion during exercise. First, the maintenance of blood glucose concentration late in exercise, a time when muscle glycogen contribution to energy metabolism is diminished. Second, a decreased rate of muscle glycogen utilisation, which would delay its depletion and hence the point of fatigue. However, the evidence of carbohydrate supplementation sparing muscle glycogen during exercise is mixed and appears to be dependent on exercise mode, intensity and duration (Tsintzas and Williams, 1998).

Following prolonged running events, muscle fibres have been shown to become glycogen depleted (Costill *et al.*, 1973; Warhol *et al.*, 1985). Glycogen depletion impairs the

force producing capability of the muscle (Chin and Allen, 1997) and may result in greater muscle injury (Tee *et al.*, 2007). Supplementation with carbohydrate during recovery from neuromuscular impairment caused by exercise has been shown to improve the rate of repletion of muscle glycogen stores (Millard-Stafford *et al.*, 2008), and improve subsequent exercise performance (Fallowfield *et al.*, 1995; Wong *et al.*, 2000). However, the effect of carbohydrate supplementation on the force producing capability of muscle following prolonged exercise and specifically load carriage has not been established.

2.7.2. Protein Supplementation

Protein supplements currently of research interest include whey protein or isolates (i.e. amino acids), branched chain amino acids (BCAA) and β-Hydroxy-β-Methylbutyrate (HMB). The findings of research concerning the effects of BCCA and HMB on reducing neuromuscular impairment are mixed (Howatson and van Someren, 2008). However, amino acid ingestion has been shown to improve some aspects of neuromuscular function and physical performance (Buckley *et al.*, 2008; Howatson and van Someren, 2008; van Loon, 2007).

Whey protein supplements provide a relatively high proportion of essential amino acids and have a similar amino acid composition to human skeletal muscle (Ha and Zemel, 2003). The benefits of amino acid ingestion during and following resistance exercise are well documented and include improved muscle hypertrophy (Hayes and Cribb, 2008) maintenance of a positive protein balance (Hawley *et al.*, 2006) and reduction in the concentration of plasma markers of muscle damage (van Loon, 2007). Amino acid consumption has also recently been shown to improve recovery of the force producing capability of muscles following intense resistance exercise. Buckley *et al.* (2008) observed a ~23 % decrease in isometric MVC force of the knee extensors following 100 maximal eccentric contractions. During the following 24 h, isometric MVC force did not recover to pre-exercise values when flavoured water was consumed. In contrast, consumption of 25 g of whey protein hydrolysate immediately after exercise resulted in complete recovery of isometric MVC force by 6 h post-exercise.

However, resistance and endurance exercise have different effects on protein breakdown and synthesis (Tipton and Wolfe, 1998) which may result in differences in the effects of amino acid supplementation and its impact on neuromuscular function. The effects

of whey protein supplementation on the physiological responses during prolonged exercise have not been investigated (Hawley *et al.*, 2006; Howatson and van Someren, 2008). Following exercise, ingestion of amino acids promote and provide building blocks for *de novo* protein synthesis and reduce protein degradation, thus providing a catabolic environment and improving muscle protein accretion (Koopman *et al.*, 2007).

During the second phase of muscle injury the activation Ca^{2+} dependent proteolytic and phospholipolytic pathways degrade the structural and contractile myofibre proteins (Armstrong, 1990; Kuipers, 1994). This results in an increase in contractile protein degradation and turnover which peaks between 24 and 72 hours after muscle injury (Warren *et al.*, 2002). Nosaka (2007) suggested the greater rate of protein synthesis and reduced protein breakdown when amino acids are ingested will reduce the magnitude of muscle damage and improve the rate of recovery. Therefore, it is reasonable to assume that the provision of protein supplements in the days following load carriage may enhance recovery from muscle injury, in turn reducing neuromuscular impairment. However, no studies have examined the effect of protein supplementation on the neuromuscular recovery following prolonged exercise with load carriage.

2.8. Review of the Literature Summary

Load carriage increases metabolic cost, cardiovascular strain and muscle recruitment compared to unloaded walking (section 2.2). Neuromuscular impairment following load carriage has been shown following some, but not all, load carriage tasks (section 2.3.3). This is surprising, as reductions in muscles force producing capability have been shown following a range of running, cycling and skiing exercise, lasting up to 48 hours after the initial exercise bout (section 2.4). However, recovery of neuromuscular function in the days following load carriage has not been investigated.

Walking on a downhill gradient, compared to level and uphill gradients, causes greater neuromuscular impairment, as greater emphasis is placed on the eccentric component of the stretch shortening cycle of the lower limbs, (section 2.4). However, the metabolic and cardiovascular responses during load carriage on a negative gradient have only been measured over 20 minutes (section 2.2.5) and the effect on neuromuscular function has not been investigated.

A reduction in neuromuscular function impairs endurance and high intensity exercise and may increase the risk of musculoskeletal injury which is detrimental to individuals or organisations undertaking load carriage (section 2.6). Hence, identification of the physiological characteristics of metabolic and neuromuscular performance during load carriage would be valuable for the selection and training of load carriers.

Interventions to decrease the metabolic cost and neuromuscular impairment caused by prolonged exercise would be beneficial to load carriers. Carbohydrate and protein supplements have the potential to reduce the metabolic and neuromuscular demands during load carriage and recovery of neuromuscular function after exercise (section 2.7). However, the effects of carbohydrate and protein supplements on metabolic and neuromuscular responses to load carriage have not been investigated.

2.9. Aims of Experimental Chapters

The purpose of this thesis is to investigate the physiological responses to load carriage, with particular reference to neuromuscular function. The aims of the experimental work will be addressed through a field study (Chapter 3) and three major laboratory studies, which will be presented as seven experimental chapters (Chapters 4 - 10). The aims of the experimental chapters of this thesis are to:

1. Establish the cardiovascular and neuromuscular responses to 280 minutes of load carriage in an occupational setting with trained load carriers, carrying a total load of 31 kg (Chapter 3).

2. Examine the intra and inter day reliability of dynamic contractions of the knee, trunk and shoulder extensors and flexors using isokinetic dynamometry and compare the variability in the parameters calculated from the isokinetic measurements (i.e. maximum peak torque, mean peak torque, maximum work). (Chapter 4).

3. Examine the intra and inter day reliability of voluntary and electrically stimulated isometric contractions of the *m. quadriceps femoris* and determine the most appropriate parameters for assessing changes in neuromuscular function following 120 minutes of exercise (Chapter 5).

4. Compare the metabolic and cardiovascular responses during 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) with no load and load carriage (25 kg backpack) (Chapter 6).

5. Compare the metabolic and cardiovascular responses during 120 minutes of load carriage (25 kg backpack) at 6.5 $km \cdot h^{-1}$ on level (0 %) and a negative gradient (-8 %) (Chapter 6).

6. Investigate changes in neuromuscular function following 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) with no load and load carriage (25 kg backpack) at 0, 24, 48 and 72 hours after exercise (Chapter 7).

7. Investigate changes in neuromuscular function following 120 minutes of load carriage (25 kg backpack) on level (0 %) and a negative (-8 %) gradients at 0, 24, 48 and 72 hours after exercise (Chapter 7).

8. Determine the relationships of individual physiological characteristics with metabolic and neuromuscular performance during 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) carrying a 25 kg backpack (Chapter 8).

9. Compare the effects of separate carbohydrate and whey protein beverages on the metabolic, cardiovascular and neuromuscular responses during 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) carrying a 25 kg backpack on level (0 %) gradient (Chapter 9).

10. Examine the effects of separate carbohydrate and whey protein beverages on the changes in neuromuscular function following 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) carrying a 25 kg backpack on level (0 %) gradient at 0, 24, 48 and 72 hours after exercise (Chapter 10).

Chapter 3. Physiological Responses of Royal Marine Recruits to Load Carriage in the Field

3.1. Introduction

Load carriage is a requirement of military personnel (Knapik *et al.*, 1996), members of the emergency services (McLellan and Selkirk, 2004) and is undertaken recreationally (Ainslie *et al.*, 2005; Lobb, 2004). In these settings, individuals typically carry absolute loads set by the requirements of the task (e.g. required equipment) rather than based on an individuals physical capability (Haisman, 1988; Lobb, 2004; McLellan and Selkirk, 2004). The physiological responses to load carriage depend on a combination of load mass, speed of movement, gradient, terrain and environmental conditions (Ainslie *et al.*, 2005; Knapik *et al.*, 1996; Pandolf *et al.*, 1977).

A set pace (4 $km \cdot h^{-1}$) road march carrying 40.7 kg has been shown to elicit a cardiovascular strain of 117 ± 13 $beats \cdot min^{-1}$ (Scott and Ramabhai, 2000). Knapik *et al.* (1997) studied the effects of different load mass (34, 48, 61 kg) during a maximal effort 20 km road march. Time to completion of the march increased with load mass. However, due to the greater speed of movement, heart rate on completion of the march was higher for the 34 kg load (154 $beats \cdot min^{-1}$) than the 48 kg (143 $beats \cdot min^{-1}$) and 61 kg (141 $beats \cdot min^{-1}$). Marksmanship performance also decreased following all marches, but there was no difference between loads. The decrease in marksmanship performance was attributed to fatigue of the supporting muscles, elevated post-exercise respiration or fatigue-induced tremors.

The most accurate assessment of muscle fatigue (i.e. a reduction in neuromuscular function) is a measure of the changes in the force producing capability of a muscle or muscle groups (Warren *et al.*, 1999). The vertical jump test is a useful measure of neuromuscular performance in the field as it has a low skill component, can be easily repeated and equipment is easily transportable (Welsh *et al.*, 2008). However, Knapik *et al.* (1991) observed a decrease in marksmanship ability and an 82 % elevation in perceived fatigue following a maximal effort 20 km road march carrying 46 kg, but no change in vertical jump power following the march. Also, Ainslie *et al.* (2003) showed no decrease in vertical jump performance following a self paced 21 km hill walk carrying 9.5 kg (mean duration 7 h 28 min). These findings are

surprising as Clarke *et al.* (1955), showed decreases in strength of the knee, trunk and ankle flexors and extensors and the shoulder elevators following a 12.1 km road march at 4 $km \cdot h^{-1}$ carrying 13, 18 and 27 kg loads. Also, neuromuscular function has been shown to be impaired following a range of other prolonged exercise events (i.e. ≥ 30 minutes) (Millet and Lepers, 2004). Both previous studies that used the vertical jump to assess changes in neuromuscular function have allowed participants to be self paced (Ainslie *et al.*, 2003; Knapik *et al.*, 1991). The effect on vertical jump performance following load carriage when walking at a set pace (typical scenario in the occupational setting) has not been previously investigated.

The relationship between physiological strain and body mass during prolonged load carriage in the field has not been investigated. In a laboratory-based study, Bilzon *et al.* (2001) showed when participants carried an absolute load of 18 kg during 4 minutes of treadmill running (9.5 $km \cdot h^{-1}$) there was a strong inverse relationship between oxygen uptake (relative to body mass) and body mass (r=-0.87). However, there was no relationship between oxygen uptake (relative to body mass) and maximal aerobic fitness. It has been suggested that this relationship makes heavier individuals better performers of occupational tasks such as load carriage (Vanderburgh, 2008). These findings suggest the physical strain experienced during load carriage to be greater for lighter individuals, potentially predisposing them to greater risk of neuromuscular impairment. However, the relationship between body mass and changes in neuromuscular function following load carriage is unknown.

The aims of this study were to; (1) Quantify the cardiovascular strain and changes in neuromuscular function of a Troop of British Royal Marine recruits during 19.3 km of load carriage (absolute load of 31.0 kg) in the field, (2) Investigate the relationship between body mass and cardiovascular strain during the load carriage task and (3) Investigate the relationship between body mass and changes in neuromuscular function following the load carriage task.

3.2. Methods

3.2.1. Participants

Twenty-seven Royal Marine recruits (mean ± SD, age 22 ± 3 years, height 1.78 ± 0.04 m, body mass 77.1 ± 6.1 kg, body fat 11.5 ± 3.2 %, predicted $\dot{V}O_2$max 52.6 ± 2.6 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in the study. The recruits were in week-25 of a 32 week recruit training course at the Commando Training Centre Lympstone. Royal Marine recruits were selected as participants for the study as they were experienced load carriers and had the ability to complete a prolonged load carriage event at a set pace as a group. Ethical approval for all procedures and protocols was provided by the Ministry of Defence Research Ethics Committee (MoDREC), UK. All protocols were performed in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. Participants provided written informed consent and were free from any musculoskeletal injury prior to commencing the study

3.2.2. Preliminary Measures

Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) (± 0.01 kg) and stature (Invicta Stadiometer Model IP1465, Leicester, UK) (± 0.005 m) were measured whilst wearing shorts and underwear. Skinfold measurements were taken at the *Biceps*, *Triceps*, *Sub Scapular* and *Iliac Crest* on the right side of the body using Harpenden Skinfold Callipers (Body Care, Southam, UK) [2]. Two measurements were taken at each site and if there was a difference > 5 mm the measurements were repeated. Percentage body fat was estimated following the assessment of skinfold thickness at four anatomical sites using previously described methods (Durnin and Womersley, 1974; Siri, 1956)

Participants completed a Multistage Fitness Test (MSFT) to exhaustion to determine their maximal aerobic capacity (Ramsbottom *et al.*, 1988) [2]. Participants ran between two parallel lines 20 m apart, in time to an audio signal to provide pacing. The time between the audio signals progressively decreased therefore increasing the required pace of each shuttle. The end of the test for each individual was determined if they failed to complete 3 consecutive

[2] MSFT and skinfold thickness data were collected by the Institute of Naval Medicine as part of a wider project examining training progression during Royal Marine Recruit training.

shuttles or voluntarily stopped. Participants wore heart rate monitors (Polar Team System ®, Polar, Kempele, Finland) and maximum heart rate (HRmax) was recorded as the peak heart rate during the test. A resting heart rate (HRrest) was measured whist participants slept overnight, and was recorded as the lowest value during a 30 second rolling average.

3.2.3. Experimental Protocol

Participants were monitored during a 19.3 km load carriage event (duration 280 min). Participants carried a total load of 31.0 kg on the shoulders (21.5 kg backpack, 9.5 kg webbing) whilst wearing boots (1.8 kg), and helmet, (1.7 kg) and carrying a rifle (5.0 kg). The load carriage event started at 06:00 hours approximately one hour after participants had consumed breakfast. All participants walked as a group and the speed was paced by instructors from the Commando Training Centre. Participants wore heart rate monitors (Polar Team System ®, Polar, Kempele, Finland) and carried a Global Positioning System (GPS) (Garmin® eTrex Legend™, Garmin, International Inc, Kansas, USA) for the duration of the load carriage. The vertical jump test was conducted the evening before and immediately after load carriage. Body mass was measured before each vertical jump test and on the morning before the load carriage task.

3.2.4. Heart Rate (HR)

Heart rate was recorded every 5 seconds using downloadable heart rate monitors (Polar Team System ®, Polar, Kempele, Finland). Heart rates were expressed as an absolute value, a percentage of maximum heart rate (%HRmax), and percentage of heart rate reserve (%HRR) (Equation 3.1) (Howley, 2001).

Equation 3.1

%HRR = (Mean HR During Exercise – HRrest) / (HRmax – HRrest)

The level of physical activity was classified using Howley's (2001) recommended zones for physical activity (Table 3.1).

Table 3.1 – Classification of physical activity intensity based on zones defined by percent heart rate reserve (%HRR) (Adapted from Howley, 2001).

Intensity	%HRR
Very light	<20
Light	20-39
Moderate	40-59
Hard	60-84
Very hard	≥85

3.2.5. Global Positioning System (GPS)

One participant carried a GPS (Garmin® eTrex Legend™, Garmin International Inc, Kansas, USA) which recorded the position of the group every 5 seconds. Data were downloaded on completion of the load carriage using Map Source software (Map Source™, Garmin International Inc, Kansas, USA). Speed of movement, altitude and distance covered were calculated.

3.2.6. Vertical Jump Test

The vertical jump test was performed to determine the effects of load carriage on neuromuscular performance. The test was completed the evening before and immediately after the load carriage event. The vertical jump technique was demonstrated by the investigator before pre and post testing. During pre-testing only, the participants were coached while completing 5 maximal jumps as a familiarisation (pilot data showed this was optimal number of jumps to become familiar with the technique without causing fatigue). Participants completed 3 maximal effort jumps and the mean score was recorded for the performance in each session. Participants stood on a pressure sensitive jump mat (University of Chichester, Chichester, UK) wearing standard issue military boots, trousers and shirt. The counter movement jump was performed from an upright starting position moving to approximately 90° knee flexion using their legs and arms to drive upwards. Vertical jump time was recorded to 0.001 second from the pressure sensitive jump mat. Vertical jump height and power were calculated using Equations 3.2 and 3.3.

Equation 3.2 (Bosco *et al.*, 1983)

Vertical Jump Height (m) = (9.81 × Jump Time $(s)^2$) / 8

Equation 3.3 (Sayers *et al.*, 1999)

Vertical Jump Power (W) = [(60 × Jump Height (cm)) + (45.3 × Body Mass (kg))] - 2055

3.2.7. Body Mass, Fluid Consumption and Estimated Sweat Loss

Body mass (Model 880, Seca Ltd., Birmingham, UK) (± 0.01 kg) was recorded wearing boots, trousers and shirt immediately before the pre and post vertical jump tests to account for changes in body mass when calculating vertical jump power (Equation 3.2). Participants were weighed wearing underwear only on the morning before and immediately after load carriage (after completing the vertical jump test).

Approximately, every 50 minutes during the load carriage, a 10 minute water break was taken (total 4 stops). The fluid consumption of a sub-sample of participants (n=14) was recorded by monitoring the volume of water consumed from their 1 L bottles.

Sweat loss was estimated for the sub-sample of participants (n=14), taking into account change in body mass, fluid intake, breakfast consumed, urine lost (Equation 3.4). The mass of breakfast was calculated as the mean of weighed plated meals measured at breakfast from the canteen (0.43 kg). Based on data collected during laboratory based investigations, urine losses were estimated at 0.21 L (0.21 kg) (Freund *et al.*, 1991).

Equation 3.4

Estimated Sweat Loss = (PreBodyMass – PostBodyMass) – (Meal + FluidIntake) + UrineLoss

It is acknowledged that assumptions have been made and that there are limitations in estimating body mass changes and sweat loss using this method (Maughan *et al.*, 2007). The nature of the collection of data in the field did not allow precise measurement of urine and respiratory losses and the release of water into the body pool during substrate oxidation could

not be accounted for. However, changes in body mass following prolonged exercise have been shown to be an accurate and reliable method of measuring changes in total body water (Baker *et al.*, 2009).

3.2.8. Environmental Conditions

Ambient temperature and humidity data were obtained retrospectively from the Met Office (Exeter, Devon, UK). The nearest site for data recording was Exeter Airfield (latitude 50:73 N, longitude 03:42 W, altitude 32 m), located 12.2 km and 7.75 km from the start and finish of the load carriage event, respectively. Environmental temperature (°C) and humidity (%) were recorded at 30 minute intervals.

3.2.9. Statistical Analysis

SPSS for windows V15 (SPSS, Chicago, Illinois) was used for statistical analyses. Distribution of the data was assessed using a Kolmogorov-Smirnov test. Data were normally distributed and differences were assessed separately using paired t-tests. Relationships between variables were made using Pearsons correlations. Correlations were classified as very strong ($\geq$ 0.90), strong (0.70 – 0.89), moderate (0.50 – 0.69) or weak ($\leq$ 0.49) (Fallowfield *et al.*, 2005). Data are presented as mean ± standard deviation (SD). Statistical significance was set at $P<0.05$.

All participants completed the load carriage assessment (n=27). However, one participant did not perform the vertical jump test after load carriage. Due to failure of heart rate monitors to record or non-compliance of participants, data for heart rate comparisons were made using n=19. Resting and maximum heart rates were unable to be obtained for a further seven participants therefore correlations made with HRmax and HRR during load carriage are for n=12.

3.3. Results

The GPS recorded 17.8 km of the 19.3 km load carriage assessment. Missing data were due to excessive overhead tree coverage resulting in loss of signal to the tracking satellites. Average speed during load carriage including water stops was 5.2 $km \cdot h^{-1}$ and excluding water stops was 5.7 $km \cdot h^{-1}$. Altitude varied between 17 and 200 m above sea level. From the GPS data, 74 minutes of uphill and 60 minutes downhill walking sections were identified (all sections >10 minute periods); no consistent sections of walking on a level gradient could be identified. During uphill and downhill walking, average speed was 5.5 and 6.0 $km \cdot h^{-1}$ and total vertical distance climbed and descended was 279 and 376 m, respectively. Ambient temperature and humidity were 13.4 ± 1.3 °C and 83.6 ± 8.8 %, respectively.

Heart rate during load carriage was 145 ± 10 $beats \cdot min^{-1}$ including water stops and was 2 % higher excluding water stops (148 ± 11 $beats \cdot min^{-1}$, $P<0.001$). This represented 72 ± 5 %HRmax (64 ± 5 %HRR) including water stops and 73 ± 5 %HRmax (66 ± 5 %HRR) excluding water stops. Using the uphill and downhill sections identified from the GPS data, heart rate during uphill walking was 153 ± 11 $beats \cdot min^{-1}$ (i.e. 76 ± 5 %HRmax, HRR 70 ± 6 %HRR) and 7 ± 1 % lower during downhill walking (i.e. 71 ± 4 %HRmax, 143 ± 11 $beats \cdot min^{-1}$, 63 ± 6 %HRR, $P<0.001$).

The relationship of body mass and $\dot{V}O_2max$ with heart rate (%HRR) during load carriage was examined in a sub-sample of participants. There was a *strong* negative relationship between body mass and %HRR (r=-0.72, $P=0.009$) (Figure 3.1). There was no relationship between relative $\dot{V}O_2max$ (relative to body mass, assessed by multistage fitness test) and %HRR during load carriage (r=-0.40, $P=0.198$) (Figure 3.2) but there was a strong relationship between absolute $\dot{V}O_2max$ and %HRR during load carriage (r=-0.71, $P=0.009$)

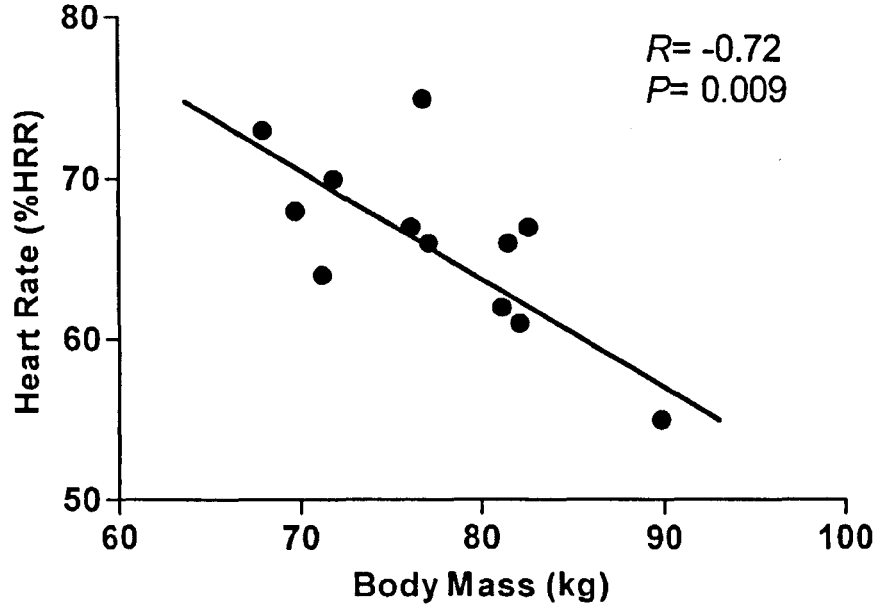


Figure 3.1 – Relationship between body mass and heart rate (%HRR) measured during load carriage, walking 19.3 km at 5.2 km·h-1 carrying 31.0 kg (n=12).

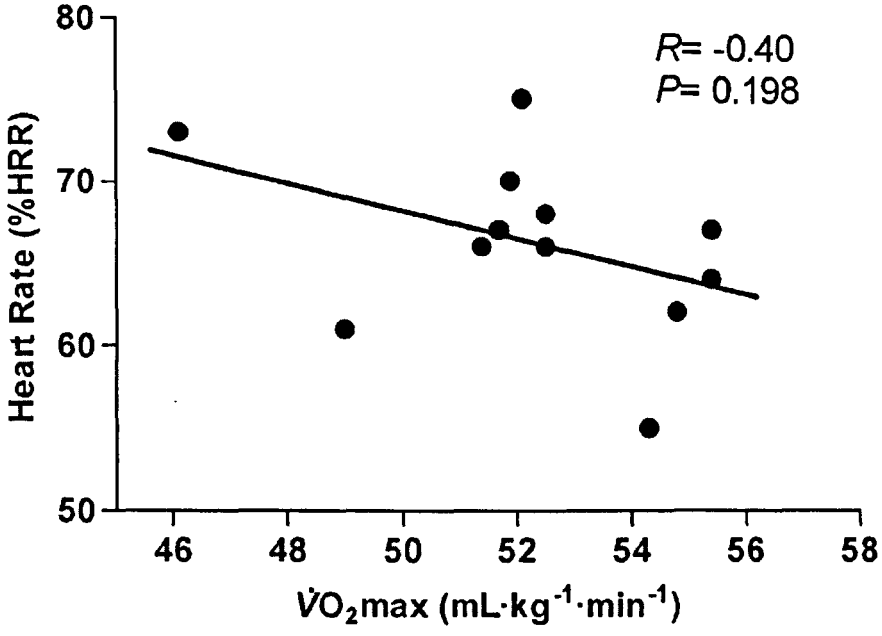


Figure 3.2 – Relationship between predicted $\dot{V}$O2max (mL·kg^{-1}·min^{-1}) measured during the multistage fitness test and heart rate (%HRR) measured during load carriage, walking 19.3 km at 5.2 km·h-1 carrying 31.0 kg (n=12).

Vertical jump height decreased by 7 ± 8 % following load carriage (0.37 ± 0.05 vs. 0.34 ± 0.06 m, $P<0.001$). There was a 4 ± 5 % decrease in vertical jump power following load carriage (3802 ± 444 vs. 3654 ± 524 W, $P<0.001$) (Figure 3.3). There was a *moderate* relationship between the change in vertical jump power (pre-post) and body mass (r=-0.65, $P=0.021$) (Figure 3.4). There was a *strong* relationship between %HRR during load carriage and change in vertical jump power (r=0.70, $P=0.012$) (Figure 3.5) and change in vertical jump height (r=0.71, $P=0.009$). However, there was no relationship between change in vertical jump power and pre vertical jump power (r=0.44, $P=0.154$) or aerobic fitness (i.e. MSFT performance) (r=0.36, $P=0.258$). But there was a moderate relationship between absolute $\dot{V}O_2$max and change in vertical jump power (r=-0.61, $P=0.035$).

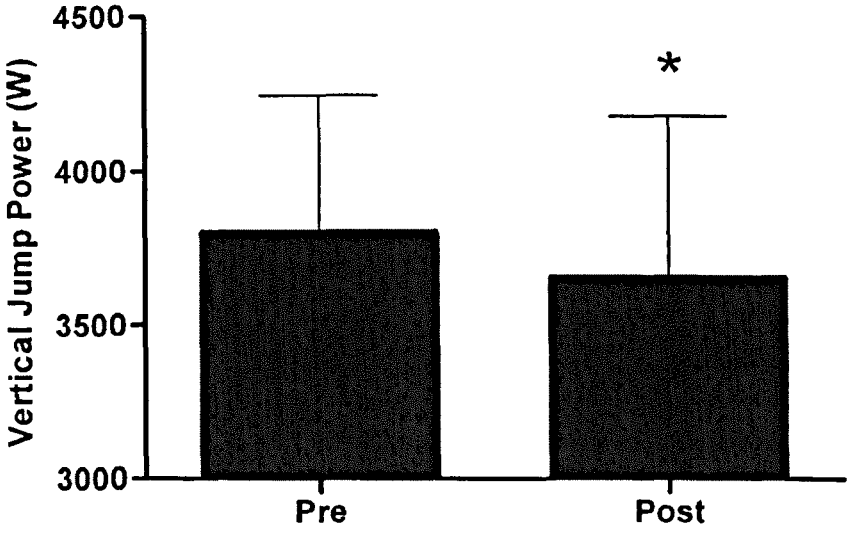


Figure 3.3 - Vertical jump power before and after load carriage, walking 19.3 km at 5.2 km·h-1 carrying 31.0 kg. * significant decrease pre vs. post P=0.009 (n=26).

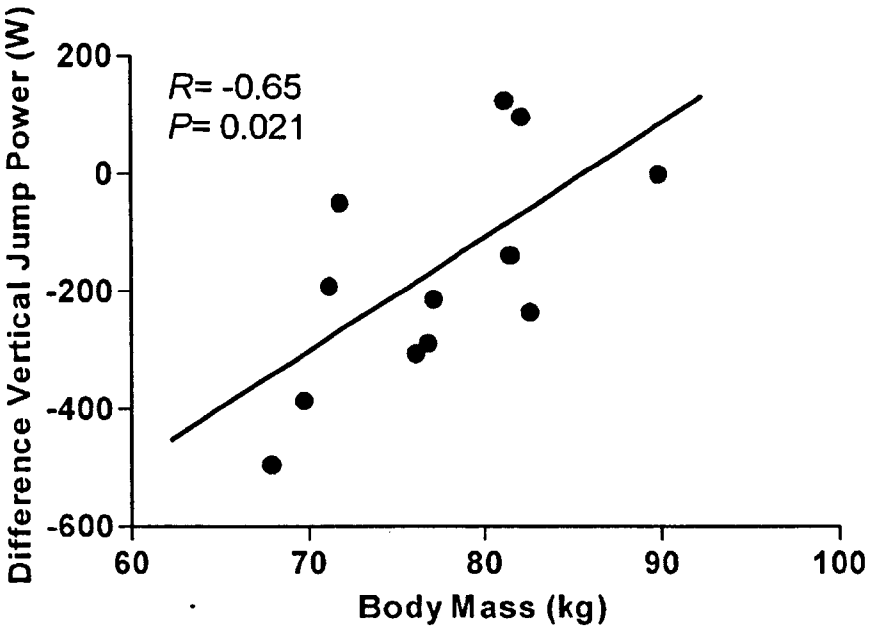


Figure 3.4 – Relationship between body mass and change in vertical jump power (indication of a change in neuromuscular function) measured during load carriage, walking 19.3 km at 5.2 km·h^{-1} carrying 31.0 kg (n=12).

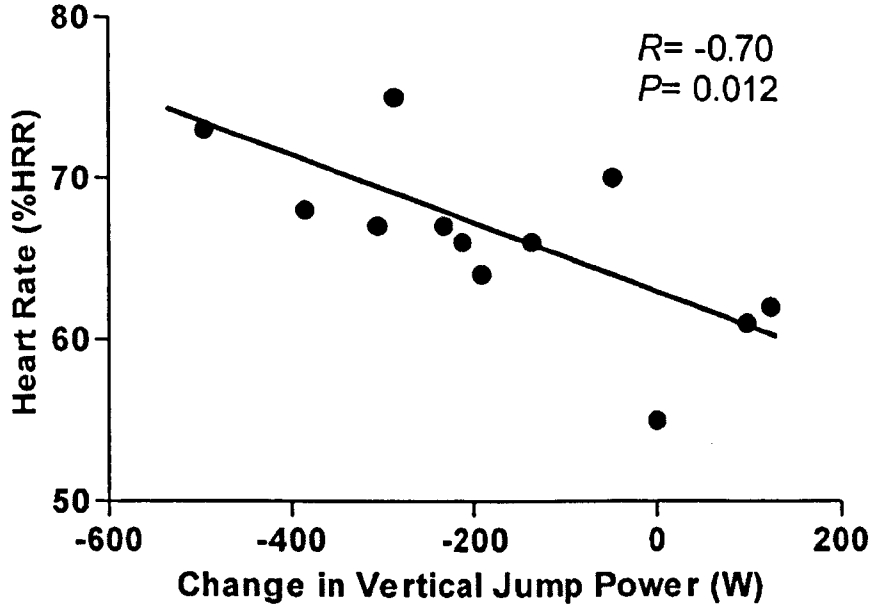


Figure 3.5 – Relationship between change in vertical jump power and heart rate (%HRR) measured during load carriage, walking 19.3 km at 5.2 km·h^{-1} carrying 31.0 kg (n=12).

After accounting for fluid and food intake and urine losses, body mass decreased by 3.9 ± 0.8 kg following load carriage (77.3 ± 7.1 vs. 73.4 ± 6.9 kg, $P<0.001$). The decrease was equivalent to 5.1 ± 1.0 % of pre load carriage body mass. Fluid (water) intake over the duration of the load carriage was 2.24 ± 0.65 L (0.48 ± 0.41 $L \cdot h^{-1}$). The decrease in body mass was greater than the intake of fluid during load carriage ($P<0.001$).

3.4. Discussion

This study investigated the cardiovascular strain and changes in neuromuscular function as a result of 19.3 km of a load carriage event carrying 31.0 kg. Participants operated at 72 ± 5 %HRmax (66 ± 5 %HRR) during load carriage, which increased during uphill walking and decreased during downhill walking, despite respective slower and faster walking speeds. A unique finding for a prolonged load carriage task in the field was that there were strong negative correlations between body mass and absolute $\dot{V}O_2$max with cardiovascular strain during load carriage (%HRR). However, there was no relationship between aerobic fitness (relative $\dot{V}O_2$max) and cardiovascular strain (%HRR) during load carriage. Following load carriage, there was a 7 ± 8 % decrease in vertical jump height and a 4 ± 5 % decrease in vertical jump power. Two novel findings were that there were moderate negative correlations between body mass and absolute $\dot{V}O_2$max with change in vertical jump power following load carriage. There was also a strong positive relationship between change in vertical jump power and cardiovascular strain (%HRR) during load carriage. However, there were no relationships between change in vertical jump power and pre vertical jump power or relative $\dot{V}O_2$max.

Using Howley's (2001) classification criteria (Table 3.1), the cardiovascular strain during load carriage (excluding water stops) (66 ± 5 %HRR) and uphill (70 ± 6 %HRR) and downhill (63 ± 6 %HRR) sections corresponded to a 'hard' exercise intensity. When water stops were included in the analysis there was a small (2 %, $P<0.001$) reduction in mean heart rate during load carriage with exercise intensity still classified as 'hard'. Although the stops provided an opportunity to consume water and remove backpacks, they resulted in a relatively small reduction in overall cardiovascular strain. The classification criteria (Table 3.1) are based on periods of up to 1 hour of physical activity, extrapolating the exercise intensity to categories for an 8 hour working day this load carriage event compares with the highest category of physical activity ('very heavy') (Howley, 2001). These classification criteria (range 1 to 8 hours) demonstrate the high physiological strain experienced by participants during the 4.5 hours of load carriage.

Using previously validated equations to calculate the oxygen cost of submaximal exercise incorporating the variables of participant body mass, heart rate and $\dot{V}O_2$max, the exercise intensity during load carriage was approximately 33.5 ± 4.6 $mL \cdot kg^{-1} \cdot min^{-1}$ (Keytel *et al.*, 2005). However, this estimation is made using equations validated during running,

cycling, rowing and stepping (i.e. non load carrying activity). Previously validated equations to estimate the energy cost of load carriage can also be used to estimate the oxygen cost of load carriage (Pandolf *et al.*, 1977), but the only physiological variable included in these equations is participant body mass. The equations of Pandolf *et al.* (1977) estimate that exercise intensity during load carriage in the present study was approximately 19.1 ± 0.6 $mL \cdot kg^{-1} \cdot min^{-1}$. It is likely the true oxygen cost of the load carriage task in the present study is at a point between 19.1 ± 0.6 and 33.5 ± 4.6 $mL \cdot kg^{-1} \cdot min^{-1}$.

Mean heart rate was higher during uphill compared to downhill sections, despite a higher speed during downhill walking. Similar results have been shown in laboratory and field trials; both heart rate and oxygen uptake ($\dot{V}O_2$) have shown corresponding increases and decreases with gradient during load carriage (Pandolf *et al.*, 1977; Santee *et al.*, 2001). Compared to walking on a level gradient, downhill walking places greater emphasis on the eccentric component of the stretch shortening cycle of the supporting muscles. Eccentric contractions result in greater neuromuscular impairment compared to concentric contractions and therefore greater reductions in a muscles force producing capability (Newham *et al.*, 1983). Carrying a load requires additional force and control from the muscles to maintain movement and posture and has been shown to increase muscle fibre recruitment of the shoulders (Holewijn, 1990), trunk (Bobet and Norman, 1984) and lower limbs (Ghori and Luckwill, 1985; Han *et al.*, 1992), which may increase fatigue or damage of these muscles compared to unloaded walking. However, the effect of gradient during load carriage on neuromuscular impairment in these specific muscle groups could not be established in the present study.

The best measurements to use to quantifying functional impairment are those that directly measure the force producing capability of muscle (Warren *et al.*, 1999). Functional impairment of the muscle has been measured following a range of endurance events using maximal voluntary contractions of specific muscle groups (primarily *m. quadriceps femoris*) (for review see Millet and Lepers, 2004). The present study showed a 7 ± 8 % decrease in vertical jump height and a 4 ± 5 % decrease in vertical jump power following the load carriage event indicating the presence of neuromuscular impairment, in particular to the lower limbs (Warren *et al.*, 1999). Reductions in neuromuscular function have been shown to last for hours or days after an initial bout of activity (Byrne *et al.*, 2004). However, due to the requirement

of participants in this study to continue with the military training programme, further measurements of neuromuscular function on subsequent days could not be obtained. Also, due to the vertical jump test being a general measure of muscular power (Sayers *et al.*, 1999), the muscle groups most prone to fatigue or damage could not be identified in the present study.

The participants in the present study were in week 25 of a 32 week military training programme, during which the load mass and intensity and duration of the load carriage tasks were gradually increased. By undertaking load carriage bouts in the weeks leading up to the load carriage bout in the present study, participants are likely to have benefited from the *repeated bout effect*. This phenomenon refers to the adaptation where a single bout of eccentric exercise (i.e. load carriage) protects against muscle damage from subsequent eccentric bouts (McHugh, 2003). Therefore, the neuromuscular impairment observed in the present study may have been greater in less well trained individuals or those unfamiliar with load carriage.

The decrease in vertical jump height observed in the present study is in contrast to previous findings (Ainslie *et al.*, 2003; Knapik *et al.*, 1991). Ainslie *et al.* (2003) showed no change in vertical jump performance following a self paced 21 km recreational hill walk carrying 9.5 kg (mean duration 7 h 28 min). However, the physical strain appears to be higher for the participants in the present study. The participants in the study of Ainslie *et al.* (2003) walked a similar distance (21.0 vs. 19.3 km); however, the load carried was lighter (9.5 kg vs. 31.0 kg), completion time was slower (mean 448 vs. 280 min) and mean heart rate during load carriage was lower (132 ± 21 vs. 145 ± 11 beats·min^{-1}). Knapik *et al.* (1991) also showed no change in vertical jump performance following a maximal effort 20 km road march carrying 46 kg. However, Knapik *et al.* (1991) did not describe if participants were initially familiarised with the vertical jump test technique, therefore a learning effect may have occurred between pre and post testing. Also, the duration of load carriage was less in the current study (280 ± 0 vs. 314 ± 70 mins), therefore there may have been a difference in exercise intensity.

Byrne & Eston (2002a) showed that neuromuscular impairment causes greater decreases in vertical jump performance for the squat than the countermovement and drop jump techniques. The authors suggested this may be because the countermovement and drop jumps incorporate an active pre-stretch phase which enhances the concentric (propulsive) muscle

action, which is not present in the squat jump technique. The present study applied a countermovement jump technique, therefore it is reasonable to assume that reductions in performance would also be observed if using a squat or drop jump technique. However, neither Ainslie *et al.* (2003) or Knapik *et al.* (1991) described the vertical jump technique adopted in their studies.

Estimated fluid loss was not matched by water intake in the present study, which would suggest that participants became dehydrated during load carriage (Maughan *et al.*, 2007). Dehydration results in a decreased plasma volume, therefore a reduced stroke volume with a compensatory rise in heart rate to maintain cardiac output. Depending on the environmental conditions and clothing, peripheral blood flow is also reduced to compensate, which may cause core body temperature to rise more rapidly, therefore placing further strain on the circulatory and thermoregulatory systems (Sawka, 1992). In the present study, body mass decreased 5.1 ± 1.0 % of pre load carriage body mass. A decrease equal to 4.3 % of body mass has been shown to reduce walking endurance by 48 % and $\dot{V}O_2$max by 22 % (Craig and Cummings, 1966). Dehydration also degrades muscular performance. In a recent review, Judelson *et al.* (2007) showed dehydration caused decreases in muscular strength (~2 %), power (~3 %) and high intensity endurance (~10 %). Therefore, dehydration may have contributed to the decrease in vertical jump performance following load carriage. These findings indicate the presence of dehydration is associated with an increased cardiovascular strain during load carriage and might affect subsequent endurance and muscular performance.

Following load carriage, individuals are often required to perform tasks (Knapik *et al.*, 1997) or further bouts of load carriage (Lobb, 2004). Neuromuscular impairment may have a negative effect on the ability of individuals to undertake these tasks other than just the consequence of decreased muscular strength. Chen *et al.* (2007) demonstrated the metabolic consequences during endurance exercise from a decrease in the force producing capability of the *m. quadriceps femoris*. The study showed a 21 % decrease in force production of the knee extensors immediately after a 30 minute downhill run (-15 % gradient) with corresponding increases in minute ventilation (4-9 %), heart rate (4-7 %) and the respiratory exchange ratio (4-9 %); these responses were apparent for the following 3 days as force production of the knee extensors recovered. In addition, Deschenes *et al.* (2000) showed neuromuscular efficiency (measured by EMG) was compromised when force producing capability of the

muscle was reduced following exercise induced muscle damage. During repeated (10x) 10 m sprints, Twist & Eston (2005) observed decreases in sprint times 0.5, 24 and 48 hours after an eccentric muscle damage protocol (10 x 10 maximal vertical jumps, interspersed 1 minutes rest).

The present study showed that during load carriage there was a strong inverse relationship between body mass and cardiovascular strain but no relationship between relative $\dot{V}O_2$max and cardiovascular strain. Similarly, during treadmill running (9.5 $km \cdot h^{-1}$) carrying an 18 kg backpack in the laboratory, Bilzon *et al.* (2001) showed a strong inverse relationship between oxygen uptake (relative $\dot{V}O_2$) and body mass ($r=-0.87$, $P<0.01$), but no relationship between relative $\dot{V}O_2$max and oxygen uptake ($\dot{V}O_2$) ($r=0.12$, $P>0.01$). The strong relationship between the respiratory and cardiovascular system and the changes that occur during exercise (Åstrand, 1956), suggest that reciprocal trends in heart rate and $\dot{V}O_2$ would have been observed by Bilzon *et al.* (2001) and in the present study. In addition the present study showed a strong inverse relationship between absolute $\dot{V}O_2$max and cardiovascular strain during load carriage.

There were moderate positive relationships between body mass and change in vertical jump power and absolute $\dot{V}O_2$max and change in vertical jump power following load carriage. But there was no relationship between maximal aerobic fitness (relative to body mass) or pre-vertical jump power and decrease in vertical jump performance following load carriage. This has not been previously demonstrated and suggests that decrements in neuromuscular function are greater for lighter individuals and those with a lower absolute $\dot{V}O_2$max. There was also a relationship between cardiovascular strain during load carriage and the decrease in vertical jump performance. However, it is unclear if the decrease in neuromuscular function was a cause or consequence of the higher cardiovascular strain.

Interestingly, selection tests for the military and emergency services currently only measure aerobic fitness relative to body mass (e.g. MSFT or timed run) (Bilzon *et al.*, 2001; Vanderburgh, 2008; Vanderburgh and Crowder, 2006). These measures of aerobic fitness favour lighter individuals (Vanderburgh, 2008). However, results of the current study show no relationship between aerobic fitness (relative to body mass) and the physiological demands of load carriage. The findings suggest that lighter individuals are at a disadvantage during load

carriage tasks when an absolute load is carried (as in an occupational setting) as a higher cardiovascular strain and oxygen cost are experienced and greater decrements in neuromuscular function is evident when undertaking the same load carriage tasks.

In conclusion, cardiovascular strain during prolonged load carriage with an absolute load was deemed to be high and the event caused a decrease in neuromuscular function, both of which are likely to have been exacerbated by dehydration. The reduction in the force producing capability of the muscle is likely to have negative consequences on strength, sprint performance, endurance exercise and neuromuscular control tasks. The reduction in neuromuscular function may also be apparent in specific muscle groups in the hours or days following load carriage, but these were not assessed in the present study. Individuals with lower body mass and absolute $\dot{V}O_2max$ appear to be at a greater disadvantage during load carriage with an absolute load as they experience a greater cardiovascular strain and decreases in neuromuscular function.

Chapter 4. Intra- and Interday Reliability of Isokinetic Contractions of Knee, Trunk and Shoulder Extensors and Flexors

4.1. Introduction

Chapter 3 showed that a 19.3 km load carriage event (5.2 $km \cdot h^{-1}$, carrying 31.0 kg) caused neuromuscular impairment. The vertical jump technique did not allow for the force producing capability of individual muscle groups to be measured. The impact of the load carriage bout on the functional capability of the muscles could not be accurately measured in the following days as participants were undertaking a military training programme, which involved repeated days of vigorous exercise. This chapter will assess the reliability of a series of laboratory based tests over a timescale that is common for measuring recovery of neuromuscular function following two hours of exercise (immediately post, 24, 48, 72 hours). Thus, a battery of tests to measure multiple muscle groups over successive days will be examined in an environment where vigorous physical activity can be controlled.

Exercise of an unaccustomed, high intensity or prolonged nature can result in muscle fatigue and damage (i.e. neuromuscular impairment) (Appell *et al.*, 1992). Neuromuscular impairment is associated with a reduction of the functional capacity of the muscle, which has been observed to persist for hours and days following an initial bout of activity (Warren *et al.*, 1999). Changes in neuromuscular function are most accurately assessed by measuring the amount force or torque (i.e. force per unit angular rotation) a muscle or muscle groups can produce (Warren *et al.*, 1999), torque is accurately measured using isokinetic dynamometry (Dvir, 1995).

The *m. quadriceps femoris* are prone to fatigue and injury following physical activities and are relatively easy to access compared with other muscle groups. Therefore the *m. quadriceps femoris* are frequently used in studies assessing neuromuscular impairment following exercise (Bentley *et al.*, 2000; Lepers *et al.*, 2001; Martin *et al.*, 2004a; Millet and Lepers, 2004). However, dynamic whole body physical activity requires the use of a range of muscle groups. Overuse of these muscle groups is likely to result in neuromuscular impairment (Appell *et al.*, 1992). This has adverse neuromuscular (Byrne *et al.*, 2004) and

metabolic (Tee *et al.*, 2007) consequences which can impact on athletic or occupational performance in the days following an exercise bout (Warren *et al.*, 2002).

Isokinetic dynamometry has been used to assess the decreases and recovery of torque producing capability of the muscle following activities known to cause neuromuscular impairment (Eston *et al.*, 1996; Lepers *et al.*, 2001; Nottle and Nosaka, 2005; Paddon-Jones *et al.*, 2000) A typical method is to measure the maximal amount of torque or work a muscle group can produce before the activity. Recovery of the torque or work producing capacity of the muscle is then measured in the following hours and days (e.g. Byrne and Eston, 2002a; Eston *et al.*, 1996; Gauche *et al.*, 2006). Knapik & Ramos (1980) suggested with different isokinetic test velocities that the motor tasks become more dissimilar, requiring different patterns of neural recruitment and co-ordination. Therefore, multiple test velocities are commonly used to assess neuromuscular function under a range of different conditions.

The reliability of isokinetic dynamometers has been previously assessed to measure extensors and flexors of the knees (Impellizzeri *et al.*, 2008; Pierce *et al.*, 2006; Symons *et al.*, 2005), trunk (Byl and Sadowsky, 1993; Dervišević *et al.*, 2007; Madsen, 1996) and shoulders (Mayer *et al.*, 1994; Orri and Darden, 2008). These studies have assessed both intra and inter – day reliability normally using a test and single re-test design or a multiple re-test design. However, the reliability of the measurements of the knee, trunk and shoulder extensors and flexors over a time scale suitable for assessing changes in neuromuscular function following muscle damage and the subsequent recovery (i.e. immediately post, 24, 48, 72 hours) has not been examined.

The aim of this study was to examine the reliability of dynamic contractions of the extensors and flexors of the knee, trunk and shoulder using isokinetic dynamometry 2, 24, 48 and 72 hours after baseline. A secondary aim was to compare the reliability of the parameters calculated from the isokinetic measurements (i.e. maximum peak torque, mean peak torque, maximum work) for each muscle group.

4.2. Methods

4.2.1. Participants

Ten healthy male participants (mean ± SD, age 30 ± 8 years, height 1.79 ± 0.05 m, body mass 79.4 ± 8.3 kg) volunteered to participate in this study. Ethical approval for all procedures and protocols was provided by the University of Chichester Ethics Committee. All protocols were performed in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. Participants provided written informed consent and were free from any musculoskeletal injury prior to commencing the study.

Participants were instructed to abstain from any vigorous and unaccustomed physical activity 24 hours before and for the duration of the study to avoid muscle damage and arrive in the laboratory in a rested state. Before baseline testing, participants were questioned to ensure they were adequately rested and free from muscle injury and completed a muscle soreness questionnaire. The questionnaire provided a map of the body divided into 12 segments (Corlett and Bishop, 1976); the perception of muscle soreness in each segment was rated on a visual analogue scale from 0 (no soreness) to 10 (unbelievable soreness). All participants reported the lowest rating of 0 (no soreness) for all body segments before the baseline testing session.

Calibration of all dynamometers was checked prior to the experiment using the dynamometers inbuilt calibration procedure. Calibrated weights were attached to the testing arm in increasing increments and the applied torque (from calibrated weights) was compared to the resultant torque (recorded by the dynamometer).

4.2.2. Preliminary Measures and Familiarisation

Participants Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) (± 0.01 kg) and stature (Avery Berkel, Smethwick, UK) (± 0.005 m) were measured whilst wearing shorts and underwear.

At least 5 days prior to beginning the experimental protocol, participants were familiarised with all test procedures by completing one complete cycle of the experimental protocol (described in detail below). A test procedure was repeated if the experimenter or participant thought that a maximal effort was not given or a learning effect was still apparent in the final contractions.

4.2.3. Experimental Protocol

Participants completed the voluntary contractions described below at 0 (baseline), 2, 24, 48 and 72 hours to assess reliability of test procedures. The test order is described below and was kept the same on each occasion (Figure 4.1) and conducted at approximately the same time of day to control for diurnal variation in torque producing capability of the muscles (Sedliak *et al.*, 2008). During all tests, standard verbal encouragement was provided (Keating and Matyas, 1996). Slow to fast test velocities were chosen (details described below) as there are known variations in motor unit recruitment patterns and muscle fibre composition between individuals and between muscle groups in any one individual (Perrin, 1993).

Knee and shoulder extension and flexion data were recorded (Cybex II isokinetic dynamometer, Cybex, Measham, UK) using HUMan Assessment Computer (HUMAC) software V40 (Computer Sports Medicine Inc, Norwood, USA) at 100 Hz and exported to Microsoft Excel 2002 for Windows (Microsoft, Redmond, Washington) for analysis. Data were corrected for the effect of gravity (Fillyaw *et al.*, 1986). Trunk extension and flexion data were recorded (Isokinetic trunk strength dynamometer, Akron Therapy Products, Ipswich, UK) at 100 Hz using Akron software V2.4 (Akron Therapy Products, Ipswich, UK) and exported for analysis in Microsoft Excel 2002 for Windows. Data were not corrected for the effect of gravity due to the limitations of the dynamometer. Although this is not considered best practice (Keating and Matyas, 1996), as data were not corrected in each session the test-retest reliability can still be accurately assessed. Caution would be required if extension/flexion ratios were calculated or if comparisons of parameters (e.g. peak torque, work) are made with data which has been corrected for the effect of gravity.

Slower test velocities were tested first for all isokinetic contractions to increase reproducibility of results between tests (Wilhite *et al.*, 1992). For all contractions, the angular velocity was calculated every 0.01 seconds during the movement and data were removed if they were not collected during the isokinetic phase of the movement or showed torque overshoot (Perrin, 1993).

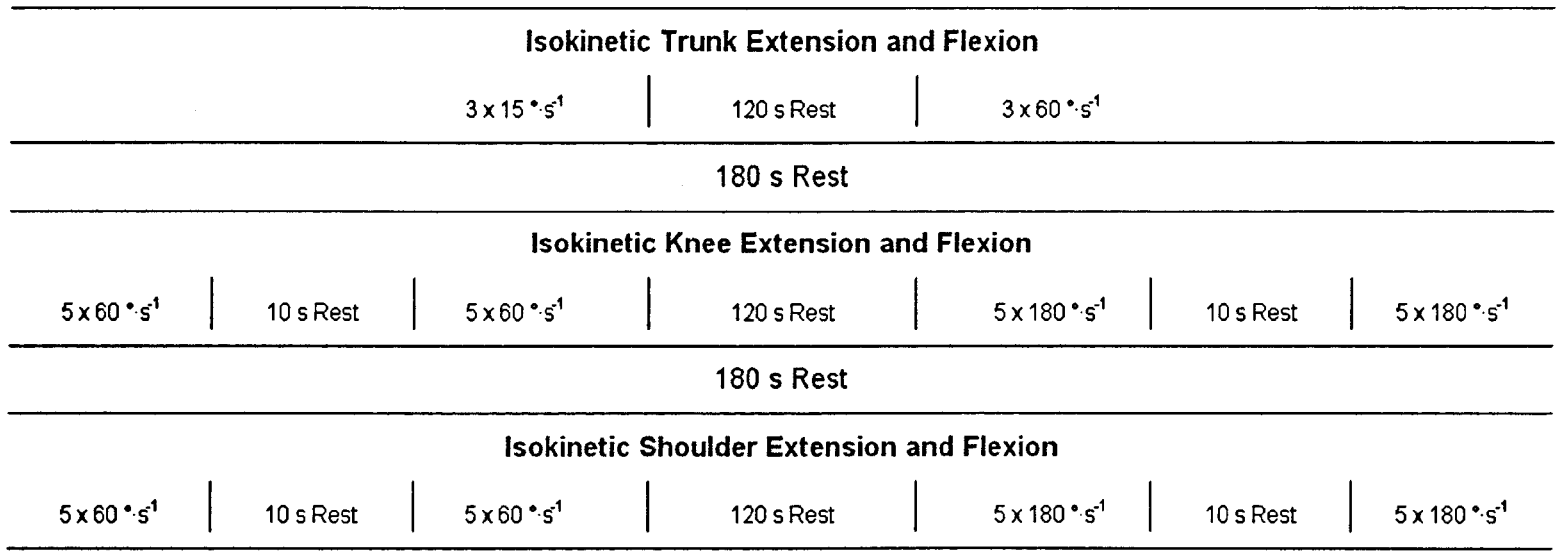


Figure 4.1 – Test battery schematic of isokinetic contractions of the trunk (15 and 60 °·s^{-1}), knee (60 and 180 °·s^{-1}) and shoulder (60 and 180 °·s^{-1}) extensors and flexors at 0 (baseline), 2, 24, 48 and 72 h.

4.2.4. Isokinetic Knee Extension and Flexion

Participants were seated in the test chair of a Cybex II isokinetic dynamometer (Cybex, Measham, UK) with their knee at 90° flexion secured using a seat belt style strap across their chest and hips (Figure 4.2). The Cybex long input adapter, adjustable arm and shin pad (Cybex, Measham, UK) were attached to the dynamometer's point of rotation and to the ankle of the non-dominant leg via a Velcro cuff as instructed in the Cybex user manual. The dominant leg was placed behind the restraining bar to prevent movement during measurement. The point of rotation of the dynamometer arm was aligned, using a laser pointer, with the *lateral femoral epicondyle* (Dvir, 1995). Participant range of motion was restricted by mechanical stops at 70° (flexion) and 0° (extension) of the knee to prevent hyper extension or flexion. Three sub-maximal (self-perceived 50 % effort) contractions (full extension and flexion) were completed to familiarise participants to the experimental set up in each test session. The test protocol consisted of two sets of five maximal dynamic contractions of the knee extensors and flexors at 60 and 180 $°\cdot s^{-1}$, each separated by 30 s rest (Figure 4.1).

