Reducing muscular fatigue in trail running : mechanisms and strategies

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Reducing muscular fatigue in trail running - mechanisms and strategies

Réduction de la fatigue musculaire en trail – mécanismes et strategies

Thesis

In partial fulfilment of the requirements for the

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SUMMARY (ENGLISH)

The aim of this thesis was to analyse strategies to reduce muscular fatigue in trail running and potentially draw conclusions on the underlying mechanisms. Trail running is a new and upcoming sport that induces a combination of fatigue and muscle damage in the main locomotor muscles. To obtain conclusive evidence on the effect of intervention studies a preliminary descriptive study was undertaken to characterise typical fatigue and damage. Subsequently a model was developed and validated that would allow the investigation of interventions in an applied field setting. A popular current strategy in trail running is the use of compression garments; therefore the effect of these on performance was studied as an intervention. Furthermore, prior heating is anecdotally considered beneficial and recent research has suggested a potential mechanism to link this with reduced muscle damage. Therefore a controlled laboratory study was conducted, examining the effects of passive heating on functional consequences of downhill running in an untrained population. In synopsis, the research conducted for this thesis provides descriptive evidence and a validated terrain model to further investigate fatigue reduction strategies in trail running. Additionally it adds to the current literature in disproving a positive effect of compression garments on performance and demonstrating the functional link between heating and eccentric-induced muscle damage reduction.

SUMMARY (FRENCH)

L'objectif de ce travail de thèse a été d'analyser les stratégies de réduction de la fatigue musculaire en course de trail et potentiellement d'identifier certains paramètres d'influence de cette fatigue. La course de trail est un nouveau sport en essor qui induit une combinaison spécifique de fatigue et dommages musculaires des principaux muscles locomoteurs. Afin de pouvoir conduire des études interventionnelles, une étude descriptive préliminaire a été conduite pour caractériser la fatigue spécifique et les dommages musculaires induits par ce type d'épreuve de trail. Ensuite, la reproductibilité du trail comme modèle de fatigue a été verifiée afin de pouvoir l'utiliser dans un contexte d'intervention. Enfin, deux études visant à réduire la fatigue induite par le trail ont été conduites. D'une part l'utilisation des vêtements de compression - très à la mode en trail - a été analysée comme stratégie d'optimisation de la performance. D'autre part, a aussi été etudié l'effet d'un réchauffement préalable du muscle sur les dommages musculaires : Dans cette optique, une étude contrôlée en laboratoire a été menée, examinant les effets d'un réchauffement passif sur les conséquences fonctionnelles de course en descente chez une population non-entraînée. En résumé, les travaux conduits au sein de cette thèse fournissent une description de la fatigue en trail, et valident l'utilisation du trail comme modèle reproductible de terrain pour investiguer les stratégies de réduction de la fatigue. De plus, ils relativisent l'effet positif des vêtements de compression sur la performance et montrent le lien fonctionnel entre le réchauffement musculaire et la réduction des dommages musculaires induits par un travail excentrique.

DECLARATION OF ORIGINALITY AND AUTHORSHIP

I, Christopher Easthope Schmidt, hereby declare that this thesis and the work reported herein was composed by and originated entirely from me. Information derived from the published and unpublished work of others has been acknowledged in the text and references are given in the list of sources.

Signed the 4 - 5 - 2013 in Nice, France

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List of Abbreviations

5-HT	Serotonin
ACH	Acetylcholine
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate kinase
AP	Action potential
ATP	Adenosine triphosphate
BAF	British Athletic Foundation
BBB	Blood brain barrier
BCAA	Branched chain amino acids
BF	Blood flow
Ca^{2+}	Ionic Calcuim
CaP_i	precipitated Calcium – inorganic Phosphate compound
CB	Cross bridge
CG	Central governor
CGM	Central Governor Model
CK	Creatine kinase
CMJ	Counter movement jump
CO	Cardiac output
CO2	Carbon dioxide
DA	Dopamine
DE	Delta efficiency
DHPR	Dihydropyridine receptor
DOMS	Delayed-onset muscle soreness
E/D	Exercise·distance^{-1} ratio
E-C	Excitation – contraction
EE	Energy expenditure
EIMD	Exercise-induced muscle damage
EMG	Electromyography
Estim	Percutaneous muscle stimulation
f-TRP	unbound plasma Tryptophan
GE	Gross efficiency
H^+	Ionic Hydrogen
H_2O	Water molecule
Hb	Haemoglobin
$Hb0_2$	Oxy-haemoglobin
HCO_3^-	Bicarbonate ion
HDEMG	High-density electromyography
HHb	Deoxy-haemoglobin
HR	Heart rate
HSP	Heat shock protein
IAAF	International Association of Athletics Federations
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
Il-6	Interleukin 6
IOD	Inter-optode distance
ITT	Interpolated twitch technique
IAU	International Association of Ultrarunners
JNK	c-Jun N-terminal kinase
K^+	Ionic Kalium
Lac	Lactate

LDH	Lactate dehydrogenase
LFF	Low frequency fatigue
LNAA	Large neutral amino acid
Mg^{2+}	Ionic Magnesium
MHC	Myosin heavy chain
MUM	Mountain ultra marathon
MVC	Maximal voluntary contraction
MVIC	Maximal isometric voluntary contraction
mVO_2	Rate of muscular oxygen consumption
NA	Noradrenaline
Na^+	Ionic Sodium
NIRS	Near infra-red spectroscopy
NMJ	Neuromuscular junction
OIA	Outdoor Industry Association
OF	Outdoor Foundation
PCr	Phosphocreatine
PHE	Phenylalanine
Pi	inorganic Phosphate
PPA	Peak-to-peak amplitude
PPD	Peak-to-peak duration
PNS	Peripheral nerve stimulation
QF	M. Quadriceps Femoris
RER	Respiratory exchange ratio
RH	Relative humidity
RMS	Root mean square
ROI	Region of interest
ROS	Reactive oxygen species
RPE	Rate of perceived exertion
RyR	Ryanodine receptor
sEMG	Surface electromyography
SR	Sarcoplasmic Reticulum
STP	Standard temperature and pressure
TMS	Transcranial magnetic stimulation
TNF	Tumour necrosis factor
TRP	Tryptophan
TWC	Trail World Championship
TYR	Tyrosine
$\dot{V}CO_2$	Rate of carbon dioxide elimination
$\dot{V}e$	Minute ventilation
VL	M. Vastus Lateralis
$\dot{V}O_2$	Rate of oxygen uptake
$\dot{V}O_2max$	Maximal rate of oxygen uptake
$\dot{V}O_2peak$	Peak rate of oxygen uptake
VT1	First ventilatory threshold
VT2	Second ventilatory threshold
UTMB	Ultra Trail de Mont Blanc
WMRA	World Mountain Running Association
WR	Work rate
WSER	Western States Endurance Race

Standard ISI notation is used throughout for the units. Country codes conform to the ISO-3166 standard.

LIST OF THESIS RELEVANT PUBLICATIONS AND CONFERENCE PRESENTATIONS

ARTICLE | **Easthope CS**, Nosaka K, Caillaud C, Vercruyssen F, Brisswalter J (2013). Reproducibility of performance and physiological measures in trail runs. J Sci and Med Spo Epub.

ARTICLE | Vercruyssen F, **Easthope CS**, Hausswirth C, Bernard T, Bieuzen F, Gruet M, Brisswalter J (2012). Influence of wearing compression stockings on performance indicators and physiological responses following a prolonged trail running exercise. Eur J Spo Sci Epub: 1-7.

ARTICLE | **Easthope CS**, Hausswirth C, Louis J, Lepers R, Vercruyssen F, Brisswalter J (2010). Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes. Eur J Appl Physiol 110:1107–1116.

ORAL | **Easthope CS**, Hausswirth C, Louis J, Lepers R, Vercruyssen F, Brisswalter J (2010). Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes. International Society of Electrophysiology and Kinesiology Conference, June 16-19, Aalborg, Denmark.

POSTER | **Easthope CS**, Mavros Y, Burdon C, Fiatarone Singh MA, Brisswalter J, Caillaud C (2012). Does prior heat exposure reduce consequences of eccentric exercise-induced muscle damage?. European Congress of Sport Sciences, July 4-7, Brugges, Belgium.

Chapter 1

Trail Running – What is it all about?

The best runner leaves no tracks.

- *Tao Te Ching*

1.1. WHAT IS TRAIL RUNNING?

Trail running, once a fringe sport frowned upon by many, has moved more and more to the central focus of recreational and competitive runners alike in the past decades[1–3]. This increase in interest can be attributed to various factors throughout society including an oversaturation of the road running scene, an increased drive to experience solitude and natural surroundings, an emerging body of research on the detrimental effects of prolonged road racing, increased accessibility, equipment advances, and more. Trail running as a locomotion modality is nowhere near new to humankind and has been attributed a prominent place in the evolution of humans as early hunter-gatherers[4–7]. Yet the recent resurgence of participation in recreational running and organised competitive events invites a new response to the question: "What is it all about?"

1.1.1. DEFINITION

As there is lacking consensus between local, national and international groups as to what actually comprises a trail run, the creation of a working definition becomes slightly less straightforward. Trail running as a budding sport is still in the "Sturm und Drang"-phase, characterised by sport sociologists as the time in which a sport is not yet institutionalised and abides by a set of loosely defined rules which are generally accepted by the participants and are roughly similar throughout all games[8]. The further analysis will focus on competition-type encounters in order to simplify the definition process. Trail running competitions are currently being hosted by a plethora of groups including classical athletic associations, manufacturers, local interest groups, and adventure tourism hosts. One of the earliest classical athletic

associations to officially sanction and employ the term "trail running" was the British Athletic Federation (BAF) in a 1995 memorandum establishing trail races as:

"In the context of athletics, trail races are primarily along footpaths and bridle paths marked on Ordnance Survey maps as 'public rights of way'. They are 'highways' to which pedestrians have unrestricted access in English law. Towpaths, forest drives, farm cart tracks and paths in parks and so on, from which motorised traffic is excluded, are also trails when the owners' permission is obtained."[9]

Trail running as a formal sport was thus established, and hence began the sprouting of further, trail-specific organisations – each with their slightly modified idea of what defines a trail race. In 1996 the American Trail Running Association was created, followed by associations in France, Germany, Italy, England, Ireland, Australia and South Africa. Additionally, local trail running clubs spawned, hosting local competitions and providing a social hub for trail runners. Manufacturers and adventure tourism companies jumped on the bandwagon, organising events explicitly linked with a company name or location and using this as a marketing asset. This has led to a large number of very diverse events grouped under the banner of "trail running".

A biannual Trail World Championship (TWC) event hosted by the International Association of Ultra Runners (IAU) was inaugurated in 2007.The IAU was granted patronage by the International Association of Athletics Federations in 1988, when the 100 km distance became officially recognised as a running event. It is responsible for race certification, record keeping and the organisation of global competitions for all distances exceeding the marathon. The TWC has therefore generally been around 70 km in length with about 2500 m climb. The 2012 IAU Trail World Championship was the most popular ever with teams from over 20 countries competing. The IAU is also responsible for certifying trail races and distributing "quality" or "qualification" labels.

Additionally, there is a trail running series with associated national and world championships hosted by XTERRA. The XTERRA series concentrates mainly on shorter distances, with participants completing a number of races between 5 and 42 km to gain points for qualification for the 21 km world championships. In a sense, this series complements the IAU competition as it covers a completely different running duration. Interestingly, XTERRA was originally conceived by the Hawaiian company TEAM Unlimited LLC in response to a request to "amplify television exposure for the Hawaiian Islands". Starting with a single mediatised adventure event in 1988, TEAM quickly expounded on its "sports fuelling tourism" concept and created the XTERRA off-road triathlon series, a strongly televised and sponsored event series culminating in the XTERRA World Championships on Hawaii. A 2007 spin-off is the XTERRA Trail Run Series, which focuses on the same values of media presence and sponsoring.

To further complicate the setting, the International Association of Athletics Federations (IAAF) has provided an extension to the cross country section of its IAAF Distance Running Manual describing Mountain Races (Rule 250.1) and has endorsed the World Mountain Running Association (WMRA) as organiser of a qualification series and world championship. The definition of mountain running seems very similar to the definitions of trail running that have been described so far (< 20% macadamised surface), yet the IAAF also defines distance (12 km) and climb (either +1200 m or +750 m & -750 m) giving a closer guideline to be respected. A more precise definition has been decided at the 2013 WMRA conference and this will be proposed to reinstitute the existing auxiliary rule as a rule in its own right at the next IAAF meeting[10].

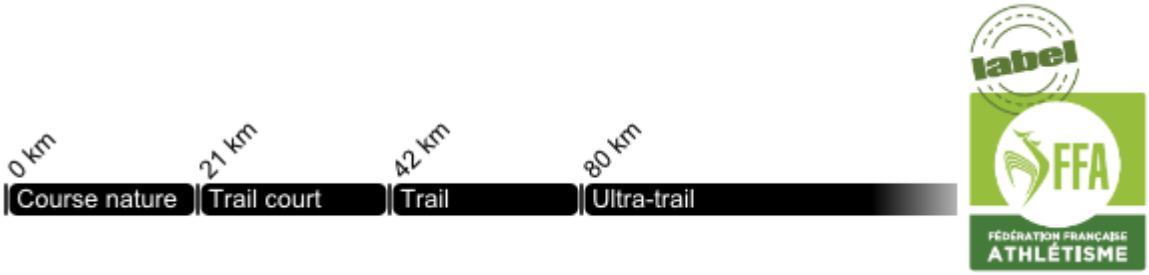


Figure 1.1: *Trail distinctions as defined by the French Federation of Athletics.*

From the large divergence of events termed trail running competitions, it becomes difficult to distil strict distance and elevation criteria for the trail to segregate this from other forms of off-road running such as orienteering, hashing, cross-country, or fell running. The definitions proposed by most classical athletic associations tend to eschew precise distance and elevation concepts and prefer to concentrate on terrain denominators. The French Federation of Athletics, for instance, regards "trail" to incorporate anything from 5 km to open end under condition that over 75% of the distance is run off paved surfaces (Fig. 1.1). This seems the only way to assimilate all the different forms of trail under a single term – a criterion that is not distance-based, but terrain-based. Therefore, for the rest of this text trail run shall be defined as any footrace fulfilling the following:

1) To qualify as a trail run, the main partition of the course should be on footpaths or cross country, minimising time on roads and other surfaced paths. Footpaths can vary from large cart tracks to steep mountain single-tracks – the only common factor is that the surface is rough, enforcing a certain adaptation of stride and footfall to compensate.

2) In terms of climb, it is difficult to nominate an absolute value, as different distances entail different absolute climbs and descents. When analysing popular races, it becomes evident that while the absolute values diverge, climb expressed as a ration of distance remains comparable (around 50 $m \cdot km^{-1}$) in the majority of cases (Fig. 1.2). This ratio may be considered one of the performance determinants of the race and to an extent gives an idea of the "toughness" of a trail competition. To give an idea, the IAAF mountain race definition proposes an E/D ratio of up to 100 $m \cdot km^{-1}$ on 12 km distance.

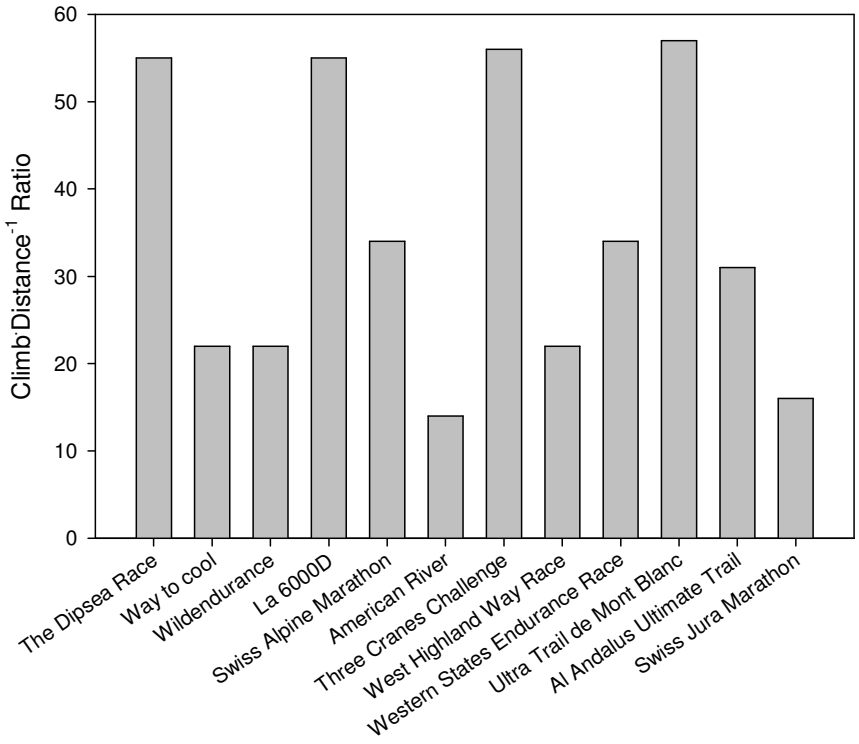


Figure 1.2: *The climb·distance^{-1} ratio of various trail races featured in peer-reviewed publications. Races are sorted by distance, increasing from left to right.*

3) The running distance and associated exercise duration is one of the most variable parameters encountered in trail races and is largely overshadowed in this definition by terrain and climb ratio. Yet exercise duration is one of the most important parameters when analysing performance, strain, intensity, and recuperation, therefore it should not go unmentioned. The shortest competitive trail races are generally longer than 10 km, and until about 55 to 60 km distance the population is largely shared. Longer distances such as the 100 miles (161 km) have been termed mountain ultra-marathons (MUM) or ultra-trails and are mainly run by a highly specialised population. These events fulfil all the earlier mentioned criteria, yet the rift in population and significant increase in exercise duration leads us to consider them as a specialised sub-class of trail run. The same applies to multi-stage events such as the famous Marathon des Sables. Although

the terrain and climb prerogatives are fulfilled, the drastic differences in exercise duration make it difficult to see these events on the same footing.

4) Multi-sport, orienteering and map-reading skill components should not be part of the performance-determining parameters as this introduces a new component to be taken into account that is difficult to control.

1.2. THE HERITAGE OF TRAIL RUNNING

While the historical prerogative for trail races lies in the early reaches of biped locomotion history, organised trail running competitions have rapidly become more popular in the last two decades. While as little as 30 years ago trail running was mainly an unknown niche sport, data collected for instance in 2009 by the outdoor foundation for a special report, indicate that nowadays over 4.7 million people (around 1.7% of the US population) compete annually in the USA alone[1]. The most popular distances are fun runs and 5 km runs, together accounting for 63% of the total participation volume while marathon distances and longer only comprised 8.6% of the volume. A perhaps superficial Google web trend analysis shows that the term "trail run*" is nowadays roughly 50% more likely to be used in the search engine than in 2004 and has been featured in US newspaper headings with increasing frequency since 2008 (Fig. 1.3).

Figure 1.3: *A Google Trend analysis of the term "trail run" and related terms.*

1.2.1. Evolution and trail running

A series of publications[4–6] from the turn of the millennium has re-evaluated ideas advanced in anthropology in the 1980s[7] concerning the probable connection between human locomotion capacity as an early hunter-gatherer culture and their ability to survive. These papers propose that trail running capability - i.e. the ability to move quickly over rugged terrain for a prolonged period of time - is directly linked to survivability through a number of factors. Early humans were neither very fast nor very strong compared to their adversaries, therefore it seems probable that the species hunted through persistence; running stronger and faster prey to the ground through continuous harassment (refer to McDougall[11] for a highly entertaining narrative on the topic). Secondly, enhanced trail capability enrolled the early humans to source food more easily through enlarging the forage radius and distance a group could cover in a given time. Thirdly, through similar mechanics a stronger trail running group could better flee when danger was near. This triple, implicit connection of trail capabilities to survivability is in the authors' opinion so consequential that trail capability may have become one of the key factors in evolution[12].

1.2.2. Trail running before the 20th century

As early humans, having learned to master their environment, gradually left their nomadic ways, it could be expected that trail capability became less of an important trait than, for instance, farming. While this may remain true in the early stages of societal development, at some point societies became larger and better organised and the need to communicate across distances arose. Fast messengers were valued and once again trail capability rose to an important position in society. Even nowadays the exploits of famous fast messengers are retained. Pheidippides for example, who ran from Sparta to Athens in 2 days (246 km) and on another, better known occasion from Marathon to Athens (42 km) to deliver his famous message, "νικωμεν! - Joy, we win!" before dying[13,14]. The Aztec, Mayan and Native Indian cultures are widely respected for their messengers, who could on some occasions deliver

messages faster than men on horses. The Iroquois messenger Sharp Shins should be mentioned here, who was reported to run 90 miles from sunrise to sunset[15]. Also Big Hawk Chief, who is reputed to have covered 120 miles in around 20 hours as part of a wager, leaving the evaluation committee stranded on their horses in the desert and arriving quite a while before them[16]. These and other feats have been transmitted orally over the centuries and may or may not have been subject to gross exaggeration; yet the fact alone that they have been maintained in the collective conscience indicates their importance in the respective cultures. To this day there are isolated cultures that hold trail running in great importance to social status such as the Tarahumara in Northern Mexico[11,17].

1.2.3. TRAIL RUNNING IN MODERN SOCIETY

A short look at the development of participant numbers and records of popular races can give an idea of the progression that trail running has experienced in the past two decades. A few races quickly crystallised as benchmarks of performance and have sparked scientific investigation. Without claiming to offer a complete or exclusive insight, a few of the more popular trail races are explained in the following.

Western States Endurance Race (WSER)

The WSER claims on its website to be the “oldest and most prestigious trail run”. While this may not be entirely exact in terms of heritage, the first run was completed earlier than most other ultra-distance races and the competition has secured a large following to this day. The race counts as one of the four races featured in the “Grand Slam of Ultrarunning”[18], a competition series in the US. Originally an endurance horse race, the first runner joined the horses in 1974 and completed the 160 km race in a gruelling 23 hours and 42 minutes. In 1977 the race was officially inaugurated and 14 runners signed up, three of which actually finished. In 1979 a qualifying standard and participation cap were put into place to curb popular demand and the race has been run more or less at maximum capacity (400 starters) ever since (Fig. 1.4). The current records for male and female were established 2012 with 14h 46m and 16h 47m

respectively. Five peer-reviewed publications have used this races as an intervention model in the last 10 years investigating mainly population statistics and modelling, as well as water and salt ingestion during the race[3,19–22].

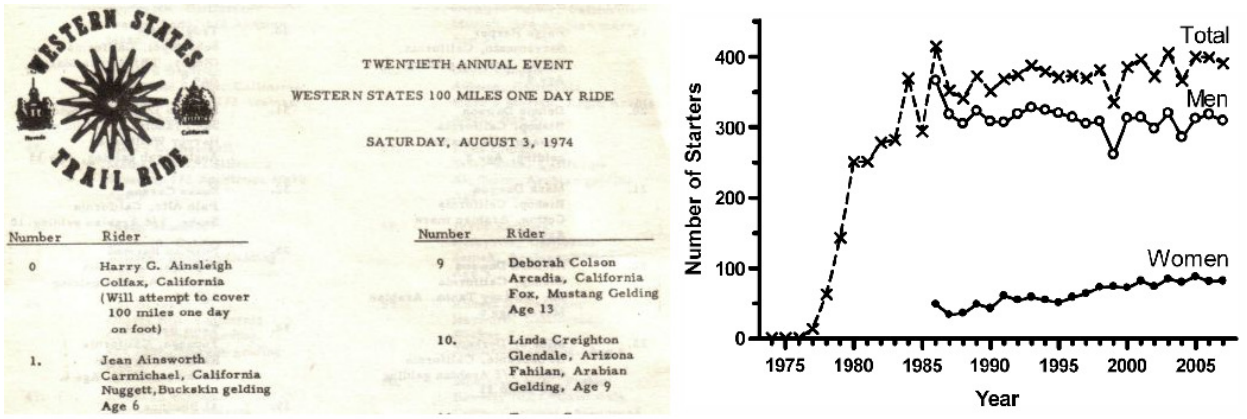


Figure 1.4: *Participation adherence in the WSER from inception until 2007[3]. On the left the original starting manifest with the first WSER runner ever documented.*

The Dipsea Race

Some time before trail running even existed as an idea let alone an expression in Western society, some men from San Francisco's Olympic Club engaged in a wager as to who of them could reach the newly opened Dipsea Inn the fastest. From this was born the Dipsea race, inaugurated in 1905 with over 100 participants. Since then the race has been in almost continual institution and is now in its 103rd iteration. The participant cap was first set in 1977 and is now at 1500 runners. The race features a unique head start system aiming to mitigate differences in performance and age. Due to the head start system together with a penalty system for former champions, the 12 km long race is at the moment championed by a 74-year-old with a time of 47 minutes. This is arguably the first ever recorded competitive trail run, yet no peer-reviewed publications have used this race as a model.

The Ultra-Trail of Mont Blanc (UTMB)

The originally 155 km Ultra-Trail of Mont Blanc was inaugurated in 2003 and the participant cap of 2300 runners was reached five years later, leading to the creation of a second, half-loop race.

To give an idea of the popularity of this course: in 2008, the 2000 registrations allowed were supposedly completed within 7 minutes of the launch of the registration site. This was a good 5 months before the actual race (anecdotal evidence). To make for a more democratic selection process, a qualification series is in place since 2010 that awards starting positions based on performance. The evolution of records is rather difficult to contemplate, as the course length has been changed a number of times and in some years the course had to be extended or terminated early due to adverse weather conditions. The current record holder of the full distance is Kilian Jornet, who completed the 2011 course (170 km) in 20h 36m. The UTMB has also sparked a number of scientific publications concerning neuromuscular consequences[23], changes in spring mass behaviour[24], and changes in erythrocyte count[25].

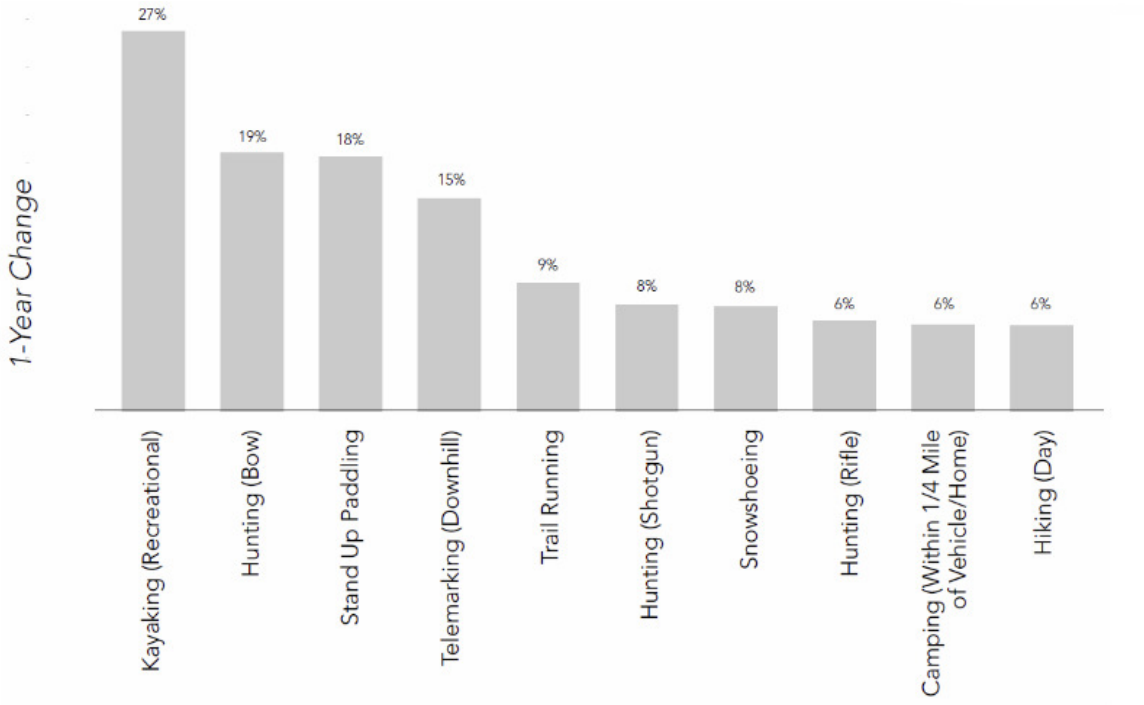
1.2.4. POPULATION STATISTICS

As mentioned in the introduction trail running is becoming more and more popular in Western culture. The steady increase in competitive events and net starters throughout the world can be interpreted as a direct index of this popularity. But there are more subtle indications that can underscore this direct index and give feedback on the number of adherents. In this sense, consumer industry research provides a rich source of information. The Outdoor Foundation (OF) is a not-for-profit association founded by the Outdoor Industry Association (OIA) to be a driving force behind the development and furthering of outdoor recreation in America. The OIA itself was founded in 1989 and represents over 1300 outdoor companies, compiling annual reports and trend analyses on the American outdoor recreation economy. According to its 2012 report, the annual spending of Americans on trail-related sports in the past years has steadily increased by around 5% per annum, culminating in a massive US$ 80,628,545,863 spent in 2012. This breaks down into 15% spent on gear and 85% spent on trail-related travelling costs. These figures naturally incorporate all trail-related sports including hiking, and trail running only makes for a small percentage. Nonetheless, the 2012 trend reports place trail running as the 5th fastest-growing outdoor activity with a 9% popularity increase and a 15.5% increase in participation from 2011 to 2012, while general participation in outdoor activities increased only

by 2.3%[26] (Fig. 1.5). In addition, a special report was released in 2010 covering trail running as a separate discipline. According to the report, participant numbers in America have grown from 4.6 million in 2006 to 4.8 million in 2009, with an 82.6% crossover to road running. New adherents made up 13.1% of the cohort and the main motivations given were “it’s relaxing”, and, ”it’s a great way to exercise”. Furthermore, the data collected goes on to describe demographic distribution, crossover sport participation and annual participation. According to the report, trail running in the US is most popular in Caucasian males aged 25 to 44 with a college education and an annual income of over US$ 100,000[1].

From a more classical scientific point of view, there are only few studies on participation development in trail running[2,3,20,27–29]. In 2010, Hoffmann et al.[2] published a historic participation analysis for 161km ultra-marathons in North America. This only represents a part of all trail races – and one of the less popular according to the 2009 OF report – yet the participation numbers are conclusive: The number of 161 km trail events has increased exponentially from 0 in 1978 to over 50 in 2008. In the same period, the number of 161 km road events has remained constant, mirroring demand. Over 2500 finishes were recorded in 161 km trail races in 2008 (Fig. 1.6).

Recently, two articles gave insight on the position of trail running as a trending sport, elucidating participation in multi-stage ultra-marathon events such as the famous Marathon des Sables in Morocco[27,28]. As shown in figure 1.6 there has been exponential growth in the ultra-marathon sector between 1992 and 2010, mainly fuelled by growing numbers of competitors from France, the UK, Germany and the USA. Also the number of competitions held has increased following a similar pattern, 2002 registering only 3 events, compared with 26 in 2009 and 22 in 2010. Ultra-marathons, however, remain rather less popular than trail running, the American Ultrarunning Association putting participation at a ballpark figure of 70,000 worldwide, compared with the 4.8 million trail runners in America alone reported in the OF’s 2009 trail special report.



	2006 in 000's	2007 in 000's	2008 in 000's	2009 in 000's	2010 in 000's	2011 in 000's	3 Year Change
Adventure Racing	725	698	920	1,089	1,339	1,065	15.8%
Trail Running	4,558	4,216	4,857	4,833	5,136	5,610	15.5%

Figure 1.5: *Growth in trail running in North America compared to other outdoor sports*[1,26].

Concerning single events, a paper published by Hoffman and Wegelin in 2009[3] directly regards participation and performance trends in the WSER. Main results include an increase in age and performance of the participants with unchanging participation since introduction of the cap. In conclusion, a synthesis of the data from consumer reports, scientific publications and independent organisation surveys make a strong case for an overall increase in trail running popularity, both competitive and recreational. Increased spending, high positions on consumer trend analyses, an increased number of finishers and increased event density can be seen as strong markers that trail running is indeed permeating the fabric of traditional road running.

1.3. TRAIL RUNNING AS A MODEL IN SCIENCE

As argued above, trail running seems to be gaining more and more adherents annually. However, there is not much dedicated scientific literature or investigation on the physiology of trail running. The lack of literature can probably be attributed to a number of factors, including the only recent increase in popularity, the often demanding conditions in the field for conducting investigations, the perceived overlap with existing knowledge on long-distance endurance and the difficulties in simulating this kind of exercise. Nonetheless, the specifics of trail running offer a unique opportunity to investigate long-duration exercise in a challenging and hostile environment.

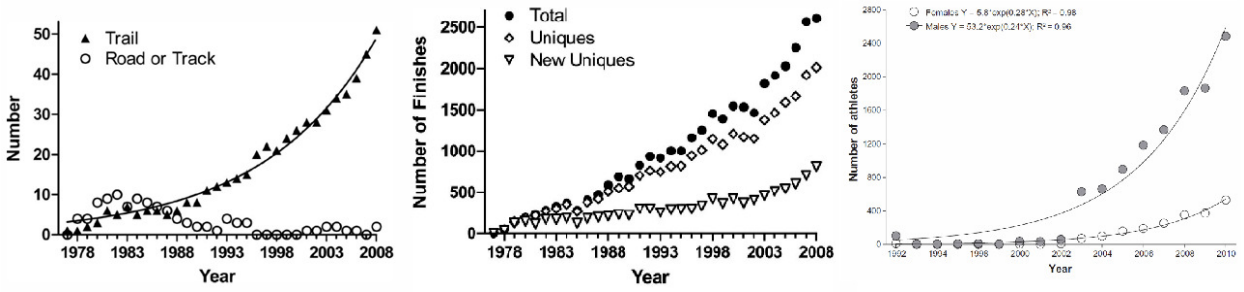


Figure 1.6: *Data drawn from the studies of Hoffman et al.*[2] *and Shoak et al.*[27] *indicate the growth of participation in trail running.*

1.3.1. PUBLICATION FREQUENCY

To give a general idea of the publication frequency, a simple search on the internet portal of the US National Library of Medicine (http://www.pubmed.gov) using the search term **(trail run[All Fields] OR trail runners[All Fields] OR trail running[All Fields]) OR (ultra[All Fields] AND marathon[All Fields])** was performed. While investigations on related fields such as orienteering and cross-country running are thus disregarded, employment of this highly directive search term also gives feedback about the employability of the term “trail”. In a preliminary search 141 results were recovered. This includes a large percentage of road races and a few unrelated hits; therefore a sounding process was necessary to eliminate these

investigations. This resulted in a final count of 31 trail-specific publications conforming to this search in this specific index (Fig. 1.7).

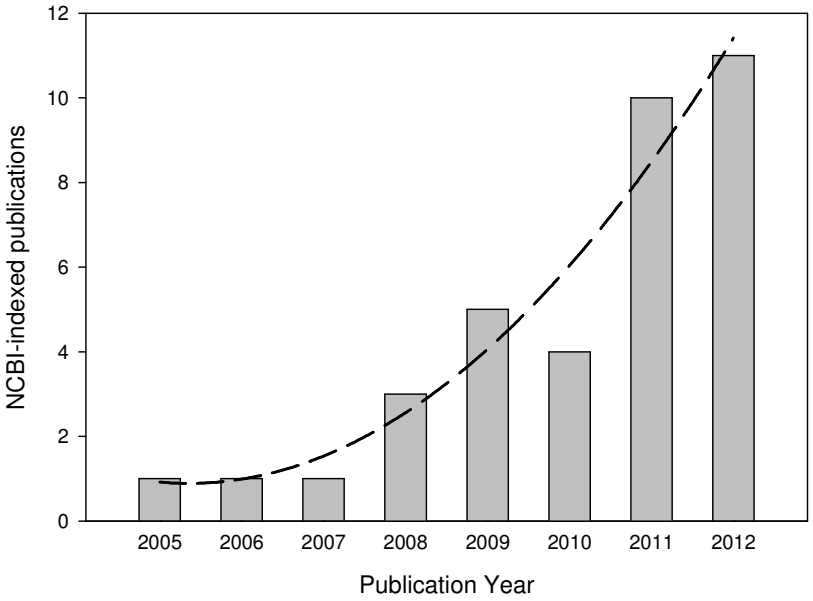


Figure 1.7: *The amount of publications concerning trail races indexed by the US National Library of Medicine as a function of time (year). Quadratic modeling results in a correlation index of R^2=0.93 (p=0.01), suggesting that publication frequency will continue to increase.*

Of these publications, 80% are based on competition analysis, 10% are simulation-based, and 10% rely on an intervention field setting. The competition analysis papers mainly regard distances of over 80 km, thus qualifying for the MUM label given in the definition (17 publications), while only a few publications concentrate on shorter distances (5 studies). Controlled studies generally focussed on shorter, more manageable distances (< 20 km, 4 studies), and the simulation studies employed similarly short distances (< 20 km, 2 studies). Of the long-distance field trials several investigated multi-day trails (6 publications).

Not included in the listed publications are a series of papers (5 publications) published on the 2009 Trans Europe Footrace, a 4500 km run across all of Europe[30–34] and a case study of an 8500 km run[35]. These publications were excluded due to the extreme duration of the race coupled with highly variant elevation data for the individual running legs.

1.4. Conclusion

Trail running has evolved since its beginnings in the early nineties into a fully-fledged sport with multiple world series and a large, dedicated fan base. This has led to an increase in scientific investigation centring mainly on the development of neuromuscular fatigue[23,36–38] and associated strategies of amelioration, be it through supplementation[39,40], hydration[41–44], pain suppression[21,32] or prior conditioning etc. The following fundamental statements set the framework of the present investigation:

- Trails can be defined as any run over 10 km that is completed mainly on unsurfaced paths, has a significant elevation difference during the course and does not incorporate any other modality.
- Trail running is becoming increasingly popular in Western culture, indicated by increasing revenue, increasing number of finishers per year, increasing number of events per year.
- There is only little trail-specific scientific knowledge available, which is mainly of a descriptive nature.
- The combination of increasing popularity and little prior knowledge makes trail running a particularly interesting model to investigate as:
    - A growing population and industry is engaged and R&D and health prevention should be backed up with independent research.
    - Increasing numbers of trail participants offer a possibility of conducting higher-powered studies on physiological and mental processes during long duration exercise.
- Trail running offers a unique opportunity to investigate the effects of prolonged exercise on neuromuscular performance and cognitive drive.