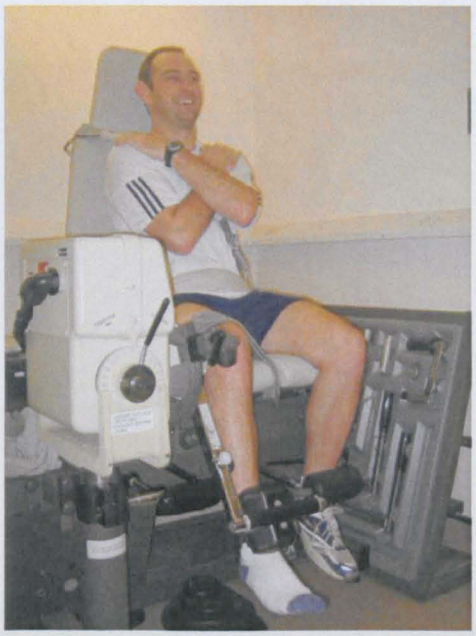


Figure 4.2 – Position of participant in the Cybex II isokinetic dynamometer during isokinetic assessment of the knee extensors and flexors

4.2.5. Isokinetic Trunk Extension and Flexion

Participants were positioned standing upright (trunk fully extended, 0 °) in an isokinetic trunk strength dynamometer (Akron Therapy Products, Ipswich, UK) (Figure 4.3). Movement was restricted to the use of the abdominal and back muscles only between extension (5 °) and flexion (50 °) of the start position. Straps were placed across the participant's upper and lower legs and hips and a frame positioned around the shoulders. The point of rotation of the dynamometer was aligned with the L5-S1 vertebrae, using a laser pointer (Dvir, 1995). This was located by identifying the highest point of the iliac crest and measuring 5 cm dorsally and 5 cm laterally from this point. Mechanical stops were applied at 5 ° and 50 ° of the participant's vertical position to limit their range of movement and prevent hyper extension or flexion. Three sub-maximal (self-perceived 50 % effort) contractions (full extension and flexion) were completed to familiarise participants to the experimental set up in each test session. The test protocol consisted of one set of three maximal dynamic contractions of the trunk extensors and flexors at 15 and 60 $°\cdot s^{-1}$, each separated by 30 s rest (Figure 4.1).

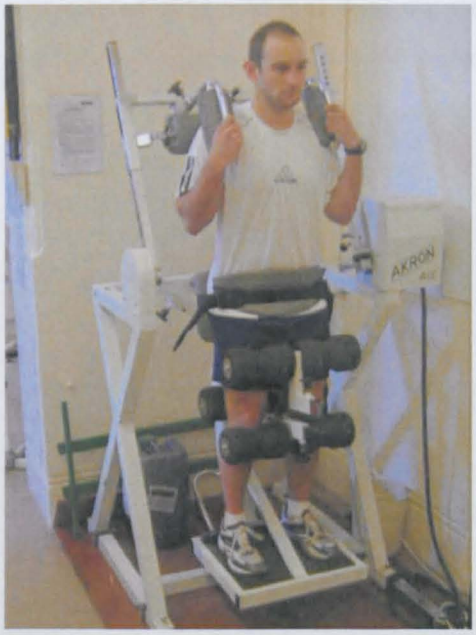


Figure 4.3 – Positioning of participant in the Akron isokinetic trunk strength dynamometer during isokinetic assessment of the trunk extensors and flexors.

4.2.6. Isokinetic Shoulder Extension and Flexion

Participants lay in a supine position on a custom made testing couch placed parallel to a Cybex II isokinetic dynamometer (Cybex, Measham, UK). The Cybex offset input adapter, shoulder testing accessory and neutral handgrip (Cybex, Measham, UK) were attached to the dynamometer. Participants griped the handle in their right hand; the adapter length was adjusted so their right arm was fully extended (0 °) (i.e. minimal flexion in the elbow) (Figure 4.4). **Participant's movement was restricted** by securing Velcro straps across the upper legs and hips with the left arm placed across the chest. The point of rotation of the dynamometer arm was aligned with the right *Acromiale* using a laser pointer (Dvir, 1995). Due to laboratory restrictions participants were tested on their right arm only, but very little difference in strength exists between dominant and non- dominant arms for flexion (0.9 to 1.2 Nm) or extension (0.1 to 0.9 Nm) (Perrin, 1993). Mechanical stops were placed the end of the range of movements to prevent hyper extension and flexion. Range of motion was between 0 ° and 180 °. Three sub-maximal (self-perceived 50 % effort) contractions (full extension and flexion) were completed to familiarise participants to the experimental set up in each test session. The test protocol consisted of two sets of five maximal dynamic contractions of the shoulder extensors and flexors at 60 and 180 $°\cdot s^{-1}$, each separated by 30 s rest (Figure 4.1).

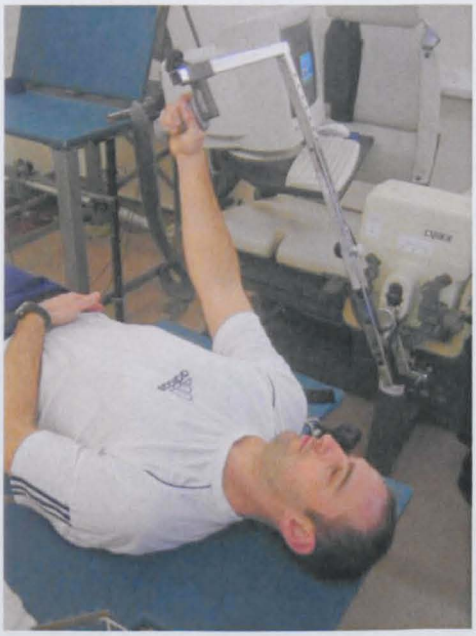


Figure 4.4 – Position of participant in the Cybex II isokinetic dynamometer during isokinetic assessment of the shoulder extensors and flexors.

4.2.7. Calculated Variables

The following variables were calculated for all maximal dynamic contractions, after removal of data not collected during the isokinetic phase (<1 % of all data) and correction for gravity (knee and shoulder only): (a) maximum peak torque, the maximum torque value recorded in all contractions at the specified speed; (b) mean peak torque, the mean of all maximum torque values recorded in all contractions; (c) maximum work, the area under the curve of each contraction and the maximum value were reported.

4.2.8. Environmental Conditions

Environmental temperature was monitored using a dry bulb thermometer (Fisher Scientific, Loughborough, UK). No differences in environmental temperature were observed between baseline tests at 2, 24, 48 and 72 hour test periods respectively (21.2 ± 2.5, 22.2 ± 2.6, 21.6 ± 1.0, 21.6 ± 1.5, 22.3 ± 1.9 °C, $P>0.05$).

4.2.9. Statistical Analysis

Statistical analysis was undertaken using SPSS for Windows V15 (SPSS, Chicago, Illinois) and Microsoft Excel 2002 for Windows (Microsoft, Redmond, Washington). Normal distribution of the data was verified using a Kolmogorov-Smirnov test.

Repeatability of the baseline measurements were compared to variables measured at 2, 24, 48 and 72 hours using Bland & Altman's 95 % Limits of Agreement (LoA) (Bland and Altman, 1987, 1995, 2003, 2007). Data were examined for heteroscedasticity using Pearsons correlation coefficient. Heteroscedasticity was present in the majority of comparisons (as is the case for the majority of variables measured on the ratio scale), therefore, log ratio LoA were calculated and used for analysis of all data sets (Atkinson and Nevill, 1998; Nevill and Atkinson, 1997). Logarithms for each data point were calculated and individual participant differences [baseline – test (2, 24, 48, 72 hours)] were plotted against respective individual means. Mean differences (bias) between baseline and 2, 24, 48, and 72 hour tests were assessed using paired t-tests with statistical significance set at $P<0.05$. The 95 % LoA were produced by calculating the standard deviation of the differences between tests 1 and test 2 and multiplying by 1.96. In addition 95 % confidence intervals (CI) were calculated around the limits of agreement by multiplying the standard error of the differences by the t-test test statistic.

4.3. Results

Absolute values of maximum peak torque, mean peak torque and maximum work at 0 (baseline), 2, 24, 48 and 72 hours are presented in Tables 4.1, 4.2 and 4.3. Reliability of maximum peak torque, mean peak torque and maximum work between 0 hours (baseline) and 2, 24, 48 and 72 hours are presented in Tables 4.4, 4.5. 4.6.

The mean peak torque of the knee extension 60 $°\cdot s^{-1}$ at baseline vs. 2 hours is provided as an example for interpretation of the results. The bias is a measure of the mean difference between measurements; in this example the mean bias for the torque at baseline vs. 2 hours is 2.1 % (P>0.05). The 95 % LoA illustrate the variation that can be expected for an individual in this population with 95 % confidence. In this example, there is 95 % confidence that the torque a participant produces during knee extension at 60 $°\cdot s^{-1}$ could increase or decrease by 11.6 % between measurement at baseline and at 2 hours. The upper and lower 95 % LoA can be calculated by adding or subtracting the 95 % LoA value to the bias respectively (13.7 % upper LoA and -9.5 % lower LoA). The upper and lower 95 % CI show the variation around the respective LoA with 95 % confidence. The variation could be as great as a -13.9 % decrease (lower 95 % CI) or a 21.0 % increase (upper 95 % CI). The bias, 95 % LoA and 95 % CI are also illustrated in Figure 4.5 for the mean peak torque knee extension 60 $°\cdot s^{-1}$ and 180 $°\cdot s^{-1}$ at baseline vs. 2 hours. A strong agreement between mean peak torque measurements with a small positive non-significant bias is illustrated in Figure 4.5(A) and a weaker agreement between peak torque measurements with a significant negative bias is illustrated in Figure 4.5(B).

Knee extension (60 $°\cdot s^{-1}$). There was no significant bias at 2, 24, 48 and 72 hours for the maximum peak torque and a small positive non-significant bias for mean peak torque (Table 4.4). There was also a non-significant bias for maximum work at 2, 24, and 72 hours respectively (3.6, 2.3, 5.1, P>0.05), however there was significant bias at 48 hours (5.9, P=0.041). Reliability (as shown by 95 % LoA and 95% CI) was similar for the maximum peak torque and mean peak torque but was consistently higher for maximum work respectively at all time points (Table 4.4).

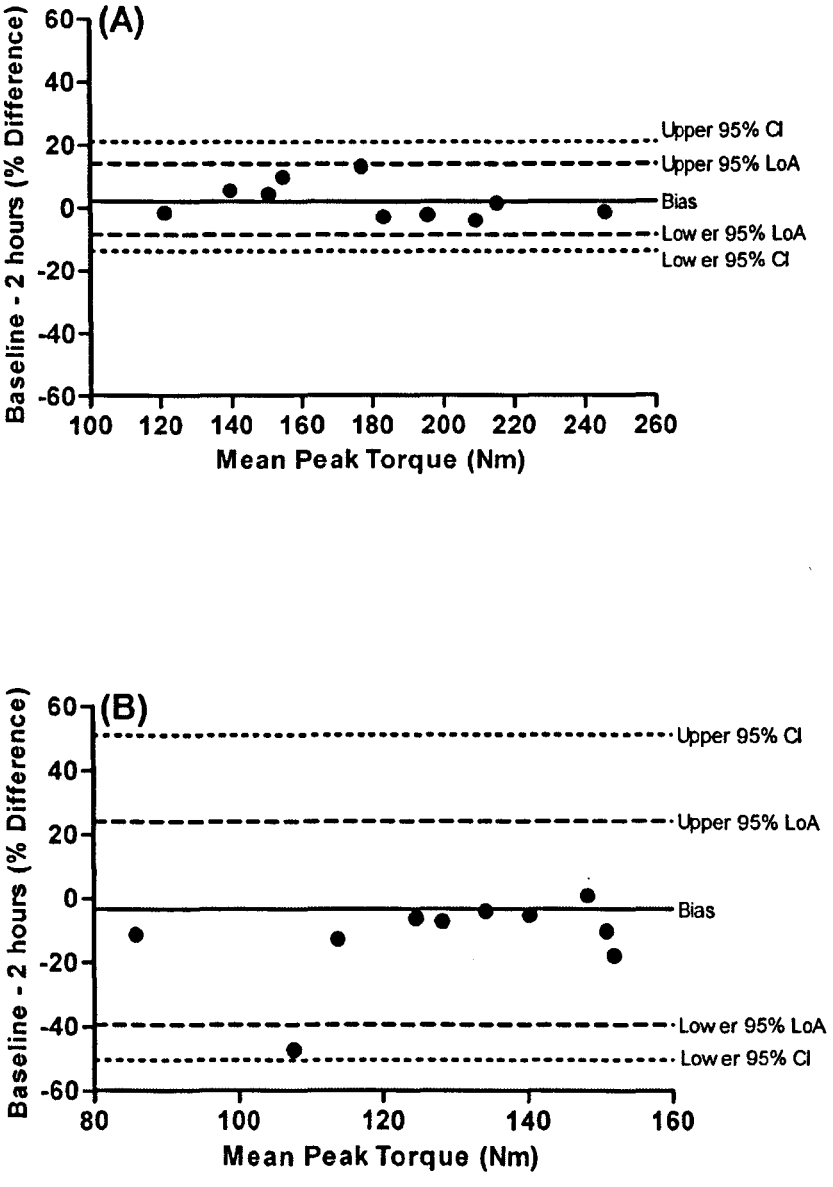


Figure 4.5 – Bland-Altman plots of the agreement between mean peak torque at baseline (0 hours) and 2 hours for knee extension (A) 60 °·s^{-1} (B) knee extension 180 °·s^{-1}. Each data point is presented individually (mean baseline and 2 hour values vs. percentage differences between baseline and 2 hour values). The mean bias (——), 95 % limits of agreement (– – –) and upper and lower confidence intervals of the 95 % limits of agreement (·····) are displayed.

Knee extension (180 °·s^{-1}). There was similar non-significant bias for the maximum peak torque at 2, 24, 48 and 72 hours (Table 4.4) The reliability (as demonstrated by the 95 % LoA and CI) was typically stronger across all time points for the maximum peak torque compared to the mean peak torque and maximum work (Table 4.4). A typical example was at 72 hours when the 95 % LoA were 13.3 for the maximum peak torque, 25.6 for the mean peak torque and 40.3 for the maximum work.

Knee flexion (60 °·s^{-1}). At 2, 24, 48 and 72 hours there was a non-significant positive bias for the maximum peak torque (0.3, 1.3, 3.2, 0.9, P>0.05). The bias was greater for the mean peak torque (3.7, 3.3, 5.0, 2.2 P>0.05) and the maximum work (2.7, 2.9 5.4, 3.4, P>0.05). The 95 % LoA and 95 % CI were similar for the maximum peak torque and mean peak torque but higher for the maximum work (Table 4.4).

Knee flexion (180 °·s^{-1}). There was significant positive bias for the maximum peak torque at 2 (3.7, P=0.031) and 24 (6.4, P=0.047) hours, and maximum work at 48 hours (13.3, P=0.047). For all parameters the reliability (95 % LoA and 95 % CI) was similar at 2, 24, 48 and 72 hours (Table 4.4).

Trunk extension (15 °·s^{-1}). There was a small non-significant bias at 2, 24, 48 and 72 hours for maximum peak torque, mean peak torque and maximum work (Table 4.5). There was variation in the 95 % LoA and 95 % CI across time points but no obvious trend (Table 4.5). The erratic variation is due to individual outlying data points for each variable which increase the 95 % LoA and 95 % CI, such as that shown in Figure 4.5(B) for knee extension.

Trunk extension (60 °·s^{-1}). There was a small non-significant bias at 2, 24, 48 and 72 hours respectively for maximum peak torque (-1.2, -1.3, -0.3, -4.8, P>0.05) and mean peak torque (-0.3, -1.6, 0.9, -2.8, P>0.05). The non-significant bias was generally greater for the maximum work (-0.5, -8.3, 5.5, -11.8, P>0.05). Also, the 95 % LoA and 95 % CI were similar for maximum peak torque and mean peak torque but higher for maximum work (Table 4.5).

Trunk flexion (15 °·s^{-1}). There was a similar, small, non-significant bias at 2, 24, 48 and 72 hours for maximum peak torque, mean peak torque and maximum work which did not systematically change (Table 4.5). The 95 % LoA and 95 % CI show reliability of the measures became slightly poorer over time for all parameters (Table 4.5). For example, reliability of the maximum peak torque compared to baseline was strongest at 2 hours c,

decreasing at 24 hours {1.0 (± 8.8) [-11.8 - 15.6]} and again at 48 hours {-0.5 (± 16.6) [-22.4 - 27.5]} and 72 hours {-2.6 (± 17.3) [-24.7 - 26.0]}

Trunk flexion (60 °·s^{-1}). There was non-significant bias at 2, 24, 48 and 72 hours respectively for maximum peak torque, mean peak torque and maximum work (Table 4.5). However, unlike trunk flexion (15 °·s^{-1}) there was no systematic change in reliability for maximum peak torque or mean peak torque, as shown by 95 % LoA and 95 % CI (Table 4.5). The reliability of the maximum work systematically became poorer over time and was lowest at 2 hours {1.1 (± 20.7) [-25.3 - 36.9}, increasing at 24 hours {-3.1 (± 31.4)[-37.6 - 50.5]} and 48 hours {-1.9 (± 42.7)[-44.7 - 74.0]} and peaked at 72 hours {3.2 (± 49.7) [-46.1 - 97.7]}.

Shoulder extension (60 °·s^{-1}). There was a small non-significant positive bias at 2, 24, 48 and 72 hours respectively for maximum peak torque, mean peak torque and maximum work (Table 4.6). Reliability (95 % LoA and 95 % CI) of measurements was similar for maximum peak torque, mean peak torque and maximum work at 2, 24, 48 and 72 hours (Table 4.6).

Shoulder extension (180 °·s^{-1}). There was no significant bias for maximum peak torque, mean peak torque and maximum work (Table 4.6). Like shoulder extension 60 °·s^{-1}, reliability was similar for the maximum peak torque, mean peak torque and maximum work at 2, 24, 48 and 72 hours (Table 4.6).

Shoulder flexion (60 °·s^{-1}). There was similar non-significant bias at 2, 24, 48 and 72 hours respectively for maximum peak torque (-1.4, -1.5, 1.1, -4.4, P>0.05) and mean peak torque (-2.3, -3.2, -0.2, -3.0, P>0.05). However, for maximum work, although there was similar non-significant bias at 2, 24 and 72 hours respectively (1.8, 2.5, -1.6, P>0.05) there was a significant positive bias at 48 hours (5.7, P=0.040). The 95 % LoA and 95 % CI were similar for maximum peak torque and mean peak torque but were lower for maximum work (Table 4.6).

Shoulder flexion (180 °·s^{-1}). There was non-significant bias at 2, 24, 48 and 72 hours for maximum peak torque, mean peak torque and maximum work (Table 4.6). Reliability of the measurements was similar for maximum peak torque and mean peak torque but generally higher for maximum work, as shown by 95 % LoA and 95 % CI (Table 4.6).

Table 4.1 – Maximum peak torque, mean peak torque and maximum work during isokinetic contractions of the knee extensors and flexors at test velocities of 60 and 180 °·s^{-1} (n=10) at 0 (baseline) 2, 24, 48 and 72 hours using an isokinetic dynamometer. Data are presented as mean ± SD.

Measurement		Baseline (0 h)	2 h	24 h	48 h	72 h
Knee Extension (60 °·s^{-1})	Maximum Peak Torque (Nm)	198 ± 40	199 ± 40	197 ± 44	197 ± 40	202 ± 40
	Mean Peak Torque (Nm)	178 ± 40	181 ± 37	179 ± 42	183 ± 38	184 ± 37
	Maximum Work (J)	143 ± 34	148 ± 32	146 ± 32	151 ± 32	150 ± 33
Knee Extension (180 °·s^{-1})	Maximum Peak Torque (Nm)	142 ± 28	142 ± 28	143 ± 25	143 ± 27	145 ± 28
	Mean Peak Torque (Nm)	112 ± 27	111 ± 26	115 ± 21	115 ± 25	117 ± 24
	Maximum Work (J)	129 ± 28	129 ± 27	132 ± 25	132 ± 27	132 ± 26
Knee Flexion (60 °·s^{-1})	Maximum Peak Torque (Nm)	125 ± 24	125 ± 20	126 ± 21	128 ± 20	125 ± 19
	Mean Peak Torque (Nm)	110 ± 21	113 ± 16	113 ± 19	115 ± 18	112 ± 20
	Maximum Work (J)	105 ± 21	107 ± 17	107 ± 18	109 ± 16	107 ± 17
Knee Flexion (180 °·s^{-1})	Maximum Peak Torque (Nm)	93 ± 13	96 ± 12	99 ± 14	98 ± 17	98 ± 16
	Mean Peak Torque (Nm)	83 ± 13	85 ± 11	87 ± 12	87 ± 16	88 ± 17
	Maximum Work (J)	20 ± 5	21 ± 4	22 ± 5	23 ± 4	22 ± 5

Table 4.2 – Maximum peak torque, mean peak torque and maximum work during isokinetic contractions of the trunk extensors and flexors at test velocities of 15 and 60 °·s^{-1} (n=8) at 0 (baseline) 2, 24, 48 and 72 hours using an isokinetic dynamometer. Data are presented as mean ± SD.

Measurement		Baseline (0 h)	2 h	24 h	48 h	72 h
Trunk Extension (15 °·s^{-1})	Maximum Peak Torque (Nm)	288 ± 38	285 ± 51	289 ± 42	278 ± 48	273 ± 58
	Mean Peak Torque (Nm)	276 ± 48	273 ± 50	273 ± 46	265 ± 54	266 ± 58
	Maximum Work (J)	1119 ± 172	1101 ± 219	1074 ± 221	1101 ± 227	1125 ± 309
Trunk Extension (60 °·s^{-1})	Maximum Peak Torque (Nm)	276 ± 53	272 ± 51	275 ± 61	277 ± 60	266 ± 64
	Mean Peak Torque (Nm)	266 ± 56	264 ± 53	265 ± 62	270 ± 62	261 ± 65
	Maximum Work (J)	215 ± 58	213 ± 54	198 ± 44	228 ± 66	192 ± 59
Trunk Flexion (15 °·s^{-1})	Maximum Peak Torque (Nm)	259 ± 44	258 ± 44	259 ± 41	257 ± 45	253 ± 50
	Mean Peak Torque (Nm)	252 ± 44	250 ± 44	252 ± 44	247 ± 49	245 ± 47
	Maximum Work (J)	1010 ± 247	978 ± 196	961 ± 187	946 ± 190	1049 ± 409
Trunk Flexion (60 °·s^{-1})	Maximum Peak Torque (Nm)	296 ± 34	290 ± 37	291 ± 34	290 ± 40	299 ± 39
	Mean Peak Torque (Nm)	287 ± 37	280 ± 41	283 ± 37	281 ± 42	288 ± 49
	Maximum Work (J)	263 ± 63	268 ± 79	257 ± 69	260 ± 68	273 ± 71

Table 4.3 – Maximum peak torque, mean peak torque and maximum work during isokinetic contractions of the shoulder extensors and flexors at test velocities of 15 and 60 °·s^{-1} (n=10) at 0 (baseline) 2, 24, 48 and 72 hours using an isokinetic dynamometer. Data are presented as mean ± SD.

Measurement		Baseline (0 h)	2 h	24 h	48 h	72 h
Shoulder Extension (60 °·s^{-1})	Maximum Peak Torque (Nm)	101 ± 20	101 ± 18	103 ± 22	106 ± 26	103 ± 23
	Mean Peak Torque (Nm)	89 ± 16	90 ± 17	90 ± 18	92 ± 20	91 ± 18
	Maximum Work (J)	163 ± 33	164 ± 31	165 ± 30	169 ± 33	165 ± 34
Shoulder Extension (180 °·s^{-1})	Maximum Peak Torque (Nm)	85 ± 15	86 ± 17	83 ± 15	88 ± 21	87 ± 17
	Mean Peak Torque (Nm)	76 ± 13	77 ± 15	75 ± 14	79 ± 18	77 ± 14
	Maximum Work (J)	40 ± 8	42 ± 9	41 ± 8	42 ± 10	41 ± 8
Shoulder Flexion (60 °·s^{-1})	Maximum Peak Torque (Nm)	76 ± 13	75 ± 14	75 ± 15	78 ± 19	74 ± 16
	Mean Peak Torque (Nm)	67 ± 12	65 ± 11	65 ± 13	67 ± 15	65 ± 13
	Maximum Work (J)	114 ± 21	115 ± 19	116 ± 22	121 ± 27	112 ± 22
Shoulder Flexion (180 °·s^{-1})	Maximum Peak Torque (Nm)	54 ± 9	55 ± 6	56 ± 8	53 ± 7	55 ± 7
	Mean Peak Torque (Nm)	46 ± 9	48 ± 6	48 ± 6	46 ± 6	48 ± 6
	Maximum Work (J)	23 ± 5	24 ± 3	25 ± 4	23 ± 4	24 ± 4

Table 4.4 – Ratio (%) limits of agreement (LoA) for the baseline value (time 0 hours) compared to 2, 24, 48 and 72 hours during isokinetic contractions of the knee extensors and flexors at angular velocities of 60 and 180 °·s^{-1} (n=10). Data are presented as bias (± 95% LoA) [lower 95% confidence interval – upper 95% confidence interval]. * Denotes significant Bias (P<0.05).

Measure		2 h	24 h	48 h	72 h
Knee Extension (60 °·s^{-1})	Maximum Peak Torque (Nm)	0.5 (± 15.6) [-19.7 - 25.7]	-1.0 (± 14.8) [-20.1 - 22.7]	-0.3 (± 12.9) [-17.4 - 20.3]	2.3 (± 13.7) [-16.1 - 24.7]
	Mean Peak Torque (Nm)	2.1 (± 11.6) [-13.9 - 21.0]	0.6 (± 16.0) [-20.0 - 26.5]	3.2 (± 13.9) [-15.6 - 26.2]	4.2 (± 15.7) [-16.9 - 30.6]
	Maximum Work (J)	3.6 (± 22.8) [-24.6 - 42.4]	2.3 (± 20.3) [-23.2 - 36.2]	5.9* (± 16.1) [-16.0 - 33.5]	5.1 (± 25.2) [-25.8 - 48.9]
Knee Extension (180 °·s^{-1})	Maximum Peak Torque (Nm)	-0.5 (± 8.3) [-12.0 – 12.5]	1.1 (± 14.5) [-18.0 – 24.7]	0.8 (± 6.3) [-8.3 - 10.7]	2.0 (± 13.3) [-15.9 – 23.7]
	Mean Peak Torque (Nm)	-0.4 (± 12.5) [17.0 – 19.6]	3.6 (± 25.6) [27.2 - 47.3]	3.1 (± 16.6) [-18.7 – 30.7]	4.8 (± 25.6) [26.3 - 49.1]
	Maximum Work (J)	3.9 (± 39.3) [-37.8 – 73.5]	-0.4 (± 48.1) [-45.8 – 82.7]	2.0 (± 35.4) [-36.2 - 63.0]	2.3 (± 40.3) [-39.4 - 72.7]
Knee Flexion (60 °·s^{-1})	Maximum Peak Torque (Nm)	0.3 (± 21.2) [-25.5 - 35.2]	1.3 (± 15.4) [-18.9 - 26.4]	3.2 (± 19.8) [-22.0 - 36.5]	0.9 (± 22.3) [-26.1 - 37.8]
	Mean Peak Torque (Nm)	3.7 (± 18.8) [-20.6 - 35.3]	3.3 (± 10.4) [-11.4 - 20.4]	5.0 (± 18.4) [-19.2 - 36.5]	2.2 (± 20.7) [-23.7 - 36.7]
	Maximum Work (J)	2.7 (± 31.1) [-32.5 - 56.2]	2.9 (± 27.1) [-29.0 - 49.2]	5.4 (± 31.5) [-31.0 - 61.0]	3.4 (± 34.4) [-34.5 - 63.4]
Knee Flexion (180 °·s^{-1})	Maximum Peak Torque (Nm)	3.7* (± 9.2) [-9.5 - 18.8]	6.4* (± 18.3) [-17.9 - 38.0]	5.1 (± 21.1) [-21.9 - 41.4]	5.1 (± 23.9) [-24.6 - 46.4]
	Mean Peak Torque (Nm)	2.5 (± 16.1) [-18.7 - 29.2]	5.4 (± 17.6) [-17.9 - 35.4]	3.7 (± 26.6) [-28.0 - 49.4]	4.9 (± 26.5) [-27.2 - 51.0]
	Maximum Work (J)	4.0 (± 41.9) [-39.5 - 78.7]	9.8 (± 48.1) [-40.2 - 101.7]	13.3* (± 39.9) [-32.6 - 90.3]	10.2 (± 54.3) [-43.7 - 115.8]

Table 4.5 – Ratio (%) limits of agreement (LoA) for the baseline value (time 0 hours) compared to 2, 24, 48 and 72 hours during isokinetic contractions of the trunk extensors and flexors at angular velocities of 15 and 60 °·s^{-1} (n=10). Data are presented as bias (± 95% LoA) [lower 95% confidence interval – upper 95% confidence interval].

Measure		2 h	24 h	48 h	72 h
Trunk Extension (15 °·s^{-1})	Maximum Peak Torque (Nm)	-1.8 (± 16.7) [-23.4 - 26.0]	0.7 (± 19.7) [-24.7 - 34.6]	-4.1 (± 19.2) [-27.8 - 27.3]	-6.4 (± 31.8) [-40.1 - 46.2]
	Mean Peak Torque (Nm)	-1.1 (± 8.3) [-13.0 - 12.4]	-0.3 (± 18.1) [-23.7 - 30.4]	-4.2 (± 21.9) [-30.4 - 31.9]	-4.6 (± 22.1) [-30.8 - 31.6]
	Maximum Work (J)	-2.4 (± 22.0) [-29.2 - 34.4]	-5.0 (± 29. 0) [-37.0 - 43.2]	-2.6 (± 14.6) [-21.8 - 21.4]	-2.3 (± 42.9) [-45.1 - 73.8]
Trunk Extension (60 °·s^{-1})	Maximum Peak Torque (Nm)	-1.2 (± 20.5) [-26.8 - 33.4]	-1.3 (± 16.3) [-22.6 - 26.0]	-0.3 (± 21.2) [-26.9 - 35.9]	-4.8 (± 33.5) [-40.2 - 51.6]
	Mean Peak Torque (Nm)	-0.3 (± 21.8) [-27.4 - 37.0]	-1.6 (± 15.3) [-21.8 - 23.8]	0.9 (± 25.5) [-30.0 - 45.6]	-2.8 (± 35.5) [-40.5 - 58.6]
	Maximum Work (J)	-0.5 (± 45.8) [-45.8 - 82.8]	-8.3 (± 35.7) [-44.0 - 50.1]	5.5 (± 64.4) [-52.6 - 135.2]	-11.8 (± 51.0) [-54.6 - 71.3]
Trunk Flexion (15 °·s^{-1})	Maximum Peak Torque (Nm)	-0.2 (± 5.5) [-8.4 - 8.7]	1.0 (± 8.8) [-11.8 - 15.6]	-0.5 (± 16.6) [-22.4 - 27.5]	-2.6 (± 17.3) [-24.7 - 26.0]
	Mean Peak Torque (Nm)	-0.9 (± 4.4) [-7.6 - 6.2]	0.7 (± 10.2) [-13.9 - 17.7]	-2.3 (± 19.7) [-26.8 - 30.5]	-3.3 (± 21.5) [-29.3 - 32.4]
	Maximum Work (J)	-2.4 (± 17.1) [-24.4 - 26.0]	-3.8 (± 23.7) [-31.7 - 35.6]	-5.6 (± 29.3) [-37.6 - 42.9]	0.3 (± 47.9) [-46.6 - 88.5]
Trunk Flexion (60 °·s^{-1})	Maximum Peak Torque (Nm)	-1.9 (± 19.5) [-26.3 - 30.7]	-2.2 (± 16.6) [-23.7 - 25.2]	-2.1 (± 23.4) [-30.2 - 37.4]	0.9 (± 22.9) [-27.7 - 40.7]
	Mean Peak Torque (Nm)	-2.7 (± 20.2) [-27.7 - 30.9]	-2.3 (± 15.2) [-22.3 - 22.8]	-2.4 (± 23.1) [-30.2 - 36.5]	-0.6 (± 29.9) [-34.8 - 51.6]
	Maximum Work (J)	1.1 (± 20.7) [-25.3 - 36.9]	-3.1 (± 31.4) [-37.6 - 50.5]	-1.9 (± 42.7) [-44.7 - 74.0]	3.2 (± 49.7) [-46.1 - 97.7]

Table 4.6 – Ratio (%) limits of agreement (LoA) for the baseline value (time 0 hours) compared to 2, 24, 48 and 72 hours during isokinetic contractions of the shoulder extensors and flexors at angular velocities of 60 and 180 °·s^{-1} (n=10). Data are presented as bias (± 95% LoA) [lower 95% confidence interval – upper 95% confidence interval].

Measure		2 h	24 h	48 h	72 h
Shoulder Extension (60 °·s^{-1})	Maximum Peak Torque (Nm)	0.2 (± 14.7) [-19.0 - 23.9]	1.6 (± 24.3) [-27.4 - 42.2]	4.2 (± 27.7) [-28.7 - 52.1]	1.8 (± 25.3) [-28.2 - 44.3]
	Mean Peak Torque (Nm)	0.8 (± 17.3) [-21.3 - 29.1]	0.9 (± 18.9) [-22.8 - 32.0]	3.2 (± 23.9) [-25.9 - 43.7]	2.5 (± 22.5) [-25.1 - 40.4]
	Maximum Work (J)	0.8 (± 19.3) [-23.3 - 32.5]	1.2 (± 15.6) [-19.1 - 26.8]	3.3 (± 13.3) [-14.9 - 25.4]	0.9 (± 14.8) [-18.5 - 25.0]
Shoulder Extension (180 °·s^{-1})	Maximum Peak Torque (Nm)	0.3 (± 18.4) [-22.8 - 30.3]	-3.1 (± 20.0) [-26.9 - 28.5]	2.4 (± 23.8) [-26.4 - 42.6]	1.2 (± 15.7) [-19.2 - 26.8]
	Mean Peak Torque (Nm)	2.0 (± 11.3) [-13.6 - 20.4]	-1.3 (± 9.2) [-13.9 - 13.2]	3.3 (± 20.1) [-22.2 - 37.0]	1.8 (± 11.4) [-13.8 - 20.3]
	Maximum Work (J)	2.9 (± 19.6) [-22.0 - 35.8]	0.8 (± 19.0) [-23.0 - 32.0]	3.7 (± 26.5) [-28.0 - 49.2]	1.9 (± 16.4) [-19.5 - 29.0]
Shoulder Flexion (60 °·s^{-1})	Maximum Peak Torque (Nm)	-1.4 (± 9.1) [-13.8 - 12.8]	-1.5 (± 25.6) [-30.8 - 40.2]	1.1 (± 33.3) [-35.2 - 57.6]	-4.4 (± 33.5) [-38.9 - 49.6]
	Mean Peak Torque (Nm)	-2.3 (± 15.9) [-22.3 - 22.8]	-3.2 (± 32.8) [-37.6 - 50.3]	-0.2 (± 25.6) [-29.9 - 42.0]	-3.0 (± 23.6) [-30.1- 34.7]
	Maximum Work (J)	1.8 (± 12.9) [-15.6 - 22.8]	2.5 (± 13.4) [-15.6 -24.5]	5. 7* (± 15.3) [-15.2 - 31.8]	-1.6 (± 12.3) [-17.8 - 17.9]
Shoulder Flexion (180 °·s^{-1})	Maximum Peak Torque (Nm)	2.1 (± 18.8) [-21.8 - 33.3]	4.1 (± 30.5) [-31.1 - 57.2]	-1.6 (± 23.5) [-29.0 - 36.5]	2.0 (± 24.4) [-27.3 - 42.9]
	Mean Peak Torque (Nm)	4.6 (± 19.2) [-20.3 - 37.2]	4.7 (± 30.3) [-30.5 - 57.6]	0.2 (± 25.6) [-29.6 - 42.6]	5.1 (± 33.4) [-32.7 - 64.3]
	Maximum Work (J)	7.3 (± 28.9) [-27.5 - 59.0]	10.3 (± 53.3) [-43.1 - 113.8]	0.8 (± 33.1) [-35.2 - 56.8]	6.1 (± 44.2) [-39.7 - 86.9]

4.4. Discussion

This study examined the reliability of isokinetic contractions of the extensors and flexors of the knee, trunk and shoulder. The novelty of this study was that the reliability was examined over a timescale suitable for assessing recovery of neuromuscular function following a two hour bout of exercise. The mean change (bias) between baseline measures and those made at 2, 24, 48 and 72 hours was generally small (0 to 8 %) but in a small minority of cases moderate (9 to 16 %). However, the 95 % LoA and 95 % CI indicated poor reliability for some parameters. For all contractions, the faster test velocities showed poorer intra and inter day reliability due to the more frequent occurrence of significant mean bias between the baseline and subsequent test sessions. In general, the faster test velocities also showed consistently poorer reliability than the slow test velocities, indicated by the wider 95 % LoA and CI. During extension and flexion of the knee, trunk and shoulders, maximum work showed the greatest variation and maximum peak torque the smallest variation.

The majority of studies investigating the reliability of isokinetic dynamometry have used correlation methods (e.g. Pearsons r) (Perrin, 1993). However, Atkinson & Nevill (1998) proposed that these methods have limitations as they can not detect systematic bias and are dependent on the range of values in a sample (i.e. a decrease in the spread of the data decreases the correlation therefore reliability). Intraclass correlation (ICC) methods are now more popular and have the advantage over Pearsons that more than one re-test can be compared to a test and can be calculated to detect the presence of systematic bias. Various categories of agreement based on the ICC, ranging from 'questionable' (0.7 to 0.8) to high (>0.9) have been proposed (Fallowfield *et al.*, 2005). However, like Pearsons Correlation, ICC is limited as it is affected by the range of values in a sample and does not take into account changes of individuals within a group. These limitations are not present when using the LoA method (Atkinson and Nevill, 1998).

Peak torque during knee extension and flexion (60 $°\cdot s^{-1}$) in the present study was 198 ± 40 and 125 ± 24 Nm at baseline, respectively. These values were similar to the 210 and 133 Nm measured during extension and flexion in healthy male participants (age 21 to 30 years) (Freedson *et al.*, 1993). Peak torque during knee extension and flexion (180 $°\cdot s^{-1}$) in the present study was 142 ± 28 and 93 ± 13 Nm at baseline, respectively, which is comparable to the 142

and 85 Nm measured during extension and flexion, respectively, in male college track athletes (age 18 to 21 years) (Appen and Duncan, 1986).

Impellizzeri *et al.* (2008) assessed the reliability of peak torque during concentric contractions of the left and right knee extensors and flexors at 60 and 180 °·s^{-1} across 3 test sessions, each separated by 96 h using ICC and LoA. There was minimal difference in repeatability between the left and the right side. The ICC were high for the left knee extensors at 60 °·s^{-1} (0.95) and 180 °·s^{-1} (0.98) and the flexors at 60 °·s^{-1} (0.93) and 180 °·s^{-1} (0.98). LoA were calculated using the mean squared error from the two way ANOVA, although statistically valid (Atkinson and Nevill, 1998), this method is different from the current study. It does not allow direct comparison of each test to baseline measures or the assessment of how reliability may change over time, which was important in addressing the aims of the present study. The LoA of Impellizzeri *et al.* (2008) for peak torque were similar to the present study (Table 4.4) for knee extension at 60 °·s^{-1} (13.0 %), knee extension at 180 °·s^{-1} (12.5 %), knee flexion at 60 °·s^{-1} (18.6 %) and knee flexion at 180 °·s^{-1} (13.9 %).

These data are supported by Nevill & Atkinson (1997) who report the ratio 95 % LoA for isokinetic knee extensions and flexions over a range of test velocities. Nevill & Atkinson (1997) show a similar 95 % LoA to the present study; for 60 °·s^{-1} knee extension (19 vs. 12.9-15.6 %) and flexion (29 vs. 15.4 -22.3 %) and for 180 °·s^{-1} knee extension (16 vs. 6.3 – 14.5 %) and flexion (14 vs. 9.2 – 23.9 %). The small random changes over time of the LoA in the present study are likely to have been caused by a single outlying individual as illustrated in Figure 4.5(B). The example data in Figure 4.5(B) compares baseline and 2 hours for knee extension 180 °·s^{-1} {-13.4* (± 43.3) [-50.4 - 51.1]}. If the outlying participant is removed the mean bias, 95 % LoA and CI are reduced to {-8.5* (± 12.5) [-24.0 – 10.1]}.

The present study showed slightly poorer reliability of work than the peak torque for all contractions. Symons *et al.* (2005) also showed wider 95 % ratio LoA were for work (39.9 %) compared to peak torque (32.5 %) during a single test retest of measurements of the knee extensors at 90 °·s^{-1} in older men (72 ± 5 years) on separate days. Compared to knee extensors (60 °·s^{-1}) at 24 h in the present study, Symons *et al.* (2005) data show wider 95 % LoA for peak torque (14.8 vs. 32.5) and work (20.3 vs. 39.9). This may be due to the differences between participant characteristics or, as suggested by Symons *et al.* (2005), the absence of a familiarisation session (which was used in the present study).

Pierce *et al.* (2006) assessed the test-retest reliability of the knee extensors and flexors in children with cerebral palsy at 15, 90 and 180 °·s^{-1}. Their results showed stronger reliability at higher test speeds as assessed by interclass correlation coefficients and LoA and the authors suggested that this likely because of the specialist population. However, there was a positive mean bias and wide 95 % LoA even for those measurements which showed strong interclass correlation coefficients. These data show the importance of establishing the reliability of measures in the population under test and support Atkinson & Nevill (1998) arguments for the use of more complex statistical procedures than just interclass correlation coefficients.

Peak torque during trunk extension (15 °·s^{-1}) in the present study was 288 ± 38 Nm at baseline, which is comparable to the 250 Nm measured in healthy male individuals (mean age 23 years) (Thorstensson and Nilsson, 1982). The test velocity was consistent between studies but unlike the present study, Thorstensson & Nilsson (1982) measures were taken whilst participants lay on their side. Peak torque during trunk extension (60 °·s^{-1}) during the present study was 276 ± 53 Nm at baseline, which is similar to the 255 Nm measured in healthy male participants (age 21 to 30 years) at the same test velocity (Freedson *et al.*, 1993). Mean torque during trunk flexion (15 °·s^{-1}) in the present study was 252 ± 44 Nm at baseline, which is slightly greater than the 197 Nm measured in a group of female runners (mean age 30 years) (Tis *et al.*, 1992), but females isokinetic strength is generally lower than males (Perrin, 1993). Peak torque during trunk flexion (60 °·s^{-1}) in the present study was 296 ± 34 Nm at baseline which is comparable to the 250 Nm measured in healthy male participants (mean 43 years of age) (Thompson *et al.*, 1985).

In comparison to the knee extensors and flexors, very few studies have assessed reliability of contractions of the trunk extensors and flexors using LoA and reliability of contractions at 15 °·s^{-1} have not been previously investigated. The LoA for trunk extension and flexion at 60 °·s^{-1} in the current study were similar to those previously reported for extension at 30 °·s^{-1} (~14 %) and 180 °·s^{-1} (~18 %) trunk flexion 30 °·s^{-1} (~20 %) and 180 °·s^{-1} (~26 %) (aproximate percentage values calculated from absolute limits presented in original summary data) (Dervišević *et al.*, 2007). However, Nevill & Atkinson (1997) reported wider LoA at 60 °·s^{-1} for both trunk extension (58 %) and flexion (54 %), the reasons for these differences are not clear as the authors do not describe the procedures or equipment used to collect the data.

These findings provide support to the reliability of the protocol and dynamometer used in the present study.

Byl & Sadowsky (1993) presented high ICC for the peak toque of the trunk extensors over 3 consecutive days at 60 °·s^{-1} (0.97), 90 °·s^{-1} (0.97) and 120 °·s^{-1} (0.95). ICC were poorer for flexion at 60 °·s^{-1} (0.93), 90 °·s^{-1} (0.90) and 120 °·s^{-1} (0.90). These findings are in contrast to the findings of the current study which showed that the reliability of peak torque for trunk extension and flexion was similar for both 15 and 60 °·s^{-1}. Byl & Sadowsky (1993) present lower ICC for work compared to peak torque during extension and flexion across all test velocities. These findings support the current study which showed poorer reliability for work compared to peak torque for both extension and flexion at 15 and 60 °·s^{-1}.

The present study showed reliability of peak torque was similar for trunk extension at 15 and 60 °·s^{-1}, but stronger reliability for flexion at 15 °·s^{-1} compared to 60 °·s^{-1} (Table 4.5). Previous studies have shown weaker reliability at higher test speeds for both extension and flexion of the trunk, however test velocities were higher (60 to 180 °·s^{-1}) and different dynamometers were used (Dervišević *et al.*, 2007; Madsen, 1996).

Peak torque during shoulder extension (60 °·s^{-1}) in the present study was 101 ± 20 Nm at baseline, which is between the 85 Nm and 118 Nm measured in non-athletic healthy male participants by Shklar & Dvir (1995) (age 22 to 30 years) and Cahalan *et al.* (1991) (age 21 to 41 years), respectively. Similarly, peak torque during shoulder extension (180 °·s^{-1}) in the present study was 85 ± 15 Nm at baseline, which is between the 73 Nm and 103 Nm measured in non-athletic healthy male participants by Shklar & Dvir (1995) (age 22 to 30 years) and Cahalan *et al.* (1991) (age 21 to 41 years), respectively. Peak torque during shoulder flexion (60 °·s^{-1}) in the present study was 76 ± 13 Nm at baseline, which is comparable to the 67 Nm measured in non-athletic healthy male participants (age 21 to 41 years) (Cahalan *et al.*, 1991). Peak torque during shoulder flexion (180 °·s^{-1}) in the present study was 54 ± 9 Nm at baseline, which is comparable to the 54 Nm measured in non-athletic healthy male participants (age 22 to 30 years) (Shklar and Dvir, 1995).

Reliability of shoulder extension and flexion using isokinetic dynamometry has not been previously assessed using the LoA method. Orri & Darden (2008) examined the test-retest reliability of shoulder extension and flexion (60 °·s^{-1}) across 3-5 days using the iSAM

9000 dynamometer. ICC showed strong reliability for extension of the left (0.94) and right (0.98) shoulder and flexion of the left (0.95) and right (0.95) shoulder. However, Mayer *et al.* (1994) examined the reliability of shoulder extension and flexion at 60, 180, 240, 300 $°\cdot s^{-1}$ after a 14 day interval. The reliability between measurements was deemed to be low and was similar for shoulder extension (16.3 %) and flexion (17.2 %). These findings differ from the current study as reliability of shoulder extension was slightly stronger than flexion, as assessed by the 95 % LoA and CI. This may have been due to differences in the participant population, test velocity or time between tests (Keating and Matyas, 1996).

Timm *et al.* (1992) assessed the mechanical and physiological reliability of three dynamometers to assess the knee extensors and flexors (Biodex, Cybex 340, Merac System) and trunk extensors and flexors (Cybex TEF, Cybex Torso, Cybex Liftask) over a range of test velocities (60 to 500 $°\cdot s^{-1}$). Mechanical reliability was measured by attaching a standardised loaded input shaft to the dynamometer arm and allowing the arm to move through a 90° range of motion 5 times. Physiological reliability was assessed by participants (86 males, 86 females, age 16-34 years) completing 2 sets of 5 maximal contractions separated by 48 hours. Mechanical test-retest reliability (assessed by ICC) of the peak torque assessed on the Cybex 340 was very strong at 60 $°\cdot s^{-1}$ (1.000) and 180 $°\cdot s^{-1}$ (1.000). The ICC of the physiological reliability of the peak torque were also strong at 60 $°\cdot s^{-1}$ for the knee extensors (0.999) and flexors (0.999) and at 180 $°\cdot s^{-1}$ for the knee extensors (0.999) and flexors (0.979). A similar pattern was observed for the other dynamometers and during trunk extension and flexion. The authors concluded that differences tended to be found in the physiological testing therefore the source of variability when using isokinetic dynamometry is due to the participants rather than the dynamometers.

In summary, isokinetic dynamometry has been used to successfully measure the effect and recovery from activities causing neuromuscular impairment (Eston *et al.*, 1996; Lepers *et al.*, 2001; Nottle and Nosaka, 2005). The present study showed maximum peak torque is the most reliable parameter to assess changes in the force producing capability of the knee, trunk and shoulder extensors over a time period to measure changes in neuromuscular function following two hours of exercise. There was generally only small and non-significant changes in the parameters over time for the group data (mean bias), but some of the measurements showed high individual variability. The variability during all isokinetic measurement is likely

to arise from the participants rather than the dynamometer, therefore adequate familiarisation and coaching is required to maintain reliability. Differences in reliability exist between test populations investigated; therefore reliability should be assessed in the population under investigation. Greater caution should be taken interpreting results at higher test velocities due to the poorer reliability between tests over time.

The results of the present study, and previously published findings, suggest the maximum peak torque at both fast and slow isokinetic test velocities during contractions of the knee, trunk and shoulder extensors and flexors is the most reliable measure for the assessment of changes in neuromuscular function in the 72 hours following exercise.

Chapter 5. Intra- and Interday Reliability of Voluntary and Electrically Stimulated Isometric Contractions of the *m. quadriceps femoris*

5.1. Introduction

Chapter 4 assessed the reliability of a battery of voluntary contractions to quantify neuromuscular performance of the shoulder, trunk and knee extensors and flexors immediately post and in the days following a two hour bout of exercise. The force producing capability of a muscle group provides information regarding the severity or neuromuscular impairment and the time course of recovery. However, the measures described in Chapter 4 do not detail the origin of the neuromuscular impairment (i.e. central or peripheral) or the peripheral mechanisms responsible for the reduction in force producing capability. This limitation is addressed in the present chapter by developing and assessing the reliability of a battery of voluntary and electrically stimulated isometric contractions of the *m. quadriceps femoris*.

The *m. quadriceps femoris* have been the subject of considerable research, as they experience high levels of neuromuscular impairment following a range of endurance events (Bentley *et al.*, 2000; Millet and Lepers, 2004; Skof and Strojnik, 2006). They are a large and easily accessible muscle, therefore commonly assessed in laboratory protocols designed to induce muscle damage and to quantify the mechanisms responsible for changes in neuromuscular function (Edwards *et al.*, 1977b; Martin *et al.*, 2004a).

The force producing capability of the *m. quadriceps femoris* can be assessed using an isometric maximal voluntary contraction (MVC) (Edwards *et al.*, 1977b; Millet and Lepers, 2004). This provides information regarding the voluntary ability of an individual to produce maximal muscle force. However, greater detail of the changes in the contractile properties of the muscle and the contribution of peripheral and central mechanisms can be obtained through electrical stimulation of the muscle *in situ* (Allen, 2001; Merton, 1954; Vøllestad, 1997). This can be applied either by surface electrodes placed on the muscle belly (percutaneous stimulation) or by directly stimulating its innervating nerve (Paillard *et al.*, 2005).

One of the most common electrical stimulation procedures is a single, twitch applied to the resting muscle (Edwards *et al.*, 1977a; Millet and Lepers, 2004). In previous studies of the effect of exercise on neuromuscular function, the most frequently calculated parameters from

a twitch or tetani of stimulations are the peak force; contraction time; half relaxation time; maximal rate of force development and maximal rate of force decrease (Bentley *et al.*, 2000; Millet *et al.*, 2003a; Millet *et al.*, 2003b; Place *et al.*, 2004) and rate constants of contraction and relaxation (Jones *et al.*, 2009). These parameters describe changes in the force generating capacity of the muscle (peak force) and the excitation-contraction coupling process (contraction time and maximal rate of force development) and muscle relaxation (half relaxation time, maximal rate of force decrease), independently of central control. These parameters are important as Warren *et al.* (2002) suggested that approximately 57 to 75 % of the strength loss following muscle damaging exercise can be accounted for by failure of the excitation-contraction coupling process

Stimulating the muscle at rest using a tetani of 0.5-2.0 seconds duration at a low (10-20 Hz) and high (50-100 Hz) frequency, and expressing this as a ratio (low frequency force : high frequency force), has been used to assess for the presence of low frequency fatigue (LFF) or high frequency fatigue (HFF) (Jones, 1996). A decrease in the ratio is indicative of LFF, and indicates a reduction in release of Ca^{2+} from the sarcoplasmic reticulum (Chin *et al.*, 1997; Westerblad *et al.*, 1993) or redistribution of sarcomere lengths (Jones, 1996), an effect which can last for days (Edwards *et al.*, 1977a; Westerblad *et al.*, 1993). An increase in the ratio indicates HFF, which is associated with K^+ accumulation in the t-tubules and interfibre spaces of the muscle (Jones, 1996). HFF is only apparent for a short time (60 to 120 s) after high intensity exercise (Strojnik and Komi, 1998; Tomazin *et al.*, 2008), Therefore, HFF is not a parameter of concern when measuring recovery of neuromuscular function over hours or days. The peak force and maximal rate of force development have also been calculated for low and high frequency stimulations (Millet *et al.*, 2003a; Millet *et al.*, 2003b).

Merton (1954) proposed the interpolated twitch technique (ITT) as a measurement of the origin of fatigue (i.e. central or peripheral), where an electrical stimulus is applied to the muscle during the isometric plateau and immediately after an MVC. Single twitches, high and low frequency tetani and two or three superimposed twitches (doublet and triplet, respectively) have been used as electrical stimuli for the ITT (Paillard *et al.*, 2005). Oskouei *et al.* (2003) recommended the use of a doublet as it was effected less by posttetanic potentiation than a single twitch.

Vøllestad (1997) suggested that the physiological mechanisms responsible for exhaustion are different from those of fatigue as measured by an MVC. Exhaustion can be

assessed by measuring endurance time whilst maintaining a target force during an isometric contraction. However, the exact physiological mechanisms responsible for task failure during an isometric hold are unclear (Hunter *et al.*, 2004). The ability to exert a steady force during an isometric contraction (steadiness) represent control of motor unit output (Christou and Carlton, 2002) and an increase of force fluctuations represents a decrease in neuromuscular function (Lavender and Nosaka, 2006). Changes in the steadiness of an isometric contraction have been used to quantify changes in neuromuscular function following activities known to cause neuromuscular impairment (Lavender and Nosaka, 2006; Saxton *et al.*, 1995). However, the reliability of isometric holds has not been investigated.

The reliability of the peak force of resting and potentiated twitches and doublets and MVC of the *m. quadriceps femoris* has been investigated, following 1 minute and 3 to 5 days rest (Place *et al.*, 2007). However, to assess changes in neuromuscular function following a protocol or activity which induces neuromuscular impairment, the measures discussed above must be repeatable over a suitable timescale (e.g. 2, 24, 48, 72 hours). Morton *et al.* (2005) have investigated the reliability of MVC and voluntary activation using a single twitch during isometric contractions of the *m. quadriceps femoris* at 2, 6, 24, 48, 72 and 168 hours. However, the repeatability of the resting twitch, potentiated doublet, low and high frequency tetani, isometric hold and the use of a doublet to assess voluntary activation have not been examined across such a timescale.

Therefore, the aim of this study was to examine the intra and inter day reliability of voluntary and electrically stimulated isometric contractions of the *m. quadriceps femoris*. A secondary aim was to determine the most appropriate parameters for assessing recovery of neuromuscular function following an exercise that is likely to cause neuromuscular impairment.

5.2. Methods

5.2.1. Participants

Ten healthy male participants (mean ± SD, age 30 ± 8 years, height 1.79 ± 0.05 m, body mass 79.4 ± 8.3 kg) volunteered to participate in this study. Ethical approval for all procedures and protocols was provided by the University of Chichester Ethics Committee. All protocols were performed in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. Participants provided written informed consent and were screened to ensure they were free from any musculoskeletal injury prior to commencing the study.

Participants were instructed to abstain from any vigorous and unaccustomed physical activity 24 hours before the start and during the study and arrive in the laboratory in a rested state. Before baseline testing, participants were questioned to ensure they were adequately rested and free from muscle injury and completed a muscle soreness questionnaire. The questionnaire provided a map of the body divided into 12 segments (Corlett and Bishop, 1976). The perception of muscle soreness in each segment was rated on a visual analogue scale from 0 (no soreness) to 10 (unbelievable soreness). All participants reported the lowest rating of 0 (no soreness) for all body segments before each baseline testing session.

Mechanical calibration of the isometric chair setup was conducted prior to the experiment by hanging calibrated weights from the strain gauge and plotting actual force against recorded force.

5.2.2. Preliminary Measures and Familiarisation

Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) (± 0.01 kg) and stature (Avery Berkel, Smethwick, UK) (± 0.005 m) were measured whilst wearing shorts and underwear.

At least 5 days prior to beginning the experimental protocol, participants were familiarised with all test procedures by completing 3 maximal voluntary contractions, a 50 % isometric hold to exhaustion and all electrical stimulation procedures (described in detail below). Also, current required to stimulate an involuntary maximal twitch force (i.e. increases in current caused no further increase in force of the twitch) and sub-maximal twitch force (5 % MVC force) were recorded and kept constant in all subsequent test sessions.

5.2.3. Experimental Protocol

Participants completed the muscle testing protocol described below at 0 (baseline), 2, 24, 48 and 72 hours to assess reliability of test procedures. The test order was the same on each occasion (Figure 5.1) and conducted at approximately the same time of day to control for diurnal variation in force producing capability of the muscles (Sedliak *et al.*, 2008).

For all tests, participants were secured in a custom built chair for testing the *m. quadriceps femoris* with hip and knee at 90° flexion (Figure 5.2A). Velcro straps were placed around the participants' chest and waist to restrict movement of the upper body and hips. A cuff was placed around the participants' ankle (proximal to the fibular notch and medial malleolus) and attached to an s-beam load cell (RS 250kg, Tedea Huntleigh, Cardiff, UK) via a steel chain at the base of the chair. The force produced from the *m. quadriceps femoris* was recorded on a computer at 1000 Hz (Chart 4 V4.1.2, AD Instruments, Oxford, UK). Two custom made saline soaked electrodes (9 x 18 cm) were placed just above the patella and over the muscle belly of the *m. quadriceps femoris* in the proximal third part of the thigh of the non-dominant leg (Figure 5.2B). The position of the electrodes was marked using permanent pen to ensure accurate placement on subsequent tests. For all electrically evoked test procedures, stimulation was provided through an electrical muscle stimulator (Model DS7A, Digitimer Limited, Welwyn Garden City, UK) and multiple pulses were controlled by a NeuroLog pulse generator (Digitimer Limited, Welwyn Garden City, UK).

When participants were first seated in the chair they conducted three 5 second sub-maximal contractions (~200 N) to become accustomed to the experimental set up. The order of the tests shown in Figure 5.1 and the measurements are described below.

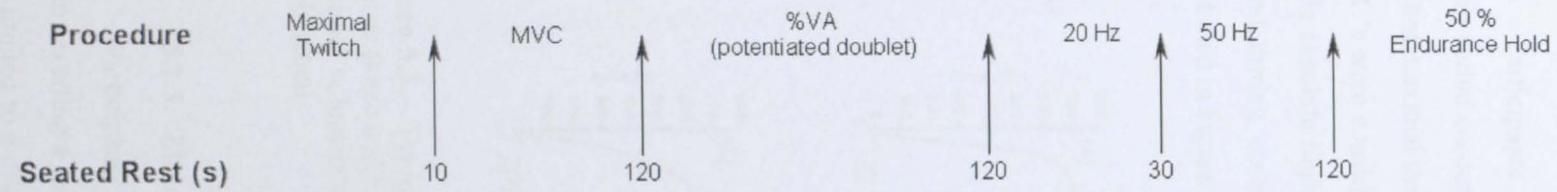
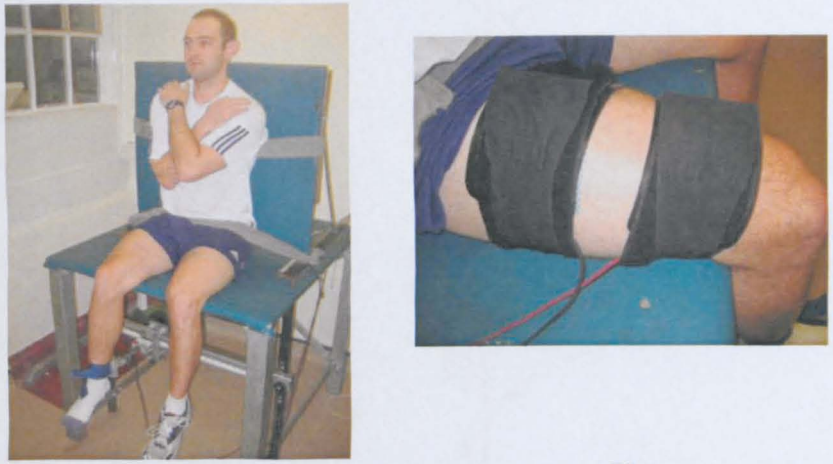


Figure 5.1 – Test order of isometric contractions of the *m. quadriceps femoris* muscles by voluntary activation and surface electrical stimulation. Tests were conducted in an isometric chair (knee and hip angle 90°), in the same order at 0 (baseline), 2, 24, 48 and 72 hours.



(A) **(B)**

Figure 5.2 – (A) Positioning of the participant in the isometric chair (B) and placement of saline soaked electrodes just above the patella and over the muscle belly of the quadriceps in the proximal third part of the thigh of the non-dominant leg.

5.2.4. Maximal Voluntary Contraction (MVC)

Participants produced a 3 to 5 second maximal voluntary contraction (MVC) with strong verbal encouragement. Only if the experimenter or participant confirmed that the effort was not maximal the procedure was repeated following 2 minutes rest. Approximately 90 % of MVC's were considered a maximal effort on the first attempt. Force calculations included the single absolute highest value and the highest mean force values over a 0.25 and 0.50 s time period during contractions. A typical example of the force recorded during a MVC is illustrated in Figure 5.3 A.

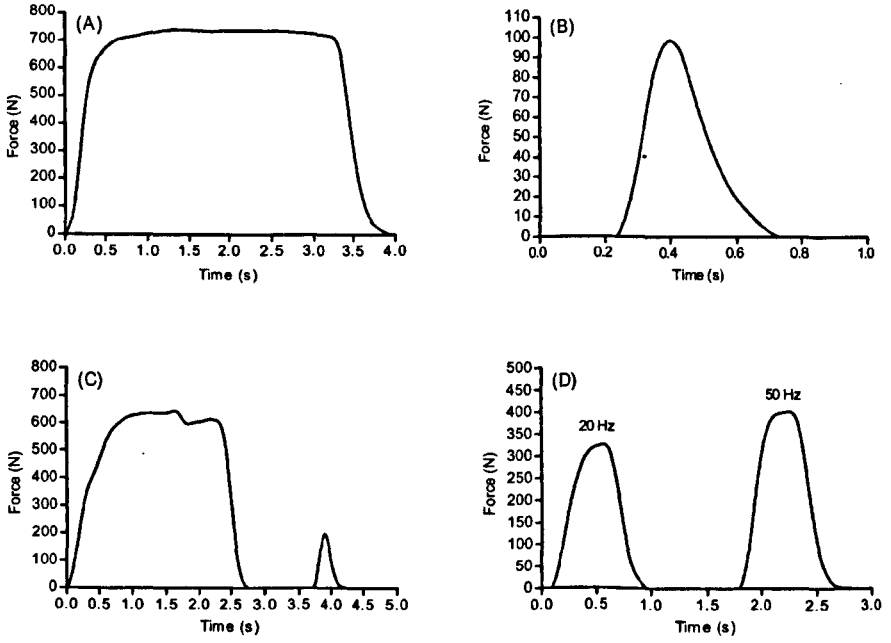


Figure 5.3 – Typical responses recorded during isometric contractions of the *m. quadriceps femoris* (knee and hip angle 90°) during voluntary and electrically stimulated contractions: (A) maximal voluntary contraction, (B) resting twitch, (C) potentiated doublet and (D) 20 Hz and 50 Hz tetani.

5.2.5. Electrically Evoked Twitch

Participants were instructed to relax then take up the slight tension in the connecting chain (exerting a minimal force on the strain gauge, $\leq$ 3 N). A single 50 μs pulse of 100 mA and limited to a maximum of 400 V was applied to the *m. quadriceps femoris*. In each of the test sessions, the current was increased in 50 mA increments until the maximal twitch current

was reached (group mean ± SD; 830 ± 54 mA). The maximal twitch was recorded and the following parameters measured: (a) peak force (N), the maximal force value of the twitch; (b) contraction time (s), the time between the first derivation from baseline and peak force; (c) half relaxation time (s), the time taken to fall from peak force to half of the value during the relaxation phase; (d) maximal rate of force development ($N \cdot s^{-1}$), the highest value of the first derivative of the force signal; (e) maximal rate of force decrease ($N \cdot s^{-1}$), the lowest value of the first derivative of the force signal; (f) rate constant for contraction ($\cdot s^{-1}$), maximal rate of force development/peak force and (g) rate constant for relaxation ($\cdot s^{-1}$), maximal rate of force decrease/peak force. Findings of previous studies examining the reliability of resting and potentiated twitches are mixed (Kufel *et al.*, 2002; Morton *et al.*, 2005). The parameters were recorded from a electrically evoked twitch from rest as the peak force has been shown to be more reliable than using a potentiated twitch over a similar timescale when using surface rather than nerve stimulation (Morton *et al.*, 2005). A typical example of the force recorded during an electrically evoked twitch is illustrated in Figure 5.3 B.

5.2.6. Interpolated Doublet (% Voluntary Activation)

Participants were instructed to produce an MVC (as described above). A doublet pulse (two maximal single twitches separated by a 10 ms gap) was applied to the *m. quadriceps femoris* during the plateau phase of the contraction, and immediately after the MVC when participants returned to rest (potentiated doublet). Percent voluntary activation (%VA) was calculated as follows:

Equation 5.1

$$\%VA = 100 - (\text{Superimposed MVC Doublet} / \text{Post MVC Doublet}) \times 100$$

A doublet stimulation was used as the resultant force is greater than a twitch and therefore a stronger measure to assess %VA (Paillard *et al.*, 2005). The same parameters as for the resting twitch (described above a-e) were calculated for the potentiated doublet. A typical example of the force recorded during a MVC with an interpolated and potentiated doublet is illustrated in Figure 5.3 C.

5.2.7. 20 Hz and 50 Hz Stimulation

Participants were instructed to relax then take up slight tension in the connecting chain (exerting a minimal force on the strain gauge, $\leq$ 3 N). 20 Hz and 50 Hz stimulations (0.5 s duration), with 30 second rest between stimulations, were applied to the *m. quadriceps femoris* using the sub-maximal twitch current (group mean ± SD; 433 ± 65 mA). A sub-maximal current was used as it has been shown to give a reliable estimate of contractile properties (Edwards *et al.*, 1977a) and is more tolerable for participants. A ratio of the forces at 20 Hz and 50 Hz was calculated, a reduction in the ratio indicates the presence of LFF and an increase HFF (Jones, 1996). The same parameters as for the resting twitch (described above a-e) were calculated for the 20 Hz and 50 Hz stimulations. However, the contraction time (b) was calculated as the time taken to rise between 20 and 90 % of the peak force. A typical example of the force recorded during 20 and 50 Hz stimulations is illustrated in Figure 5.3 D.

5.2.8. 50 % Isometric Hold

Participants were instructed to produce a voluntary submaximal contraction equating to a force of 50 % of MVC peak force until exhaustion. MVC peak force was taken as the single absolute highest value attained in the test session. The participants exerted a force between 105 % and 95 % of the 50 % MVC peak force throughout the test, whilst observing their effort on a computer screen. Exhaustion was defined as the point when 95 % of 50 % MVC peak force could no longer be maintained. Throughout the test, standard commands and advice on whether to increase or decrease the force exerted was given every 5 to 10 seconds until the participant stopped their contraction. The following parameters were calculated: (a) duration (s), time from the point that 50 % force is first achieved to when force declines to < 95 % of the 50 % MVC force; (b) impulse (Ns), area under the curve; (c) mean force (N), mean force for the duration of the contraction; and (d) steadiness, calculated using the following formula (Hunter *et al.*, 2002):

Equation 5.2

Steadiness = (SD Contraction Force / Mean Contraction Force) x 100

Steadiness was calculated for the whole contraction and sub-divisions of time for total durations of 5, 10, 20, 30 s, from five seconds after the start of the contraction (to allow all participants to establish a steady contraction).

5.2.9. Environmental Conditions

Environmental temperature was monitored using a dry bulb thermometer (Fisher Scientific, Loughborough, UK). No differences in environmental temperature were observed between baseline tests and 2, 24, 48 and 72 hour test periods, respectively (21.2 ± 2.5, 22.2 ± 2.6, 21.6 ± 1.0, 21.6 ± 1.5, 22.3 ± 1.9 °C, $P>0.05$).

5.2.10. Statistical Analysis

Statistical analysis was undertaken using SPSS for Windows V15 (SPSS, Chicago, Illinois) and Microsoft Excel 2002 for Windows (Microsoft, Redmond, Washington). Normal distribution of the data was verified using a Kolmogorov-Smirnov test.

Repeatability of the baseline contractile parameters and measurements were assessed at 2, 24, 48 and 72 hours after baseline using Bland & Altman's 95 % Limits of Agreement (LoA) (Bland and Altman, 1987, 1995, 2003, 2007). Data were examined for heteroscedasticity using Pearsons correlation coefficient. Heteroscedasticity was present in the majority of comparisons (as is the case for the majority of variables measured on the ratio scale), therefore, log ratio LoA were calculated and used for analysis of all datasets (Atkinson and Nevill, 1998; Nevill and Atkinson, 1997). Logarithms for each data point were calculated and individual participant differences [baseline – test (2, 24, 48, 72 hours)] were plotted against respective individual means. Mean differences (bias) between baseline and 2, 24, 48, and 72 hour tests were assessed using paired t-tests with statistical significance set at $P<0.05$. The 95 % LoA were produced by calculating the standard deviation of the differences between tests 1 and test 2 and multiplying by 1.96. In addition, 95 % confidence intervals (CI) were calculated around the limits of agreement by multiplying the standard error of the differences by the t-test test statistic. All data are shown as percent ratio LoA for ease of interpretation and presented as mean bias (± 95 % LoA) [lower 95 % CI – upper 95 % CI].

5.3. Results

The single maximum value of the MVC at baseline vs. 2 hours is provided as an example for interpretation of the results {0.9 (± 14.2) [-17.9 - 24.0]}. The bias is a measure of the mean difference between measurements; in this example the mean bias for the force at baseline vs. 2 hours is 0.9 % (P>0.05). The 95 % LoA illustrate the variation that can be expected for this population with 95 % confidence. In this example, there is 95 % confidence that the force an individual participant produces during an MVC could increase or decrease by 14.2 % between measurement at baseline and at 2 hours. The upper and lower 95 % LoA can be calculated by adding or subtracting the 95 % LoA value to the bias respectively (15.1 % upper LoA and -13.3 % lower LoA). The upper and lower 95 % CI show variation around the respective LoA with 95 % confidence. The variation could be as great as a -17.9 % decrease (lower 95 % CI) or a 24.0 % increase (upper 95 % CI). The bias, 95 % LoA and 95 % CI are also illustrated in Figure 5.4 for the twitch and doublet for baseline vs. 2 hours.

The MVC peak force at baseline was 661 ± 124 N (single maximum value), 657 ± 122 N (0.25 second divisions) and 654 ± 121 N (0.5 second divisions). There was no significant mean bias compared to baseline for any of the MVC parameters at 2, 24, 48 and 72 hours and the magnitude of the bias was similar across parameters and time points (Table 5.4). The 95 % LoA and 95 % CI (Table 5.4) show that the repeatability of these measures compared to baseline were similar for the single maximum value, 0.25 s divisions and 0.50 s divisions at 2, 24, 48 and 72 hours.

There was no significant mean bias or systematic change in voluntary activation during the MVC compared to baseline between 2, 24, 48 and 72 hours (Table 5.4). There was low variability (95 % LoA [lower and upper 95 % CI]) in voluntary activation between baseline and all time points (Table 5.4).

The initial mean peak force of the single twitch was 76 ± 21 N and there was no significant mean change compared to baseline at 2, 24, 48 and 72 hours respectively (Table 5.5). The peak force of the doublet was higher than the single twitch at baseline (190 ± 43 vs. 76 ± 21 N, P<0.001). There was also no significant mean bias in doublet peak force compared to baseline at 2, 24, 48 and 72 hours (Table 5.5).

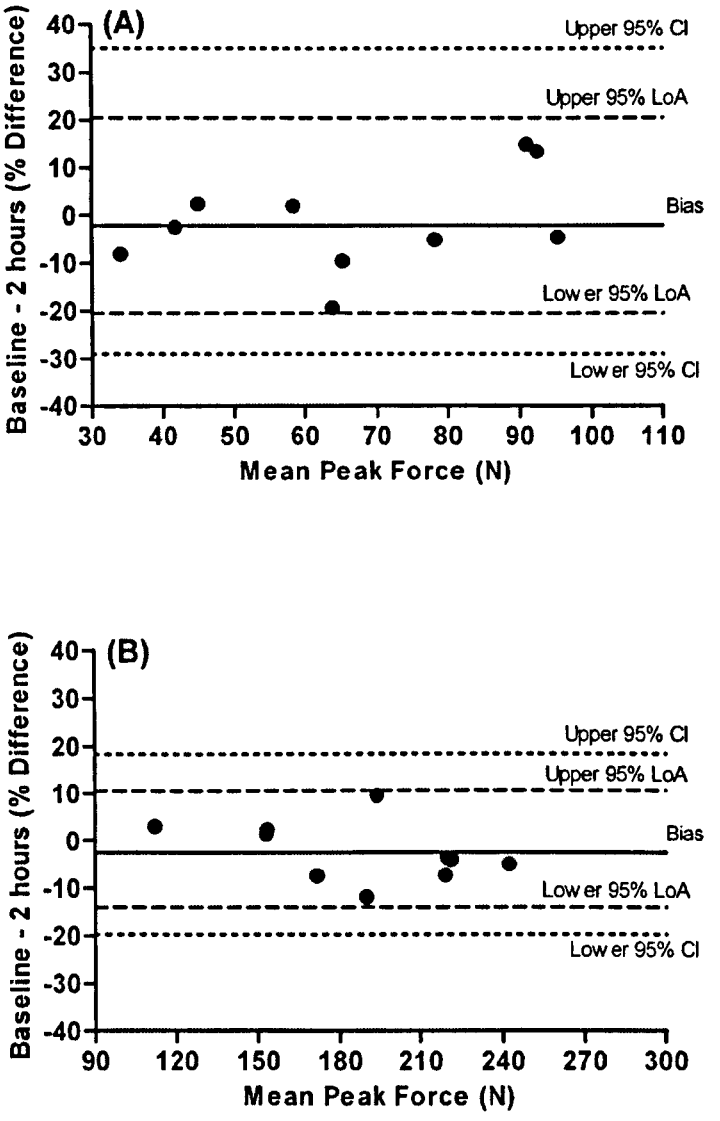


Figure 5.4 – Bland-Altman plots of the agreement between peak force at baseline (0 Hours) and 2 hours for **(A)** stimulated twitch and **(B)** doublet. Each data point is presented individually (mean baseline and 2 hour values vs. percentage differences between baseline and 2 hour values). The mean bias (——), 95 % limits of agreement (– – –) and upper and lower confidence intervals of the 95 % limits of agreement (·····) are displayed.

Figure 5.4 illustrates the greater individual variation for the twitch compared to doublet peak force for baseline vs. 2 hours, which is also apparent for baseline vs. 24, 48 and 72 hours. The variation from baseline is similar at 2, 24, 48, and 72 hours for both the twitch and the doublet, therefore there was no systematic change over time. The greater variation for the twitch compared to the doublet is also apparent in contraction time, half relaxation time, maximal rate of force development and maximal rate of force decrease.

Compared to baseline at 24 and 48 hours, there was a significant mean bias for the peak force of the 20 Hz stimulation (9.2 and 9.1 %, $P<0.05$) and 50 Hz stimulation (8.7 and 9.1 %, $P<0.05$). There was no significant bias for both the 20 Hz and 50 Hz stimulations at 2 hours or 72 hours, compared to baseline. There was a large variation in the peak force of the 20 Hz stimulation, which was lowest at 24 hours and highest at 72 hours (Table 5.6). A similar degree of variation was apparent for the 50 Hz stimulations, which was similarly lowest at 2 hours and highest at 72 hours (Table 5.6), maximal rate of force development at 24 hours (7.5 %, $P=0.002$), and the maximal rate of force decrease at 24 hours (8.0 %, $P=0.028$). Significant mean bias compared to baseline was also apparent during the 50 Hz stimulation for the maximal rate of force development at 24 hours (7.5 %, $P=0.028$) and maximal rate or force decrease at 72 hours (11.5 %, $P=0.002$). The variation in the contraction time, half relaxation time, maximal rate of force development and maximal rate of force decrease during the 20 Hz and 50 Hz stimulations was high and similar to the peak force measurements (Table 5.6). There was no systematic change in the variation of the measurements between 2, 24, 48, and 72 hours (Table 5.6).

Significant changes in mean bias occurred more frequently for the 20 Hz stimulation and 50 Hz stimulation parameters compared to the twitch and doublet parameters. The variation (95 % LoA [lower and upper 95 % CI]) was similar for the twitch, 20 Hz and 50 Hz stimulations, but lower for the doublet. A typical example is the comparison between peak force recorded at baseline and 24 hours (Table 5.6). The variation in the rate constant of contraction was greater for the 20 Hz and 50 Hz stimulations compared to the twitch and doublet. However, the variation in the rate constant of relaxation was similar for the doublet, 20 Hz and 50 Hz stimulations but greater for the twitch.

Despite the large variation in peak force of the 20 Hz and 50 Hz stimulations when expressed as the 20:50 Hz ratio reliability was improved. Compared to the individual 20 Hz

and 50 Hz peak force (Table 5.6) the 20:50 Hz ratio showed smaller mean bias, 95 % LoA , and 95 % CI at 2 h {1.5 (± 15.4) [-18.7 - 26.8]}, 24 h {0.4 (± 10.3) [-13.6 - 16.8]}, 48 h {0.0 (± 10.9) [-14.8 - 17.3]} and 72 h {-1.2 (± 9.1) [-13.8 - 13.1]}.

The mean duration of the 50 % endurance hold was 80 ± 26 s at baseline and there was no significant mean bias at 2, 24, 48, and 72 hours (Table 5.1). However, there was still a relatively low reliability, illustrated by the 95 % LoA and CI (Table 5.4). There was no significant bias for the mean force during the contraction, and compared to the duration the variability in mean force was lower. The 95 % LoA [lower and upper 95 % CI] systematically decreased from 16.2 [-21.0 - 25.7] at 2 hours to 10.0 [-14.3 - 15.2] at 72 hours. There was no significant bias for impulse during the 50 % endurance hold and the variation was similar to the duration. This would be expected as impulse is calculated from the duration and mean force during the contraction. Although there was no significant mean bias in the steadiness during the 50 % endurance hold, there was very low reliability (95 % LoA) for the whole contraction and the sub-divisions of time when compared to baseline at 2, 24, 48 and 72 hours (Table 5.4).

Table 5.1 – Contractile parameters of the *m. quadriceps femoris* muscles during a maximal voluntary contraction and 50 % of MVC force endurance hold to exhaustion at 0 (baseline) 2, 24, 48 and 72 hours during isometric contractions (knee and hip angle 90°). Data are presented as mean ± SD, n=10.

	Measure	Baseline (0 h)	2 h	24 h	48 h	72 h
MVC	Single MaxValue (N)	661 ± 124	666 ± 116	642 ± 109	658 ± 125	658 ± 133
	0.25 s Divisions (N)	657 ± 122	664 ± 117	639 ± 109	655 ± 121	655 ± 131
	0.50 s Divisions (N)	654 ± 121	656 ± 117	636 ± 108	652 ± 123	648 ± 130
	VA (%)	97 ± 2	98 ± 2	98 ± 3	97 ± 3	97 ± 3
50 % Endurance Hold	Duration (s)	80 ± 26	78 ± 27	82 ± 29	85 ± 27	86 ± 29
	Impulse (Ns)	25413 ± 7420	24243 ± 7257	24998 ± 6945	26146 ± 6686	26434 ± 6884
	Mean Force (N)	322 ± 56	320 ± 49	314 ± 51	318 ± 53	321 ± 59
	Steadiness	4.3 ± 2.7	4.0 ± 2.5	3.2 ± 2.8	3.5 ± 3.0	3.5 ± 2.9
	Steadiness (30 s)	2.4 ± 1.1	4.1 ± 4.5	2.7 ± 2.2	2.4 ± 2.0	2.5 ± 2.0
	Steadiness (20 s)	2.9 ± 2.0	2.7 ± 1.6	2.6 ± 1.3	4.5 ± 8.0	5.1 ± 7.3
	Steadiness (10 s)	2.6 ± 2.1	2.4 ± 2.0	3.4 ± 4.1	2.1 ± 1.2	3.7 ± 5.3
	Steadiness (5 s)	3.2 ± 2.5	4.1 ± 2.9	2.2 ± 1.5	2.5 ± 1.7	2.6 ± 1.6

Table 5.2 – Contractile parameters of the *m. quadriceps femoris* muscles during electrically stimulated twitch and doublet contractions at 0 (baseline) 2, 24, 48 and 72 hours during isometric contractions (knee and hip angle 90°). Data are presented as mean ± SD, n=10.

	Measure	Baseline (0 h)	2 h	24 h	48 h	72 h
Twitch	Peak Force (N)	76 ± 21	75 ± 23	80 ± 24	72 ± 17	74 ± 17
	Contraction Time (s)	0.177 ± 0.022	0.178 ± 0.025	0.182 ± 0.025	0.176 ± 0.021	0.182 ± 0.021
	Half Relaxation Time (s)	0.103 ± 0.018	0.094 ± 0.010	0.097 ± 0.018	0.092 ± 0.009	0.095 ± 0.010
	Maximal Rate of Force Development ($N \cdot s^{-1}$)	626 ± 164	648 ± 194	668 ± 183	624 ± 148	644 ± 155
	Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	-475 ± 99	-519 ± 145	-545 ± 180	-513 ± 128	-513 ± 132
	Rate Constant for Contraction ($\cdot s^{-1}$)	8.0 ± 0.4	8.3 ± 0.3	8.2 ± 0.5	8.4 ± 0.4	8.5 ± 0.3
	Rate Constant for Relaxation ($\cdot s^{-1}$)	-6.2 ± 0.9	-6.7 ± 0.7	-6.7 ± 1.1	-7.0 ± 0.8	-6.8 ± 0.9
Doublet	Peak Force (N)	190 ± 43	185 ± 38	192 ± 40	190 ± 39	191 ± 39
	Contraction Time (s)	0.187 ± 0.010	0.188 ± 0.010	0.189 ± 0.004	0.189 ± 0.008	0.188 ± 0.010
	Half Relaxation Time (s)	0.098 ± 0.010	0.098 ± 0.008	0.098 ± 0.009	0.096 ± 0.009	0.096 ± 0.009
	Maximal Rate of Force Development ($N \cdot s^{-1}$)	1611 ± 320	1562 ± 327	1665 ± 353	1637 ± 323	1669 ± 328
	Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	-1315 ± 338	-1284 ± 336	-1351 ± 372	-1353 ± 328	-1380 ± 341
	Rate Constant for Contraction ($\cdot s^{-1}$)	8.5 ± 0.4	8.5 ± 0.2	8.7 ± 0.2	8.6 ± 0.3	8.8 ± 0.4
	Rate Constant for Relaxation ($\cdot s^{-1}$)	-6.9 ± 0.9	-6.9 ± 0.8	-7.0 ± 0.9	-7.1 ± 0.8	-7.2 ± 0.9

Table 5.3 – Contractile parameters of the *m. quadriceps femoris* muscles during electrically stimulated 20 and 50 Hz contractions at 0 (baseline) 2, 24, 48 and 72 hours during isometric contractions (knee and hip angle 90°). Data are presented as mean ± SD, n=10.

	Measure	Baseline (0 h)	2 h	24 h	48 h	72 h
20 Hz	Peak Force (N)	255 ± 79	266 ± 100	282 ± 95	278 ± 82	275 ± 81
	20:90 Contraction Time (s)	0.216 ± 0.026	0.225 ± 0.030	0.221 ± 0.027	0.226 ± 0.019	0.223 ± 0.014
	Half Relaxation Time (s)	0.171 ± 0.035	0.162 ± 0.020	0.161 ± 0.010	0.166 ± 0.018	0.162 ± 0.010
	Maximal Rate of Force Development ($N \cdot s^{-1}$)	1101 ± 284	1104 ± 350	1185 ± 307	1141 ± 285	1143 ± 302
	Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	-1421 ± 518	-1481 ± 619	-1551 ± 603	-1520 ± 516	-1543 ± 552
	Rate Constant for Contraction ($\cdot s^{-1}$)	4.4 ± 0.3	4.3 ± 0.4	4.3 ± 0.5	4.2 ± 0.4	4.2 ± 0.4
	Rate Constant for Relaxation ($\cdot s^{-1}$)	-5.5 ± 0.5	-5.5 0.5	-5.4 ± 0.5	-5.4 ± 0.5	-5.5 ± 0.5
50 Hz	Peak Force (N)	314 ± 108	321 ± 128	342 ± 119	338 ± 103	336 ± 113
	20:90 Contraction Time (s)	0.203 ± 0.027	0.199 ± 0.022	0.206 ± 0.028	0.210 ± 0.023	0.200 ± 0.023
	Half Relaxation Time (s)	0.169 ± 0.010	0.175 ± 0.013	0.176 ± 0.022	0.167 ± 0.013	0.194 ± 0.047
	Maximal Rate of Force Development ($N \cdot s^{-1}$)	1535 ± 460	1545 ± 533	1645 ± 499	1619 ± 453	1633 ± 473
	Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	-1732 ± 691	-1777 ± 789	-1882 ± 773	-1901 ± 718	-1890 ± 746
	Rate Constant for Contraction ($\cdot s^{-1}$)	4.9 ± 0.4	4.9 ± 0.4	4.9 ± 0.4	4.8 ± 0.3	4.9 ± 0.5
	Rate Constant for Relaxation ($\cdot s^{-1}$)	-5.4 ± 0.4	-5.5 ± 0.5	-5.4 ± 0.5	-5.5 ± 0.5	-5.6 ± 0.5
20:50 Hz Ratio		0.85 ± 0.06	0.86 ± 0.04	0.86 ± 0.04	0.86 ± 0.05	0.84 ± 0.04

Table 5.4 - Ratio (%) limits of agreement (LoA) for the baseline value (time 0 h) compared to 2, 24, 48 and 72 h for the *m. quadriceps femoris* during a maximal voluntary contraction and 50 % endurance hold to exhaustion during isometric contractions (knee and hip angle 90°). Data are presented as mean bias (± 95% LoA) [lower 95% confidence interval – upper 95% confidence interval] (n=10). Upper and lower 95% CI were too large therefore could not be accurately calculated for steadiness.

Measure		2 h	24 h	48 h	72 h
MVC	Single Maximum Value (N)	0.9 (± 14.2) [-17.9 - 24.0]	-2.7 (± 16.4) [-23.1 - 23.0]	-0.6 (± 11.9) [16.5 - 18.4]	-0.9 (± 9.8) [-14.2 - 14.6]
	0.25 s Divisions (N)	1.0 (± 14.3) [-17.8 - 24.2]	-2.6 (± 15.4) [-22.0 - 21.6]	-0.5 (± 11.9) [-16.4 - 18.5]	-0.7 (± 9.9) [-14.2 - 14.8]
	0.50 s Divisions (N)	0.3 (± 15.0) [-19.2 - 24.5]	-2.7 (± 15.2) [-21.9 - 21.1]	-0.4 (± 11.9) [-16.3 - 18.5]	-1.2 (± 11.8) [-16.9 - 17.3]
	Voluntary Activation (%)	0.9 (± 6.9) [-9.0 - 12.0]	0.8 (± 6.4) [-8.4 - 10.9]	-0.2 (± 6.0) [-8.8 - 9.2]	-0.4 (± 9.1) [-12.9 - 13.9]
50 % Hold	Duration (s)	-4.3 (± 25.8) [-32.9 - 36.6]	1.2 (± 26.9) [-30.0 - 46.3]	5.3 (± 31.0) [-30.6 - 59.9]	5.8 (± 23.1) [-23.3 - 46.0]
	Impulse (Ns)	-4.6 (± 33.8) [-39.2 - 49.7]	-1.2 (± 34.6) [-37.7 - 56.5]	4.0 (± 36.5) [-35.7 - 68.4]	5.1 (± 26.8) [-27.2 - 51.7]
	Mean Force (N)	-0.3 (± 16.2) [-21.0 - 25.7]	-2.4 (± 16.4) [-22.8 - 23.4]	-1.2 (± 13.8) [-19.2 - 20.7]	-0.7 (± 10.0) [-14.3 - 15.2]
	Steadiness (Total)	-5.5 (± 157.1)	-29.3 (± 243.7)	-23.0 (± 272.7)	-22.0 (± 125.2)
	Steadiness (30 s)	24.0 (± 310.9)	2.5 (± 217.1)	-9.8 (± 186.9)	-6.4 (± 293.8)
	Steadiness (20 s)	-5.5 (± 164.9)	-6.2 (± 86.7)	-4.6 (± 357.8)	21.1 (± 265.5)
	Steadiness (10 s)	-7.9 (± 66.9)	14.6 (± 708.6)	-11.2 (± 129.2)	10.4 (± 159.2)
	Steadiness (5s)	25.5 (± 285.5)	-27.8 (± 374.8)	-15.6 (± 341.0)	-12.8 (± 248.5)

Table 5.5 - Ratio (%) limits of agreement (LoA) for the baseline value (time 0 h) compared to 2, 24, 48 and 72 h for the *m. quadriceps femoris* during an electrically stimulated isometric twitch and doublet contractions (knee and hip angle 90°). Data presented as mean bias (± 95% LoA) [lower 95% confidence interval – upper 95% confidence interval] (n=10). * Significant bias ($P<0.05$).

	Measure	2 h	24 h	48 h	72 h
Twitch	Peak Force (N)	-1.1 (± 19.7) [-25.1 - 30.5]	4.4 (± 24.5) [-25.6 - 46.5]	-4.2 (± 35.4) [-40.1 - 53.0]	-1.6 (± 29.8) [-34.3 - 47.4]
	Contraction Time (s)	0.4 (± 14.7) [-19.1 - 24.6]	2.3 (± 15.4) [-18.4 - 28.3]	-0.4 (± 14.0) [-19.0 - 22.4]	2.5 (± 12.2) [-14.5 - 23.0]
	Half Relaxation Time (s)	-7.1 (± 18.5) [-29.0 - 21.4]	-5.1 (± 31.6) [-38.5 - 46.4]	-9.6 (± 38.6) [-46.0 - 51.3]	-7.1 (± 26.4) [-35.8 - 34.5]
	Maximal Rate of Force Development ($N \cdot s^{-1}$)	2.4 (± 21.3) [-24.5 - 38.9]	6.3 (± 21.4) [-21.7 - 44.3]	0.3 (± 31.6) [-35.0 - 54.6]	3.6 (± 27.5) [-29.4 - 51.9]
	Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	7.2 (± 34.4) [-32.8 - 70.9]	11.6 (± 46.2) [-38.7 - 103.2]	7.1 (± 18.1) [-17.6 - 39.1]	7.1* (± 18.6) [-18.2 - 40.3]
	Rate Constant for Contraction ($\cdot s^{-1}$)	4.2* (± 9.1) [-9.2 – 19.6]	2.1 (± 7.1) [-8.4 – 13.8]	5.3* (± 12.1) [-12.0 – 26.1]	6.0* (± 10.7) [-9.7 – 24.4]
	Rate Constant for Relaxation ($\cdot s^{-1}$)	9.1* (± 23.0) [-21.3 – 51.2]	7.2 (± 37.9) [-35.4 – 77.9]	12.4 (± 41.2) [-34.8 – 93.8]	9.6 (± 28.5) [-26.3 – 62.8]
Doublet	Peak Force (N)	-2.5 (± 13.4) [-19.7 - 18.4]	1.3 (± 10.4) [-13.1 - 18.1]	0.1 (± 13.9) [-18.1 - 22.4]	0.9 (± 14.6) [-18.3 - 24.7]
	Contraction Time (s)	0.8 (± 9.1) [-11.9 - 15.2]	1.4 (± 9.5) [-11.9 - 16.7]	1.1 (± 11.1) [-14.1 - 19.0]	0.5 (± 7.7) [-10.4 - 12.6]
	Half Relaxation Time (s)	-0.1 (± 12.4) [-16.6 - 19.7]	-0.4 (± 13.7) [-18.3 - 21.5]	-2.1 (± 8.8) [-14.1 - 11.6]	-2.7* (± 6.2) [-11.4 - 6.8]
	Maximal Rate of Force Development ($N \cdot s^{-1}$)	-3.3 (± 11.1) [-17.8 - 13.8]	3.1 (± 11.6) [-13.1 - 22.2]	1.6 (± 10.8) [-13.3 - 19.0]	3.7 (± 12.5) [-13.6 - 24.4]
	Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	-2.5 (± 16.3) [-22.9 - 23.1]	2.1 (± 15.5) [-18.3 - 27.7]	3.2 (± 13.1) [-14.7 - 24.9]	5.2* (± 12.7) [-12.6 - 26.6]
	Rate Constant for Contraction ($\cdot s^{-1}$)	-0.8 (± 10.7) [-15.2 – 16.0]	1.8 (± 11.9) [-14.4 – 21.0]	1.5 (± 9.8) [-12.3 – 17.3]	2.8 (± 10.4) [-11.8 – 19.7]
	Rate Constant for Relaxation ($\cdot s^{-1}$)	-0.1 (± 16.7) [-21.3 – 26.9]	0.8 (± 17.8) [-21.8 -30.0]	3.1 (± 11.8) [-13.3 – 22.6]	4.2* (± 7.1) [-6.3 – 16.0]

Table 5.6 - Ratio (%) limits of agreement (LoA) for baseline (time 0 h) compared to 2, 24, 48 and 72 h for the *m. quadriceps femoris* during electrically stimulated 20 and 50 Hz contractions during isometric contractions (knee and hip angle 90°). Data presented as mean bias (± 95% LoA) [lower 95% confidence interval – upper 95% confidence interval] (n=10). * Significant bias ($P<0.05$).

Measure	Parameters	2 h	24 h	48 h	72 h
20 Hz	Peak Force (N)	1.8 (± 30.9) [-32.9 - 54.4]	9.2* (± 21.9) [-19.7 - 48.4]	9.1* (± 23.3) [-21.1 - 50.9]	8.4 (± 32.2) [-29.7 - 67.0]
	20:90 Contraction Time (s)	-3.9 (± 26.7) [-33.4 - 38.6]	-2.2 (± 21.8) [-27.9 - 32.7]	-4.7(± 23.3) [-31.1 - 31.9]	-3.4(± 25.8) [-32.3 - 37.8]
	Half Relaxation Time (s)	-4.3 (± 19.7) [-27.5 - 26.3]	-4.5 (± 36.2) [-40.8 - 54.2]	-2.0 (± 19.2) [-25.3 - 28.7]	-4.1 (± 33.9) [-39.0 - 50.6]
	Maximal Rate of Force Development ($N \cdot s^{-1}$)	-1.2 (± 16.9) [-22.5 - 25.8]	7.5* (± 10.8) [-8.2 - 26.0]	3.9 (± 21.4) [-23.1 - 40.3]	3.9 (± 21.4) [-23.1 - 40.3]
	Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	1.7 (± 34.7) [-35.8 - 61.2]	8.0* (± 19.9) [-18.5 - 43.1]	7.5 (± 23.5) [-22.4 - 49.0]	9.1 (± 36.2) [-32.4 - 75.8]
	Rate Constant for Contraction ($\cdot s^{-1}$)	-2.9 (± 20.1) [-26.9 – 28.8]	-1.5 (± 18.2) [-23.9 – 27.5]	-4.8 (± 20.2) [-28.4 – 26.6]	-4.2 (± 25.4) [-32.5 – 35.9]
	Rate Constant for Relaxation ($\cdot s^{-1}$)	-0.1 (± 11.0) [-15.0 – 17.5]	-1.1 (± 7.6) [-11.7 – 10.8]	-1.4 (± 9.3) [-14.1 – 13.1]	0.6 (± 12.7) [-16.3 – 21.0]
50 Hz	Peak Force (N)	0.2 (± 25.4) [-29.4 - 42.3]	8.7* (± 19.1) [-17.1 - 42.4]	9.1* (± 19.2) [-16.8 - 43.1]	9.7 (± 32.1) [-28.7 - 68.8]
	20:90 Contraction Time (s)	1.9 (± 25.9) [-29.2 - 46.5]	-1.5 (± 13.0) [-18.8 - 19.5]	-4.0 (± 20.7) [-28.7 - 29.3]	1.4 (± 13.7) [-17.2 - 24.0]
	Half Relaxation Time (s)	3.1 (± 14.7) [-16.6 - 27.5]	-3.3 (± 60.1) [-53.3 - 100.4]	-9.2 (± 68.2) [-59.4 - 103.0]	8.2 (± 61.3) [-48.4 - 126.7]
	Maximal Rate of Force Development ($N \cdot s^{-1}$)	-0.5 (± 23.1) [-27.9 - 37.1]	7.5* (± 18.6) [-17.5 - 40.0]	6.1 (± 23.9) [-23.9 - 47.9]	8.0 (± 25.3) [-23.9 - 53.2]
	Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	-2.9 (± 39.7) [-42.1 - 62.8]	-2.9 (± 39.7) [-42.1 - 62.8]	4.7 (± 30.3) [-30.4 - 57.7]	11.5** (± 16.8) [-12.4 - 41.8]
	Rate Constant for Contraction ($\cdot s^{-1}$)	-0.4 (± 15.4) [-20.5 – 24.8]	-1.2 (± 17.8) [-23.7 – 28.0]	-2.1 (± 19.3) [-25.9 – 29.4]	-0.4 (± 17.6) [-22.8 – 28.7]
	Rate Constant for Relaxation ($\cdot s^{-1}$)	0.6 (± 7.0) [-9.6 – 11.8]	-0.6 (± 5.9) [-9.2 – 8.9]	1.6 (± 14.6) [-18.0 – 26.0]	2.1 (± 8.5) [-10.3 – 16.2]

5.4. Discussion

This study examined the repeatability of voluntary and electrically stimulated contractions to measure the contractile properties of the *m. quadriceps fermoris* from baseline at 2, 24, 48 and 72 h. The repeatability of a single twitch, potentiated doublet, VA with doublet stimulation, 20 Hz and 50 Hz stimulations and isometric endurance hold had not previously been compared over a time scale suitable for measuring changes in neuromuscular function following exercise. The main findings of the present study were that MVC force showed no significant mean bias and had similar reliability (95 % LoA and CI) compared to the single maximum value, 0.25 or 0.5 s divisions. The contractile parameters (peak force, contraction times and relaxation times) during the doublet stimulation showed greater reliability (95 % LoA and CI) than the parameters of the single twitch and 20 and 50 Hz stimulations. Measurement of VA using the interpolated doublet was repeatable across time as there was no significant mean bias and high reliability (95 % LoA and CI). Endurance time and steadiness during the 50 % isometric hold showed no significant mean bias, but consistently poor reliability at all time points (i.e. 2, 24, 48, 72 h), illustrated by the wide 95 % LoA.

MVC force in the present study was approximately 650 N, which is comparable to the ~600 N observed by Edwards *et al.* (1977b) for healthy male participants (body mass 80 kg). There was no significant bias in MVC at 2, 24, 48 or 72 hours compared to baseline for the single maximum value, 0.25 or 0.5 s divisions. This supports previous findings which showed no mean difference in MVC peak force during isometric contractions of the *m. quadriceps femoris* at 2, 6, 24, 48, 72, 168 hours (Morton *et al.*, 2005). Place *et al.* (2007) also showed strong intra day and inter day repeatability of the peak force of MVC of the *m. quadriceps femoris*. The 95 % LoA showed no change over time for the single maximum value, 0.25 or 0.5 s divisions. In the best case (single maximum value at 72 hours) the 95 % CI indicates 95 % confidence that muscle damage is present if an individual participant MVC has decreased by more than 14.2 %. However, due to variation within a population a researcher can be 95 % confident that a change of less than 14.2 % from baseline for a group of participants is a true result providing appropriate statistical analysis are used to assess the group data. An MVC is one of the most commonly used technique to assess recovery of neuromuscular function in the hours or days following exercise (Lattier *et al.*, 2004a, 2004b; Lepers *et al.*, 2002; Martin *et al.*, 2005; Millet *et al.*, 2003b; Millet *et al.*, 2003c; Place *et al.*, 2004). The method of analysis

to determine the peak force in these studies was not stated. However, the results of the present study show that repeatability of MVC peak force was similar using a single maximum value, 0.25 s and 0.5 s divisions, therefore any of the analysis could be used to evaluate recovery of neuromuscular function.

Place *et al.* (2007) showed twitch and doublet peak force to be 17 and 31 percent of MVC force, respectively, which is comparable to the 12 and 29 percent of MVC force in the present study. If the differences in twitch peak force are taken into consideration the contraction time (~0.180 s) and half relaxation time (~0.095 s) in the present study are similar to those observed by Gauche *et al.* (2006) of 0.070 s and 0.050 s respectively, when the twitch peak force was half that of the present study (~34 vs. 75 N). The twitch maximal rate of force development and decrease in the present study (Table 5.2) are comparable to the 894 and 316 $N \cdot s^{-1}$ observed by Millet *et al.* (2003c).

There was no significant mean bias for the peak force of the electrically stimulated twitch and doublet at 2, 24, 48 or 72 hours compared to baseline. These are consistent with previous findings that show peak force of resting and potentiated twitches to be repeatable over this time scale (Morton *et al.*, 2005). However, in the present study, the lower 95 % LoA and 95 % CI for the doublet indicate that the random variation in peak force between baseline and 2, 24, 48 and 72 hours was greater for the twitch. Contrary to these results, Place *et al.* (2007) presented similar intra day (1 minute) and inter day (3-5 days) reliability (Interclass Correlation Coefficients) for the peak force of electrically stimulated twitch and doublet. Similar to the trends for peak force, the parameters of contraction time, half relaxation time, maximal rate of force development, and maximal rate of force decrease demonstrated stronger reliability for the doublet compared to the twitch. The reliability of the rate constant of contraction was similar between the twitch and doublet but the variability in of the rate constant for relaxation was greater for the twitch. The current study supports the notion that in general a potentiated doublet is a more reliable measure than a resting twitch to assess changes in muscle contractile properties over time. Many researchers use a resting or potentiated twitch to measure changes in neuromuscular function (Lattier *et al.*, 2004a, 2004b; Millet *et al.*, 2003c; Place *et al.*, 2004). Millet *et al.* (2003c) showed a decrease in MVC ($P<0.01$) but no change in twitch peak force, contraction time, maximal rate of force development and maximal rate of force relaxation following a 140 km cycle race. However, changes in these

parameters may have occurred, but due to the large variability of the twitch parameters may been un-detected. Lepers *et al.* (2001) calculated the twitch parameters from the mean of 5 single twitches and showed reductions in peak force ($P<0.01$), half relaxation time and the rate of force development following a 30 minute cycling exercise at 80 % of maximal power output. These results suggest that reliability may be improved by averaging the response of five compared to single stimuli. However, this concept was not investigated in the current study. If doublet stimulations had been used, there would have been lower variability and changes in these parameters may have been observed.

The peak force of 20 and 50 Hz contractions was approximately 40 and 51 percent of MVC force in the present study which is similar to the 48 and 60 percent of MVC force observed by Skurvydas *et al.* (2003). The 20:50 Hz ratio of approximately 0.85 observed in the present study is slightly higher than the 0.79 observed by Skurvydas *et al.* (2003) and approximately 0.78 recorded by Edwards *et al.* (1977a).

The peak force and maximal rate of force development of low (20Hz) and high (50-80Hz) frequency stimulations have previously been used to describe changes in the contractile properties of muscle (Martin *et al.*, 2005; Millet *et al.*, 2003a; Millet *et al.*, 2003b). The current study assessed the repeatability of all parameters that have previously been calculated for electrically stimulated contractions. The parameters of the 20 Hz and 50 Hz stimulations demonstrated some significant mean bias (i.e. poor repeatability) and large 95 % LoA and 95 % CI (i.e. high variation). However, the peak force of 20 and 50 Hz stimulations are more frequently expressed as a ratio to determine the presence of LFF or HFF (Jones, 1996). When data in the present study were expressed as a ratio there was no significant mean bias at 2, 24, 48 and 72 hours compared to baseline and the 95 % LoA and 95 % CI were reduced. These findings suggest that only peak force of 20 and 50 Hz stimulations expressed as a ratio should be used to describe changes in the contractile properties of muscle. This is keeping with the majority of published studies, as rather than changes in individual parameters of low and high frequency stimulations a ratio is commonly used to measure changes in the excitation contraction coupling process (Martin *et al.*, 2005; Millet *et al.*, 2003a; Millet *et al.*, 2003c; Ratkevicius *et al.*, 1998).

The participants in the present study had an initial voluntary activation of 97 % which is considered normal as even highly trained participants fail to constantly achieve maximal

activation (Allen *et al.*, 1995b). The ITT has been widely used to assess voluntary activation of the *m. quadriceps femoris* (for reviews see Millet and Lepers, 2004; Paillard *et al.*, 2005; Shield and Zhou, 2004). There has been recent debate as to whether the relationship between the superimposed and voluntary force is linear or non-linear ('S' shaped) due to the shortening of the muscle during contraction and potentiation during the stimulus and the effect this has on the accuracy of the calculation of percentage voluntary activation (de Haan *et al.*, 2009a, 2009b; Kooistra *et al.*, 2007; Taylor, 2009a, 2009b). However, the authors of these discussions acknowledge that it is currently the only feasible technique to determine whether the site of neuromuscular impairment is central or peripheral. Allen *et al.* (1995b) and Behm *et al.* (1996) both raised concern that activation deficits were not being observed because the interpolated twitch was masked by the noise of the MVC. Therefore, in the current study a doublet stimulation rather than a twitch was used to calculate voluntary activation (Oskouei *et al.*, 2003).

The measurement of voluntary activation was repeatable as there was no significant mean bias and small 95 % LoA and 95 % CI at 2, 24, 48 and 72 hours. Similarly, eight healthy male participants with a mean voluntary activation of 94.6 ± 4.1 % showed no mean difference and similar 95 % CI in voluntary activation compared to baseline over the same timescale (Morton *et al.*, 2005). Place *et al.* (2007) reported a decrease in voluntary activation following a standardised fatiguing contraction. The procedure was repeated 3-5 days after the initial fatiguing contraction and similar voluntary activation was observed, suggesting that the ITT is also repeatable in a fatigued state. In direct comparisons of assessment of voluntary activation using nerve or surface stimulation across a range of joint angles provides identical findings (Newman *et al.*, 2003; Rutherford *et al.*, 1986). Thus, the results of the present study and previous published findings suggest that the ITT is a reliable measure to assess the origin of neuromuscular impairment (central or peripheral) over time using percutaneous stimulation.

Isometric holds are used to assess changes in physiological mechanisms responsible for time to task failure (exhaustion) (Hunter *et al.*, 2004; Vøllestad *et al.*, 1997) and steadiness (Hunter *et al.*, 2002; Lavender and Nosaka, 2006). A 60 % MVC endurance hold duration of 95 s has previously been observed in endurance trained runners in a rested state (Nicol *et al.*, 1991) which is similar to the data of the present study (Table 5.1) Tracy & Enoka (2002)

measured a steadiness value of 2.96 during a 10 second isometric hold at 50 % MVC force which is similar to the values recorded in the present study (Table 5.1).

Endurance time showed no significant mean bias at 2, 24, 48 and 72 hours, however, the 95 % LoA and 95 % CI indicated that there was a poor reliability for individuals between trials. A decrease in steadiness during an isometric hold indicates a reduction in neuromuscular function (Lavender and Nosaka, 2006; Maluf and Enoka, 2005). This may be due to increased recruitment of large motor units to compensate for the force loss, impairment of the excitation contraction coupling process, increased muscle temperature (Saxton *et al.*, 1995) or increased synchronisation of motor unit firing (Hamlin and Quigley, 2001). Although there was no significant mean bias in steadiness at 2, 24, 48 and 72 hours compared to baseline, there was a poor reliability (as shown by the 95 % LoA). There was no improvement in the reliability of the steadiness measurement by using smaller time periods during the hold. Therefore, when assessing recovery of neuromuscular function over a time scale ranging from 2 to 72 hours changes in steadiness should be interpreted with caution. As the isometric hold force was set at 50 % of the MVC of each test session, the impulse was calculated to attempt to account changes in duration and force between days. The impulse showed no significant mean bias compared to baseline, and a similar variation to the duration. When using an isometric endurance hold to measure exhaustion of the *m. quadriceps femoris* the most repeatable parameters are duration and impulse but all parameters demonstrate a poor reliability between trials.

In summary, this study demonstrated that both voluntary and electrically stimulated contractions of the *m. quadriceps femoris* were repeatable over a time scale suitable for assessing recovery of neuromuscular function in healthy trained male participants. Although all measures showed random variation, this variation was consistent over time (i.e. 2, 24, 48, 72 hours). For certain parameters the random variation may be greater than the change observed in some experimental protocols. However, the inclusion of a control condition for comparison further strengthens an experimental design as the random variation would be present in both experimental and control groups.

The present study, and previously published findings, suggest the following measurements should be used to assess recovery of the *m. quadriceps femoris* following fatiguing or damaging exercise: (1) The MVC peak force or the mean peak force over 0.25 or

0.5 s divisions to assess the voluntary maximal force production of the muscle; (2) The ITT to provide an indication of the origin of fatigue (central or peripheral); (3) The potentiated doublet to assess changes in the contractile properties of the muscle; and (4) Low (20 Hz) and high (50 Hz) frequency stimulations expressed as a ratio to detect the presence of LFF.

Chapter 6. Physiological Responses to Load Carriage during Level and Downhill Treadmill Walking

6.1. Introduction

Chapter 3 showed that during load carriage in the field (19.3 km, carrying 31 kg) participants operated at 72 ± 5 %HRmax (66 ± 5 %HRR), a cardiovascular strain classified as 'hard' (Howley, 2001). Participants in Study 3 were undertaking load carriage in a large group in the field during a military training assessment, limiting the physiological measures that could be taken during exercise. The physiological strain of exercise was monitored using heart rate but this was highly variable due to the varying terrain and speed of movement. To quantify the physiological responses in greater detail, the present chapter will examine the cardiovascular and metabolic responses during load carriage in a controlled laboratory setting.

Epstein *et al.* (1988) first showed that when a 40 kg load was carried in a backpack at 4.5 $km \cdot h^{-1}$ on a +5 % gradient for 120 min, $\dot{V}O_2$ increased between 20 and 120 minutes from 52.1 ± 0.6 to 56.2 ± 0.6 % $\dot{V}O_2max$ ($P<0.01$). This increase was not apparent when carrying a 25 kg load under the same conditions. Epstein *et al.* (1988) suggested that $\dot{V}O_2drift$ occurred when the work rate was increased above 50 % $\dot{V}O_2max$. However, more recent data does not support this hypothesis. During a 12 km walk at 0 % gradient, increases in $\dot{V}O_2$ have been shown carrying loads of 31.5 kg at 5.7 $km \cdot h^{-1}$ (36.6 ± 1.5 to 40.4 ± 2.0 % $\dot{V}O_2max$, $P<0.05$), 49.4 kg at 4.0 $km \cdot h^{-1}$ (26.6 ± 0.8 to 29.7 ± 0.9 % $\dot{V}O_2max$, $P<0.05$) and 49.4 kg at 5.7 $km \cdot h^{-1}$ (41.7 ±1.1 to 50.1 ± 1.7 % $\dot{V}O_2max$, $P<0.05$), but not when walking unloaded at any speed (Patton *et al.*, 1991). However, Sagiv *et al.* (1994) found no increase in $\dot{V}O_2$ during 240 min of walking at 4.5 $km \cdot h^{-1}$ carrying 38 kg and 50 kg. Sagiv *et al.* (1994), compared with previous studies, this may have been due to an improved backpack design which utilised hip and chest belts and shoulder straps allowing better mechanical efficiency. Further examination of Sagiv *et al.* (1994) data shows that participants had relatively high maximum oxygen uptake values ($\dot{V}O_2max$ 65.2 ± 5.0 $mL \cdot kg^{-1} \cdot min^{-1}$) and walked at a relatively low speed (4.5 $km \cdot h^{-1}$) carrying loads of 38 kg and 50 kg resulting in $\dot{V}O_2$ of only 14 ± 4 and 19 ± 5 $mL \cdot kg^{-1} \cdot min^{-1}$, respectively.

Patton *et al.* (1991) and Warber *et al.* (2000) have also shown increases in heart rate (HR) during bouts of prolonged load carriage which is indicative of cardiovascular drift (Coyle and Gonzalez-Alonso, 2001). However, Sagiv *et al.* (1994) showed no cardiovascular drift when participants carried 38 kg and 50 kg during 240 minutes of treadmill walking at 4.5 $km \cdot h^{-1}$. The similarities in changes in $\dot{V}O_2$ and heart rate are unsurprising due to the relationship between the respiratory and cardiovascular system and the changes that occur during exercise (Åstrand, 1956).