1.5. CHAPTER 1 BIBLIOGRAPHY

1. Outdoor Industry Foundation. *A Special Report on Trail Running*. Boulder, Colorado: Outdoor Industry Foundation; 2010:1–12.

2. Hoffman MD, Ong JC, Wang G. Historical analysis of participation in 161 km ultramarathons in North America. *Int J Hist Sport*. 2010; 27(11):1877–1891.

3. Hoffman MD, Wegelin JA. The Western States 100-Mile Endurance Run: participation and performance trends. *Med Sci Sports Exerc*. 2009; 41(12):2191–2198.

4. Bramble DM, Lieberman DE. Endurance running and the evolution of Homo. *Nature*. 2004; 432(7015):345–352.

5. Lieberman DE, Bramble DM, Raichlen DA et al. Brains, Brawn, and the Evolution of Human Endurance Running Capabilities. In: Grine FE, Fleagle JG, Leakey RE, eds. *The First Humans – Origin and Early Evolution of the Genus Homo*. Vertebrate Paleobiology and Paleoanthropology. Netherlands: Springer; 2009:77–92.

6. Lieberman DE, Bramble DM. The evolution of marathon running : capabilities in humans. *Sports Med*. 2007; 37(4-5):288–290.

7. Devine J. The Versatility of Human Locomotion. *Am Anthropol*. 1985; 87(3):550–570.

8. Pociello C. *Sports et société: approche socio-culturelle des pratiques*. Paris: Vigot; 1981.

9. Trail Running Association. A guide to organising trail races. 2001.

10. World Mountain Running Association. WMRA Championships - Technical Regulations. 2011.

11. McDougall C. *Born to run: a hidden tribe, superathletes, and the greatest race the world has never seen*. New York: Vintage Books; 2011.

12. Lieberman DE, Bramble DM, Raichlen DA et al. The evolution of endurance running and the tyranny of ethnography: a reply to Pickering and Bunn (2007). *J Hum Evol*. 2007; 53(4):439–442.

13. Plutarch LM. On the Glory of the Athenians. In: *Moralia*. Vol IV. Loeb Classical Library; 1936:87–102.

14. Lucian of S. *The Works of Lucian of Samosata*. Adelaide: University of Adelaide @ eBooks; 2007.

15. Fenton WN. The Journal of James Emlen Kept on a Trip to Canandaigua, New York. *Ethnohistory*. 1965; 12(4):279–342.

16. Nabokov P. *Indian running: native American history & tradition*. Santa Fe, New Mexico: Ancient City Press; 1987.

17. Lumholtz C. *Unknown Mexico a record of five years' exploration among the tribes of the western Sierra Madre in the tierra caliente of Tepic and Jalisco and among the Tarascos of Michoacan*. New York: C. Scribner's Sons; 1902.

18. Boeder RB. *Beyond the Marathon : The Grand Slam of Trail Ultrarunning*. Vienna: Old Mountain Press; 1996.

19. Nieman DC, Dumke CI, Henson DA et al. Immune and oxidative changes during and following the Western States Endurance Run. *Int J Sports Med*. 2003; 24(7):541–547.

20. Hoffman MD. Performance trends in 161-km ultramarathons. *Int J Sports Med*. 2010; 31(1):31–37.

21. Hoffman MD, Lee J, Zhao H et al. Pain perception after running a 100-mile ultramarathon. *Arch Phys Med Rehabil*. 2007; 88(8):1042–1048.

22. Hoffman MD. Ultramarathon trail running comparison of performance-matched men and women. *Med Sci Sports Exerc*. 2008; 40(9):1681–1686.

23. Millet GY, Tomazin K, Verges S et al. Neuromuscular Consequences of an Extreme Mountain Ultra-Marathon. *PLoS ONE*. 2011; 6(2):e17059.

24. Morin JB, Tomazin K, Edouard P et al. Changes in running mechanics and spring-mass behavior induced by a mountain ultra-marathon race. *J Biomech*. 2011; 44(6):1104–1107.

25. Robach P, Boisson R-C, Vincent L et al. Hemolysis induced by an extreme mountain ultra-marathon is not associated with a decrease in total red blood cell volume. *Scand J Med Sci Sports*. 2012:[Epub ahead of print].

26. Outdoor Foundation. *Outdoor Recreation Participation Topline Report*. Boulder, Colorado: Outdoor Industry Foundation; 2012:10.

27. Abou Shoak M, Knechtle B, Rüst et al. European dominance in multistage ultramarathons: an analysis of finisher rate and performance trends from 1992 to 2010. *Open Access Sports Med*. 2013; 4:9–18.

28. Knoth C, Knechtle B, Rüst CA et al. Participation and performance trends in multistage ultramarathons—the "Marathon des Sables" 2003–2012. *Extrem Physiol Med*. 2012; 1(1):1–13.

29. Eichenberger E, Knechtle B, Rüst CA et al. The aspect of nationality and performance in a mountain ultra-marathon-the "Swiss Alpine Marathon." *J Hum Sport Exerc*. 2012; 7(4):748–762.

30. Murray A, Costa RJ. Born to run. Studying the limits of human performance. *BMC Med*. 2012; 10(1):76–79.

31. Schütz U, Schmidt-Trucksäss A, Knechtle B et al. The Transeurope Footrace Project: longitudinal data acquisition in a cluster randomized mobile MRI observational cohort study on 44 endurance runners at a 64-stage 4,486 km transcontinental ultramarathon. *BMC Med*. 2012; 10(1):78.

32. Freund W, Weber F, Billich C et al. Ultra-Marathon Runners Are Different: Investigations into Pain Tolerance and Personality Traits of Participants of the TransEurope FootRace 2009. *Pain Pract*. 2013 [ePub].

33. Freund W, Faust S, Birklein F et al. Substantial and reversible brain gray matter reduction but no acute brain lesions in ultramarathon runners: experience from the TransEurope-FootRace Project. *BMC Med*. 2012; 10(1):170–181.

34. Millet GP, Millet GY. Ultramarathon is an outstanding model for the study of adaptive responses to extreme load and stress. *BMC Med*. 2012; 10(1):77–79.

35. Millet GY, Morin J-B, Degache F et al. Running from Paris to Beijing: biomechanical and physiological consequences. *Eur J Appl Physiol*. 2009; 107(6):731–738.

36. Fourchet F, Millet GP, Tomazin K et al. Effects of a 5-h hilly running on ankle plantar and dorsal flexor force and fatigability. *Eur J Appl Physiol*. 2012; 112(7):2645–2652.

37. Martin V, Kerhervé H, Messonnier LA et al. Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run. *J Appl Physiol*. 2010; 108(5):1224–1233.

38. Millet GY, Martin V, Lattier G et al. Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *J Appl Physiol*. 2003; 94(1):193–198.

39. Gauche E, Lepers R, Rabita G et al. Vitamin and mineral supplementation and neuromuscular recovery after a running race. *Med Sci Sports Exerc*. 2006; 38(12):2110–2117.

40. Miller PC, Bailey SP, Barnes ME et al. The effects of protease supplementation on skeletal muscle function and DOMS following downhill running. *J Sports Sci*. 2004; 22(4):365–372.

41. Casa DJ, Stearns RL, Lopez RM et al. Influence of hydration on physiological function and performance during trail running in the heat. *J Athl Train*. 2010; 45(2):147–156.

42. Lopez RM, Casa DJ, Jensen KA et al. Examining the Influence of Hydration Status on Physiological Responses and Running Speed During Trail Running in the Heat With Controlled Exercise Intensity. *J Strength Cond Res*. 2011; 25(11):2944–2954.

43. Stearns RL, Casa DJ, Lopez RM et al. Influence of hydration status on pacing during trail running in the heat. *J Strength Cond Res*. 2009; 23(9):2533–2541.

44. Costa RJ, Teixeira A, Rama L et al. Water and sodium intake habits and status of ultra-endurance runners during a multi-stage ultra-marathon conducted in a hot ambient environment: an observational field based study. *Nutr J*. 2013; 12:13–29.

Chapter 2

The Aetiology of Fatigue

I'm pretty tired... Think I'll go home now.

- Forrest Gump

2.1. BACKGROUND

The single most important factor in determining trail performance is the capability of the runner to avoid and resist (neuromuscular and mental) fatigue. A runner that is fatigued is more likely to run inefficiently and injure himself on the course, is less capable of making positive strategic decisions, and of effectively actively monitoring physiological needs such as nutrition and hydration[1–6]. In order to develop strategies to help runners better resist both cognitive and neuromuscular fatigue, a better understanding of the fatigue processes specific to trail running is necessary. To this end some of the more popular fatigue models shall be presented and their relevance to trail running discussed in the following. Fatigue is generally defined as a "fully reversible decrease in muscular force output induced by intensive contractions"[7,8]. This definition of fatigue insinuates that fatigue is present from the moment that voluntary force output decreases. In the history of fatigue investigation, this perspective on fatigue is a rather modern approach. The prestigious Ciba symposium 82, which laid much of the foundation for modern fatigue research, defined fatigue as "as a failure to maintain the required force or power output" as late as 1981[9]. In effect, fatigue has been shown in all mammalian muscles and in all different kinds of contractions. This includes concentric (dynamic shortening muscle), isometric (static equidistant muscle) and eccentric (dynamic lengthening muscle) contractions and is valid for intermittent and sustained contractions. Generally speaking, intermittent contractions tend to cause less fatigue due to the short recuperation periods between contractions, while sustained contractions over 50% maximal voluntary contraction force (MVC) hamper the blood flow, causing ischemia and accelerating the rate of fatigue onset[10,11].

2.2. History of fatigue investigation and classical concepts

The earliest observers of human performance must have already noticed a progressive decline of performance in intensely-used muscles, although this did not become part of scientific discourse until Berzelius described high levels of lactic acid in the muscles of an exhausted stag in 1807[12,13]. Needham gives a detailed account of the history of muscle research in his *Machina Carnis* [14] tracing the aetiology of fatigue from Berzelius over Mosso's 1904 book *La fatica*[15], creating fatigue symptoms using electrical stimulation and the seminal paper of Hill and Kupalov[16], indicating that muscular performance decrease was directly linked with lactic acid accumulation and dispersion[16]. These early studies gave rise to the notion that fatigue is a mainly peripheral phenomenon and strongly linked to lactic acid concentration. This idea was first challenged by Eberstein and Sandrow[17], who perfused fatigued muscle fibres with caffeine and observed an acceleration of force production recovery, suggesting that fatigue may instead be linked to a failure of the excitation-contraction (E-C) coupling. Soon after, Bergström et al.[18] perfected a muscle biopsy technique, which allowed the extraction of muscle samples for biochemical analysis at specific time points during exercise. This facilitated more detailed analysis of the biochemical changes involved in the fatigue process and in turn led to research identifying the causes of fatigue as linked to glycogen depletion[19] and phosphate metabolite and H^+ accumulation[20]. These muscle-centric changes are collectively known as peripheral causes of fatigue, as they are locally restricted changes in the force producing units. Investigations of these peripheral changes are often based on electrical stimulation that circumvents possible confounders in the activation process.

When the development of fatigue in an integrated physiological system is to be described, the activation process from the supraspinal level through the central nervous system (CNS) needs to be taken into account. Changes in α motor neuron drive may precede and modify peripheral fatigue manifestations and can account for an important part of the fatigue process. While this was in some form evident to the pioneers of fatigue research[15,21], direct identification and

quantification of changes in the motor drive output that is finally effectuated at the level of the motor end plates, let alone at other points in the activation cascade, has proven to be technically challenging. Until appropriate methods were developed (notably fine-wire electromyography[22]), studies of changes in motor output were rather empirical and employed indirect markers such as hypnosis, prior mental conditioning and using conditioning cues[23]. This geographical and temporal dissociation of the phenomena has historically led to a distinctly dualistic perspective on fatigue, dividing processes into peripheral and central components. More recently, models have been proposed that re-unify the components and focus on the (afference and feed-forward based) interaction between central and peripheral components of fatigue[24–29].

2.3. Peripheral fatigue

Peripheral fatigue models focus on explaining fatigue processes in the actual movement effectuators, i.e. at a level lower than the neuromuscular junction. The notion that the primary source of muscle force output reduction lies in this area can be traced to the first experiments describing fatigue, in which isolated muscle fibres were continually stimulated electrically and a decline in force output was observed[16]. Since performance reduction was observed at a lower hierarchical level than the motor endplates and early examinations disclosed no changes in neuronal motor output[30,31], it was only logical to investigate the muscle itself to identify the processes that lead to a reduction in force. Peripheral fatigue has been the focus of much investigation and numerous factors that are in some form implicated in the development of fatigue have been identified. Studies examining peripheral fatigue are often based on models such as electrically stimulated in-vivo muscles, isolated muscles, isolated single fibres and in some cases skinned fibres which allow the reduction of confounders but incur certain disadvantages (Table 2.1 taken from Allen 2008). To further elucidate the potential action points of fatigue in the periphery, a schematic of the mechanics of activation shall be presented in the following.

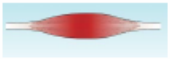
Muscle in vivo 	Advantages	All physiological mechanisms present Fatigue can be central or peripheral All types of fatigue can be studied Stimulation patterns appropriate for fiber types and stage of fatigue
	Disadvantages	Mixture of fiber types Complex activation patterns Produces correlative data; hard to identify mechanisms Experimental interventions very limited
Isolated muscle 	Advantages	Central fatigue eliminated Dissection simple
	Disadvantages	Mixture of fiber types Inevitable extracellular gradients of O_2, CO_2, K^+, lactic acid Mechanisms of fatigue biased by presence of extracellular gradients Drugs cannot be applied rapidly because of diffusion gradients
Isolated single fiber	Advantages	Only one fiber type present Force and other changes (ionic, metabolic) can be unequivocally correlated Fluorescent measurements of ions, metabolites, membrane potential, etc. possible Easy and rapid application of extracellular drugs, ions, metabolites, etc.
	Disadvantages	Dissection difficult Environment different to in vivo K^+ accumulation and other in vivo changes absent Prone to damage at physiological temperatures Small size makes analysis of metabolites difficult
Skinned fiber	Advantages	Precise solutions can be applied Possible to study myofibrillar properties, SR release and uptake, AP/Ca^{2+} release coupling Metabolic and ionic changes associated with fatigue can be studied in isolation
	Disadvantages	Relevance to fatigue can be questionable May lose important intracellular constituents Relevant metabolites to study must be identified in other systems

Table 2.1: *Models used to investigate fatigue. Replicated from Allen, 2008*[8]

2.3.1. THE EXCITATION-CONTRACTION COUPLING

From the arrival of an action potential (AP) at the motor endplate and the actual contraction of the muscle, a cascade of processes must take place, each component of which is potentially susceptible to fatigue. Motor endplates are distributed rather homogenously along the length of the muscle in order to achieve a simultaneous arrival of the AP. Arriving at the motor endplates, the AP leads to a secretion of acetylcholine (ACH), which incurs a membrane depolarisation. This travels longitudinally along the surface membrane of the muscle (2 to 6 $m \cdot s^{-1}$)[32] and transversely into the transverse tubules (T-system; 0.3 $m \cdot s^{-1}$)[33]. The depolarisation of the T-system stimulates dihydropyridine receptors (DHPRs) that are located along the T-system, which in turn activate the ryanodine receptors (RyR) located in the membrane of the sarcoplasmic reticulum (SR). The RyR receptors have multiple channels through which calcium

(Ca^{2+}) from the sarcoplasmic reticulum (SR) is released into the myoplasm. With Ca^{2+} release, the first phase of the E-C coupling is complete (Fig 2.2).

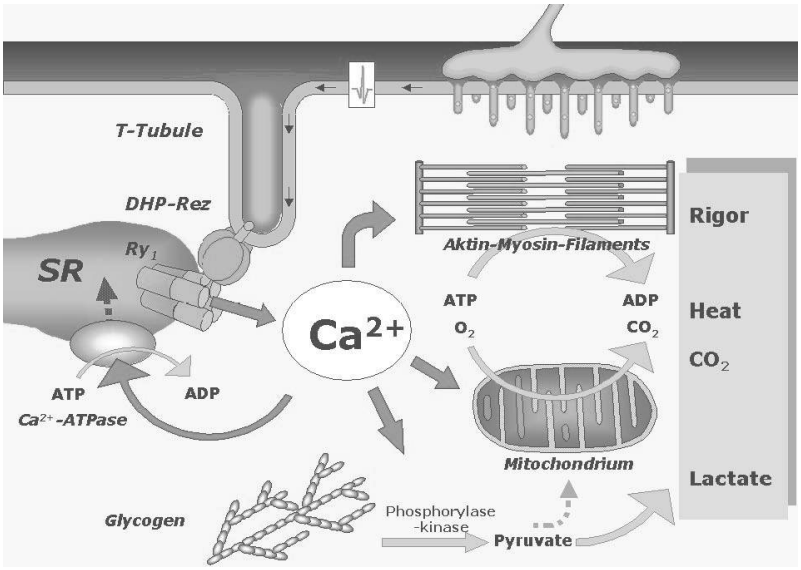


Figure 2.2: *Schematics of the excitation-contraction coupling.*

The increase of the myoplasmic Ca^{2+} concentration has a number of effects: (1) the actual contraction is initiated, (2) the oxidative processes of the mitochondrium are stimulated and adenosine tri-phosphate (ATP) production is enhanced, (3) the energy dependent SR Ca^{2+} pump is activated, removing Ca^{2+} from the myoplasm. Contraction initiation from this point is a rather well-investigated process – concentration increase of Ca^{2+} facilitates increased binding of Ca^{2+} to troponin C, which in turn shifts the tropomyosin compound freeing the myosin binding sites and allowing the formation and cycling of cross bridges from actin to myosin filaments. This is the direct origin of muscular movement as the actin and myosin filaments in the muscle slide past each other shortening the serial-connected sarcomeres. Many of the described processes are energy driven and this energy is primarily derived from hydrolysis of adenosine tri-phosphate (ATP) into adenosine di-phosphate (ADP) and inorganic phosphate (P_i). Rephosphorylation takes place through one of three pathways: (1) Aerobic: oxygen and glucose dependent mitochondrial process, (2) Anaerobic glycolysis: oxygen independent glucose dependent cytosolic pathway, (3) Phosphocreatine (PCr): oxygen independent donation of P_i to ADP molecules via kinase interaction. A simplified concept of predominant rephosphorylation

mechanisms in function of exercise time are represented in figure 2.3. Both the accumulation of ADP and related products as well as the rephosphorylation sites of ADP into ATP are further points at which fatigue processes may latch.

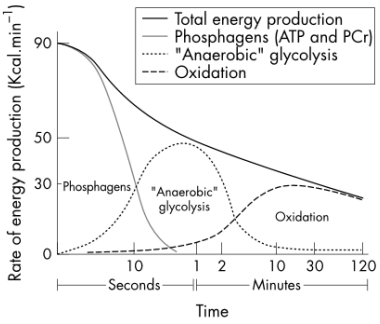


Figure 2.3: *Simplified concept of ATP re-synthesis processes over exercise duration. Figure reproduced from Howald et al. (1978)*[34]*.*

2.3.2. Potential fatigue processes affecting the propagation phase

The propagation of the AP over the surface membrane into the T-system can be impaired by changes in membrane potential and sodium (Na^+) and potassium (K^+) gradients that may develop during intense work that leads to fatigue. In prolonged exercise, such as trail running, this is further provoked through sweat loss-induced dehydration. Effectively, the main influences on propagation are associated with increases of leaked ions such as K^+ and Na^+, which impede the conductivity of the T-system channels. While in vitro studies using skinned and stimulated fibres have identified that high extra-cellular K^+ concentrations (7 to 13 mMol) create a graded reduction in force production (-30 to -100%), it is not certain that this is pertinent during exercise[35–38]. Physiological extracellular K^+ concentrations at rest lie at around 4 mMol[36] and can locally increase up to 10 mMol in-vitro, but have not been shown to exceed 6 mMol in muscle during exercise in-vivo[39]. Indirect conclusions concerning propagation velocity in humans have been drawn from measures obtained using peripheral nerve stimulation (PNS). As the electrical stimulus is induced into the α motor neuron, a direct muscular response (M-wave) is elicited at around 3-6ms delay along with a delayed monosynaptic response (H-reflex, 45 to 56 ms). The shape of the M-wave in fatigued and non-fatigued muscles is very similar, leading to the conclusion that longitudinal propagation

properties remain unaffected by fatigue[10,11,40,41]. Studies employing a linear array of electrodes (high density electromyography – HDEMG), have on the other hand observed an decrease of propagation velocity with fatigue[42–45]. More studies are required to arrive at a definite conclusion, and any potential changes are likely to be task dependent[44]. In any case, a number of dedicated systems exist to ensure that K^+ leakage does not become too pertinent and critically degrade propagation velocity.

2.3.3. Factors influencing calcium release and calcium re-uptake

Once the AP has franchised the T-system, the next steps in the cascade leading to the release of Ca^{2+} from the SR are the activation of the voltage-sensitive DHPRs and the subsequent ryanodine receptor reaction. This sensitive part of the E-C coupling is termed the triad junction due to its three principle actors; the SR, the T-system and the DHPR-RyR sensor complex. In contrast to the actual processes in the T-system itself, the release of Ca^{2+} into the myoplasm is technically relatively straightforward to measure and provides an interesting outcome measure. In short, the interaction between the DHPRs and the associated RyRs calcium-release channels is currently a focal point of fatigue research[46] and seems more bi-directional and complex than first assumed (for a detailed review consult Bannister[47]). Investigations of the triad junction indicate that the rate of Ca^{2+} release is dependent on myriad factors including the concentration[48,49] and sensitivity of DHPRs and RyRs[50,51] and stimulation type (caffeine, AP, etc). Acutely, the Ca^{2+} rate of release is reduced by a decrease in amplitude of the arriving AP, increase of cytoplasmic ionic magnesium (Mg^{2+}) levels[52–54], decrease in myoplasmic ATP concentration[55,56], and a decrease in SR Ca^{2+} levels[57,58]. Calcium reuptake into the SR is central to keeping the diffusion gradient stable. The reuptake rate is negatively affected by fatigue processes, namely by increased levels of inorganic phosphate and ADP and the associated decreases in ATP[55,59–61]. Inorganic phosphate can seep into the SR through the ionic chloride channels at high intracellular concentrations, leading to CaP_i precipitation effectively lowering free Ca^{2+} concentration and blocking the calcium release channels[49]. This mechanism is also susceptible to oxidative stress and increased reactive oxygen species (ROS) such as hydrogen

peroxide[62]. Especially in prolonged exercise where the mitochondria adopt a high rate of ATP rephosphorylation, the development of ROS is an important contributor to the development of fatigue. Not only do ROS increase the influx of P_i into the SR, but they are also directly associated with c-Jun N-terminal kinase (JNK) -mediated necrotic cell death via the Tumour Necrosis Factor (TNF) Receptor super family[63–65]. Furthermore, ROS are implicated in the mediation of the inflammatory response, being secreted by myokines to induce mononuclear cell apoptosis[66]. The lack of Ca^{2+} re-uptake into the SR has also been demonstrated to be central in prolonged endurance exercise, as Ca^{2+} content has been shown to increase to over 30% of resting level following runs over 20 km[67,68]. Increased Ca^{2+} concentration and associated membrane leakage trigger signalling cascades via calpain activation, which ultimately result in further cell necrosis and mononuclear cell invasion[69].

2.3.4. ATP SUPPLY AND METABOLIC BY-PRODUCTS

Adenosin tri-phospate (ATP) is the muscle cell's primary source of energy. An ATP depletion lead to a loss in force production, as the unlatching of myosin cross bridges (CB) becomes impeded and cycling becomes impossible. Additionally, a number of ATP or ADP to P_i ratio sensitive mechanisms that can hinder the E-C coupling exist. A decrease in the ATP to ADP ratio for instance leads to a down-regulation of the Ca^{2+} release at the RyR site, a reduction in CB cycling velocity, a mitigation of CB catching force, a reduction in Ca^{2+} sensitivity, a decrease in Ca^{2+} resorption rate and increased CaP_i precipitation in the SR. A fall of the ATP to ADP ratio also leads to an increase in adenosine monophosphate (AMP), which gives a strong signal to the AMP-activated protein kinase (AMPK) to curb energy depletion and reactivate catabolic ATP synthesis pathways[70]. ATP production is principally dependent on ready supplies of ADP, P_i, PCr, substrates and oxygen among others. Potential limitations in the substrates required for ATP synthesis can lead to a shift in synthesis pathways leading to a less optimal ratio of ATP to waste products. ATP during prolonged effort is mainly synthesised in the mitochondria through the aerobic pathway, this being by far the most efficient ATP synthesis process[71]. Without going into too much detail, mitochondrial abundance is one of the potentially limiting factors. Type 1

muscle fibres (red muscle) tend to represent the primary fibre type in endurance runners and exhibit greater mitochondrial abundance. Further bottlenecks are oxygen and nutrient supply. Oxygen supply is dependent on oxygen uptake at the level of the lungs, cardiac output and diffusion capacity (capillarisation) at the muscle site. A number of different energy sources can be used at the nutrient level, the main sources in prolonged exercise being composed of carbohydrates and fat. Glycogen is stored in the muscle cells themselves and primarily in the liver, providing a fast and highly effective energy source. Triglycerides and fatty acids on the other hand are a less optimal energy source, as cross-membrane transport is energy driven, and fatty acids must undergo oxidation before they can enter the Krebs cycle[72,73]. Substrate utilisation is finely regulated using different messaging cascades, the most central and well-known being the AMPK-insulin cascade[71]. The aerobic ATP synthesis pathway is also considered the main source of radical oxygen species (ROS) which have been shown to incite signalling cascades leading to inflammation and protein degradation. As the muscle becomes fatigued, the ATP synthesis provided by the aerobic pathway alone is no longer sufficient to meet the cell's energy demands and the anaerobic pathway is re-stimulated. By-products of the anaerobic pathway include lactate (Lac), P_i, and H^+ ions, both of which have detrimental effects on the force generating capacities of the cell. As $[H^+]$ increases, the pH of the cell drops decreasing Ca^{2+} sensitivity and Ca^{2+} resorption in the SR. Increased [Lac] was for a long time considered the primary instigator of fatigue, yet more recent studies indicate that the concentrations reached during exercise (< 30 mMol) are far below the threshold for force depression (50 mMol), Ca^{2+} release inhibition and Ca^{2+} sensitivity depression[74]. As lactate diffuses through the cell membrane, a diffusion gradient is created draws cellular water (H_2O) into the extracellular matrix. This leads to a drop in intracellular $[H_2O]$ and an associated inhibition of force production. In short exercise this may be negligible, yet in prolonged exercise global dehydration frequently onsets and the muscular drainage process is exacerbated. In summary, the metabolic component of fatigue can be seen as the driving mechanic of the process. The compounds stemming from the metabolisation and rephosphorylation of ATP, mainly H^+, Mg^{2+}, ROS, and P_i, result in the processes ultimately leading to contraction failure through various interrelated pathways[75].

2.3.5. Myosin cross bridge latching and cycling

As already touched upon in the preceding section, the final possible site of fatigue is within the latching and cycling of the myosin cross bridges (CB) themselves. The process can be differentiated into a number of phases starting with the Ca^{2+} induced shift of the troponin complex, MHC latching (weak phase), ATP hydrolysis and power stroke, ATP binding (strong phase) and MHC release, Ca^{2+} regulation and movement of the troponin complex. Fatigue has been found to slow CB cycling, reduce maximal CB latching force and reduce myofibrillar Ca^{2+} sensitivity therefore decreasing the number of open binding sites for a given amount of Ca^{2+} [8]. Discretely, Ca^{2+} sensitivity is reduced by an increase in $[P_i]$, [ROS][76] or $[H^+]$. Increases in $[P_i]$ have also been associated with reduction in latching force, which may lead to a "slipping" of the myosin heads, making each cycle less effective. Increases of [ADP] have been shown to negatively impact CB cycling velocity, although the precise mechanisms are not yet fully elucidated.

2.3.6. Methods used to determine peripheral fatigue in trail running

During trail running, the determination of peripheral fatigue is difficult due to limited available non-invasive methodologies. In order to stabilise and maximise motor drive, techniques such as evoked contractions and twitch trains of different lengths are frequently employed. The main muscle groups investigated are the knee extensors and the plantar flexors, both of which have shown force depression post trail running in function of exercise duration[77–81]. Stimulation is generally applied either neutrally (PNS) or percutaneously (Estim) at various frequencies. Millet et al.[79] for instance employed PNS at stimulation frequencies of 20 and 80 Hz after a 30 km trail run (E/D = 27) in the knee extensors. Different frequencies were use in stimulation to try and identify low frequency fatigue (LFF), which is associated with peripheral alterations. They observed a depression in peak-to-peak amplitude of the electromyographic response to PNS (M-wave), and a decrease in mechanical twitch response amplitude and contraction time, although no changes were reported in the rate of twitch force development or relaxation. Both

stimulation frequencies exhibited a similar decline in evoked force, leading to an unchanged frequency ratio[79]. Recently, LFF was observed for the first time in trail running[77], yet this remains a novel occurrence and was recorded after a 161 km race, so might represent a special case. Similar methodologies have been used following mountain ultra marathons[77] and prolonged trails[81]. Apart from these non-invasive strategies, it is feasible to recover tissue samples using muscle biopsies, although there is currently no published study examining tissue samples in trail runners. This would allow protein analysis, although the samples are probably prone to streaming due to the damaging nature of the exercise and there is an impact of the procedure itself[82,83]. Similar peripheral fatigue has also been extensively investigated in prolonged flat running[84–86] of similar duration, although it has been suggested that the greater eccentric strain encountered during trail running evokes a specific type of peripheral fatigue[77,87]. Further investigation is warranted to determine whether or not trail running results in a different peripheral fatigue profile than other types of endurance exercise.

2.4. CENTRAL FATIGUE

In the preceding, the peripheral processes leading to a reduction in force production have been discussed on the prerogative that the neuronal drive arriving at the neuro-muscular junction (NMJ) remains maximal throughout exercise. In real-life exercise this has been shown to be only feasible in short maximal contractions and in most cases there are myriad factors that influence the synchronisation and amplitude of the activation pattern that reaches the NMJ[15,88]. A progressive reduction in motor neural drive through prolonged or repeated contractions has been termed central fatigue[89]. Simply put, during a fatigue task, as the muscle becomes fatigued evoked force output decreases at a lower rate than the voluntary force output (Fig. 2.4).

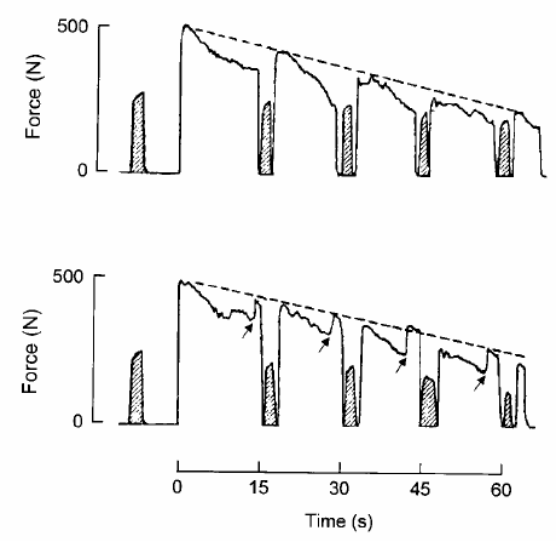


Figure 2.4: *Voluntary and evoked torque decline. The upper panel depicts a strong voluntary torque decline, while the evoked torque stays almost stable. In the bottom panel, participants were exhorted to produce a "super effort" at the end of each contraction. This led to a more pronounced decline of evoked force. Reproduced from Bigland-Ritchie, 1981*[90]

This leads to the conclusion that the motor drive to the muscles is not invariably maximal, but is reduced progressively while fatigue occurs. While physiologists concur that both peripheral and central factors are involved in fatigue processes, there has been prolonged discussion over which site is the limiting factor. Although demonstrations of central fatigue reach back as far as the end of the 19th century[15], quantification has proven difficult due to limited tools available. A number of techniques were pioneered in the mid 1950s which allowed a first ingression on the topic of central fatigue, namely the interpolated twitch technique and the first insights on surface electromyography (sEMG) recordings and force production[22,30]. This revitalised the topic by enabling qualitative assessment of the motor drive, and subsequently studies on central fatigue gained momentum. In the same period, Bigland and Lippold[22] first deployed fine wire EMG and the identification and analysis of single motor unit action potentials (MUAPs) became feasible. Contrary to what is assumed in peripheral fatigue models, motor drive is not invariably maximal and is subject to modulation depending on various factors. This has already been demonstrated in some of the early experiments on fatigue (Fig. 2.5).

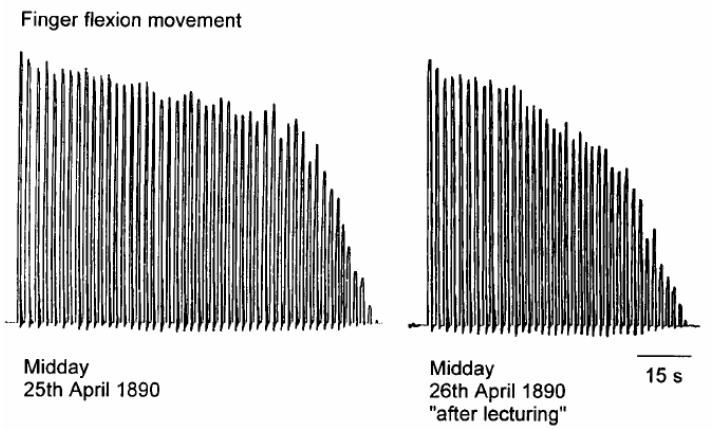


Figure 2.5: *Finger flexion moment in a submaximal task before and after a cognitive task (lecturing). Note that the decline in moment is stronger and onset is earlier post-intervention, although the cognitive intervention did not involve any muscle-fatiguing task. Reproduced from Mosso, 1904*[15]

One of the most accessible indications that motor drive is not a fixed constant are the adaptations engendered through training in both regulatory domains; firing frequency and recruitment rate. Additionally training has been demonstrated to increase synchronicity and optimise global motor strategies. Results include contra-lateral increases of force in untrained muscle[91,92], increases in EMG surface potential following training (although this is often not considered a valid indicator[36]), increases in the ability to sustain high discharge frequencies[93],increase of doublet discharges[94]and an increase in rate of force development[94,95]. Neither muscle cross sectional area[96] nor maximal evocable force[97,98] increase as much as maximal voluntary force. Also, imagined training has also been reported to increase MVC in the abductor digiti minimi, although there is no peripheral training component involved[99]. A number of studies suggest that increases in task force are also produced by the optimisation of motor patterns[100,101]

2.4.1. Characteristics and investigation methods in central fatigue

The original twitch interpolation method introduced by Merton[30] relies on the decline of a superimposed twitch with increasing activation of the muscle compared to a control twitch at rest. This method has been refined over the past and nowadays twitches of under 0.1% of

maximal force can be resolved.[102,103] Different muscles have been identified to have different maximal voluntary activation ratios[104,105] and no muscle has been recorded to achieve 100% voluntary activation. A number of methodological constraints must be respected when relying on voluntary activation measurement and this has led to an unreliable reputation of the measure which is not fully justified[7]. More precisely, central fatigue manifests itself mainly as a drop in the firing rate of the recruited motor units[106,107], but motor unit recruitment is also reduced[108,109]. This leads to the observation of decreased voluntary activation[107,110,111] and is accompanied by an increase in force fluctuation[20,112] leading to a higher control cost and interfering with accurate task performance. The site of origin for the decline in motor drive is not completely determined to-date, and it seems probable that there are interactions between multiple sites at multiple levels that contribute to down-regulation of firing rates and recruitment.

2.4.2. The muscle wisdom hypothesis – spinal factors

The "muscle wisdom" hypothesis relies on a matching of motor drive decline to changes in the mechanical properties of the contractile element. As the contractile element is activated, tetanic fusion frequency drops as muscle relaxation speed increases. Therefore, an inhibition of motor drive would engender no loss of force and represents an energy optimisation strategy. When the regulative mechanisms become overloaded, regulation precision is compromised and stronger down-regulation could lead to a drop in force production[95]. Research on the auto-regulative mechanisms on a spinal level abounded in the years following the proposal of this hypothesis and identified numerous pathways that may be involved. Generally, any of the afferents from muscle receptors could potentially be implicated, especially those modulating drive through spinal reflex inhibition loops such as the spindle-driven Ia afferents and Golgi-driven Ib afferents. Also, the group III and IV afferents, which respond to numerous changes in the muscle including biochemical parameters, muscle stretch, pressure, contraction and nociceptor activation[113–117], play a role in motor drive regulation[109,118]. Finally there may be a form of pre-synaptic inhibition leading to inhibited motor drive as a function of metabolite

accumulation and local concentration changes. While there is varying evidence for implication of each of these mechanisms, so far no consensus has been reached as to how precisely this reflexive regulation functions. A number of weaknesses of the muscle wisdom hypothesis have surfaced which make this form of regulation rather improbable. For instance, changes in the mechanical properties of the contractile unit through temperature changes[95,119] and unit length[120,121] did not have the predicted impact on firing rates. Furthermore, interactions between firing rate and muscle relaxation time are different in intermittent[122] and submaximal[123,124] contractions, suggesting that reflexory regulation is task dependent. Although the "muscle wisdom" hypothesis itself seems unlikely at this point in time, the research conducted has illuminated various reflexory contributors to central fatigue.