There is limited research investigating the effect of negative (downhill) gradients on load carriage. During short duration load carriage (< 20 minutes) $\dot{V}O_2$ is reduced when walking on a negative gradient compared to walking on a level (0 %) gradient (Santee *et al.*, 2001). The gradient resulting in the lowest $\dot{V}O_2$ varies between individuals but the mean optimum gradient for lowest $\dot{V}O_2$ is -8 % (Santee *et al.*, 2001). Further decreases in gradient cause $\dot{V}O_2$ to increase (up to -12 % has been investigated) (Santee *et al.*, 2001). These findings are similar to unloaded walking where $\dot{V}O_2$ is lowest between -6 and -15 % (Wanta *et al.*, 1993). During prolonged walking on negative gradients, a greater $\dot{V}O_2$drift over time has been observed compared to level walking (Davies and Barnes, 1972; Johnson *et al.*, 2002). Walking on a negative gradient places greater emphasis on the eccentric component of the stretch shortening cycle in the supporting muscles of the lower limb (e.g. the quadriceps) (Nicol *et al.*, 2006). Davies & Barnes (1972) suggest that the additional recruitment of muscle fibres during eccentric contractions to maintain stability and position when walking may lead to an increased $\dot{V}O_2$drift over time. The effect of load carriage on $\dot{V}O_2$ and heart rate during prolonged walking on negative gradients is unknown.

This study had two main aims: (1) to compare the physiological changes during 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) with no load and load carriage (25 kg backpack) and (2) to investigate the physiological differences of 120 minutes of load carriage (25 kg backpack) on level (0 %) and a negative gradient (-8 %). It was hypothesised that: (1) load carriage on a level gradient would cause a higher $\dot{V}O_2$ and heart rate at baseline and increase $\dot{V}O_2$ and cardiovascular drift compared to walking with no load and (2) load carriage on a -8 % gradient (downhill) would reduce $\dot{V}O_2$ and heart rate at baseline but increase $\dot{V}O_2$ and cardiovascular drift compared to load carriage on a level gradient.

6.2. Methods

6.2.1. Participants

Ten healthy male participants (age 30 ± 8 years, height 1.79 ± 0.05 m, body mass 79.4 ± 8.3 kg, body fat 8.4 ± 3.7 %, $\dot{V}O_2max$ 55.1 ± 5.6 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in the study. Participants had a range of previous recreational experience of carrying load in backpacks. Ethical approval for all procedures and protocols was provided by the University of Chichester Ethics Committee. All protocols were performed in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. Participants provided written informed consent and were free from any musculoskeletal injury prior to commencing the study. Participants were instructed to refrain from any vigorous physical activity in the day prior to treadmill walking and avoid consumption of caffeine, sports drinks or food two hours prior to the commencement of exercise.

6.2.2. Preliminary Measures

Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) (± 0.01 kg) and stature (Avery Berkel, Smethwick, UK) (± 0.005 m) were measured whilst wearing shorts and underwear. Skinfold thickness was measured at the *Chest, Axilla, Triceps, Sub Scapular, Abdomen, Iliac Crest and Thigh* on the right side of the body using Harpenden Skinfold Callipers (Body Care, Southam, UK). Two measurements were taken at each site and if there was a difference > 1 mm, the measurements were repeated. Percentage body fat was estimated following the assessment of skinfold thickness at seven anatomical sites using previously described methods (Jackson and Pollock, 1978; Siri, 1956).

Participants completed an incremental exercise test to exhaustion on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK) to assess maximal oxygen uptake ($\dot{V}O_2max$). All data collection procedures are described in detail in the experimental protocol. The $\dot{V}O_2max$ protocol consisted of running at a speed of 9 $km \cdot h^{-1}$ on a gradient of 1 %; gradient increased by 1 $\% \cdot min^{-1}$ during the first 5 minutes. Speed then increased at 0.1 $km \cdot h^{-1}$ every 5 seconds until volitional exhaustion. In at least the last 3 minutes of the test, expired gases were collected in 1 minute samples using the Douglas bag method. The final bag was only used if the collection time was at least 30 seconds and contained > 65 L of expired gas. Heart rate was monitored throughout the test and recorded at

5 s intervals. A capillary blood sample was taken 4 minutes after the end of the test and plasma lactate concentration measured. Participants were deemed to have reached $\dot{V}O_2$max if they obtained at least two of the following criteria; an increase in $\dot{V}O_2$ of <2.1 $mL \cdot kg^{-1} \cdot min^{-1}$ between the last 2 expired gas collections, plasma lactate > 5.5 $mmol \cdot L^{-1}$ or respiratory exchange ratio (RER) >1.15 (Howley *et al.*, 1995). All participants attained $\dot{V}O_2$max.

6.2.3. Experimental Protocol

The study was a three way cross over randomised design, where each participant performed the following conditions on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK): (1) 120 minutes level walking at 6.5 $km \cdot h^{-1}$ and 0 % gradient carrying no load [Level Walking (LW)], (2) 120 minutes level walking at 6.5 $km \cdot h^{-1}$ and 0 % gradient carrying a 25 kg backpack [Level Walking with Load Carriage (LWLC)], (3) 120 minutes downhill walking at 6.5 $km \cdot h^{-1}$ and -8 % gradient carrying a 25 kg backpack [Downhill Walking with Load Carriage (DWLC)]. Walking speed was kept constant between test conditions and an absolute load used to reflect realistic occupational requirements (e.g. military load carriage). The exercise model of 120 minutes walking at 6.5 $km \cdot h^{-1}$ carrying a 25 kg load, replicates the duration, pace and load during the final load carriage assessment of British Infantry training (Rayson *et al.*, 2000). This model was selected as an acceptable load carriage task for the participants in the present study (i.e. physically fit but a mixed level of load carriage experience). This decision was made as compared to recruits undertaking British Infantry training (Brown *et al.*, 2008), participants in the present study had greater aerobic fitness (53.1 ± 4.2 vs. 55.1 ± 5.6 $mL \cdot kg^{-1} \cdot min^{-1}$) and were heavier (69.0 ± 8.5 vs. 79.4 ± 8.3 kg), with a lower fat mass (14.2 ± 4.1 vs. 8.4 ± 3.7 %).

Whilst participants walked on the treadmill, the measures described below were taken at 5, 15, 30, 45, 60, 75, 90, 105, 120 minutes of exercise (excluding RPE, initial measurement taken at minute 15). Measurements recorded at minute 5 were taken as a baseline and changes over time were calculated from this time point. $\dot{V}O_2$ and cardiovascular drift (heart rate) were calculated as the difference between the values measured at 5 minutes (baseline) and 120 minutes.

6.2.4. Metabolic Measures

Two minute collections of expired gases were made using Douglas bags (Plysu Protection Systems Limited, Milton Keynes, UK). The Douglas bags were flushed with room air and fully evacuated prior to gas collection. Respiratory gas fractions (O_2 and CO_2) were analysed (Series 1400 gas analyser, Servomex plc., Crowborough, UK) and volume of expired air measured (Harvard dry gas meter, Harvard Apparatus Ltd., Edenbridge, UK). The gas analyser was calibrated using a two point calibration: O_2 and CO_2 were zeroed using 100 % nitrogen gas (Linde Gas UK Ltd., West Bromwich, UK); O_2 was spanned to 20.95 % using room air and CO_2 was spanned to 5.66 % using a known gas mixture (15.20 % O_2, 5.66 % CO_2, 79.14 % N) (Linde Gas UK Ltd., West Bromwich, UK). To calibrate the gas meter room air was pumped through in 35 L increments up to 175 L using a 7 L syringe (Model 4900, Hans Rudolph Inc., Kansas City, USA). Known volume was plotted against measured volume to obtain a correction factor (1.021 to 1.034). Expired gas volumes were corrected (measured volume x correction factor). Volume of oxygen uptake ($\dot{V}O_2$), using the Haldane transformation, and respiratory exchange ratio (RER) ($\dot{V}CO_2/\dot{V}O_2$) were calculated. Data are presented as standard temperature (0 °C) and pressure (100.3 kPa) of dry gas (STPD).

6.2.5. Heart Rate (HR)

Heart rate was recorded every five seconds using downloadable heart rate monitors (Polar Vantage NV, Polar Electro Oy, Kempele, Finland). Average heart rates were calculated over one minute time intervals every five seconds.

6.2.6. Plasma Glucose and Lactate

Capillary blood samples were taken from the finger and analysed for plasma lactate and glucose (YSI 2300 Stat Plus, Yellow Springs Instruments Co. Inc., Yellow Springs, USA). No lysing agent was used in the analyser; therefore plasma (rather than blood) lactate and glucose were measured.

6.2.7. Stride Frequency

The number of steps taken in 1 minute were measured. Steps were counted from one foot and recorded each time it made contact with the treadmill (to the nearest half step).

6.2.8. Ratings of Perceived Exertion (RPE)

Participants were asked to rate their level of perceived exertion on a 15 point Borg Scale (Borg, 1982). The baseline reading of RPE was taken at minute 15 and at 15 minute intervals until completion of the test.

6.2.9. Additional Measures

Participants were weighed immediately prior to, and following, the treadmill walks to determine changes in body mass. Participants consumed water ad libitum during the experiment to reflect procedures in the occupational setting and intake was recorded and accounted for in the calculation of post exercise body mass. Environmental temperature was monitored using a dry bulb thermometer (Fisher Scientific, Loughborough, UK) and controlled using the laboratory air conditioning (South East Cooling Ltd., Bognor Regis, UK). No differences in environmental temperature were observed between conditions [21.31 ± 0.78 °C (LW), 21.28 ± 1.06 °C (LWLC), 21.11 ± 0.50 °C (DWLC)].

6.2.10. Statistical Analysis

SPSS for windows V15 (SPSS, Chicago, Illinois) was used for statistical analyses. Distribution of the data was assessed using Kolmogorov-Smirnov test for normality. Data were normally distributed and differences between groups and over time (5 vs. 120 minutes) were assessed using 2 way repeated measures ANOVA. If sphericity was violated, the Greenhouse-Geisser correction was used. When differences were observed they were examined using pre-planned paired t-tests to control for the number of comparisons made between data points. Comparisons were made between (1) LW vs. LWLC and (2) LWLC vs. DWLC, to ensure only one variable (i.e. load or gradient) was changed between compared conditions. The results are presented as mean ± standard deviation (SD). Statistical significance was set at $P<0.05$.

6.3. Results

6.3.1. Level Walking (LW) vs. Level Walking with Load Carriage (LWLC)

$\dot{V}O_2$ during LWLC was 41 ± 17 % higher than LW at minute 5 (23.0 ± 2.7 vs. 16.4 ± 0.7 mL·kg^{-1}·min^{-1}, P<0.001) and 50 ± 19 % higher at minute 120 (26.9 ± 3.3 vs. 17.9 ± 0.5 mL·kg^{-1}·min^{-1}, P<0.001). There was a greater absolute increase in $\dot{V}O_2$ over the 120 minutes during LWLC compared to LW (3.9 ± 2.3 vs. 1.6 ± 0.6 mL·kg^{-1}·min^{-1}, P=0.018) (Figure 6.1A). However, when expressed as a percentage change from the baseline value (5 minutes), there was no difference in the change in $\dot{V}O_2$ over the 120 minutes LWLC and LW (10 ± 4 vs. 17 ± 10 %, P=0.680) (Figure 6.1B).

Similarly, HR during LWLC was 25 ± 7 % higher than LW at minute 5 (116 ± 13 vs. 93 ± 8 beats·min^{-1}, P<0.001) and 43 ± 16 % higher at minute 120 (141 ± 23 vs. 99 ± 12 beats·min^{-1}, P<0.001). There was a greater increase in HR over the 120 minutes during LWLC (116 ± 13 to 141 ± 23 beats·min^{-1}) compared during LW (96 ± 10 to 99 ± 12 beats·min^{-1}) (P=0.001) (Figure 6.2). This was accompanied by a higher plasma lactate concentration during LWLC at minute 5 (1.40 ± 0.32 vs. 0.90 ± 0.30 mmol·L^{-1}, P<0.001) and minute 120 (0.84 ± 0.25 vs. 0.56 ± 0.20 mmol·L^{-1}, P=0.001). RPE was higher during LWLC at minute 5 (10 ± 2 vs. 8 ± 2, P=0.001) and increased over the 120 minute duration for both LWLC (10 ± 2 to 14 ± 2, P=0.003) and LW (8 ± 2 to 9 ± 2, P=0.003).

Plasma glucose concentration was lower during LWLC compared to LW at minute 5 (4.21 ± 0.45 vs. 3.69 ± 0.59 mmol·L^{-1}, P=0.026) and minute 120 (4.40 ± 0.30 vs. 4.00 ± 0.44 mmol·L^{-1}, P=0.023). There was no difference in RER between LWLC and LW at minute 5 (0.90 ± 0.09 vs. 0.86 ± 0.06, P=0.099). However, RER decreased during LWLC from 0.90 ± 0.09 to 0.83 ± 0.04 (P=0.021) which was not apparent during LW (0.86 ± 0.06 to 0.84 ± 0.07, P=0.234).

A greater reduction in body mass was measured following LWLC compared to LW (1.45 ± 0.16 vs. 0.81 ± 0.19 kg, P<0.001). Participants did consume more water during LWLC (0.42 ± 0.35 vs. 0.16 ± 0.21 L, P=006), however, this was less than the reduction in body mass (P<0.001).

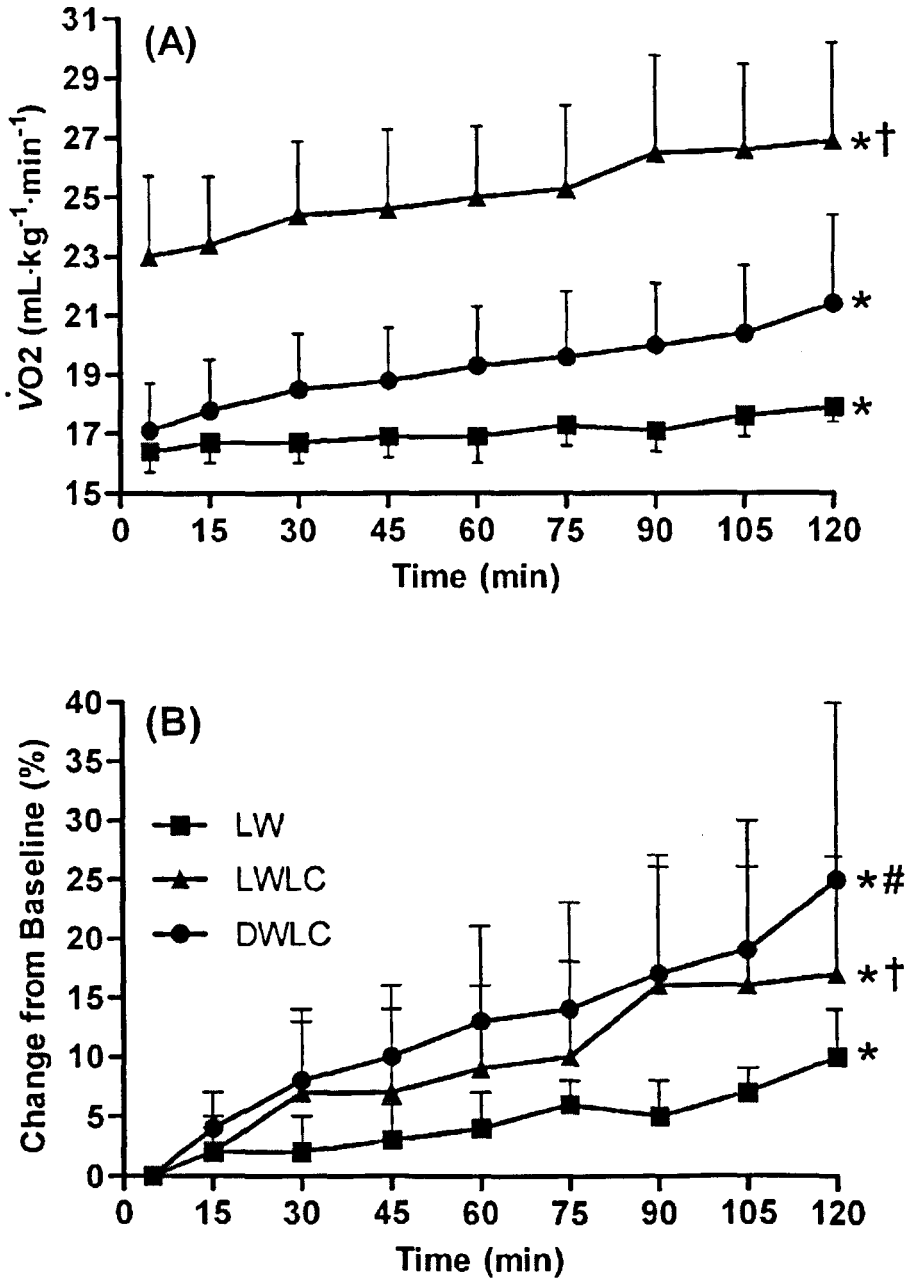


Figure 6.1 – (A) Oxygen uptake (B) Percentage change in oxygen uptake from baseline value (minute 5). During 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) with level walking carrying no load (LW, ■), level walking carrying 25 kg backpack (LWLC, ▲) and downhill walking carrying a 25 kg backpack (DWLC, ●). (*) $\dot{V}O_2$ increased above baseline during walking, (†) increase was greater during LWLC vs. LW (#) increase was greater during DWLC vs. LWLC (#) ($P<0.05$)

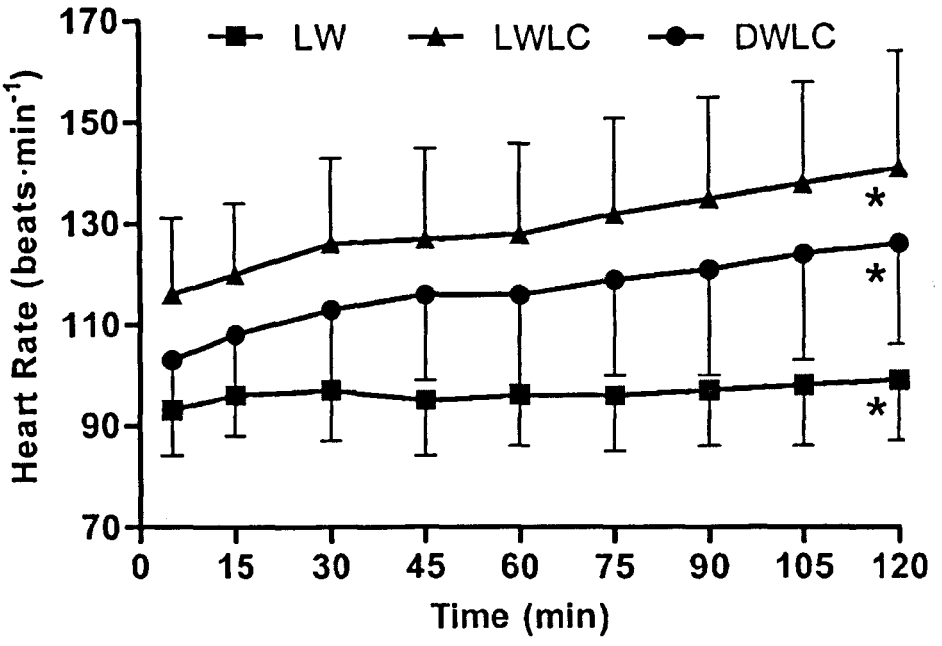


Figure 6.2 – Heart rate (beats·min^{-1}) during 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) with level walking carrying no load (LW, ■), level walking carrying 25 kg backpack (LWLC, ▲) and downhill walking carrying a 25 kg backpack (DWLC, •). (*) HR increased above baseline during walking.

Stride frequency was 2 ± 2 steps higher during LWLC at minute 5 (64 ± 3 vs. 62 ± 3 steps·min^{-1}, P=0.029) and 3 ± 2 steps higher at minute 120 (65 ± 3 vs. 62 ± 3 steps·min^{-1}, P=0.001). As participants maintained the same walking speed this indicates that stride length was reduced during LWLC. There was no change in stride frequency between minute 5 and 120 for both LWLC (64 ± 3 to 65 ± 3 steps·min^{-1}, P=0.708) or LW (62 ± 3 to 62 ± 3 steps·min^{-1}, P=0.708).

6.3.2. Level Walking Load Carriage (LWLC) vs. Downhill Walking with Load Carriage (DWLC)

$\dot{V}O_2$ during DWLC was 25 ± 8 % lower than during LWLC at minute 5 (17.1 ± 1.6 vs. 23.0 ± 2.7 mL·kg^{-1}·min^{-1}, P<0.001) and 20 ± 6 % lower at minute 120 (21.4 ± 3.0 vs. 26.9 ± 3.3 mL·kg^{-1}·min^{-1}, P<0.001). $\dot{V}O_2$ increased between 5 and 120 min for both DWLC (17.1 ± 1.6 to 21.4 ± 3.0 mL·kg^{-1}·min^{-1}, P<0.001) and LWLC (23.0 ± 2.7 to 26.9 ± 3.3 mL·kg^{-1}·min^{-1}, P<0.001) but there was no difference between conditions (P=0.411) (Figure 6.1A). However,

the percentage change in $\dot{V}O_2$ from the baseline during the 120 minutes was greater for DWLC compared to LWLC (25 ± 15 vs. 17 ± 10 %, P=0.027) (Figure 6.1B). When divided into four time periods (5-30, 30-60, 60-90, 90-120), the percentage increase between conditions was similar in the first 3 stages, but during the final 30 minute stage (i.e. 90-120 minutes) the percentage increase in $\dot{V}O_2$ was greater for DWLC than LWLC (7 ± 6 vs. 2 ± 3 %, P=0.044).

HR was 11 ± 9 % lower during DWLC at minute 5 (103 ± 9 vs. 116 ± 13 beats·min^{-1}, P=0.003) and 11 ± 9 % lower during DWLC at minute 120 (126 ± 21 vs. 141 ± 23 beats·min^{-1}, P=0.004). HR increased over time for both DWLC (103 ± 9 to 126 ± 21, P=0.001) and LWLC (116 ± 13 to 141 ± 23, P=0.001) but there was no difference between conditions (P=0.719) (Figure 6.2). When expressed as a percentage change from the baseline value (5 min), there was no difference in the change in heart rate over the 120 minutes between DWLC and LWLC (22 ± 14 vs. 22 ± 15 %, P=0.936). There was no difference in RPE between DWLC and LWLC at minute 15 (9 ± 2 vs. 10 ± 2, P=0.094). RPE increased over the 120 min walk for both DWLC (9 ± 2 to 13 ± 3, P=0.008) and LWLC (10 ± 2 to 14 ± 2, P=0.003). However, at minute 120, RPE was lower during DWLC (13 ± 3 vs. 14 ± 2, P=0.014).

Plasma lactate concentration was lower during DWLC compared to LWLC at minute 5 (1.04 ± 0.27 vs. 1.40 ± 0.32 mmol·L^{-1}, P=0.021) but there were no differences at minute 120 (0.92 ± 0.32 vs. 0.84 ± 0.25 mmol·L^{-1}, P=0.477). Of note, plasma lactate declined between minute 5 and minute 105 for DWLC (1.04 ± 0.27 to 0.65 ± 0.18 mmol·L^{-1}, P=0.003) and LWLC (1.40 ± 0.32 to 0.83 ± 0.21 mmol·L^{-1}, P<0.001). However, during the final 15 minutes of DWLC only, plasma lactate concentration increased from 0.66 ± 0.18 to 0.92 ± 0.32 mmol·L^{-1} (P=0.008).

There was no difference in stride frequency between DWLC and LWLC at minute 5 (64 ± 3 vs. 64 ± 3 steps·min^{-1}, P=1.000). However, over the duration of DWLC there was an increase in stride frequency from 64 ± 3 steps·min^{-1} at minute 5 to 66 ± 4 steps·min^{-1} at minute 120 (P=0.043) indicating a shortening of stride length, which was not apparent during LWLC. This is despite the fact that during DWLC two participants reduced their stride length by 1 and 3 steps·min^{-1}, suggesting that there was individual adaptation to the task.

There was no difference in RER between DWLC and LWLC at minute 5 (0.86 ± 0.05 vs. 0.90 ± 0.09, P=0.128). RER did not change over the duration of DWLC (0.86 ± 0.05 (min 5) to 0.85 ± 0.04 (min 120), P=0.490). During LWLC, RER decreased over the 120 minutes from 0.90 ± 0.09 to 0.83 ± 0.04 (P=0.021). There were no differences in plasma glucose concentration between DWLC and LWLC at 5 minutes (4.19 ± 0.54 vs. 4.21 ± 0.45 mmol·L^{-1}, P=0.914) or 120 minutes (4.36 ± 0.53 vs. 4.40 ± 0.30 mmol·L^{-1}, P=0.836)

There were no differences between DWLC and LWLC for change in body mass (pre-post, corrected for fluid intake) (1.39 ± 0.15 vs. 1.45 ± 0.16 kg, P=0.556) or fluid intake during the treadmill walking (0.32 ± 0.34 vs. 0.42 ± 0.35 L, P=0.171).

6.4. Discussion

In the present study, observations were made during 120 minutes of treadmill walking at 6.5 km·h^{-1}. Carrying a 25 kg backpack increased $\dot{V}O_2$ and heart rate and caused a greater $\dot{V}O_2$ and cardiovascular drift over time compared to unloaded walking. Carrying a 25 kg backpack on a -8 % (downhill) gradient decreased $\dot{V}O_2$ compared to a level gradient. However, a novel finding was that despite the lower $\dot{V}O_2$ during exercise, the increase in $\dot{V}O_2$ over time was similar on level and -8 % gradients. However, the percentage change in $\dot{V}O_2$ from the baseline during the 120 minutes was greater for DWLC compared to LWLC. These data confirm the hypothesis that (1) load carriage on a level gradient causes a higher $\dot{V}O_2$ and heart rate at baseline and increases $\dot{V}O_2$ and cardiovascular drift compared to walking with no load. But only partly support the hypothesis that (2) load carriage on a -8 % gradient (downhill) reduces $\dot{V}O_2$ and heart rate at baseline but increases $\dot{V}O_2$ and cardiovascular drift compared to load carriage on a level gradient.

Level walking carrying a 25 kg backpack increased $\dot{V}O_2$ compared to walking unloaded, which is in agreement with previous findings (Patton *et al.*, 1991; Quesada *et al.*, 2000). The initial work rate during LWLC in the current study was 42.1 %$\dot{V}O_2$max, which is similar to that of participants walking at 6.0 km·h^{-1} carrying a backpack of 31 % of body weight (23.6 ± 3.6 kg) which elicited an initial work rate of 40 %$\dot{V}O_2$max (Quesada *et al.*, 2000). The oxygen cost at 60 minutes during LWLC in the present study (25.0 ± 2.4 mL·kg^{-1}·min^{-1}) was similar to that estimated for the load carriage task (distance 19.3 km, speed 5.2 km·h^{-1}, load 31 kg) examined in Chapter 3 (between 19.1 ± 0.6 and 33.5 ± 4.6 mL·kg^{-1}·min^{-1})

The addition of carrying load on a level gradient caused a greater absolute increase in $\dot{V}O_2$ over time. Epstein *et al.* (1988) and Patton *et al.* (1991) have previously shown that $\dot{V}O_2$drift increases with the load carried. However, Patton *et al.* (1991) showed that with lighter loads (5.2 kg) $\dot{V}O_2$drift was not apparent during 12 km of treadmill walking at 5.7 km·h^{-1}. During unloaded level walking, the present study showed an increase in $\dot{V}O_2$ over time. The reason for this is unclear; compared with LW in the current study, the participants of Patton *et al.* (1991) study were of a similar aerobic fitness (55.1 ± 5.6 vs. 58.5 ± 1.5mL·kg^{-1}·min^{-1}) and were working at a similar initial exercise work rate (30.0 ± 3.5 vs. 29.5 ± 0.9 %$\dot{V}O_2$max. The

main difference from Patton *et al.* (1991) was that the treadmill speed of the current study was faster (6.5 vs. 5.7 $km \cdot h^{-1}$).

$\dot{V}O_2$ while carrying 25 kg walking downhill was lower than carrying 25 kg during level walking at minute 5 (17.1 ± 1.6 vs. 23.0 ± 2.7 $mL \cdot kg^{-1} \cdot min^{-1}$) and minute 120 (21.4 ± 3.0 vs. 26.9 ± 3.3 $mL \cdot kg^{-1} \cdot min^{-1}$). Santee *et al.* (2001) showed a similar decrease in $\dot{V}O_2$ when participants carried a 18.1 kg backpack at 4.8 $km \cdot h^{-1}$ on 0 and -12 % gradients (10.4 ± 0.8 vs. 15.6 ± 1.1 $mL \cdot kg^{-1} \cdot min^{-1}$). Despite the lower $\dot{V}O_2$ at 5 and 120 minutes, the increase in $\dot{V}O_2$ over time (absolute value) was similar between LWLC and DWLC. When expressed as a percentage increase from baseline, the increase in $\dot{V}O_2$ was higher whilst carrying load downhill. These differences in $\dot{V}O_2$drift with load carriage on level and negative gradients have not been previously observed.

Epstein *et al.* (1988) suggested $\dot{V}O_2$drift would only occur if individuals were working above 50 %$\dot{V}O_2$max. However, Patton *et al.* (1991) found that exercise intensities as low as 26.5 %$\dot{V}O_2$max caused a drift in $\dot{V}O_2$. The data of the present study supports these more recent findings and show that $\dot{V}O_2$drift occurred when participants walked at 31.3 ± 0.89, 45.9 ± 2.6 and 35.2 ± 2.5 %$\dot{V}O_2$max for LW, LWLC and DWLC, respectively. This suggests that the reason for $\dot{V}O_2$drift during load carriage may be more subtle than simply a function of the exercise work rate (%$\dot{V}O_2$max) at which individuals are exercising.

Carrying a load requires additional force and control from the muscles to maintain movement and posture and has been shown to increase muscle fibre recruitment of the shoulders (Holewijn, 1990), trunk (Bobet and Norman, 1984) and lower limbs (Ghori and Luckwill, 1985). The increase in muscle fibre recruitment will increase the demand for oxygen and therefore cause $\dot{V}O_2$ to rise. This may in part account for the higher $\dot{V}O_2$ during LWLC compared with LW.

During prolonged exercise muscle fibres become fatigued and/or damaged, reducing the force they are able to produce (Millet and Lepers, 2004). To compensate, additional motor units are recruited to maintain movement on the treadmill at the required speed and to support the load. This additional recruitment will increase oxygen demand and may drive an upward drift in $\dot{V}O_2$. The higher stride frequency during LWLC requires participants to take an

average of an additional 2 steps (i.e. 2 stretch shortening cycles) per minute. The combination of the greater force required from muscle fibres and higher stride frequency when carrying a load will increase muscle fatigue and therefore $\dot{V}O_2$drift which may account for some of differences between the LWLC and LW. Although a quantitative measurement of fatigue was not taken, the qualitative ratings of perceived exertion were greater for LWLC compared to LW and were observed to increase over time in both conditions.

During prolonged walking, $\dot{V}O_2$drift has been observed at -5 %, -15 % and -25 % but not on 0 % gradients (Johnson *et al.*, 2002). Davies & Barnes (1972) suggested that the increase in $\dot{V}O_2$ when walking on negative gradients may be due to the recruitment of additional muscle fibres in the supporting muscles (e.g. quadriceps) to maintain stride length and position on the treadmill during eccentric contractions. Type II muscle fibres are preferentially recruited during eccentric muscle actions (Nardone *et al.*, 1989), and muscle fibres become fatigued and damaged more rapidly during eccentric compared to concentric exercise (Clarkson and Newham, 1995). Therefore, we speculate that during DWLC a greater number of type II fibres were recruited initially, which become fatigued and/or damaged more rapidly due to the more severe eccentric muscle actions. The demand for oxygen will increase as additional motor units are recruited and may be further increased by the progressive switch from type II to type I fibres. This could add to the greater percentage increase in $\dot{V}O_2$ observed during DWLC, especially during the final 30 minutes of the treadmill walking. Studies of the mechanisms responsible for $\dot{V}O_2$drift during 45 minute bouts of downhill running concluded that muscle damage could not account for the increase in $\dot{V}O_2$ (Westerlind *et al.*, 1994). However, these studies used serum creatine kinase as a measure of muscle damage which has been shown to be a poor indicator of force losses of the muscle (Warren *et al.*, 1999). More recently, decreases in running economy following downhill running have been shown to be accompanied by decreases in the ability of the knee extensors to produce force (maximal voluntary contraction) (Chen *et al.*, 2007). Also, Dick & Cavanagh (1987) showed a 10 % increase in $\dot{V}O_2$ during a 40 minute downhill run at 44 %$\dot{V}O_2$max with a corresponding 23 % increase in iEMG, but no change in $\dot{V}O_2$ or iEMG during 40 minutes of level running at 66 %$\dot{V}O_2$max. The authors concluded that the $\dot{V}O_2$drift observed during downhill running was due to muscle fibre damage and the recruitment of additional muscle fibres. Following four hours of treadmill load carriage (3 % grade, 5.6 km·h^{-1}, 29.6 kg

backpack) Warber *et al.* (2000) showed that squat jump performance (maximum number of squats with 45.5 kg at 25 repetitions·min^{-1}, 75 squat maximum) decreased by 53 %. Potentially, fatigue of leg, hip and back muscles may have occurred during load carriage and contributed to this loss in strength. These findings suggest that neuromuscular impairment may be linked to the changes in $\dot{V}O_2$drift during prolonged load carriage. Further work is suggested to examine neuromuscular impairment resulting from prolonged load carriage.

During treadmill running, a poorer economy and a greater metabolic cost has been shown when stride frequency deviates from optimum (Cavanagh and Williams, 1982). More recently, during a 60 minute steady speed run (at individual 10 km race pace), Hunter & Smith (2007) observed a 3 % increase in $\dot{V}O_2$ and a corresponding decrease of 1-2 % in stride frequency. The decrease in stride frequency was attributed to fatigue during the run. Stride frequency increased during DWLC but stayed constant during the LW and LWLC. The change in stride frequency would have contributed to the increase in $\dot{V}O_2$ during DWLC and may explain some of the differences in $\dot{V}O_2$drift from LWLC. Stride frequency is a very crude measure of gait and more subtle changes in walking pattern may have occurred but were not measured in this study.

Participants' diets were not controlled between conditions; therefore discussions of substrate oxidation must be interpreted with significant caution. However, participants arrived in the laboratory in a rested state and did not consume any food or fluid (other than water) at least two hours prior to starting the treadmill walking. There was no change in plasma glucose concentration over time in any condition. However, during LWLC there was a decrease in RER indicating a change from carbohydrate to fat as an energy source (Jeukendrup and Wallis, 2005); this was not apparent during LW and DWLC. This may suggest that endogenous glycogen stores were reduced to a greater extent during LWLC compared to the other conditions. Reduced glycogen stores has been associated with a decrease in running economy (Kirwan *et al.*, 1988). This may contribute to the higher $\dot{V}O_2$drift during LWLC.

Carbohydrate produces 5.02 kcal·L$^{-1}O_2$ where as fat only produces 4.85 kcal·L$^{-1}O_2$ (Jeukendrup and Wallis, 2005). Assuming a negligible contribution from protein metabolism, at the start of LWLC (RER=0.90) 64 % and 36 % of energy was derived from carbohydrate and fat respectively, providing 4.96 kcal·L$^{-1}O_2$. At 120 minutes (RER=0.83), 43 % and 57 %

of energy was derived from carbohydrate and fat respectively, providing 4.92 kcal·L^{-1}O2. Therefore, if $\dot{V}O_2$ remained constant throughout the 120 minutes of treadmill walking, to supply the same amount of energy at minute 120 as minute 5 would only require an additional 0.01 $LO_2 \cdot min^{-1}$. $\dot{V}O_2$ increased by 0.20 $L \cdot min^{-1}$ between minute 5 and 120, respectively. Therefore the additional O_2 requirements for fat oxidation could only account for 5 % of the increase in $\dot{V}O_2$. However, RER is only an indication of whole body substrate oxidation, therefore glycogen depletion may have occurred locally in individual muscle fibres (Costill *et al.*, 1973). This could further contribute to muscle fatigue and increases in $\dot{V}O_2$drift during LWLC.

Legg *et al.* (1997) suggested that during load carriage localised muscular discomfort is more likely to have an ischemic or anaerobic origin rather than limitations associated with aerobic processes. A combination of higher exercise work rate and the strain imposed on individual muscle groups is likely to account for the higher plasma lactate during LWLC compared to LW. Following a cycling based endurance training programme, a reduction in $\dot{V}O_2$drift was strongly correlated with a decrease in blood lactate across a range heavy exercise work intensities ($r=0.81$; $P<0.001$) (Casaburi *et al.*, 1987). Plasma lactate decreased during the 120 minutes of LWLC and there was a strong positive relationship (calculated using Pearson's correlation coefficient) between $\dot{V}O_2$drift and plasma lactate concentration ($r=0.80$; $P=0.005$) which was not apparent during LW ($r=-0.25$; $P=0.487$). Plasma lactate concentration was lower during DWLC compared to LWLC, potentially due to the lower exercise work rate (%$\dot{V}O_2$max). During DWLC there was no change in plasma lactate concentration over the duration of the exercise due to the increase between 105 and 120 minutes and there was no relationship between $\dot{V}O_2$drift and plasma lactate ($r=0.06$; $P=0.868$). Similarly, no relationship was found between blood lactate concentration and $\dot{V}O_2$drift during downhill running (Westerlind *et al.*, 1994). It appears plasma lactate concentration is related to the $\dot{V}O_2$drift observed during LWLC, but not during LW and DWLC. It is unclear as to whether lactate is responsible for, or as a result of the $\dot{V}O_2$drift during LWLC.

The heart rate at minute 60 during LWLC in the present study (68 ± 6 %HRmax) was similar to the average heart rate measured in Chapter 3 during the 19.3 km load carriage event in the field walking at 5.2 $km \cdot h^{-1}$ carrying a 31 kg load (72 ± 5 %HRmax). In all test

conditions a progressive increase in heart rate was observed over the 120 minutes (Figure 6.2), which is an indication of cardiovascular drift (Coyle and Gonzalez-Alonso, 2001). This has been shown in other studies of prolonged load carriage (Patton *et al.*, 1991; Warber *et al.*, 2000). Cardiovascular drift occurs due to a decrease in stroke volume and an increase in heart rate, associated with a rise in core body temperature (Coyle and Gonzalez-Alonso, 2001). The increase in heart rate during all conditions is apparent (Figure 6.2); however, core temperature was not measured in this study. During a 12 km walk (6 $km \cdot h^{-1}$) carrying 30 kg load in 21-24 °C ambient temperature, core body temperature and heart rate have been shown to increase by 1.5 ± 0.7 °C and 61 ± 17 $beats \cdot min^{-1}$, respectively (Shoenfeld *et al.*, 1978). Also, during load carriage (14 kg, 4.4 $km \cdot h^{-1}$, 5 % gradient) in the heat (35°C), corresponding rises in core body temperature and heart rate have been observed (Byrne *et al.*, 2005). These data suggest that an increase in core body temperature may have been responsible for the cardiovascular drift observed during the exercise. However, following a cycling based endurance training programme, Casaburi *et al.* (1987) found no relationship between a reduction in $\dot{V}O_2$drift and rise in core body temperature across a range of work heavy exercise intensities ($r=0.15$ $P>0.05$). Therefore, it is unlikely that the differences in $\dot{V}O_2$drift observed in the present study were affected by changes in core body temperature.

The decrease in body mass indicates sweat loss during all conditions. Although the limitations of estimating hydration status by measuring changes in body mass are acknowledged (Maughan *et al.*, 2007), it has been shown to be an accurate and reliable method of measuring changes in total body water (Baker *et al.*, 2009). Compared to LW, estimated sweat loss was higher during LWLC and was not matched by fluid replacement. This suggests that participants became progressively dehydrated which would resulted in a hyperosmotic hypovolemic state, reducing stroke volume, increasing heart rate and therefore cardiovascular drift (Coyle and Gonzalez-Alonso, 2001). Estimated fluid loss was also greater than fluid intake during DWLC, which would exacerbate cardiovascular drift as discussed previously.

In conclusion, carrying load on a level gradient increased $\dot{V}O_2$ and HR and caused greater absolute $\dot{V}O_2$ and cardiovascular drift compared to carrying no load. Potential mechanisms for the differences include; changes in substrate utilisation, dehydration and neuromuscular impairment caused by supporting the load during locomotion. Compared to a

level gradient, carrying load on a -8 % (downhill) gradient decreased $\dot{V}O_2$ and HR but increased the percentage $\dot{V}O_2$drift. This is likely to be due to increased muscle fibre recruitment and greater neuromuscular impairment caused by walking on a negative gradient resulting in a change in stride frequency and walking economy. The higher $\dot{V}O_2$ and cardiovascular drift increased the physiological strain during load carriage, which would be exacerbated with task duration. Further studies are required to explore the potential mechanisms for changes in $\dot{V}O_2$ and cardiovascular parameters and the effects these may have on individuals when carrying loads.

Chapter 7. Neuromuscular Function Following Load Carriage during Level and Downhill Treadmill Walking

7.1. Introduction

Previous investigations of physiological responses to load carriage have primarily focused upon the metabolic and cardiovascular demands (Epstein *et al.*, 1988; Pandolf *et al.*, 1977; Patton *et al.*, 1991; Sagiv *et al.*, 1994) and, to a lesser extent, changes in muscle activation when walking unloaded and with backpacks (Bobet and Norman, 1984; Ghori and Luckwill, 1985; Han *et al.*, 1992; Harman *et al.*, 1992). However, the effect of load carriage on neuromuscular function has received little attention since Clarke *et al.* (1955) initial study. Chapter 3 showed that a 19.3 km load carriage event carrying 31.0 kg caused a 7 ± 8 % reduction in vertical jump height, indicating neuromuscular impairment. Two hours of load carriage on level and downhill gradients caused $\dot{V}O_2$drift, potential mechanisms included neuromuscular impairment and its negative impact on mechanical efficiency (Chapter 6).

Following load carriage, there is often a requirement to conduct other tasks or subsequent bouts of load carriage in both recreational (Lobb, 2004) and occupational settings (Knapik *et al.*, 1991; Knapik *et al.*, 1997). A decrease in neuromuscular function is likely to have an adverse effect on the ability to perform these tasks (Byrne *et al.*, 2004; Tee *et al.*, 2007). For example, Knapik *et al.* (1997) observed a decrease in marksmanship performance following a maximal effort 20 km road march with a range of load combinations (34, 48 and 61 kg). The decrease in performance was attributed to fatigue of the supporting skeletal musculature, elevated post-exercise respiration or fatigue induced muscle tremors.

Functional impairment of skeletal muscle is most accurately assessed by directly measuring the ability of a muscle or muscle group to produce force (Warren *et al.*, 1999). In the literature, following an exercise protocol or athletic event, reduction in functional capability of muscle is often referred to as neuromuscular fatigue (Andersson *et al.*, 2008; Lepers *et al.*, 2002; Strojnik and Komi, 1998) or exercise induced muscle damage (Byrne *et al.*, 2004; Howatson and van Someren, 2008). Both result in a reduction in the force producing capability of the muscle, usually the former refers only to immediate consequences and the latter to the effects evident during the following hours or days. Neuromuscular impairment has

been shown to occur following a range of endurance exercise events (Lepers *et al.*, 2002; Millet and Lepers, 2004; Millet *et al.*, 2002). Changes in neuromuscular function can be assessed using voluntary and electrically stimulated contractions (Jones, 1996; Paillard *et al.*, 2005; Shield and Zhou, 2004). However, these techniques have not been used to assess neuromuscular function following prolonged load carriage tasks. The present study will address this using the battery of voluntary and electrically stimulated tests developed in Chapters 4 and 5.

Clarke *et al.* (1955) measured decreases in strength of the knee, trunk, ankle flexors and extensors and the shoulder elevators following a 12.1 km road march at 4 $km \cdot h^{-1}$ carrying loads of 13, 18 or 27 kg, suggesting the presence of neuromuscular impairment. However, the authors acknowledge that there was uncontrolled variation due to extreme strength scores which caused large standard deviations in the results. This could have arisen from conducting the experiment in the field. Also, the equipment used to measure strength changes used a wire tension dynamometer with plate weights and therefore measured on a discrete rather than continuous scale (Clarke *et al.*, 1954). Contrary to these findings, Knapik *et al.* (1991) showed no change in neuromuscular function using a vertical jump test measured before and after a 20 km maximal effort road march.

Load carriage is undertaken on a range of terrains and gradients (Pandolf *et al.*, 1977). The stretch shortening cycle of the supporting muscles (e.g. the *m. quadriceps femoris*) during walking involves concentric and eccentric contractions. Walking on a downhill gradient places greater emphasis on the eccentric component in the *m. quadriceps femoris* during the stretch shortening cycle (Tee *et al.*, 2007). Muscle damage and functional impairment are greater when eccentric contractions are performed (Hamlin and Quigley, 2001; Newham *et al.*, 1983). Neuromuscular impairment has been shown to be greater during downhill compared to level running (Dick and Cavanagh, 1987). However, changes in neuromuscular function following load carriage on a negative gradient have not been investigated.

The aims of this study were twofold. First, to investigate changes in neuromuscular function following 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) with no load and load carriage (25 kg backpack). Second, to compare changes in neuromuscular function following 120 minutes of load carriage (25 kg backpack) on level (0 %) and a negative (-8 %) gradients. It was hypothesised that: (1) load carriage on a level gradient would cause greater decreases in neuromuscular function (i.e. force producing capability) compared to walking with no load;

(2) load carriage on a -8 % gradient (downhill) would cause greater decreases in neuromuscular function compared to load carriage on a level gradient.

7.2. Methods

7.2.1. Participants

Ten healthy male participants (age: 30 ± 8 years, height: 1.79 ± 0.05 m, body mass: 79.4 ± 8.3 kg, body fat: 8.4 ± 3.7 %) volunteered to participate in the study. Participants had a range of previous recreational experience of carrying load in backpacks. Ethical approval for all procedures and protocols were provided by the University of Chichester Ethics Committee. All protocols were performed in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. Participants provided written informed consent and were screened to ensure they were free from any musculoskeletal injury prior to commencing the study. Participants were instructed to avoid consumption of caffeine, sports drinks or food two hours prior to arriving in the laboratory and refrain from any vigorous physical activity in the day prior to, and for 72 hours following, treadmill walking.

7.2.2. Preliminary Measures

Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) (± 0.01 kg) and stature (Avery Berkel, Smethwick, UK) (± 0.005 m) were measured whilst wearing shorts and underwear. Skinfold thickness was measured at the *Chest, Axilla, Triceps, Sub Scapular, Abdomen, Iliac Crest and Thigh* on the right side of the body using Harpenden Skinfold Callipers (Body Care, Southam, UK). Two measurements were taken at each site and if there was a difference > 1 mm, the measurements were repeated. Percentage body fat was estimated following the assessment of skinfold thickness at seven anatomical sites using previously described methods (Jackson and Pollock, 1978; Siri, 1956)

At least 5 days prior to beginning the experimental protocol, participants were familiarised with all test procedures. Participants completed 3 maximal voluntary isometric contractions, and all electrical stimulation procedures (described in detail in Chapters 4 and 5). Also, the electrical current required to stimulate an involuntary maximal twitch force (i.e. increases in current caused no further increase in force of the twitch) (group mean ± SD; 830 ± 54 mA) and sub-maximal twitch force (5 % MVC force) (group mean ± SD; 433 ± 65 mA) were recorded and kept constant in all subsequent test sessions. Participants also completed 1 cycle of the isokinetic experimental protocol (described in detail in Chapter 4). A test procedure was repeated if the experimenter or participant thought that a maximal effort was not given or if the force continued to increase in the final two contractions of a set.

7.2.3. Experimental Protocol

The study was a three way cross over randomised design, where each participant performed the following conditions on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK), at least 7 days apart: (1) 120 minutes level walking (0 % gradient) at 6.5 $km \cdot h^{-1}$ carrying no load [Level Walking (LW)], (2) 120 minutes level walking (0 % gradient) at 6.5 $km \cdot h^{-1}$ carrying a 25 kg backpack [Level Walking with Load Carriage (LWLC)], (3) 120 minutes downhill walking (-8 % gradient) at 6.5 $km \cdot h^{-1}$ and carrying a 25 kg backpack [Downhill Walking with Load Carriage (DWLC)]. Walking speed was kept constant between test conditions and an absolute load carried, to reflect realistic occupational requirements (e.g. military load carriage).

Participants completed the muscle testing protocol (Figure 7.1) before commencing load carriage (baseline) and at 0 (immediately post), 24, 48 and 72 hours following load carriage. The test order was the same on each occasion (Figure 7.1) and conducted at approximately the same time of day to control for diurnal variation in force producing capability of the muscles (Sedliak *et al.*, 2008). Three minutes rest were provided between each of the test procedures.

The methodology of the muscle testing protocol has previously been described in detail for all isometric contractions of *m. quadriceps femoris* (Chapter 5) and isokinetic contractions of the knee, trunk and shoulder extensors and flexors (Chapter 4). Briefly, responses to isometric contractions of the *m. quadriceps femoris* during MVC, VA, potentiated doublet, and the 20:50 Hz ratio were recorded. Peak torque was measured at two test velocities during isokinetic contractions of the knee (60 and $180° \cdot s^{-1}$), shoulder (60 and $180° \cdot s^{-1}$) and trunk (15 and $60° \cdot s^{-1}$) extensors and flexors. Selection of the measurements was based on those that would best measure changes in neuromuscular function whilst determining the mechanisms responsible and that showed the greatest reliability (Chapters 4 and 5).

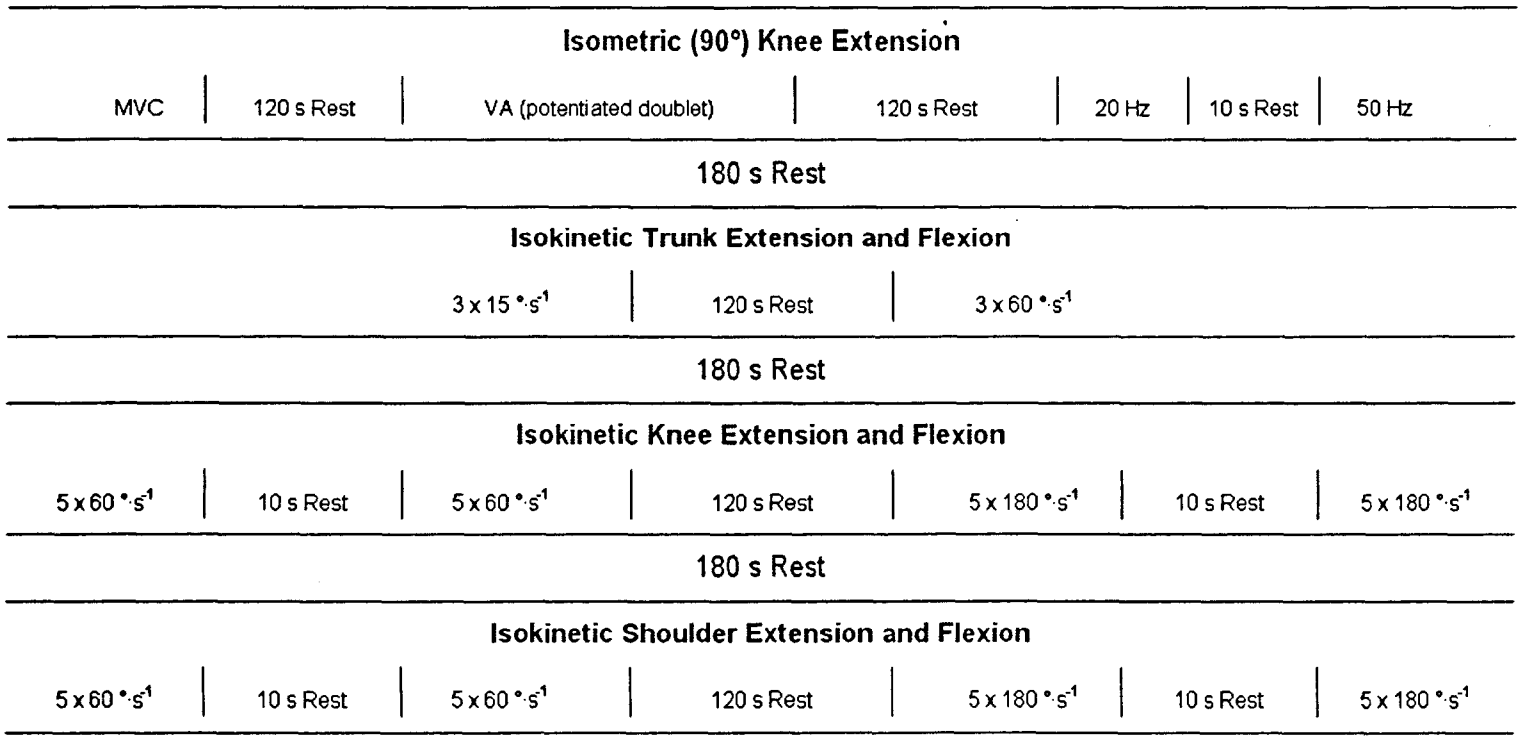


Figure 7.1 – Schematic of test battery of isometric and isokinetic contractions conducted before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 km·h^{-1}) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients.

7.2.4. Environmental Conditions

Environmental temperature was monitored using a dry bulb thermometer (Fisher Scientific, Loughborough, UK). No differences in environmental temperature were observed between pre tests and 0, 24, 48 and 72 hour test periods respectively, for LW (21.2 ± 1.5, 21.9 ± 2.6, 22.3 ± 1.5, 22.5 ± 1.8, 22.0 ± 1.3 °C, $P>0.05$), LWLC (22.4 ± 1.6, 22.4 ± 1.6, 22.7 ± 2.0, 22.7 ± 2.0, 21.8 ± 2.2 °C, $P>0.05$) or DWLC (21.5 ± 1.6, 21.5 ± 1.3, 21.5 ± 1.5, 21.6 ± 1.0, 21.3 ± 2.5 °C, $P>0.05$). There were no differences in environmental temperature between conditions ($P>0.05$).

7.2.5. Statistical Analysis

Statistical analysis was undertaken using SPSS for Windows V15 (SPSS, Chicago, Illinois). Normal distribution of the data was verified using a Kolmogorov-Smirnov test. Comparisons were made between LW and LWLC and LWLC and DWLC to ensure only one variable (i.e. load or gradient) was changed between each condition. Pre-planned paired t-tests were used to compare variables between experimental conditions (LW vs. LWLC and LWLC vs. DWLC) at the pre-exercise time point. Changes in variables across time were examined by comparing changes from pre-exercise values only. Differences from pre-exercise values were calculated at each time point following exercise (i.e. delta values at 0, 24, 48, 72 h). Pre-planned one sample t-tests were used to compare if delta values (at 0, 24, 48 and 72 h) differed from zero (i.e. pre-exercise) for each condition. If changes over time were apparent in both conditions, the magnitude of the change was compared using paired t-tests on the delta values at that time point (i.e. 0, 24, 48 and 72 h). Using pre-planned t-tests rather than a 2 way (condition x time) repeated measures Analysis of Variance (ANOVA) reduces the possibility of a type II statistical error. The calculation of a 2 way ANOVA using data in the present study is likely to cause type II statistical error due to a lack of statistical power, caused by a high number of unnecessary comparisons on a relatively small population sample (n=10). Although using multiple t-tests increases the risk of a type I statistical error, this is offset by the greater reduction of the possibility of type II error for analysis of the data in the present study. Statistical significance was set at $P<0.05$. Data are presented as mean ± standard deviation (SD).

7.3. Results

7.3.1. Level Walking (LW) vs. Level Walking Load Carriage (LWLC)

Peak torque (60 °·s^{-1}) of the knee flexors decreased immediately after LWLC (13 ± 10 %, *P*=0.003) and remained below the pre value at 24 h (11 ± 10 %, *P*=0.010), returning to pre values at 48 and 72 h (Figure 7.2). Peak torque (180 °·s^{-1}) of the knee flexors decreased by 13 ± 14 % immediately after LWLC (*P*=0.016) and then recovered at 24 h and remained at pre values at 48 and 72 h. There was no decrease in peak torque of the knee flexors at the low (60 °·s^{-1}) or high (180 °·s^{-1}) test velocities following LW (Table 7.1).

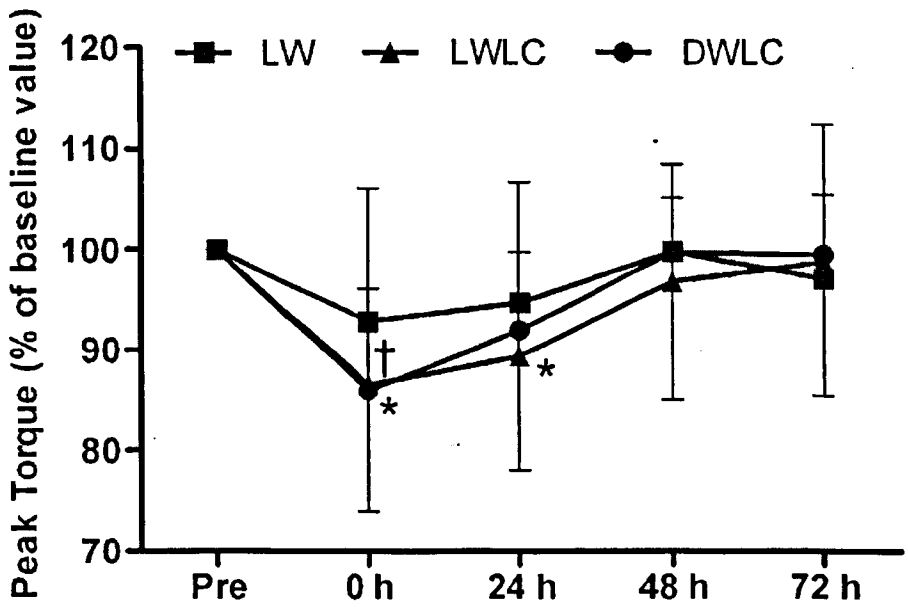


Figure 7.2 – Peak torque of the knee flexors during isokinetic contractions at (60 °·s^{-1}), measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 km·h^{-1}) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients (n = 10). Data are presented as percentage change from baseline. Symbols show difference from pre measurement for LWLC (* *P*<0.05) and DWLC († P<0.05).

Peak torque (60 °·s^{-1}) of the knee extensors decreased by 14 ± 14 % from baseline immediately (0 h) after LWLC (*P*=0.017). The peak torque made a small recovery towards baseline value at 24 h (12 ± 15 %, *P*=0.038) and 48 h (6 ± 7 %, *P*=0.023), but was still below baseline at 72 h (7 ± 8 %, *P*=0.028) (Table 7.1). Similarly, peak torque (180 °·sec^{-1}) of the

knee extensors decreased by 6 ± 7 % from baseline immediately after LWLC (P=0.009), but then recovered at 24 h and remained at pre values at 48 and 72 h. There was no decrease in peak torque of the knee extensors at the low (60 °·s^{-1}) or high (180 °·s^{-1}) test velocities following LW (Table 7.1).

Following LWLC, peak torque (15 °·s^{-1}) of the trunk extensors decreased by 8 ± 7 % (P=0.038) and returned to baseline values at 24, 48 and 72 h (Table 7.2). There was no change in peak torque (15 °·s^{-1}) of the trunk extensors following LW. There was no change in peak torque (60 °·s^{-1}) of the trunk extensors following LWLC or LW (Table 7.2).

Peak torque (15 °·s^{-1}) of the trunk flexors decreased following LWLC (10 ± 8 %, P=0.008) returning to pre values at 24, 48 and 72 h, there was no change following LW (P>0.05) (Figure 7.3). Similarly, at the higher test velocity, peak torque (60 °·s^{-1}) of the trunk flexors decreased by 10 ± 11 % from pre values following LWLC (P=0.044) and returned to pre values at 24, 48 and 72 h, there was no change following LW (P>0.05) (Table 7.2).

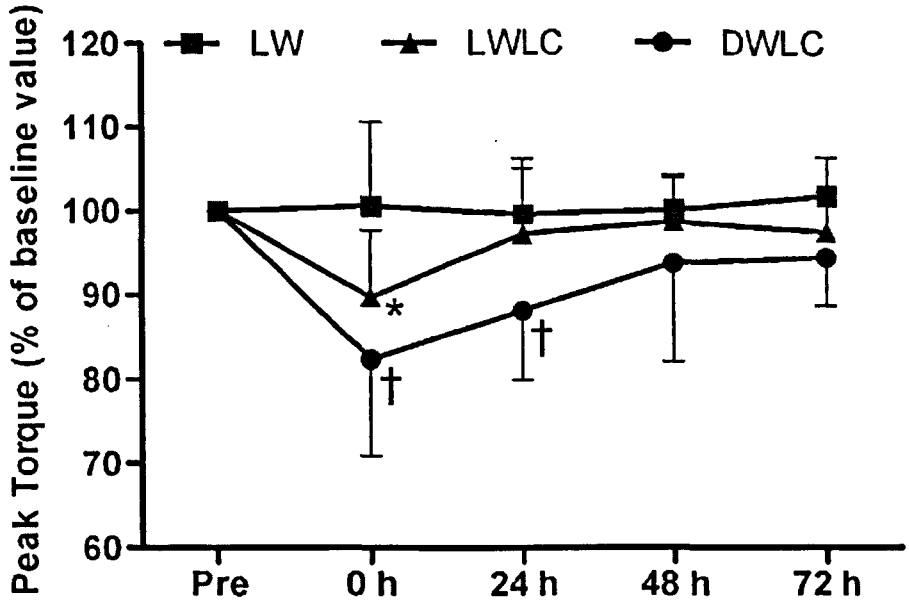


Figure 7.3 – Peak torque of the trunk flexors during isokinetic contractions (15 °·s^{-1}), measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 km·h^{-1}) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients (n = 10). Data are presented as percentage change from baseline. Symbols show difference from pre measurement for LWLC (* P<0.05) and DWLC († P<0.05).

Following LWLC peak torque (60 °·s^{-1}) of the shoulder extensors decreased by 7 ± 9 % (P=0.043) at 0 h and remained below pre values at 24 h (6 ± 7 %, P=0.014) returning to pre values at 48 and 72 h (Figure 7.4). There was no change in peak torque (180 °·s^{-1}) of the shoulder extensors following LWLC (Table 7.3). There were no changes in peak torque of the shoulder extensors following LW at the low (60 °·s^{-1}) or high (180 °·s^{-1}) test velocities (P>0.05) (Table 7.3).

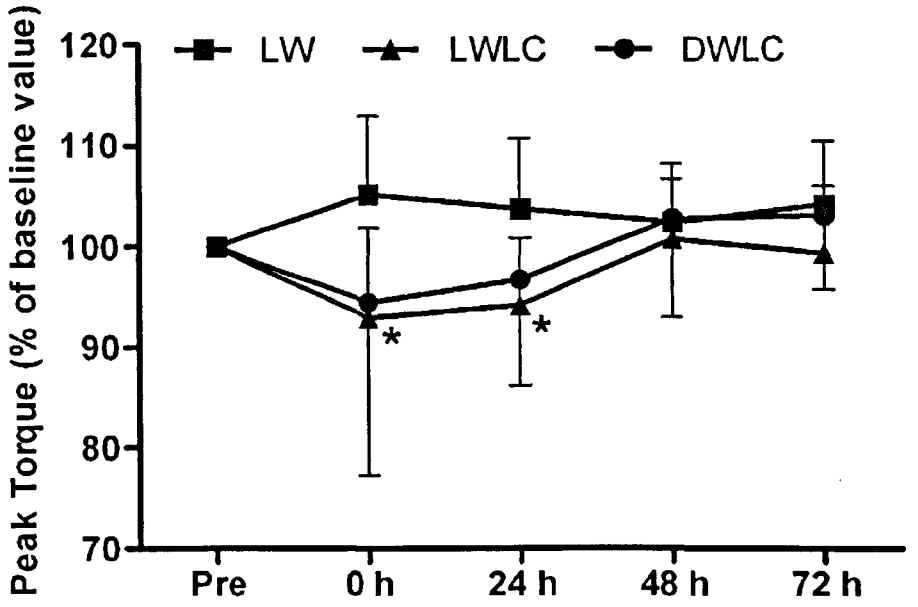


Figure 7.4 – Peak torque of the shoulder extensors during isokinetic contractions (60 °·s^{-1}), measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 km·h^{-1}) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients (n = 10). Data are presented as percentage change from baseline. Symbol shows difference from pre measurement for LWLC (* P<0.05).

Peak torque (60 °·s^{-1}) of the shoulder flexors was reduced following LWLC at 0 h (6 ± 7 %, P=0.015) but returned to pre-values at 24, 48 and 72 h (Table 7.3). However, there was no change in peak torque following LW. There was no change in peak torque of the shoulder flexors (60 °·s^{-1}) following LWLC or LW (Table 7.3).

Isometric MVC force of the *m. quadriceps femoris* decreased immediately after LWLC at 0 h (15 ± 11 %, P=0.006), the force recovered at 24 h but then fell below pre-values at 48 h (7 ± 8 %, P=0.030) and 72 h (7 ± 8 %, P=0.007) (Figure 7.5). There was no change in isometric MVC force from the pre value following LW (P>0.05). VA during the MVC decreased immediately after LWLC (95 ± 5 vs. 91 ± 10 %, P=0.037) and returned to baseline at 24 h. At 48 h following LWLC VA was greater than the pre value (P=0.022) but was similar to pre value at 72 h (Table 7.4). There was no change in VA at 0 or 48 h after LW. However, at 24 and 72 h after LW, VA was lower than the pre values (P<0.05) (Table 7.4).

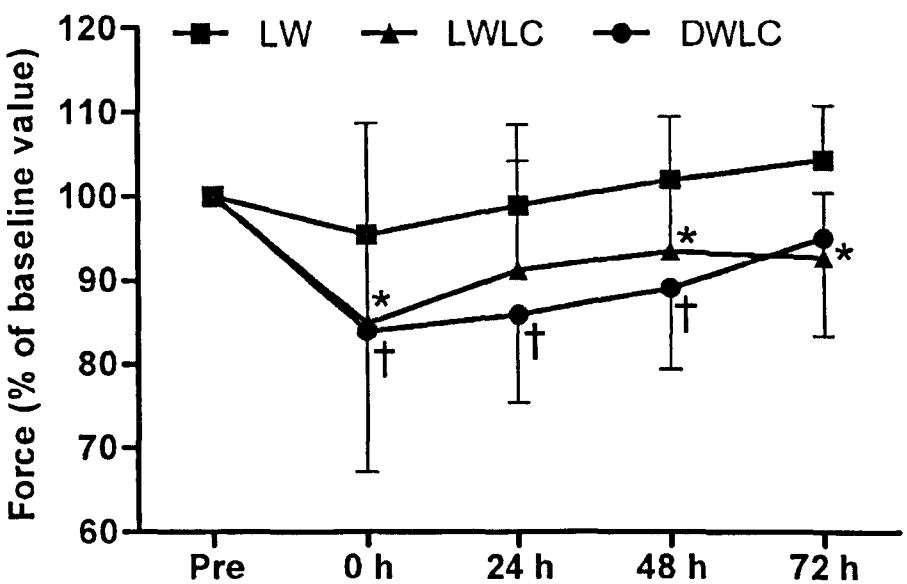


Figure 7.5 – Force of the *m. quadriceps femoris* during isometric MVC, measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 km·h^{-1}) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients (n = 10). Data are presented as percentage change from baseline. Symbols show difference from pre measurement for LWLC (* P<0.05) and DWLC († P<0.05).

Doublet peak force did not change following LWLC or LW (P>0.05) (Table 7.4). Doublet contraction time decreased immediately after LWLC (0.191 ± 0.013 vs. 0.181 ± 0.008 s, P=0.012) and returned to pre values at 24, 48 and 72 hours, there was no change following LW (Table 7.4). Similarly, doublet half relaxation time decreased immediately after LWLC (0.095 ± 0.008 vs. 0.092 ± 0.007 s, P=0.023) and returned to pre values at 24, 48 and 72 hours, there was no change following LW (Table 7.3). Doublet maximal rate of force development

and doublet maximal rate of force decrease, rate constant for contraction and rate constant for relaxation did not change following LWLC or LW (P>0.05) (Table 7.4).

The pre value of the ratio of 20:50 Hz stimulations was higher for LWLC than LW at baseline (0.88 ± 0.04 vs. 0.84 ± 0.03, P=0.025). Following LWLC the 20:50 Hz ratio decreased at 0 h (0.84 ± 0.04, P=0.028), returning to the pre value at 24 h (0.84 ± 0.04, P=0.050) and dropping below pre values at 48 h (0.83 ± 0.04, P=0.022) and 72 h (0.85 ± 0.04, P=0.004). There was no change in the 20:50 Hz ratio following LW (Table 7.4).

7.3.2. Level Walking Load Carriage (LWLC) vs. Downhill Walking Load Carriage (DWLC)

Peak torque (60 °·s^{-1}) of the knee extensors decreased immediately after DWLC by 14 ± 17 % (P=0.045) and LWLC by 14 ± 14 % (P=0.017), there was no difference in the decrease between conditions (P=0.708). Following DWLC knee extensor peak torque (60 °·s^{-1}) returned to pre values at 24, 48 and 72 h, but remained below pre values for LWLC at 24 h (12 ± 15 %, P=0.038), 48 h (6 ± 7 %, P=0.023) and 72 h (7 ± 8 %, P=0.028). Peak torque (180 °·s^{-1}) of the knee extensors decreased by 11 ± 13 % and 6 ± 7 % of the pre value for DWLC and LWLC respectively (P<0.05), there was no difference between conditions (P=0.156). Following DWLC, peak torque (180 °·s^{-1}) remained below the pre value at 24 h (7 ± 8 %, P=0.025) but recovered to pre value level at 48 and 72 h (Table 7.1). Following LWLC, peak torque (180 °·s^{-1}) returned and remained at baseline value at 24, 48 and 72 h (Table 7.1).

Following DWLC, peak torque (60 °·s^{-1}) of the knee flexors decreased compared to pre values at 0 h only (14 ± 12 %, P=0.005) and returned to baseline at 24, 48 and 72 h (Figure 7.2). Following LWLC, peak torque (60 °·s^{-1}) of the knee flexors decreased at 0 h (13 ± 10 %, P=0.003) and 24 h (11 ± 10 %, P=0.010) to baseline at 24, 48 and 72 h (Figure 7.2). There was no differences between conditions (P=0.860). Similarly, peak torque (180 °·s^{-1}) of the knee flexors decreased by 11 ± 10 % and 13 ± 14 % at 0 h after LWLC and DWLC respectively (P<0.05), there was no difference between conditions (P=0.982). Peak torque (180 °·s^{-1}) remained below pre values at 24 h for DWLC only (11 ± 10 %, P=0.010) and then returned to baseline at 48 and 72 h (Figure 7.2).

There was no change in peak torque of the trunk extensors at low (15 °·s^{-1}) or high (60 °·s^{-1}) test velocities following DWLC (Table 7.2). However, immediately after LWLC peak

torque (15 °·s^{-1}) decreased by 8 ± 7 % of pre values (P=0.038), returning to baseline levels at 24, 48 and 72 hours. There was no change in peak torque (60 °·s^{-1}) following LWLC.

Peak torque (15 °·s^{-1}) of the trunk flexors decreased by 18 ± 11 % immediately after DWLC (P=0.007) and remained 12 ± 8 % below the pre value at 24 h (P=0.012). Peak torque (15 °·s^{-1}) of the trunk flexors also decreased by 10 ± 8 % following LWLC (P=0.008), but there was no difference from DWLC (P=0.095) (Figure 7.3). After the initial decrease following LWLC peak torque (15 °·s^{-1}) recovered to pre values at 24, 48 and 72 h. Peak torque (60 °·s^{-1}) of the trunk flexors decreased immediately after DWLC and LWLC by 9 ± 10 % and 10 ± 11 % respectively (P<0.05), but there was no difference between conditions (P=0.773). Peak torque (60 °·s^{-1}) recovered to pre values at 24, 48 and 72 h for both LWLC and DWLC.

There was no change in peak torque (60 °·s^{-1}) of the shoulder extensors following DWLC (Table 7.3). However, peak torque (60 °·s^{-1}) decreased by 7 ± 9 % immediately after LWLC (P=0.043) and remained 6 ± 7 % below pre values at 24 h (P=0.014), returning to baseline at 48 and 72 hours (Figure 7.4). Peak torque (180 °·s^{-1}) of the shoulder extensors increased at 48 h following DWLC (P=0.021) but did not change following LWLC. Peak torque (60 °·s^{-1}) of the shoulder flexors decreased by 11 ± 15 % and 6 ± 7 % following DWLC and LWLC respectively (P<0.05), there was no difference between conditions (P=0.326). There was no change in peak torque (180 °·s^{-1}) of the shoulder flexors for DWLC or LWLC.

The pre test isometric MVC force was higher for DWLC compared to LWLC (726 ± 156 vs. 692 ± 141 N, P=0.002). The change in MVC force over time is illustrated in (Figure 7.5). MVC force decreased by 16 ± 17 % and 15 ± 11 % immediately after DWLC and LWLC respectively (P<0.05), there was no difference between conditions (P=0.480). At 24 h, MVC force remained below pre value for DWLC (14 ± 11 %, P=0.011) but recovered for LWLC (9 ± 13 %, P=0.090). However, at 48 h MVC force was 11 ± 10 and 7 ± 8 % below pre value levels for LWLC and DWLC respectively (P<0.05). At 72 h, MVC force following DWLC returned to pre value but remained 7 ± 8 % below baseline for LWLC (P=0.007). Voluntary activation during the MVC did not change following DWLC but decreased immediately after LWLC (P=0.037) and was higher than pre values at 48 h (P=0.022) (Table 7.5).

Doublet peak force decreased after DWLC only (12 ± 8 %, P=0.002) and returned and remained above pre value at 24, 48 and 72 h. Doublet contraction time decreased after DWLC

(0.191 ± 0.013 vs. 0.181 ± 0.008, P=0.004) and LWLC (0.188 ± 0.008 vs. 0.179 ± 0.009, P=0.012) and returned to pre values at 24, 48 and 72 h, there was no difference between conditions (P=0.301). Doublet half relaxation time decreased immediately following DWLC (P<0.001) and recovered at 24 h, remaining above pre values at 48 and 72 h (P<0.05) (Table 7.5). Doublet half relaxation time also decreased immediately after LWLC (P=0.023) but returned and remained at pre values at 24, 48 and 72 h, there were no differences between conditions (P=0.559) (Table 7.5). Doublet maximal rate of force development decreased immediately after DWLC (1666 ± 350 vs. 1481 ± 285 $N \cdot s^{-1}$, P=0.007) and remained below baseline at 24 h (1554 ± 264 $N \cdot s^{-1}$, P=0.029) recovering to baseline values at 48 and 72 h. Following DWLC there were increases in the rate constants for contraction (P=0.021) and relaxation (P<0.001) (Table 7.4). There was no change in doublet maximal rate of force development, rate constant for contraction or rate constant for relaxation following LWLC (Table 7.4). Doublet maximal rate of force decrease did not change following DWLC or LWLC (Table 7.4).

The pre value of the ratio of 20:50 Hz stimulations was lower for DWLC than LWLC at baseline (0.85 ± 0.03 vs. 0.88 ± 0.04, P=0.032). At 0 h the 20:50 Hz ratio decreased for both DWLC and LWLC (P<0.05), there was no difference between conditions (P=0.185). The 20:50 Hz ratio returned to the pre value at 24 h for both DWLC and LWLC but dropped below pre value at 48 and 72 h for LWLC only (P<0.05) (Table 7.5).

Table 7.1 – Peak torque of the knee extensors and flexors during isokinetic contractions at 60 and 180 °·s^{-1} measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 km·h^{-1}) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients (n = 10). Difference from pre measurement (* P<0.05, ** P<0.01). Difference in pre measurement between LWLC and DWLC († P<0.05).

Variable	Condition	Peak Torque (Nm)				
		Pre	0 h	24 h	48 h	72 h
Extension (60 °·s^{-1})	LW	191 ± 37 †	179 ± 39	181 ± 41	194 ± 43	187 ± 37
	LWLC	205 ± 36	176 ± 38 *	181 ± 49 *	194 ± 43 *	191 ± 43 *
	DWLC	211 ± 42	178 ± 45 *	180 ± 50	194 ± 42	203 ± 30
Extension (180 °·s^{-1})	LW	136 ± 30	134 ± 28	134 ± 29	138 ± 30	138 ± 26
	LWLC	138 ± 35	131 ± 38 **	135 ± 41	140 ± 36	142 ± 32
	DWLC	150 ± 23	132 ± 26 *	138 ± 24*	143 ± 23	149 ± 20
Flexion (60 °·s^{-1})	LW	121 ± 23	113 ± 28	114 ± 24	120 ± 21	117 ± 21
	LWLC	125 ± 24	109 ± 26 **	112 ± 27 *	121 ± 23	122 ± 19
	DWLC	124 ± 16	107 ± 20 **	114 ± 21	123 ± 17	122 ± 11
Flexion (180 °·s^{-1})	LW	93 ± 18	91 ± 19	94 ± 17	96 ± 18	94 ± 16
	LWLC	96 ± 21	85 ± 23 *	89 ± 20	95 ± 21	96 ± 21
	DWLC	100 ± 17	88 ± 18 **	89 ± 18 *	98 ± 15	99 ± 14

Table 7.2 – Peak torque of the trunk extensors and flexors during isokinetic contractions at 15 and 60 °·s^{-1} measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 km·h^{-1}) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients (n = 8). Difference from pre measurement (* P<0.05, ** P<0.01).

Variable	Condition	Peak Torque (Nm)				
		Pre	0 h	24 h	48 h	72 h
Extension (15 °s^{-1})	LW	279 ± 47	257 ± 50	257 ± 45	259 ± 59	271 ± 56
	LWLC	263 ± 59	242 ± 61 *	259 ± 70	272 ± 44	254 ± 52
	DWLC	270 ± 52	234 ± 73	245 ± 70	258 ± 57	280 ± 54
Extension (60 °s^{-1})	LW	267 ± 50	261 ± 65	251 ± 66	263 ± 70	273 ± 67
	LWLC	270 ± 58	250 ± 67	260 ± 73	273 ± 59	272 ± 59
	DWLC	274 ± 54	246 ± 75	253 ± 61	261 ± 53	281 ± 66
Flexion (15 °s^{-1})	LW	256 ± 40	256 ± 41	254 ± 39	256 ± 44	261 ± 43
	LWLC	260 ± 41	235 ± 52 **	253 ± 50	257 ± 46	253 ± 41
	DWLC	276 ± 59	228 ± 61 **	243 ± 56 *	255 ± 46	260 ± 52
Flexion (60 °s^{-1})	LW	296 ± 32	282 ± 49	285 ± 34	289 ± 38	287 ± 44
	LWLC	296 ± 36	268 ± 51 *	279 ± 52	293 ± 34	293 ± 35
	DWLC	293 ± 42	267 ± 56 *	270 ± 43	288 ± 45	298 ± 41

Table 7.3 – Peak torque of the shoulder extensors and flexors during isokinetic contractions at 60 and 180 °·s^{-1} measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 km·h^{-1}) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients (n = 10). Difference from pre measurement (* P<0.05). Difference in pre measurement between LWLC and DWLC († P<0.05).

Variable	Condition	Peak Torque (Nm)				
		Pre	0 h	24 h	48 h	72 h
Extension (60 °·s^{-1})	LW	96 ± 17 †	101 ± 19	100 ± 20	99 ± 19	101 ± 21
	LWLC	101 ± 17	95 ± 22 *	96 ± 20 *	102 ± 20	100 ± 19
	DWLC	103 ± 14	98 ± 23	100 ± 17	107 ± 19	107 ± 19
Extension (180 °·s^{-1})	LW	81 ± 15	84 ± 16	84 ± 14	84 ± 16	84 ± 15
	LWLC	85 ± 13	82 ± 18	81 ± 18	84 ± 16	83 ± 15
	DWLC	83 ± 12	85 ± 16	85 ± 15	89 ± 16 *	87 ± 16
Flexion (60 °·s^{-1})	LW	66 ± 13	73 ± 15	74 ± 18	71 ± 13	72 ± 15
	LWLC	70 ± 17	65 ± 16 *	70 ± 19	73 ± 20	70 ± 15
	DWLC	74 ± 17	65 ± 14 *	72 ± 16	73 ± 15	75 ± 16
Flexion (180 °·s^{-1})	LW	50 ± 7	51 ± 9	52 ± 9	52 ± 8	54 ± 8
	LWLC	51 ± 9	50 ± 9	50 ± 8	50 ± 9	51 ± 8
	DWLC	53 ± 9	51 ± 6	51 ± 6	55 ± 7	55 ± 8

Table 7.4 – Voluntary and electrically stimulated isometric contractions of the *m. quadriceps femoris* measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) with no load (LW) or load carriage (25 kg backpack) on a level (LWLC) and downhill (DWLC) gradients (n = 10, unless stated). Difference from pre measurement (* P<0.05, ** P<0.01, *** P<0.001). Difference in pre measurement between LW and LWLC ($^{\#}$ P<0.05, $^{\#\#}$ P<0.01). Difference in pre measurement between LWLC and DWLC († P<0.05).

Measure	Cond	Pre	0 h	24 h	48 h	72 h
MVC (N)	LW	660 ± 155	617 ± 111	647 ± 134	670 ± 141	687 ± 150
	LWLC	692 ± 141	584 ± 126 **	624 ± 124	650 ± 150 *	648 ± 164 **
	DWLC	726 ± 156 $^{\#\#}$	592 ± 114 *	617 ± 128 *	638 ± 112 *	680 ± 119
VA (%)	LW	98 ± 3	96 ± 6	95 ± 5 *	96 ± 5	96 ± 5 *
	LWLC	95 ± 5	91 ± 10 *	97 ± 4	99 ± 2 *	97 ± 4
	DWLC	97 ± 4	94 ± 12	95 ± 8	94 ± 8	94 ± 8
Doublet Peak Force (N)	LW	174 ± 40	167 ± 43	178 ± 36	186 ± 33	187 ± 29
	LWLC	180 ± 41	166 ± 35	177 ± 36	179 ± 35	176 ± 33
	DWLC	191 ± 42	166 ± 32 **	180 ± 29	186 ± 37	187 ± 34
Doublet Contraction Time (s)	LW	0.186 ± 0.011	0.182 ± 0.012	0.188 ± 0.008	0.192 ± 0.012	0.187 ± 0.010
	LWLC	0.191 ± 0.013	0.181 ± 0.008 *	0.185 ± 0.012	0.189 ± 0.007	0.186 ± 0.005
	DWLC	0.188 ± 0.008	0.179 ± 0.009 **	0.188 ± 0.007	0.190 ± 0.015	0.188 ± 0.010
Doublet Half Relaxation Time (s)	LW	0.096 ± 0.010	0.099 ± 0.015	0.097 ± 0.010	0.098 ± 0.011	0.097 ± 0.010
	LWLC	0.095 ± 0.008	0.092 ± 0.007 *	0.097 ± 0.012	0.096 ± 0.008	0.094 ± 0.006
	DWLC	0.096 ± 0.008	0.092 ± 0.008 ***	0.099 ± 0.009	0.099 ± 0.010 *	0.099 ± 0.009 *

Measure	Cond	Pre	0 h	24 h	48 h	72 h
Doublet Maximal Rate of Force Development ($N \cdot s^{-1}$)	LW	1536 ± 332	1482 ± 371	1552 ± 338	1575 ± 268	1608 ± 264
	LWLC	1588 ± 324	1450 ± 289	1546 ± 313	1549 ± 311	1569 ± 302
	DWLC	1666 ± 350	1481 ± 285 **	1554 ± 264 *	1606 ± 326	1626 ± 289
Doublet Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	LW	-1255 ± 239	-1199 ± 347	-1269 ± 253	-1306 ± 238	-1318 ± 209
	LWLC	-1308 ± 291	-1238 ± 214	-1263 ± 269	-1270 ± 263	-1298 ± 217
	DWLC	-1359 ± 283	-1266 ± 235	-1246 ± 212	-1277 ± 253	-1291 ± 226
Rate Constant for Contraction ($\cdot s^{-1}$)	LW	8.9 ± 0.4	8.9 ± 0.4	8.7 ± 0.4	8.5 ± 0.4	8.6 ± 0.5
	LWLC	8.9 ± 0.4	8.7 ± 0.4	8.7 ± 0.4	8.7 ± 0.2	8.9 ± 0.4
	DWLC	8.7 ± 0.4	8.9 ± 0.4 *	8.6 ± 0.5	8.7 ± 0.4	8.7 ± 0.3
Rate Constant for Relaxation ($\cdot s^{-1}$)	LW	-7.3 ± 1.0	-7.2 ± 1.1	-7.2 ± 0.9	-7.1 ± 0.9	-7.1 ± 0.9
	LWLC	-7.3 ± 0.9	-7.5 ± 0.8	-7.2 ± 1.0	-7.1 ± 0.7	-7.4 ± 0.7
	DWLC	-7.2 ± 0.8	-7.7 ± 0.8 *	-6.9 ± 0.9	-6.9 ± 0.9	-7.0 ± 0.9 *
20:50 Hz Ratio (n=9)	LW	0.84 ± 0.03 #	0.84 ± 0.04	0.85 ± 0.04	0.84 ± 0.05	0.85 ± 0.06
	LWLC	0.88 ± 0.04	0.84 ± 0.04 *	0.84 ± 0.05	0.83 ± 0.06 *	0.85 ± 0.02 *
	DWLC	0.85 ± 0.03 †	0.80 ± 0.04 **	0.83 ± 0.04	0.85 ± 0.04	0.85 ± 0.04

7.4. Discussion

This study investigated changes in neuromuscular function following 120 minutes of load carriage during level and downhill (-8 %) treadmill walking. A novel aspect of this study was to investigate these changes using voluntary and electrically stimulated contractions 0, 24, 48 and 72 h after load carriage, in a controlled laboratory setting. No changes in neuromuscular function were observed following walking for 120 minutes at 6.5 $km \cdot h^{-1}$ on a 0 % gradient. However, the addition of carrying a 25 kg backpack caused decreases in the force produced by the knee extensors during isokinetic and isometric contractions, which were still apparent up to 72 h following the load carriage bouts. Decreases in the force producing capability of the knee extensors during all isometric and isokinetic contractions were similar for LWLC, and DWLC. However, there were differences in the timescale of recovery between conditions at the fast and slow isokinetic test velocities. There were decreases in the force produced during isokinetic contractions, of the knee extensors and the knee, trunk and shoulder flexors (LWLC and DWLC) and shoulder extensors (LWLC), immediately post and in the days following the load carriage bout.

These findings confirm the hypothesis that load carriage on a level gradient causes greater decreases in neuromuscular function compared to walking with no load. However, the hypothesis that load carriage on a -8 % gradient (downhill) causes greater decreases in neuromuscular function compared to load carriage on a level gradient was not supported.

The present study supports Clarke *et al.* (1955), which showed decreases in force producing capability of the knee and trunk flexors and extensors following a 12.1 km road march at 4 $km \cdot h^{-1}$ carrying loads 13, 18 and 27 kg. However, as highlighted in the introduction, Clarke *et al.* (1955) acknowledged there was large uncontrolled variation in strength scores and equipment used to measure force producing capability of the muscles. The present study also included an unloaded walking control condition to examine the effect of load carriage and investigated the effect of downhill walking with load carriage immediately post exercise and on the subsequent days of recovery.

In comparison to the present study, Clarke *et al.* (1955) observed smaller decreases in knee extensor (8 %) and flexor (6 %) peak torque but comparable changes in reduction of trunk extensors and flexors torque (11 %). Compared to LWLC, changes in knee extensor and flexor torque were similar after DWLC (Table 7.1). However, the force producing capability

of the trunk and shoulder extensors decreased after LWLC only. Following DWLC there was a trend for greater decreases in peak torque of the trunk (Table 7.2) and shoulder (Table 7.3) flexors. Levine *et al.* (2007) showed a greater sagittal range of motion of the lumber spine when walking downhill compared to a level gradient. It is likely that this would also be apparent during DWLC, placing additional strain on the trunk flexors supporting the backpack, causing greater fatigue or acute muscle injury. In addition, strain may have been removed from the trunk extensors, reducing the neuromuscular impairment.

7.4.1. Voluntary Contractions Knee Extensors

The addition of carrying load during the 120 minutes of treadmill walking caused a decrease in the force produced by the knee extensors, during both isokinetic (60 and 180 $°\cdot s^{-1}$) and isometric contractions. This was probably due to a greater recruitment of muscle fibres required to maintain posture and movement on the treadmill when carrying a load (Ghori and Luckwill, 1985). This probably resulted in higher force production by the muscle fibres and more fibres being exposed to damage during the stretch shortening cycle, therefore impairing their ability to produce force following LWLC.

Decreases in the force produced by the knee extensors has been observed following prolonged running, cycling and ski skating (Millet and Lepers, 2004). Prolonged running events are more comparative to load carriage than cycling or ski-skating events as the muscles perform similar stretch shortening cycles (Nicol *et al.*, 2006). Isometric knee extensor torque during an MVC has been shown to decrease between 15 and 63 % following prolonged (> 30 min) running (Gauche *et al.*, 2006; Millet *et al.*, 2003a; Place *et al.*, 2004). However, these activities were of a higher exercise intensity and duration to load carriage. Place *et al.* (2004) showed no decrease in knee extensor torque during an MVC after two hours of running at approximately 55 % $\dot{V}O_2$max. Participant's work rate during the run was higher than that during load carriage (25–40 % $\dot{V}O_2$max) (Patton *et al.*, 1991). However, decrease in knee extensor force of the MVC following DWLC and LWLC were 16 and 15 %, respectively, possibly due to the greater recruitment and subsequent damage to muscle fibres during load carrying activities.

Following DWLC, at 0 hours, there was no difference in the decrease in force produced during isometric or isokinetic contractions of the knee extensors compared to LWLC. This is surprising, as neuromuscular impairment has been shown to be greater when

running on negative compared to level gradients (Dick and Cavanagh, 1987). Walking on a downhill gradient places greater emphasise on the eccentric component of stretch shortening cycle and greater forces are absorbed by the *m. quadriceps femoris* (Tee *et al.*, 2007). Typically, the decrease in force producing capacity of a muscle or muscle group is greater following eccentric compared to concentric contractions (Hamlin and Quigley, 2001; Newham *et al.*, 1983). Also, although the force produced during isokinetic and isometric contractions returned to pre-exercise value at 72 hours following DWLC they were still suppressed following LWLC, suggesting greater severity of muscle injury following LWLC. Carrying a load during level walking alters gait and increases knee and femur ranges of motion (Attwells *et al.*, 2006). These changes may result in greater exposure of *m. quadriceps femoris* or additional fibres at different muscle lengths to mechanical damage during the eccentric component of the stretch shortening cycle. Load carriage also increases ground reaction forces (Tilbury-Davis and Hooper, 1999), therefore increasing the amount of force that the supporting muscles must absorb. These biomechanical changes have only been examined in level gradients and may be different with load carriage on downhill gradients. These differences could potentially explain the greater neuromuscular impairment following LWLC compared to DWLC (as shown by the slower recovery of force producing capability over time).

MVC force showed a bimodal pattern of recovery following LWLC as it recovered at 24 h and dropped back below pre-exercise value at 48 and 72 h. Dousset *et al.* (2007) suggested that this is due to the varying effects of different mechanisms responsible for the force loss over the time course of recovery. The initial exercise bout (i.e. load carriage) causes mechanical damage resulting in damage to the muscle structure and the initial loss of force, which begins to recover over the following hours (Clarkson and Hubal, 2002). The initial mechanical overload on the muscle fibres induces an increase in intracellular calcium concentration resulting in a loss of cellular Ca^{2+} homeostasis, which triggers a chain of events after cessation of exercise (Armstrong, 1990). The increase in intracellular Ca^{2+} leads to the activation of a number of Ca^{2+} dependent proteolytic and phospholipolytic pathways which degrade the structural and contractile myofibre proteins as well as the myofibre membrane (Armstrong, 1990; Kuipers, 1994). This results in additional losses in force producing capability, recovery from which can take up to 8 days after the initial exercise bout (Warren *et al.*, 2002). This suggests that despite a similar decrease in MVC force immediately after

exercise, the initial injury to muscle fibres may have been more severe following DWLC compared to LWLC, resulting in a slower recovery in the initial stages of the muscle injury process. Hence MVC force remaining below pre-exercise values 24 hours after DWLC only.

Interestingly, knee extensor and flexor isokinetic torque recovered more quickly at the slower isokinetic velocity following LWLC but following DWLC the faster velocity showed slower recovery (Table 7.1). This suggests different fibres or mechanisms were affected during recovery from LWLC and DWLC. There is debate as to whether different isokinetic test velocities recruit different fibre types (i.e. type I or type II) (Friden *et al.*, 1983a; Perrin, 1993). Friden *et al.* (1983a) suggested that type II fibres are primarily responsible for development of tension at higher angular velocities. During sub-maximal exercise (i.e. load carriage in the present study) type I fibres are primarily responsible for generating power during locomotion (Sargeant, 1994). Both type I and type II muscle fibres become fatigued and damaged more rapidly during eccentric compared to concentric exercise (Clarkson and Newham, 1995). However, type II muscle fibres are preferentially recruited during eccentric muscle actions (Nardone *et al.*, 1989) and there is evidence to suggest that type II muscle fibres suffer greater damage than Type I muscle fibres following eccentric contractions (Friden *et al.*, 1983b). Therefore, a greater number of type II fibres may have been initially recruited during DWLC which became damaged more rapidly due to the eccentric muscle actions when carrying a load downhill. The greater damage to the Type II fibres may be reflected in the slower recovery of neuromuscular function at the higher isokinetic test velocity following DWLC. Type II fibres may not have been recruited as readily during LWLC, therefore more reliance may have been placed on the Type I fibres, hence a greater impairment and the slower recovery at the slow isokinetic test velocity.

7.4.2. Electrical Stimulation of the Knee Extensors

The decrease in the force produced by the knee extensors was accompanied by a decrease in VA immediately after LWLC (Table 7.5), suggesting that part of neuromuscular impairment was due to central mechanisms (Shield and Zhou, 2004). The decreases in VA was less than that observed following a 30 km running race (98 vs. 93 %) (Millet *et al.*, 2003a), 300 min treadmill run at 55 % maximal aerobic velocity (99 vs. 78 %) (Place *et al.*, 2004) and 65 km ultramarathon (82 vs. 60 %) (Millet *et al.*, 2002). The reduced voluntary activation may have originated from a supraspinal site and/or the spinal level (Gandevia, 2001), but these could not be distinguished in the present study. There was also a small decrease in VA

following LW at 24 and 72 h; however this was not great enough to cause a reduction in MVC force. Racinais *et al.* (2008), suggested this fall in voluntary activation represents a protective mechanism to protect the muscle from further peripheral damage. Hence, the neuromuscular impairment following load carriage is likely to have had both central and peripheral origins.

Warren *et al.* (2002) showed in mouse muscle that approximately 57 to 75 % of the strength loss during the first 72 h of muscle injury can be attributed to failure of the excitation-contraction coupling pathway. The decrease in the 20:50 Hz ratio following LWLC and DWLC shows the presence of LFF (Jones, 1996). LFF indicates a reduction in Ca^{2+} release from the sarcoplasmic reticulum and/or a redistribution of sarcomere lengths (i.e. popping sarcomere theory) (Jones, 1996). This damage to the structure of the muscle fibre results in impairment of the excitation-contraction coupling process (Allen *et al.*, 2008; Chin *et al.*, 1997). This confirms a peripheral component to the neuromuscular impairment following LWLC and DWLC. During prolonged exercise (such as load carriage) this damage is most likely due to eccentric contractions and the shock wave rather than metabolic changes (Millet and Lepers, 2004). Reductions in the force and Ca^{2+} have also been shown to be closely associated with reduced muscle glycogen concentration (Chin and Allen, 1997). Interestingly, the 20:50 Hz ratio recovered 24 h after DWLC and LWLC but after an intermediate recovery was still below pre-exercise values following LWLC at 72 h, suggesting the peripheral damage to the muscle structure was still present at 72 h. Comparisons between conditions for the 20:50 Hz ratio should be interpreted with caution as the pre-exercise values were greater for LWLC than DWLC.

The change in doublet parameters also indicates disruption to the excitation-contraction coupling process and damage of muscle fibres. Doublet peak force decreased after DWLC, this decrease is a common symptom of muscle fatigue and is caused by a combination of maximum force generating capacity (damage to the muscle structure), reduced myofibrillar Ca^{2+} sensitivity and reduced Ca^{2+} release, reducing the capability of the cross bridges to form strong binding (Allen *et al.*, 1995a). Potentiation (causing an increase in doublet force) and fatigue (causing a reduction in peak force) associated effects are simultaneously present following prolonged exercise (Rassier and Macintosh, 2000). Therefore, the change in peak force of an electrically stimulated contraction is the sum of the potentiated and fatigue effects (Rassier and Macintosh, 2000). Although peak torque of stimulated contractions has been shown to decrease following prolonged exercise (Lepers *et al.*, 2002; Millet *et al.*, 2003a)

increases have been observed following an ultramarathon (Millet *et al.*, 2002) and ski-skating marathon (Millet *et al.*, 2003b). Therefore, the mechanisms responsible for the decrease in doublet peak torque following DWLC may have also been affected to a lesser extent immediately after LWLC or LW but obscured by the effects of potentiation.

The faster half relaxation time immediately after DWLC and LWLC and increase in rate constants of contraction and relaxation following DWLC, are surprising as fatigued muscles generally show a slowing of contraction and relaxation velocity (Allen *et al.*, 1995a). Millet *et al.* (2002) also showed a faster half relaxation time of a electrically stimulated twitch following a 65-km ultramarathon (68.9 to 57.8 ms). The changes in the relaxation time following exercise due to neuromuscular impairment (i.e. a slowing) may have been masked by potentiation, which increases the speed the half relaxation time (O'Leary *et al.*, 1997).

Compared to the pre exercise value, half relaxation time was slower at 48 and 72 h following DWLC. Lepers *et al.* (2002) showed similar findings in recovery from 5 h cycling exercise and suggested this may be due to a delayed impairment of the processes that govern the half-relaxation time (i.e. Ca^{2+} movements). In their review of the metabolic factors of fatigue Allen *et al.* (1995a) showed multiple mechanisms are responsible for the slower cross bridge detachment, the most important of which are a fall in ATP and PCr, slower uptake of Ca^{2+} into the sarcoplasmic reticulum and reduced sensitivity of myofibrillar proteins to Ca^{2+}. However, However, Booth *et al.* (1997) argue that the decrease in Ca^{2+} uptake is not associated with a slowing of relaxation following exhaustive exercise. Irrespective of the mechanisms, a slowing in relaxation time will slow the rate at which rapidly alternating movements (e.g. running) can be performed (Allen *et al.*, 1995a).

The slower contraction time of the doublet immediately after DWLC and LWLC shows a slowing of the muscle contraction. These data provide further support of impairment to the excitation contraction coupling process, probably reflecting a reduced Ca^{2+} release (Martin *et al.*, 2005).

7.4.3. Voluntary Contractions of Knee Flexors

The decrease in the knee flexor peak torque immediately after LWLC and DWLC was of a similar magnitude to the knee extensors and there was no change following LW. Harman *et al.* (1992) showed no change in muscle activity of the knee flexors with increases in backpack load from 6 to 47 kg. However, their data were only collected over a short time

period (several steps on a force platform). The pattern of muscle fibre recruitment may have changed over the 2 h for LWLC to support the additional load causing the observed neuromuscular impairment. The mechanical stress experienced by the knee flexors is likely to be similar during DWLC and LWLC as the additional force during the eccentric contractions when walking on a negative gradient is likely to be absorbed by the *m. quadriceps femoris.* Similarly to the knee extensors; recovery of peak torque at the slower test velocity was faster following DWLC but recovery at the faster test velocity was quicker following LWLC. This suggests differences between conditions in the mechanisms responsible for the functional impairment, similar to those discussed above for the knee extensors.

7.4.4. Voluntary Contractions of Trunk Extensors and Flexors

The trunk extensors showed a decrease in force producing capability after LWLC at the slow test velocity only, but recovered to pre-exercise value at 24 h; there were no changes following LW or DWLC. The trunk flexors decreased after LWLC and DWLC only. Al Khabbaz *et al.* (2008) showed that *m. rectus abdominis* activity increased when a backpack load was carried compared to unloaded walking. Similar increases with the addition of load have been shown for the paraspinal muscles (Cook and Neumann, 1987). The increased muscle activity with load carriage is likely to account for the greater neuromuscular impairment observed following LWLC compared to LW. The decrease in trunk flexor peak torque was the same for LWLC and DWLC immediately after load carriage at the faster test velocity and recovered in both conditions by 24 h. However, peak torque the slower test velocity following DWLC and recovered to pre-exercise value at 48 h compared to 24 h following LWLC, indicating greater neuromuscular impairment following DWLC. These data coupled with the observation that trunk extensor torque decreased after LWLC only, suggests that trunk flexors support more of the load when carrying a backpack on a negative gradient, resulting in greater neuromuscular impairment.

7.4.5. Voluntary Contractions of Shoulder Extensors and Flexors

The addition of carrying load when walking caused a decrease in peak torque of the shoulder extensors, which returned to pre-exercise value 48 h post. However, this was not apparent after DWLC. Peak torque of the shoulder flexors decreased after LWLC and DWLC only and recovered to pre-exercise value at 24 h in both conditions. The addition of carrying a load has been shown to increase activity of the supporting muscles (e.g. *m. trapezius*) (Holewijn, 1990). This additional work would account for the decreases in force production of

the shoulder extensors and flexors when load is added. The time course of recovery of shoulder extensor and flexor peak torque was faster than the knee and trunk extensors and flexors following LWLC. This may be due to different contributions from the muscle groups during load carriage. The shoulders perform a less dynamic movement during load carriage compared to the knee extensors and flexors. Therefore, the origin of damage to the knee and trunk extensors and flexors may have been due to greater mechanical stress compared to a metabolic origin of shoulders (e.g. accumulation of metabolites) (Tee *et al.*, 2007). Rucksack palsy has also been widely reported during load carriage, it has been hypothesised that the pressure from the backpack exerts pressure on the C5 and C6 nerve roots of the upper brachial plexus, causing numbness, paralysis and cramping (Knapik *et al.*, 2004). This may account for the some of the immediate loss in neuromuscular function of the shoulder extensors and flexors following load carriage. Pressure from the backpack straps may have also restricted blood flow to parts of the shoulder extensors and flexors resulting in local anaerobic processes and accumulation of metabolites also contributing to the neuromuscular impairment (Legg *et al.*, 1997).

7.4.6. Consequences of Neuromuscular Impairment

In addition to the decrement in strength, neuromuscular impairment has been shown to have a negative impact on endurance activities, causing increases in $\dot{V}O_2$ and heart rate at a set running pace (Chen *et al.*, 2007), decreases in insulin sensitivity and increased resting metabolic rate (Tee *et al.*, 2007). Reductions in neuromuscular function have also been shown to decrease motor control during skilled tasks (Byrne *et al.*, 2004) and sprint performance (Twist and Eston, 2005). A reduction in the force producing capability may also expose muscle groups to a greater risk of muscle strain due to their reduced ability to absorb force (Mair *et al.*, 1996). The decreases in neuromuscular function shown in the present study suggests that following load carriage the ability of individuals to undertake endurance, strength and skilled tasks and greater risk of muscle strain may be impaired up to 72 h after the load carriage bout. Moreover, load carriage in the field involves movement over a range of terrain and gradients (Pandolf *et al.*, 1977). Place *et al.* (2004) suggested that running on a treadmill may provide greater shock absorption compared to the ground, therefore decrease the muscular stress. The same is likely to also be true of load carriage and the neuromuscular impairment observed in the present study such that the consequences to functional performance may be exacerbated on open ground.

7.4.7. Conclusion

In conclusion, the addition of carrying load on a level gradient caused decreases in the force producing capability of the knee, trunk and shoulder extensors and flexors, suggesting neuromuscular impairment of these muscle groups. Interestingly, load carriage on a downhill gradient caused similar decrements in the force producing capacity of the knee extensors and the knee, trunk and shoulder flexors to carrying load on a level gradient. The most prolonged decrements in strength were observed in the knee extensors, which did not return to pre-exercise value at 72 h following load carriage on a level gradient. Electrically evoked isometric contractions revealed the neuromuscular impairment of the knee extensors was due to a combination of central and peripheral mechanisms, including disruption of the excitation contraction coupling process. Neuromuscular impairment is likely to have negative impact on strength, endurance and motor control tasks and may be exacerbated during load carriage in the field.

Chapter 8. Physiological Determinants of Metabolic and Neuromuscular Performance during Load Carriage

8.1. Introduction

Chapter 3 showed participant body mass to be inversely related to heart rate reserve during, and change in vertical jump power following, 19.2 km of load carriage in the field. The findings indicated lighter individuals experienced a greater cardiovascular strain and neuromuscular impairment during load carriage. However, body mass may not have been the only determinant of the physiological response to load carriage, as greater lean body mass is associated with higher muscular strength and absolute maximal oxygen uptake ($\dot{V}O_2max$) (Mattila *et al.*, 2007).

Understanding the physiological determinants of performance can provide information of use for training and selection of individuals to undertake physical activity. This is particularly important in occupational settings, where selection and training of individuals for load carriage tasks is undertaken. Physiological determinants of prolonged exercise performance, for example, are assessed by either a best effort time to completion (Coyle, 1995) or a physiological response (e.g. $\dot{V}O_2$) during set pace exercise (Lyons *et al.*, 2005). The physiological determinants of performance of a range of athletic events have been previously investigated, including running (Farrell *et al.*, 1979), cycling (Coyle *et al.*, 1988), race walking (Hagberg and Coyle, 1983) and cross country ski racing (Mahood *et al.*, 2001). The strongest predictors of performance during running, cycling and race walking events are maximal oxygen uptake ($\dot{V}O_2max$), oxygen uptake and velocity at lactate threshold, sub maximal economy and muscle fibre type (Coyle, 1995). The aim of many athletic events is to complete in the fastest time. However, occupational activities such as load carriage usually require maintenance of a steady pace and the ability to continue to perform exercise on completion (i.e. maintain physical reliability) (Knapik *et al.*, 1996).

Load carriage results in unique metabolic and neuromuscular responses compared to other unloaded endurance events. Bilzon *et al.* (2001), showed that the additional metabolic demand (relative $\dot{V}O_2$) when carrying an absolute load is inversely related to body mass and greater decreases in running performance (18 kg backpack run to exhaustion) were observed

for lighter individuals. However, the authors showed no relationship between body mass and the metabolic demand or time to exhaustion during unloaded running. In addition, compared to running and walking, load carriage results in greater recruitment of muscle fibres of upper and lower body to support the load and maintain stability during movement (Bobet and Norman, 1984; Ghori and Luckwill, 1985; Holewijn, 1990).

Previous investigations have established the physiological determinants of load carriage to predict performance outcomes (i.e. best effort time to completion) (Simpson *et al.*, 2006; Williams and Rayson, 2006) and maximal load carrying capacity (Rayson *et al.*, 1993). These physiological determinants included body mass, $\dot{V}O_2$max and muscular strength. Models to predict the metabolic demand of load carriage in the field have only been developed using descriptive variables including gradient, speed, terrain, body mass and load mass (Epstein *et al.*, 1987; Pandolf *et al.*, 1977; Soule *et al.*, 1978). Lyons *et al.* (2005) investigated the relationships of anthropometric measurements and aerobic fitness with the metabolic demand of carrying loads of 20 and 40 kg in a backpack, walking for 20 minutes at 4 $km \cdot h^{-1}$ on a range of gradients (0, 3, 6, and 9 %). Correlations showed $\dot{V}O_2$ during load carriage most strongly related to absolute $\dot{V}O_2$max ($r=-0.64$, $P<0.01$) and a lean body mass/fat mass ratio + backpack mass ($r=-0.52$, $P<0.01$). However, the contribution of muscular strength as a physiological determinant of the metabolic demands of load carriage has not been investigated.

During sub-maximal endurance exercise, a slow and steady increase energy cost of the task can occur, therefore increasing oxygen uptake ($\dot{V}O_2$drift) (Gaesser and Poole, 1996). $\dot{V}O_2$drift has been observed during load carriage of durations $\geq$ 2 hours (Epstein *et al.*, 1988; Patton *et al.*, 1991; Reading *et al.*, 1996). However, the physiological determinants of $\dot{V}O_2$drift during load carriage have not been investigated.

Neuromuscular impairment, characterised by a reduction in the force producing capacity of a muscle or muscle group has been shown following prolonged exercise (Millet and Lepers, 2004). Decreases in strength have also been shown following 12.1 km road marches at 4 $km \cdot h^{-1}$ carrying loads 13, 18 and 27 kg (Clarke *et al.*, 1955) and in Chapter 7. Neuromuscular impairment has a negative impact on an individual's ability to undertake strength, endurance and motor control tasks (Byrne *et al.*, 2004; Tee *et al.*, 2007). However,

the physiological determinants of the neuromuscular impairment following load carriage have not been investigated.

The aim of this study was to examine the relationship between individual physiological characteristics with metabolic and neuromuscular performance during 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) carrying a 25 kg backpack. Three measurements of physical performance will be examined (1) $\dot{V}O_2$ during load carriage, (2) $\dot{V}O_2$drift during load carriage, and (3) change in neuromuscular function following load carriage (percentage change in maximal voluntary force produced by the *m. quadriceps femoris*).

8.2. Methods

8.2.1. Participants

Twenty one healthy male participants (age 25 ± 7 years, height 1.80 ± 0.06 m, body mass 77.8 ± 7.3 kg, percentage body fat 8.1 ± 4.2 %, $\dot{V}O_2max$ 57.2 ± 4.8 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in the study. Participants had a range of previous recreational experience of carrying load in backpacks. Ethical approval for all procedures and protocols was provided by the University of Chichester Ethics Committee. All protocols were performed in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. Participants provided written informed consent and were free from any musculoskeletal injury prior to commencing the study. Participants were instructed to refrain from any vigorous physical activity in the day prior to treadmill walking and avoid consumption of caffeine, sports drinks or food two hours prior.

8.2.2. Preliminary Measures

Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) (± 0.01 kg) and stature (Avery Berkel, Smethwick, UK) (± 0.005 m) were measured whilst wearing shorts and underwear. Skinfold measurements were taken at the *Chest, Axilla, Biceps, Triceps, Sub Scapular, Iliac Crest, Supraspinale and Abdomen* on the right side of the body using Harpenden Skinfold Callipers (Body Care, Southam, UK). Two measurements were taken at each site and if there was a difference > 1 mm the measurements were repeated. Percentage body fat was estimated following the assessment of skinfold thickness at seven anatomical sites (*Chest, Axilla, Triceps, Sub Scapular, Abdomen, Iliac Crest* and *Thigh*) using previously described methods (Jackson and Pollock, 1978; Siri, 1956).

Participants completed an incremental exercise test to exhaustion on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK) to assess maximal oxygen uptake ($\dot{V}O_2max$). Detailed methodology was described in Chapter 5.

8.2.3. Familiarisation

At least 5 days prior to beginning the experimental protocol, participants were familiarised with all test procedures. Participants completed three MVCs and one cycle of the isokinetic experimental protocol (Figure 8.1). If the experimenter or participant thought that a sub-maximal effort was not given or participants were not adequately familiarised for a

particular test it was repeated. A test procedure was repeated if the experimenter or participant thought that a maximal effort was not given or a learning effect was still apparent in the final contractions.

8.2.4. Experimental Protocol

Participants walked on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK) for 120 minutes at 6.5 $km \cdot h^{-1}$ and 0 % gradient carrying a 25 kg backpack. The speed and absolute load was chosen to reflect realistic occupational requirements (e.g. military load carriage). Whilst participants walked on the treadmill approximately two minute collections of expired gases were taken at 5, 15, 30, 45, 60, 75, 90, 105 and 120 minutes of exercise. Before and immediately post load carriage, participants completed the isokinetic contractions (Figure 8.1).

The methods in the present study have previously been described in detail for metabolic measures (Chapter 6) isometric contractions of *m. quadriceps Femoris* (Chapter 5) and isokinetic contractions of the knee, trunk and shoulder extensors and flexors (Chapter 4). Briefly, volume of oxygen uptake ($\dot{V}O_2$) was calculated using the Haldane transformation (Wilmore and Costill, 1973) from approximately two minute collections (inspiration to inspiration) of expired gases using the Douglas bag method. Isometric force of the *m. quadriceps femoris* was recorded during an MVC in an isometric chair. Peak torque was measured at two test velocities during isokinetic contractions of the knee (60 and $180° \cdot s^{-1}$), shoulder (60 and $180° \cdot s^{-1}$) and trunk (15 and $60° \cdot s^{-1}$) extensors and flexors.

8.2.5. Environmental conditions

Environmental temperature was monitored using a dry bulb thermometer (Fisher Scientific, Loughborough, UK). Environmental temperature during load carriage was (20.8 ± 1.0 °C). No differences in environmental temperature were observed between pre and post tests (21.1 ± 1.7 °C vs. 21.2 ± 1.6 °C, P=0.225).