2.4.3. Contributors to central fatigue – supraspinal factors

Supraspinal factors play an integral role in central fatigue and are defined as a reduction in output from the motor cortex. Transcranial magnetic stimulation (TMS) has recently provided the research landscape with a ready tool to investigate the effects stimulation of the motor cortex itself in fatigued states[125–127]. Briefly, supraspinal stimulation leads to a lower evoked force than peripheral stimulation in a fatigued state, indicating that drive modulation occurs not only at spinal sites, but also at supraspinal sites. Supraspinal motor drive modulation is a rather contemporary research field and the discrete mechanisms driving central fatigue are yet to be concurred upon[89]. At the moment, supraspinal factors are presumed to be mechanisms either acting upon the motor cortex and reducing its drive output ("supra" motor cortical), or factors reducing the sensitivity of the corticospinal neurons to excitation. Mechanisms of the second group would insinuate that a given drive output from the motor cortex would result in less motor drive in the corticospinal neurons[7]. As changes in the conduction properties of corticospinal motor neurons have been observed during voluntary contractions[128,129], this could be a likely interaction. On the other hand, supra motor cortical effects such as neurotransmission factors, motor cortical inhibition and afference modulation[130,131] have also been observed in animal models and it is difficult to distinguish between the two with the

methods available at the moment. Supraspinal factors in central fatigue are hence not yet fully elucidated in their causality, yet their existence is unequivocally accepted[132].

2.4.4. The "central fatigue hypothesis" – neurotransmission factors

First proposed in 1987, the "central fatigue hypothesis" takes a different approach on the origin of a decline in motor drive[133]. Regrouping a series of changes in blood composition and the blood brain barrier (BBB), the theory basically hinges upon certain amino acids gaining unregulated access to the brain and initiating a series of up-regulations which ultimately lead to decreased motor drive. More precisely, the large neutral amino acid (LNAA) transporter located in the BBB is, during normal operation, almost saturated with transporting branched chain amino acids (BCAAs) into the brain circuit. During exercise, blood BCAA concentration drops and competing aromatic amino acids can use the free valences of the LNAA-transporter. Additionally, in the case of Tryptophan (TRP), the mobilisation of free fatty acids from the adipose tissue during exercise leads to an increase in unbound plasma TRP (f-TRP), that increases the probability of transport[134]. Three compounds especially are transported, TRP, Phenylalanine (PHE), and Tyrosine (TYR)[135]. Both TRP and TYR are the limiting factors in the catalysed synthesis pathway of serotonin (5-HT)[135]. Increases in 5-HT are associated with feelings of tiredness and lethargy and have been suggested to decrease motor drive and motor unit recruitment[136]. Since its proposition, the "central fatigue hypothesis" has been modified to include mediators in the form of changes in the noradrenaline (NA) and dopamine (DA) systems. Both systems are central to motivation, memory, reward and attention processes and are dependent on TYR for synthesis. Evidence for this hypothesis stems mainly from the animal domain, although several studies have attempted to modify central fatigue (or at least 5-HT concentration) through supplementation or pharmacological intervention in humans. In a 2007 review, Meeusen and Watson conclude that, "although the rationale for a central fatigue hypothesis is solid, the largely inconsistent findings of many manipulation studies make it difficult to draw any conclusions regarding the role of central neurotransmission in the fatigue process."[137]

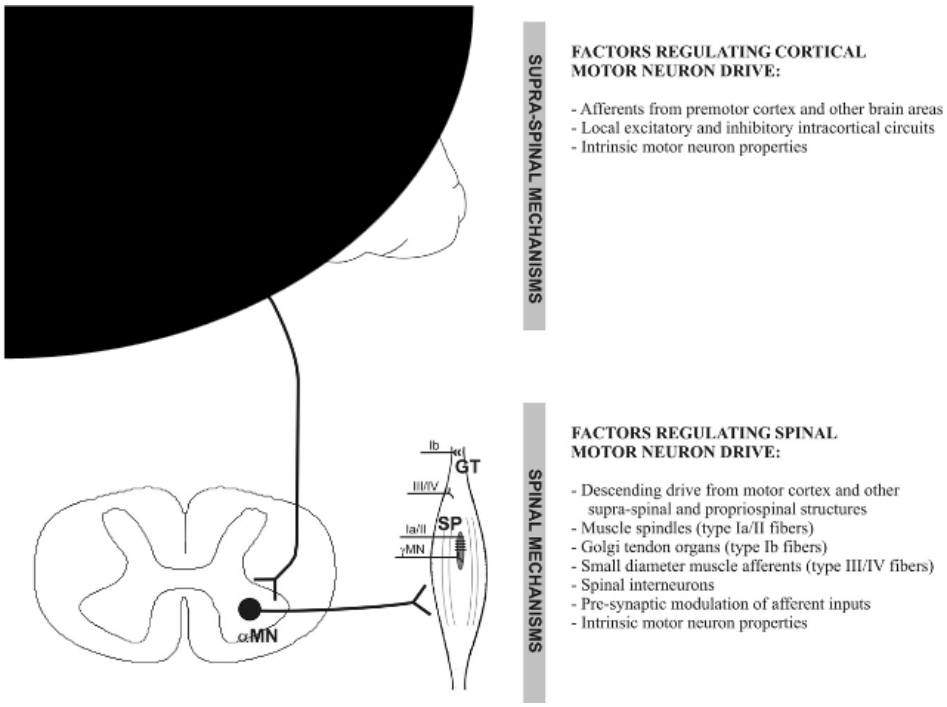


Figure 2.6: *Contributors to central fatigue. Replicated from Ranieri and Lazzaro, 2012*[132]

2.4.5. METHODS USED TO DETERMINE CENTRAL FATIGUE IN TRAIL RUNNING

Central fatigue in trail running seems to be a main focal point, due to some of the more recent observations concerning peripheral fatigue. The main components of central fatigue are recapitulated above in figure 2.6. Recently, neuromuscular fatigue in the knee extensors has for instance been measured after running races of 10[67], 20[67], 30[79] 42[138,139], 50[140], 55[80], 65[81] and 166[77] km distance. Lab studies also assessed neuromuscular fatigue after 2, 5 and 24 hour treadmill runs[84–86,141], 45 mins of simulated trail running[142] and 90 mins of intermittent versus continuous running[143]. Earlier studies have assessed similar variables, and have recently been reviewed[87]. Although there is a need for more studies examining force loss in prolonged exercise of over 450 mins, it seems that knee extensor force loss increases with increasing exercise duration in a non-linear fashion, levelling off around the 8 hour mark (Fig. 2.7).

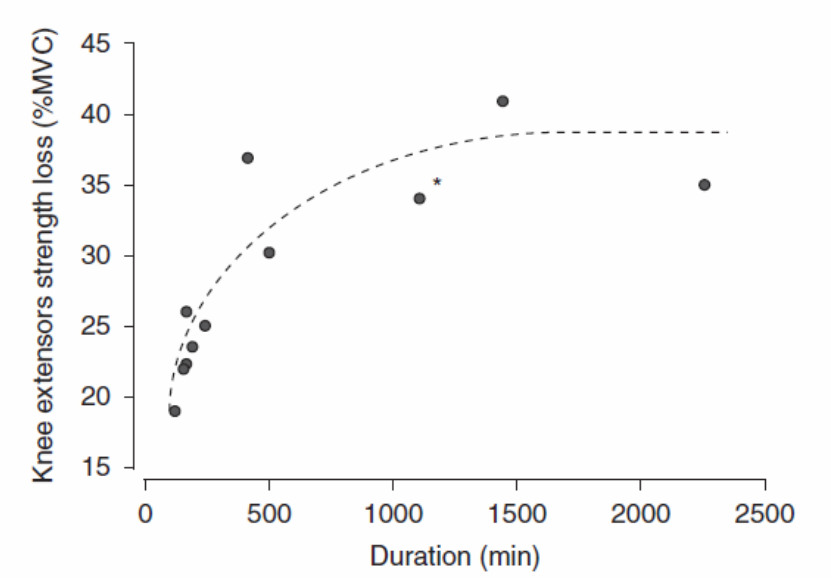


Figure 2.7: *Knee extensor strength loss in running endurance tasks of different duration. Reproduced from Millet, 2011*[25]

In addition to the generic decreases in knee extensor force production, indicating that a form of fatigue has been induced, a number of indexes were assessed that are conclusive as to the origins of the measured decrease. Primarily, contractile element properties were examined using the M-wave, mechanical twitch properties and peripheral stimulation at high and low frequencies as presented earlier. Also, although suffering from high inter-individual variability, an accumulation of indirect plasma markers of muscle damage has been reported after prolonged running, supporting the presented data on peripheral alterations[80,85,140,144–147]. In summary, peripheral alterations are present, but – following the logic of Marcora et al.[27,148] that muscle contractions during running are not at a high percentage of maximal force – should not inherently lead to termination of exercise.

Therefore central activation changes must play a focal role in prolonged running exercise. Using the interpolated twitch technique and M-wave normalised EMG, it has been shown that central factors play a large role in muscle fatigue[79,81,84,85,141,143]. As previously discussed, central fatigue spans several areas, the biochemical, spinal and supraspinal. On the biochemical plane, Ohta et al.[149] reported increases in serum serotonin and f-TYR levels, suggesting a form of biochemically

driven inhibition. Another potential pathway could be the reduction of cerebral blood flow due to a reduction in arterial CO_2 and the competition for oxygen[150]. Spinal mechanisms have been investigated using the Hoffmann reflex (H-reflex), a monosynaptic spinal reflectory action induced through electrical stimulation of the Ia afferents, which has been observed to be depressed following prolonged (90 mins) running exercise[143]. Furthermore, Millet and Lepers[87] observed that post-exercise voluntary activation depression was more pronounced in modalities involving muscle damage such as running, therefore maybe implicating nociceptor and metaboreceptor afferences in voluntary drive regulation. This indicates that group III and IV spinal inhibition or pre-synaptic inhibition is present. Supraspinal changes are therefore likely not only to be driven by biochemical changes, but also by afferent muscular feedback[89]. With the advent of TMS, the contribution of supraspinal and spinal factors can be better differentiated. Momentarily there is still a lack of data in this field but Ross et al.[139], for instance, reported that post-marathon motor evoked potential is depressed by as much as 67%, indicating a substantial central component.

2.5. Fatigue in prolonged exercise

To recapitulate, the theories presented above mainly address the question of how fatigue develops in an isometrically contracted muscle performing maximally. This model is chosen as it limits possible confounders such as motion artefacts, changes in sarcomere overlap and shifts on the force-velocity relationship. In these well-controlled models already, the precise mechanisms of fatigue are hard to unequivocally determine. When regarding an endurance exercise paradigm using dynamic and intermittent contractions, the system complexity increases substantially. More components are involved and have an impact on the development of fatigue such as substrate availability, the lungs, and the heart. This leads to more intricate model designs and adds complexity to the interpretation of results. Therefore a number of theories have been advanced as to how fatigue develops in endurance exercise. These shall be briefly presented in the following.

2.5.1. The "catastrophe theory"

The explanation reaching back the furthest in time was probably coined by AV Hill[16,21] based on the experiments of Fletcher and Hopkins[151], storing frog muscles in oxygenated and anaerobic environments. Simply put, an accumulation of peripheral metabolites was observed in both sets, yet reached a maximum more quickly in the anaerobic set, leading to the idea that exercise was terminated due to metabolite accumulation. Simplifying and fast forwarding the history of fatigue models, it can be stated that this concept was transferred to the integrative human system principally by Edwards[152] and termed the "catastrophe theory" in the 1980s. "Catastrophe" in this case describes the depletion of ATP leading ultimately to the inability to contract. Historically, the catastrophe model centres on a limit in oxygen transmission due to a bottleneck in cardiac output (CO). CO is thought to plateau due to insufficient oxygen supply to the myocardium, thus mitigating the heart's potential to contract and increase CO. This leads to an upper limit in oxygen supply to the muscles and therefore in aerobic ATP resynthesis. Anaerobic glycolysis becomes more strongly implicated in resynthesis and metabolites build up, leading to the mechanisms discussed in the section covering peripheral fatigue (Section 2.3). The original model interpreted lactate accumulation as a peripheral governor that hindered the muscle from continuing to contract, thus avoiding muscle rigor onset (Fig. 2.8).

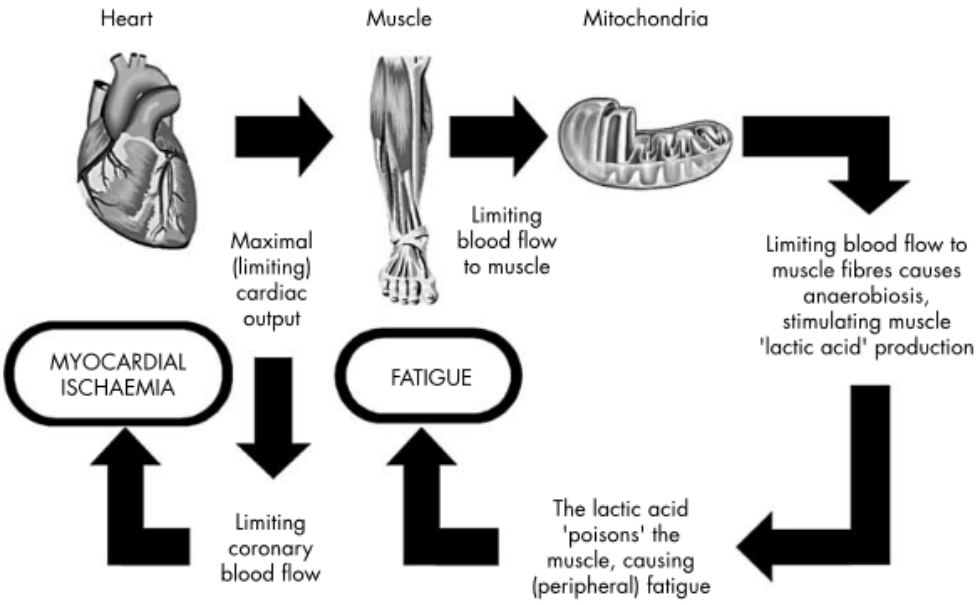


Figure 2.8: *The catastrophe model of fatigue. Limits in CO bottleneck coronary blood flow leading to a plateau in cardiac output. Due to the limited oxygen supply, muscles switch to anaerobic glycolysis and metabolites accumulate leading to fatigue and ultimately task failure. Reproduced from Noakes and Gibson, 2004*[153]

In contrast to the fatigue models discussed in the first section, this model is more applicable to endurance exercise, as it takes into account the different limiting factors and cross-references various physiological systems (coronary and muscular). Since the original invocation, a number of modifications have been made to adjust the concept to new findings, yet the basic paradigm remains unchanged. In some form or another an accumulation[154] or depletion of a substrate[155] often induced by a lack of oxygen, leads to the breakdown or bottlenecking of a system resulting in exercise termination (fatigue).

2.5.2. TELEO-ANTICIPATORY MODELS (CGM)

Probably the earliest model to challenge the catastrophe model of fatigue was the central governor model (CGM) proposed by Noakes at the turn of the 21st century[24,156,157]. Instead of

seeing the disruption of peripheral homeostasis in the muscle as the reason for exercise termination, Noakes argues for a protective mechanism that terminates exercise before catastrophe occurs – the "central governor". While its authors trace the CGM theory back to A.V. Hill, various modifications have been made to the original model due to the strong degree of circumspection raised through scepticism encountered in the scientific community[158–161]. In general, the CGM model attempts to resolve a number of contradictions and omissions in the catastrophe model using a cerebral feed-forward regulation mechanism. As catastrophic failure and the onset of skeletal muscle rigor have to date seldom been observed as a result of exercise, the notion of a governor of some form persists. This governor must therefore in some way effect a limitation of ATP depletion in order to maintain homeostasis and to hinder the onset of muscle rigor (Fig. 2.9).

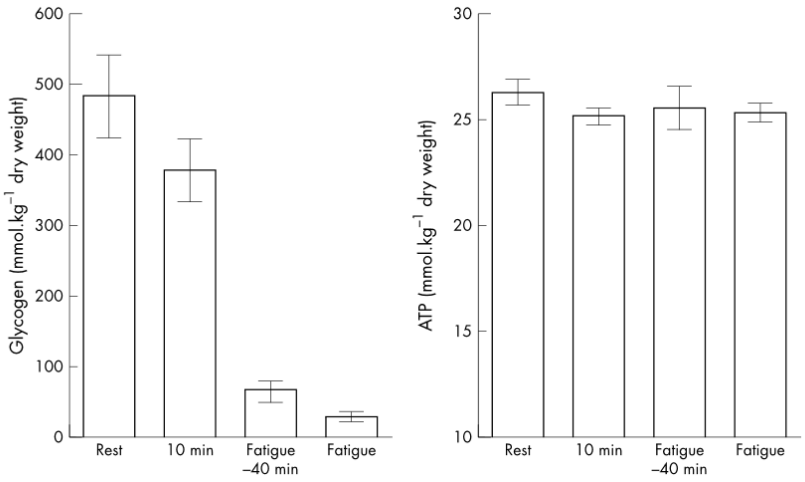


Figure 2.9: *Glycogen and ATP concentrations during prolonged exercise. Note that although glycogen concentrations fall drastically, ATP remains at a stable concentration. Thus there is some form of exercise intensity regulation active to stabilise ATP concentrations. Reproduced from Febbraio and Dancey, 1999*[162].

As presented in the peripheral fatigue section, it is now known that lactate accumulation plays only a minimal role in contraction impediment. This has also been confirmed in patients with McArdle's syndrome[163–165]. Hence the original idea proposed by Hill that lactate accumulation prevents the onset of rigor through creating an acidic environment that prevented contraction

loses the regulative element which must in some form be replaced. The CGM advances that the regulator is not a peripheral impediment of contraction, but resides in the subconscious brain and interfaces afferent neural drive from exercising muscles, cardiac and pulmonary systems, blood oxygenation receptors, metaboreceptors, motivational factors and task-dependant factors in a conglomerate fashion to determine motor drive. This conceptualisation allows for explanation of exercise regulation and termination, especially in varying environments and with varying motivational status and pharmaceutical intervention[153,161]. Also it explains the reduction in motor drive that has frequently been observed as fatigue develops. In Table 2.10, a recent comparison is made between the catastrophe model and the CGM model concerning some specific cases.

Table 2. The explanation for some common phenomena observed in athletes according to either the A.V. Hill or central governor model.

Phenomenon	A.V. Hill model	Central governor model
Pacing	One pace for all distances	Variable pacing
End spurt	Impossible	Possible
Homeostasis	Fatigue due to loss of homeostasis	Homeostasis maintained under all conditions as a result of behaviour modification
Skeletal muscle activation	Exercise terminates with 100% muscle activation	Never 100% muscle activation during exercise
Drug effects	Act on heart, lungs, muscles, but not brain	Explains effects of drugs acting on brain
RPE	A measure of the exercise intensity	A measure of the relative exercise duration

Note: RPE, rating of perceived exertion.

Table 2.10: *Differences of the CGM and catastrophe model in specific circumstances. Retrieved from Noakes, 2012*[156].

The central governor model is diagrammatically presented in figure 2.11 with the causality chain for motor drive generation. Especially during endurance exercise, evidence is accumulating that fatigue is a composite process which is actively regulated to optimise results. For instance, the beneficial effects of external focus strategies and cortically functioning pharmaceutics would also not be explainable[156,166,167]. Changes in motivation could not generate more performance. Pacing strategies such as negative splits (increasing speed throughout the race) or final bursts would not be possible using an only peripherally regulated model[29,168] although it has been argued both that pacing does not take place in high-level sporting competitions[169] and that pacing involves conscious decision making and therefore exercise planning cannot take place solely in the subconscious[170–172]. Effectively, in the CGM,

many of the factors discussed in the section on central fatigue are present. The CG is populated with information from various sites throughout the system via afferences (Group III and IV) and biochemical environment changes, which provide sufficient information to determine the general status. From this status report a subconscious teleoanticipatory function is assumed, in which the CG matches the current snapshot to an optimal performance template and, depending on their congruence subconsciously modulates motor drive and a conscious representation of fatigue. Based on the consciousness of fatigue, the model additionally allows for a voluntary modulation of motor drive, which can be impacted by external factors such as motivation boosts or psychological strategies.

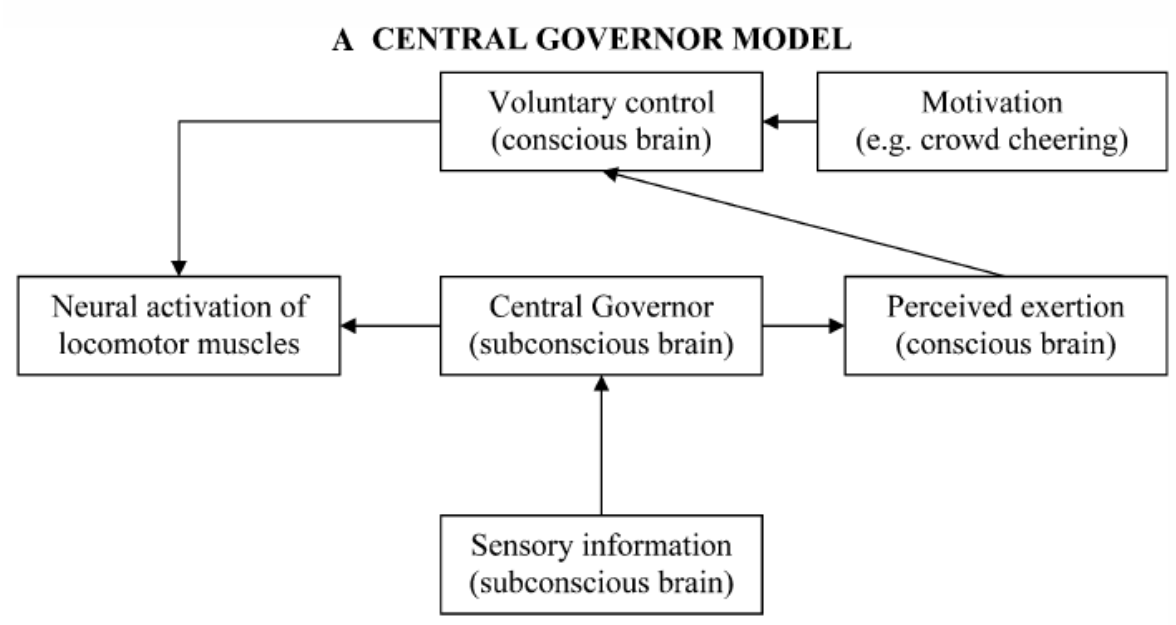


Figure 2.11: *The Central Governor model. Replicated from Marcora, 2008*[173]

Since the inception of the CGM, numerous studies have been conducted to clarify if the CGM predictions hold true and to examine in more detail the causative links between the different components of the model. For instance, Marcora et al. have introduced Brehm's general motivation theory to the model, explaining why motor drive is ultimately limited by motivation[27,174], and has proposed a dissociation of the sensation of fatigue from the afferences[175]. Nybo et al. conducted a series of very interesting experiments on hyperthermia

and exercise, determining that exercise in hot environments is terminated earlier[176,177], while rate of perceived exertion (RPE) is consistently higher[178] and is accompanied by cerebral perturbations[179,180]. These results support the concept that both motor drive and RPE are afference-mediated. Amann et al.[181] impeded afferences pharmaceutically and reported higher peripheral fatigue markers and improved performance, suggesting that there is a negative feedback loop linking afference and motor drive[181–183]. Additionally, they have demonstrated a link between muscle afferents and cardiovascular control[184], supporting the presence of an interconnected regulation mechanism. In the most recent iteration of the teleo-anticipatory model that started out with the CGM, fatigue is defined as an emotion rather than a physiological process that leads to the regulation of exercise[185,186].

2.5.3. THE FLUSH MODEL

The CGM model represented the first rift from peripheral driven models to a more integrative view, yet more recent models have been proposed that are better adapted to trail running. Recently, Millet[25] proposed an RPE-regulated fatigue model that combines peripheral, central and motivational factors in a framework specifically reflecting the constraints of prolonged endurance exercise (> 4 hours). Studies on ultra-long endurance have evidenced that both central and neuromuscular alterations play a role in fatigue aetiology in this task type.

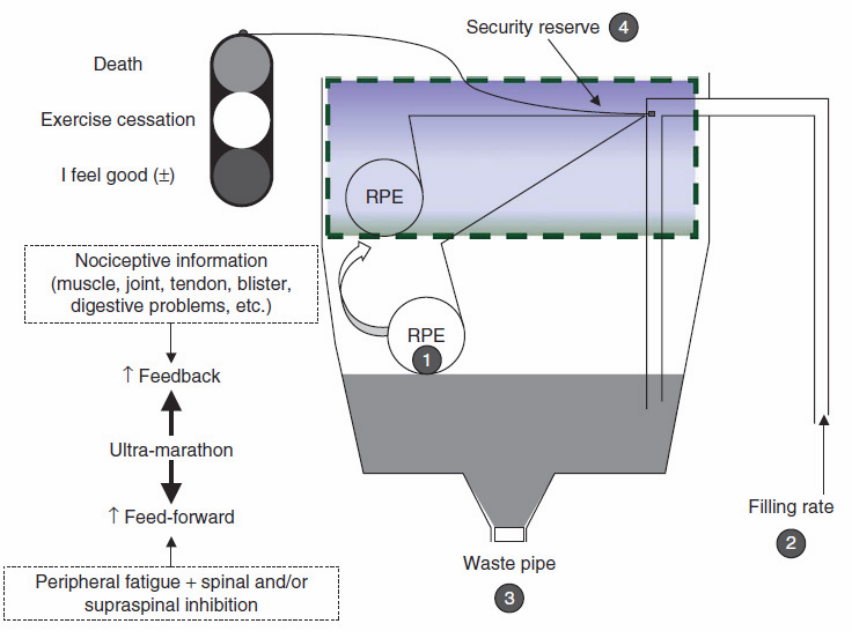


Figure 2.12: *The Flush model. The Flush model consists of 4 main components: 1) The "ball-cock", 2) the filling rate, 3) the waste pipe and 4) the security reserve. Reproduced from Millet, 2011*[25]

Drawing from the data presented in the fatigue sections and the observation that runners tend to adopt a positive racing strategy in which running speed declines throughout the effort, the Flush model depicts the accumulation of fatigue and the regulation of pace. In coherence with other teleoanticipatory regulative models, the flush model assumes that the athlete endeavours to keep the rate of perceived exertion (RPE) under a maximal tolerable level until the end of the exercise. Effectively the model consists of 4 main parts: the regulator ("ball-cock"), the filling rate and waste pipe, and the security reserve (Fig. 2.12). The "ball-cock" regulator represents the RPE, which can increase or decrease depending on the water accumulation level. The athlete plans exercise in a feed-forward manner in order to avoid the RPE (1) mounting into the security reserve (4). If RPE does reach this level, it results in premature exercise cessation or catastrophic failure. RPE rises in function of the filling rate (2) and the evacuation rate (3). The filling rate is determined by the energy output of the athlete based on his iterative planning of the race[187,188]. The planning is iterative as it relies on both feed-forward and feed-back mechanisms, each of which aims towards constant optimisation.

Feedback mechanisms can be mainly considered the group III and IV afferences described in the central fatigue section (Section 4), indirectly projecting to the anterior cingulate cortex[28,189]. Feed-forward mechanisms rely on the experience of the athlete and his planning competence[188]. Both of these process groups can be affected by environmental factors such as hypoxia[183,190], hypoglycaemia[191] or heat[177,192], leading to adapted exercise planning and/or cut-off. The other determinant of RPE rise is the evacuation rate (3). The most obvious process to increase evacuation rate is rest, yet there are other possibilities such as nutritional strategies, carbohydrate mouthwash[193], cooling mouthwash[194], and dissociation strategies[167]. The initial RPE level is dictated by preceding circumstances such as sleep deprivation[195,196], mental fatigue[174] and pre-fatigue, thus feed-forward influencing the initial filling rate. One interesting characteristic of the flush model is that the RPE sensor can become deregulated through pharmaceutical manipulation (amphetamines etc.[181,197]) leading to a transgression into the security reserve.

The flush model, at this given moment in time, represents what the author would regard as the most applied fatigue model for trail running. While certainly it rests a representation and detailed mechanics are not represented, the model supplies an idea of why prolonged endurance is terminated and how exercise is optimised to avoid termination yet produce the best performance. To better understand fatigue in trail running though, it is also necessary to take into account the exercise-induced muscle damage occurring on the downhill segments of the courses.

2.6. Eccentric muscle damage

Exercise-induced muscle damage (EIMD) is frequently encountered during daily life and is, as such, a well-described phenomenon. Since the first description of EIMD[198] in the early 20th century, a growing body of literature has sought to describe and find cures for EIMD. The induction of muscle damage is mainly effectuated through lengthening (eccentric) contractions of the muscle. This occurs quite frequently during everyday life, for instance when descending a

staircase, or setting something down. When regarding the classical force-velocity relationship of a muscle fibre, the eccentric contraction is capable of maintaining around 1.4 times the maximal isometric force output. The exact molecular mechanics of the eccentric contraction are not fully clarified, but it is assumed that there is a form of slippage between the heavy myosin chains (MHC) and the actin filaments, leading to partial and then complete rupture of the connections. Therefore a very slow cycling of myosin cross bridges (CB) is necessary in all muscle contractions, independent of type. This cycling speed is minimal during eccentric contractions, leading to a higher maximal force. Higher maximal muscle forces also lead to higher forces on the structural components of the myofibril, which can lead to eccentric-induced muscle damage.

Exercise-induced muscle damage is already a topic in flat running, as in the elongation phase of the stretch-shortening cycle an eccentric contraction is effectuated. This is all the more pertinent in trail running, as the large downhill component exacerbates the eccentric section of the contraction, leading to a greater strain on the muscle.

2.6.1. Muscle fatigue and muscle damage

Within this framework it is important to understand the difference between peripheral muscle fatigue and muscle damage. While, at first glance, these two processes may seem similar, there are distinct differences in the mechanics and functional parameters. As a very general distinction, the aetiology is different, EIMD being induced through mechanical myofibrillar stress, while peripheral fatigue is mainly caused by metabolite accumulation in some form. EIMD creates structural damage that is generally visible using light microscopy. While both insults incur lysosomal activity, there is a marked bimodal inflammatory response following EIMD. While these differences can be observed experimentally, the easiest way to tell the two processes apart is through the functional consequences.

2.6.1.1. FUNCTIONAL CONSEQUENCES OF EIMD

The occurrence of EIMD leads to a number of functional consequences that hamper performance and, depending on severity, quality of life. Common effects are swelling, reduced range of motion, pain and reduced force capacity[199–202]. A recent review has proposed discrimination into mild (< 20%), moderate (20 to 50%) and severe (> 50%) muscle damage depending on initial force depression[203]. Protocols examining downhill running, as it is encountered in trail races, generally induced mild to moderate muscle damage[204–206]. Maximal force can remain reduced for 48 (mild) to 168 (severe) hours. Following exercise, the muscle develops tenderness to palpitation and pressure, referred to as delayed onset muscle soreness (DOMS), as it onsets around 6 to 8 hours after exercise cessation and peaks between 48 and 72 hours post-exercise, depending on the severity[207,208]. Swelling and the associated decrease in range of motion and increase in passive stiffness peaks at around 24 hours post-exercise and declines at around 4 days, again depending on the severity of the damage[208]. This is accompanied by increases in bulk proxy damage markers such as creatine kinase (CK), lactate dehydrogenase (LDH) and plasma interleukin-6 (Il-6). In figure 2.13, an overview of the different phases that damaged muscles go through is presented. As is evident through the first two phase blocks, metabolic fatigue declines rather quickly (hours), while muscle damage persists over a longer period (days). The generation and response mechanisms to EIMD will be further iterated in the following.

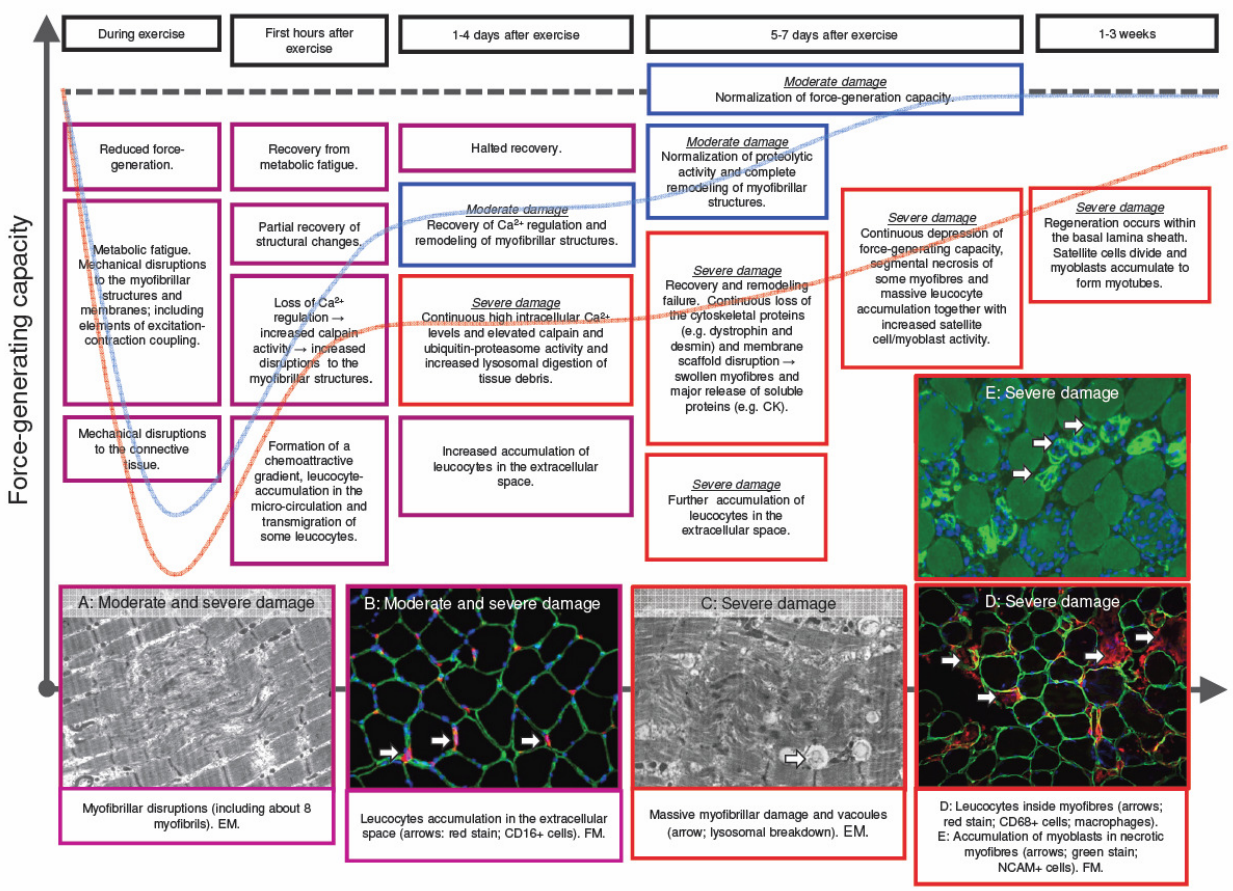


Figure 2.13: *Functional consequences of exercise-induced muscle damage. Replicated from Paulsen, 2012*[203]

2.6.2. Mechanisms of EIMD

As previously described, EIMD is characterised by a bimodal response, which, in effect, is related to the different kinetics of the main processes underlying the functional changes. In the following, these processes shall be briefly presented.

2.6.2.1. Mechanical induction

Eccentric contractions result in a high strain on the structural components of the contractile unit, which leads to morphological damage. The high strain developed by eccentric contractions can be traced to two interrelated properties. Firstly, the contractile unit is capable of producing

the highest forces during eccentric contractions. Secondly, the recruitment profile is different, fewer units being recruited for the same amount of force production[209]. In combination, this suggests that a high contractile load is placed on a relatively small number of fibres, independent of the absolute load amount[209]. While there is still insufficient and contradictory evidence, the "sarcomere popping theory" proposed by Morgan[210] is accepted by general consensus to explain the ensuing muscle damage. Morgan[210] proposed that sarcomere lengthening is non-uniform and that certain sarcomeres stretch more than others. Given the serial nature of the myofibril and the force-length relationship of the sarcomere[211] (Fig. 2.14), this could explain muscle damage apparition in relatively focused areas as it is seen using light microscopy on tissue samples. As the myofibril lengthens non-uniformly, the stronger sarcomeres move toward more optimal striation spacing, while the weaker sarcomeres are progressively disadvantaged. As force production declines in the weaker sarcomeres and they elongate, the load is transferred to the passive structures. In Morgan's terminology, the sarcomeres have "popped"[212]. There is some indirect evidence at hand that supports this concept, namely muscle damage being exacerbated at longer sarcomere lengths[213,214] and higher contraction speeds[201]. Light microscope analysis of damaged muscle tissue indicates that EIMD is frequently "localised" – i.e. not homogenously distributed throughout the sample but focused on certain areas[215]. This supports the idea that the passive structures carrying the load can rupture, leading to a transverse propagation of damage due to the higher loads incurred on surrounding structures. After an intense bout of eccentric exercise, a disruption of up to 50% of the muscle volume has been reported[216]. Direct evidence for the sarcomere popping theory was presented by Talbot and Morgan[217], although this was not reproduced in follow-up experiments by Telley et al.[218] who longitudinally labeled rabbit psoas muscle and observed the changes in striation distance during eccentric contractions. Although irregular striation distances were recorded, there was no evidence of sarcomere popping, potentially due to insufficient stretch and activation[219] .

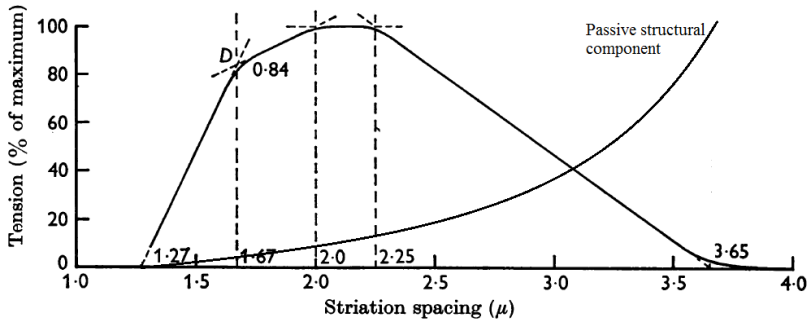


Figure 2.14: *The force-length relationship of the sarcomere. Based upon Gordon et al. 1966*[211] *and modified to include an approximation of the passive structural component.*

Following EIMD induction, there is a distinct loss of desmin staining[220–222220,223] in damaged muscle samples. Desmin is a protein related to structural integrity and is localised near the z-disk in sarcomeres. The loss of desmin supports the light microscope imagery, in which the bulk of the damage is seen around the z-disk[224]. The mechanical disruption documented to accompany EIMD through electron micrographs[216,224–226] (Fig. 2.15) and in some cases T2 magnetic resonance imaging[227–229] ensues an inflammatory reaction and remodelling process that can take multiple weeks to complete.

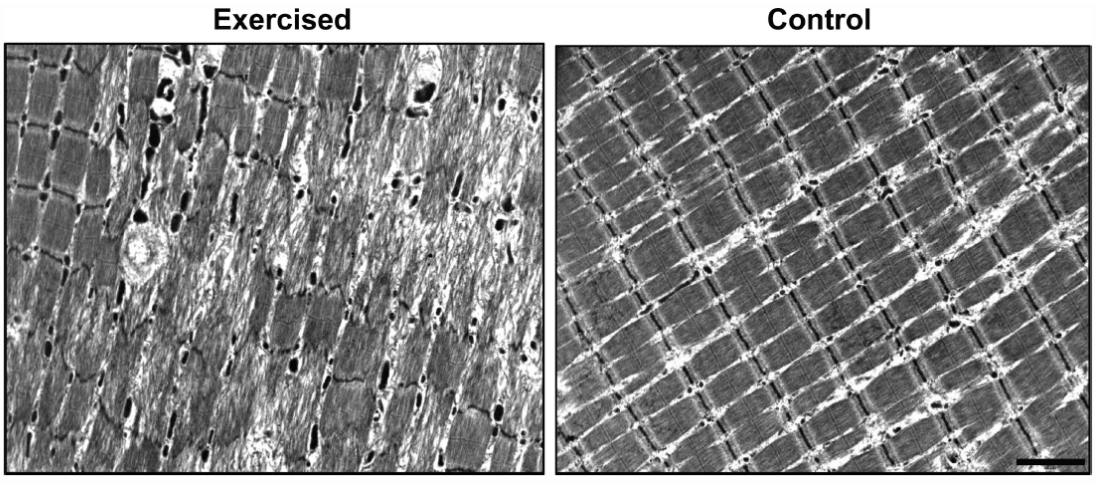


Figure 2.15: *Electron micrographs from control and exercise samples (1h post exercise). Reproduced from Paulsen et al., 2009*[226]

2.6.2.2. BIOCHEMICAL RESPONSE TO EIMD

Since the first description of EIMD in the early 20th century, a large body of literature has been developed leading to a well-founded, if not complete, understanding of the inflammation processes involved. Several complete and recent reviews[199,203,207,230–233] already cover this topic, therefore only an outline will be given here. Generally, the aetiology of EIMD is considered bimodal[208,234,235] and can be divided into a number of intertwined responses following the mechanical induction: disturbance of Ca^{2+} homeostasis, inflammatory response and stress protein signalling. The sum of these responses ultimately leads to protein degradation and subsequent restructuring of the damaged tissue. Mechanical disruption of the muscle membrane and the sarcoplasmic reticulum (SR) increases permeability and leads to a severe Ca^{2+} leakage[221,236–238] into the intracellular space. In addition to the leakage, intracellular Ca^{2+} is further augmented by the activation of strain-dependent trans-membrane calcium channels[221]. This sudden influx of calcium is probably the first signal for an inflammation response triggering Ca^{2+} dependent degradation pathways, notably calpains[69,222,239]. Calpains are a neutral protease that is mainly concentrated around the z-disk in human muscles. Activated by Ca^{2+}, calpains have been observed to play a role in the cleaving of cytostructural proteins such as desmin. As mentioned earlier, desmin plays a large role in the inter-myofibrillar connections and its suppression post-damage is considered one of the hallmarks of EIMD. On the other hand, neither actin nor myosin are targets for calpain degradation and are not suppressed post-damage. This may explain why muscle damage is primarily localised around the z-disks[69].

Calpains are also implicated in type III cell death (necrosis) signalling, and has been suspected of playing a key role in lysosome rupture[240]. Following calpain activation, a lysosomal reaction ensues and frequent ruptures of the lysosomal membranes lead to necrosis followed by an invasion of fluid, neutrophiles, leukocytes and macrophages[208,239,241,242] – a classical inflammatory response. Without delving deeper into the mechanisms and kinetics of the inflammation processes, the rise in bulk plasma markers such as inflammatory cytokines can be retraced to this phase in the biochemical response to EIMD. The inflammation response also

engenders an influx of xanthine oxidase, which is associated with the initiation of a myokine regulation cascade and a concurrent increase of reactive oxygen species (ROS)[243–246].

Finally, EIMD has been linked to increases in heat shock protein (HSP) expression[226,247,248], a highly-conserved, ubiquitous stress response pathway. HSPs were originally discovered in drosophila following heat shock[249] and have since been found to be active in most mammalian species[250]. The heat stress protein family consists of a large number of HSP, which are classified according to their molecular weight. Each class or family of HSPs has specific functions to which they are adapted and expressed accordingly. For instance, small HSPs specialise in the inhibition of protein aggregation, HSP60 and 70 families assist in protein folding and refolding, and the HSP90 family stabilises substrate proteins[251]. Eccentric muscle damage is mainly associated with changes in HSP27 and the inducible form of HSP72[252]. HSP27 belongs to the family of "small heat shock proteins" and is believed to play a role in maintaining the cytostructure. Inducible HSP72 is a rather generic HSP and is implicated in numerous processes. HSPs are considered molecular chaperones in that they assist with protein folding and prevent protein agglomeration by facilitating cross-membrane transport of damaged proteins to the proteasome. Heat shock proteins have been found to respond to a large bandwidth of different stressors such as heat[253–255], infection and inflammation[251], exercise[256], hypoxia[257,258], ROS[257,259], and mechanical stress[260]. When comparing the inducing stressors to the mechanisms involved in muscle damage, it already becomes apparent that HSP expression will be up-regulated almost throughout the whole process of EIMD.

2.6.2.3. Post-recovery adaptations to a single bout of EIMD

Three basic concepts underlie the adaptations to EIMD: Structural adaptations, biochemical adaptations and neural changes. Generally it is accepted that regeneration is driven by the migration of satellite cells into the damaged regions[261–263], which then differentiate and either form new myofibres or fuse with existing ones[203,264]. A number of researchers have proposed that EIMD induces structural remodelling favouring longitudinal sarcogenesis, thus lengthening the muscle fibre and making it less susceptible to muscle damage at similar contraction

lengths[265–268]. This is indirectly supported through the common observation that there is a shift in the optimal contraction angle toward longer lengths. In a series of studies, Yu et al. [265] directly observed the pattern of sarcogenesis, which conforms well to the proposed hypothesis. A further structural hypothesis proposes that weaker fibres are restructured or eliminated following EIMD, reducing non-uniformity and thereby sarcomere popping[269,270]. Considering biochemical changes in the muscle, it is mainly believed that neutrophil invasion is accelerated and HSP response is ameliorated[226,271,272]. An elevated kinetic of the HSP response could already reduce the amount of structural damage limiting Ca^{2+} leakage and the ensuing destructive cascade[215,226,252,271,273]. Additionally raised HSP levels post exercise have a beneficial effect on protein synthesis, increasing synthesis rate by assisting in the folding and rapidly chaperoning the synthesised proteins[274,275]. An blunted neutrophil response may be related to less lysosomal membrane rupture and reduces the magnitude of the inflammation reaction[272]. As a final point, it is often proposed that activation strategies of the damaged muscles are modified to spread the load of the contraction over more motor units, thus reducing the individual strain[198,276,277]. This can lead to less sarcomere popping and limits the muscle damage in its very genesis.

2.6.3. The repeated bout effect

As the induction of EIMD in a muscle engenders adaptations on a number of levels, it seems probable that a subsequent bout of the same stimulus will result in relatively less EIMD. This adaptation effect is well documented in the literature[271,278–281], yet the underlying mechanisms are unclear. Recent reviews[203,207,231] conclude that multiple mechanisms are probably implicated which account for all system levels differentiated above. Functionally, the reduction in EIMD is dependent on the initial training status[282], the magnitude of the first stimulus[202,276,283,284] and the inter-bout delay before the second stimulus[284–286]. Functional adaptations include reduced force depression and accelerated recovery, reduced pain sensation, increased range of motion, and reduced swelling.

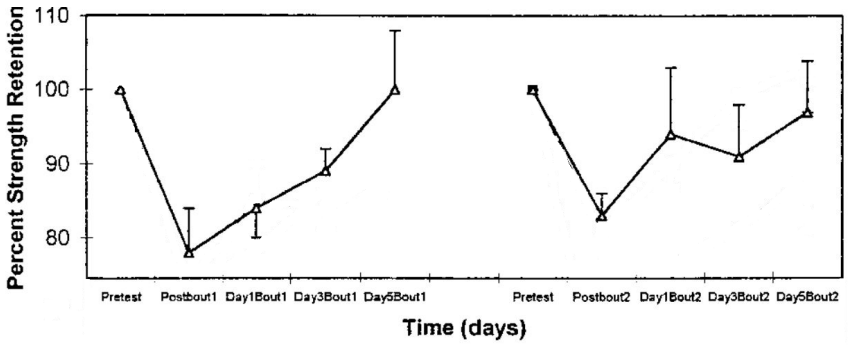


Figure 2.16: *Force reduction in two bouts of downhill running exercise interspersed by two weeks rest. Adapted from Eston et al., 2000*[287]

Studies investigating downhill running[279,287–289] generally record a force depression of < 20% of maximal isometric force in the knee extensors in the initial bout, classifying them as "mild" inducers of EIMD. The employed downhill running protocols, however, were not of a prolonged type (< 1 h) and not at very high intensity (< 80% $\dot{V}O_2$max). Force returned to baseline values within 5 days post-exercise in most cases. In a second bout of exercise, force depression is limited and recuperation accelerated (Fig. 2.16). Also, muscle soreness is attenuated and plasma CK response is mitigated (Fig. 2.17). This indicates that downhill running, even at a low intensity over a relatively short time, leads to the same type of adaptations as moderate or severe EIMD, albeit in lesser form.

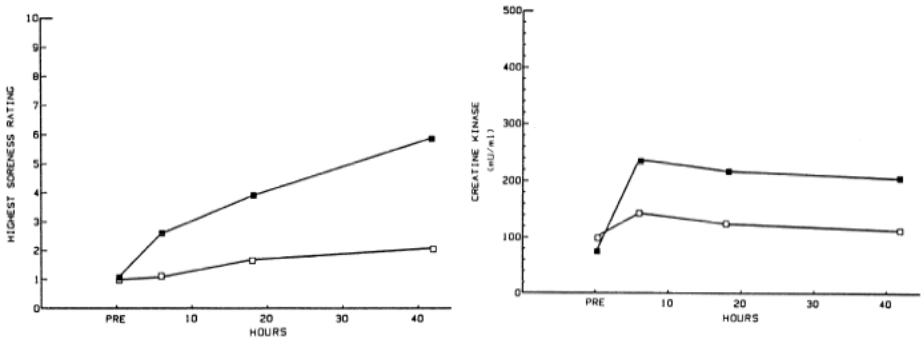


Figure 2.17: *Muscle soreness and CK profile in two bouts of downhill running exercise interspersed with six weeks rest. Replicated from Byrnes et al., 1985*[279]

2.7. Conclusion: Fatigue in trail running

Although trail running is still a rather recent field of investigation, a number of studies have examined the effects of trail running on neuromuscular fatigue. As presented in the introductory section, most of the published investigations examined changes in parameters brought about through a competitive trail race. The studies examining trail races are not exclusively focussed on fatigue in trail running, but often on the impact of a competition on nutrition[290–295] and hydration status[296–298], hyponatremia[299–303], and running kinematics[304]. An intervention approach is less common, notably being employed by some studies on vitamin supplementation and hydration[80,305–307] and some studies on cooling as a recovery strategy using trail running (field and simulated) as an exercise model[142,308,309]. That being said, the bulk of studies on trail running do revolve around fatigue or neuromuscular damage markers. It has been proposed that trail running elicits greater changes in neuromuscular alteration that running comparable distances with no elevation changes[77,85], indicating that trail running indeed results in a specific fatigue profile. Specifically, the studies conducted by Millet et al.[77,79,81,87] and Gauche et al.[80] enable the conception of a voluntary force profile induced by trails of different distances in the knee extensors (see Fig. 2.7). The plateau of force depression after around 8 hours exercise duration and the moderate loss relative to the required locomotive muscle force indicate that central fatigue is involved to a certain extent. Supporting this, while muscle damage markers are frequently elevated following trail running, they remain less potent than after other intense eccentric exercise types[203,259,310,311]. Furthermore, using peripheral nerve stimulation and evoked contractions, there has been little difference recorded in high and low frequency fatigue[77,79] and mechanical twitch properties[77,79]. Regarding central fatigue, not a whole lot more is known about trail running. Peripheral twitch techniques indicate that there is a reduction in voluntary activation of around 10-20%[77,79]. Furthermore, in a study comparing damaging and non-damaging prolonged exercise, Millet and Lepers[87] observed that post-exercise voluntary activation depression was more pronounced in the damaging modalities. To date, no studies using TMS in trail running have been published, therefore it is mainly speculative as to what the supraspinal contribution to fatigue is. In

conclusion, there is a distinct lack of literature on the characteristics of trail-specific fatigue, although the fatigue profile is distinctly different from running in the flat.

2.8. CHAPTER 2 BIBLIOGRAPHY

1. Tomporowski PD. Effects of acute bouts of exercise on cognition. *Acta Psychol (Amst)*. 2003; 112(3):297–324.

2. Collardeau M, Brisswalter J, Vercruyssen F et al. Single and choice reaction time during prolonged exercise in trained subjects: influence of carbohydrate availability. *Eur J Appl Physiol*. 2001; 86(2):150–156.

3. McMorris T, Davranche K, Jones G et al. Acute incremental exercise, performance of a central executive task, and sympathoadrenal system and hypothalamic-pituitary-adrenal axis activity. *Int J Psychophysiol Off J Int Organ Psychophysiol*. 2009; 73(3):334–340.

4. Labelle V, Bosquet L, Mekary S et al. Decline in executive control during acute bouts of exercise as a function of exercise intensity and fitness level. *Brain Cogn*. 2013; 81(1):10–17.

5. Lambourne K, Tomporowski P. The effect of exercise-induced arousal on cognitive task performance: a meta-regression analysis. *Brain Res*. 2010; 1341:12–24.

6. Pontifex MB, Hillman CH. Neuroelectric and behavioral indices of interference control during acute cycling. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol*. 2007; 118(3):570–580.

7. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev*. 2001; 81(4): 1725–1789.

8. Allen DG, Lamb GD, Westerblad H. Skeletal Muscle Fatigue: Cellular Mechanisms. *Physiol Rev*. 2008; 88(1):287–332.

9. Edwards RHT. Human Muscle Function and Fatigue. In: Porter R, Whelan J, eds. *Novartis Foundation Symposia*. Ciba Foundation Symposium 82 - Human Muscle Fatigue: Physiological Mechanisms. Chichester, UK: John Wiley & Sons, Ltd.; 1981:1–18.

10. Bigland-Ritchie B, Cafarelli E, Vøllestad NK. Fatigue of submaximal static contractions. *Acta Physiol Scand Suppl*. 1986; 556:137–148.

11. Bigland-Ritchie B, Furbush F, Woods JJ. Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors. *J Appl Physiol*. 1986; 61(2):421–429.

12. Kompanje EJO, Jansen TC, van der Hoven B et al. The first demonstration of lactic acid in human blood in shock by Johann Joseph Scherer (1814–1869) in January 1843. *Intensive Care Med*. 2007; 33(11):1967–1971.

13. Berzelius JJ. *Föreläsningar i djurkemien*. Stockholm; 1806.

14. Needham DM, Needham DM. *Machina Carnis: The Biochemistry of Muscular Contraction in Its Historical Development*. Cambridge: Cambridge University Press; 1971.

15. Mosso A. *La fatica*. Milan: Treves; 1891.

16. Hill AV, Kupalov P. Anaerobic and aerobic activity in isolated muscle. *Proc R Soc Lond Ser B Contain Pap Biol Character*. 1929; 105(737):313–322.

17. Eberstein A, Sandow A. Fatigue mechanisms in muscle fibers. In: Gutman E, Hink P, eds. *The Effect of Use and Disuse on the Neuromuscular Functions*. Amsterdam: Elsevier; 1963:515–526.

18. Bergström J, Hermansen L, Hultman E et al. Diet, Muscle Glycogen and Physical Performance. *Acta Physiol Scand*. 1967; 71(2-3):140–150.

19. Burke RE, Levine DN, Tsairis P et al. Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J Physiol*. 1973; 234(3):723–748.3.

20. Dawson MJ, Gadian DG, Wilkie DR. Muscular fatigue investigated by phosphorus nuclear magnetic resonance. *Nature*. 1978; 274(5674):861–866.

21. Hill AV. *Muscular activity*. Published for the Johns Hopkins University by Williams & Wilkins; 1926.

22. Bigland B, Lippold OCJ. Motor unit activity in the voluntary contraction of human muscle. *J Physiol*. 1954; 125(2):322–335.

23. Ikai M, Steinhaus AH. Some factors modifying the expression of human strength. *J Appl Physiol*. 1961; 16(1):157–163.

24. Noakes TD. 1996 J.B. Wolffe Memorial Lecture. Challenging beliefs: ex Africa semper aliquid novi. *Med Sci Sports Exerc*. 1997; 29(5):571–590.

25. Millet GY. Can Neuromuscular Fatigue Explain Running Strategies and Performance in Ultra-Marathons?: The Flush Model. *Sports Med*. 2011; 41(6):489–506.

26. Knicker AJ, Renshaw I, Oldham AR et al. Interactive processes link the multiple symptoms of fatigue in sport competition. *Sports Med*. 2011; 41(4):307–328.

27. Marcora SM, Staiano W. The limit to exercise tolerance in humans: mind over muscle? *Eur J Appl Physiol*. 2010; 109(4):763–770.

28. Amann M, Secher NH. Point: Afferent feedback from fatigued locomotor muscles is an important determinant of endurance exercise performance. *J Appl Physiol*. 2010; 108(2):452–454.

29. Tucker R, Noakes T. The physiological regulation of pacing strategy during exercise: a critical review. *Br J Sports Med*. 2009; 43(6):e1–e9.

30. Merton PA. Voluntary strength and fatigue. *J Physiol*. 1954; 123(3):553–564.

31. Merton PA. Problems of Muscular Fatigue. *Br Med Bull*. 1956; 12(3):219–221.

32. Blijham PJ, Hengstman GJD, Ter Laak HJ et al. Muscle-fiber conduction velocity and electromyography as diagnostic tools in patients with suspected inflammatory myopathy: a prospective study. *Muscle Nerve*. 2004; 29(1):46–50.

33. Nakajima S, Gilai A. Radial propagation of muscle action potential along the tubular system examined by potential-sensitive dyes. *J Gen Physiol*. 1980; 76(6):751–762.

34. Howald H, Von Glutz G, Billeter R. Energy stores and substrate utilization in muscle during exercise. In: Landry F, Orban W, eds. *The Third International Symposium on Biochemistry of Exercise*. Miami: Symposia Specialists, Inc.; 1978:75–86.

35. Overgaard K, Nielsen OB. Activity-induced recovery of excitability in K+-depressed rat soleus muscle. *Am J Physiol-Regul Integr Comp Physiol*. 2001; 280(1):R48–R55.

36. Sejersted OM, Sjøgaard G. Dynamics and consequences of potassium shifts in skeletal muscle and heart during exercise. *Physiol Rev*. 2000; 80(4):1411–1481.

37. Balog EM, Fitts RH. Effects of fatiguing stimulation on intracellular Na+ and K+ in frog skeletal muscle. *J Appl Physiol*. 1996; 81(2):679–685.

38. Thompson LV, Balog EM, Riley DA et al. Muscle fatigue in frog semitendinosus: alterations in contractile function. *Am J Physiol-Cell Physiol*. 1992; 262(6):C1500–C1506.

39. West W, Hicks A, McKelvie R et al. The relationship between plasma potassium, muscle membrane excitability and force following quadriceps fatigue. *Pflügers Arch Eur J Physiol*. 1996; 432(1): 43–49.

40. Sandiford SD, Green HJ, Duhamel TA et al. Muscle Na-K-pump and fatigue responses to progressive exercise in normoxia and hypoxia. *Am J Physiol-Regul Integr Comp Physiol*. 2005; 289(2): R441–R449.

41. Baker AJ, Kostov KG, Miller RG et al. Slow force recovery after long-duration exercise: metabolic and activation factors in muscle fatigue. *J Appl Physiol*. 1993; 74(5):2294–2300.

42. Farina D, Fortunato E, Merletti R. Noninvasive estimation of motor unit conduction velocity distribution using linear electrode arrays. *Ieee Trans Biomed Eng*. 2000; 47(3):380–388.

43. Farina D, Pozzo M, Merlo E et al. Assessment of Average Muscle Fiber Conduction Velocity From Surface EMG Signals During Fatiguing Dynamic Contractions. *Ieee Trans Biomed Eng*. 2004; 51(8):1383–1393.

44. Houtman CJ, Stegeman DF, Dijk JPV et al. Changes in muscle fiber conduction velocity indicate recruitment of distinct motor unit populations. *J Appl Physiol*. 2003; 95(3):1045–1054.

45. Farina D, Arendt-Nielsen L, Merletti R et al. Assessment of single motor unit conduction velocity during sustained contractions of the tibialis anterior muscle with advanced spike triggered averaging. *J Neurosci Methods*. 2002; 115(1):1–12.

46. MacIntosh BR, Holash RJ, Renaud J-M. Skeletal muscle fatigue - regulation of excitation-contraction coupling to avoid metabolic catastrophe. *J Cell Sci*. 2012; 125(9):2105–2114.

47. Bannister RA. Bridging the myoplasmic gap: recent developments in skeletal muscle excitation-contraction coupling. *J Muscle Res Cell Motil*. 2007; 28(4-5):275–283.

48. Baylor SM, Hollingworth S. Sarcoplasmic reticulum calcium release compared in slow-twitch and fast-twitch fibres of mouse muscle. *J Physiol*. 2003; 551(1):125–138.

49. Allen DG, Lamb GD, Westerblad H. Impaired calcium release during fatigue. *J Appl Physiol*. 2008; 104(1):296–305.

50. Delbono O, Meissner G. Sarcoplasmic reticulum Ca 2+ release in rat slow-and fast-twitch muscles. *J Membr Biol*. 1996; 151(2):123–130.

51. Lamb GD, Junankar PR, Stephenson DG. Raised intracellular [Ca2+] abolishes excitation-contraction coupling in skeletal muscle fibres of rat and toad. *J Physiol*. 1995; 489(Pt 2):349–362.

52. Lamb GD, Stephenson DG. Effect of Mg2+ on the control of Ca2+ release in skeletal muscle fibres of the toad. *J Physiol*. 1991; 434(1):507–528.

53. Laver DR, O'Neill ER, Lamb GD. Luminal Ca2+–regulated Mg2+ Inhibition of Skeletal RyRs Reconstituted as Isolated Channels or Coupled Clusters. *J Gen Physiol*. 2004; 124(6):741–758.

54. Meissner G, Darling E, Eveleth J. Kinetics of rapid calcium release by sarcoplasmic reticulum. Effects of calcium, magnesium, and adenine nucleotides. *Biochemistry (Mosc)*. 1986; 25(1):236–244.

55. Inesi G, de Meis L. Regulation of steady state filling in sarcoplasmic reticulum. Roles of back-inhibition, leakage, and slippage of the calcium pump. *J Biol Chem*. 1989; 264(10):5929–5936.

56. Nakamura J, Tajima G, Sato C et al. Substrate regulation of calcium binding in Ca2+-ATPase molecules of the sarcoplasmic reticulum. *J Biol Chem*. 2002; 277(27):24180–24190.

57. Posterino GS, Lamb GD. Effect of sarcoplasmic reticulum Ca2+ content on action potential-induced Ca2+ release in rat skeletal muscle fibres. *J Physiol*. 2003; 551(1):219–237.

58. Dutka TL, Cole L, Lamb GD. Calcium phosphate precipitation in the sarcoplasmic reticulum reduces action potential-mediated Ca2+ release in mammalian skeletal muscle. *Am J Physiol-Cell Physiol*. 2005; 289(6):C1502–C1512.

59. Fryer MW, Owen VJ, Lamb GD et al. Effects of creatine phosphate and P (i) on Ca2+ movements and tension development in rat skinned skeletal muscle fibres. *J Physiol*. 1995; 482(Pt 1):123–140.

60. Posterino GS, Fryer MW. Mechanisms underlying phosphate-induced failure of Ca2+ release in single skinned skeletal muscle fibres of the rat. *J Physiol*. 1998; 512(1):97–108.

61. Allen DG, Westerblad H. Role of phosphate and calcium stores in muscle fatigue. *J Physiol*. 2001; 536(3):657–665.

62. Scherer NM, Deamer DW. Oxidative stress impairs the function of sarcoplasmic reticulum by oxidation of sulfhydryl groups in the Ca2+-ATPase. *Arch Biochem Biophys*. 1986; 246(2):589–601.

63. Morgan MJ, Kim Y-S, Liu Z. TNFα and reactive oxygen species in necrotic cell death. *Cell Res*. 2008; 18(3):343–349.

64. Choi K, Kim J, Kim GW et al. Oxidative stress-induced necrotic cell death via mitochondira-dependent burst of reactive oxygen species. *Curr Neurovasc Res*. 2009; 6(4):213–222.

65. Fiers W, Beyaert R, Declercq W et al. More than one way to die: apoptosis, necrosis and reactive oxygen damage. *Oncogene*. 1999; 18(54):7719–7730.

66. Simon H-U, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. *Apoptosis*. 2000; 5(5):415–418.

67. Overgaard K, Fredsted A, HYLDAL A et al. Effects of running distance and training on Ca2+ content and damage in human muscle. *Med Sci Sports Exerc*. 2004; 36(5):821–829.

68. Overgaard K, Lindstrøm T, Ingemann-Hansen T et al. Membrane leakage and increased content of Na+-K+ pumps and Ca2+ in human muscle after a 100-km run. *J Appl Physiol*. 2002; 92(5): 1891–1898.

69. Belcastro AN. Skeletal muscle calcium-activated neutral protease (calpain) with exercise. *J Appl Physiol*. 1993; 74(3):1381–1386.

70. Hardie DG. Minireview: The AMP-Activated Protein Kinase Cascade: The Key Sensor of Cellular Energy Status. *Endocrinology*. 2003; 144(12):5179–5183.

71. Rivas DA, Fielding RA. Skeletal Muscle. In: Benjamin Caballero, ed. *Encyclopedia of Human Nutrition (Third Edition)*. Waltham: Academic Press; 2013:193–199.

72. Jeukendrup AE, Saris WH, Wagenmakers AJ. Fat metabolism during exercise: a review. Part I: fatty acid mobilization and muscle metabolism. *Int J Sports Med*. 1998; 19(4):231–244.

73. Jeukendrup AE, Saris WH, Wagenmakers AJ. Fat metabolism during exercise: a review--part II: regulation of metabolism and the effects of training. *Int J Sports Med*. 1998; 19(5):293–302.

74. Westerblad H, Allen DG, Lännergren J. Muscle fatigue: lactic acid or inorganic phosphate the major cause? *Physiology*. 2002; 17(1):17–21.

75. Allen DG, Lannergren J, Westerblad H. Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Exp Physiol*. 1995; 80(4):497–527.

76. Reid MB. Invited Review: redox modulation of skeletal muscle contraction: what we know and what we don't. *J Appl Physiol*. 2001; 90(2):724–731.

77. Millet GY, Tomazin K, Verges S et al. Neuromuscular Consequences of an Extreme Mountain Ultra-Marathon. *Plos One*. 2011; 6(2):e17059.

78. Fourchet F, Millet GP, Tomazin K et al. Effects of a 5-h hilly running on ankle plantar and dorsal flexor force and fatigability. *Eur J Appl Physiol*. 2012; 112(7):2645–2652.

79. Millet GY, Martin V, Lattier G et al. Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *J Appl Physiol Bethesda Md 1985*. 2003; 94(1):193–198.

80. Gauche E, Lepers R, Rabita G et al. Vitamin and mineral supplementation and neuromuscular recovery after a running race. *Med Sci Sports Exerc*. 2006; 38(12):2110–2117.

81. Millet GY, Lepers R, Maffiuletti NA et al. Alterations of neuromuscular function after an ultramarathon. *J Appl Physiol Bethesda Md 1985*. 2002; 92(2):486–492.

82. Morin J-B, Samozino P, Féasson L et al. Effects of muscular biopsy on the mechanics of running. *Eur J Appl Physiol*. 2009; 105(2):185–190.

83. Malm C, Nyberg P, Engström M et al. Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J Physiol*. 2000; 529(Pt 1):243–262.

84. Place N, Lepers R, Deley G et al. Time course of neuromuscular alterations during a prolonged running exercise. *Med Sci Sports Exerc*. 2004; 36(8):1347–1356.

85. Martin V, Kerhervé H, Messonnier LA et al. Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run. *J Appl Physiol*. 2010; 108(5):1224–1233.

86. Millet GY, Banfi JC, Kerherve H et al. Physiological and biological factors associated with a 24 h treadmill ultra-marathon performance. *Scand J Med Sci Sports*. 2011; 21(1):54–61.

87. Millet GY, Lepers R. Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Med*. 2004; 34(2):105–116.

88. Gandevia SC, Allen GM, Butler JE et al. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol*. 1996; 490(Pt 2):529–536.

89. Taylor JL, Todd G, Gandevia SC. Evidence for a supraspinal contribution to human muscle fatigue. In: *Proceedings of the Australian Physiological Society*.Vol 36.; 2005:83–89.

90. Bigland-Ritchie B. EMG and Fatigue of Human Voluntary and Stimulated Contractions. In: Porter R, Whelan J, eds. *Ciba Foundation Symposium 82 - Human Muscle Fatigue: Physiological Mechanisms*. John Wiley & Sons, Ltd.; 1981:130–156.

91. Darcus HD, Salter N. The effect of repeated muscular exertion on muscle strength. *J Physiol*. 1955; 129(2):325–336.

92. Scripture EW, Smith TL, Brown EM. On the education of muscular control and power. *Stud Yale Psychol Lab*. 1894; 2:114–119.

93. Grimby L, Hannerz J, Hedman B. The fatigue and voluntary discharge properties of single motor units in man. *J Physiol*. 1981; 316(1):545–554.

94. Van Cutsem M, Duchateau J, Hainaut K. Changes in single motor unit behaviour contribute to the increase in contraction speed after dynamic training in humans. *J Physiol*. 1998; 513(1):295–305.

95. Marsden CD, Meadows JC, Merton PA. "Muscular wisdom" that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. *Adv Neurol*. 1983; 39:169–211.

96. Booth FW, Thomason DB. Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. *Physiol Rev*. 1991; 71(2):541–585.

97. Davies CT, Dooley P, McDonagh MJ et al. Adaptation of mechanical properties of muscle to high force training in man. *J Physiol*. 1985; 365(1):277–284.

98. McDonagh MJ, Hayward CM, Davies CT. Isometric training in human elbow flexor muscles. The effects on voluntary and electrically evoked forces. *J Bone Joint Surg Br*. 1983; 65(3):355–358.

99. Yue G, Cole KJ. Strength increases from the motor program: comparison of training with maximal voluntary and imagined muscle contractions. *J Neurophysiol*. 1992; 67(5):1114–1123.

100. Herbert RD, Dean C, Gandevia SC. Effects of real and imagined training on voluntary muscle activation during maximal isometric contractions. *Acta Physiol Scand*. 1998; 163(4):361–368.

101. Harridge SD, Kryger A, Stensgaard A. Knee extensor strength, activation, and size in very elderly people following strength training. *Muscle Nerve*. 1999; 22(7):831–839.

102. Gandevia SC, Allen GM, McKenzie DK. Fatigue: neural and muscular mechanisms. In: *Advances in experimental medicine and biology*. New York: Plenum Press; 1995:281–294.

103. Allen GM, Gandevia SC, McKenzie DK. Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. *Muscle Nerve*. 1995; 18(6):593–600.

104. Belanger AY, McComas A t. Extent of motor unit activation during effort. *J Appl Physiol*. 1981; 51(5):1131–1135.

105. Newman SA, Jones G, Newham DJ. Quadriceps voluntary activation at different joint angles measured by two stimulation techniques. *Eur J Appl Physiol*. 2003; 89(5):496–499.

106. Bellemare F, Woods JJ, Johansson R et al. Motor-unit discharge rates in maximal voluntary contractions of three human muscles. *J Neurophysiol*. 1983; 50(6):1380–1392.

107. Bigland-Ritchie B, Johansson R, Lippold OC et al. Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J Physiol*. 1983; 340(1):335–346.

108. Duchateau J, Hainaut K. Effects of immobilization on contractile properties, recruitment and firing rates of human motor units. *J Physiol*. 1990; 422(1):55–65.

109. Garland SJ, Garner SH, McComas AJ. Reduced voluntary electromyographic activity after fatiguing stimulation of human muscle. *J Physiol*. 1988; 401(1):547–556.

110. Edwards RH, Hill DK, Jones DA. Effect of fatigue on the time course of relaxation from isometric contractions of skeletal muscle in man. *J Physiol*. 1972; 227(2):26–27.

111. Gollnick PD, Körge P, Karpakka J et al. Elongation of skeletal muscle relaxation during exercise is linked to reduced calcium uptake by the sarcoplasmic reticulum in man. *Acta Physiol Scand*. 1991; 142(1):135–136.

112. Loiselle DS, Walmsley B. Cost of force development as a function of stimulus rate in rat soleus muscle. *Am J Physiol-Cell Physiol*. 1982; 243(5):C242–C246.

113. Clarke RW, Harris J, Houghton AK. Spinal 5-HT-receptors and tonic modulation of transmission through a withdrawal reflex pathway in the decerebrated rabbit. *Br J Pharmacol*. 1996; 119(6):1167–1176.

114. Mense S, Stahnke M. Responses in muscle afferent fibres of slow conduction velocity to contractions and ischaemia in the cat. *J Physiol*. 1983; 342(1):383–397.

115. Kaufman MP, Rybicki KJ. Discharge properties of group III and IV muscle afferents: their responses to mechanical and metabolic stimuli. *Circ Res*. 1987; 61(4 Pt 2):I60–I65.

116. Hayes SG, Kindig AE, Kaufman MP. Comparison between the effect of static contraction and tendon stretch on the discharge of group III and IV muscle afferents. *J Appl Physiol*. 2005; 99(5): 1891–1896.

117. Ellaway PH, Murphy PR, Tripathi A. Closely coupled excitation of γ-motoneurones by group III muscle afferents with low mechanical threshold in the cat. *J Physiol*. 1982; 331(1):481–498.

118. Woods JJ, Furbush F, Bigland-Ritchie B. Evidence for a fatigue-induced reflex inhibition of motoneuron firing rates. *J Neurophysiol*. 1987; 58(1):125–137.

119. Bigland-Ritchie B, Thomas CK, Rice CL et al. Muscle temperature, contractile speed, and motoneuron firing rates during human voluntary contractions. *J Appl Physiol*. 1992; 73(6): 2457–2461.

120. Vander Linden DW, Kukulka CG, Soderberg GL. The effect of muscle length on motor unit discharge characteristics in human tibialis anterior muscle. *Exp Brain Res*. 1991; 84(1):210–218.

121. Bigland-Ritchie BR, Furbush FH, Gandevia SC et al. Voluntary discharge frequencies of human motoneurons at different muscle lengths. *Muscle Nerve*. 1992; 15(2):130–137.

122. Vøllestad NK, Sejersted I, Saugen E. Mechanical behavior of skeletal muscle during intermittent voluntary isometric contractions in humans. *J Appl Physiol*. 1997; 83(5):1557–1565.

123. Garland SJ, Enoka RM, Serrano LP et al. Behavior of motor units in human biceps brachii during a submaximal fatiguing contraction. *J Appl Physiol*. 1994; 76(6):2411–2419.

124. Garland SJ, Griffin L, Ivanova T. Motor unit discharge rate is not associated with muscle relaxation time in sustained submaximal contractions in humans. *Neurosci Lett*. 1997; 239(1):25–28.

125. Barker A, Jalinous R, Freeston I. Non-invasive stimulation of human motor cortex. *Lancet*. 1985;(1):1106–1107.

126. Gruet M, Temesi J, Rupp T et al. Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue. *Neuroscience*. 2013; 231:384–399.

127. Millet GY, Bachasson D, Temesi J et al. Potential interests and limits of magnetic and electrical stimulation techniques to assess neuromuscular fatigue. *Neuromuscul Disord*. 2012; 22, Supplement 3(0):181–186.

128. Taylor JL, Butler JE, Allen GM et al. Changes in motor cortical excitability during human muscle fatigue. *J Physiol*. 1996; 490(Pt 2):519–528.

129. Butler JE, Taylor JL, Gandevia SC. Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *J Neurosci*. 2003; 23(32):10224–10230.

130. Jankowska E, Padel Y, Tanaka R. Disynaptic inhibition of spinal motoneurones from the motor cortex in the monkey. *J Physiol*. 1976; 258(2):467–487.

131. Kasser RJ, Cheney PD. Characteristics of corticomotoneuronal postspike facilitation and reciprocal suppression of EMG activity in the monkey. *J Neurophysiol*. 1985; 53(4):959–978.

132. Ranieri F, Di Lazzaro V. The role of motor neuron drive in muscle fatigue. *Neuromuscul Disord*. 2012; 22:S157–S161.

133. Newsholme EA, Acworth IN, Blomstrand E. Amino acids, brain neurotransmitters and a functional link between muscle and brain that is important in sustained exercise. *Adv Myochem*. 1987; 1:127–133.

134. Chaouloff F, Kennett GA, Serrurrier B et al. Amino acid analysis demonstrates that increased plasma free tryptophan causes the increase of brain tryptophan during exercise in the rat. *J Neurochem*. 1986; 46(5):1647–1650.