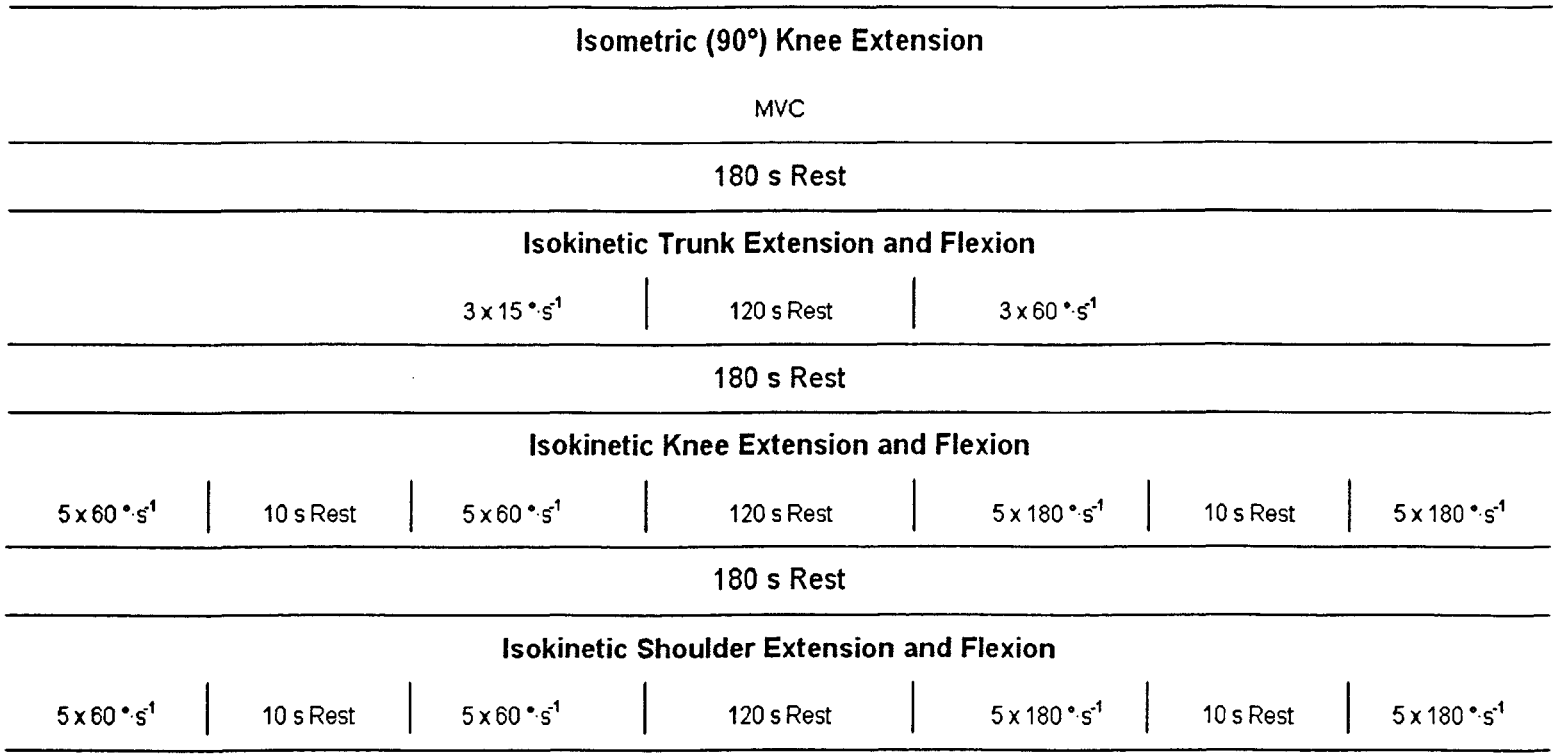


Figure 8.1 – Schematic of test battery of isometric and isokinetic contractions conducted before and immediately after 120 minutes of treadmill walking (6.5 km·h^{-1}) with a 25 kg backpack on a level gradient.

8.2.6. Statistical Analysis

Statistical analysis was undertaken using SPSS for Windows V15 (SPSS, Chicago, Illinois). Normal distribution of the data was verified using a Kolmogorov-Smirnov test. Relationships between dependent variables ($\dot{V}O_2$, $\dot{V}O_2$drift, and change in knee extensor force) were investigated using Pearson's bivariate correlations (2 tailed). Correlations were classified as very strong (>0.90), strong (0.70 – 0.89), moderate (0.50 – 0.69) or weak (<0.49) (Fallowfield *et al.*, 2005). Linearity and absence of outliers in independent variables were confirmed (Fallowfield *et al.*, 2005). Independent variables were checked, using Pearson's bivariate correlations, for the presence of multicolinearity ($r \geq \pm 0.9$) and singularity ($r = \pm 1.0$) (Fallowfield *et al.*, 2005), if present the variable with the strongest bivariate correlation (Pearsons r) with the dependent variable was used in the regression equation. Assumptions for model 1 regression were met (Winter *et al.*, 2001). Hierarchical (blockwise entry) of independent variables were used to develop regression equations to examine the relationships between $\dot{V}O_2$, $\dot{V}O_2$drift, and change in knee extensor force and the independent variables. A ratio of a maximum of five participants to one independent variable was selected (i.e. maximum four independent variables) for the multivariable models (Fallowfield *et al.*, 2005; Winter *et al.*, 2001). Decisions of entry of independent variables into the model were based on published literature (i.e. Lyons *et al.*, 2005; Lyons *et al.*, 2003; Rayson *et al.*, 1993; Simpson *et al.*, 2006; Williams and Rayson, 2006) and prior observations (i.e. single variable correlations). Paired t-tests were used to examine differences over time. Statistical significance was set at $P<0.05$.

8.3. Results

$\dot{V}O_2$ at baseline (minute 5) was 24.3 ± 2.6 mL·kg^{-1}·min^{-1} and increased to 27.5 ± 2.8 mL·kg^{-1}·min^{-1} at the end of load carriage (minute 120) (P<0.001). Relationships between $\dot{V}O_2$ during load carriage and dependent variables were based on the $\dot{V}O_2$ measurements made at 45 minutes. Analysis showed the mean $\dot{V}O_2$ at 45 minutes had strongest correlations with all other time points (r=0.83 to 0.94, P<0.001), therefore was judged to be most representative of the $\dot{V}O_2$ during load carriage and used to produce the multivariable model. There were no relationships between the dependent variables ($\dot{V}O_2$ during load carriage, $\dot{V}O_2$drift and, %Δ MVC Force).

The independent variables that showed the strongest relationships with $\dot{V}O_2$ during load carriage were age (r=-0.82, P<0.001) and body mass (r=-0.82, P<0.001). The other anthropometric measurements showed slightly weaker correlations, there were strong relationships with fat free mass (r=-0.76, P<0.001), height (r=-0.66, P=0.001) and waist circumference (r=-0.61, P=0.003) and a moderate relationship with the supraspinale skinfold (r=-0.45, P=0.43). However, there were also strong relationships between body mass and age (r=0.57, P=0.003), height (r=0.72, P<0.001), fat free mass (r=0.93, P<001), waist circumference (r=0.59, P=005) and the supraspinale skinfold (r=0.57, P=006). Of the strength measurements, only knee extensor torque 180 °·s^{-1} and knee flexor torque 60 °·s^{-1} showed independent relationships with $\dot{V}O_2$ during load carriage (r=-0.74 and -0.48, P<0.05, respectively).

The final multivariable model for $\dot{V}O_2$ during load carriage contained 3 variables and accounted for 85 % of the variance in $\dot{V}O_2$ during load carriage (Model 1 and Figure 8.2). Addition of further independent variables caused an unacceptable increase in multicolinearity (Tolerance and VIF values). Body mass accounted for 82 % of the total variance (P<0.001). Relative $\dot{V}O_2$max and knee flexion 60 °·s^{-1} only explained an additional 1 % and 2 % of the variance respectively (P>0.05), but strengthened the model. The beta weights in the standardised formula show that body mass was the strongest independent predictor of $\dot{V}O_2$ during load carriage (P<0.001).

Model 1 - $\dot{V}O_2$ during load carriage

$\dot{V}O_2 = 40.64 - 0.23$ (Body Mass) + 0.10 (Relative $\dot{V}O_2max$) -0.02 (Knee Flexor Torque 60 °·s^{-1})

R= 0.85 R^2= 0.73 Adj R^2= 0.68 SEE= 1.41 mL·kg^{-1}·min^{-1} (P<0.001)

Standardised Model

$\dot{V}O_2 = 40.64 - 0.68$ (Body Mass) + 0.18 (Relative $\dot{V}O_2max$) -0.02 (Knee Flexor Torque 60 °·s^{-1})

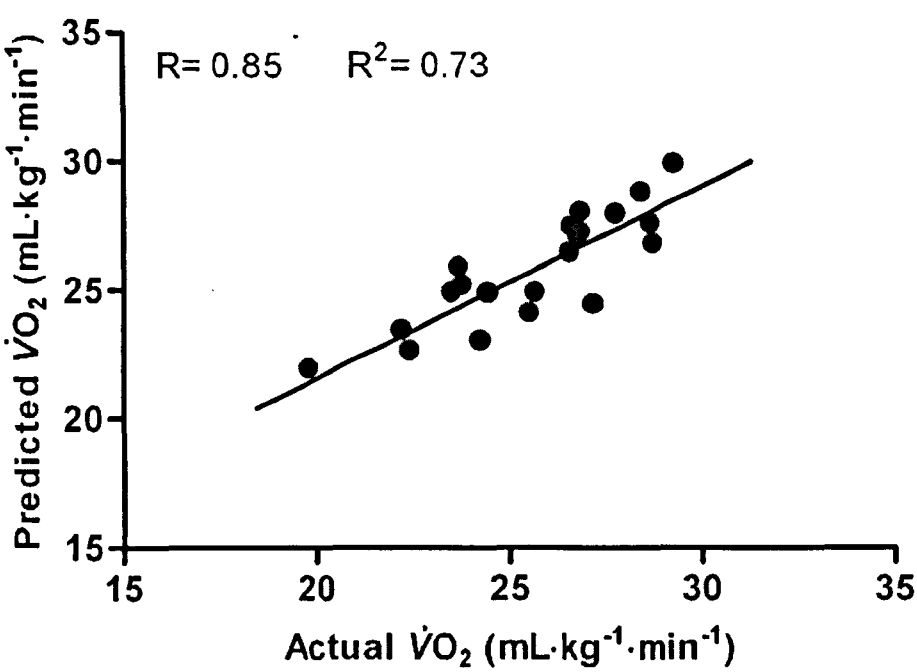


Figure 8.2 – Actual $\dot{V}O_2$ measured at minute 45 of 120 minutes of treadmill walking (6.5km·h^{-1}) carrying a 25 kg backpack compared to predicted $\dot{V}O_2$ during load carriage using the model [$\dot{V}O_2 = 40.64 - 0.23$ (Body Mass) + 0.10 (Relative $\dot{V}O_2max$) -0.02 (Knee Flexor Torque 60 °·s^{-1})] (n=21).

There was a moderate independent relationship between $\dot{V}O_2$drift during load carriage and absolute $\dot{V}O_2$max (r=-0.49, *P*=0.026), however, there was no relationship with relative $\dot{V}O_2$max (r=-0.25, *P*=0.245). There was a moderate relationship between $\dot{V}O_2$drift during load carriage and isometric knee extension force (r=-0.47, *P*=0.033) and trunk flexor torque 60 $°\cdot s^{-1}$ (r=-0.48, *P*=0.037). But the other strength measurements showed no relationship with $\dot{V}O_2$drift.

The multivariable model explained 63 % of the variance in $\dot{V}O_2$drift during load carriage (Model 2 and Figure 8.3). Absolute $\dot{V}O_2$max explained 43 % of the variance, isometric MVC force explained a further 19 % of the variance (*P*=0.036) and trunk flexor torque 60 $°\cdot s^{-1}$ only explained a further 1 % of the variance (*P*=0.053), but strengthened the model. The beta weights showed no single independent variable was a better predictor of $\dot{V}O_2$drift during load carriage.

Model 2 - $\dot{V}O_2$drift during load carriage

$\dot{V}O_2$Drift = 6.09 – 1.18 (Absolute $\dot{V}O_2$max) + 0.01 (Isometric MVC Force) - 0.01 (Trunk Flexor Torque 60 $°\cdot s^{-1}$)

R= 0.63 R^2= 0.40 Adj R^2= 0.28 SEE= 1.77 $mL\cdot kg^{-1}\cdot min^{-1}$ (*P*=0.048)

Standardised Model

$\dot{V}O_2$Drift 6.09 – 0.31 (Absolute $\dot{V}O_2$max) + 0.40 (Isometric MVC Force) - 0.16 (Trunk Flexor Torque 60 $°\cdot s^{-1}$)

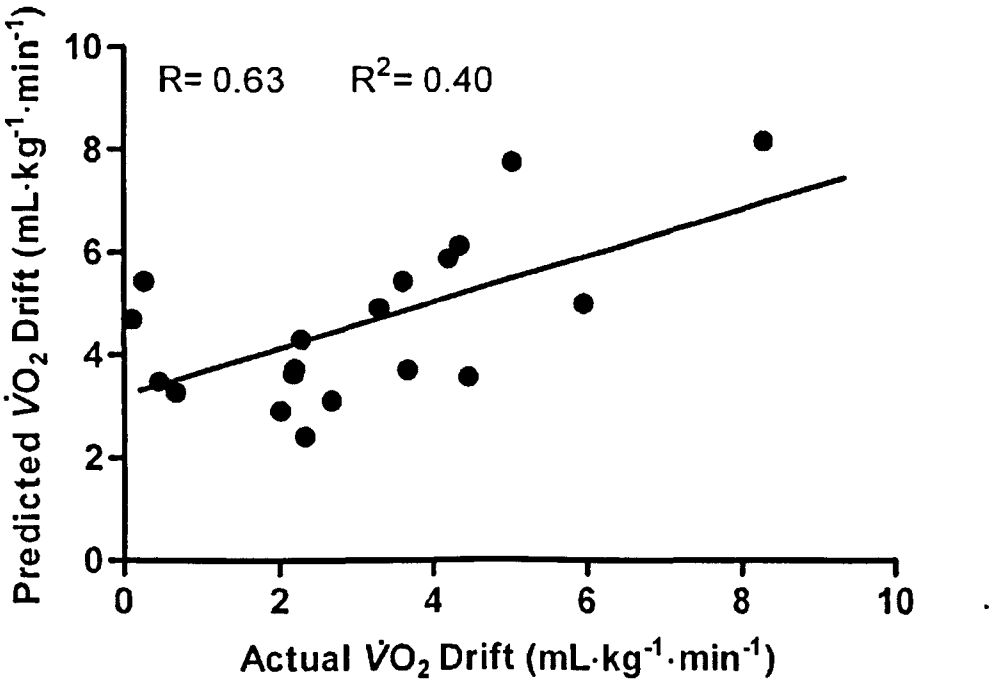


Figure 8.3 – Actual $\dot{V}O_2$drift during 120 minutes of treadmill walking (6.5km·h^{-1}) carrying a 25 kg backpack compared to predicted $\dot{V}O_2$drift during load carriage using the model [$\dot{V}O_2$Drift = 5.47 – 0.97 (Absolute $\dot{V}O_2$max) + 0.01 (Isometric MVC Force) - 0.01 (Trunk Flexor Torque 60 °·s^{-1}) – 0.02 (Shoulder Extensor Torque 60 °·s^{-1})] (n=19).

Isometric MVC force decreased by 119 ± 78 N (17 ± 9 %) following load carriage (P<0.005). The %Δ MVC force showed moderate relationships with knee flexor torque 60 °·s^{-1} (r=0.46, P=0.038) and shoulder flexor torque 60 °·s^{-1} (r=0.53, P=0.046), there were no other independent relationships with the other strength measurements.

The multivariable model explained 72 % of the variance in %Δ MVC force (Model 3 and Figure 8.4). Shoulder flexion torque 60 °·s^{-1} explained 48 % of the variance (P=0.036) and knee flexion torque 60 °·s^{-1}, relative $\dot{V}O_2$max and trunk flexion torque 60 °·s^{-1} each explained a further 8 % of the variance. The beta weights showed that no single variable was than another as a better predictor of %Δ MVC force.

Model 3 - %Δ MVC force following load carriage

%Δ MVC Force = -23.60 + 0.13 (Shoulder Flexor Torque 60 °·s^{-1}) + 0.11 (Knee Flexor Torque 60 °·s^{-1}) – 0.78 (Relative $\dot{V}$ O$_2$max) + 0.1 (Trunk Flexor Torque 60 °·s^{-1})

R= 0.72 R^2= 0.51 Adj R^2= 0.37 SEE= 7.67 % (*P*=0.030)

Standardised Model

%Δ MVC Force = -23.60 + 0.19 (Shoulder Flexor Torque 60 °·s^{-1}) + 0.30 (Knee Flexor Torque 60 °·s^{-1}) – 0.41 (Relative $\dot{V}$ O$_2$max) + 0.36 (Trunk Flexor Toque 60 °·s^{-1})

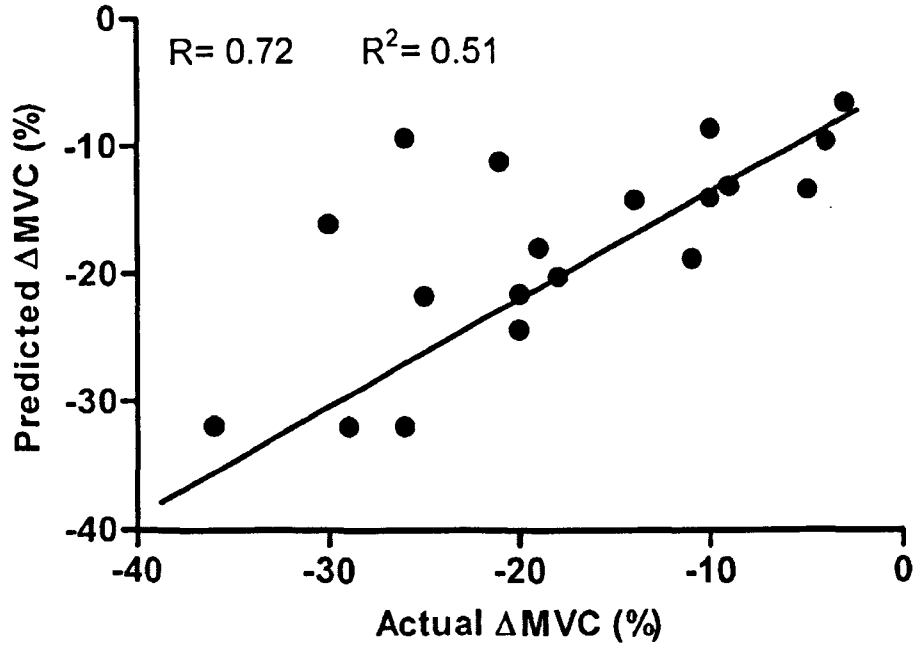


Figure 8.4 – Actual %ΔMVC following 120 minutes of treadmill walking (6.5km·h^{-1}) carrying a 25 kg backpack compared to predicted %ΔMVC following load carriage using the model [%Δ MVC Force = -23.60 + 0.13 (Shoulder Flexor Torque 60 °·s^{-1}) + 0.11 (Knee Flexor Torque 60 °·s^{-1}) – 0.78 (Relative $\dot{V}$ O$_2$max) + 0.1 (Trunk Flexor Torque 60 °·s^{-1})] (n=19).

Table 8.1 – Values of independent variables (mean ± SD) and individual correlations (2 tailed Persons r) between independent variables following load carriage (120 minutes of treadmill walking at 6.5km·h^{-1} carrying 25 kg). Only skins fold measurements with significant correlations (P<0.05) with one of the dependent variables are presented. Isokinetic trunk measurements are n=19 all other variables are n=21. Statistically significant correlation between dependent and independent variables (* P<0.05).

Independent Variable	Values	$\dot{V}$ O_2 during (ml·kg^{-1}·min^{-1})	$\dot{V}$ O_2drift (ml·kg^{-1}·min^{-1})	ΔMVC force (% change)
Height (m)	1.80 ± 0.05	-0.66 **	-0.23	0.19
Body Mass (kg)	77.8 ± 5.5	-0.82 **	-0.34	0.23
Fat Free Mass (kg)	66.3 ± 5.7	-0.76 **	-0.29	0.31
Relative $\dot{V}$ O_2max (ml·kg^{-1}·min^{-1})	57.2 ± 4.8	0.41	-0.25	- 0.31
Absolute $\dot{V}$ O_2max (L·min^{-1})	4.50 ± 0.56	-0.31	-.0.49 *	- 0.07
Supraspinale (mm)	100 ± 30	-0.45 *	-0.35	-0.18
Waist Circumference (mm)	783 ± 127	-0.61 **	-0.04	-0.27
Isometric Knee Extensor Force (N)	694 ± 120	-0.03	0.47 *	-0.03
Knee Extensor Torque 60 °·s^{-1} (Nm)	219 ± 41	-0.32	-0.37	0.05
Knee Extensor Torque 180 °·s^{-1} (Nm)	143 ± 30	-0.47 *	0.25	0.42
Knee Flexor Torque 60 °·s^{-1} (Nm)	130 ± 26	-0.48 *	-0.01	0.46 *
Knee Flexor Torque 180 °·s^{-1} (Nm)	93 ± 21	-0.36	-0.32	- 0.04
Shoulder Extensor Torque 60 °·s^{-1} (Nm)	106 ± 19	-0.18	0.28	0.43
Shoulder Extensor Torque 180 °·s^{-1} (Nm)	86 ± 13	-0.19	0.10	0.28
Shoulder Flexor Torque 60 °·s^{-1} (Nm)	72 ± 15	-0.23	-0.11	0.53 *
Shoulder Flexor Torque 180 °·s^{-1} (Nm)	51 ± 7	-0.30	-0.02	0.26
Trunk Extensor Torque 15 °·s^{-1} (Nm)	269 ± 52	0.08	0.16	0.41
Trunk Extensor Torque 60 °·s^{-1} (Nm)	267 ± 60	-0.06	0.28	0.34
Trunk Flexor Torque 15 °·s^{-1} (Nm)	266 ± 37	-0.37	0.38	0.39
Trunk Flexor Torque 60 °·s^{-1} (Nm)	293 ± 37	-0.23	-0.48 *	0.42

8.4. Discussion

This study investigated the relationships of physiological characteristics with metabolic and neuromuscular performance over 120 minutes of treadmill walking (6.5 km·h^{-1}) carrying a 25 kg back pack. Body mass was the strongest independent predictor of $\dot{V}O_2$ during load carriage, relative $\dot{V}O_2$max and knee flexor torque also provided a small contribution to the multivariable model. A novel aspect of this study was to investigate the physiological characteristics which contribute to $\dot{V}O_2$drift during load carriage. The multivariable model showed the physiological characteristics contributing to $\dot{V}O_2$drift were absolute $\dot{V}O_2$max, isometric MVC force, isokinetic trunk flexor and shoulder extensor torque. The present study was the first to attempt to establish the physiological determinants of neuromuscular performance during endurance exercise. Analysis showed that shoulder flexor torque, knee flexor torque, trunk flexor torque and relative $\dot{V}O_2$max best explained the variation in the neuromuscular impairment following load carriage

The initial $\dot{V}O_2$ during load carriage was 42.8 ± 4.3 % $\dot{V}O_2$max. A similar work rate was observed in participants walking at 6.0 km·h^{-1} carrying a backpack of 31 % of body mass (23.6 ± 3.6 kg) which elicited an initial exercise intensity of 40.0 % $\dot{V}O_2$max (Quesada *et al.*, 2000). The multivariable model for $\dot{V}O_2$ during load carriage was developed using the measurement at 45 minutes as it showed strongest correlations with all other time points (r=0.83 to 0.94, P<0.001). As the present study has shown, $\dot{V}O_2$drifts upwards during load carriage and the 45 minute collection best represents the 'average' $\dot{V}O_2$. The final multivariable model showed body mass to be the strongest independent predictor of $\dot{V}O_2$ during load carriage. The inverse linear relationship (r=-0.82, P<0.001) indicates heavier participants experienced a lower $\dot{V}O_2$ during load carriage. The findings of the present support those of Bilzon *et al.* (2001), which showed no relationship between body mass and $\dot{V}O_2$ during unloaded treadmill running (9.5 km·h^{-1}) (r=-0.47, P>0.05), however, the addition of a 18 kg backpack resulted in a strong inverse linear relationship between body mass and $\dot{V}O_2$ (r=-0.87, P<0.01).

Whilst carrying 20 and 40 kg at 4 km·h^{-1} on 0, 3, 6 and 9 % gradients measures of body composition (FFM) have been shown to have a stronger relationship with $\dot{V}O_2$ during load carriage than body mass (Lyons *et al.*, 2005). However, the results of the present study

showed a slightly stronger correlation between $\dot{V}O_2$ during load carriage and body mass (r=-0.82, $P<0.001$) than FFM (r=-0.76, $P<0.001$). The participants in the present study and Lyons *et al.* (2005) have similar physiological characteristics, therefore the differences in findings may be due to variations in the load carriage protocol (i.e. load and treadmill velocity). In agreement with the results of the present study, Frykman & Harman (1995) showed a strong relationship between 3.2 km best effort load carriage performance time carrying a 34 kg backpack and body mass (r=-0.60, $P<0.05$), but no relationship with fat free mass (r=-0.31, $P>0.05$). Age, height, supraspinale skinfold thickness and waist circumference also correlated with $\dot{V}O_2$ during load carriage. However, the variables also showed co-linearity with body mass, suggesting that their relationship with $\dot{V}O_2$ during load carriage was not due to the anthropometric measures or participant age or height *per se.*

Relative $\dot{V}O_2$max is an important determinant of performance ability in athletic events such as running and cycling (Coyle, 1995). However, relative $\dot{V}O_2$max made a minimal contribution to explaining the total variance in the multivariable model and neither relative nor absolute $\dot{V}O_2$max showed any independent correlations with $\dot{V}O_2$ during load carriage, which may be due to the relationship between body mass and $\dot{V}O_2$max. As a result of the scaling methods, relative $\dot{V}O_2$max is generally higher for lighter individuals (Nevill *et al.*, 1992). The addition of carrying an absolute load causes a greater increase the metabolic demand for lighter individuals (Bilzon *et al.*, 2001). Therefore the addition of load is likely to negate an individual's higher aerobic capacity during load carriage, hence its poor relationship with $\dot{V}O_2$ during load carriage. Harman & Frykman (1995) demonstrated this effect during a best effort 3.2 km load carriage event. Unloaded there was a strong relationship between performance time and relative $\dot{V}O_2$max (r=-0.59, $P<0.05$) but not absolute $\dot{V}O_2$max (r=-0.41, $P>0.05$). In comparison, when carrying a 34 kg backpack there was a very strong inverse relationship between performance time and absolute $\dot{V}O_2$max (r=-0.84, $P<0.05$) but no relationship with relative $\dot{V}O_2$max (r=-0.01, $P>0.05$).

Knee extensor (180 $°\cdot s^{-1}$) and flexor (60 $°\cdot s^{-1}$) torque both showed a moderate inverse linear relationship with $\dot{V}O_2$ during load carriage, indicating stronger participants experienced a lower metabolic demand during load carriage. Carrying a backpack load has been shown to increase muscle activation of the knee extensors (Ghori and Luckwill, 1985) and flexors

(Harman *et al.*, 1992). It is likely that weaker individuals will need to recruit additional muscle fibres to support the load of the backpack during load carriage contributing to a higher exercise $\dot{V}O_2$. However, the shoulder and trunk extensors and flexors showed no correlation with $\dot{V}O_2$ during load carriage but have also been shown to experience greater activation with the addition of carrying a backpack (Al-Khabbaz *et al.*, 2008; Bobet and Norman, 1984; Holewijn, 1990). This may be due to the different contributions from the muscle groups during load carriage. The shoulder and trunk extensors and flexors are primarily static and act to support and stabilise the load where as the knee extensors and flexors perform a dynamic action to maintain the participant's position on the treadmill.

Between minute 5 and minute 120 of load carriage $\dot{V}O_2$ increased by 13 %, indicating the presence of $\dot{V}O_2$drift (Gaesser and Poole, 1996). Similarly, Patton *et al.* (1991) observed an 8 % increase in $\dot{V}O_2$ during 12 km of treadmill walking at 5.7 $km \cdot h^{-1}$ carrying a 31.5 kg backpack ($P<0.05$). Absolute $\dot{V}O_2$max showed a moderate negative linear relationship with $\dot{V}O_2$drift during load carriage ($r=-0.49$, $P=0.026$) and explained 43 % of the variance in $\dot{V}O_2$drift in the multivariable model. Indicating participants with a greater absolute $\dot{V}O_2$max experienced a smaller amount of $\dot{V}O_2$drift during load carriage. Similarly, performance time during a 3.2 km best effort load carriage task showed strong correlations with absolute $\dot{V}O_2$max carrying 34 kg ($r=-0.84$, $P<0.05$) and 61 kg ($r=-0.74$, $P<0.05$) but no relationship with relative $\dot{V}O_2$max (Harman and Frykman, 1995). The inclusion of absolute $\dot{V}O_2$max rather than relative $\dot{V}O_2$max or body mass only in the multivariable model suggests a combination of a greater body mass and aerobic training are advantageous in reducing $\dot{V}O_2$drift.

There was a positive linear relationship between isometric MVC force of the knee extensors and $\dot{V}O_2$drift, explaining 20 % of the variance in the multivariable model. The greater force producing capacity of the knee extensors may reflect a higher percentage of type II fibres (Hakkinen *et al.*, 1985), consequently, these participants will have a lower percentage of type I fibres. Due to the additional demand placed upon each of the type I fibres they are likely to fatigue more rapidly than those participants with a higher percentage of type I fibres. As fibres become fatigued and their force producing capacity decreases, additional motor units will be recruited to maintain position on the treadmill, increasing the demand for oxygen, potentially increasing $\dot{V}O_2$drift. In support of this explanation, Dick & Cavanagh (1987)

showed a 10 % increase in $\dot{V}O_2$ during a 40 minute downhill run at 44 % $\dot{V}O_2$max with a corresponding 23 % increase in IEMG, but no change in $\dot{V}O_2$ or IEMG during 40 minutes of level running at 66 % $\dot{V}O_2$max. The authors concluded that the $\dot{V}O_2$drift observed during downhill running was due to muscle fibre damage and the recruitment of additional muscle fibres.

There was a moderate negative correlation between trunk flexor torque (60 °·s^{-1}) and $\dot{V}O_2$drift (r=-0.48, P=0.037). This relationship is opposite to that of the isometric force of the knee extensors. As discussed above, this may be due to the roles of the different muscle groups during load carriage (i.e. contribution of dynamic movement of knee extensors to maintain position on the treadmill and the trunk flexors to stabilising and supporting the load). Individuals with weaker trunk flexors are likely to less efficient at stabilising the load, resulting in greater movement, potentially increasing fatigue of these supporting muscles. During 3 hours of unloaded walking changes in gait have been observed to occur as a result of muscle fatigue (Yoshino *et al.*, 2004) and changes in gait from an optimum increase $\dot{V}O_2$ during running (Cavanagh and Williams, 1982). Therefore, fatigue of the trunk muscles during load carriage may reduce stability of the load, altering gait, causing increased $\dot{V}O_2$drift.

Knee extensor isometric MVC force decreased by 17 ± 9 % from the pre value following the load carriage. Similar decreases (8–34 %) have been observed following prolonged running, cycling and ski-skating exercises (Millet and Lepers, 2004). Isokinetic torque (60 °·s^{-1}) of the shoulder, knee and trunk flexors all contributed to the multivariable model to explain %Δ MVC force following load carriage. Interestingly, there was no relationship between any of the isometric or isokinetic knee extensor measurements with %Δ MVC force; the reason for this is unclear. The correlations show a positive relationship between the strength of the shoulder, trunk and knee flexors and %Δ MVC force. Indicating weaker participants experience greater neuromuscular impairment of the knee extensors following load carriage. As discussed above, weaker individuals are likely to be less efficient stabilising a load and potentially experience greater fatigue in these muscle groups. When the muscles supporting the load become fatigued their capacity to absorb the force will be reduced (Mair *et al.*, 1996) Consequently more force will to be absorbed by the lower limbs (i.e. *m. quadriceps femoris*) as the foot strikes the treadmill causing greater neuromuscular impairment of the knee extensors.

Despite having no individual correlation with %Δ MVC force following load carriage, relative $\dot{V}O_2$max explained an additional 8 % of the variance in the multivariable model. The model showed a negative linear relationship between relative $\dot{V}O_2$max and %Δ MVC force, indicating individuals with a greater relative $\dot{V}O_2$max experienced greater neuromuscular impairment. As discussed previously, the scaling of relative $\dot{V}O_2$max to body mass means lighter individuals usually attain higher relative $\dot{V}O_2$max values (Nevill *et al.*, 1992). However, the inclusion of relative $\dot{V}O_2$max rather than body mass in the model suggests the relationship between $\dot{V}O_2$max and neuromuscular impairment is not entirely due to differences in body mass.

Most previous studies investigating the physiological determinants of load carriage have used time to complete a load carriage event as a performance measure. Simpson *et al.* (2006) found no relationships between anthropometric measurements, $\dot{V}O_2$max or peak torque of the knee and hip extensors and flexors with time to complete a 3.2 and 29 km best effort load carriage task carrying a 20 kg backpack. However, Williams & Rayson (2006) showed age, height and shuttle run time (measure of relative $\dot{V}O_2$max) to be significant predictors of a 3.2 km best effort load carriage test carrying 25 kg (Adj $R^2 = 0.40$, $P<0.0005$). When carrying a 15 kg backpack, significant predictor variables included gender, stature, percent fat and shuttle run time (Adj $R^2 = 0.81$, $P<0.0005$). Williams *et al.* (2002) showed that a modified military training program improved the time to complete a 3.2 km back packing time trial carrying 15 kg. The authors attributed the improved performance to a combination of improvements in strength and aerobic endurance. In addition, improvements in strength variables (back extension, upright pull and incremental lift) have also been correlated with changes in load carriage performance during training (Williams and Rayson, 2006). Load carriage in the present study is likely to be of a lower intensity than maximal effort tasks described above. Therefore the physiological mechanisms responsible for performance may also vary. However, previous findings do support the present data suggesting that a combination of greater body mass, muscular strength and aerobic fitness contribute to load carrying ability.

Interestingly, occupational groups engaged in load carriage activities (e.g. military and emergency services) currently use selection tests which measure aerobic performance relative

to body mass (e.g. multistage fitness test or timed run), which favour lighter individuals (Bilzon *et al.*, 2001; Vanderburgh, 2008). However, the present study has shown that metabolic and neuromuscular performance is poorer for lighter individuals and those with a high relative $\dot{V}O_2$max.

The models in the present study describe the physiological determinants of load carriage performance. Although the models show a strong association between actual and predicted values in the test population (R = 0.63 - 0.85) their ability to predict physiological outcome in the wider population is poorer (Adj R^2= 0.37 – 0.68). Also, the models in the present study were developed using one load carriage protocol, further research is required to assess whether changes in speed, gradient, duration and load mass change the physiological determinants. Therefore, caution must be taken if using these models to predict physiological outcomes (i.e. $\dot{V}O_2$, $\dot{V}O_2$drift, %Δ MVC force) of load carriage in the wider population.

In summary, metabolic and neuromuscular performance during load carriage are determined by a combination of anthropometric, aerobic fitness and strength characteristics. In agreement with previous research, the present study showed body mass had the greatest influence on the metabolic cost of load carriage, relative $\dot{V}O_2$max and knee flexor strength also provided a small contribution. However, the determinants of $\dot{V}O_2$drift and %Δ MVC force during load carriage were a combination of strength and aerobic fitness parameters and not body mass. The findings suggest individuals with the most efficient metabolic and neuromuscular performance during load carriage are those with a large body mass, high absolute $\dot{V}O_2$max and strong trunk, shoulder and knee flexors. From an applied perspective these findings indicate that selection and training of individuals for load carriage requires individuals to have, or development of, a combination of aerobic fitness, muscular strength and body mass.

Chapter 9. Differential Effects of Carbohydrate and Protein Supplementation on the Physiological Responses to Load Carriage

9.1. Introduction

Chapter 6 showed that load carriage increases $\dot{V}O_2$ and $\dot{V}O_2$drift over time compared to walking unloaded. The potential mechanisms suggested for the $\dot{V}O_2$drift during exercise included a change in substrate utilisation and neuromuscular impairment resulting in a poorer walking economy. In support of these assertions, a reduction in neuromuscular function was observed immediately post and in the days following load carriage (Chapter 7). However, neuromuscular responses during load carriage were not measured in Chapter 6, a shortfall which will be addressed in the present chapter by measuring muscle activation using EMG.

Physical performance is likely to be improved during and following load carriage if the metabolic demand (i.e. $\dot{V}O_2$ and $\dot{V}O_2$drift) and neuromuscular impairment during exercise can be reduced. The use of dietary supplements has been shown to improve performance during exercise and aid recovery (for reviews see Howatson and van Someren, 2008; Jeukendrup, 2004; Maughan *et al.*, 2004) and their use has been documented in occupational groups (Arsenault and Kennedy, 1999; Flakoll *et al.*, 2004) and sports performers (Maughan *et al.*, 2004). Carbohydrate and whey protein mixtures are two commonly used and commercially available supplements (Howatson and van Someren, 2008).

The effects of carbohydrate supplementation during endurance exercise are well documented and include improved endurance capacity (time to exhaustion), exercise performance (i.e. time to complete a set distance) and potentially the sparing of muscle glycogen during prolonged exercise (>2 h) (for reviews see Ivy, 1999; Jeukendrup, 2004; Peters, 2003). The infusion of a glucose solution (18 % concentration) at an equal volume to sweat losses has been shown to attenuate $\dot{V}O_2$ and cardiovascular drift during cycling at a constant work rate (70 %$\dot{V}O_2$max) (Hamilton *et al.*, 1991). However, Brisswalter *et al.* (2000) observed no attenuation in $\dot{V}O_2$drift during a 120 minute run at ventilatory threshold (81 %$\dot{V}O_2$max) with consumption of a carbohydrate solution (5.4 %) compared to a placebo.

In an attempt to reduce the negative effects of muscle injury following exercise, whey proteins are sometimes consumed prior to or during prolonged exercise (Howatson and van

Someren, 2008). This practice improves whole body net protein balance by increasing the rate of protein synthesis (Kumar *et al.*, 2009). Early consumption also ensures amino acids are immediately available following exercise to promote and provide building blocks for *de novo* protein synthesis and reduce protein degradation (Koopman *et al.*, 2007). This may improve repair of muscle tissue and therefore recovery of neuromuscular function (Nosaka, 2007), which would be of particular benefit as further exercise is often conducted after an initial load carriage bout (Knapik *et al.*, 1996; Lobb, 2004). The benefits of amino acid ingestion during and following resistance exercise are well documented (for review see Hayes and Cribb, 2008). However, although the effect of consumption of carbohydrate and protein mixtures have been studied (Saunders *et al.*, 2007; Valentine *et al.*, 2008), the effect of ingesting whey protein supplements alone on the physiological responses during prolonged exercise have received very little attention (Hawley *et al.*, 2006; Howatson and van Someren, 2008).

Byrne *et al.* (2005) studied the effect of a carbohydrate-electrolyte beverage compared to a placebo on the physiological responses to load carriage (14 kg backpack, three 60-minute cycles of walking at 4.4 $km \cdot h^{-1}$, + 5 % gradient, separated by 15 minutes of seated rest) in extreme ambient conditions (35°C, 55 % humidity). The carbohydrate beverage increased blood glucose concentration but had no effect on mean heart rate or core body temperature during exercise. No measurements of $\dot{V}O_2$, cardiovascular drift or substrate oxidation were made. The effect of carbohydrate supplementation during load carriage in milder environmental conditions has not been investigated.

The aim of this study was to examine the effect of commercially available carbohydrate and protein beverages on physiological responses to 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) carrying a 25 kg backpack. It was hypothesised that: (1) Carbohydrate supplementation would reduce $\dot{V}O_{2drift}$ compared to placebo and protein beverages. (2) Carbohydrate supplementation would have no effect on cardiovascular drift compared to placebo and protein beverages. (3) Protein supplementation would have no effect on $\dot{V}O_2$ and cardiovascular drift compared to a placebo beverage.

9.2. Methods

9.2.1. Participants

Ten healthy male participants (age 28 ± 9 years, height 1.82 ± 0.07 m, body mass 81.5 ± 10.5 kg, body fat 16.4 ± 3.2 %, $\dot{V}O_2max$ 55.0 ± 5.5 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in the study. Participants had a range of previous recreational experience of carrying load in backpacks. Ethical approval for all procedures and protocols was provided by the University of Chichester Ethics Committee. All protocols were performed in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. Participants provided written informed consent and were free from any musculoskeletal injury prior to commencing the study. Participants were instructed to refrain from any vigorous physical activity in the day prior to treadmill walking and to only consume a standardised light meal on the day of each test session but to avoid consumption of caffeine, sports drinks or food 3 hours prior to beginning the treadmill walk.

9.2.2. Preliminary Measures

Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) and stature (Avery Berkel, Smethwick, UK) (± 0.005 m) were measured whilst wearing shorts and underwear. Skinfold thickness was measured at the *Chest, Axilla, Triceps, Sub Scapular, Abdomen, Iliac Crest and Thigh* on the right side of the body using Harpenden Skinfold Callipers (Body Care, Southam, UK). Two measurements were taken at each site and if there was a difference > 1 mm, the measurements were repeated. Percentage body fat was estimated following the assessment of skinfold thickness at seven anatomical sites using previously described methods (Jackson and Pollock, 1978; Siri, 1956).

Participants completed an incremental exercise test to exhaustion on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK) to assess maximal oxygen uptake ($\dot{V}O_2max$). Detailed methodology has been described previously in Chapter 6.

9.2.3. Experimental Protocol

The study was a repeated measures three way cross over randomised design. Participants walked for 120 minutes at 6.5 $km \cdot h^{-1}$ and 0 % gradient carrying a 25 kg backpack on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK). During each of the 3 treadmill walking sessions, participants consumed 500 mL of a commercially

available beverage (250 mL at the start and 250 mL at 60 minutes of walking) mixed as directed by the manufacturers' guidelines:

(1) Placebo (PLA); 490 mL water and 10 mL sugar free orange cordial (Tesco, Dundee, UK), [nutritional content per 500 mL; Energy 1 Kcal, Carbohydrate 0.1 g, Protein 0 g, Fat 0 g).

(2) Carbohydrate (CHO) (6.4 % carbohydrate concentration); 490 mL water, 10 mL sugar free orange cordial (Tesco, Dundee, UK), 34 g Super Soluble Maxijul (SHS International Limited, Liverpool, UK) [nutritional content per 500 mL; Energy 130 Kcal, Carbohydrate (100 % glucose) 32 g, Protein 0 g, Fat 0 g].

(3) Protein (PRO) (7.0 % whey protein concentration); 500 mL water and 44 g orange flavoured Maximuscle Promax (Maximuscle Limited, Hemel Hempstead, UK) [nutritional content per 500 mL; Energy 176 Kcal, Carbohydrate 3 g, Protein 36 g, Fat 3 g].

In addition to the commercially available beverages participants consumed 200 mL of water at 30 minutes and 200 mL of water at 90 minutes of walking. The drinking regime was designed through pilot work to ensure participants consumed volumes of the beverages recommended by manufactures and that gastrointestinal comfort was maintained for the duration of exercise. The load was evenly distributed in the backpack and remained constant between conditions. The backpack had adjustable shoulder straps and a fixed height waist strap that could be tightened, but no sternum strap, participants adjusted the strapping to achieve a comfortable fit. Walking speed was kept constant between test conditions and an absolute load used to reflect realistic occupational requirements (e.g. military load carriage).

9.2.4. Measures during Treadmill Walking

Whilst participants walked on the treadmill, measurements of expired gases ($\dot{V}O_2$ and RER), stride frequency, plasma lactate and glucose and RPE were taken at 5, 15, 30, 45, 60, 75, 90, 105 and 120 minutes of exercise. Measurements recorded at minute 5 were taken as a baseline and changes over time were calculated from this time point. $\dot{V}O_2$ and cardiovascular drift (heart rate) were calculated as the difference between the values measured at 5 minutes (baseline) and 120 minutes. The methodologies of these parameters are described in detail in Chapter 6.

9.2.5. Electromyography (EMG)

Electromyography recordings were taken at minute 7 (baseline) and minute 107. The *m. rectus femoris* (RF), *m. vastus lateralis* (VL), *m. semitendinosus* (ST), *m. biceps femoris* (BF) were selected for analysis. Electrode positions were selected by determining muscle belly by palpating the muscle when relaxed and contracted (Gregoire *et al.*, 1984). To ensure accurate positioning in subsequent trials, electrode positions were marked and recorded on lines running either from the centre of the base of the *patella* to the *iguinal crease* (RF and VL only) or along the mid-line between the ST and BF at the insertion at the tibiofemoral joint to the base of the *m. gluteus maximus* (ST and BF only). The site for electrode attachment was shaved and dead skin removed with an abrasive patch then cleaned with an alcohol wipe. Electrodes (Bangoli DE-2.1 Parallel Bar Electrodes, Delsys INC, Boston, USA) were positioned using one layer of thin medical tape (Transpore 3M Surgical Tape, 3M, Minnesota, USA) and secured using a larger patch of water proof medical tape (Leukoplast Sleek, BSN Medical, Hull, UK). The electrode wires were attached to the upper leg using medical tape and passed through the waist band of the participants' shorts to reduce movement. The EMG activity was recorded (Bangoli 8-Channel EMG System, Delsys INC, Boston, USA) at 1000 Hz for 60 seconds at minutes 7 and 107 during treadmill walking.

EMG data were processed using Delsys EMGworks Analysis V3.6 (Delsys INC, Boston, USA) and Microsoft Excel 2002 for Windows (Microsoft, Redmond, Washington). Raw EMG signals were filtered using a 2^{nd} order Butterworth bandpass filter (low 10 Hz, High 350 Hz) (as recommended by the guidelines of the Journal of Electromyography and Kinesiology). The Root Mean Square (RMS) of the filtered data was calculated using a moving window (window length 0.05 s, window overlap 0.025 s) (Pincivero *et al.*, 2000; Tseng *et al.*, 2007). RMS data were divided into individual steps (using the stride frequency counts at 5 and 105 minutes) and a single peak RMS calculated for each step. Peak RMS values recorded at each time point (7 and 107 minutes) were ranked and the top and bottom 10 % of values were removed to exclude outlying values. The arithmetic mean of the remaining peak RMS values was calculated to provide an average peak RMS values for each muscle at the beginning (minute 7) or end (minute 107) of load carriage. To ease interpretation of changes in muscle activation, all data were expressed as a percentage of the baseline (minute 7) value (i.e. all pre-values at 100 %).

9.2.6. Food Diary

Participants were instructed to consume a similar light meal at least 3 hours prior to each session of treadmill walking. Participants recorded all food and beverages (with estimated mass or portion size) consumed on the day of testing, prior to the treadmill walking. Food diaries were analysed for energy content (kcal), carbohydrate, fat and protein mass (g) using Microdiet Plus for Windows V1.2 (Downlee Systems Ltd, Derbyshire, UK).

There were no differences between the PLA, CHO or PRO conditions for dietary intake of the light standardised meal prior to load carriage of total energy (266 ± 157 vs. 259 ± 145 vs. 277 ± 147 kcal, P=0.521), protein (11 ± 6 vs. 11 ± 6 vs. 10 ± 6 g, P=0.347), fat (3 ± 3 vs. 3 ± 3 vs. 3 ± 3 g, P=0.612) or carbohydrate (51 ± 37 vs. 49 ± 36 vs. 55 ± 34 g, P=0.411).) During load carriage the dietary supplements provided the following nutritional quantities; PLA (Energy 1 Kcal, Carbohydrate 0.1 g, Protein 0 g, Fat 0 g), CHO (Energy 130 Kcal, Carbohydrate 32 g, Protein 0 g, Fat 0 g) and PRO (Energy 176 Kcal, Carbohydrate 3 g, Protein 36 g, Fat 3 g, Sodium 0.2 g).

9.2.7. Additional Measures

Participants were weighed (in shorts and underwear only) immediately prior to, and following, the treadmill walks to determine changes in body mass, which were corrected for urine loss and fluid intake. Environmental temperature was monitored using a dry bulb thermometer (Fisher Scientific, Loughborough, UK) and controlled using the laboratory air conditioning (South East Cooling Ltd., Bognor Regis, UK). No differences in environmental temperature were observed between conditions [21.21 ± 0.45 °C (PLA), 21.10 ± 0.60 °C (CHO), 21.18 ± 0.20 °C (PRO), P=0.656].

9.2.8. Statistical Analysis

SPSS for windows Version 16 (SPSS, Chicago, Illinois) was used for statistical analyses. Distribution of the data was assessed using Kolmogorov-Smirnov test for normality. Data were normally distributed and differences between groups and over time were assessed using 2 way repeated measures ANOVA. Dietary intake of energy, carbohydrate, fat and protein before load carriage was assessed between conditions using a one way ANOVA. If sphericity was violated, the Greenhouse-Geisser correction was used. If the ANOVA revealed significant interaction effects, differences were examined using pre-planned paired t-tests; either for between conditions or over time (i.e. baseline vs. 120 minutes) or between

conditions using delta values (i.e. 120 minutes – baseline). The results are presented as mean ± standard deviation (SD). Statistical significance was set *a priori* at $P<0.05$.

9.3. Results

9.3.1. Oxygen Uptake

There was no difference in $\dot{V}O_2$ between conditions at the start (minute 5) of load carriage (P>0.05). $\dot{V}O_2$ increased between minutes 5 and 120 during PLA (22.7 ± 2.5 to 24.1 ± 2.3 $mL \cdot kg^{-1} \cdot min^{-1}$, P=0.002), CHO (22.9 ± 2.0 to 24.6 ± 1.6 $mL \cdot kg^{-1} \cdot min^{-1}$, P=0.003), and PRO (22.4 ± 2.5 to 26.2 ± 2.6 $mL \cdot kg^{-1} \cdot min^{-1}$, P<0.001) (Figure 9.1A). However, the increase over time during CHO (8 ± 5 %) was less than during PLA (14 ± 6 %, P=0.036) and PRO (17 ± 4 %, P=0.002), but there was no difference in the increases between PLA and PRO (P=0.084) (Figure 9.1). When divided into quartiles (i.e. 5-30, 30-60, 60-90, 90-120), CHO showed an initial rise in $\dot{V}O_2$ between minute 5 and 30 (5 ± 4 %, P=0.008), but did not increase further (i.e. plateaued) between 30 and 60 minutes (-1 ± 5 %, P=0.657), 60 and 90 minutes (3 ± 5 %, P=0.640) and 90 and 120 minutes (1 ± 3 %, P=0.609). There were no plateaus in the quartiles of PLA or PRO (P<0.05).

9.3.2. Heart Rate

Heart rate increased between minutes 5 and 120 by 16 ± 10 % during PLA (118 ± 24 to 136 ± 24, P<0.001), 12 ± 6 % during CHO (118 ± 20 to 131 ± 19, P<0.001), and 18 ± 6 % during PRO (114 ± 19 to 143 ± 20, P<0.001). There were no differences in the increases between conditions (P=0.251).

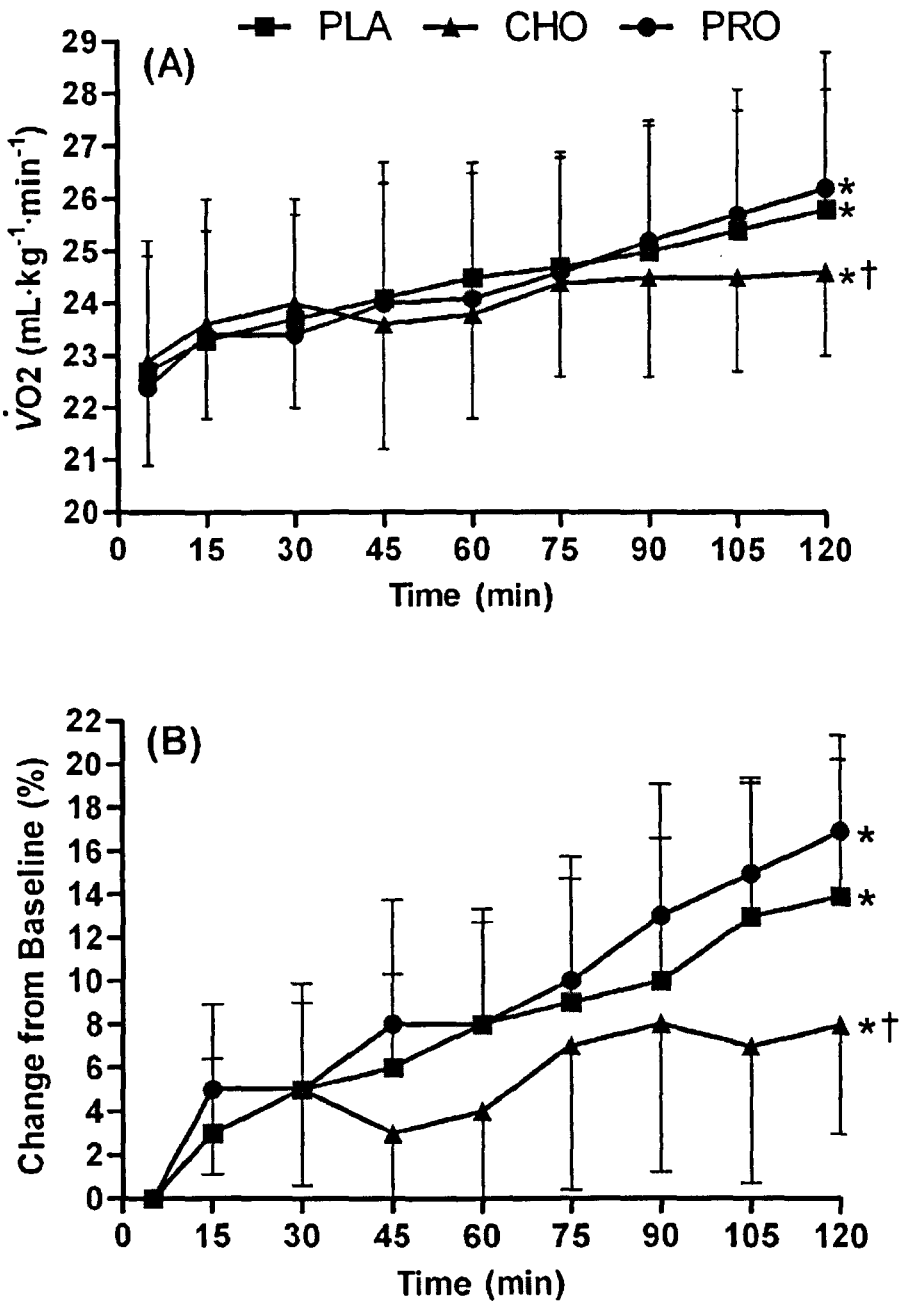


Figure 9.1 – (A) Oxygen uptake ($mL \cdot kg^{-1} \cdot min^{-1}$) (B) Percentage change in oxygen uptake from baseline value (minute 5). During 120 minutes of treadmill walking at 6.5 $km \cdot h^{-1}$ (n=10) on a level gradient (0 %) carrying a 25 kg backpack with consumption of 250mL (at 0 and 60 minutes) of either placebo beverage (PLA, ■), carbohydrate (6.4 %) beverage (CHO, ▲) or protein (7 %) beverage (PRO, ●). Symbols indicate an increase in $\dot{V}O_2$ between minutes 5 and 120 ($P<0.05$) (*) and a difference in $\dot{V}O_2$drift during CHO compared to PLA and PRO ($P<0.05$) (†).

9.3.3. Respiratory Exchange Ratio (RER)

There were no differences in RER between conditions at minute 5 (P>0.05). Figure 9.2 shows RER decreased between minutes 5 and 120 during PLA (0.96 ± 0.05 to 0.87 ± 0.04, P<0.001) and PRO (0.92 ± 0.04 to 0.86 ± 0.04, P<0.001) only, but not during CHO (0.94 ± 0.05 to 0.91 ± 0.05, P=0.056). As a result, RER at the end of load carriage (minute 120), was higher in CHO than PLA (P=0.011) and PRO (P=0.002). There was no difference in RER at minute 120 between PLA and PRO (P=0.746).