135. Fernstrom JD. Branched-chain amino acids and brain function. *J Nutr*. 2005; 135(6):1539S–1546S.

136. Cooper JR, Bloom FE, Roth R. *The biochemical basis of neuropharmacology*. 8th ed. New York: Oxford University Press; 2003.

137. Meeusen R, Watson P. Amino acids and the brain: do they play a role in “central fatigue”? *Int J Sport Nutr Exerc Metab*. 2007; 17:S37–46.

138. Petersen K, Hansen CB, Aagaard P et al. Muscle mechanical characteristics in fatigue and recovery from a marathon race in highly trained runners. *Eur J Appl Physiol*. 2007; 101(3):385–396.

139. Ross EZ, Middleton N, Shave R et al. Human, Environmental & Exercise: Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. *Exp Physiol*. 2006; 92(2):417–426.

140. Mastaloudis A, Traber MG, Carstensen K et al. Antioxidants did not prevent muscle damage in response to an ultramarathon run. *Med Sci Sports Exerc*. 2006; 38(1):72–80.

141. Saldanha A, Nordlund Ekblom MM, Thorstensson A. Central fatigue affects plantar flexor strength after prolonged running. *Scand J Med Sci Sports*. 2008; 18(3):383–388.

142. Hausswirth C, Louis J, Bieuzen F et al. Effects of Whole-Body Cryotherapy vs. Far-Infrared vs. Passive Modalities on Recovery from Exercise-Induced Muscle Damage in Highly-Trained Runners. *Plos One*. 2011; 6(12):e27749.

143. Racinais S, Girard O, Micallef JP et al. Failed Excitability of Spinal Motoneurons Induced by Prolonged Running Exercise. *J Neurophysiol*. 2007; 97(1):596–603.

144. Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. *Eur J Appl Physiol*. 2007; 99(4):443–447.

145. Ostrowski K, Hermann C, Bangash A et al. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *J Physiol*. 1998; 513(3):889–894.

146. Papassotiriou I, Alexiou VG, Tsironi M et al. Severe aseptic inflammation caused by long distance running (246 km) does not increase procalcitonin. *Eur J Clin Invest*. 2008; 38(4):276–279.

147. Koller A, Mair J, Schobersberger W et al. Effects of prolonged strenuous endurance exercise on plasma myosin heavy chain fragments and other muscular proteins. Cycling vs running. *J Sports Med Phys Fitness*. 1998; 38(1):10–17.

148. Marcora SM, Bosio A, de Morree HM. Locomotor muscle fatigue increases cardiorespiratory responses and reduces performance during intense cycling exercise independently from metabolic stress. *Am J Physiol-Regul Integr Comp Physiol*. 2008; 294(3):R874–R883.

149. Ohta M, Hirai N, Ono Y et al. Clinical biochemical evaluation of central fatigue with 24-hour continuous exercise. *Rinsho Byori*. 2005; 53(9):802–809.

150. Nybo L, Rasmussen P. Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. *Exerc Sport Sci Rev*. 2007; 35(3):110–118.

151. Fletcher WM, Hopkins FG. Lactic acid in amphibian muscle. *J Physiol*. 1907; 35(4):247–309.

152. Edwards RH. Biochemical bases of fatigue in exercise performance: catastrophe theory of muscular fatigue. In: Knuttgen H, Vogel K, Poortmans J, eds. *Biochemistry of exercise*.Vol 13. Champain, IL: Human Kinetics; 1983:3–28.

153. Noakes TD, St Clair Gibson A. Logical limitations to the “catastrophe” models of fatigue during exercise in humans. *Br J Sports Med*. 2004; 38(5):648–649.

154. Margaria R, Edwards HT, Dill DB. The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Am J Physiol Content*. 1933; 106(3): 689–715.

155. Coggan A, Coyle EF. Carbohydrate Ingestion During Prolonged Exercise: Effects on Metabolism and Performance. *Exerc Sport Sci Rev*. 1991; 19(1):1–40.

156. Noakes TD. The Central Governor Model in 2012: eight new papers deepen our understanding of the regulation of human exercise performance. *Br J Sports Med*. 2012; 46(1):1–3.

157. Noakes TD, St Clair Gibson A, Lambert EV. From catastrophe to complexity: a novel model of integrative central neural regulation of effort and fatigue during exercise in humans: summary and conclusions. *Br J Sports Med*. 2005; 39(2):120–124.

158. Bassett DR Jr, Howley ET. Maximal oxygen uptake: "classical" versus "contemporary" viewpoints. *Med Sci Sports Exerc*. 1997; 29(5):591–603.

159. Noakes TD. Maximal oxygen uptake: "classical" versus "contemporary" viewpoints: a rebuttal. *Med Sci Sports Exerc*. 1998; 30(9):1381–1398.

160. Shephard RJ. Is it Time to Retire the Central Governor? *Sports Med*. 2009; 39(9):709–721.

161. Noakes TD. Is it time to retire the A.V. Hill Model?: A rebuttal to the article by Professor Roy Shephard. *Sports Med Auckl Nz*. 2011; 41(4):263–277.

162. Febbraio MA, Dancey J. Skeletal muscle energy metabolism during prolonged, fatiguing exercise. *J Appl Physiol*. 1999; 87(6):2341–2347.

163. Shulman RG, Rothman DL. The "glycogen shunt" in exercising muscle: a role for glycogen in muscle energetics and fatigue. *Proc Natl Acad Sci*. 2001; 98(2):457–461.

164. Vissing J, Haller RG. The effect of oral sucrose on exercise tolerance in patients with McArdle's disease. *N Engl J Med*. 2003; 349(26):2503–2509.

165. Nielsen OB, Paoli F, Overgaard K. Protective effects of lactic acid on force production in rat skeletal muscle. *J Physiol*. 2001; 536(1):161–166.

166. Lohse KR, Sherwood DE. Defining the Focus of Attention: Effects of Attention on Perceived Exertion and Fatigue. *Front Psychol*. 2011; 2:332.

167. Raglin JS. The psychology of the marathoner. *Sports Med*. 2007; 37(4):404–407.

168. Albertus Y, Tucker R, St Clair Gibson A et al. Effect of distance feedback on pacing strategy and perceived exertion during cycling. *Med Sci Sports Exerc*. 2005; 37(3):461–468.

169. Weir JP, Beck TW, Cramer JT et al. Is fatigue all in your head? A critical review of the central governor model. *Br J Sports Med*. 2006; 40(7):573–586.

170. Gibson ASC, Baden DA, Lambert MI et al. The conscious perception of the sensation of fatigue. *Sports Med*. 2003; 33(3):167–176.

171. Tucker R. The anticipatory regulation of performance: the physiological basis for pacing strategies and the development of a perception-based model for exercise performance. *Br J Sports Med*. 2009; 43(6):392–400.

172. Thomas K, Stone MR, Thompson KG et al. The effect of self- even- and variable-pacing strategies on the physiological and perceptual response to cycling. *Eur J Appl Physiol*. 2011; 112(8): 3069–3078.

173. Marcora SM. Do we really need a central governor to explain brain regulation of exercise performance? *Eur J Appl Physiol*. 2008; 104(5):929–931.

174. Marcora SM, Staiano W, Manning V. Mental fatigue impairs physical performance in humans. *J Appl Physiol*. 2009; 106(3):857–864.

175. Marcora S. Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs. *J Appl Physiol*. 2009; 106(6):2060–2062.

176. Nybo L. Hyperthermia and fatigue. *J Appl Physiol*. 2008; 104(3):871–878.

177. Nybo L, Nielsen B. Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol*. 2001; 91(3):1055–1060.

178. Nybo L, Nielsen B. Perceived exertion is associated with an altered brain activity during exercise with progressive hyperthermia. *J Appl Physiol*. 2001; 91(5):2017–2023.

179. Nielsen B, Nybo L. Cerebral changes during exercise in the heat. *Sports Med Auckl Nz*. 2003; 33(1):1–11.

180. Nybo L, Secher NH. Cerebral perturbations provoked by prolonged exercise. *Prog Neurobiol*. 2004; 72(4):223–261.

181. Amann M, Proctor LT, Sebranek JJ et al. Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol*. 2009; 587(1): 271–283.

182. Amann M, Proctor LT, Sebranek JJ et al. Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise. *J Appl Physiol*. 2008; 105(6):1714–1724.

183. Amann M, Romer LM, Subudhi AW et al. Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. *J Physiol*. 2007; 581(1):389–403.

184. Amann M, Blain GM, Proctor LT et al. Group III and IV muscle afferents contribute to ventilatory and cardiovascular response to rhythmic exercise in humans. *J Appl Physiol*. 2010; 109(4): 966–976.

185. Noakes TD. Fatigue is a Brain-Derived Emotion that Regulates the Exercise Behavior to Ensure the Protection of Whole Body Homeostasis. *Front Physiol*. 2012; 3:82.

186. Swart J, Lindsay TR, Lambert MI et al. Perceptual cues in the regulation of exercise performance - physical sensations of exercise and awareness of effort interact as separate cues. *Br J Sports Med*. 2012; 46(1):42–48.

187. Ulmer H-V. Concept of an extracellular regulation of muscular metabolic rate during heavy exercise in humans by psychophysiological feedback. *Experientia*. 1996; 52(5):416–420.

188. Lambert MI, Dugas JP, Kirkman MC et al. Changes in running speeds in a 100 km ultra-marathon race. *J Sports Sci Med*. 2004; 3:167–73.

189. Smirmaul B de PC. Sense of effort and other unpleasant sensations during exercise: clarifying concepts and mechanisms. *Br J Sports Med*. 2012; 46(5):308–311.

190. Millet GY, Aubert D, Favier FB et al. Effect of acute hypoxia on central fatigue during repeated isometric leg contractions. *Scand J Med Sci Sports*. 2009; 19(5):695–702.

191. Nybo L. CNS fatigue and prolonged exercise: effect of glucose supplementation. *Med Sci Sports Exerc*. 2003; 35(4):589–594.

192. Tucker R, Marle T, Lambert EV et al. The rate of heat storage mediates an anticipatory reduction in exercise intensity during cycling at a fixed rating of perceived exertion. *J Physiol*. 2006; 574(3):905–915.

193. Chambers ES, Bridge MW, Jones DA. Carbohydrate sensing in the human mouth: effects on exercise performance and brain activity. *J Physiol*. 2009; 587(8):1779–1794.

194. Mündel T, Jones DA. The effects of swilling an l (-)-menthol solution during exercise in the heat. *Eur J Appl Physiol*. 2010; 109(1):59–65.

195. Martin BJ. Effect of sleep deprivation on tolerance of prolonged exercise. *Eur J Appl Physiol*. 1981; 47(4):345–354.

196. Oliver SJ, Costa RJ, Laing SJ et al. One night of sleep deprivation decreases treadmill endurance performance. *Eur J Appl Physiol*. 2009; 107(2):155–161.

197. Swart J, Lamberts RP, Lambert MI et al. Exercising with reserve: evidence that the central nervous system regulates prolonged exercise performance. *Br J Sports Med*. 2009; 43(10):782–788.

198. Hough T. Ergographic studies in muscular soreness. *Am J Physiol*. 1902; 7(1):76–92.

199. Clarkson PM, Sayers SP. Etiology of Exercise-Induced Muscle Damage. *Can J Appl Physiol*. 1999; 24(3):234–248.

200. Clarkson PM, Tremblay I. Exercise-induced muscle damage, repair, and adaptation in humans. *J Appl Physiol Bethesda Md 1985*. 1988; 65(1):1–6.

201. Chapman D, Newton M, Sacco P et al. Greater muscle damage induced by fast versus slow velocity eccentric exercise. *Int J Sports Med*. 2006; 27(8):591–598.

202. Chen TC, Chen HL, Lin MJ et al. Muscle damage responses of the elbow flexors to four maximal eccentric exercise bouts performed every 4 weeks. *Eur J Appl Physiol*. 2009; 106(2):267–275.

203. Paulsen G, Mikkelsen UR, Raastad T et al. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev*. 2012; 18(1):42–97.

204. Braun WA, Dutto DJ. The effects of a single bout of downhill running and ensuing delayed onset of muscle soreness on running economy performed 48 h later. *Eur J Appl Physiol*. 2003; 90(1-2): 29–34.

205. Malm C, Sjödin B, Sjöberg B et al. Leukocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. *J Physiol*. 2004; 556(3):983–1000.

206. Feasson L, Stockholm D, Freyssenet D et al. Molecular adaptations of neuromuscular disease-associated proteins in response to eccentric exercise in human skeletal muscle. *J Physiol*. 2002; 543(1):297–306.

207. Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol*. 2001; 537(Pt 2):333–345.

208. MacIntyre DL, Reid WD, Lyster DM et al. Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise. *J Appl Physiol Bethesda Md 1985*. 1996; 80(3): 1006–1013.

209. Enoka RM. Eccentric contractions require unique activation strategies by the nervous system. *J Appl Physiol*. 1996; 81(6):2339–2346.

210. Morgan DL. New insights into the behavior of muscle during active lengthening. *Biophys J*. 1990; 57(2):209–221.

211. Gordon AM, Huxley AF, Julian FJ. The variation in isometric tension with sarcomere length in vertebrate muscle fibres. *J Physiol*. 1966; 184(1):170–192.

212. Morgan DL, Proske U. Popping sarcomere hypothesis explains stretch-induced muscle damage. *Clin Exp Pharmacol Physiol*. 2004; 31(8):541–545.

213. Talbot JA, Morgan DL. The effects of stretch parameters on eccentric exercise-induced damage to toad skeletal muscle. *J Muscle Res Cell Motil*. 1998; 19(3):237–245.

214. Lieber RL, Friden J. Muscle damage is not a function of muscle force but active muscle strain. *J Appl Physiol*. 1993; 74(2):520–526.

215. Lauritzen F, Paulsen G, Raastad T et al. Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans. *J Appl Physiol*. 2009; 107(6):1923 –1934.

216. Fridén J, Sjöström M, Ekblom B. Myofibrillar Damage Following Intense Eccentric Exercise in Man. *Int J Sports Med*. 1983; 4(3):170–176.

217. Talbot JA, Morgan DL. Quantitative analysis of sarcomere non-uniformities in active muscle following a stretch. *J Muscle Res Cell Motil*. 1996; 17(2):261–268.

218. Telley IA, Stehle R, Ranatunga K et al. Dynamic behaviour of half-sarcomeres during and after stretch in activated rabbit psoas myofibrils: sarcomere asymmetry but no "sarcomere popping." *J Physiol*. 2006; 573(1):173–185.

219. Morgan DL. Sarcomere popping requires stretch over a range where total tension decreases with length. *J Physiol*. 2006; 574(2):627–628.

220. Barash IA, Peters D, Fridén J et al. Desmin cytoskeletal modifications after a bout of eccentric exercise in the rat. *Am J Physiol - Regul Integr Comp Physiol*. 2002; 283(4):R958 –R963.

221. Lieber RL, Thornell LE, Fridén J. Muscle cytoskeletal disruption occurs within the first 15 min of cyclic eccentric contraction. *J Appl Physiol*. 1996; 80(1):278–284.

222. Belcastro AN, Parkhouse W, Dobson G et al. Influence of exercise on cardiac and skeletal muscle myofibrillar proteins. *Mol Cell Biochem*. 1988; 83(1):27–36.

223. Lieber RL, Schmitz MC, Mishra DK et al. Contractile and cellular remodeling in rabbit skeletal muscle after cyclic eccentric contractions. *J Appl Physiol*. 1994; 77(4):1926 –1934.

224. Fridén J, Sjöström M, Ekblom B. A morphological study of delayed muscle soreness. *Experientia*. 1981; 37(5):506–507.

225. Newham DJ, McPhail G, Mills KR et al. Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci*. 1983; 61(1):109–122.

226. Paulsen G, Lauritzen F, Bayer ML et al. Subcellular movement and expression of HSP27, alphaB-crystallin, and HSP70 after two bouts of eccentric exercise in humans. *J Appl Physiol*. 2009; 107(2):570–582.

227. Shellock FG, Fukunaga T, Mink JH et al. Exertional muscle injury: evaluation of concentric versus eccentric actions with serial MR imaging. *Radiology*. 1991; 179(3):659–664.

228. Mair J, Koller A, Artner-Dworzak E et al. Effects of exercise on plasma myosin heavy chain fragments and MRI of skeletal muscle. *J Appl Physiol*. 1992; 72(2):656–663.

229. Marqueste T, Giannesini B, Fur YL et al. Comparative MRI analysis of T2 changes associated with single and repeated bouts of downhill running leading to eccentric-induced muscle damage. *J Appl Physiol*. 2008; 105(1):299–307.

230. Morton JP, Kayani AC, McArdle A et al. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. *Sports Med Auckl Nz*. 2009; 39(8):643–662.

231. Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil*. 2002; 81(11):S52–S69.

232. Fridén J, Lieber RL. Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components. *Acta Physiol Scand*. 2001; 171(3):321–326.

233. Stupka N, Tarnopolsky MA, Yardley NJ et al. Cellular adaptation to repeated eccentric exercise-induced muscle damage. *J Appl Physiol*. 2001; 91(4):1669–1678.

234. Ingalls CP, Warren GL, Williams JH et al. E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *J Appl Physiol Bethesda Md 1985*. 1998; 85(1):58–67.

235. Faulkner JA, Jones DA, Round JM. Injury to skeletal muscles of mice by forced lengthening during contractions. *Q J Exp Physiol Camb Engl*. 1989; 74(5):661–670.

236. Duan C, Delp MD, Hayes DA et al. Rat skeletal muscle mitochondrial [Ca2+] and injury from downhill walking. *J Appl Physiol*. 1990; 68(3):1241–1251.

237. Lowe DA, Warren GL, Hayes DA et al. Eccentric contraction-induced injury of mouse soleus muscle: effect of varying [Ca2+]. *J Appl Physiol*. 1994; 76(4):1445–1453.

238. Yasuda T, Sakamoto K, Nosaka K et al. Loss of sarcoplasmic reticulum membrane integrity after eccentric contractions. *Acta Physiol*. 1997; 161(4):581–582.

239. Belcastro AN, Shewchuk LD, Raj DA. Exercise-induced muscle injury: a calpain hypothesis. *Mol Cell Biochem*. 1998; 179(1-2):135–145.

240. Golstein P, Kroemer G. Cell death by necrosis: towards a molecular definition. *Trends Biochem Sci*. 2007; 32(1):37–43.

241. Abrams GD. Response of the body to injury: inflammation and repair. *Pathophysiol Clin Concepts Dis Process*. 1997:38–58.

242. Raj DA, Booker TS, Belcastro AN. Striated muscle calcium-stimulated cysteine protease (calpain-like) activity promotes myeloperoxidase activity with exercise. *Pflügers Arch*. 1998; 435(6):804–809.

243. MacIntyre DL, Reid WD, McKenzie DC. Delayed muscle soreness. *Sports Med*. 1995; 20(1):24–40.

244. MacIntyre DL, Sorichter S, Mair J et al. Markers of inflammation and myofibrillar proteins following eccentric exercise in humans. *Eur J Appl Physiol*. 2001; 84(3):180–186.

245. Hellsten Y, Hansson H-A, Johnson L et al. Increased expression of xanthine oxidase and insulin-like growth factor I (IGF-I) immunoreactivity in skeletal muscle after strenuous exercise in humans. *Acta Physiol Scand*. 1996; 157(2):191–197.

246. Hellsten Y, Frandsen U, Orthenblad N et al. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. *J Physiol*. 1997; 498(Pt 1):239–248.

247. Thompson HS, Scordilis SP, Clarkson PM et al. A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. *Acta Physiol Scand*. 2001; 171(2):187–193.

248. Thompson HS, Maynard EB, Morales ER et al. Exercise-induced HSP27, HSP70 and MAPK responses in human skeletal muscle. *Acta Physiol Scand*. 2003; 178(1):61–72.

249. Ritossa F. A new puffing pattern induced by heat shock and DNP in Drosophila. *Experientia*. 1962; 18(12):571–573.

250. Welch WJ. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiol Rev*. 1992; 72(4):1063–1081.

251. Csermely P, Yahara I. Heat shock proteins. In: Kéri G, Tóth I, eds. *Molecular pathomechanisms and new trends in drug research*. London; New York: Taylor & Francis; 2003:67–75.

252. Paulsen G, Vissing K, Kalhovde JM et al. Maximal eccentric exercise induces a rapid accumulation of small heat shock proteins on myofibrils and a delayed HSP70 response in humans. *Am J Physiol - Regul Integr Comp Physiol*. 2007; 293(2):R844 –R853.

253. Sandström ME, Siegler JC, Lovell RJ et al. The effect of 15 consecutive days of heat–exercise acclimation on heat shock protein 70. *Cell Stress Chaperones*. 2008; 13(2):169–175.

254. Marshall HC, Ferguson RA, Nimmo MA. Human resting extracellular heat shock protein 72 concentration decreases during the initial adaptation to exercise in a hot, humid environment. *Cell Stress Chaperones*. 2006; 11(2):129–134.

255. Sandström ME, Madden LA, Taylor L et al. Variation in basal heat shock protein 70 is correlated to core temperature in human subjects. *Amino Acids*. 2009; 37(2):279–284.

256. Madden LA, Sandström ME, Lovell RJ et al. Inducible heat shock protein 70 and its role in preconditioning and exercise. *Amino Acids*. 2008; 34(4):511–516.

257. Taylor L, Midgley AW, Chrismas B et al. The effect of acute hypoxia on heat shock protein 72 expression and oxidative stress in vivo. *Eur J Appl Physiol*. 2010; 109(5):849–855.

258. Taylor L, Midgley AW, Chrismas B et al. Daily hypoxia increases basal monocyte HSP72 expression in healthy human subjects. *Amino Acids*. 2011; 40(2):393–401.

259. Kinnunen S, Hyyppä S, Lappalainen J et al. Exercise-induced oxidative stress and muscle stress protein responses in trotters. *Eur J Appl Physiol*. 2005; 93(4):496–501.

260. U Feige D. *Stress-inducible cellular responses*. Birkhauser Basel; 1996.

261. Bondesen BA, Mills ST, Kegley KM et al. The COX-2 pathway is essential during early stages of skeletal muscle regeneration. *Am J Physiol-Cell Physiol*. 2004; 287(2):C475–C483.

262. Bondesen BA, Mills ST, Pavlath GK. The COX-2 pathway regulates growth of atrophied muscle via multiple mechanisms. *Am J Physiol-Cell Physiol*. 2006; 290(6):C1651–C1659.

263. Shen W, Li Y, Zhu J et al. Interaction between macrophages, TGF-β1, and the COX-2 pathway during the inflammatory phase of skeletal muscle healing after injury. *J Cell Physiol*. 2008; 214(2): 405–412.

264. Close GL, Kayani A, Vasilaki A et al. Skeletal muscle damage with exercise and aging. *Sports Med*. 2005; 35(5):413–427.

265. Yu J-G, Fürst DO, Thornell L-E. The mode of myofibril remodelling in human skeletal muscle affected by DOMS induced by eccentric contractions. *Histochem Cell Biol*. 2003; 119(5):383–393.

266. Lynn R, Morgan DL. Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. *J Appl Physiol*. 1994; 77(3):1439 –1444.

267. Whitehead NP, Allen TJ, Morgan DL et al. Damage to human muscle from eccentric exercise after training with concentric exercise. *J Physiol*. 1998; 512(2):615–620.

268. Ploutz-Snyder LL, Tesch PA, Dudley GA. Increased vulnerability to eccentric exercise-induced dysfunction and muscle injury after concentric training. *Arch Phys Med Rehabil*. 1998; 79(1): 58–61.

269. Armstrong RB. Initial events in exercise-induced muscular injury. *Med Sci Sports Exerc*. 1990; 22(4):429–435.

270. Foley JM, Jayaraman RC, Prior BM et al. MR measurements of muscle damage and adaptation after eccentric exercise. *J Appl Physiol Bethesda Md 1985*. 1999; 87(6):2311–2318.

271. Thompson HS, Clarkson PM, Scordilis SP. The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. *Acta Physiol Scand*. 2002; 174(1):47–56.

272. Pizza FX, Davis BH, Henrickson SD et al. Adaptation to eccentric exercise: effect on CD64 and CD11b/CD18 expression. *J Appl Physiol Bethesda Md 1985*. 1996; 80(1):47–55.

273. Touchberry C, Gupte A, Bomhoff G et al. Acute heat stress prior to downhill running may enhance skeletal muscle remodeling. *Cell Stress Chaperones*. 17(6):693–705.

274. Sugiyama Y, Suzuki A, Kishikawa M et al. Muscle develops a specific form of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. *J Biol Chem*. 2000; 275(2):1095–1104.

275. Magaudda L, Di Mauro D, Trimarchi F et al. Effects of physical exercise on skeletal muscle fiber: ultrastructural and molecular aspects. *Basic Appl Myol*. 2004; 14(1):17–21.

276. Nosaka K, Clarkson PM. Muscle damage following repeated bouts of high force eccentric exercise. *Med Sci Sports Exerc*. 1995; 27:1263–1263.

277. Paddon-Jones D, Muthalib M, Jenkins D. The effects of a repeated bout of eccentric exercise on indices of muscle damage and delayed onset muscle soreness. *J Sci Med Sport Sports Med Aust*. 2000; 3(1):35–43.

278. Brown SJ, Child RB, Day SH et al. Exercise-induced skeletal muscle damage and adaptation following repeated bouts of eccentric muscle contractions. *J Sports Sci*. 1997; 15(2):215–222.

279. Byrnes WC, Clarkson PM, White JS et al. Delayed onset muscle soreness following repeated bouts of downhill running. *J Appl Physiol*. 1985; 59(3):710 –715.

280. Chen TC, Nosaka K, Sacco P. Intensity of eccentric exercise, shift of optimum angle, and the magnitude of repeated-bout effect. *J Appl Physiol*. 2007; 102(3):992 –999.

281. McHugh MP, Tetro DT. Changes in the relationship between joint angle and torque production associated with the repeated bout effect. *J Sports Sci*. 2003; 21(11):927–932.

282. Falvo MJ, Schilling BK, Bloomer RJ et al. Repeated bout effect is absent in resistance trained men: an electromyographic analysis. *J Electromyogr Kinesiol Off J Int Soc Electrophysiol Kinesiol*. 2009; 19(6):e529–535.

283. Chen TC, Chen H-L, Lin M-J et al. Potent protective effect conferred by four bouts of low-intensity eccentric exercise. *Med Sci Sports Exerc*. 2010; 42(5):1004–1012.

284. Chen HL, Nosaka K, Chen TC. Muscle damage protection by low-intensity eccentric contractions remains for 2 weeks but not 3 weeks. *Eur J Appl Physiol*. 2012; 112(2):555–565.

285. Nosaka K, Sakamoto K, Newton M et al. How long does the protective effect on eccentric exercise-induced muscle damage last? *Med Sci Sports Exerc*. 2001; 33(9):1490–1495.

286. Cleary MA, Kimura IF, Sitler MR et al. Temporal pattern of the repeated bout effect of eccentric exercise on delayed-onset muscle soreness. *J Athl Train*. 2002; 37(1):32–36.

287. Eston RG, Lemmey AB, McHugh P et al. Effect of stride length on symptoms of exercise-induced muscle damage during a repeated bout of downhill running. *Scand J Med Sci Sports*. 2000; 10(4):199–204.

288. Smith LL, McKune AJ, Semple SJ et al. Changes in serum cytokines after repeated bouts of downhill running. *Appl Physiol Nutr Metab Physiol Appliquée Nutr Métabolisme*. 2007; 32(2):233–240.

289. Rowlands AV, Eston RG, Tilzey C. Effect of stride length manipulation on symptoms of exercise-induced muscle damage and the repeated bout effect. *J Sports Sci*. 2001; 19(5):333–340.

290. Case S, Evans D, Tibbets G et al. Dietary intakes of participants in the IditaSport Human Powered Ultra-marathon. *Alaska Med*. 1995; 37(1):20–24.

291. Case S, Stuempfle K, Hughes S et al. Dietary Intake, Gastrointestinal Symptoms, and Cognitive Function During the 2000 Iditasport Human Powered Ultra Marathon. *Med Sci Sports Exerc*. 2001; 33(5):S72.

292. Stuempfle K, Hughes S, Case S et al. Dietary Intakes of Participants in the 1994-1998 Iditasport Human Powered Ultra-Marathon. *Med Sci Sports Exerc*. 1999; 31(5):S80.

293. Glace BW, Murphy CA, McHugh MP. Food intake and electrolyte status of ultramarathoners competing in extreme heat. *J Am Coll Nutr*. 2002; 21(6):553–559.

294. Machefer G, Groussard C, Zouhal H et al. Nutritional and plasmatic antioxidant vitamins status of ultra endurance athletes. *J Am Coll Nutr*. 2007; 26(4):311–316.

295. Rehrer NJ, Brouns F, Beckers EJ et al. Physiological changes and gastro-intestinal symptoms as a result of ultra-endurance running. *Eur J Appl Physiol*. 1992; 64(1):1–8.

296. Singh NR, Peters EM. Markers of Hydration Status in a 3-Day Trail Running Event. *Clin J Sport Med Off J Can Acad Sport Med*. 2013:[ePub].

297. Singh NR, Denissen EC, McKune AJ et al. Intestinal Temperature, Heart Rate, and Hydration Status in Multiday Trail Runners. *Clin J Sport Med*. 2012; 22(4):311–318.

298. Knechtle B, Knechtle P, Rüst CA et al. Regulation of electrolyte and fluid metabolism in multi-stage ultra-marathoners. *Horm Metab Res*. 2012; 44(12):919–926.

299. Lebus DK, Casazza GA, Hoffman MD et al. Can changes in body mass and total body water accurately predict hyponatremia after a 161-km running race? *Clin J Sport Med*. 2010; 20(3):193–199.

300. Hew-Butler T, Hoffman MD, Stuempfle KJ et al. Changes in copeptin and bioactive vasopressin in runners with and without hyponatremia. *Clin J Sport Med*. 2011; 21(3):211–217.

301. Hoffman MD, Stuempfle KJ, Rogers IR et al. Hyponatremia in the 2009 161-km Western States Endurance Run. *Int J Sports Physiol Perform*. 2012; 7(1):6–10.

302. Bruso JR, Hoffman MD, Rogers IR et al. Rhabdomyolysis and hyponatremia: a cluster of five cases at the 161-km 2009 Western States Endurance Run. *Wilderness Environ Med*. 2010; 21(4):303–308.

303. Cuthill JA, Ellis C, Inglis A. Hazards of ultra-marathon running in the Scottish Highlands: exercise-associated hyponatraemia. *Emerg Med J*. 2009; 26(12):906–907.

304. Morin JB, Tomazin K, Edouard P et al. Changes in running mechanics and spring-mass behavior induced by a mountain ultra-marathon race. *J Biomech*. 2011; 44(6):1104–1107.

305. Lopez RM, Casa DJ, Jensen KA et al. Examining the Influence of Hydration Status on Physiological Responses and Running Speed During Trail Running in the Heat With Controlled Exercise Intensity. *J Strength Cond Res*. 2011; 25(11):2944–2954.

306. Stearns RL, Casa DJ, Lopez RM et al. Influence of hydration status on pacing during trail running in the heat. *J Strength Cond Res Natl Strength Cond Assoc*. 2009; 23(9):2533–2541.

307. Casa DJ, Stearns RL, Lopez RM et al. Influence of hydration on physiological function and performance during trail running in the heat. *J Athl Train*. 2010; 45(2):147–156.

308. Pournot H, Bieuzen F, Louis J et al. Time-Course of Changes in Inflammatory Response after Whole-Body Cryotherapy Multi Exposures following Severe Exercise. *Plos One*. 2011; 6(7):e22748.

309. Clements JM, Casa DJ, Knight JC et al. Ice-water immersion and cold-water immersion provide similar cooling rates in runners with exercise-induced hyperthermia. *J Athl Train*. 2002; 37(2):146–150.

310. Denissen EC, De Waard AH, Singh NR et al. Low markers of muscle damage and inflammation following a 3–day trail run. *South Afr J Sports Med*. 2012; 24(1):15–21.

311. Kraemer WJ, Fragala MS, Watson G et al. Hormonal responses to a 160-km race across frozen Alaska. *Br J Sports Med*. 2008; 42(2):116–120.

Chapter 3
Methods

3.1. General investigation design

Before presenting the experimental studies conducted, the overlying rationale shall be depicted, followed by a more detailed overview of the employed methodology. As trail running is a rather new field of study, there is still a distinct lack of descriptive literature on the subject. Therefore, before embarking on intervention-based investigation, it was paramount to develop a descriptive basis to ensure that the intervention models effectively targeted the subject matter. To this end, classical fatigue indices following a trail run were assessed in different populations. The results allowed for the designation of an appropriate population for further studies. After this descriptive information was compiled, an investigation model was developed and tested for reproducibility. In the intervention-based section, the focus was placed on during-effort and pre-effort strategies that could improve performance and recovery. As a during-effort strategy, the effects of lower limb compression garments on performance and fatigue indexes were evaluated using the prior validated intervention model. In a final study, a completely laboratory-based intervention study was conducted on passive heat exposure as a pre-race strategy of minimising exercise-induced muscle damage. During these studies, a number of common techniques were used, which shall be elaborated in the following.

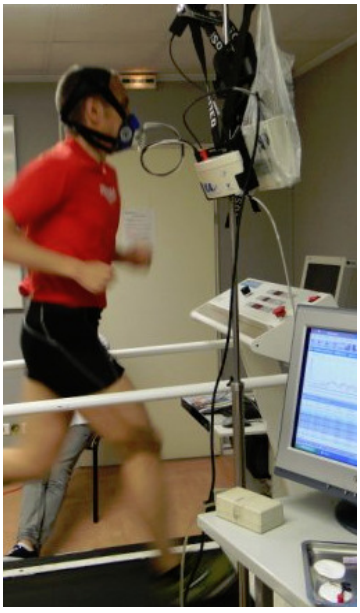
3.1.1. Subjects

In all studies barring the final study on muscle damage, a population of experienced male trail runners from 20 to 55 years of age was investigated. Several minimal requirements for participation in the studies were established, which included > 50 $km \cdot wk^{-1}$ training in the preceding 3 months, > 2 years trail running experience, no smoking, no history of any lower limb muscle-skeletal injuries in the past year, no history of coronary or pulmonary disease, no type of regular medication or supplementation, etc. For the final study on heat exposure, a less trained cohort was desired in order to maximise muscle damage and training effects. Therefore, the population sourced from university Health Science students was subject to quite different criteria. Students were required to have no experience in any sport involving eccentric contractions of the lower limb, no history of heat sickness or heat conditioning, no lower limb

muscle-skeletal injures in the past year, no intake of regular medication or supplementation and no history of pulmonary or coronary disease. Additionally, a regular alcohol intake of > 5 standard drinks per week or smoking in the past year were considered distinct exclusion criteria. Subject characteristics throughout the trail studies (masters excluded) were similar, averaging to a mean age of 33.4 ± 4 years, a weight of 71.7 ± 6 kg, a height of 178.9 ± 5 cm and a $\dot{V}O_2$max of 59.7 ± 7 $mL \cdot min^{-1} \cdot kg^{-1}$. Respectively, with no significant differences in groups, subjects for the heat study averaged an age of 27.1 ± 4 years, weighed 69.2 ± 11 kg, were 174.1 ± 8 cm tall and displayed an average $\dot{V}O_2$max of 48.8 ± 9 $mL \cdot min^{-1} \cdot kg^{-1}$.

3.2. Metabolic responses

3.2.1. Maximal oxygen consumption and ventilatory threshold



Maximal oxygen uptake ($\dot{V}O_2$max) is considered the single best indicator of aerobic and cardiovascular fitness, which plays an important role in trail running performance. Conceptually, the assessment of oxygen consumption relies on gas volume and component analysis and is effectuated by collecting and analysing fractions of the expired gas. This type of measurement was pioneered by Douglas in 1911 by collecting expired gases in sealed canvas bags and has remained the golden standard of respiratory exchange measurement for over a century[1,2]. Due to convenience and certain constraints in using the Douglas bag method (notably the intermittency of the method and subsequent determination of $\dot{V}O_2$peak instead of $\dot{V}O_2$max), computerised systems known as metabolic carts now frequently replace the original method (see image above). These systems rely on the same principles and analyse samples either on a breath-by-breath basis or temporal basis using a mixing chamber. All systems used during the presented studies (Cosmed K4 B2, Rome, Italy; Medgraphics Ultima, Norfolk, UK) used breath cycle detection, which results in a

higher resolution measurement but increases susceptibility to artefacts. By analysing volume and expired gas fractions, the metabolic cart allows assessment of:

- Minute ventilation ($\dot{V}_e$): the volume of air that is expired in 1 minute in standard temperature and pressure (STP). Values in males range from 5 to 8 $L \cdot min^{-1}$ at rest to over 100 $L \cdot min^{-1}$ during heavy exercise[3].
- Oxygen consumption ($\dot{V}O_2$): the volume of O_2 that is consumed during 1 minute, calculated reversely by assessing the difference of inspired and expired O_2 fraction and multiplying with the minute ventilation (STP).
- Carbon dioxide elimination ($\dot{V}CO_2$): the amount of carbon dioxide produced during 1 minute of exercise, assessed from in/out fraction differences and the V_e.
- Respiratory exchange ratio (RER): the ratio of $\dot{V}CO_2$ / $\dot{V}O_2$ which is used as a rough measure of the main energy repletion processes[4]. An RER of < 0.7 is associated with fat oxidation, 0.7 < RER < 1 defines mixed metabolisation of carbohydrates and fats while 1 < RER designates a pure carbohydrate-driven metabolism[5–7].

The determination of maximal oxygen uptake was conducted using an incremental step protocol in which work output of the subject was continually increased until volitional exhaustion[8–11]. As there is an influence of testing modality on $\dot{V}O_2max$, especially in trained subjects, the exercise modality is generally chosen to reflect the subject matter[12]. In most of the presented studies, testing was therefore conducted on a treadmill at a 4% gradient to simulate variant environmental conditions and wind resistance[13]. In the first descriptive study, since a comparison between young and master athletes was to be made, an ergometer test was opted for as this is less susceptible to age-dependent differences in musculoskeletal stiffness. Initial speed and increment duration varied between studies and was generally chosen to ensure that the test would be terminated in less than 20 minutes. Subsequent analysis of the $\dot{V}O_2$ data entailed smoothing with moving averages and identification of the highest 30 s average from the data set once a plateau ($\dot{V}O_2$ time slope < 0.05 $L \cdot min^{-1}$)[14] during the last 30 s in $\dot{V}O_2$ had

been reached. Further criteria were an RER of greater than 1.1 and a heart rate above 90% of the age-predicted maximum[15].

The ventilatory thresholds (VT1 and VT2) were determined using the ventilatory equivalent breakpoint method pioneered by Wasserman[16,17]. These points of disproportionate increase in $\dot{V}O_2$ are considered important indicators of aerobic fitness[18]. The first ventilatory threshold was defined as the first increase in the $\dot{V}_e / \dot{V}O_2$ equivalent that is not proportional to the increase in the $\dot{V}_e$ / CO_2 equivalent and corresponds to the onset of HCO_3^- buffering of lactic acid production[19]. This is often interpreted as the point at which aerobic mechanisms alone can no longer sustain the necessary ATP concentration to continue the effort. The second ventilatory threshold (VT2) is reached at a high work intensity at which blood lactate accumulation increases sharply and the clearance mechanisms are overwhelmed. In an attempt to buffer acidosis, hyperventilation is induced, which leads to a substantial increase in both ventilatory equivalents[19,20].

The ventilatory equivalent method used in the presented studies relies on the graphical determination of breakpoints in the $\dot{V}_e$ / CO_2 to $\dot{V}_e$ / O_2 graph. As this determination can vary between interlocutors, analysis was in all cases completed by two experienced parties (blinded), and in case of a substantial disagreement a third party was invited. The values determined for VT1 & VT2 were then transferred into the temporal domain and the corresponding heart rate and speed or power output was noted.

3.2.2. HEART RATE

Throughout the presented studies, HR was recorded using Polar or Garmin heart rate monitors that function using a chest-based sensor which telemetrically transferred the data real-time to a wrist-based recording unit. These devices have been validated in comparison to more sophisticated ECG units and are frequently used in the literature to monitor HR during exercise[21]. Data collection commenced generally at least 15 min before begin of exercise and

the subjects autonomously set markers at exercise initiation and cession. After collection data was transferred to a computer for analysis. Marker positions were verified and the data cut and filtered to correspond to the investigated exercise segment and eliminate any potential transmission defects. Using the maximal heart rate (HR) determined during incremental exercise along with the HR corresponding to VT1 and VT2, exercise intensity could be discriminated into moderate, heavy, and severe exercise, using guidelines defined elsewhere[22].

3.2.3. LOCOMOTION EFFICIENCY

As stated earlier, $\dot{V}O_2max$ is considered a reliable indicator of aerobic fitness, yet it does not correlate well with running performance, as maximal oxygen uptake is only one component in a complex weave of processes that result in running performance. One further important indicator of performance is locomotion efficiency[23,24], defined as the amount of mechanical work that can be completed at a given oxygen consumption. Locomotion efficiency is a compound measure that represents system properties on a number of levels including gross motor coordination, activation and contractile properties, burst synchronisation, mechanical properties of the muscle-tendon unit, substrate availability and feedback loop optimisation[25,26]. As locomotion efficiency can already be seen as an integrative measure of sorts, it is not surprising that it correlates well with running performance[27,28]. There are a number of ways to calculate locomotion efficiency, two of the most common being known as gross efficiency (GE) and delta efficiency (DE). Both rely on a similar design, in which the subject completes an exercise similar to the target modality for around 5 to 10 minutes after a metabolic steady state has been attained, while respiratory exchange is consistently monitored. Gross efficiency is calculated as the quotient of external work rate and internal energy consumption, in this case estimated from oxygen consumption and respiratory exchange ratio[29]. Delta efficiency on the other hand is calculated from a series of gross efficiencies which is subjected to linear regression analysis. Delta efficiency is often considered the most robust method of determining running economy[30]. To summarise:

$$Gross\ efficiency\ [\%] = \frac{Work\ rate\ (WR\ [J])}{Energy\ expenditure\ (EE\ [J])}\ x\ 100$$

$$Delta\ efficiency\ [\%] = \frac{\Delta Work\ rate\ (WR\ [J])}{\Delta Energy\ expenditure\ (EE\ [J])}\ x\ 100$$

3.2.4. Blood lactate concentration

Probably the most historical of fatigue indexes, lactate analysis has been in existence since the very beginning of fatigue studies. Most of the modern hand-held analysers are based on reflectance photometry or amperometric techniques, both of which show good correlation with classical laboratory methods[31,32]. In the following studies, blood was sampled from the earlobe into a self-vacuuming Lactate Pro (ARKRAY, Kyoto, Japan) device, which had been calibrated prior to use (see image to the right). Operating temperatures were respected and results were returned by the amperometric-based unit within 60 seconds. The Lactate Pro was shielded from excessive temperature changes (e.g. direct sunlight) throughout each study.

3.2.5. Near infra-red spectroscopy

Investigating the muscular oxygen consumption and the oxygenation of the blood can be very informative regarding the energy demands of the region of interest (ROI). Recently, it has become popular to use near infra-red spectroscopy (NIRS) to elucidate these values. NIRS relies on the chromophoric properties of haemoglobin (Hb), which change between its oxygen-free (HHb) and oxygen-bound state (HbO_2). Simply speaking, a NIRS system consists of an emitter that emits low-energy photons, which are recorded again by a detector. Placing both emitter and detector on the skin, different tissue depths are probed by varying the inter-optode

distance (IOD). Deep-penetrating photons are prone to increased scattering and absorption, while shallow protons tend to leave the ROI before being detected. Therefore, the mean photon path is a curved shape, penetrating roughly half the depth of the IOD[33,34]. Once the IOD exceeds roughly 5 cm, the signal becomes too weak and is eclipsed by noise, therefore limiting NIRS penetration depth. This behaviour is described by an equation derived from the modified Beer-Lambert law:

$$\Delta c = \frac{\Delta OD}{\varepsilon \; x \; L \; x \; DPF}$$

In this, Δc represents a change in concentration of the chromophore, ΔOD is the change in optical density, ε is the chromophore extinction coefficient, L is the inter-optode distance and the DPF is the differential pathlength factor which accounts for tissue-induced scattering. By using two wavelengths which correspond to an elevated extinction coefficients in either deoxy- or oxy- haemoglobin, a relatively robust assessment of changes in components can be made using a set of linear equations[35].

The Portamon system used in the presented studies uses a single compound detector and 3 emitters (IOD: 30, 35, 40 mm). This results in penetration depths of 1 to 2 cm. The wavelengths used are 760 and 850 nm, which are some of the commonly encountered frequencies in commercial spectroscopes and correspond with elevated extinction coefficients in deoxy- and oxy- haemoglobin, respectively. Both wavelengths are also absorbed by oxy- and deoxy-myoglobin, which exerts a confounding influence[36,37], but do not seem to negatively impact the measurement of muscular $\dot{V}O_2$ consumption ($m\dot{V}O_2$) and blood flow (BF)[38]. While NIRS gives conclusive information on HbO_2 and HHb concentrations, the assessment of $m\dot{V}O_2$ and BF requires an occlusion of some sort (venous or arterial) to control blood influx. In the presented case, a venous occlusion was elicited at 70mmHg and the early time derivatives of total Hb were analysed to divulge blood flow. Muscular $\dot{V}O_2$ consumption was regarded as the time derivate of HHb. Occlusions were duplicated in each testing session with the subjects sitting in a reclined position with their legs stretched out. The NIRS was attached and marked over the muscle belly of the M. Vastus Lateralis (VL), around 15 to 20 cm above the patella. Before

commencing measurement, the system was securely attached and shielded from external light. The duplicate occlusions were separated by 2 minutes rest and only the first 20 seconds of occlusion time were analysed, while the BF influx was still relatively linear.

3.3. Psychological parameters

3.3.1. Rate of perceived exertion

While there are a plethora of objective markers to describe exercise intensity, it is also possible to quantify effort through the individual perception of the subject. Borg devised a scale of a "rate of perceived exertion", which has remained in popular use over the past 40 years[39,40]. Interestingly, RPE in well-trained subjects correlates well with more objective indices of exercise intensity[41,42]. In the presented studies, RPE was collected in order to give conclusive information on how the subject assessed the exercise. The original Borg scale ranging from 6 to 20 was used for in-exercise assessment using a visual scale detailing both numeric and expressive gradients for subjects to gesture-indicate their perceived exertion: 6 anchors for "very, very light" and 20 anchors for "very, very hard".

3.4. Muscular function

3.4.1. Maximal voluntary isometric contraction

The golden standard measurement for neuromuscular fatigue, if adhering to the definition of fatigue as a "loss in maximal force generating capacity", is the maximal voluntary isometric contraction (MVIC)[43]. In the presented studies, the main muscle group of interest was the Quadriceps Femoris muscle group (QF), consisting of the M. Rectus Femoris, M. Vastus Lateralis, M. Vastus Medialis and M. Vastus Intermedius. Together with the triceps surae muscle groups, these muscles provide the main propulsive force during running[44] and are classically tested for fatigue during and after running[45–47]. The QF is a rather large and superficial muscle group, therefore absolute effects are more pronounced and the muscle is easily accessible for surface electromyography, superficial stimulation or tissue sampling.

Specific activation is a common everyday task and subjects therefore do not need a long training phase to synchronise activation patterns[48–50]. Isometric force testing is probably the easiest genre of force testing to investigate[51]. While not being fully representative of the dynamic contraction encountered in locomotion, it simplifies a number of possible confounders. Notably, there is less change in muscle length[52–54] and less skin-muscle motion, making the collection of EMG data substantially easier.

Testing in the presented studies was conducted with an isokinetic dynamometer (Biodex System 2 & System 3, NY, USA) into which subjects were strapped by use of two cross-shoulder attachment belts and a fixation sling for the leg (see image above). A standardised position was adopted by the subjects in which their arms were crossed over the chest and the hands gripped the contra-lateral shoulder. The dynamometer was then positioned by an experienced operator to ensure that, during contraction, the axis of the knee was precisely aligned with the axis of the dynamometer itself. A complete leg extension was assigned with a 0° angle and a 90° -flexed leg was assigned as 90°. As, depending on segment mass and angle, there is a component of force eschewed by counteracting gravity, a correction was made by determining the mass moment at 0° and 90° and performing a quasi-linear correction using the rotational circumference of the mass lever. Testing was performed at 70° and 90° flexion, depending on the protocol. Subjects were fully briefed using standard terminology ("extend the leg as hard and fast as possible!")[55–57] and completed a warm-up before commencing the three second MVICs. The warm-up consisted of 20 contractions (1 s contraction, 1 s rest) at 50% MVIC followed by 3 ramp contractions over 3 seconds to 70% MVIC. Three minutes rest were given before subjects commenced MVIC testing. During MVIC, subjects were continuously verbally motivated and their position was closely monitored by the operator to foreclose any torsional motion of the pelvis or use of the

arms to enhance force production. Moment [Nm] was captured at an acquisition rate of 100 Hz and saved for further analysis.

3.4.2. PERCUTANEOUS ELECTRIC STIMULATION

Peripheral electrical stimulation is useful in elucidating the difference between maximal voluntary contraction, and maximal evoked contraction, and allows assessment of a voluntary activation (VA) ratio using a number of methods[58,59]. Peripheral stimulation can be applied either percutaneously by attaching large electrodes directly on the proximal and distal ends of the muscle (Estim), or as neuromuscular stimulation, by stimulating the alimenting α motor neurones, which then in turn activate the muscle (peripheral nerve stimulation, PNS). Both methods were employed during the presented studies using a Digitimer DS7A (Welwyn Garden City, England) rectangular pulse generator and shall be further elaborated upon after describing the common principles. Peripheral stimulation relies on an electrical pulse that traverses the epidermal layer and innervates the tissue underneath. By modulating the amount, shape, duration, frequency and intensity of the invoked pulses, the muscle response can be changed. The pulses used in the presented studies were 400 V and rectangular in shape with a duration of 200 μs. For twitch elicitation, a 100 Hz doublet profile was chosen[60], as this provides a good compromise between twitch amplitude and distinction and subject discomfort (which ensues performance decrease[61]). Other studies have employed single twitches or short pulse trains, yet results from an unpublished pilot study indicated that twitch amplitude gains were less pronounced and offset by a distinct increase in subject discomfort at more than 5 sequential twitches. Intensity calibration technique varied, depending on the technique used. Following data collection, voluntary activation was estimated using the interpolated twitch technique (ITT)[58] by contrasting the twitch amplitudes[62].

$$VA\ [\%] = \left[1 - \left({Superimposed\ twitch}/{Control\ twitch}\right)\right] x\ 100$$

Neural stimulation has the benefit that it incorporates the neuromuscular junction into the assessed circuit and aliments the muscle with a physiological supra-maximal stimulus. Placement of the stimulation node was determined by excitability at low intensities and was marked to ensure identical placement at each testing session. Calibration of the 100 Hz doublet stimulation intensity was assessed using real-time analysis of the M-wave, intensity being increased until the M-wave amplitude no longer increased and the H-wave was no longer discernible. During MVC testing a 100 Hz doublet was superimposed on the MVIC around 400 ms after the rate of force development stagnated, i.e. a stable force plateau had been reached (> 250 ms)[60]. A control doublet was given 2 seconds after finishing the contraction in order to avoid differences in force potentiation between the two conditions.

Peripheral percutaneous stimulation induces the current straight over the muscle, directly effectuating a depolarisation of the muscle membrane and t-tube system. The placement of the stimulation nodes is less critical than in neural stimulation and the sustained twitch intensities are somewhat higher (400 - 800 mA). However, this type of stimulation conveys a rather large component to the antagonists, which have a confounding effect on the estimation of VA[63]. In the presented study, two large oval carbon-rubber electrodes (8 x 13 cm) were placed proximally and distally over the quadriceps. Stimulation intensity was calibrated by repeatedly giving doublets and increasing intensity gradually until either 70% MVIC was obtained, or the subject declined further increase[64]. The MVIC protocol consisted of a doublet 5 s before contraction onset, a superimposed doublet at 1 s after contraction onset and 3 post-contraction doublets at 5 s, 10 s and 15 s after contraction end.

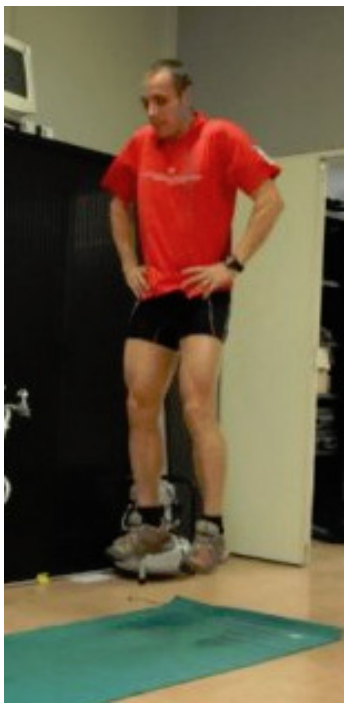
3.4.3. Surface electromyography

Surface electromyography (sEMG) is used to quantify onset, duration and amplitude of the depolarisation stimulus that is transferred to the muscle. This is usually done by attaching two gel-based (silver chloride) electrodes on the muscle body of interest (double differential method, inter-electrode distance = 20 mm) and running the signal difference through a series of amplifiers and filters to enhance the signal-to-noise ratio. The electrode placement on the

three superficial muscles of the QF is well-known and guided mainly by anatomical landmarks, although there are some approaches that rely on identification of the motor point (innervation zone). For the presented studies, the classical guidelines of Basmajian[65] and SENIAM[66,67] were followed. In all studies a similar methodology was adapted. Individual subjects' test-retest sessions were at similar times during the day and electrode placement was marked as to render it invariable. Subjects were asked not to use skin care products on the areas that were to be treated 24 hours pre-session and were asked to arrive well-hydrated. Skin preparation consisted of removing dead skin particles (and hair where necessary) with a razor, alcohol swabbing, scrubbing with abrasive cream and a final pass with an alcohol swab to ensure residue removal. A short moment was respected for the alcohol to fully evaporate before the electrodes were placed and connected to the EMG unit (Noraxon TeleMyo 2400T, Scottsdale, USA). Skin impedance was verified to be less than 5 kΩ. A mass electrode was attached to a bony landmark in the vicinity of the recording site (iliac crest, patella). The signal (pre-amplification: common-mode rejection ratio = 100 dB; Z input = 10 GΩ; gain = 600; bandwidth frequency = 6 - 1600 Hz) was verified to be responsive and noise-free and was sampled at 1000 Hz during testing. Post-acquisition, a 50 Hz notch filter was applied along with a 2nd order Butterworth band-pass filter from 6 to 500 Hz. The maximal root mean squares (RMS) were calculated by squaring the signal, averaging and then calculating the square root within a moving linear envelope of 500 ms. The highest RMS was retained for further processing.

The EMG recordings were also used to determine the properties of the M-wave elicited by the doublets given at rest. Key points of interest were the peak-to-peak amplitude (PPA) and peak-to-peak duration (PPD), which have been shown to closely relate to neuromuscular fatigue[68]. As the muscle and alimenting motor neurons grow fatigued, the PPA decreases and PPD increases due to a loss in action potential transmission and a decreasing ability to propagate action potentials[69]. This can be related to a decrease in blood ammonia concentration[70], or a reduced Na^+ and K^+ gradient across the sarcolemma[71].

3.4.4. COUNTER MOVEMENT JUMP



The counter movement jump (CMJ) allows a simple functional assessment of explosive force production of the knee extensors. The outcome measure is maximal jumping height from a standardised starting position. As this is a functional assessment, it is susceptible to changes anywhere in the motor skill, neuromuscular and mechanical chain. CMJ height was tested using a Bosco Ergojump System, which records flight time of the subject and then, using an internal algorithm, displays the jump height. While this is less accurate than using a force plate or motion capture system, it is highly portable and frequently used in field testing[72]. While the loss of accuracy using the Bosco system is negligible, large variability of jumping height can be procured by non-standard jumping techniques. Therefore initial position and landing technique were closely monitored by the operator throughout testing and the subjects were extensively familiarised before testing. To initialise the jump, subjects placed their hands on their hips and took a shoulder-wide stance with knee and hip angles both at around 150°. After the initial position was deemed satisfactory, they were free to initiate the jumping motion at their own choosing. The jumping motion consisted of descending the centre of mass as needed and directly jumping as high as possible while keeping the hands on the hips (see image above). Landing was to be completed on the toes with an almost full leg extension in order to recreate (as close as possible) the take-off position without compromising the knees.

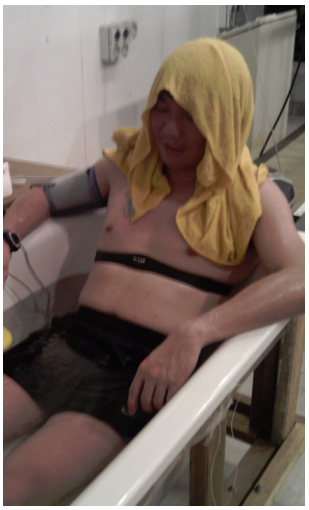
3.4.5. CREATINE KINASE AND LACTATE DEHYDROGENASE

Throughout studies, both creatine kinase (CK) and lactate dehydrogenase (LDH) were assessed in blood that was drawn from the antecubital veins using a vacutainer system. Samples were shuffled and directly centrifuged (3000 $Rot \cdot min^{-1}$, 4 °C, 10 min) to separate the phases. Plasma was then aliquoted and stored at -80 °C until analysis in duplicate (first thaw) for CK and LDH.

Analysis was effectuated using an automated Roche Hitachi 911 chemistry analyser (Roche Diagnostics Corporation, Indianapolis, USA), which uses IFCC-recommended spectrophotometric analysis to determine CK activity. The analysis kits were acquired directly from the manufacturer.

3.5. Thermal indexes

3.5.1. Core temperature



Core temperature was monitored during passive heating by means of a rectal thermometer. During passive heating, participants were immersed up to the waist (see image on the right) in hot water (41.5 °C) in a hot and humid environment (27 °C and 60% RH). Heart rate and RPE were monitored as described earlier and additionally core temperature was recorded. Blood pressure was verified every 5 minutes using a commercial automated blood pressure unit. To ensure accurate measurement of temperatures, each thermocouple wire was sheathed and then cross checked against a platinum resistance thermometer (Leeds & Northrup Type 8926, Minworth, UK) connected to a temperature bridge readout (Leeds & Northrup 8078, Minworth, UK) and the temperature-voltage curve defined for the thermal region of interest. During recording, a two channel voltage logger was used with two thermocouples: one for water temperature and one for core temperature. Post recording, voltages curves were cross-referenced with the calibration and temperatures calculated.

3.6. CHAPTER 3 BIBLIOGRAPHY

1. Douglas C. A method for determining the total respiratory exchange in man. *J Physiol*. 1911; 42:17–18.

2. Bassett DR, Howley ET, Thompson DL et al. Validity of inspiratory and expiratory methods of measuring gas exchange with a computerized system. *J Appl Physiol*. 2001; 91(1):218–224.

3. Blackie SP, Fairbarn MS, McElvaney NG et al. Normal values and ranges for ventilation and breathing pattern at maximal exercise. *Chest*. 1991; 100(1):136–142.

4. Goedecke JH, Gibson ASC, Grobler L et al. Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *Am J Physiol Endocrinol Metab*. 2000; 279(6):E1325–E1334.

5. Krogh A, Lindhard J. The relative value of fat and carbohydrate as sources of muscular energy. *Bioch J*. 1920; 14:290–363.

6. Christensen E, Hansen O. Arbeitsfähigkeit und Ernährung. *Scand Arch Physiol*. 1939; 81:160–171.

7. Jansson E. On the significance of the respiratory exchange ratio after different diets during exercise in man. *Acta Physiol Scand*. 1982; 114(1):103–110.

8. Weltman A, Snead D, Stein P et al. Reliability and validity of a continuous incremental treadmill protocol for the determination of lactate threshold, fixed blood lactate concentrations, and VO2max. *Int J Sports Med*. 2008; 11(1):26–32.

9. Billat VL, Hill DW, Pinoteau J et al. Effect of protocol on determination of velocity at VO2 max and on its time to exhaustion. *Arch Physiol Biochem*. 1996; 104(3):313–321.

10. Noakes TD, Myburgh KH, Schall R. Peak treadmill running velocity during the VO2 max test predicts running performance. *J Sports Sci*. 1990; 8(1):35–45.

11. Jones NL, Makrides L, Hitchcock C et al. Normal standards for an incremental progressive cycle ergometer test. *Am Rev Respir Dis*. 1985; 131(5):700–708.

12. Buchfuhrer MJ, Hansen JE, Robinson TE et al. Optimizing the exercise protocol for cardiopulmonary assessment. *J Appl Physiol*. 1983; 55(5):1558–1564.

13. Jones AM, Doust JH. A 1% treadmill grade most accurately reflects the energetic cost of outdoor running. *J Sports Sci*. 1996; 14(4):321–327.

14. Yoon B-K, Kravitz L, Robergs R. VO2max, Protocol Duration, and the VO2 Plateau. *Med Sci Sports Exerc*. 2007; 39(7):1186–1192.

15. Howley ET, Bassett DR Jr, Welch HG. Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc*. 1995; 27(9):1292–1301.

16. Wasserman K, Whipp BJ, Koyl SN et al. Anaerobic threshold and respiratory gas exchange during exercise. *J Appl Physiol*. 1973; 35(2):236–243.

17. Wasserman K, Stringer WW, Casaburi R et al. Determination of the anaerobic threshold by gas exchange: biochemical considerations, methodology and physiological effects. *Z Kardiol*. 1994; 83 Suppl 3:1–12.

18. McArdle WD, Katch FI, Katch VL. *Exercise Physiology: Nutrition, Energy, and Human Performance*. Baltimore: Lippincott Williams & Wilkins; 2010.

19. Davis JA. Anaerobic threshold: review of the concept and directions for future research. *Med Sci Sports Exerc*. 1985; 17(1):6–21.

20. Skinner JS, McLellan TH. The transition from aerobic to anaerobic metabolism. *Res Q Exerc Sport*. 1980; 51(1):234–248.

21. Hausswirth C, Le Meur Y, Couturier A et al. Accuracy and repeatability of the Polar RS800sd to evaluate stride rate and running speed. *Int J Sports Med*. 2009; 30(5):354–359.

22. Lucia A, Hoyos J, Carvajal A et al. Heart rate response to professional road cycling: the Tour de France. *Int J Sports Med*. 1999; 20:167–172.

23. Powers SK, Dodd S, Deason R et al. Ventilatory Threshold, Running Economy and Distance Running Performance of Trained Athletes. *Res Q Exerc Sport*. 1983; 54(2):179–182.

24. Bassett DR, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc*. 2000; 32(1):70–84.

25. Morgan DW, Martin PE, Krahenbuhl GS. Factors Affecting Running Economy. *Sports Med*. 1989; 7(5):310–330.

26. Saunders PU, Pyne DB, Telford RD et al. Factors affecting running economy in trained distance runners. *Sports Med*. 2004; 34(7):465–485.

27. Conley DL, Krahenbuhl GS. Running economy and distance running performance of highly trained athletes. *Med Sci Sports Exerc*. 1980; 12(5):357–360.

28. Williams KR, Cavanagh PR. Relationship between distance running mechanics, running economy, and performance. *J Appl Physiol*. 1987; 63(3):1236–1245.

29. Brouwer E. On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. *Acta Physiol Pharmacol Neerl*. 1957; 6:795–802.

30. Gaesser G, Brooks M. Muscular efficiency during steady-rate exercise: effects of speed and work rate. *J Appl Physiol*. 1975; 38(6):1137–1139.

31. Mc Naughton L, Thompson D, Philips G et al. A comparison of the lactate Pro, Accusport, Analox GM7 and Kodak Ektachem lactate analysers in normal, hot and humid conditions. *Int J Sports Med*. 2002; 23:130–135.

32. Tanner RK, Fuller KL, Ross ML. Evaluation of three portable blood lactate analysers: Lactate Pro, Lactate Scout and Lactate Plus. *Eur J Appl Physiol*. 2010; 109(3):551–559.

33. Cui W, Kumar C, Chance B. Experimental study of migration depth for the photons measured at sample surface. In: *Proc SPIE*. Vol 1431; 1991:180–191.

34. Hongo K, Kobayashi S, Okudera H et al. Noninvasive cerebral optical spectroscopy: depth-resolved measurements of cerebral haemodynamics using indocyanine green. *Neurol Res*. 1995; 17(2):89–93.

35. Sassaroli A, Fantini S. Comment on the modified Beer–Lambert law for scattering media. *Phys Med Biol*. 2004; 49(14):N255–N257.

36. Hoofd L, Colier W, Oeseburg B. A Modeling Investigation to the Possible Role of Myoglobin in Human Muscle in Near Infrared Spectroscopy (NIRS) Measurements. In: Dunn JF, Swartz HM, eds. *Oxygen Transport to Tissue XXIV*. Advances in Experimental Medicine and Biology. New York: Springer; 2003:637–643.

37. Spires J, Lai N, Zhou H et al. Hemoglobin and Myoglobin Contributions to Skeletal Muscle Oxygenation in Response to Exercise. In: LaManna JC, Puchowicz MA, Xu K, et al., eds. *Oxygen Transport to Tissue XXXII*. Advances in Experimental Medicine and Biology. New York: Springer; 2011:347–352.

38. Van Beekvelt MCP, van Engelen BGM, Wevers RA et al. In vivo quantitative near-infrared spectroscopy in skeletal muscle during incremental isometric handgrip exercise. *Clin Physiol Funct Imaging*. 2002; 22(3):210–217.

39. Borg G. Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med*. 1970; 2(2):92–98.

40. Borg GAV. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*. 1982; 14(5):377–381.

41. Borg G, Hassmén P, Lagerström M. Perceived exertion related to heart rate and blood lactate during arm and leg exercise. *Europ J Appl Physiol*. 1987; 56(6):679–685.

42. Löllgen H, Ulmer HV, Nieding G v. Heart rate and perceptual response to exercise with different pedalling speed in normal subjects and patients. *Eur J Appl Physiol Occup Physiol*. 1977; 37(4):297–304.

43. Vøllestad NK. Measurement of human muscle fatigue. *J Neurosci*. 1997; 74(2):219–227.

44. Hamner SR, Seth A, Delp SL. Muscle contributions to propulsion and support during running. *J Biomech*. 2010; 43(14):2709–2716.

45. Millet GY, Lepers R. Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Med*. 2004; 34(2):105–116.

46. Millet GY, Tomazin K, Verges S et al. Neuromuscular Consequences of an Extreme Mountain Ultra-Marathon. *PLoS ONE*. 2011; 6(2):e17059.

47. Fourchet F, Millet GP, Tomazin K et al. Effects of a 5-h hilly running on ankle plantar and dorsal flexor force and fatigability. *Eur J Appl Physiol*. 2012; 112(7):2645–2652.

48. Kues JM, Rothstein JM, Lamb RL. Obtaining reliable measurements of knee extensor torque produced during maximal voluntary contractions: an experimental investigation. *Phys Ther*. 1992; 72(7):492–501.

49. Murray MP, Gardner GM, Mollinger LA et al. Strength of Isometric and Isokinetic Contractions Knee Muscles of Men Aged 20 to 86. *Phys Ther*. 1980; 60(4):412–419.

50. Lohse KR, Sherwood DE. Defining the Focus of Attention: Effects of Attention on Perceived Exertion and Fatigue. *Front Psychol*. 2011; 2:332.

51. Masuda K, Masuda T, Sadoyama T et al. Changes in surface EMG parameters during static and dynamic fatiguing contractions. *J Electromyogr Kinesiol*. 1999; 9(1):39–46.

52. Bazzy AR, Korten JB, Haddad GG. Increase in electromyogram low-frequency power in nonfatigued contracting skeletal muscle. *J Appl Physiol*. 1986; 61(3):1012–1017.

53. Inbar GF, Allin J, Kranz H. Surface EMG spectral changes with muscle length. *Med Biol Eng Comput*. 1987; 25(6):683–689.

54. Potvin JR, Bent LR. A validation of techniques using surface EMG signals from dynamic contractions to quantify muscle fatigue during repetitive tasks. *J Electromyogr Kinesiol*. 1997; 7(2):131–139.

55. Marchant DC, Greig M, Scott C. Attentional focusing instructions influence force production and muscular activity during isokinetic elbow flexions. *J Strength Cond Res*. 2009; 23(8):2358–2366.

56. Capa RL, Audiffren M. How does achievement motivation influence mental effort mobilization? Physiological evidence of deteriorative effects of negative affects on the level of engagement. *Int J Psychophysiol*. 2009; 74(3):236–242.

57. Jung M-C, Hallbeck MS. Quantification of the effects of instruction type, verbal encouragement, and visual feedback on static and peak handgrip strength. *Int J Ind Ergonom*. 2004; 34(5):367–374.

58. Merton PA. Voluntary strength and fatigue. *J Physiol*. 1954; 123(3):553–564.

59. Allen GM, Gandevia SC, McKenzie DK. Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. *Muscle Nerve*. 1995; 18(6):593–600.

60. Shield A, Zhou S. Assessing voluntary muscle activation with the twitch interpolation technique. *Sports Med*. 2004; 34(4):253–267.

61. Button DC, Behm DG. The effect of stimulus anticipation on the interpolated twitch technique. *J Sports Sci Med*. 2008;(7):520–524.

62. Krishnan C, Williams GN. Quantification method affects estimates of voluntary quadriceps activation. *Muscle Nerve*. 2010; 41(6):868–874.

63. Krishnan C, Williams GN. Error associated with antagonist muscle activity in isometric knee strength testing. *Eur J Appl Physiol*. 2010; 109(3):527–536.

64. Périard JD, Thompson CCMW. Central and Peripheral Fatigue During Passive and Exercise-Induced Hyperthermia. *Med Sci Sports Exerc*. 2011; 43(9):1657–65.

65. Basmajian JV. *Muscles alive, their functions revealed by electromyography*. Baltimore: Lippincott Williams & Wilkins; 1978.

66. Hermens HJ, Freriks B, Disselhorst-Klug C et al. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol*. 2000; 10(5):361–374.

67. Hermens HJ, Freriks B. The state of the art on sensors and sensor placement procedures for surface electromyography: a proposal for sensor placement procedures. *Deliverable of the SENIAM Project*. 1997.

68. Place N, Lepers R, Deley G et al. Time course of neuromuscular alterations during a prolonged running exercise. *Med Sci Sports Exerc*. 2004; 36(8):1347–1356.

69. Overgaard K, Nielsen OB. Activity-induced recovery of excitability in K+-depressed rat soleus muscle. *Am J Physiol Regul Integr Comp Physiol*. 2001; 280(1):R48–R55.

70. Mutch BJ, Banister EW. Ammonia metabolism in exercise and fatigue: a review. *Med Sci Sports Exerc*. 1983; 15(1):41–50.

71. Pastene J, Germain M, Allevard AM et al. Water balance during and after marathon running. *Eur J Appl Physiol Occup Physiol*. 1996; 73(1-2):49–55.

72. García-López J, Peleteiro J, Rodgríguez-Marroyo JA et al. The validation of a new method that measures contact and flight times during vertical jump. *Int J Sports Med*. 2005; 26(4):294–302.

Chapter 4
DESCRIPTIVE STUDY

4.1. INTRODUCTION

This first descriptive study was designed to investigate and quantify the physiological reactions of two populations of different ages to a trail running competition. A young (30 years) and older cohort (46 years) were recruited and muscular performance and cycling economy were assessed before and in the 3 days following the competition. This collection of data allowed the quantification of fatigue parameters that would be of importance in designing the following experiments. The trail competition chosen for investigation was a 55 km race with 3000 m climb, as in this initial experiment we wanted to choose a common competition distance that was long enough to ensure differential effects. The concept governing this study was to quantify the differences between age groups in trail to subsequently define age-selection criteria for further studies. Changes in both neuromuscular function and gait adaptation (running economy) were of interest, as was exercise-intensity distribution during the effort. In synthesis, the older cohort achieved similar performance in the race and demonstrated similar amounts of fatigue and muscle damage, yet recuperated more slowly than the young cohort. Cycling economy was reduced post-race in both groups, and delta economy showed a greater depression in the older cohort. From a descriptive perspective, the fatigue encountered was more pronounced than in equidistant flat road races and enabled envisioning shorter races as valid intervention models. These results also led to the exclusion of older (> 46 years) subjects from the following studies, irrespective of training status, as they would potentially distort recuperation results. The study was published in late 2010 in the *European Journal of Applied Physiology*.

4.2. EFFECTS OF A TRAIL RUNNING COMPETITION ON MUSCULAR PERFORMANCE AND EFFICIENCY IN WELL-TRAINED YOUNG AND MASTER ATHLETES

Eur J Appl Physiol
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ORIGINAL ARTICLE

Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes

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Abstract

To determine the acute effects of a trail running competition and the age-dependent differences between young and master athletes, 23 subjects (10 young (30.5±7 yrs) 13 master (45.9±5.9yrs) participated in a 55km trail running competition. The study was conceived as an intervention study compromising pre, post 1h, 24h 48h and 72h measurements. Measurements consisted of blood tests, ergometer cycling and MVC contractions. Parameters monitored included MVC, Twitch- and M-wave properties, EMG (RMS) of the vastus lateralis, two locomotion efficiency calculation and muscle damage markers in the blood (CK, LDH). Results indicate post-race increases in CK and LHD, decreases in MVC values (-32% *vs.* -40% in young and master), decrease in EMG, increase in contraction time and concomitant decrease in peak twitch values, and a decrease in locomotion efficiency (-4.7% *vs.* -6.3% in young and master). Masters showed greater fatigue and muscle damage than young, but managed to achieve similar race times. This study shows that trail runs are more detrimental to muscle function than level runs, and gives indication that training may not halt muscle deterioration through aging, but can help maintain performance level.

Keywords: trial running, ultra long distance, master, eccentric contractions, muscle damage, efficiency

Introduction

While the popularity of running trail events has increased over the past five years (Hoffman and Wegelin 2009), limited information is available concerning the physiological responses of the runner occurring during this type of contest. Trails can be defined as ultra long distance runs lasting over than 5 hours in duration which are performed in a mountain context involving extensive vertical displacement (both uphill and downhill). One of the main performance determining components of trail runs is exercise duration. In general, ultra endurance exercises such as marathon running, road cycling, or Ironman triathlon are well-known to impose a strenuous physical load on the organism, which leads to decreases in locomotion efficiency an concomitant substrate changes (Brisswalter et al. 2000 ; Fernström et al. 2007), thermal stress and dehydration (Sharwood et al. 2004), oxidative stress (Nieman et al. 2004; Suzuki et al. 2006) and, specifically in running events, structural muscle damage (Overgaard et al. 2002; Suzuki et al. 2006). The second major characteristic of trail running events is the large proportion of eccentric work performed during the downhill segments of the race. Eccentric contractions involve force generation in a lengthening muscle, and are known to procure severe structural damage on muscles, affecting their contractile and recuperative properties (Nicol et al. 2006). Several studies in the last decade have investigated the effects of long distance runs performed on level courses. Results show a structural disruption of the sarcomere, an increased release of muscular enzymes into the plasma and a substantial impairment in maximal force generating capacity (Lepers et al. 2000a ; Millet et al. 2002, 2003; Overgaard et al. 2002; Place et al. 2004) or a decrease in post-race locomotion efficiency (Millet et al. 2000, 2009), indicating that muscles are progressively damaged during the exercise. Specifically, maximal isometric knee extension force has been reported to decrease by 24% after a 30-km running race (Millet et al. 2003), by 28% after 5h of treadmill running (Place et al. 2004) and by 30% after a 65-km ultra-marathon (Millet et al. 2002). Recently, Millet et al. (2009) reported a 6.2 % decrease in running efficiency three weeks after a 8500-km run between Paris and Beijing performed in 161 days. Gauche et al. (2006) have reported that maximal voluntary force decreased by 37% at the end of a prolonged trail run. Repeated eccentric contractions may also affect locomotion efficiency, as demonstrated by Braun and Dutto (2003), who observed a decrease of 3.2% in running efficiency 48h after a 30-min downhill run. In a similar vein, Moysi et al. (2005) found a 6% decrease in cycling efficiency after 10 series of 25 repetitions of squats, an eccentric exercise. Repeated eccentric contractions, independent of their context, seem to induce a decrease in locomotion efficiency, even if efficiency is evaluated in concentrically dominated cycling. Based upon the reviewed literature, it was assumed that trail-running races would accentuate muscle damage when compared to level running, due to the large proportion of eccentric contractions occurring in the successive downhill segments of courses and therefore lead to both a decrease in muscular performance and locomotion efficiency. Few studies so far have analyzed physiological aspects of trail running. The existing studies mainly focused on the

origin of the decline in contraction capacity (e.g. Miles et al. 2006; Gauche et al. 2006) or on pacing strategies during the race (Stearns et al. 2009). To our knowledge, only limited data is available on the impact of this type of events on locomotion efficiency (Millet et al. 2000).