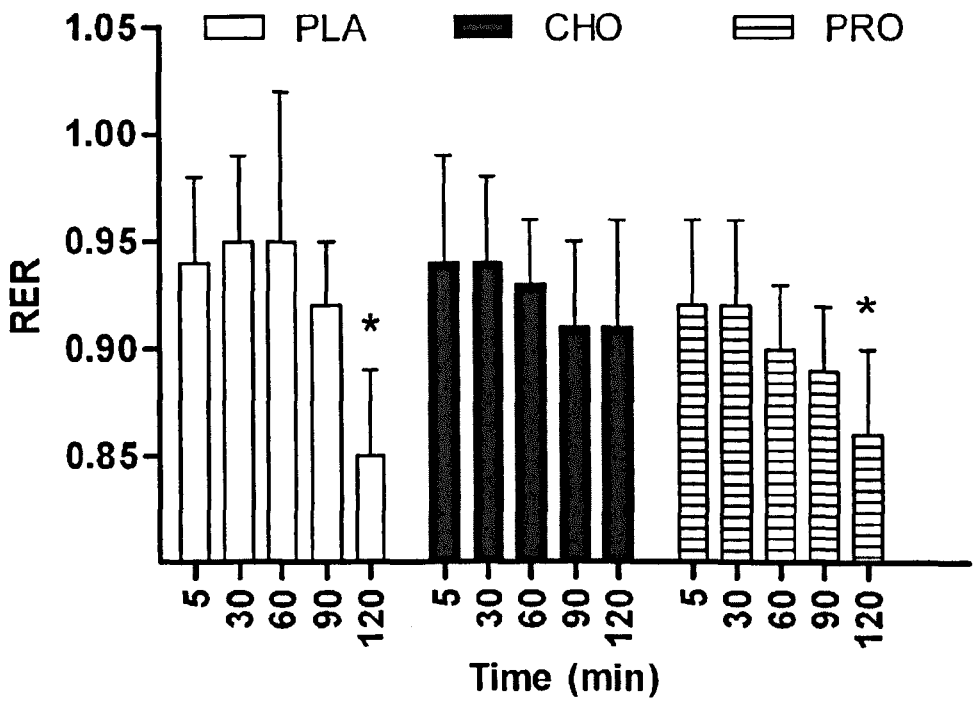


Figure 9.2 – Respiratory exchange ratio (RER) during 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) on a level gradient (0 %) carrying a 25 kg backpack with consumption of 250 mL of either placebo beverage (PLA, □), carbohydrate (6.4 %) beverage (CHO, ■) or protein (7 %) beverage (PRO, ▤) at 0 and 60 minutes of the load carriage task. Symbol (*) indicates a reduction in RER between minutes 5 and 120 (P<0.001)

9.3.4. Plasma Glucose and Lactate

The beverages containing the supplements (PLA, CHO, PRO) were consumed at 0 minutes (250 mL) and 60 minutes (250 mL) of treadmill walking. Plasma glucose at minute 5 was higher in CHO (4.49 ± 0.41 mmol·L^{-1}) than PLA (4.14 ± 0.48 mmol·L^{-1}, P=0.021) or PRO (4.09 ± 0.45 mmol·L^{-1}, P=0.029), likely to be due to the consumption of 250 mL of CHO supplement at minute 0. There was no change in plasma glucose between minutes 5 and 120 for PLA, CHO or PRO (P=0.317). However, Figure 9.3 shows that following consumption of the CHO beverage at 0 minutes plasma glucose increased from 4.49 ± 0.41 mmol·L^{-1} at 5 minutes to a peak of 4.84 ± 0.73 mmol·L^{-1} at 15 minutes (P=0.037) and then gradually declined to 4.23 ± 0.41 mmol·L^{-1} at 45 minutes (P=0.036). At 60 minutes, the second CHO beverage was consumed and plasma glucose increased from 4.11 ± 0.37 mmol·L^{-1} at 60 minutes to a peak of 4.96 ± 0.51 mmol·L^{-1} at 75 minutes (P=0.004), gradually returning to the 60 minute (pre-consumption) concentration at 90 minutes (4.73 ± 0.47 mmol·L^{-1}, P=0.657). However, the consumption of PLA and PRO beverages caused no change from minute 5 concentration at any time point (P>0.05).

Plasma lactate decreased between minutes 5 and 120 during PLA (1.34 ± 0.54 to 0.81 ± 0.25 mmol·L^{-1}, P=0.002), CHO (1.35 ± 0.64 to 1.17 ± 0.43 mmol·L^{-1}, P=0.021) and PRO (1.17 ± 0.43 to 0.71 ± 0.22 mmol·L^{-1}, P=0.006). However, there was no difference in the rate of decrease between conditions (P=0.721).

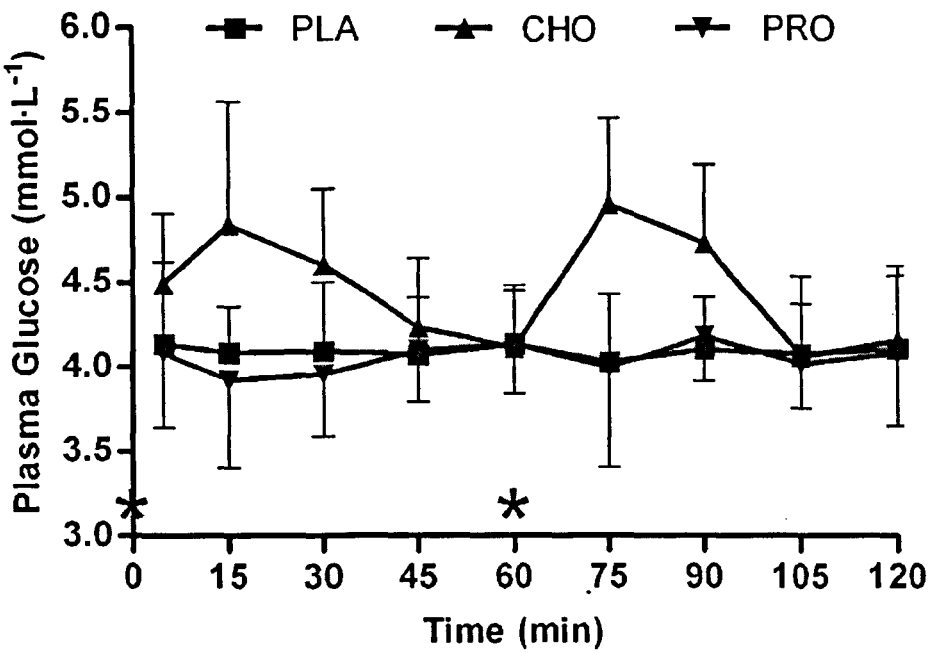


Figure 9.3 – Plasma glucose (mmol·L-1) during 120 minutes of treadmill walking at 6.5 km·h-1 (n=10) on a level gradient (0 %) carrying a 25 kg backpack with consumption of 250 mL (at 0 and 60 minutes) of either placebo beverage (PLA, ■), carbohydrate (6.4 %) beverage (CHO, ▲) or protein (7 %) beverage (PRO, ●). Symbol (*) indicates point at which beverage was consumed.

9.3.5. EMG

The EMG data are illustrated in Figure 9.4. Due to the movement during walking and the accumulation of sweat a clean raw signal could not be recorded for some muscles. If a clean raw signal could not be recorded for a muscle in one test condition (i.e. PLA, CHO or PRO) the muscle was excluded from the final analysis to maintain a repeated measures design. Therefore, results are presented for RF (n=8), VL (n=10), ST (n=7) and BF (n=6). There were no changes in mean peak RMS between minute 7 (set as 100 %) and minute 107 (expressed as percentage of baseline) during PLA, CHO or PRO respectively, for the RF (95 ± 35, 88 ± 26, 94 ± 22 %, P=0.176), VL (86 ± 39, 96 ± 39, 95 ± 19 %, P=0.161), ST (91 ± 26, 103 ± 36, 97 ± 44 %, P=0.527), or BF (84 ± 23, 86 ± 22, 105 ± 18 %, P=0.328).

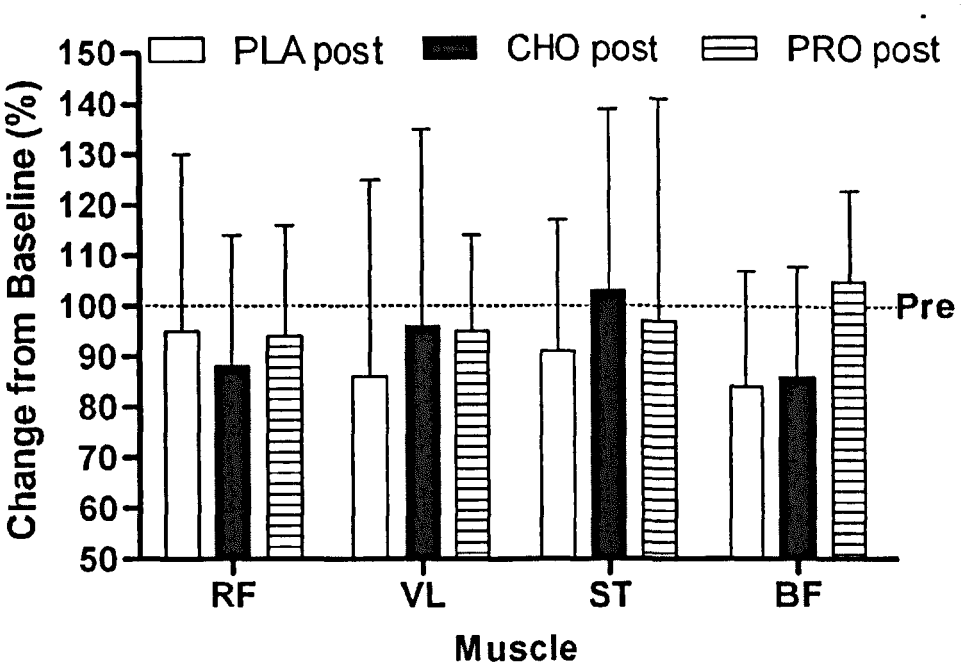


Figure 9.4 – Percentage change in the mean RMS value from baseline (minute 7) of the *m. rectus femoris* (RF) (n=8), *m. vastus lateralis* (VL) (n=10), *m. semitendinosus* (ST) (n=7) and, *m. biceps femoris* (BF) (n=6) after 107 minutes of treadmill walking at 6.5 km·h-1 on a level gradient (0 %) carrying a 25 kg backpack with consumption of 250 mL (at 0 and 60 minutes) of either placebo beverage (PLA, □), carbohydrate (6.4 %) beverage (CHO, ■) or protein (7 %) beverage (PRO, ⊟) Dashed line (·····) represents the pre RMS value set as 100%.

9.3.6. Stride Frequency

There was no difference in stride frequency at minutes 5 or 120 for any condition (P=0.293). There was no change in stride frequency over time for PLA (64 ± 4 to 63 ± 3), CHO (64 ± 3 to 64 ± 4), PRO (63 ± 3 to 64 ± 3) (P=0.378).

9.3.7. RPE

RPE increased between minutes 15 and 120 during PLA from (11 ± 2 to 12 ± 2), PRO (11 ± 2 to 12 ± 2) and CHO (11 ± 2 to 12 ± 2) (P=0.013), but there was no difference between conditions (P=0.379).

9.3.8. Body Mass

There was no difference in the decrease in body mass following load carriage between conditions; PLA (1.5 ± 0.3 kg), CHO (1.5 ± 0.2 kg) and PRO (1.5 ± 0.4 kg) (P=0.844). As fluid intake was controlled, participants consumed 900 mL of fluid (500 mL supplement and 400 mL water) in each condition.

9.4. Discussion

The present study was the first to examine the effects of carbohydrate and protein supplements on $\dot{V}O_2$ and cardiovascular drift during load carriage (120 minutes, 6.5 km·h^{-1} and carrying a 25 kg backpack). When a placebo beverage was consumed $\dot{V}O_2$ and heart rate increased between 5 and 120 minutes. Consumption of a carbohydrate beverage reduced $\dot{V}O_2$drift compared to the placebo and protein beverages but had no effect on $\dot{V}O_2$ and heart rate at baseline or cardiovascular drift during exercise. Compared to consumption of the placebo, the protein beverage had no effect on $\dot{V}O_2$ and heart rate at baseline or $\dot{V}O_2$ and cardiovascular drift during load carriage. These data confirm the hypotheses that: (1) Carbohydrate supplementation reduces $\dot{V}O_2$drift compared to placebo and protein beverages. (2) Carbohydrate supplementation has no effect on cardiovascular drift compared to placebo and protein beverages (3) Protein supplementation has no effect on $\dot{V}O_2$ and cardiovascular drift compared to a placebo beverage.

9.4.1. Metabolic Responses

The initial exercise intensity in the present study (e.g. minute 5 during PLA 41.2 %$\dot{V}O_2$max) was similar to that observed by Quesada *et al.* (2000) (40.0 %$\dot{V}O_2$max) whilst participants carried a 23.6 kg backpack at 6.0 km·h^{-1}. During PLA, $\dot{V}O_2$ and HR increased between minutes 5 and 120 by 14 and 16 %, respectively. Similarly, Patton *et al.* (1991) observed a 10 % increase in both $\dot{V}O_2$ and HR between minutes 10 and 145 minutes whilst participants walked on treadmill at a slower speed of 5.7 km·h^{-1} (0 % gradient) carrying a 26.3 kg backpack.

The present study is the first to show attenuation in $\dot{V}O_2$drift with ingestion of a carbohydrate solution. Between minutes 5 and 120 $\dot{V}O_2$drift was lower during CHO compared to PLA and PRO (Figure 9.1). When the data were divided into shorter time periods (across quartiles), after the initial 5 ± 4 % rise in $\dot{V}O_2$ between 5 and 30 minutes, $\dot{V}O_2$ plateaued in the subsequent quartiles. Previously, Brisswalter *et al.* (2000) observed no attenuation in $\dot{V}O_2$drift during a 120 minute run at ventilatory threshold (81 %$\dot{V}O_2$max) with consumption of a carbohydrate solution (5.4 %) compared to a placebo. However, compared to the present study exercise intensity was greater (41 vs. 81 %$\dot{V}O_2$max) and participants

were more highly trained ($\dot{V}O_2$max 55.0 ± 5.5 vs. 66.8 ± 3.9 mL·kg^{-1}·min^{-1}). In Brisswalter *et al.* (2000) study the carbohydrate supplement was provided per kg of body mass resulting in a greater volume of supplement consumed (500 vs. ~1264 mL), this difference in fluid volume was made smaller when water from the present study is included (900 vs. 1264 mL). In addition, compared to the present study, the concentration of the carbohydrate was lower (6.4 vs. 5.4 %), but due to the larger supplement volume resulted in a greater total mass of carbohydrate being consumed (32 vs. 61 g) by the participants of Brisswalter *et al.* (2000).

Hamilton *et al.* (1991) observed $\dot{V}O_2$drift during 120 minutes of cycling at a constant work rate (initial work rate 70 %$\dot{V}O_2$max) when participants consumed a volume of water equal to sweat loss. In a second trial, participants received an intravenous infusion of the same volume of glucose solution (18 %) to maintain blood glucose at 10.1 ± 0.4 mmol·L^{-1} and $\dot{V}O_2$drift was attenuated and RER remained constant. However, the delivery of glucose solution via intravenous infusion resulted in blood glucose concentration being maintained at approximately 10 mmol·L^{-1}; a level not normally physiologically attainable in healthy individuals during exercise (Jeukendrup, 2004). The 0.85 mmol·L^{-1} rise in plasma glucose concentration between 60 and 75 minutes during CHO is consistent with the normal elevation of 0.5 to 1.0 mmol·L^{-1} of blood glucose concentration observed during exercise (Jeukendrup, 2004). In support of these data, Burstein *et al.* (1994) also observed a constantly elevated blood glucose concentration when a carbohydrate beverage (7.2 % concentration) was ingested compared to water during 4 days of load carriage (134 km, 10-15 kg backpack). Potential mechanisms for the effects of carbohydrate supplementation are discussed below.

To reflect representative conditions of individuals undertaking load carriage in occupational and recreational settings, participants consumed a light meal before treadmill walking. From the food diaries it was evident that there were no differences in the total energy, carbohydrate, fat or protein consumed prior to each test condition. It is likely that glycogen stores would not have been depleted prior to treadmill walking as participants avoided vigorous physical activity in the day prior to treadmill walking and consumed a light meal on the morning before load carriage (i.e. no overnight fast) (Jeukendrup, 2004). There were also no differences in RER at baseline (minute 5) which indicates that the contribution of carbohydrate and fat as energy sources were similar between conditions at the start of treadmill walking (Jeukendrup and Wallis, 2005).

RER decreased during PLA and PRO (Figure 9.3), indicating a shift from carbohydrate to fat as an energy source (Jeukendrup and Wallis, 2005). However, during CHO there was no change in RER between minutes 5 and 120 of load carriage (Figure 9.3), indicating the maintenance of CHO as the primary energy source. This finding is in keeping with findings in prolonged (~15 km) running studies (Brisswalter *et al.*, 2000). These data suggest that endogenous glycogen stores were possibly reduced to a greater extent during PLA and PRO compared to CHO. Reduced glycogen stores have been associated with a decrease in running economy (Kirwan *et al.*, 1988). Therefore, reduced glycogen stores may account for the greater $\dot{V}O_2$drift during PLA and PRO. Kirwan *et al.* (1988) did not indicate any mechanisms for the effect reduced glycogen stores had on running economy, but stated that not all the difference could be accounted for by changes in substrate oxidation.

Carbohydrate provides 5.02 kcal·$L^{-1}O_2$ where as fat provides 4.85 kcal·$L^{-1}O_2$ (Jeukendrup and Wallis, 2005). Therefore, the lower yield of energy per unit of O_2 when fat is used as an energy source during the later stages of load carriage may account for some of the rise in $\dot{V}O_2$ during PLA and PRO compared to CHO. Using previously derived equations (Jeukendrup and Wallis, 2005), a comparison between the CHO and PLA conditions can be made (PRO is not discussed as there was no difference from PLA) assuming a negligible contribution from protein metabolism. At the start of PLA (RER=0.96) 85 % and 15 % of energy was derived from carbohydrate and fat respectively, providing 4.99 kcal·$L^{-1}O_2$. At 120 minutes (RER=0.87), 54 % and 46 % of energy was derived from carbohydrate and fat respectively, providing 4.94 kcal·$L^{-1}O_2$. Therefore, if $\dot{V}O_2$ remained constant throughout PLA, to supply the same amount of energy at minute 120 as minute 5 would require an additional 0.011 $LO_2 \cdot min^{-1}$. Where as, at the start of CHO (RER=0.94) 80 % and 20 % of energy was derived from carbohydrate and fat respectively, providing 4.99 kcal·$L^{-1}O_2$. At 120 minutes (RER=0.91), 69 % and 31 % of energy was derived from carbohydrate and fat respectively, providing 4.97 kcal·$L^{-1}O_2$. Therefore, if $\dot{V}O_2$ remained constant throughout CHO, to supply the same amount of energy at minute 120 as minute 5 would only require an additional 0.004 $LO_2 \cdot min^{-1}$. Therefore, the additional requirement for O_2 for substrate oxidation at the end of PLA was 64 % greater than CHO. However, between minutes 5 and 120 $\dot{V}O_2$ increased by 0.250 $L \cdot min^{-1}$ during PLA and by 0.145 $L \cdot min^{-1}$ during CHO. Thus, the additional O_2 requirements for fat oxidation could only account for 4.4 and 2.2 % of the total increase in $\dot{V}O_2$ during PLA and CHO, respectively.

Several other mechanisms may also contribute to the attenuation of $\dot{V}O_2$drift with carbohydrate supplementation. RER is a measure of whole body substrate oxidation, but glycogen depletion may have occurred locally in individual muscle fibres (Costill *et al.*, 1973) which has been suggested as a cause of muscle fatigue (Hargreaves, 2004). However, no changes in EMG activity were observed in any condition in the present study (discussed in detail below). There is debate in the literature regarding the effectiveness of exogenous carbohydrate ingested during exercise and its role in sparing muscle glycogen stores, which appears to be highly dependent on exercise mode, intensity and duration and the amount of carbohydrate provided (Tsintzas and Williams, 1998). It is possible that the provision of CHO may have spared muscle glycogen stores in the present study, therefore reducing neuromuscular impairment in the later stages of load carriage. The provision of CHO may have also spared liver glycogen and/or promoted glycogenesis, due to the sub-maximal exercise intensity (40 – 48 %$\dot{V}O_2$max), (Jeukendrup, 2004). These processes ensure the greater availability of glucose in the later stages of load carriage, thus maintaining higher rates of carbohydrate oxidation. In addition, carbohydrate ingestion during prolonged exercise has been shown to attenuate the rise of Inosine 5'-Monophosphate (IMP), which is a marker of the mismatch between ATP re-synthesis and degradation (Spencer *et al.*, 1991). Therefore, the exogenous carbohydrate may have maintained higher rates of ATP synthesis, potentially reducing neuromuscular impairment. However, it should be noted that Spencer *et al.* (1991) participants cycled at ~70 %$\dot{V}O_2$max, which was a different exercise mode and a higher exercise intensity than the present study. These effects of carbohydrate ingestion may be particularly beneficial if load carriage duration was extended or further exercise was conducted following load carriage.

The 2007 American College of Sports Medicine position stand on exercise and fluid replacement recommends that ~30-60 g·h^{-1} of carbohydrate will maintain blood glucose and sustains exercise performance, but there is variation between individuals and exercise intensity (Sawka *et al.*, 2007). However, Jeukendrup (2004) concluded that performance benefits are observed with up to 16 g·h^{-1} of carbohydrate, with no greater improvement with higher concentrations. The present study showed performance benefits by providing a typical volume of a commercially available sports drink, supplying an average of 17 g·h^{-1} of carbohydrate. Figure 9.2 shows that plasma glucose concentration peaked 15 minutes after the ingestion of the carbohydrate beverage and gradually declined towards baseline as glucose was oxidised or

synthesised to glycogen. These data suggest that more regular feeding of carbohydrate (e.g. at 15 minute intervals) may be more beneficial at maintaining a consistently higher plasma glucose concentration. This may be especially important if exercise intensity were higher when more energy substrate would be required. However, additional consumption of a carbohydrate beverage or an increase in the carbohydrate concentration may cause gastrointestinal discomfort and the uptake of glucose may be limited by gastric emptying or uptake from the intestine (Jeukendrup and Jentjens, 2000).

9.4.2. Neuromuscular Function

As discussed previously, CHO may have contributed to a reduction in neuromuscular impairment. However, no differences in activation of the RF, VL, ST and BF muscles were observed using EMG over the duration of load carriage in any condition. Indicating there was no neuromuscular impairment over the duration of load carriage. In support of these findings; Bobet & Norman (1982), also showed no change in average EMG activity (*m. tibialis anterior, m. vastus lateralis, m. biceps femoris, m. erector spinae and m. trapezius*) during 120 minutes of load carriage (20, 25 and 32 kg backpack). However, Dick & Cavanagh (1987) showed a 10 % increase in $\dot{V}O_2$ at 44 % $\dot{V}O_2$max with a corresponding 23 % increase in IEMG during 40 minutes of downhill treadmill running, but no change in $\dot{V}O_2$ or IEMG during 40 minutes of level running at 66 % $\dot{V}O_2$max. The authors concluded that the $\dot{V}O_2$drift observed during downhill running was due to muscle fibre damage and the recruitment of additional muscle fibres.

Neuromuscular impairment is most accurately measured by changes in the force producing capability of muscle or muscle group (Warren *et al.*, 1999). Clarke *et al.* (1955), showed decreases in strength of the knee, trunk and ankle flexors and extensors and the shoulder elevators following a 12.1 km road march at 4 $km \cdot h^{-1}$ carrying loads 13, 18 and 27 kg. In support of these data, Quesada *et al.* (2000) observed declines in knee extension moment peaks following 40 minutes of treadmill walking carrying 11.8 and 23.6 kg backpacks. Their data suggested that the knee extensors and flexors made a substantial contribution to supporting the load, which was not maintained at the end of load carriage, possibly due to knee extensor fatigue.

Therefore it is surprising that no changes in muscle activation were observed during load carriage in the present study. However, there was large variation (standard deviations)

associated with the mean data for each of the muscle groups (Figure 9.4), which were due to a highly variable individual response. A typical example is *m. vastus lateralis* during PLA; between 7 and 107 minutes, the group RMS decreased to 86 ± 39 % from baseline (n=10). However, four of the participants showed an increase to 126 ± 23 % of the pre value. While the other six participants showed a decrease to 60 ± 17 % of the pre value. Similar changes were apparent in the other muscle groups measured in the present study. Bobet & Norman (1982) also observed high inter participant variability but only measured EMG responses from four participants, therefore trends between groups of individuals seen in the present study may not have been observed. These data suggests individual adaptations and changes in muscle recruitment during load carriage, possibly due to fatigue or injury of the supporting muscles (Clarke *et al.*, 1955) or muscular discomfort (Legg *et al.*, 2003). This may have also occurred in other muscle groups (e.g. shoulder, trunk or ankle extensors and flexors), but were not measured in the present study. These trends support Quesada *et al.* (2000) earlier work suggesting a possible change in muscle recruitment over time to support the load, which may vary between individuals.

The individual changes and small sample size of the EMG data in the present study may have resulted in potential changes in neuromuscular function not being detected. Further investigation would be beneficial with a larger test population to examine individual and group responses during exercise.

9.4.3. Cardiovascular Drift

Interestingly, CHO had no effect on cardiovascular drift, which suggests different mechanisms are responsible for cardiovascular and $\dot{V}O_2$drift. Hamilton *et al.* (1991) showed cardiovascular drift during 120 minutes of cycling at a constant work rate at an initial exercise intensity 70 %$\dot{V}O_2$max when participants consumed no fluid. In a second trial water was consumed at 20 minute intervals to match sweat losses and cardiovascular drift was attenuated, but $\dot{V}O_2$drift was apparent in both trials. In a subsequent experiment (described previously), participants were infused with an identical volume of glucose solution (18 %) to maintain blood glucose at 10.1 ± 0.4 mmol·L^{-1} and both HR and $\dot{V}O_2$ were prevented from rising and RER was maintained during the 120 minutes of cycling. These findings suggest that dehydration is responsible for cardiovascular drift during exercise and, as discussed previously, the availability of carbohydrate as an energy substrate is a major contributor to $\dot{V}$

O_2drift. In the present study body mass decreased by 1.5 kg in all conditions which was not matched by fluid intake (0.9 L), suggesting participants became dehydrated (Baker *et al.*, 2009). This would support the concept that cardiovascular drift is caused by dehydration. Likewise, Byrne *et al.* (2005), observed cardiovascular drift during three 60 minute periods of load carriage (14 kg) in the heat (35 °C), despite consuming fluid body mass decreased by 0.3 ± 0.4 % from the start of load carriage (indicating dehydration). However, it should be noted that the environmental temperature in the present study was lower (~21 °C) and there was no difference between conditions.

These findings suggest if fluid consumption matched sweat losses, cardiovascular drift could be attenuated during load carriage. However, pilot work for the present study revealed that participants found it uncomfortable to consume 1.5 L of fluid during load carriage. The current International Olympic Committee guidelines recommend the consumption of a volume of fluid to match sweat losses (Coyle, 2004). However, Noakes (2007) argues that *ad libitum* consumption of fluid is adequate to maintain health and performance and is more tolerable during exercise. The present study could not address these issues further, but shows consideration is required as to whether individuals should follow an enforced or *ad libitum* fluid consumption regime. Load carriers may also be hesitant to carry additional load due to the increased physiological strain (Knapik *et al.*, 1996). However, using previously validated equations (Borghols *et al.*, 1978), carrying an additional 1.5 kg of load (i.e. 1.5 L fluid) would increase heart rate by 2 beats·min^{-1} and $\dot{V}O_2$ by 0.05 L·min^{-1}. But consumption of this fluid during load carriage could attenuate a cardiovascular drift of approximately 18 beats·min^{-1} over two hours.

9.4.4. Whey Protein Supplementation during Load Carriage

The present study is the first to assess the effects of a whey protein supplement on the aerobic and cardiovascular responses during prolonged exercise. The ingestion of Branched Chain Amino Acids (BCAA) (a component of whey protein) has been suggested to attenuate the onset of central fatigue during prolonged exercise but there is debate as to their effectiveness (Newsholme and Blomstrand, 2006). PRO had no effect on the parameters measured during load carriage compared to PLA. The use of protein as energy substrate is minimal when individuals have adequate availability of carbohydrate, but reaches its highest rate when individuals are glycogen depleted (Lemon and Nagle, 1981). Participants in the

present study were likely to have started load carriage with close to optimal glycogen stores (as discussed previously). Thus, under the conditions of the present study PRO is likely to have had little impact on the physiological changes during load carriage. However, in some circumstances when load carriage must be undertaken where individuals are in negative energy balance and glycogen stores may be depleted (e.g. sustained military deployments; see Nindl *et al.*, 2002) supplementation with protein may be beneficial during load carriage, this requires further investigation.

Wagenmakers *et al.* (1991) hypothesised that excessive BCAA metabolism could drain the tricarboxylic acid cycle in the primary BCAA aminotransferase reaction, leading to a reduced flux in the tricarboxylic acid cycle and a reduced ability to oxidise blood glucose and fatty acids. Hence, the consumption of the BCAA in PRO may have impaired exercise performance. However, compared to PLA, PRO had no effect on the metabolic, cardiovascular or neuromuscular parameters measured in the present study. It is possible the provision of amino acids during exercise may be beneficial during recovery. During endurance exercise muscle protein synthesis decreases and breakdown is increased (Wolfe, 2000b). Therefore, consumption of whey protein at the start of exercise may be beneficial for providing amino acids for protein synthesis during and following exercise. Indeed, a positive net protein balance during exercise can only be achieved when amino acid availability is increased (i.e. ingestion of proteins) (Kumar *et al.*, 2009). In addition, the delivery of amino acids to the muscle is higher if they are ingested prior to or during exercise due to the increased muscle blood flow (Wolfe, 2001). Following exercise, rates of protein synthesis have been shown to increase between 10 and 80 % (Carraro *et al.*, 1990), but a positive protein balance is only possible with the provision of additional amino acids (Rodriguez *et al.*, 2007). It has also been suggested that increasing protein availability during exercise may attenuate protein breakdown during exercise, providing a potentially less catabolic environment during post-exercise recovery (Rodriguez *et al.*, 2007). This will also ensure the availability of amino acids during recovery, when more beneficial effects have been observed the earlier amino acids are available (Nosaka, 2007). Thus, providing some support to practitioners who recommend the consumption of protein during endurance exercise (Howatson and van Someren, 2008). However, the effects of protein supplementation during exercise on neuromuscular function during post exercise recovery have not been established.

9.4.5. Conclusion

In conclusion, the present study showed that when a placebo beverage is consumed during 120 minutes of treadmill walking at 6.5 $km \cdot h^{-1}$ carrying a 25 kg backpack, $\dot{V}O_2$ and HR increase and RER decreased over time. Ingestion of a carbohydrate beverage reduced $\dot{V}O_2$drift and attenuated the decline in RER. However, the reduction in oxygen cost can not be completely accounted for by the higher rates of carbohydrate oxidation. The supply of exogenous carbohydrate may have spared muscle and liver glycogen or maintained higher rates of ATP re-synthesis this would potentially improve neuromuscular function, and therefore walking economy, later in exercise. However, no differences were observed in muscle activation from the EMG data. Trends in the EMG data indicate that there were specific individual changes in muscle recruitment, possibly due to muscle injury or fatigue, but these were not evident in the mean group response. There were no differences in cardiovascular drift between conditions, which is probably because participants became dehydrated as fluid losses were not matched by fluid intake. The consumption of a protein beverage had no effect on the physiological parameters measured during this study. But the ingestion of protein may be of benefit in situations when glycogen stores are depleted prior to exercise and ensures a positive protein balance during and following exercise which is likely to aid recovery.

Chapter 10. Improved Time Course of Recovery of Neuromuscular Function following Load Carriage with Carbohydrate and Whey Protein Supplementation

10.1. Introduction

Neuromuscular impairment was shown in Royal Marine Recruits immediately after 19.2 km of load carriage in the field (Chapter 3). The reduction in neuromuscular function was shown to last for up to 72 h following 120 minutes of load carriage walking at 6.5 $km \cdot h^{-1}$ on a level treadmill gradient carrying a 25 kg backpack (Chapter 7). This is likely to impair performance during endurance (Chen *et al.*, 2007) and high intensity exercise (Twist and Eston, 2005) and motor control tasks (Byrne *et al.*, 2004). A reduction in the force producing capability may also expose muscle groups to a greater risk of muscle sprain due to their reduced ability to absorb force (Mair *et al.*, 1996). Load carriage as is commonly conducted in environments where repeated days or weeks of physical work and exercise are undertaken when optimal neuromuscular function is required (e.g. military training and deployments), therefore the recovery of neuromuscular function is of particular importance. Thus, strategies to improve the recovery of neuromuscular function following exercise would be beneficial to individuals and organisations undertaking load carriage.

During prolonged exercise ingestion of carbohydrates improves exercise performance (Jeukendrup, 2004) and delays the onset of fatigue (Coyle, 1992). Byrne *et al.* (2005) observed an improved rate of completion of three 60 minute load carriage bouts (14kg) in 35°C ambient temperature when a 6.4 % carbohydrate-electrolyte beverage was consumed (50 % completion) compared to water (21 % completion). Stewart *et al.* (2007) showed that supplementation with a glucose solution compared to water caused a lesser reduction in maximal force producing capability of the quadriceps at the end of a cycle to exhaustion at 60 $\dot{V}O_2$peak. During recovery carbohydrate supplementation in the hours and days following exercise improves subsequent exercise performance (Millard-Stafford *et al.*, 2008). However, the effect of carbohydrate supplementation on recovery of the force producing capability of muscle groups following load carriage is unknown.

The consumption of protein and amino acids following prolonged exercise to improve recovery of neuromuscular function and structure has recently been a topic of significant interest (Rasmussen and Richter, 2009). Whey protein supplements provide a relatively high proportion of essential amino acids and have a similar amino acid composition to human skeletal muscle (Ha and Zemel, 2003). The benefits of whey protein supplementation following resistance exercise to improve muscle hypertrophy and function are well documented (Hayes and Cribb, 2008). However, it appears that resistance and endurance based exercise have different effects on protein turnover (Tipton and Wolfe, 1998), which may result in differences in the recovery of muscle structure and function. After endurance exercise protein breakdown and synthesis are both increased but protein balance is still slightly negative (Wolfe, 2000b). Ingestion of protein during and following exercise results in a positive protein balance as amino acids are provided for protein synthesis and their presence may also attenuate protein breakdown (Kumar *et al.*, 2009). The rate of muscle protein synthesis and breakdown are central in determining both strength and neuromuscular function (Wolfe, 2000b). Therefore, the maintenance of positive protein balance in the muscle should improve neuromuscular function. However, the effect of protein supplementation on recovery of the force producing capability of muscle groups following load carriage has not been investigated.

The aim of this study was to compare the effects of a placebo beverage (flavoured water) to commercially available carbohydrate and whey protein supplements on the recovery of neuromuscular function in the days following (0, 24, 48, 72 h) 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) carrying a 25 kg backpack. It was hypothesised that: (1) compared to a placebo consumption of a carbohydrate supplement would result in earlier recovery of muscle force producing capability following load carriage (2) compared to a placebo consumption of a whey protein supplement would result in earlier recovery of muscle force producing capability following load carriage.

10.2. Methods

10.2.1. Participants

Ten healthy male participants (age 28 ± 9 years, height 1.82 ± 0.07 m, body mass 81.5 ± 10.5, body fat 16.4 ± 3.2 %, $\dot{V}O_2max$ 55.0 ± 5.5 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to participate in the study. Participants had a range of previous recreational experience of carrying load in backpacks. Ethical approval for all procedures and protocols were provided by the University of Chichester Ethics Committee. All protocols were performed in accordance with the ethical standards laid down in the 2004 Declaration of Helsinki. Participants provided written informed consent and were screened to ensure they were free from any musculoskeletal injury prior to commencing the study. In the day prior to load carriage participants were instructed to refrain from any vigorous physical activity. On the day of load carriage participants consumed a standardised light meal and avoided consumption of caffeine, sports drinks or food three hours prior to exercise. In the days following load carriage, participants were instructed to maintain a normal diet (recorded in a food diary, described in detail below), keeping consistency between test conditions, and refrain from any vigorous physical activity.

10.2.2. Preliminary Measures

Body mass (Seca Model 880, Seca Ltd., Birmingham, UK) (± 0.01 kg) and stature (Avery Berkel, Smethwick, UK) (± 0.005 m) were measured whilst wearing shorts and underwear. Skinfold thickness was measured at the *Chest, Axilla, Triceps, Sub Scapular, Abdomen, Iliac Crest and Thigh* on the right side of the body using Harpenden Skinfold Callipers (Body Care, Southam, UK). Two measurements were taken at each site and if there was a difference > 1 mm, the measurements were repeated. Percentage body fat was estimated following the assessment of skinfold thickness at seven anatomical sites using previously described methods (Jackson and Pollock, 1978; Siri, 1956).

At least 5 days prior to beginning the experimental protocol, participants were familiarised with all test procedures. Participants completed 3 maximal voluntary isometric contractions, and all electrical stimulation procedures (described in detail below). Also, the electrical current required to stimulate an involuntary maximal twitch force (group mean ± SD; 830 ± 67 mA) and sub-maximal twitch force (5 % MVC force) (group mean ± SD; 420 ± 77 mA) were recorded and kept constant in all subsequent test sessions. Participants also

completed 1 cycle of the isokinetic experimental protocol (described in detail below). A test procedure was repeated if the experimenter or participant thought that a maximal effort was not given or if the force continued to increase in the final two contractions of a set.

10.2.3. Experimental Protocol

The study was a repeated measures three way cross over randomised design. Participants walked for 120 minutes at 6.5 $km \cdot h^{-1}$ and 0 % gradient carrying a 25 kg backpack on a motorised treadmill (Woodway Ergo ELG 70, Cranlea & Co, Birmingham, UK). The load was evenly distributed in the backpack and remained constant between conditions. The backpack had adjustable shoulder straps and a fixed height waist strap that could be tightened, but no sternum strap, participants adjusted the strapping to achieve a comfortable fit. Walking speed was kept constant between test conditions and an absolute load used to reflect realistic occupational requirements (e.g. military load carriage). During each of the 3 treadmill walking sessions participants consumed 500 mL of a commercially available beverage (250 mL at the start and 250 mL at 60 minutes of walking) mixed as directed by manufacturers guidelines;

(1) Placebo (PLA); 490 mL water and 10 mL sugar free orange cordial (Tesco, Dundee, UK), [nutritional content per 500 mL; Energy 1 Kcal, Carbohydrate 0.1 g, Protein 0 g, Fat 0 g)

(2) Carbohydrate (CHO) (6.4 % carbohydrate concentration); 490 mL water, 10 mL sugar free orange cordial (Tesco, Dundee, UK), 34 g Super Soluble Maxijul (SHS International Limited, Liverpool, UK) [nutritional content per 500 mL; Energy 130 Kcal, Carbohydrate (100 % glucose) 32 g, Protein 0 g, Fat 0 g],

(3) Protein (PRO) (7.0 % protein concentration); 500 mL water and 44 g orange flavoured Maximuscle Promax (Maximuscle Limited, Hemel Hempstead, UK) [nutritional content per 500 mL; Energy 176 Kcal, Carbohydrate 3 g Protein 36 g, Fat 3 g, Sodium 0.2 g].

In addition to the commercially available beverages participants consumed 200 mL of water at 30 minutes and 200 mL water at 90 minutes of walking. Immediately after and in the evening after (~1900 h) each muscle testing session (described below), participants consumed 500 mL of the allocated supplement (i.e. PLA, PRO, CHO). Rather than providing supplement per body mass of the participants, absolute volumes of the supplement were provided to attempt to maintain ecological validity of consuming commercially available supplements.

Participants completed the muscle testing protocol of voluntary and electrically stimulated contractions (see Chapter 7) before commencing load carriage (pre-exercise) and at 0 (immediately post), 24, 48 and 72 hours following load carriage. The test order was the same on each occasion and conducted at approximately the same time of day to control for diurnal variation in force producing capability of the muscles (Sedliak *et al.*, 2008). Three minutes rest was provided between each of the test procedures.

The methodology of the muscle testing protocol has previously been described in detail for all isometric contractions of *m. quadriceps femoris* (Chapter 5) and isokinetic contractions of the knee, trunk and shoulder extensors and flexors (Chapter 4). Briefly; responses to isometric contractions of the *m. quadriceps femoris* during MVC, VA, potentiated doublet, and the 20:50 Hz ratio were recorded. Peak torque was measured at two test velocities during isokinetic contractions of the knee (60 and 180°·s^{-1}), shoulder (60 and 180°·s^{-1}) and trunk (15 and 60°·s^{-1}) extensors and flexors.

10.2.4. Dietary Intake

Participants were instructed to consume a light meal at least 3 hours prior to treadmill walking which was to be similar between the three test sessions. Participants recorded any food or beverages (with estimated mass or portion size) consumed on the day of and for 72 hours following treadmill walking. Food diaries were analysed for energy content (Kcal), carbohydrate, fat and protein mass (g) using Microdiet Plus for Windows V1.2 (Downlee Systems Ltd, Derbyshire, UK).

Table 10.1 shows there were no differences between conditions before or after load carriage in dietary intake of energy [pre (P=0.623), 24 h (P=0.609), 48 h (P=0.968) or 72 h (P=0.452)], carbohydrate [pre (P=0.472), 24 h (P=0.504), 48 h (P=0.804) or 72 h (P=0.453)], fat [pre (P=0.762), 24 h (P=0.687), 48 h (P=0.869) or 72 h (P=0.664)] or protein [pre (P=0.393), 24 h (P=0.573), 48 h (P=0.567) or 72 h (P=0.577)].

Table 10.1 – Dietary intake of energy, carbohydrate, fat and protein measured by food diaries before (Pre) and after (24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 $km \cdot h^{-1}$ (n=10) on a level gradient (0%) carrying a 25 kg backpack. Either a placebo beverage (PLA), carbohydrate (6.4%) beverage (CHO) or protein (7 %) beverage (PRO) was consumed at 0 and 60 minutes (250 ml) during treadmill walking or twice daily (morning and evening) for the 3 days following load carriage (n = 10). Data are presented excluding the consumption of the supplement beverages.

Variable	Condition	Pre	24 h	48 h	72 h
Energy (Kcal)	PLA	266 ± 157	1494 ± 740	1484 ± 659	1600 ± 549
	CHO	259 ± 154	1547 ± 702	1468 ± 680	1532 ± 628
	PRO	277 ± 147	1611 ± 658	1481 ± 626	1613 ± 534
Carbohydrate (g)	PLA	51 ± 37	212 ± 162	217 ± 159	221 ± 108
	CHO	49 ± 36	224 ± 156	209 ± 162	207 ± 111
	PRO	55 ± 34	233 ± 150	216 ± 161	226 ± 106
Fat (g)	PLA	3 ± 3	41 ± 24	41 ± 28	52 ± 28
	CHO	3 ± 3	45 ± 28	45 ± 32	50 ± 26
	PRO	3 ± 3	46 ± 27	43 ± 23	53 ± 23
Protein (g)	PLA	11 ± 6	82 ± 26	73 ± 27	76 ± 21
	CHO	11 ± 6	77 ± 22	69 ± 23	75 ± 22
	PRO	10 ± 6	80 ± 23	69 ± 19	73 ± 21

10.2.5. Environmental Conditions

Environmental temperature was monitored using a dry bulb thermometer (Fisher Scientific, Loughborough, UK). No differences in environmental temperature were observed over time between pre tests and 0, 24, 48 and 72 hour test periods respectively, for PLA (20.4 ± 0.5, 20.7 ± 0.7, 20.6 ± 0.7, 20.9 ± 0.8, 20.9 ± 0.6 °C), CHO (20.6 ± 0.9, 20.9 ± 0.7, 20.6 ± 0.8 20.5 ± 0.9, 20.4 ± 0.8 °C) or PRO (20.6 ± 0.7, 20.9 ± 0.8, 20.6 ± 0.9, 20.5 ± 0.8, 20.4 ± 0.7 °C) (P=0.204) or between conditions (P=0.892).

10.2.6. Statistical Analysis

Statistical analysis was undertaken using SPSS for Windows V15 (SPSS, Chicago, Illinois). Normal distribution of the data was verified using a Kolmogorov-Smirnov test. Pre-planned paired t-tests were used to compare variables between experimental conditions at the pre-exercise time point. Changes in variables across time were examined by comparing changes from pre-exercise values only. Differences from pre-exercise values were calculated at each time point following exercise (i.e. delta values at 0, 24, 48, 72 h). Pre-planned one sample t-tests were used to compare if delta values (at 0, 24, 48 and 72 h) differed from zero (i.e. pre-exercise) for each condition. If changes over time were apparent in both conditions, the magnitude of the change was compared using paired t-tests on the delta values at that time point (i.e. 0, 24, 48 and 72 h). Using pre-planned t-tests rather than a 2 way (condition x time) repeated measures, Analysis of Variance (ANOVA) reduces the possibility of a type II statistical error. The calculation of a 2 way ANOVA using data in the present study is likely to cause type II statistical error due to a lack of statistical power, caused by a high number of unnecessary comparisons on a relatively small population sample (n=10). Although using multiple t-tests increases the risk of a type I statistical error, this is offset by the greater reduction of the possibility of type II error for analysis of the data in the present study. Statistical significance was set at P<0.05. Data are presented as mean ± standard deviation (SD).

10.3. Results

10.3.1. Voluntary and Electrically Stimulated Isometric Contractions of the *m. quadriceps femoris*

Isometric MVC force of the knee extensors decreased from pre-exercise value immediately after load carriage for PLA (14 ± 7 %, $P<0.001$), CHO (12 ± 10 %, $P=0.006$) and PRO (14 ± 8 %, $P<0.001$), with no difference between conditions ($P>0.05$). At 24 h following load carriage MVC force was still below pre-exercise value for PLA (12 ± 10 %, $P=0.009$), CHO (9 ± 11 %, $P=0.021$) and PRO (10 ± 9 %, $P=0.003$). By 48 h post load carriage MVC force was 10 ± 10 % below pre-exercise value for PLA ($P=0.008$), but had returned to pre-exercise value for CHO ($P=0.199$) and PRO ($P=0.099$). At 72 h post load carriage PLA returned to pre-exercise value ($P=0.145$) and both CHO ($P=0.457$) and PRO ($P=0.731$) remained at the pre-exercise value (Figure 10.1).

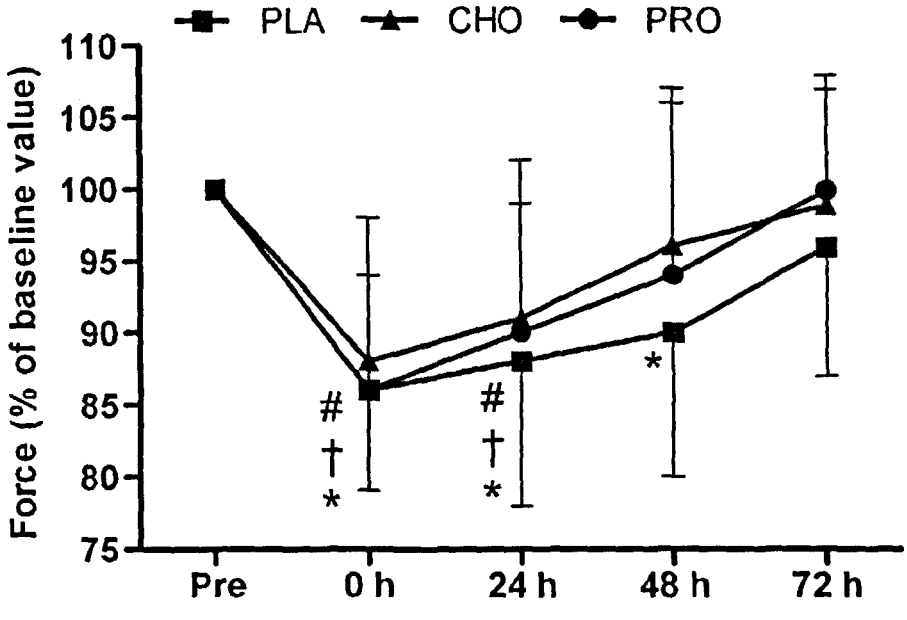


Figure 10.1 – Force of the *m. quadriceps femoris* during isometric MVC, measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) on a level gradient (0 %) carrying a 25 kg backpack with consumption of 250 mL (at 0 and 60 minutes) of a beverage containing either placebo (PLA, ■), carbohydrate (6.4 %) (CHO, ▲) or protein (7 %) (PRO, ●) consumed at 0 and 60 minutes (250 mL) during treadmill walking or twice daily (morning and evening) for the 3 days following load carriage (n = 10). Symbols show difference from pre measurement for PLA (* $P<0.05$), CHO († $P<0.05$), PRO (# $P<0.05$).

Immediately after load carriage VA decreased from 98 ± 3 % to 92 ± 6 % during PRO (P=0.005), but then recovered at 24 h (P=0.2) and was not different from pre-exercise values at 48 (P=0.181) and 72 h (P=0.452). There were no changes in VA following load carriage during PLA or CHO (Table 10.2).

The 20:50 Hz ratio was lower before exercise for PRO compared to PLA (P=0.030) and CHO (P=0.019), but there was no difference between CHO and PLA (P=0.795) (Table 10.2). Immediately after load carriage the 20:50 Hz ratio decreased after PLA only (Table 10.2); decreasing from 0.88 ± 0.04 to 0.83 ± 0.06 (P=0.012). At 24 h, 20:50 Hz ratio shortly returned to pre-exercise value (0.83 ± 0.07, P=0.072) but fell below pre-exercise value again at 48 h (0.83 ± 0.06, P=0.043), returning to the pre-exercise value again at 72 h (0.85 ± 0.06, P=0.160).

Table 10.2 shows there were no changes over time in any condition for the doublet peak force (P>0.05), contraction time (P>0.05), half relaxation time (P>0.05), maximal rate of force development (P>0.05), maximal rate of force decrease (P>0.05) or the rate constant for contraction (P>0.05). The rate constant for relaxation increased immediately after CHO only (P=0.031), returning to baseline at 24 h (P=0.772).

10.3.2. Isokinetic Contractions of the Knee Extensors

Figure 10.2 shows that peak torque (60 °·s^{-1}) of the knee extensors decreased following load carriage for PLA (6 ± 5 %, P=0.004), CHO (6 ± 4 %, P=0.005) and PRO (5 ± 6 %, P=0.043), there was no difference between conditions (P>0.05). At 24 h, peak torque (60 °·s^{-1}) of the knee extensors had returned to the pre-exercise value for PRO (P=0.082) but remained below pre-exercise value for PLA (8 ± 9 %, P=0.019) and CHO (5 ± 6 %, P=0.043). At 48 h after load carriage, peak torque (60 °·s^{-1}) of the knee extensors remained above pre-exercise value for PRO (P=0.755) and returned to pre-exercise value for the first time for CHO (P=0.561), but remained below pre-exercise value for PLA (8 ± 7 %, P=0.008). By 72 h following load carriage, peak torque (60 °·s^{-1}) of the knee extensors had returned to pre-exercise value for PLA (P=0.682) and remained above the pre-exercise value for CHO (P=0.798) and PRO (P=0.284). There was no change over time in any condition for peak torque (180 °·s^{-1}) of the knee extensors (P>0.05) (Table 10.3).

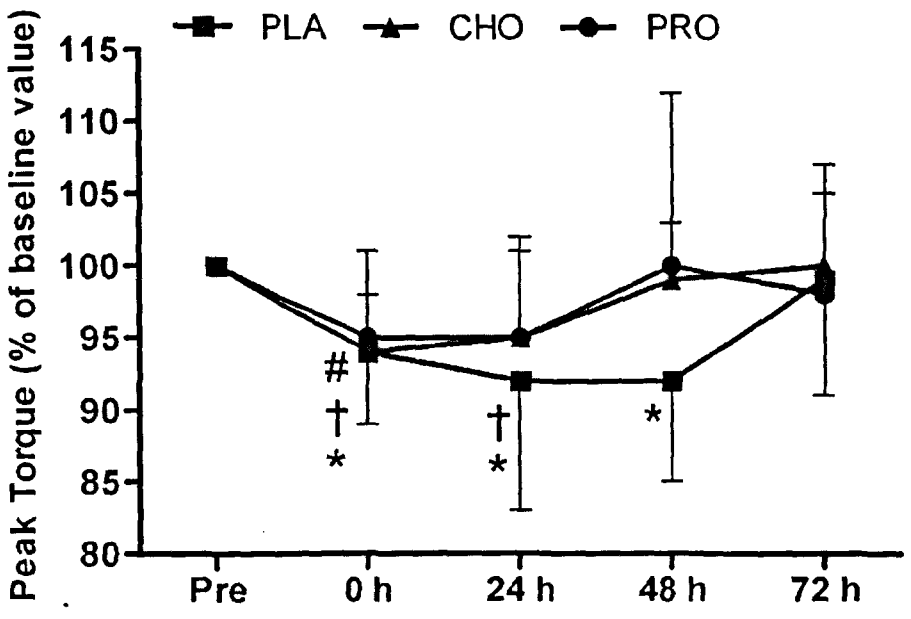


Figure 10.2 – Force of the knee extensors during isokinetic contractions at (60 °·s^{-1}), measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) on a level gradient (0 %) carrying a 25 kg backpack with consumption of 250 mL (at 0 and 60 minutes) of a beverage containing either placebo (PLA, ■), carbohydrate (6.4 %) (CHO, ▲) or protein (7 %) (PRO, ●) consumed at 0 and 60 minutes (250 mL) during treadmill walking or twice daily (morning and evening) for the 3 days following load carriage (n = 10). Symbols show difference from pre measurement for PLA (* P<0.05), CHO († P<0.05), PRO (# P<0.05).

10.3.3. Isokinetic Contractions of the Knee Flexors

Peak torque (60 °·s^{-1}) of the knee flexors decreased immediately after load carriage for PLA (9 ± 7 %, P=0.009), CHO (10 ± 9 %, P=0.012) and PRO (8 ± 6 %, P=0.005), but there were no differences between conditions (P>0.05). By 24 h after load carriage, knee flexor peak torque (60 °·s^{-1}) returned to pre-exercise value for PRO (P=0.087) but remained below pre-exercise value for PLA (8 ± 7 %, P=0.005) and CHO (7 ± 7 %, P=0.024). At 48 h after load carriage, CHO returned to pre-exercise value (P=0.101) and PRO remained at pre-exercise value (P=0.898) but PLA was still suppressed (7 ± 5 %, P=0.001). By 72 hours after load carriage, knee flexor peak torque (60 °·s^{-1}) had returned to pre-exercise value for PLA (P=0.378) and remained at pre-exercise value for CHO (P=0.201) and PRO (P=0.696). Peak torque (180 °·s^{-1}) of the knee flexors decreased immediately after load carriage for CHO (12 ±

12 %, P=0.029) but not PLA (P=0.078) or PRO (P=0.062). Knee flexors peak torque (180 °·s^{-1}) returned to pre-exercise value 24 h after load carriage for CHO (P=0.763) and remained at pre-exercise value for all conditions at 48 and 72 h (Table 10.3).

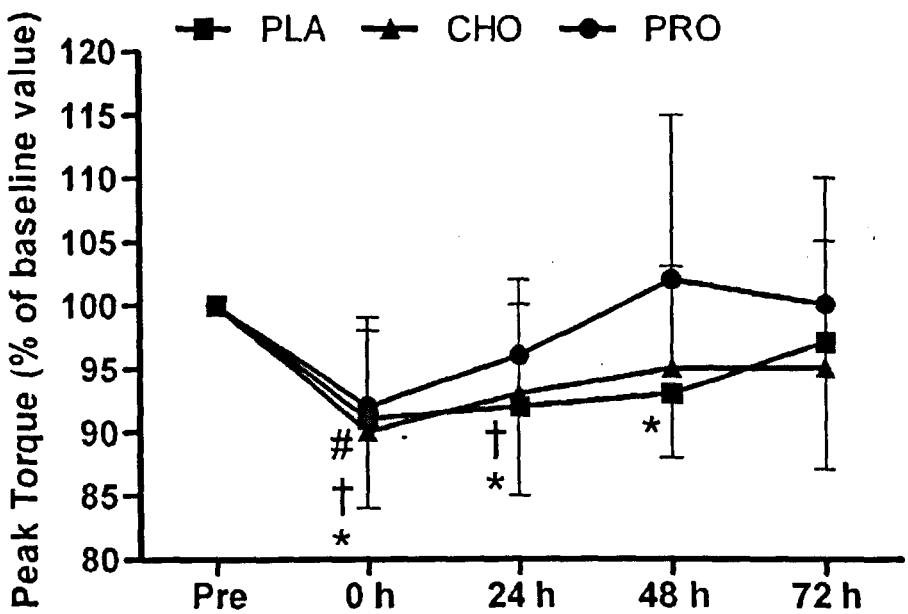


Figure 10.3 – Peak torque of the knee flexors during isokinetic contractions at (60 °·s^{-1}), measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) on a level gradient (0 %) carrying a 25 kg backpack with consumption of 250 mL (at 0 and 60 minutes) of a beverage containing either placebo (PLA, ■), carbohydrate (6.4 %) (CHO, ▲) or protein (7 %) (PRO, •) consumed at 0 and 60 minutes (250 mL) during treadmill walking or twice daily (morning and evening) for the 3 days following load carriage (n = 10). Symbols show difference from pre measurement for PLA (* P<0.05), CHO († P<0.05), PRO (# P<0.05).