A further characteristic of trail running competitions is the increasing participation of master athletes (Hoffman and Wegelin 2009). People who regularly participate in endurance training and who try to maintain their physical performance level despite the aging process (Tanaka and Seals 2008) are generally considered master athletes. In a competition context, competitors are traditionally classified as master athletes when over 40 years of age, the age at which a first decline in endurance peak performance is observed (Lepers et al. in press; Sultana et al. 2008; Tanaka and Seals 2008). The ageing process induces a great number of structural and functional transformations, which lead to an overall decline in physical capacity (Thompson 2009). The general ageing of to population procures the need to design strategies which on one hand increase functional capacity in older people (e.g. Henwood and Taaffe 2006), and on the other enhance the performance of master athletes. Supportively, recent studies have shown that master endurance athletes are able to maintain their performance despite structural changes in muscle performance or in maximal aerobic power, which are classically associated with aging (Lepers et al. in press; Tanaka and Seals 2008; Bieuzen et al. 2009; Louis et al. 2009).

In this context, the first purpose of our study was to evaluate muscle performance and efficiency of runners participating in a long distance trail competition. The second purpose was to compare the changes in these parameters between young and master runners competing in the same long distance running trail.

Materials and methods

Subjects

Eleven young and 15 well-motivated master athletes volunteered to participate in this study. The characteristics of the subjects are shown in table 1. All subjects had to be free from present or past neuromuscular and metabolic conditions that could have affected the recorded parameters. The subjects had regular training experience in long distance running prior to the study (8.4 ± 6.0 yrs for the young vs. 13.3 ± 7.8 yrs for the master runners), and had performed a training program of 72.1 ± 25.1 and 74.1 ± 23.6 km/wk for young and masters respectively during the 3 months preceding the experiment. The local ethics committee (St Germain en Laye, France) reviewed and approved the study before its initiation and all subjects gave their informed written consent before participation.

Table 1. General characteristics and performance parameters of subjects as: [Means (SD)]

	Young (n= 10)	Master (n=13)
Age (years)	30.5 (7.0)	45.9 (5.9)
Height (m)	1.8 (0.0)	1.8 (0.1)
Weight (kg)	70.6 (5.5)	70.7 (8.1)
VO_2 peak ($ml^{-1}min{\cdot}kg^{-1}$)	58.8 (6.5)	55.0 (5.8)
Marathon record (hrs:min)	03:02 (00:26)	02:58 (00:13)
Time to complete intervention (hrs:min)	06:42 (00:51)	06:51 (00:47)

Experimental procedure

The study was divided into four phases; preliminary testing and familiarization, pre-testing, trail race intervention and post-testing (see figure 1) . During the first phase, subjects were familiarized with the various laboratory techniques to be used and preliminary tests were performed. During the third phase, subjects had to perform a 55-km trail running race in a medium altitude mountain context. During the second and the fourth phases, muscle performance and efficiency were analyzed and blood samples were collected. All physiological parameters were recorded one day before (pre) and three days after the trail running race (post 1h, post 24 h, post 48 h, and post 72 h).

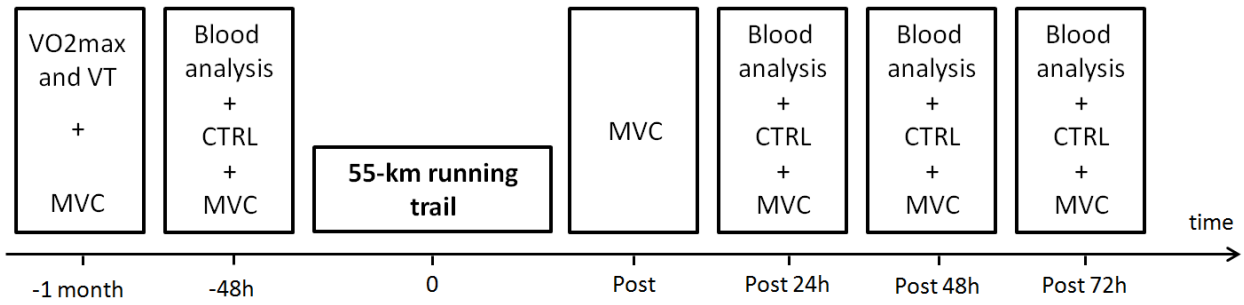

Fig. 1 A schematic representation of the experimental protocol: VO_{2max} and *VT* are the incremental cycling exercise aimed at determining maximal oxygen uptake and ventilatory threshold. *CTRL* denominates the control cycling exercise, while *MVC* represents maximal voluntary contraction and neuromuscular tests.

Preliminary session

During a preliminary session that took place one month before the experiment, 26 subjects (11 young and 15 masters) underwent an incremental cycling test at a self-selected cadence on

an electromagnetically braked ergocycle (SRM, Schoberer Rad Messtechnik, Jülich, Welldorf, Germany). In accordance with the recommendations of the ethic committee, a cycle ergometer protocol was chosen rather than a running protocol. The ergocycle allows subjects to maintain a constant power output which is independent of the selected cadence, by automatically adjusting torque to angular velocity. This test was performed in compliance with the guidelines of the French sport medicine society. The test began with a warm-up lasting 6 min at 100 W, after which the power output was increased by 30 W each minute until the subjects were exhausted. During this incremental cycling exercise, oxygen uptake (VO_2), minute ventilation (VE), and respiratory exchange ratio (RER) were continuously measured every 15 s using a telemetric system (Cosmed K4b2, Roma, Italy). The criteria used for the determination of VO_{2max} were a plateau in VO_2 despite an increase in power output, a RER above 1.1, and a heart rate (HR) above 90% of the predicted maximal HR (Howley et al. 1995). Maximal oxygen uptake (VO_{2max}) was determined as the average of the last three highest VO_2 values recorded (58.8 ± 6.5 ml/min/kg for the young vs. 55.0 ± 5.8 ml/min/kg for the masters athletes). The ventilatory threshold (VT) was determined according to the method described by Wasserman et al. (1973). The maximal aerobic power output (MAP) was the highest power output completed in 1 min (352.5 ± 41.1 W for the young vs. 347.6 ± 62.9 W for the masters athletes).

Race conditions

The running event was a 55-km trail race involving a 6000-m vertical displacement (3000-m up and 3000-m down). The starting point and finishing line were at 694-m altitude, and the highest point of the race was at 3050 m. Due to the competitive nature of the intervention, each subject was well motivated to perform maximally over the distance. From the initial group (11 young and 15 masters) only three subjects (one young and two master athletes) did not finish the course. Therefore all data presented corresponds to the finalist group (10 young and 13 master athletes). Physical activity after the race was controlled (walking activities were limited and massages were prohibited). Mean race times performed by subjects are shown in table 1.

Maximal isometric force and muscle properties

Ten minutes after the submaximal cycling exercise, the maximal voluntary isometric force of the right knee extensor (KE) muscles was determined using an isometric ergometer chair (type: J. Sctnell, Selephon, Germany) connected to a strain gauge (Type: Enertec, schlumberger, Villacoublay, France). Subjects were comfortably seated and the strain gauge was securely connected to the right ankle. The angle of the right knee was fixed at 100° (0° = knee fully extended). Extraneous movement of the upper body was limited by two harnesses enveloping the chest and the abdomen. For each testing session, the subjects were asked to perform three 2-3 s maximal isometric contractions (0 $rad \cdot s^{-1}$) of the KE muscles. The subjects were verbally

encouraged and the three trials were executed with a 1-min rest period. The best performance consecutive to the three trials was selected as the maximal isometric voluntary contraction (MVC, in Newton). In addition to MVC, the M-wave of the vastus lateralis was recorded from a twitch evoked by an electrical stimulation. Changes of neuromuscular properties were evaluated through all testing sessions (Lepers et al. 2000b; Place et al. 2004). Electrical stimulation was applied to the femoral nerve of the dominant leg according to the methodology previously described by Place et al. (2004). The following parameters of the muscular twitch were obtained: (a) peak twitch (Pt), i.e. the highest value of twitch tension production and (b) contraction time (Ct), i.e. the time from the origin of the mechanical response to Pt.

EMG recordings

During the MVC, electrical activity of the vastus lateralis (VL) muscle was monitored using bipolar surface electrodes (Blue sensor Q-OO-S, Medicotest SARL, France). The pairs of pregelled Ag/AgCl electrodes (interelectrode distance = 20 mm; area of electrode = 50 mm^2) were applied along the fibers at the height of the the muscle belly, as recommended by the SENIAM. A low skin impedance (< 5kΩ) was obtained by abrading and cleaning the area with an alcohol wipe. The impedance was subsequently measured with a multimeter (Isotech, IDM 9 N). To minimize movement artifacts, the electrodes were secured with surgical tape and cloth wrap. A ground electrode was placed on a bony site over the right anterior superior spine of the iliac crest. To ensure that the electrodes were precisely at the same place for each testing session, the electrode location was marked on the skin with an indelible marker. EMG signals were pre-amplified (Mazet Electronique Model, Electronique du Mazet, Mazet Saint-Voy, France) close to the detection site (common-mode rejection ratio = 100 dB; Z input = 10 GΩ; gain = 600; bandwidth frequency = 6-1,600 Hz). EMG data were sampled at 1000 Hz and quantified by using the root mean square (RMS). Maximal RMS EMG of VL muscle was set as the maximal 500-ms RMS value found over the 3-second MVC (i.e., 500-ms window width, 1-ms overlap) with Origin 6.1 software. During evoked stimulation performed before the MVC, peak-to-peak amplitude (PPA) and peak-to-peak duration (PPD) of the M-wave were determined for the VL muscle. Amplitude was defined as the sum of absolute values for maximum and minimum points of the biphasic (one positive and one negative deflection) M-wave. Duration was defined as the time from maximum to minimum points of the biphasic M wave.

Blood markers of muscle damages

For each evaluations series, 15 ml of blood was collected into vacutainer tubes via antecubetal venipuncture. The pre-exercise sample was preceded by a 10 minutes rest period. Once the blood sample was taken, tubes were shuffled by turning and placed on ice for 30 s before centrifugation (10 min, 3000 T/min, 4°C). The obtained plasma sample was then stored in

multiple aliquots (Ependorf type, 500 µl per samples) at -80°C until analyzed for the markers described below. All assays were performed in duplicate on first thaw. As a marker of sarcolemma disruption, muscle enzymes activity in plasma, creatine kinase (CK) and lactodeshydrogensase (LDH) were measured spectrophotometrically using commercially available reagents (Roche/Hitachi, Meylan, France).

Locomotion efficiency

Subjects were asked to perform a cycling control exercise (CTRL) at a self-selected cadence on the same ergocycle as used in the preliminary session. This cycling exercise involved 6 min at 100 W followed by 10 min at a relative power output corresponding to the ventilatory threshold. For each subject and each cycling session, metabolic data were continuously recorded to assess the efficiency in cycling.
Efficiency can be expressed as a ratio between (external) power output and the ensuing energy expenditure (EE). Efficiency may, however, be calculated in a variety of ways (Martin et al. 2005). In this study, two types of efficiency calculation were employed, gross efficiency (GE), and delta efficiency (DE). GE is defined as work rate divided by energy expenditure and calculated using the following equation (Gaesser and Brooks 1975):

$$\text{Gross efficiency (\%)} = \frac{Work\ rate\ (WR, in\ joules)}{Energy\ expenditure\ (EE, in\ joules)} \times 100$$

DE is considered by many to be the most valid estimate of muscular efficiency (Gaesser and Brooks 1975; Coyle et al. 1992). DE calculations are based upon a series of work rates which are then subjected to linear regression analysis.

$$\text{Delta efficiency (\%)} = \frac{\Delta\ Work\ rate\ (WR, in\ joules)}{\Delta\ Energy\ expenditure\ (EE, in\ joules)} \times 100$$

In order to obtain precise values for work rate utilized in the efficiency calculations, power output was assessed from the set work rate and the true cadence as monitored by the SRM crank system. EE was obtained from the rate of oxygen uptake, using the equations developed by Brouwer (1957). These equations take the substrate utilization into account, by calculating the energetic value of oxygen based on the RER value. To minimize a potential influence of the VO_2 slow component, which might vary between subject groups, the mean EE during the 3^{th} to 6^{th} minute was used in the calculations of GE and DE.

Statistical analysis

All data presented are means ± SD (tables and figures). Each dependent variable was then compared between the different testing conditions using a two-way ANOVA with repeated measures (period vs group). Newman-Keuls post-hoc tests were applied to determine the between-means differences, if the analysis of variance revealed a significant main effect for

period or interaction of group x period. For all statistical analysis, a P < 0.05 value was accepted as the level of significance.

Results

Muscular performance

In all evaluations, MVC values of master athletes were significantly lower than young's values (-1.8 ± 4.6%, see Figure 2). One hour after the intervention (post), maximal isometric strength values of knee extensors decreased significantly when compared with pre-race values, in non significantly different proportions for young (-32%) and master athletes (-40%). MVC values for young subjects returned to baseline at Post 24h, at which time the MVC reduction in masters remained significant (-13.6%). A significant decrease in EMG activity (RMS) during MVC of the vastus lateralis (VL) muscle was observed at 1h and 24h post-exercise without any differences between groups or periods. Compared with pre-race values, post-exercise MVC RMS values decreased in young by -40.2 ± 19% and in masters by -42 ± 19.2%.

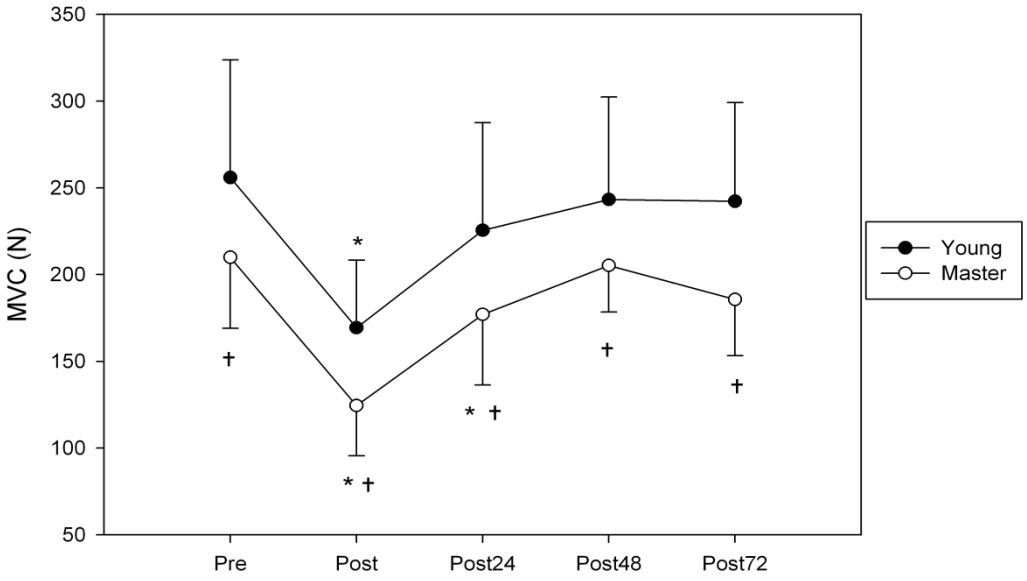

Fig. 2 Changes in knee extensors' maximal isometric strength for young and master athletes before (Pre) and 1h (Post), 24h (Post 24), 48h (Post 48) and 72h (Post 72) after the intervention. *Significantly different from pre-exercise ($P < 0.05$); †significantly different from young ($P < 0.05$).

Muscular twitch and M-wave properties

Before the race, no significant effect of age was observed on peak twitch torque (Pt) or contraction time (Ct). One hour after the race, no effect was recorded on Ct or Pt whatever the groups. Post 24h, a slower contraction time (Ct) and a lower peak twitch torque (Pt) were

recorded in both groups. Compared to pre-race values, Pt decreased by 18.2% in young and by 23.5% in masters runners at post 24. These alterations in twitch properties returned close to pre-test values in young subjects, but remained significant 48h and 72h in masters subjects (table 2).

Before the race no significant effect of age was observed on PPA or PPD values of the M-wave for VL muscle (table 2). One hour after the race a significant increase in PPD was observed in both group and this increase remains significant post 24h but only in masters athletes. Furthermore, in masters, PPA values decreased below pre-race values, 48h and 72h after the race and no effects were observed in young subjects.

Table 2 Twitch and M-wave parameters of the vastus lateralis muscle before (Pre) , 1h (Post), 24h (Post24), 48h (Post 48) and 72h (Post72) after the race.

	Variable (Units)		Pre	Post	Post 24	Post 48	Post 72
Twitch	Pt (N)	Young	36 (9)	35 (11)	29 (11)*	34 (9) †	35 (12) †
		Master	36 (11)	34 (12)	27 (12)*	28 (08)*	29 (12)*
	Ct (ms)	Young	63.3 (13.7)	63.4 (10.6)	68.8 (11.2)*	64.7 (9.5) †	66.9 (10.3) †
		Master	61.3 (15.6)	64.9 (17.4)	71.1 (12.9)*	73.2 (10.8)*	76.2 (12.7)*
M-Wave	PPA (mV)	Young	3.5 (1.4)	3.6 (1.6)	3.9 (1.5)	3.4 (1.7)	3.0 (1.4)
		Master	3.4 (1.5)	3.1 (1.3)	3.1 (1.5)	2.4 (1.4)*	2.3 (0.7)*
	PPD (ms)	Young	7.6 (1.5)	9.2 (1.2)*	7.0 (2.2)†	7.0 (2.5)	7.3 (2.8)
		Master	7.9 (1.5)	9.5 (2.5)*	9.3 (2.8)*	7.8 (2.7)	7.6 (3.3)

Mean (SD) values of 10 young and 13 master athletes are shown.
*Significantly different from pre-exercise ($P < 0.05$); †significantly different from master ($P < 0.05$);
Pt - peak twitch. *Ct* - contraction time. *HRt* - half-relaxation time. *PPA* - peak-to-peak amplitude. *PPD* - peak-to-peak duration

Blood markers of muscle damages

Twenty four hours (Post 24h) after the race, the plasma activities of CK and LDH increased significantly in comparison to pre-race values, with a greater increase for master subjects. CK and LDH values remained significantly elevated at post 48h and post 72h, without any difference between groups (Table 3).

Table 3. Changes in muscle damage indicating blood markers for young and master athletes before (Pre), 24h (Post 24h), 48h (Post 48h) and 72h (Post 72h) after the race.

Variable (Units)	Group	Normal range	Pre	Post 24h	Post 48h	Post 72h
CK (U/l)	Young	50-230	135 (26)	1470 (565)*†	909 (303)*	430 (251)*
	Master	50-230	138 (107)	1559(593)*	920 (298)*	531 (271)*
LDH (U/l)	Young	120-245	229 (52)	528 (164)*	453 (65)*	410 (65)*
	Master	120-245	194 (63)	482 (142)*	468 (105)*	473 (165)*

*Significantly different from pre-exercise ($P < 0.05$); †significantly different from masters ($P < 0.05$). *CK* - creatine kinase. *LDH* - lactate dehydrogenase

Locomotion efficiency and cycling cadence

Gross efficiency (GE), delta efficiency (DE) and cadence values are presented table 4. No significant difference in GE, DE or cadence was observed between groups before the race. After the race, results indicated a non-group specific, significant decline in GE from post 24h to post 72h (GE mean decrease in young *vs.* masters in % of pre-race values: -4.7% vs. -6.3%, respectively). In both groups VE increased post 24h, post 48h and post 72h in comparison to pre-test values (VE mean increase in young *vs.* masters in % of pre-race values: + 11.7 % vs. + 10.1%, respectively). No significant change in DE was observed in young subjects after the race. Par contrary, a significant decrease in DE was recorded in master subjects (DE decrease in masters athletes at post 24h, post 48h and post 78h in % of pre-race values: -10.6% ; -10.4% ; -1.5%, respectively).

Post-race cadence was significantly higher in all post-race evaluations for young subjects when compared with masters. Results indicate a significant increase in cycling cadence post 24h (+ 4.4%), post 48h (+ 10.6%), and post 72h (+ 17%) for young, and only in post 48h (+ 3.9%) and post 72h (+ 10.8%) for master athletes.

Table 4. Changes in efficiency, ventilation and cycling cadence for young and masters during cycling exercises performed before (Pre), 24h (Post 24), 48h (Post 48) and 72h (Post 72) after the race.

Variable (Units)		Pre	Post 24	Post 48	Post 72
GE (bpm)	Young	19.6 (2.4)	18.8 (1.5)*	18.2 (2.9) *	19 (2.1)*
	Master	19.9 (1.3)	19.1 (1.5)*	18 (1.9)*	18.8 (1.7)*
DE ($l.min^{-1}$)	Young	23.1(5)	22.3 (2.6)†	23.4(3.6)†	23.4(5.8)†
	Master	22.5(2.6)	20.1 (2.4)*	19.9 (3.7)*	20.6 (3.2)*
VE ($l.min^{-1}$)	Young	74 (9) †	81 (12)* †	85 (13)* †	82 (15)* †
	Master	83 (11)	90(11)*	94 (14)*	90 (14)*
Cad (rpm)	Young	83 (8)	86 (12)* †	92 (15)* †	97 (17)* †
	Master	82 (9)	80 (8)	85 (10)*	90 (8)*

Mean (SD) values of 10 young and 13 master athletes are shown.
*Significantly different from pre-exercise ($P < 0.05$); †significantly different from masters ($P < 0.05$)
GE - gross efficiency. *DE* - delta efficiency *VE* - minute ventilation. *Cad* - pedaling cadence

Discussion

The objective of the present study was to investigate changes in muscular performance and locomotion efficiency in well-trained endurance runners engaged in a trail running competition. The participation of two age groups of runners (young vs. masters) allowed us to additionally study the effect of aging on the previously enumerated physiological parameters. The main results of our study indicate that: (1) post-run muscular performance and locomotion efficiency decline while the associated concentrations of muscle damage indicating blood markers rise, regardless of age, and (2) there are significant differences between age groups in both muscular performance and locomotion efficiency in both pre-race and post-race conditions. Results indicate no differences between groups in blood marker concentrations.

The running event analyzed in this study was a 55-km trail race featuring a 6000-m vertical displacement (3000-m up and 3000-m down). The average race time was 06:45 ± 00:45. As stated, the main performance components of trail running are exercise duration and vertical displacement (uphill and downhill). From this perspective, trail running competitions induce an intensive physical work load on the organism. Considering the popularity of trail running and the abundance of competitions over the world, it appears important to precisely characterize the acute physiological reactions consecutive to such events. One of the most significant consequences of the race was a reduction in muscular performance. The recorded data manifests a significant decline in maximal force generating capacities in young (-32%) and

master athletes (-40%) one hour post-race. This MVC decline is in accordance with data previously reported in the literature (Gauche et al. 2006; Millet et al. 2003, 2009). The intervention seems to have decreased MVC in a slightly greater magnitude than races on level courses (Millet et al. 2009; Millet et al. 2003; Place et al. 2004), whereas one would have to adapt for workload for a precise comparison. It is generally accepted, that the eccentric muscle contractions occurring in running generate structural muscle damage leading to MVC loss (Millet et al. 2002, 2003; Overgaard et al. 2002; Place et al. 2004). After the race (post 24 to 72h), MVC values progressively returned to their pre-race level. In addition, results indicate a significant decrease in VL muscle activity (i.e. RMS values) recorded during MVC performed one hour after the race, and persisting until 72h after the race. Further parameters used to characterize muscular fatigue included muscular twitch and M-wave properties. Pt decreased significantly 24h after the race, accompanied by a concomitant increase in Ct from 24 to 72h after the race, albeit only in masters. The main explanation for these perturbations of contractile parameters could be an alteration of the excitation-contraction coupling process that can be attributed to several mechanisms including, but not limited to, reduced Ca^{2+} release from the sarcoplasmic reticulum (Westerblad et al. 1991), a decrease in blood pH and a reduced rate or force of crossbridge latching (Metzger et Moss 1990). An increase in Ct after the race could also indicate an impairment in type II muscles fibers (i.e. fast contraction fibers) which may be compensated for by the more fatigue resistant type I muscle fibers (i.e. slow contraction fibers). Twitch muscle properties were unchanged at 1 hour post-race, alterations appearing only 24h after the race and later. This phenomenon might suggest that muscle fatigue was counterbalanced by potentiation mechanisms occurring immediately after the race (Baudry et al. 2005; Shima et al. 2007; Bieuzen et al. 2009). By contrary, PPD was significantly reduced immediately after the race (post) and tended to return to basal values 24 to 72h after the race. The master group exhibited increased PPA at 24h post-race. As previously described in the literature, these increases in M-wave parameters suggest an alteration in muscle excitability; probably generated by impairments in neuromuscular propagation due to an increase in sarcolemma permeability to sodium, potassium and chloride (Lepers, 2009). These results support the assumption of muscle damage development through trail running. The data recorded for muscle damage indicating blood markers underscores this observation. A post-race increase in the plasma activity of muscle enzymes (CK and LDH), which persisted for several days after the race (table 3), was recorded. Similarly, Suzuki and al. (2006) reported a significant increase in CK and LDH activities in the plasma soon after an Ironman triathlon, which remained elevated until one day after the race. Intracellular enzymes such CK and LDH indicate muscle injury arising from myofibrillar disruption (Clarkson et al. 1992; Noakes 1987), and are classically used to assess the loss of sarcolemmal integrity after strenuous exercises (Overgaard et al. 2004). As neither CK or LDH are considered redundant indicators (Warren et al. 1999) the analysis was augmented by the acquisition of further physiological variables.

As an important determinant of performance in endurance events, locomotion efficiency is classically surveyed in athletes in order to evaluate the effects of particular training periods (Santalla et al. 2009). It has been reported, that even small increments in cycling efficiency may lead to major improvements in endurance (Moseley and Jeukendrup 2001). The efficiency of physical work is a measure of the body's effectiveness in converting chemical energy into mechanical energy. Efficiency was here calculated as described in the methods section; the quotient of work rate and energy expenditure (Gaesser and Brooks 1975). A decrease in locomotion efficiency can therefore be interpreted as either a relative increase in energy expenditure, or a relative decrease in work rate. Considering that work rate was kept constant in our study, increased energy expenditure remains the only viable option. Recorded values show a decline in GE in both groups of athletes after the race, which persisted until 72h post-race. Although commonly employed, GE has been criticized for its inclusion of energy-delivery processes that do not contribute to production of mechanical work in the denominator. Therefore, in this study, locomotion efficiency was also evaluated through DE calculation, which is considered to be the most valid estimate of muscular efficiency (Gaesser et Brooks 1975; Coyle et al. 1992; Mogensen et al. 2006). Interestingly, only DE values determined for master athletes at 24, 48 and 72h after the race declined, confirming the increase in energy expenditure to ensure a continuous power output. This phenomenon is largely related to a decline in muscular performance. In order to produce the same locomotive work as in the pre-race condition, strategies such as an increase in spatio-temporal recruitment of muscle fibers or an increase in cycling cadence, involving a concomitant increase in VE (table 4) could be engaged. The attained results provide evidence of an alteration of cycling efficiency in both groups tested, whereas the masters group suffered a greater decline.

The second aim of this study was to analyze age-related effects on muscular performance and cycling efficiency after the running trail race by comparing physiological variables recorded in young and master athletes. Race completion time did not significantly differ between groups (06:42 ± 00:51 vs. 06:51 ± 00:47, for young vs. masters respectively). Despite the structural and functional alterations typically observed during the aging process, master athletes were able to produce the same level of performance as the young group. This observation confirms the realistic possibility of preventing the age-related decline of physical performance through physical activity.

The analysis of muscular performance in the two groups of athletes shows a classical decline in maximal force generating capacity in masters (-21,8 ± 4,6%), when compared with young for all testing sessions performed before and after the race (Louis et al. 2009; Bieuzen et al. 2009). Results additionally indicate a similar decrease in MVC values at one hour post-race in both age groups which, in the master subjects only, persisted until 24h after the race, suggesting a slower recovery. Based on the results of Coggan et al. (1990), which could be confirmed by

Tarpenning et al. in 2004, the global decrease of MVC values in master athletes similar to our experimental population can be mainly explained by neural factors, such as muscle recruitment and/or specific tension. The twitch analysis based assessment of muscular function seems to confirm this hypothesis. This study is the first to present twitch and m-wave data for master athletes after a trail running competition. As previously described in studies on long-distance exercise induced fatigue in young subjects (e.g. Millet et al. 2002), Pt and Ct parameters increased 24 h after the race. The proportions were similar in both groups tested. The increase in the mechanical response to twitch persisted several days after the race in masters only, suggesting greater muscle damage in this group. Concurrently master PPD values increased proportionally to the development in the young group at 1h post-race, and returned to pre-race values in all the following testing conditions. By contrary, master PPA values decreased significantly from 48 to 72h after the race, while this decline was marginal in young. These results indicate that the aging process inevitably accentuated the decline in post-race maximal force generating capacity. Despite a similar training status in young and master athletes, the values of these parameters show a greater alteration in muscular function (i.e. contractivity and excitability) after the race, involving a slower recovery of muscle strength. However, an assessment of VL muscle activity supports the speculation that muscle activation was not impaired by aging, as MVC RMS values declined in similar proportions between groups after the race.

As depicted in table 3, CK and LDH activity in plasma increased in similar proportions after the race, indicating a similar level of muscular deterioration between groups following the trail competition. Surprisingly, despite an age-related decline in muscle strength, the competition induced reduction of MVC was similar between groups. This might support the idea that regular endurance training reinforces active muscles, and therefore limits the structural and functional changes classically associated with aging (Lexell 1995).

Results of this study show an effect of aging on cycling efficiency before and after the running race. While GE declined in similar proportions in both groups after the race, DE declined only in masters 24, 48 and 72h after the race (table 4). The GE decline in both groups could be mainly related to increases in energy-delivery processes that do not contribute to mechanical work. Variations in these processes originate through modifications in cycling kinematics (e.g. cycling cadence) or muscular contraction patterns (e.g. recruitment of subsidiary muscles, increase in antagonistic co-activation) in fatigued muscles and must be considered when regarding the GE (Braun and Dutto 2003). The decline of DE in masters could be strongly related to alterations in muscular performance, provoking an increase in muscle activity in cycling to produce the same external work. Gleeson et al. (1998) suggested that an increase in type II fiber recruitment may occur when exercise is performed in a fatigued state. In addition, if force-generating capacity was compromised, more motor units would have to be activated to achieve the same sub-

maximal force output, resulting in a concomitant increase in metabolic cost (Braun and Dutto 2003). Such an effect could contribute to the significantly higher VE shown in the present study. The results demonstrate, that master athletes reached a higher level of fatigue through the race, when compared to young athletes. However, the increase in energy-delivery processes through aging could be considered as a natural adaptation in master athletes in order to maintain performance.

Conclusion

The aim of this study was to assess physiological responses to an exhaustive trail running competition and to analyze possible differences between young and master athletes. A 55 km ultra-endurance event was used as a fatigue-generating intervention. An especially large amount of muscular fatigue was generated through the large proportion of eccentric contractions occurring during the downhill sections of the race. Results indicate an acute fatigue in all subjects (young and masters), which is mainly represented by decreases in muscle performance. Despite similar race performances between groups, the extent of decline in strength production and locomotion efficiency after the race was greater in master than in young athletes, suggesting a greater fatigue-resistance in the young subjects. The results attained in this study give indication that regular endurance training cannot halt the age related decline in muscle performance, but can help maintain performance level by generating adaptive physiological mechanisms and strategies.

References

Baudry S, Klass M, Duchateau J (2005) Postactivation potentiation influences differently the nonlinear summation of contractions in young and elderly adults. J Appl Physiol 98: 1243-1250.

Bieuzen F, Hausswirth C, Louis J, Brisswalter J (2009) Age-related changes in neuromuscular function and performance following a high-intensity intermittent task in endurance-trained men. Gerontology 414.

Braun WA, Dutto DJ (2003) The effects of a single bout of downhill running and ensuing delayed onset of muscle soreness on running economy performed 48 h later. Eur J Appl Physiol 90:29–34.

Brisswalter J, Hausswirth C, Vercruyssen F, Collardeau M, Vallier JM, Lepers R, Goubault C (2000) Carbohydrate ingestion does not influence the charge in energy cost during a 2-h run in well-trained triathletes. Eur J Appl Physiol 81(1-2):108-113.

Brouwer E (1957) One simple formulate for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals from gaseous exchange (oxygen intake and caloric acid output) and urine-N. Acta Physiol Pharmacol Neerl 6:795-802.

Clarkson PM, Nosaka K, Braun B (1992) Muscle function after exercise-induced muscle damage and rapid adaptation. Med Sci Sports Exerc 24: 512-520.

Coggan AR, Spina RJ, Rogers MA, King DS, Brown M, Nemeth PM, Holloszy JO (1990) Histochemical and enzymatic characteristics of skeletal muscle in master athletes. J Appl Physiol 68: 1896-1901.

Coyle EF, Sidossis LS, Horowitz JF, Beltz JD (1992) Cycling efficiency is related to the percentage of type 1 muscle fibers. Med Sci Sports Exerc 24(7):782-788.

Fernström N, Bakkman L, Tonkonogi M, Shabalina IG, Rozhdestvenskaya Z, Mattsson CM, Enqvist JK, Ekblom B, Sahlin K (2007) Reduced efficiency, but increased fat oxidation, in mitochondria from human skeletal muscle after 24-h ultraendurance exercise. J Appl Physiol 102(5):1844-1849.

Gaesser GA, Brooks GA (1975) Muscular efficiency during steady-rate exercise: effects of speed and work rate. J Appl Physiol 38(6):1137-1139.

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 81:1725-1789.

Gauché E, Lepers R, Rabita G, Leveque J-M, Bishop D, Brisswalter J, Hausswirth C (2006) Vitamin and mineral supplementation and neuromuscular recovery after a running race. Med Sci Sports Exerc 38(12):2110-2117.

Henwood TR, Taaffe DR (2006) Short-term resistance training and the older adult: the effect of varied programmes for the enhancement of muscle strength and functional performance. Clin Physiol Funct Imaging 26:305-313.

Hoffman MD, Wegelin JA (2009) The Western States 100-Mile Endurance Run: participation and performance trends. Med Sci Sports Exerc 41: 2191-2198.

Howley ET, Bassett DR, Welch HG (1995) Criteria for maximal oxygen uptake: review and commentary. Med Sci Sports Exerc 27:1292-1301.

Lepers R, Sultana F, Bernard T, Hausswirth C, Brisswalter J (in press) Age related changes in triathlon performances. Int J Sport Med.

Lepers R, Pousson M, Maffiuletti N, Martin A, Van Hoecke J (2000a) The effects of a prolonged running exercise upon strength characteristics. Int J Sports Med 21: 275-280.

Lepers R, Hausswirth C, Maffiuletti N, Brisswalter J, Van Hoecke J (2000b) Evidence of neuromuscular fatigue after prolonged cycling exercise. Med Sci Sports Exerc 32(11):1880–1886.

Lepers R (2008) Analysis of Hawaii ironman performances in elite triathletes from 1981 to 2007. Med Sci Sports Exerc 40(10):1828-1834.

Lepers R. (2009) Muscle fatigue following prolonged dynamic exercise. Advances in Neuromuscular Physiology of Motor Skills and Muscle Fatigue Minoru Shinohara (editor), pp: 369-390.

Lexell J (1995) Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci 50 Spec No: 11-16.

Louis J, Hausswirth C, Bieuzen F, Brisswalter J (2009) Muscle strength and metabolism in master athletes. Int J Sports Med 30(10):754-759.

Metzger JM, Moss RL (1990) Effects of tension and stiffness due to reduced pH in mammalian fast- and slow-twitch skinned skeletal muscle fibres. J Physiol 428: 737-750.

Miles MP, Walker EE, Conant SB, Hogan SP, Kidd JR (2006) Carbohydrate influences plasma interleukin-6
but not C-reactive protein or creatine kinase following a 32-km mountain trail race. Int J Sport Nutr Exerc Metab 16 :36-46.

Millet G, Lepers R, Lattier G, Martin V, Babault N, Maffiuletti N (2000) Influence of ultra-long term fatigue on the oxygen cost of two types of locomotion. Eur J Appl Physiol 83: 376-380.

Millet GY, Lepers R, Maffiuletti NA, Babault N, Martin V, Lattier G (2002) Alterations of neuromuscular function after an ultramarathon J Appl Physiol 92:486-492.

Millet GY, Martin V, Lattier G, Ballay Y (2003) Mechanisms contributing to knee extensor strength loss after prolonged running exercise. J Appl Physiol 94:193–198.

Millet GY, Morin JB, Degache F, Edouard P, Feasson L, Verney J, Oullion R (2009) Running from Paris to Beijing: biomechanical and physiological consequences. Eur J Appl Physiol.

Mogensen M, Bagger M, Pedersen PK, Fernstrom M, Sahlin K (2006) Cycling efficiency in humans is related to low UCP3 content and to type I fibres but not to mitochondrial efficiency. J Physiol 571: 669-681.

Moseley L, Jeukendrup AE (2001) The reliability of cycling efficiency. Med Sci Sports Exerc 33: 621-627.