10.3.4. Isokinetic Contractions of the Trunk Extensors

Peak torque (15 °·s^{-1}) of the trunk extensors decreased immediately after load carriage for PLA (12 ± 15 %, P=0.016), CHO (15 ± 7 %, P<0.001) and PRO (10 ± 11 %, P=0.018), but there were no differences between conditions (P>0.05). By 24 h, CHO (P=0.156) and PRO (P=0.982) both returned to pre-exercise value but PLA was still 9 ± 11 % below the pre-exercise value (P=0.040). All conditions were at pre-exercise value at 48 h and at 72 h (Table 10.4). There was no change in peak torque (60 °·s^{-1}) for the trunk extensors immediately after load carriage for PRO (P=0.079) but PLA and CHO decreased by 13 ± 15 %, (P=0.033) and

13 ± 12 %, (P=0.011), respectively. At 24 h, PRO remained at pre-exercise value (P=0.793) and CHO returned to pre-exercise value (P=0.742) but PLA was still 11 ± 9 % below pre-exercise value (P=0.008). All conditions were at pre-exercise value by 48 h remaining constant at 72 h (Table 10.4)

10.3.5. Isokinetic Contractions of the Trunk Flexors

Figure 10.4 shows that peak torque (15 °·s^{-1}) of the trunk flexors decreased immediately after load carriage for PLA (10 ± 7 %, P=0.002), CHO (8 ± 10 %, P=0.026) and PRO (9 ± 7 %, P=0.003), but there was no difference between conditions (P>0.05). All conditions had returned and remained at pre-exercise value by 24, 48 and 72 h (Table 10.4). Similarly peak torque (60 °·s^{-1}) of the trunk flexors decreased immediately after load carriage for PLA (7 ± 10 %, P=0.049), CHO (5 ± 5 %, P=0.013) and PRO (7 ± 7 %, P=0.009), but there was no difference between conditions (P=0.627). All conditions had returned to pre-exercise value by 24 h and remained constant at 48 and 72 h (Table 10.4).

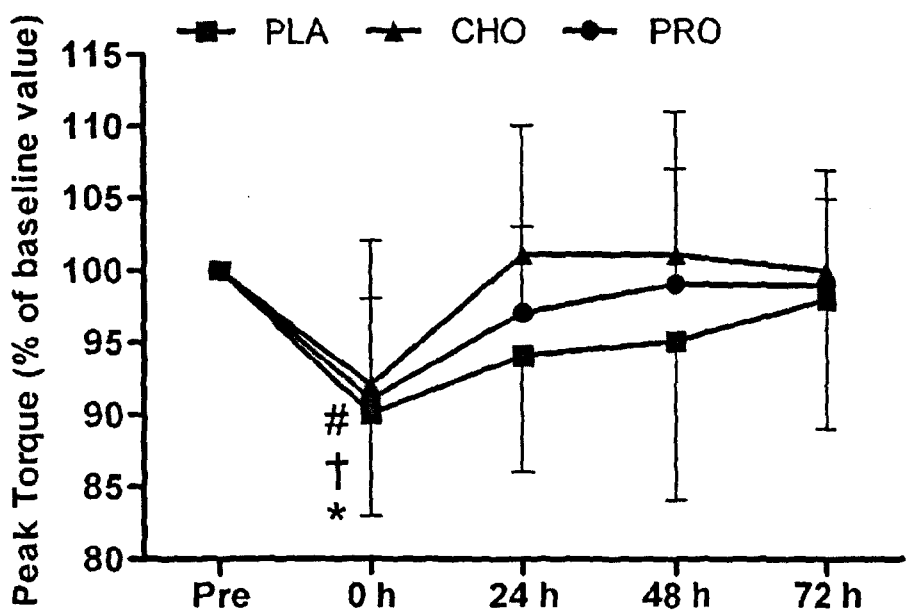


Figure 10.4 – Peak torque of the trunk flexors during isokinetic contractions (15 °·s^{-1}), measured before and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) on a level gradient (0 %) carrying a 25 kg backpack with consumption of 250 mL (at 0 and 60 minutes) of a beverage containing either placebo (PLA, ■), carbohydrate (6.4 %) (CHO, ▲) or protein (7 %) (PRO, ●) consumed at 0 and 60 minutes (250 mL) during treadmill walking or twice daily (morning and evening) for the 3 days following load carriage

(n = 10). Symbols show difference from pre measurement for PLA (* $P<0.05$), CHO († $P<0.05$), PRO (# $P<0.05$).

10.3.6. Isokinetic Contractions of the Shoulder Extensors and Flexors

There were no changes over time in any condition for the shoulder extensors (60 $°\cdot s^{-1}$) ($P>0.05$), shoulder extensors (180 $°\cdot s^{-1}$) ($P>0.05$), shoulder flexors (60 $°\cdot s^{-1}$) ($P>0.05$) or shoulder flexors (180 $°\cdot s^{-1}$) ($P>0.05$) (Table 10.5).

Table 10.2 – Voluntary and electrically stimulated isometric contractions of the m. *quadriceps femoris* measured before (Pre) and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 $km \cdot h^{-1}$ (n=10) on a level gradient (0%) carrying a 25 kg backpack. Either a placebo beverage (PLA), carbohydrate (6.4%) beverage (CHO) or protein (7 %) beverage (PRO) was consumed at 0 and 60 minutes (250 ml) during treadmill walking and twice daily (morning and evening) for the 3 days following load carriage (n = 10). Difference from pre measurement (* P<0.05, ** P<0.01, *** P<0.001). PRO different at pre-exercise from PLA and CHO († P<0.05).

Variable	Cond	Pre	0 h	24 h	48 h	72 h
MVC (N)	PLA	694 ± 117	603 ± 134 ***	615 ± 144 **	622 ± 123 **	664 ± 118
	CHO	676 ± 132	594 ± 118 **	614 ± 134 *	639 ± 107	662 ± 100
	PRO	682 ± 138	592 ± 143 ***	615 ± 158 **	639 ± 147	676 ± 129
VA (%)	PLA	98 ± 4	93 ± 11	96 ± 3	95 ± 9	96 ± 7
	CHO	97 ± 4	92 ± 9	95 ± 9	95 ± 5	97 ± 7
	PRO	98 ± 3	92 ± 6 *	93 ± 10	94 ± 9	96 ± 7
Doublet Peak Force (N)	PLA	174 ± 39	173 ± 39	170 ± 46	175 ± 50	176 ± 33
	CHO	171 ± 41	166 ± 34	171 ± 32	170 ± 29	169 ± 34
	PRO	175 ± 44	174 ± 46	171 ± 50	172 ± 42	174 ± 42
Doublet Contraction Time (s)	PLA	0.187 ± 0.007	0.181 ± 0.009	0.187 ± 0.010	0.187 ± 0.017	0.189 ± 0.011
	CHO	0.185 ± 0.006	0.184 ± 0.010	0.187 ± 0.009	0.185 ± 0.009	0.187 ± 0.006
	PRO	0.184 ± 0.011	0.182 ± 0.007	0.186 ± 0.010	0.186 ± 0.009	0.188 ± 0.008
Doublet Half Relaxation Time (s)	PLA	0.100 ± 0.009	0.098 ± 0.009	0.099 ± 0.011	0.099 ± 0.011	0.100 ± 0.012
	CHO	0.100 ± 0.011	0.096 ± 0.006	0.100 ± 0.012	0.098 ± 0.010	0.097 ± 0.008
	PRO	0.100 ± 0.013	0.095 ± 0.010	0.099 ± 0.011	0.098 ± 0.010	0.099 ± 0.009

Variable	Cond	Pre	0 h	24 h	48 h	72 h
Doublet Maximal Rate of Force Development ($N \cdot s^{-1}$)	PLA	1664 ± 402	1631 ± 379	1602 ± 406	1649 ± 412	1681 ± 310
	CHO	1650 ± 406	1594 ± 318	1647 ± 356	1618 ± 328	1613 ± 318
	PRO	1679 ± 422	1656 ± 427	1616 ± 410	1652 ± 365	1671 ± 359
Doublet Maximal Rate of Force Decrease ($N \cdot s^{-1}$)	PLA	-1326 ± 354	-1348 ± 371	-1301 ± 390	-1324 ± 389	-1339 ± 335
	CHO	-1315 ± 362	-1326 ± 294	-1311 ± 372	-1330 ± 292	-1319 ± 304
	PRO	-1350 ± 407	-1405 ± 420	-1315 ± 397	-1339 ± 383	-1343 ± 344
Rate Constant for Contraction ($\cdot s^{-1}$)	PLA	9.6 ± 0.6	9.5 ± 0.5	9.5 ± 0.7	9.6 ± 0.7	9.6 ± 0.5
	CHO	9.6 ± 0.5	9.6 ± 0.5	9.6 ± 0.5	9.5 ± 0.6	9.6 ± 0.5
	PRO	9.6 ± 0.7	9.6 ± 0.6	9.5 ± 0.6	9.7 ± 0.6	9.6 ± 0.5
Rate Constant for Relaxation ($\cdot s^{-1}$)	PLA	-7.7 ± 1.1	-7.8 ± 1.0	-7.7 ± 1.2	7.7 ± 1.3	-7.6 ± 1.3
	CHO	-7.7 ± 1.0	-8.0 ± 0.8 *	-7.6 ± 1.2	-7.9 ± 1.1	-7.9 ± 1.0
	PRO	-7.7 ± 1.4	-8.1 ± 1.0	-7.8 ± 1.2	-7.8 ± 1.2	-7.7 ± 1.0
20:50 Hz Ratio	PLA	0.88 ± 0.04	0.83 ± 0.06 *	0.83 ± 0.07	0.83 ± 0.06 *	0.85 ± 0.06
	CHO	0.87 ± 0.05	0.85 ± 0.06	0.83 ± 0.04	0.83 ± 0.03	0.85 ± 0.07
	PRO	0.84 ± 0.06 †	0.82 ± 0.07	0.81 ± 0.08	0.83 ± 0.05	0.86 ± 0.06

Table 10.3 – Peak torque of the knee extensors and flexors during isokinetic contractions at 60 and 180 °·s^{-1} measured before (Pre) and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) on a level gradient (0%) carrying a 25 kg backpack. Either a placebo beverage (PLA), carbohydrate (6.4%) beverage (CHO) or protein (7 %) beverage (PRO) was consumed at 0 and 60 minutes (250 ml) during treadmill walking and twice daily (morning and evening) for the 3 days following load carriage (n = 10). Difference from pre measurement (* P<0.05, ** P<0.01, *** P<0.001).

Variable	Condition	Peak Torque (Nm)				
		Pre	0 h	24 h	48 h	72 h
Extension (60 °·s^{-1}) (n=9)	PLA	234 ± 34	219 ± 34 **	215 ± 42 *	214 ± 33 **	231 ± 37
	CHO	232 ± 33	217 ± 26 **	220 ± 32 *	230 ± 34	233 ± 38
	PRO	230 ± 42	219 ± 42 *	219 ± 46	227 ± 40	224 ± 41
Extension (180 °·s^{-1}) (n=8)	PLA	148 ± 20	145 ± 22	141 ± 28	149 ± 23	154 ± 24
	CHO	150 ± 17	151 ± 16	152 ± 19	155 ± 21	147 ± 20
	PRO	150 ± 24	149 ± 23	140 ± 28	145 ± 24	146 ± 22
Flexion (60 °·s^{-1}) (n=9)	PLA	137 ± 24	125 ± 25 **	126 ± 23 **	128 ± 23 **	133 ± 27
	CHO	136 ± 20	123 ± 25 *	128 ± 23 *	129 ± 23	130 ± 25
	PRO	135 ± 23	123 ± 20 *	129 ± 21	136 ± 13	133 ± 14
Flexion (180 °·s^{-1}) (n=8)	PLA	95 ± 21	85 ± 20	90 ± 28	97 ± 21	102 ± 18
	CHO	97 ± 9	86 ± 17 *	96 ± 21	95 ± 17	92 ± 16
	PRO	98 ± 13	90 ± 15	96 ± 15	99 ± 19	97 ± 14

Table 10.4 – Peak torque of the trunk extensors and flexors during isokinetic contractions at 60 and 180 °·s^{-1} measured before (Pre) and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 km·h^{-1} (n=10) on a level gradient (0%) carrying a 25 kg backpack. Either a placebo beverage (PLA), carbohydrate (6.4%) beverage (CHO) or protein (7 %) beverage (PRO) was consumed at 0 and 60 minutes (250 ml) during treadmill walking and twice daily (morning and evening) for the 3 days following load carriage (n = 10). Difference from pre measurement (* P<0.05, ** P<0.01, *** P<0.001).

Variable	Condition	Peak Torque (Nm)				
		Pre	0 h	24 h	48 h	72 h
Extension (15 °·s^{-1}) (n=9)	PLA	243 ± 55	218 ± 71 *	220 ± 57 *	244 ± 54	237 ± 56
	CHO	259 ± 49	221 ± 45 *	236 ± 63	252 ± 60	256 ± 61
	PRO	242 ± 54	218 ± 62 *	243 ± 55	249 ± 73	241 ± 65
Extension (60 °·s^{-1}) (n=9)	PLA	232 ± 56	204 ± 65 *	207 ± 55**	227 ± 43	230 ± 60
	CHO	226 ± 61	196 ± 51 *	221 ± 76	218 ± 72	243 ± 75
	PRO	232 ± 77	196 ± 60	229 ± 68	244 ± 62	233 ± 81
Flexion (15 °·s^{-1}) (n=10)	PLA	281 ± 38	252 ± 39 **	264 ± 41	265 ± 45	273 ± 38
	CHO	282 ± 40	259 ± 44 *	283 ± 33	282 ± 26	280 ± 33
	PRO	272 ± 37	247 ± 35 **	263 ± 43	268 ± 36	270 ± 37
Flexion (60 °·s^{-1}) (n=10)	PLA	298 ± 36	275 ± 34 *	298 ± 28	296 ± 44	310 ± 25
	CHO	300 ± 36	284 ± 40 *	301 ± 35	300 ± 32	299 ± 40
	PRO	299 ± 36	277 ± 38 **	289 ± 43	296 ± 44	299 ± 34

Table 10.5 – Peak torque of the shoulder extensors and flexors during isokinetic contractions at 60 and 180 $^{\circ}\cdot s^{-1}$ measured before (Pre) and after (0, 24, 48 and 72 h) 120 minutes of treadmill walking at 6.5 $km\cdot h^{-1}$ (n=10) on a level gradient (0%) carrying a 25 kg backpack. Either a placebo beverage (PLA), carbohydrate (6.4%) beverage (CHO) or protein (7 %) beverage (PRO) was consumed at 0 and 60 minutes (250 ml) during treadmill walking and twice daily (morning and evening) for the 3 days following load carriage (n = 10).

Variable	Condition	Peak Torque (Nm)				
		Pre	0 h	24 h	48 h	72 h
Extension ($60\ ^{\circ}\cdot s^{-1}$)	PLA	106 ± 17	105 ± 20	108 ± 20	112 ± 22	110 ± 19
	CHO	95 ± 19	95 ± 19	98 ± 18	95 ± 18	99 ± 20
	PRO	115 ± 18	108 ± 20	109 ± 22	113 ± 23	111 ± 22
Extension ($180\ ^{\circ}\cdot s^{-1}$)	PLA	85 ± 11	84 ± 12	87 ± 13	90 ± 12	89 ± 13
	CHO	89 ± 12	91 ± 10	90 ± 9	92 ± 11	91 ± 15
	PRO	91 ± 14	87 ± 13	90 ± 15	92 ± 11	90 ± 11
Flexion ($60\ ^{\circ}\cdot s^{-1}$)	PLA	75 ± 16	73 ± 20	75 ± 18	78 ± 17	80 ± 21
	CHO	76 ± 11	76 ± 13	80 ± 18	80 ± 20	82 ± 18
	PRO	81 ± 16	75 ± 14	74 ± 20	79 ± 20	77 ± 14
Flexion ($180\ ^{\circ}\cdot s^{-1}$)	PLA	56 ± 9	51 ± 12	53 ± 8	53 ± 7	55 ± 8
	CHO	55 ± 7	53 ± 7	54 ± 9	55 ± 6	52 ± 8
	PRO	55 ± 8	53 ± 11	54 ± 10	55 ± 11	57 ± 9

10.4. Discussion

The present study is the first to show differences in the time course of recovery of the force producing capability of muscle groups following load carriage with carbohydrate and whey protein supplements. In this study the effects of carbohydrate and whey protein beverages on the recovery of neuromuscular function were investigated following 120 minutes treadmill walking at 6.5 $km \cdot h^{-1}$ carrying a 25 kg backpack. Immediately after load carriage the decrease in isokinetic torque (60 $° \cdot s^{-1}$) of the knee extensors and flexors were similar for all conditions. However, consumption of the whey protein beverage resulted in faster recovery of the peak torque of the knee extensors and flexors than both placebo and carbohydrate beverage conditions. The carbohydrate beverage resulted in faster recovery of isokinetic peak torque (60 $° \cdot s^{-1}$) of the knee extensor and flexors compared to the placebo beverage. The decrease in maximal isometric force production of the knee extensors was similar in all conditions following load carriage. Isometric knee extension force recovered to pre-exercise value 48 h after load carriage with consumption of the carbohydrate and whey protein beverages but remained below pre-exercise value until 72 h after load carriage in the placebo condition. Similarly trunk extensor peak torque (15 $° \cdot s^{-1}$) decreased following load carriage and there was no difference between conditions. Peak toque had recovered by 24 h in the whey protein and carbohydrate conditions but only returned to pre-exercise value in the placebo condition 48 h after load carriage.

These findings confirm the hypothesis that (1) compared to a placebo consumption of a carbohydrate supplement results in earlier recovery of muscle force producing capability following load carriage (2) compared to a placebo consumption of a whey protein supplement results in earlier recovery of muscle force producing capability following load carriage.

The reductions in force producing capability of the muscles measured in the present study support Clarke *et al.* (1955) data, which showed decreases in strength of the knee and trunk extensors and flexors following a 12.1 km road march at 4 $km \cdot h^{-1}$ carrying a 27 kg load. In comparison to the changes observed immediately after PLA in the present study, Clarke *et al.* (1955) observed greater decreases in knee extensor peak torque (6 vs. 8 %) but lesser decreases in knee flexor peak torque (9 vs. 6 %). Reductions were comparable for changes in trunk extensor (12 vs. 11 %) and flexor peak torque (10 vs. 11 %). No changes in force producing capability of the shoulder extensors or flexors were observed following load

carriage in the present study and these muscle groups were not measured by Clarke *et al.* (1955). Isometric knee extension force decreased by 14 % immediately after load carriage during PLA. However, Place *et al.* (2004) showed no decrease in isometric knee extensor MVC force after 2 hours of running at approximately 55 %$\dot{V}$ O_2max, despite a higher work rate during the run compared to load carriage (25–40 %$\dot{V}$ O_2max) (Patton *et al.*, 1991). Decreases in isometric MVC force of between 15 % (Martin *et al.*, 2004b) and 63 % (Gauche *et al.*, 2006) have been observed following other running based endurance exercise, but the duration and intensity of these events varied and were greater than the load carriage bout in the present study.

10.4.1. Knee Extensors

Figure 10.1 shows there was no difference between conditions in the reduction in peak torque (60 °·s^{-1}) of the knee extensors immediately after load carriage. During recovery, PRO returned to the pre-exercise value at 24 h, followed by CHO at 48 h and PLA at 72 h. Consuming whey protein supplements during resistance training has been shown to improve muscle hypertrophy (Hayes and Cribb, 2008), maintain a positive protein balance (Hawley *et al.*, 2006) and reduce the concentration of plasma markers of muscle damage (van Loon, 2007). Following endurance exercise, only the effect of amino acid ingestion on protein turnover has been investigated (Tipton and Wolfe, 1998). The effect of amino acid ingestion on recovery of the force producing capability of muscles following resistance and endurance exercise has received little attention. Buckley *et al.* (2008) observed a ~23 % decrease in isometric MVC force of the knee extensors following 100 maximal eccentric contractions. During the 24 h recovery measured by Buckley *et al.* (2008), isometric MVC force and did not recover to pre-exercise values when flavoured water consumed, but consumption of 25 g of whey protein hydrolysate immediately after exercise resulted in complete recovery of isometric MVC force by 6 h post-exercise.

Protein supplementation during and following exercise promotes and provides building blocks for *de novo* protein synthesis and reduces protein degradation, ensuring a positive protein balance (Koopman *et al.*, 2007). Maintenance of an anabolic rather than catabolic environment enhances muscle protein accretion (van Loon, 2007), probably resulting in enhanced repair of the structural muscle proteins damaged during exercise. Indeed, Nosaka (2007) suggested the greater rate of protein synthesis and reduced protein breakdown when amino acids are ingested will reduce the magnitude of muscle damage and improve the rate of

recovery. This may explain faster recovery of the force producing capability of knee extensor peak torque (60 $°\cdot s^{-1}$) during PRO compared to PLA and CHO.

The data in the present study show that carbohydrate supplementation during load carriage does not effect the force producing capability of the knee extensors immediately after load carriage. However, compared to PLA, the carbohydrate supplement showed beneficial effects in promoting faster recovery of neuromuscular function. In contrast to these findings, Nelson *et al.* (2004), showed no effect on the recovery of neuromuscular function following a 15 minute downhill run in a glycogen depleted state when a high carbohydrate diet (80 % carbohydrate) was consumed compared to no food. However, Nelson *et al.* (2004) only provided a single high carbohydrate meal immediately after exercise and subsequent dietary intake was not recorded or controlled between groups.

During prolonged exercise muscle glycogen stores have been shown to be reduced (Costill *et al.*, 1973) and fatigue coincides with depleted glycogen stores in the working muscles (Coyle, 1992). Glycogen depleted fibres exhibit higher energy deficiency due to elevated post exercise Inosine 5'-Monophosphate (IMP) concentrations (a marker of the mismatch between ATP re-synthesis and degradation) (Norman *et al.*, 1988). In addition, Chin and Allen (1997) showed the force producing capability of glycogen depleted fibres is impaired in vitro. Although these data suggest neuromuscular function may be compromised by glycogen depletion there is no experimental evidence linking muscle glycogen concentration and the force producing capability during isometric or isokinetic contractions in vivo. This is probably due to the difficulty is separating the range of biochemical and structural changes that also occur in addition to glycogen depletion following exercise (Sahlin *et al.*, 1998). Also, the energy used to produce a 3-5 s maximal contraction will not rely upon energy derived from carbohydrate stores.

The extent to which the carbohydrate supplement in the present study enhanced muscle glycogen stores is debatable. The effect of ingestion of carbohydrate during exercise in sparing muscle or liver glycogen stores appears to be dependent on exercise mode, intensity and duration (Tsintzas and Williams, 1998). The provision of the carbohydrate supplements following exercise has been shown to improve glycogen synthesis (Millard-Stafford *et al.*, 2008). But, in the present study 500 mL of the 6.4 % carbohydrate supplement was consumed twice daily in one bolus, providing 32 g of carbohydrate (~0.3 $g\cdot kg\ body\ mass^{-1}\cdot h^{-1}$ in the hour following exercise), which is considerably less than the 1.2 $g\cdot kg\ body\ mass^{-1}\cdot h^{-1}$ believed to

be optimal for restoration of muscle glycogen (Ivy, 2001). Also, the benefits of exogenous carbohydrate supplementation to restore muscle glycogen are appear to occur in the first 4-6 hours following an exercise bout (Hawley *et al.*, 2006; Millard-Stafford *et al.*, 2008). Therefore, the effects are likely to have only been observed up to 24 h following load carriage in the present study.

The preceding discussion suggests carbohydrate supplementation had a minimal effect in improving muscle glycogen concentration and if so it is unlikely to account for the improved force producing capability of the muscles in the present study. Importantly, the carbohydrate supplement would have increased blood glucose and stimulated insulin release. Insulin has been shown to increase the rate of protein synthesis at rest and attenuate the rate of protein breakdown following exercise (Wolfe, 2001). Although a completely positive protein balance can only be achieved with the provision of amino acids (Tipton and Wolfe, 2004). Therefore, CHO may have decreased the negative protein balance following exercise compared to PLA, slowing the degradation of structural proteins, which as discussed above, may have improved neuromuscular function.

The mechanisms acting on protein synthesis and breakdown may also contribute to the differences in the rates of recovery observed during CHO and PRO. Both the carbohydrate and whey protein supplements reduce the negative protein balance. But, only the supply of amino acids in the whey protein supplement restores a positive protein balance (Tipton and Wolfe, 2004). As discussed previously, this is likely to be beneficial in repairing and rebuilding the structural proteins of the muscle damaged during exercise (van Loon, 2007). This may be of particular importance in the period between 24 and 72 h after exercise when protein degradation rate is at its peak and contractile protein loss is an important component of strength losses (Warren *et al.*, 2002). Hence, the additional amino acids supplied during PRO may enhance the repair of the muscle structural proteins, resulting in earlier recovery of the knee extensor peak torque (60 $°\cdot s^{-1}$) to pre-exercise value during PRO, compared to CHO.

There was no change in knee extension peak torque (180 $°\cdot s^{-1}$) following load carriage in any condition. There is debate as to whether different isokinetic test velocities recruit different fibre types (i.e. Type I or Type II) (Friden *et al.*, 1983a; Perrin, 1993). Friden *et al.* (1983a) suggested that type II fibres are primarily responsible for development of tension at higher angular velocities. If this contention is correct, the present study's data suggests that

primarily type I fibres are recruited during load carriage and therefore exposed to greater damage than type II fibres. However, this requires further investigation.

Figure 10.1 shows isometric MVC force of the knee extensors decreased following load carriage, but there were no differences between conditions. Isometric force producing capability returned to pre-exercise value at 48 h during both CHO and PRO but during PLA returned to pre-exercise values at 72 h. The mechanisms responsible for the faster recovery during CHO and PRO are likely to be the same as discussed previously for the isokinetic contractions. However, during PRO and CHO the force producing capability during the isokinetic (60 $°\cdot s^{-1}$) contractions showed a different pattern of recovery compared to the isometric contractions. Compared to an isometric contraction; during an isokinetic (shortening) contraction fewer cross-bridges are attached as the actin binding sites move past the myosin cross bridges (Jones *et al.*, 2004). During a maximal dynamic contraction each actin-myosin complex is required to produce as much force as possible for the limited time of the cross bridge cycle (Green, 1997). This suggests that at 24 h in CHO during the isokinetic contractions binding sites were less effective, resulting in a lower torque production. Conversely, during PRO at 24 h the enhanced repair of structural proteins discussed previously may have improved the effectiveness of binding sites resulting in a peak torque equal to the pre-exercise value. These effects may have been less evident during the isometric contraction as more cross bridges are formed. However, this remains rather speculative and requires further investigation.

The 20:50 Hz ratio decreased immediately after PLA and remained below pre-exercise value until 72 h after the load carriage bout; however, there was no change in the 20:50 Hz ratio following CHO or PRO. Comparisons with the PRO condition should be interpreted with caution due to the lower pre-exercise 20:50 Hz value (Table 10.2). The decrease in the 20:50 Hz ratio following PLA shows the presence of LFF, indicating a reduction in Ca^{2+} release from the sarcoplasmic reticulum and/or a redistribution of sarcomere lengths (i.e. popping sarcomere theory) (Jones, 1996). It is believed this is as a consequence of damage to the structure of the muscle fibre and impairment of the excitation-contraction coupling process (Allen *et al.*, 2008; Chin *et al.*, 1997). LFF has been observed following a 30 km run (Millet *et al.*, 2003a), but not following a 42 km running race (Petersen *et al.*, 2007) or a 300 min treadmill run at 55 % of maximal aerobic velocity (Place *et al.*, 2004). Lepers and Millet (2004) concluded that during prolonged exercise (such as load carriage) this damage is most

likely due to the forces absorbed during eccentric contractions rather than metabolic changes. Reductions in the force and Ca^{2+} release have also been shown to be closely associated with reduced muscle glycogen concentration (Chin and Allen, 1997). As discussed previously, it is possible that the carbohydrate supplement caused a sparing muscle glycogen or enhanced glycogen synthesis during recovery, thus attenuating the LFF that were observed in PLA. Westerbald *et al.* (1993) concluded the force loss that accompanies LFF is most likely due to a structural change to one of the proteins involved in excitation-contraction coupling and the time course of recovery of LFF represents the repair or re-synthesis of this protein. As discussed previously, consumption of amino acids or carbohydrate results in a more positive protein balance both during and following exercise (Kumar *et al.*, 2009; Wolfe, 2000a). Thus, the positive protein balance during PRO and CHO may have attenuated some of the structural damage observed during PLA, resulting in the maintenance of the 20:50 Hz ratio.

Interestingly, VA decreased immediately after load carriage during PRO only and remained above pre-exercise value from 24 h onwards during recovery (Table 10.2). This is surprising as it has been suggested that branched chain amino acids (BCAA) are a beneficial nutrient in delaying the onset of central fatigue as they compete with tryoptophan for transport into the brain and consequently a reduce serotonin release (Fernstrom, 2005). BCAA constitute approximately 23 % of the whey protein supplement. However, during sustained exercise BCAAs are also taken up by the muscle and the plasma concentration decreases, giving rise to more tryoptophan crossing the blood brain barrier (Newsholme and Blomstrand, 2006). Whey proteins also contain between 20–25 % of alpha-lactalbumin, ingestion of which has been indirectly shown to increase brain serotonin activity (Markus *et al.*, 2002). Thus, the net effect of the ingestion of a large bolus (33 g) of whey protein during endurance exercise may actually increase brain serotonin activity. Evidence suggests that increases in brain serotonin activity during prolonged exercise hastens central fatigue (Davis and Bailey, 1997). Therefore, the reduction in VA immediately after load carriage during PRO may have been due to the effect of certain components in whey protein supplement on serotonin release.

10.4.2. Knee Flexors

Knee flexor peak torque (60 $°\cdot s^{-1}$) decreased immediately after load carriage, but there was no difference between conditions (Figure 10.2). The pattern of recovery was identical to the recovery of the force producing capability of the knee extensors at 60 $°\cdot s^{-1}$. As discussed previously the decrease in force producing capability was similar to that observed by Clarke *et*

al. (1955), following a 12.1 km road march at 4 km·h^{-1} carrying a load of 27 kg. However, no other research has examined the recovery of knee flexors following prolonged exercise. The differences in the rate of recovery between conditions are probably due to the same mechanisms as discussed previously for the knee extensors. Knee flexor peak torque (180 °·s^{-1}) decreased following load carriage for CHO only, then returned and remained at the pre-exercise value (Table 10.3). However, the reason for the difference between conditions is unclear.

10.4.3. Trunk Extensors and Flexors

Trunk extension peak torque (15 °·s^{-1}) decreased immediately after load carriage, but there was no difference between conditions. Both CHO and PRO returned to pre-exercise value by 24 h but PLA remained below pre-exercise value until 48 h (Table 10.4). The neuromuscular impairment caused by load carriage was probably as a result of muscle recruitment (e.g. *m. rectus abdominis* and paraspinal muscles) to support the backpack load compared to unloaded walking (Al-Khabbaz *et al.*, 2008; Cook and Neumann, 1987). The faster recovery to pre-exercise value in the CHO and PRO conditions is likely to be due to the effect of the supplements in restoring neuromuscular function, as discussed previously. At the faster test velocity (60 °·s^{-1}) peak torque decreased immediately after load carriage during PLA and CHO recovering at 24 and 48 h, respectively. There was a trend (P=0.079) for a reduction in peak torque immediately after load carriage during PRO, but this did not reach statistical significance, most probably due to a high individual variation in the response rather than an effect of the whey protein supplement.

At both the low (15 °·s^{-1}) and high (60 °·s^{-1}) isokinetic test velocities trunk flexion peak torque decreased immediately after load carriage and returned to and remained at pre-exercise value at 24 h (Table 10.4). These data indicate that due to the time course of recovery of the neuromuscular impairment of the trunk extensor muscles was more severe than the trunk flexors and the supplementation had no benefit in restoring neuromuscular function of the trunk extensors.

10.4.4. Shoulder Extensors and Flexors

There was no change in peak torque at the low (60 °·s^{-1}) or high (180 °·s^{-1}) test velocities for the shoulder extensors or flexors during recovery from load carriage in any condition. This is surprising as carrying backpacks has been shown to increase activity of the

supporting muscles (e.g. *m. trapezius*) (Holewijn, 1990). In addition Clarke *et al.* (1955), observed decreases in the strength of the shoulder elevators following a 12.1 km road march at 4 km·h^{-1} carrying a 27 kg load. Pressure from the backpack straps has also been suggested to restrict blood flow to parts of the shoulder extensors and flexors resulting in local anaerobic processes and accumulation of metabolites (Legg *et al.*, 1997), which can also contribute to neuromuscular impairment (Tee *et al.*, 2007).

10.4.5. Nutritional Intake

There were no differences in dietary intake of energy, carbohydrate, fat or protein over the 72 h that recovery of neuromuscular function was measured following load carriage (Table 10.1). This supports to the earlier conclusions that the faster recovery of neuromuscular function during PRO and CHO was due to the supplements rather than any alterations in dietary intake of nutrients. Compared to most recent Institute of Medicine recommendations (2002), the data in table 10.1 suggested that during the 72 h after the load carriage bout the participants in the present study were approximately in deficit of 1173 Kcal·day^{-1} energy, 129 g·day^{-1} carbohydrate and 37 g·day^{-1} fat, but participants did consume 16 g·day^{-1} protein above recommended guidelines. However, it has been shown that self report food diaries consistently underreport nutritional intake (Trabulsi and Schoeller, 2001). Participants maintained their normal dietary intake throughout the study and were weighed prior to each load carriage bout, the number of days between their first and last test was 41 ± 29 days. Assuming surplus energy is stored on a fat:fat free mass ratio of 75:25, a change in body mass of 1 kg can be assumed to be equivalent of ~7170 kcal (Westerterp *et al.*, 1995). If the participants had been in negative energy balance of ~1173 Kcal (as the food diaries indicate) for ~41 days (time between first and last body mass measurement) participants would have lost an average ~6.7 kg. However, there was no difference in body mass between the first and last load carriage bout (82.0 ± 10.2 vs. 82.0 ± 10.7 kg, P=0.990). These findings suggest participants consistently underreported their real food intake in all three test conditions and were not in negative energy balance or nutritional deficit during the recovery of period.

The two boluses of carbohydrate and protein beverages provided an additional 260 Kcal and 352 Kcal·day^{-1}, respectively. However, additional energy intake *per se* does not have an influence on muscle protein metabolism following exercise, but may do if individuals were in chronic energy deficit (Tipton and Wolfe, 2004). This suggests the provision of glucose (CHO) and amino acids (PRO) in the supplements rather than the provision of energy were

responsible for the differences in recovery of the force producing capability of the muscles between conditions.

10.4.6. Applied Perspective

In addition to the decrement in strength, neuromuscular impairment has a negative impact on endurance activities, causing increases in $\dot{V}O_2$ and heart rate at a set running pace (Chen *et al.*, 2007), decreases motor control during skilled tasks (Byrne *et al.*, 2004) and sprint performance (Twist and Eston, 2005). The enhanced recovery of the force producing capability of certain muscles with the consumption of the carbohydrate and protein beverages is likely to result in a faster recovery from the neuromuscular impairment. Consequently, this may have a positive effect on the endurance, motor control and sprinting tasks, however, this requires further investigation.

When muscles become fatigued (i.e. reduction in force-generating capability) the amount of force they are able to absorb before injury (i.e. muscle strains) is reduced (Mair *et al.*, 1996). During physical activity impact forces must still be absorbed, which is likely to be through other muscles or the supporting structures (i.e. connective tissue and bones). Thus, if physical activity is undertaken when muscles are in a weakened state (e.g. following load carriage) the possibility of musculoskeletal injury is likely to be increased. Therefore, the improved recovery of neuromuscular function during CHO and PRO may also reduce the risk of musculoskeletal injury in subsequent physical activity (e.g. training, competition or occupational work). In support of this contention, Flakoll *et al.* (2004) showed a reduction in injury rates when 10 g of a protein supplement was provided after exercise compared to an non-protein control during 54 day military basic training course (containing bouts of load carriage).

10.4.7. Conclusion

Load carriage resulted in similar reductions in the force producing capability of the knee, trunk and shoulder extensors immediately after load carriage, independent of the supplement consumed. However, during recovery, ingestion of a whey protein beverage resulted in the return isokinetic knee extensor and flexors peak torque (60 $°\cdot s^{-1}$) to pre-exercise values at 24 h compared to the carbohydrate beverage at 48 h and placebo beverage at 72 h. When the placebo beverage was consumed, isometric knee extension peak force and isokinetic trunk extension peak torque (15 $°\cdot s^{-1}$) returned to pre-exercise values at 48 and 72 h after load

carriage, respectively. Ingestion of the whey protein and carbohydrate beverages both resulted in recovery of isometric knee extension force at 24 h and trunk extension peak torque (15 $°\cdot s^{-1}$) at 48 h. The faster recovery of neuromuscular function following load carriage was probably due to the carbohydrate and whey protein beverages stimulating protein synthesis and attenuating protein breakdown. The maintenance of an anabolic environment is likely to have enhanced the repair of the muscle tissue damaged during exercise therefore improving their force producing capability.

Chapter 11. General Discussion

The purpose of this thesis was to investigate the physiological responses to load carriage, with particular reference to neuromuscular function. A 19.3 km load carriage event in the field was shown to cause neuromuscular impairment in a trained population (i.e. Royal Marines) in an occupational setting (i.e. military training) (Chapter 3). The use of trained load carriers in an occupational setting provided ecological validity to the findings, but detailed measures of neuromuscular function could not be made as participants were undertaking a military training program. Therefore, all further experiments were conducted in the laboratory using participants with mixed load carriage experience. To accommodate for the differences in load carriage experience, compared to the field study, the laboratory load carriage task was of a shorter duration (280 vs. 120 minutes) and a lighter load (31 vs. 25 kg). In the laboratory, the reliability of a battery of voluntary and electrically stimulated contractions was established to measure changes in neuromuscular function immediately after and in the days following a 120 minute exercise bout (Chapters 4 and 5). The physiological responses during 120 minutes of treadmill walking (LW) with load carriage on level (0 %) (LWLC) and downhill (-8 %) (DWLC) gradients were measured (Chapter 6). The test battery (Chapters 4 and 5) was used to investigate the effects of 120 minutes of load carriage on neuromuscular function at 0, 24, 48 and 72 hours after the load carriage on level (0 %) and downhill (-8 %) gradients (Chapter 7). Chapter 3 showed the physiological responses to load carriage varied between individuals of different body mass. Therefore, the physiological determinants of participants' metabolic and neuromuscular responses to the 120 minutes of load carriage on a level gradient were established (Chapter 8). The effects of carbohydrate and whey protein beverages on the metabolic, cardiovascular and neuromuscular responses during 120 minutes of load carriage on a level gradient were compared (Chapter 9). In the days following load carriage, the effect of continuing to consume carbohydrate and whey protein beverages on the recovery of neuromuscular function was measured at 0, 24, 48 and 72 hours after exercise (Chapter 10)

11.1. Physiological Responses during Load Carriage

A 19.3 km load carriage event in the field walking at 5.2 $km \cdot h^{-1}$ carrying 31 kg load elicited a cardiovascular strain of 72 ± 5 %HRmax and an estimated oxygen cost of between 19.1 ± 0.6 and 33.5 ± 4.6 $mL \cdot kg^{-1} \cdot min^{-1}$. Due to the nature of the field study (i.e. as part of military training) more detailed physiological measures could not be taken (Chapter 3).

In the laboratory, exercise intensity whilst walking at 6.5 $km \cdot h^{-1}$ carrying a 25 kg backpack was 25.0 ± 2.4 $mL \cdot kg^{-1} \cdot min^{-1}$ and 68 ± 6 %HRmax after 60 minutes of exercise, similar to the cardiovascular strain and estimated oxygen cost of the load carriage task in the field. During LW, $\dot{V}O_2$ gradually increased over time ($\dot{V}O_2$drift) (Chapter 6). LWLC caused a higher $\dot{V}O_2$ at baseline (minute 5) and greater $\dot{V}O_2$drift between minutes 5 and 120, compared to LW. However, there was no difference in the magnitude of $\dot{V}O_2$drift during DWLC compared to LWLC, despite a lower $\dot{V}O_2$ at baseline during DWLC. Identical trends were also observed for HR. The increase in $\dot{V}O_2$ and HR at baseline during LWLC compared to LW is likely to be due to an increase in muscle fibre recruitment to maintain posture and locomotion (Bobet and Norman, 1984; Ghori and Luckwill, 1985; Holewijn, 1990). The decrease in $\dot{V}O_2$ and HR at baseline during DWLC was due to the reduced workload when walking downhill and was in keeping with previous studies (Santee *et al.*, 2001).

$\dot{V}O_2$drift during load carriage was partly attributed to fatigue and/or damage to muscle fibres. As fibres become fatigued and/or damaged, additional motor units are recruited to maintain movement on the treadmill at the required speed and to support the load, increasing the demand for oxygen and potentially driving an upward drift in $\dot{V}O_2$. A change in muscle fibre recruitment may result in alterations in gait and posture, resulting in a poorer walking economy, further increasing $\dot{V}O_2$ (Cavanagh and Williams, 1982). This series of events is likely to be exacerbated when walking downhill when more emphasise is placed on the eccentric component of the stretch shortening cycle (Tee *et al.*, 2007), therefore increasing neuromuscular impairment (Clarkson and Newham, 1995). RER decreased during LWLC only, indicating a shift from carbohydrate to fat as an energy source. However, based on equations reported by Jeukendrup and Wallis (2005), the additional O_2 requirements for fat oxidation would only account for 5 % of the increase in $\dot{V}O_2$ during load carriage. Sweat losses (estimated by changes in body mass) were less than fluid intake during LWLC and DWLC, suggesting participants became dehydrated, which may have exacerbated the $\dot{V}O_2$ and HR responses over time.

A limitation of the study conducted in Chapter 6 was the lack of measurement of muscle activation during treadmill walking. Dick & Cavanagh (1987) showed a 10 % increase in $\dot{V}O_2$ during a 40 minute downhill run at 44 % $\dot{V}O_2$max with a corresponding 23 % increase in integrated electromyography signal (IEMG). However, no changes in $\dot{V}O_2$ or

IEMG were observed during 40 minutes of level running at 66 % $\dot{V}O_2$max. The authors concluded that the $\dot{V}O_2$drift observed during downhill running was due to muscle fibre damage and the recruitment of additional muscle fibres. This shortfall was addressed in Chapter 9 when EMG was used to measure changes in activation of the *m. rectus femoris, m. vastus lateralis, m. semitendinosus or m. biceps femoris* between 7 and 107 minutes of a 120 minute bout of load carriage. There was no mean change in muscle activation in any of the muscle groups measured for the 10 participants. However, individual participants' responses were highly variable for each muscle group. For example, EMG activity of the *m. vastus lateralis* increased for four participants and decreased for six participants. These findings suggest individual adaptations in muscle recruitment occurred over the duration of the load carriage bout.

Chapter 8 investigated the physiological determinants of load carriage performance. The findings showed individuals with the most efficient metabolic and neuromuscular performance during load carriage are those with a large body mass, high absolute $\dot{V}O_2$max and strong trunk, shoulder and knee flexors. Therefore improvement of, or selecting individuals with, these characteristics is likely to improve the ability to undertake load carriage tasks.

11.2. Neuromuscular Consequences of Load Carriage

Chapter 3 showed that load carriage over 19.3 km carrying a 31 kg load in the field caused a 7 ± 8 % reduction in vertical jump height, indicating the presence of neuromuscular impairment. Changes in neuromuscular function immediately post and in the days following load carriage were further investigated in the laboratory whilst carrying a 25 kg backpack during 120 minutes of treadmill walking (6.5 $km \cdot h^{-1}$) on level (0 %) and downhill (-8 %) gradients (Chapter 7). Neuromuscular function was assessed at 0, 24, 48 and 72 h following the load carriage bout using the test battery of voluntary and electrically stimulated contractions validated in Chapters 4 and 5.

Following LW there were no changes in force producing capability of any muscle group during the isometric or isokinetic contractions. However, neuromuscular impairment was apparent immediately post and in the days following LWLC and DWLC. Immediately after LWLC and DWLC, there were similar decreases in isokinetic peak torque (60 $°\cdot s^{-1}$) of the knee extensors (14 vs. 14 %) and flexors (13 vs. 14 %). Trunk extensor peak torque (15

$°\cdot s^{-1}$) decreased after LWLC only (8 %). A trend indicated the reduction in trunk flexor peak torque (15 $°\cdot s^{-1}$) immediately after LWLC was less than DWLC (10 vs. 18 %). Shoulder extensor peak torque (60 $°\cdot s^{-1}$) decreased after LWLC only (10 %). There was a trend for a lesser decrease in shoulder flexor peak torque (60 $°\cdot s^{-1}$) immediately after LWLC compared to DWLC (6 vs. 11 %).

Force producing capability of all muscle groups began to recover towards baseline in the following days. Shoulder flexor peak torque (60 $°\cdot s^{-1}$) fully recovered by 48 and 24 hours after LWLC and DWLC, respectively. Trunk flexor peak torque (15 $°\cdot s^{-1}$) fully recovered 24 and 48 hours after LWLC and DWLC, respectively. Isometric force producing capability of the knee extensors fully recovered 72 hours after DWLC but was still below baseline following LWLC. The slower time course of recovery following both LWLC and DWLC suggests that neuromuscular impairment was greatest in the lower limbs.

Differences in the time course of recovery between LWLC and DWLC suggest variations in the patterns of neuromuscular impairment. The findings indicate that a greater forward lean with load carriage on a negative gradient placed additional strain on the trunk and shoulder flexors, causing greater neuromuscular impairment. In addition, strain was removed from the trunk and shoulder extensors during DWLC; reducing the neuromuscular impairment of these muscle groups.

It was hypothesised that DWLC would cause greater neuromuscular impairment, due to the greater emphasis placed on the eccentric component of the stretch shortening cycles when walking on a negative gradient. Interestingly, there was no evidence to support this hypothesis and to the contrary the force producing capability of the knee extensors recovered to pre-exercise values by 72 hours during DWLC but not LWLC. The slower time course of recovery suggests more severe neuromuscular impairment following LWLC. However, this finding should be interpreted with caution as only six muscle groups were measured in the present study each showing different reductions and rates of recovery following exercise (Chapter 7). This result is surprising, as neuromuscular impairment has been shown to be greater during running on downhill compared to level gradients (Dick and Cavanagh, 1987).

Participants showed individual variations in the magnitude of the decrease the force producing capability of muscle groups and the time course of recovery. Chapter 8 showed neuromuscular impairment of the knee extensors during an isometric contraction following

LWLC was explained by the force producing capability of the shoulder, trunk and knee flexors and relative $\dot{V}O_2$max. Therefore, differences in force producing capability of these muscle groups and aerobic fitness between individuals are likely to account for the inter-participant differences in the neuromuscular impairment following exercise. There were also inter-participant differences in the bimodal pattern of recovery following load carriage (Dousset *et al.*, 2007), contributing to the variation in the pattern of recovery of neuromuscular function in the days following load carriage.

The decrease in the force produced by the knee extensors was accompanied by a decrease in voluntary activation during isometric contractions following LWLC at 0 hours, indicating part of the neuromuscular impairment was due to central mechanism (Shield and Zhou, 2004). However, the specific central mechanisms can not be determined form the data of the present study and require further investigation.

Peripheral mechanisms also contributed to the neuromuscular impairment of the knee extensors, as shown by changes in the electrically evoked contractions. These changes indicate different mechanisms and points of failure in the excitation-contraction coupling process and muscle relaxation. The presence of LFF following LWLC and DWLC indicates a reduction in Ca^{2+} release from the sarcoplasmic reticulum and/or a redistribution of sarcomere lengths (i.e. popping sarcomere theory) (Jones, 1996). Interestingly, LFF recovered 24 h after DWLC but did not return to baseline following LWLC, suggesting the excitation contraction coupling process was still impaired 72 hours after the initial exercise bout. Doublet peak force decreased after DWLC only, this decrease is a common symptom of neuromuscular impairment and is caused by a combination of loss of maximum force generating capacity (damage to the muscle structure), reduced myofibrillar Ca^{2+} sensitivity and reduced Ca^{2+} release (Allen *et al.*, 1995a). The slower contraction time of the doublet immediately after DWLC and LWLC show a slowing of the muscle contraction probably also reflecting a reduced Ca^{2+} release (Martin *et al.*, 2005). The more prolonged half relaxation time at 48 and 72 hours after DWLC reflects a slower rate of cross bridge detachment and may indicate slower uptake of Ca^{2+} into the sarcoplasmic reticulum and reduced sensitivity of myofibrillar proteins to Ca^{2+} (Allen *et al.*, 1995a).

Neuromuscular impairment immediately post and in the days after the load carriage bout will have negative consequences for the load carrier as physical task performance will be

impaired (Byrne *et al.*, 2004) and the risk of musculoskeletal injury may be increased (Mair *et al.*, 1996). Thus, these negative effects may be limited if neuromuscular impairment were reduced.

11.3. Effects of Nutritional Supplementation

Carbohydrate and whey protein supplements were identified as potential interventions to reduce physiological strain associated with load carriage (i.e. $\dot{V}O_2$ and cardiovascular drift and neuromuscular impairment). In the laboratory, the effects of the supplements were evaluated on the metabolic, cardiovascular and neuromuscular responses during load carriage (Chapter 9) and on neuromuscular function immediately post and in the days following exercise (Chapter 10).

Participants consumed 250 mL of either a placebo (PLA), carbohydrate (CHO) or whey protein (PRO) beverage at 0 and 60 minutes during 120 minutes of load carriage (25 kg backpack) walking (6.5 $km \cdot h^{-1}$) on a level gradient (Chapter 9). There were no differences in $\dot{V}O_2$ between conditions at the start (minute 5) of load carriage and $\dot{V}O_2$ gradually increased over the 120 minutes of load carriage in all conditions ($\dot{V}O_2$drift). However, the increase over time during CHO (8 ± 5 %) was less than during PLA (14 ± 6 %) and PRO (17 ± 4 %), but there was no difference in the increases between PLA and PRO. RER decreased between minutes 5 and 120 during PLA and PRO but not during CHO. However, the additional oxygen requirement to use fat instead of carbohydrate as an energy source could not completely account for the differences in $\dot{V}O_2$drift. Due to the relationship between muscle glycogen stores and muscle fatigue during exercise (Sahlin, 1992), it was hypothesised the carbohydrate supplement may improve neuromuscular function in the later stages of load carriage, thus maintaining a more efficient walking economy. However, this was not supported by the data as there were no differences in the EMG activity of the *m. rectus femoris, m. vastus lateralis, m. semitendinosus or m. biceps femoris* between 7 and 107 minutes of the 120 minutes of load carriage in any condition. There were no differences in cardiovascular drift between conditions, probably because participants became dehydrated as fluid losses were not matched by fluid intake in all conditions (Hamilton *et al.*, 1991). Compared to the placebo beverage the consumption of a protein beverage had no effect on the physiological parameters measured during load carriage.

In the three days following each of the 120 minute load carriage bouts participants consumed two 500 mL boluses of either a placebo, carbohydrate or whey protein beverages. Neuromuscular function was measured 0, 24, 48 and 72 h following the load carriage bout (Chapter 10) using the test battery of voluntary and electrically stimulated isometric and isokinetic contractions (Chapters 4 and 5). Immediately after load carriage for PLA, decreases in the maximal force producing capability were observed in isokinetic contractions of knee extensors (60 $°\cdot s^{-1}$) (6 ± 5 %), knee flexors (60 $°\cdot s^{-1}$) (9 ± 7 %), trunk extensors (15 $°\cdot s^{-1}$) (12 ± 15 %) and isometric knee extension (14 ± 7 %). There were no differences in the reduction of force producing capability of the muscle groups between conditions.

During recovery, isokinetic peak torque (60 $°\cdot s^{-1}$) of the knee extensors and flexors returned to pre-exercise value at 24 h during PRO, followed by CHO at 48 h and PLA at 72 h. Isometric knee extension MVC force returned to pre-exercise values at 48 h during CHO and PRO but at 72 h during PLA. Trunk extension peak torque (15 $°\cdot s^{-1}$) returned to pre-exercise value at 24 h during CHO and PRO and at 72 h during PLA. The electrically stimulated 20:50 Hz ratio decreased immediately after PLA and remained below pre-exercise value until 72 h after the load carriage bout; however, there was no change in the 20:50 Hz ratio following CHO or PRO. The reduction in the 20:50 Hz during PLA indicates an impaired release of Ca^{2+} release from the sarcoplasmic reticulum and/or a redistribution of sarcomere lengths (i.e. popping sarcomere theory) (Jones, 1996) but this remained unaffected during PRO and CHO.

After endurance exercise, protein breakdown and synthesis are both increased, but protein balance is still negative (Wolfe, 2000b). Ingestion of carbohydrate stimulates insulin release, which increases protein synthesis at rest and attenuates protein breakdown following exercise (i.e. improving protein balance) (Wolfe, 2001). However, a positive protein balance is only possible with the provision of amino acids (both during and following exercise) (Tipton and Wolfe, 2004). The amino acids promote and provide building blocks for *de novo* protein synthesis and reduce protein degradation (Koopman *et al.*, 2007). Thus ensuring the maintenance of an anabolic rather than catabolic environment and enhancing muscle protein accretion (van Loon, 2007). Westerbald *et al.* (1993) concluded that the force loss that accompanies LFF is most likely due to a structural change to one of the proteins involved in excitation-contraction coupling and the time course of recovery of LFF represents the repair or re-synthesis of this protein This suggests that the supplementations may have provided

protection or improved recovery, through maintenance or repair of the proteins involved in excitation-contraction coupling. |Thus, the faster recovery of neuromuscular function is likely to be due to CHO and PRO improving protein balance, therefore enhancing repair of muscle tissue and structural proteins damaged during exercise.

11.4. Future Research

The studies presented in this thesis have primarily focused upon load carriage of 120 minute duration at a set load and speed. This protocol provided a relevant occupational model (Rayson *et al.*, 2000) to investigate the metabolic, cardiovascular and neuromuscular responses to load carriage. Further research using similar experimental procedures with manipulation of load mass and walking speed and the inclusion female participants would be a useful expansion to the current body of knowledge. In addition, no studies have examined changes in neuromuscular function over an extended duration (e.g. weeks or months) in an occupational setting where load carriage is undertaken (e.g. emergency services and military work or training). This could be investigated using the voluntary and electrically stimulated contractions used in the present studies. Interestingly, Stewart *et al.* (2008), recently showed increasing the number of consecutive days of cycling exercise causes greater neuromuscular impairment. As load carriage is undertaken in environments where repeated days of physical work are common (e.g. military training, recreational walking), Stewart *et al.* (2008) findings add support to the importance of measuring neuromuscular function in these settings.

The enhanced recovery of the force producing capability of certain muscles with the consumption of carbohydrate and protein beverages is likely to have a positive impact on subsequent exercise performance (Byrne *et al.*, 2004) and potentially reduce the risk of musculoskeletal injury (Mair *et al.*, 1996), however, this requires further investigation. The effect of supplementation on exercise performance could be investigated by measuring neuromuscular function (i.e. voluntary and electrically stimulated contractions) in parallel with performance measures (e.g. endurance or high intensity exercise). However, the effect of the performance measure on further neuromuscular impairment would need to be considered. Establishing relationships between neuromuscular impairment and musculoskeletal injuries requires a longitudinal study measuring both parameters, which would probably be most suitably assessed in an occupational training environment where physical activity is strictly controlled. It would be of interest to examine the enhanced recovery of neuromuscular

function with carbohydrate and protein supplements during occupational work or training, where the benefit of faster recovery of neuromuscular function may be apparent to performance during repeated days of work or physical training.

11.5. Conclusion

The research presented in this thesis has shown that load carriage in an occupational setting in trained individuals causes neuromuscular impairment. Laboratory based studies showed the addition of load carriage during 120 minutes of treadmill walking increased $\dot{V}O_2$ and HR at baseline and caused greater absolute $\dot{V}O_2$ drift over time. Load carriage on a downhill gradient reduced $\dot{V}O_2$ and HR at baseline and resulted in no change in $\dot{V}O_2$ and cardiovascular drift over time compared to load carriage on a level gradient. Neuromuscular impairment of the knee, trunk and shoulder extensors and flexors was apparent immediately load carriage in the laboratory and gradually recovered in the following days. All muscle groups showed full recovery 72 hours after load carriage, except the knee extensors after load carriage on a level gradient. Different patterns in the time course of recovery were apparent following load carriage on level and downhill gradients, which was probably due to differences in posture and gait during exercise. This is important in both recreational and occupational settings as neuromuscular impairment has a negative impact on endurance, strength, power and motor control task performance and may increase the risk of musculoskeletal injury. Individuals with the most efficient metabolic and neuromuscular performance during load carriage are those with a large body mass, high absolute $\dot{V}O_2max$ and strong trunk, shoulder and knee flexors. The ingestion of a carbohydrate beverage reduces the magnitude of $\dot{V}O_2drift$ during load carriage, which can only partly be accounted for by the reduced requirement for oxygen during substrate oxidation. However, the ingestion of whey protein had no effect on the physiological responses measured during 120 minutes of load carriage. The consumption of either carbohydrate or whey protein beverages in the days following a bout of load carriage results in a faster time course of recovery of force producing capability of muscles. This is probably due to a reduction in protein turnover, thus enhanced repair of structural proteins damaged during exercise.

Chapter 12. References

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