Moysi JS, Garcia-Romero JC, Alvero-Cruz JR, Vicente-Rodriguez G, Ara I, Dorado C, Calbet JAL (2005) Effects of eccentric exercise on cycling efficiency. Can J Appl Physiol 30(3):259-275.

Nieman DC, Henson DA, McAnulty SR, McAnulty L, JD M, Ahmed A, Heward C (2004) Vitamin E and immunity after the Kona triathlon world championship. Med Sci Sports Exerc 36:1328–1335.

Noakes TD (1987) Effect of exercise on serum enzyme activities in humans. Sports Med 4: 245-267.

Overgaard K, Fredsted A, Hyldal A, Ingemann-Hansen T, Gissel H, Clausen T (2004) Effects of running distance and training on Ca2+ content and damage in human muscle. Med Sci Sports Exerc 36:821–829.

Place N, Lepers R, Deley G, Millet GY (2004) Time Course of Neuromuscular Alterations during a Prolonged Running Exercise. Med Sci Sports Exerc 36(8):1347–1356.

Santalla A, Naranjo J, Terrados N (2009) Muscle efficiency improves over time in world-class cyclists. Med Sci Sports Exerc 41: 1096-1101.

Sharwood KA, Collins M, Goedecke JH, Wilson G, Noakes TD (2004) Weight changes, medical complications, and performance during an Ironman triathlon. Br J Sports Med 38:718-724.

Shima N, McNeil CJ, Rice CL (2007) Mechanomyographic and electromyographic responses to stimulated and voluntary contractions in the dorsiflexors of young and old men. Muscle Nerve 35: 371-378.

Stearns RL, Casa DJ, Lopez RM, McDermott BP, Gunio MS, Decher NR, Seruggs IC, West AE, Armstrong LE, Maresh CM (2009) Influence of Hydration Status on Pacing During Trail Running in the Heat. J Strength Cond Res 23: 2533-2541.

Sultana F, Brisswalter J, Lepers R, Hausswirth C, Bernard T (2008) Effects of age and gender on Olympic triathlon performances. Science & Sports 23: 130–135.

Suzuki K, Peake J, Nosaka K, Okutsu M, Abbiss CR, Surriano R, Bishop D, Quod MJ, Lee H, Martin DT, Laursen PB (2006) Changes in markers of muscle damage, inflammation and HSP70 after an Ironman triathlon race. Eur J Appl Physiol 98: 525-534.

Tanaka H, Seals DR (2008) Endurance exercise performance in masters athletes: age-associated changes and underlying physiological mechanisms. J Physiol 586(1):56-63.

Tarpenning KM, Hamilton-Wessler M, Wiswell RA, Hawkins SA (2004) Endurance training delays age of decline in leg strength and muscle morphology. Med Sci Sports Exerc 36: 74-78.

Thompson LV (2009) Age-related muscle dysfunction. Exp Gerontol 44: 106-111.

Warren GL, Lowe DA, Armstrong RB (1999) Measurement tools used in the study of eccentric contraction-induced injury. Sports Med 27(1): 43-59.

Wasserman K, Whipp BJ, Koyl SN, Beaver WL (1973) Anaerobic threshold and respiratory gas exchange during exercise. J Appl Physiol 35:236-243.

Westerblad H, Lee JA, Lannergren J, Allen DG (1991) Cellular mechanisms of fatigue in skeletal muscle. Am J Physiol 261: C195-209.

Chapter 5
Reproducibility study

5.1. INTRODUCTION

After making informed choices on cohort and running distance parameters, a model was developed to test future interventions. A new group of trail runners was recruited locally to quantify and validate the reproducibility of performance, fatigue and muscle damage parameters after three circuits of a 5 km loop course that was marked in the mountains near to the laboratory. An outdoor course was chosen in order to avoid the possible caveats of simulation and to make the intervention easily adoptable and directly applicable to the study matter. Subjects completed the course 4 times in 4 weeks (7 days between runs) and parameters were assessed before and for 3 days after each repetition. Results indicate that reproducibility parameters are not compromised significantly when compared to laboratory-based reproducibility studies. The first repetition of the course resulted in significantly different values in a number of variables, indicating that at least one familiarisation bout is of paramount importance. For the following three repetitions, performance, MVC and CMJ demonstrated high to very high reproducibility. There was however significant variation in CK and lactate analysis, making these parameters unsuitable for investigating small effect sizes. The study was recently accepted for publication in the *Journal of Science and Medicine in Sport* and is currently in press.

5.2. REPRODUCIBILITY OF PERFORMANCE AND FATIGUE IN TRAIL RUNNING

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Reproducibility of performance and fatigue in trail running

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Abstract

Objective. This study aimed to test the reproducibility of running performance, neuromuscular fatigue markers and indirect muscle damage indicators in a field-based trail time-trial. **Design.** Running performance and changes in classical physiological parameters were analysed in 11 experienced trail runners before and in the days following four bouts of outdoor trail running (15.6 km), 7 days apart. **Methods.** Heart rate, running time and lactate concentration were monitored in each running bout. Maximal voluntary contraction torque (MVC), counter movement jump height (CMJ), plasma creatine kinase (CK) activity and muscle soreness were assessed before and 1, 24 and 48 hours post-race. Within-bout changes were elucidated using a two-way repeated measures ANOVA. Inter-repetition reproducibility was examined using an intraclass correlation coefficient (ICC, R) and the mean intra-subject coefficient of variation (CV) at each measurement time point. **Results.** Running time was longer ($p<0.05$) for the first bout compared with the other three bouts. Magnitude and time course of changes in CMJ, CK activity and muscle soreness were similar among all four bouts (overall peak means: -17%, +35% and 54/100 mm respectively). The acute reduction in MVC (peak mean: -17%) was attenuated exclusively in the fourth bout ($p<0.05$). The two middle bouts showed good reproducibility (ICC and CV) for running time, MVC and CMJ, but low to moderate for CK activity, muscle soreness, blood lactate and rate of perceived exertion. **Conclusions.** A short outdoor trail run is a reliable model for investigations of fatigue and muscle damage, but certain methodological precautions should be respected.

Keywords: *Physical exertion; Aerobic exercise; Reliability and Validity; Field study; Eccentric exercise*

Introduction

Trail races are off-road endurance runs covering distances from 15 to 75 km (>90 km for ultra trails) on unsurfaced mountain trails with extensive vertical displacement[1]. Distance and the climb·distance^{-1} ratio (E/D, normal range: 40 to 65 m·km^{-1}; 8–13%) are the main performance parameters[1,2]. Recent studies investigating trail races reported aspects of neuromuscular fatigue mainly assessed by maximal voluntary contraction (MVC) torque and changes in twitch and activation parameters[1–3]. For example, MVC torque of the knee extensors has been reported to decrease 23.5% after a 30 km trail run[4], 32% after a 55 km trail run[1] and 35% after a 166 km mountain ultra-marathon[2]. The neuromuscular fatigue is often accompanied by increases in self-reported muscle soreness ratings and plasma bulk damage markers, such as creatine kinase (CK)[1,2,5], lasting for several days. This is associated with an exacerbated eccentric component invoked in the downhill phases. The physiological stress profile elicited through combined fatigue and muscle damage is specific to trail running.

Participant increases[6] invite the investigation of and development of strategies to minimise neuromuscular fatigue and structural damage to the muscle. However, evaluating trail-specific interventions is challenging, as trail race simulation in a laboratory is difficult due to terrain and grade variability. It may therefore be more effective to assess strategies and modalities that could affect performance and recovery in a field setting. Under this constraint, two factors might affect reproducibility of parameters examined in field trail runs.

Firstly, studies conducted in the field are associated with higher variability induced, for example, through environmental factors (temperature, wind, humidity and surface conditions). While the test-retest reproducibility of treadmill-based protocols has been frequently evaluated[7,8], no previous study has investigated the reproducibility of variables associated with running performance, neuromuscular fatigue and muscle damage in a field-based trail run.

Secondly, it is well known that an initial bout of eccentric exercise induces a protective effect which decreases muscle damage and ameliorates recovery in subsequent bouts. This protective effect is referred to as “the repeated bout effect” and is generally observed from 2 to 6 weeks following the initial intervention in untrained muscles[9,10]. There have been several reports of diminished effect magnitude in trained muscle[11,12] yet, to the best of our knowledge, no previous study has investigated the repeated bout effect in a trail running model, especially performed by trained runners.

Classical fatigue-induction models are not suited to examining trail running as they do not take into account the rather severe gradients and variable surface encountered on typical courses. Prior trail investigations employed either treadmill simulation[13,14], which disregards the terrain component completely, or competition analysis[1,2,4], which is unsuited to intervention type investigations and involves a complicated measurement set-up. Therefore this study employed

a short (<20km) short distance trail with a medium E/D[1, 2] of 52.88. This model is straightforward to implement and has the additional advantage that it reflects a typical training distance for recreational trail runners and entails a short recuperation time.
The aim of this study was therefore to examine the feasibility of using an outdoor trail run to evaluate future intervention strategies. To this end, the reproducibility of neuromuscular fatigue and structural muscle damage markers over 4 bouts of a 15.6 km trail run was determined in experienced trail runners.

Methods

Eleven actively competitive male trail runners (age: 34.7 ± 9.8 years, body mass: 72.3 ± 6.8 kg, height: 178.4 ± 7.0 cm, maximal oxygen uptake: 60.1± 6.5 $mL \cdot min^{-1} \cdot kg^{-1}$) participated in this study. Inclusion criteria included a minimum of 2 years trail racing experience and a training volume of 40–100 $km \cdot wk^{-1}$ (mean: 60 ± 20 $km \cdot wk^{-1}$) in the 3 months preceding initial testing. For 2 days before and after each trial, the runners were requested to refrain from exercise and to adhere to a standardised nutritional routine. Written informed consent was obtained and the study was approved by the Institutional Human Research Ethics Committee.
After an initial maximal oxygen uptake ($\dot{V}O_2max$) test on a treadmill, all participants performed four bouts of trail running on the same course with 7 days rest between bouts. In each bout, running time, heart rate, post-run ratings of perceived exertion and blood lactate concentration were recorded. Immediately before (pre) and 1(post), 24 and 48 hours after the run the following parameters were assessed: maximal voluntary isometric knee extension (MVC) torque, counter movement jump (CMJ) height, plasma creatine kinase (CK) activity and muscle soreness. These variables were examined over time in each bout and each time point was compared between bouts.
Two weeks before the first bout, all participants completed a maximal incremental running protocol on a treadmill (+4%, Gymrol S2500, HEF Tecmachine, Andrezieux-Boutheon, France) in the lab while heart rate (RS800, Polar, Kemple, Finland) and pulmonary gas exchange (Oxycon Alpha, Jaeger, The Netherlands) were recorded. All instruments were calibrated before each test as described by the manufacturers. The protocol consisted of a 6 minute warm-up at 9 $km \cdot h^{-1}$ followed by an increase of 1 $km \cdot h^{-1}$ every two minutes until volitional exhaustion. Maximal heart rate (HRmax) and oxygen uptake ($\dot{V}O_2max$) were determined as the highest 30 s mean, fulfilling the classical criteria of a respiratory equivalent greater than 1.1, a HR greater than 90% of the age prediction and a plateau in $\dot{V}O_2$ despite an increase in mechanical intensity[15].
The trail time-trial consisted of 3 laps of a 5.2 km course (total distance: 15.6 km) starting close to sea level. Each lap was composed of a climbing segment (2200 m, 13%, 275 m climb) followed by a downhill segment (3000 m, -9%, 275 m descent). The course was exclusively on mountain single tracks with repeated technical sections on rocky and root-covered paths. Each

participant was weighed and equipped with a Polar RS800 heart rate monitor, 680 ml of fluid containing carbohydrates (74 $g \cdot L^{-1}$) and 2 energy gels (carbohydrates: 18 $g \cdot gel^{-1}$). All participants were asked to wear similar clothes for each bout and to aim for the best completion time possible. Starting times were staggered, allowing 20 minutes between participants.
Immediately after the run, a blood sample was taken from the ear lobe for lactate analysis (Lactate Pro, Arkray, Amstelveen, The Netherlands), participants were weighed, and RPE was verbally queried while standing using standard terminology and a 6–20 point Borg Scale[16].
MVC testing took place in the laboratory about 10 minutes drive from the time-trial course before and 1, 24 and 48 hours after the run. Following the motorised transfer, participants were securely strapped into an isokinetic dynamometer (Biodex System 3, Shirley, New York, USA) with the knee joint angle of the right leg at 90° (full leg extension = 0°). The axis of the knee joint was carefully aligned with the rotational axis of the dynamometer and all settings were kept constant throughout the experiment. Before each MVC, participants warmed up on the isokinetic dynamometer by repeating 10 one-second isometric contractions at 50% MVC (one second rest between contractions). After 3 minutes rest, in which participants were asked to indicate perceived muscle pain of the knee extensors on a 10 cm visual analogue scale visibly anchoring zero for 'no pain' and 10 for 'maximal pain', testing commenced. Participants were instructed to "extend the knee as hard and fast as possible" for the three 5-second MVC measures (55 s rest between attempts) while standardised verbal encouragement was given. The highest MVC value achieved in the three attempts was used.
Ten minutes after MVC testing, participants were positioned on an Ergo Jump system (Boscosystem, S. Rufina, Italy) and instructed to place their hands on their hips and to jump as high as possible and land with extended legs. Jumping position was standardised as described previously[17], and the participants practised extensively under supervision before the measurements. Three jumps with 30 seconds rest between attempts were then recorded. The maximum jump height achieved was used for further analysis.
Blood samples were drawn from the antecubital vein using a standard vacutainer system and centrifuged for 10 minutes to obtain plasma. Plasma samples were aliquoted and stored in a freezer (-80°C) until analysed for CK activity by a Roche Hitachi 911 chemistry analyser (Roche Diagnostics Corporation, Indianapolis, IN, USA).
A two-way repeated measures ANOVA (TIME (4) x BOUT (4)) was conducted on the absolute values of MVC torque, CMJ height, plasma CK activity, muscle soreness and lap times. A Newman-Keuls post-hoc test was used for multiple comparisons to identify differences between individual time points. Reproducibility of parameters across bouts was examined with an intraclass correlation coefficient (ICC, R) and the mean intra-individual coefficient of variation (CV) was calculated for each time point[18]. Reproducibility was judged by the R values of ICC[19]: 0–0.25: little, 0.26–0.49: low, 0.50–0.69: moderate, 0.70–0.89: high, and 0.9–1.0: very

high. The significance level was set at $p<0.05$ and all data are presented as means ± standard deviation (SD).

Results

All bouts were performed in sunny conditions at similar temperatures (20–24°C), low wind speeds (0–5 $km \cdot h^{-1}$), dry conditions and good visibility. There were no significant differences in the amount of fluid ingested during the run (444 ± 53mL), weight loss from pre (72.3 ± 7.6 kg) to post (71.2 ± 6.5 kg) run, and the number of energy gels ingested (1.5 ± 0.2) among the four bouts.

Completion time was significantly elevated in the first bout compared to other bouts ($p<0.05$, Table 1). Blood lactate was reduced following the first bout, but no significant differences were found for RPE or mean HR. The reproducibility was high for completion time, especially when the first bout was excluded, but low for mean HR, RPE and blood lactate.

Table1. Mean ± SD values for total running time, mean heart rate [%HRmax], rate of perceived exertion (RPE [6-20pt]) and blood lactate [$mmol \cdot L^{-1}$] measured immediately after the run for the first (1), second (2), third (3) and fourth (4) bouts of trail running. Reproducibility determined by an intraclass correlation coefficient (ICC, R) and coefficient of variation (CV in %) for all four bouts (1-4), the last three bouts (2-4), and the middle two bouts (2-3), is shown on the right. [a] indicates a significant difference from other bouts ($p<0.05$).

	Bout				Reproducibility					
Parameter	1	2	3	4	1–4		2–4		2–3	
					ICC	CV	ICC	CV	ICC	CV
Running time [s]	5842 ± 521[a]	5511 ± 440	5623 ± 378	5628 ± 438	0.78	3.5	0.85	2.5	0.82	2.3
Heart rate [%]	91.8 ± 4.6	89.2 ± 5.7	90.1 ± 4.8	90.1 ± 3.5	0.54	3.2	0.49	3.0	0.55	3.4
RPE [6-20 pt]	17.9 ± 2.1	16.6 ± 1.9	17.6 ± 1.7	17.9 ± 2.0	0.33	8.4	0.52	7.1	0.56	5.9
Lactate [mmol/L]	3.9 ± 1.7[a]	6.4 ± 1.1	5.3 ± 2.0	5.4 ± 1.6	0.38	27.6	0.41	22.8	0.66	16.1

The pre values for MVC torque and CMJ height showed no significant differences between bouts (Figure 1). A significant interaction effect was found for MVC torque; in contrast to bouts 1 to 3, acute torque reduction was no longer significantly different from baseline in bout 4 (Figure 1a). The reproducibility of post-exercise MVC torque was high (ICC 0.82–0.93; CV 5.3–8.7%), especially when only bouts 2–3 were considered (Table 2). No significant interaction effect was found for CMJ height, but CMJ height decreased significantly by post 24 hours in all bouts and in no case returned to baseline by 48 hours post-run. The reproducibility

of CMJ height was moderate to high (ICC 0.55–0.82; CV 3.6–7.9%), and increased with exclusion of bouts 1 and 4 (Table 2).

Table 2. Reproducibility of knee extensor maximal voluntary isometric contraction torque (MVC), counter movement jump height (CMJ), plasma creatine kinase concentrations (CK) and visual analogue scale for muscle soreness determined by an intraclass correlation coefficient (ICC, R) and coefficient of variation (CV in %) for the four bouts (1-4), the last three bouts (2-4), and middle two bouts (2-3).

		Reproducibility					
Parameter	**Time**	**1–4**		**2–4**		**2–3**	
		ICC	CV [%]	ICC	CV [%]	ICC	CV [%]
MVC [N·m]	**Pre**	0.62	10.7	0.83	6.6	0.95	4.6
	Post	0.82	8.5	0.81	8.6	0.93	5.3
	24 h post	0.84	8.7	0.83	8.1	0.84	7.5
	48 h post	0.88	7.3	0.91	6.6	0.89	7.3
CMJ [cm]	**Pre**	0.60	8.7	0.69	6.7	0.59	7.0
	Post	0.78	7.9	0.79	7.1	0.74	7.2
	24 h post	0.77	6.1	0.82	4.5	0.82	3.6
	48 h post	0.75	6.2	0.68	6.2	0.55	7.0
CK [IU·L^{-1}]	**Pre**	0.46	19.3	0.41	20.0	0.46	15.6
	Post	0.59	16.2	0.51	17.1	0.44	15.8
	24 h post	0.28	34.0	0.20	34.4	0.12	32.9
	48 h post	0.37	26.6	0.29	28.6	0.13	24.3
Muscle Soreness [mm]	**Pre**	0.89	17.10	0.88	16.1	0.89	11.00
	Post	0.16	48.8	0.29	50.3	0.67	51.5
	24 h post	0.25	47.0	0.22	52.6	0.31	49.0
	48 h post	0.51	55.0	0.55	60.1	0.61	51.4

Baseline values were similar among bouts for plasma CK activity and self-reported muscle soreness (Figure 1). Both plasma CK activity and muscle soreness were significantly increased for all post-run time points in all bouts compared to baseline. No significant differences were evident for changes in plasma CK activity (Figure 1c) and muscle soreness (Figure 1d) among the bouts. As shown in Table 2, plasma CK activity showed low to moderate reproducibility post-run (ICC 0.12–0.59; CV 15.6–34.4%). Muscle soreness reproducibility was low to moderate (ICC 0.16–0.89; CV 11–60.1%), independent of exclusions.

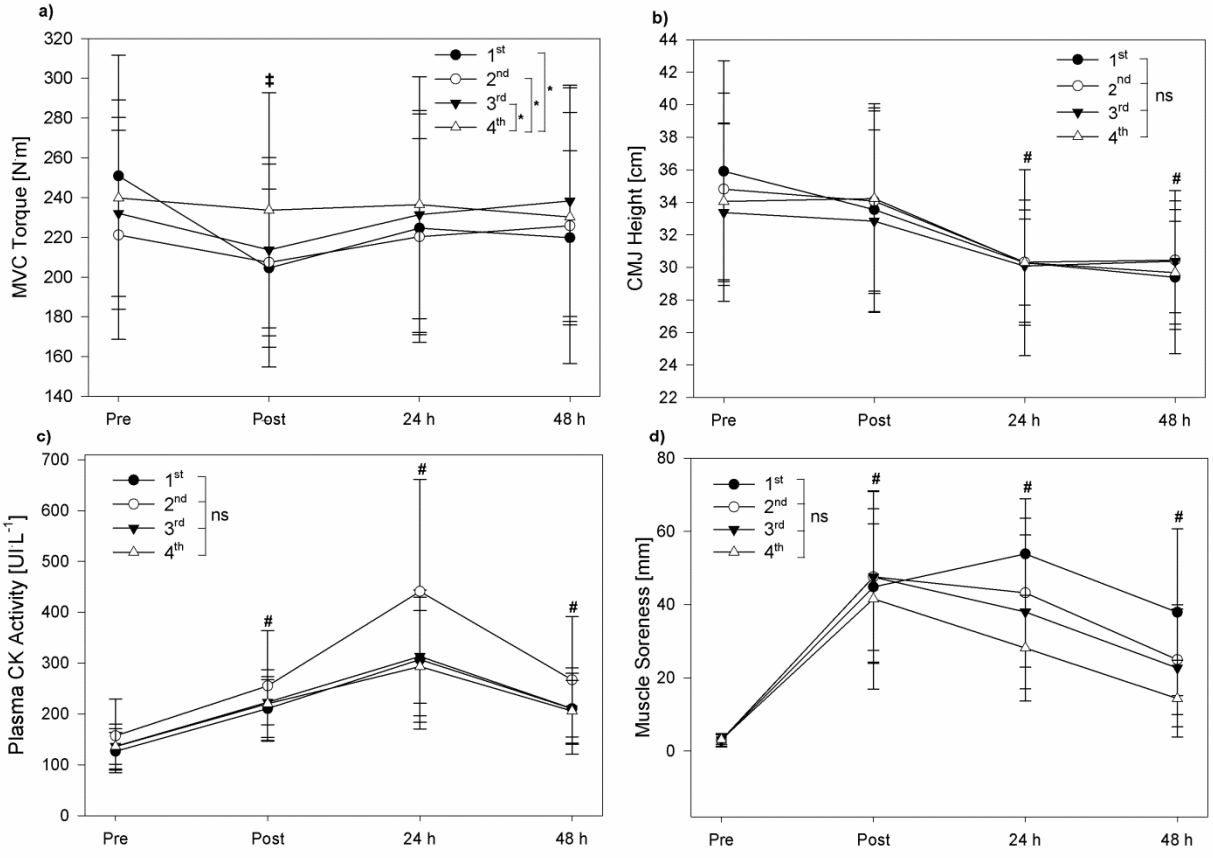
Figure 1. Changes in maximal voluntary isometric contraction torque of the knee extensors (a), counter movement jump height (b), plasma CK activity (c) and muscle soreness (d) before (Pre) and 1 hour (Post), 24 hours and 48 hours after the first (1st), second (2nd), third (3rd) and fourth (4th) trail running bouts.

* Significant (p<0.05) difference between bouts based on two-way ANOVA

Significant (p<0.05) difference from the baseline (Pre) values for all bouts

‡ Significant (p<0.05) difference from the baseline (Pre) values for 1st 2nd and 3rd bouts

ns: No significant differences between bouts



Discussion

To the best of our knowledge, this was the first investigation of reproducibility of performance, neuromuscular fatigue and indirect muscle damage indexes over four outdoor trail runs performed by experienced runners. The primary results are an increased running time in the first bout accompanied by lower blood lactate concentrations, which we attribute to the lack of

prior experience on the course. Furthermore, the indexes of neuromuscular fatigue and muscle damage were similar throughout bouts 1 to 3 and reduced in the last, maybe due to a repeated bout effect. These results indicate that the amount of bouts should be taken into account when using a short outdoor trail run as a fatigue or muscle damage model in order to evaluate intervention strategies to ameliorate performance or recuperation.

To characterise the effectiveness of the intervention, the time courses of parameters were examined. We observed a decrease in MVC torque post run (peak: -17%), which returned to baseline by 24 h in all bouts, CMJ decreases persisting until 48h (peak: -17%) and increases in plasma CK activity (peak at 24h: +35%) and muscle soreness (peak bout 1 at post: 54/100mm; peak bouts 2-4 at post 24: 47/100mm) (Figure 1). These alterations are similar to those reported in previous studies examining fatigue and muscle damage in a trained cohort following long distance trail runs (30, 55 and 166km), which report MVC reductions of 20–40%[1,2,4]. No studies examining fatigue after short distance field based trail runs exist to our knowledge.

In this study, ICC and CV values showed that reproducibility was high for running time, MVC torque and CMJ height – especially for the two middle bouts. Considerable variability existed for changes in blood lactate, plasma CK activity, RPE, and muscle soreness, making these parameters unsuitable as main outcome variables.

The reproducibility of completion time in the present study (Table 1) appears to be comparable to that of 'indoor' settings, especially when excluding bout 1. For example, Nicholson & Sleivert[20] reported a CV of 3.7% for completion time of two 10 km time trials 7 days apart on an indoor track. A review indicates that time trials (1500–5000m) run on an indoor track have average CV values of ~2.5% for completion time[21].

Despite similar running times among the last three bouts, RPE and post-run lactate concentration were largely variable (Table 1). This is not uncommon, Saunders et al.[22], for instance, reporting a CV of 10–52% in lactate concentration in two repetitions of 3 four-minute bouts of treadmill running 7 days apart. RPE reliability has been reported to decrease rapidly with increasing exercise intensity and duration[23]. The relatively low reproducibility in RPE and lactate may be further accentuated in a trail race scenario due to the continuous variation of pace and terrain-induced changes in dissociation strategies and subsequent reduction in sensitivity to physiological cues.

MVC values were well reproducible for all time points in the examined scenario (Table 2), especially when excluding the very first test and the post-exercise test in the fourth bout. This concurs with results reported by Maffiuletti et al.[24], who observed an ICC of 0.97 and CV of 5.5% for peak knee extensor MVC torque in 2 sessions 7 days apart. Changes in CMJ height following the trail run showed similar reproducibility for the two middle bouts and for the last three bouts as was reported previously[17,25]. It appears that the reproducibility of muscle function changes (MVC torque and CMJ) in outdoor trail running is comparable to that of laboratory based studies[24,25].

Plasma CK activity and muscle soreness showed only moderate reproducibility in the presented study. A large variability in the CK responses to exercise has previously been reported[11,26,27], and this is also reflected in the present study. The qualitative time profile of muscle soreness was similar in all four bouts. The reproducibility remains low in all time points, even after exclusion of bouts 1 & 4.

Reproducibility increased considerably in this study when only bouts 2 and 3 were considered. The dissimilarity of the first MVC test and running time from the others is probably related to task learning and highlights the importance of a familiarisation session in an ecological context.

The second methodological result of this reproducibility study is the attenuation of acute post-exercise MVC reduction by ~6% in the fourth bout compared to bouts 2 and 3. As proposed in the introduction, this may be caused by a repeated bout effect conferred through the earlier exercise bouts. Similar attenuation has been reported by Thompson et al. [28] in week 4 of their study on eccentric damage in the elbow flexors. Additionally, it has already been reported that eccentric-induced changes in indirect markers of muscle damage are smaller for resistance-trained individuals[11,12] and that the bulk of the protective effect is conferred within the first repetitions of a bout[10]. Therefore it was expected that a protective effect against muscle damage had already been invoked in our trained trail running population and would not be observed in the experiment. Nonetheless, MVC attenuation was observed, which leads us to believe that the amount of bouts should be limited to a maximum of three in an experimental design, even in well-trained subjects.

There are a number of limitations to the model presented, as for instance, it depends on the environmental conditions and is therefore primarily suited to climatically stable environments. Additionally the terrain and elevation will not be constant between testing sites, making inter-protocol comparisons less trustworthy.

Conclusion

The reported results indicate that if only one group is used in a cross-over design to investigate an intervention effect on trail running, it seems necessary to instigate a familiarisation bout before conducting two testing bouts. In order to evaluate fatigue and muscle damage indexes reliably, the design should optimally take into account the repeated bout effect, even if the muscle damage invoked is minimal. From the outcome measures observed in this study, MVC and CMJ decline show the highest reproducibility and are therefore best suited as main outcome measures. In contrast, the magnitude of variability for RPE, lactate, CK and muscle soreness makes these markers insensitive to small changes and more appropriate as auxiliary variables. It appears that the reproducibility of the changes in variables in the present study is not largely different from that shown in laboratory-based studies[22,24,25], indicating that an outdoor trail model is equally suited to a lab when evaluating trail running interventions. Therefore, the trail running model used in the present study can be used to investigate the

effect of an intervention or a strategy on performance or fatigue; however, methodological precautions should be taken to ensure optimal reproducibility.

Practical Implications

For athletes: The first time a trail course is run, performance is likely to be reduced and fatigue accentuated.

For researchers: Outdoor trail runs are a viable investigation model that may be used to assess trail-specific interventions when conducting prior familiarisation and limiting the accumulated eccentric stimulus.

Due to the reduction in reliability after the third bout, this model is mainly applicable for simple within-group designs regarding a single intervention versus a control condition.

Acknowledgements

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References

1. Easthope CS, Hausswirth C, Louis J et al. Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes. *Eur J Appl Physiol*. 2010; 6(110):1107–1116.

2. Millet GY, Tomazin K, Verges S et al. Neuromuscular consequences of an extreme mountain ultra-marathon. *PLoS ONE*. 2011; 6(2):e17059.

3. Nieman DC, Dumke CI, Henson DA et al. Immune and oxidative changes during and following the Western States Endurance Run. *Int J Sports Med*. 2003; 24(7):541–547.

4. Millet GY, Martin V, Lattier G et al. Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *J Appl Physiol*. 2003; 94(1):193–198.

5. Kim HJ, Lee YH, Kim CK. Biomarkers of muscle and cartilage damage and inflammation during a 200 km run. *Eur J Appl Physiol*. 2007; 99(4):443–447.

6. Hoffman MD, Ong JC, Wang G. Historical analysis of participation in 161 km ultramarathons in North America. *Int J Hist Sport*. 2010; 27(11):1877–1891.

7. Hopkins WG, Hewson DJ. Variability of competitive performance of distance runners. *Med Sci Sports Exerc*. 2001; 33(9):1588–1592.

8. Laursen PB, Francis GT, Abbiss CR et al. Reliability of time-to-exhaustion versus time-trial running tests in runners. *Med Sci Sports Exerc*. 2007; 39(8):1374–1379.

9. Nosaka K, Clarkson PM, McGuiggin ME et al. Time course of muscle adaptation after high force eccentric exercise. *Eur J Appl Physiol Occup Physiol*. 1991; 63:70–76.

10. Chen TC, Chen HL, Lin MJ et al. Muscle damage responses of the elbow flexors to four maximal eccentric exercise bouts performed every 4 weeks. *Eur J Appl Physiol*. 2009; 106(2):267–275.

11. Falvo MJ, Schilling BK, Bloomer RJ et al. Repeated bout effect is absent in resistance trained men: an electromyographic analysis. *J Electromyogr Kinesiol*. 2009; 19(6):e529–535.

12. Newton MJ, Morgan GT, Sacco P et al. Comparison of responses to strenuous eccentric exercise of the elbow flexors between resistance-trained and untrained men. *J Strength Cond Res*. 2008; 22(2):597–607.

13. Hausswirth C, Louis J, Bieuzen F et al. Effects of Whole-Body Cryotherapy vs. Far-Infrared vs. Passive Modalities on Recovery from Exercise-Induced Muscle Damage in Highly-Trained Runners. *PLoS ONE*. 2011; 6(12):e27749.

14. Pournot H, Bieuzen F, Louis J et al. Time-Course of Changes in Inflammatory Response after Whole-Body Cryotherapy Multi Exposures following Severe Exercise. *PLoS ONE*. 2011; 6(7):e22748.

15. Taylor HL, Buskirk E, Henschel A. Maximal oxygen intake as an objective measure of cardio-respiratory performance. *J Appl Physiol*. 1955; 8(1):73–80.

16. Borg GAV. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*. 1982; 14(5):377–381.

17. Markovic G, Dizdar D, Jukic I et al. Reliability and factorial validity of squat and countermovement jump tests. *J Strength Cond Res*. 2004; 18(3):551–555.

18. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull*. 1979; 86(2):420–428.

19. Munro BH. Statistical analysis: understanding the results. *Clin Nurse Spec*. 1989; 3(3):113.

20. Nicholson RM, Sleivert GG. Indices of lactate threshold and their relationship with 10-km running velocity. *Med Sci Sports Exerc*. 2001; 33(2):339–342.

21. Currell K, Jeukendrup AE. Validity, reliability and sensitivity of measures of sporting performance. *Sports Med*. 2008; 38(4):297–316.

22. Saunders PU, Pyne DB, Telford RD et al. Reliability and variability of running economy in elite distance runners. *Med Sci Sports Exerc*. 2004; 36(11):1972–1976.

23. Lamb KL, Eston RG, Corns D. Reliability of ratings of perceived exertion during progressive treadmill exercise. *Br J Sports Med*. 1999; 33(5):336–339.

24. Maffiuletti NA, Bizzini M, Desbrosses K et al. Reliability of knee extension and flexion measurements using the Con-Trex isokinetic dynamometer. *Clin Physiol Funct Imaging*. 2007; 27(6):346–353.

25. Hori N, Newton RU, Kawamori N et al. Reliability of performance measurements derived from ground reaction force data during countermovement jump and the influence of sampling frequency. *J Strength Cond Res*. 2009; 23(3):874–882.

26. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. *Br Med Bull*. 2007; 81-82:209–230.

27. Nosaka K, Clarkson PM. Variability in serum creatine kinase response after eccentric exercise of the elbow flexors. *Int J Sports Med*. 1996; 17(2):120–127.

28. Thompson HS, Clarkson PM, Scordilis SP. The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. *Acta Physiol Scand*. 2002; 174(1):47–56.

Chapter 6

Intervention study 1

6.1. INTRODUCTION

Subsequent to the development and validation of an outdoor testing model, it was possible to begin the methodologically sound evaluation of interventions. Due to the recently skyrocketing popularity of lower limb compression garments (CG), these were analysed for performance benefits. Subjects were recruited from the same sources as earlier studies and were familiarised by running the course twice before completing two randomised experimental sessions with and without CG. Muscular performance, blood flow and tissue oxygenation were measured before and after each session and performance, HR, RPE and lactate concentrations were collected throughout the run. The results, in a nutshell, are that CGs had no effect on the assessed parameters in the used model. In light of the large body of contradictory results on the matter, it would not be surprising if the used model was not sensitive enough to discern the effects. Nonetheless, a 15 km field-based trail run is a completely representative functional evaluation and can provide insight for athletes competing in these kinds of competitions. The study was published in the *European Journal for Sport Science* in January 2013.

6.2. THE INFLUENCE OF WEARING COMPRESSION STOCKINGS ON PERFORMANCE INDICATORS AND PHYSIOLOGICAL RESPONSES FOLLOWING A PROLONGED TRAIL RUNNING EXERCISE

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ORIGINAL ARTICLE

The influence of wearing compression stockings on performance indicators and physiological responses following a prolonged trail running exercise

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Abstract

The objective of this study was to investigate the effects of wearing compression stockings (CS) on performance indicators and physiological responses during prolonged trail running. Eleven trained runners completed a 15.6-km trail run at a competition intensity whilst wearing or not wearing CS. Run time, counter movement jump (CMJ), maximal voluntary contraction (MVC) and the oxygenation profile of vastus lateralis muscle using near-infrared spectroscopy (NIRS) method were measured before and following exercise. Run time, heart rate (HR), blood lactate concentration and ratings of perceived exertion were evaluated during the CS and non-CS sessions. No significant difference in any dependent variables was observed during the run sessions. Run times were 5681.1 ± 503.5 and 5696.7 ± 530.7 s for the non-CS and CS run times, respectively. The relative intensity during CS and non-CS runs corresponded to a range of 90.5-91.5% HRmax and performance times were comprised between 94 and 95 min. Although NIRS measurements such as muscle oxygen uptake and muscle blood flow significantly increased following exercise (+ 57.7% and + 42.6%, + 59.2% and + 32.4 %, respectively for the CS and the non-CS, $P < 0.05$), there was no difference between CS and non-CS conditions. The findings suggest that competitive runners do not gain any practical or physiological benefits from wearing CS during prolonged off-road running.

Key words: compression socks, trail running, performance indicators, muscle oxygenation, physiological responses

Introduction

During the two last decades, road runners have been wearing compression garments (CGs) during race particularly with the use of compression socks (CS) in an attempt to enhance their performance (e.g. Paula Radcliffe, Lornah Kiplagat). Since the intensive development of technological clothing in the area of endurance sports, the wearing of CS has been widely used by on and off-road runners for training and racing. Based on anecdotal reports, runners often comment on their leg's feeling with a lower perception of strain in the calf when exercising with CS. These statements are in line with a previous study indicating that knee-length CGs are more comfortable with less possibilities of wrinkling when compared to thigh-length CGs (Benkö, Cooke, McNally, & Mollan, 2001). Paradoxically, a lack of clear evidence of CS benefits was reported for performance, indicators of muscle power (e.g. countermovement jump) or metabolic adaptations in trained runners (Ali, Creasy, & Edge, 2011; Goh, Laursen, Dascombe, & Nosaka, 2011; Ménétrier, Mourot, Bouhaddi, Regnard, & Tordi, 2011; Sperlich et al., 2010, 2011; Varela-Sanz, Espana, Carr, Boullosa, & Esteve-Lanao, 2011). However, little is known about the analysis of selected physiological variables (e.g. muscle oxygenation) and performance responses (e.g. maximal voluntary contraction) following a prolonged running exercise (> 1-h) close to the race intensity. Interestingly, using Near-Infrared Spectroscopy (NIRS) method, Dascombe, Hoare, Sear, Reaburn, & Scanlan, (2011) have demonstrated that wearing CGs positively influenced a number of peripheral circulatory measures within the vastus lateralis during a time to exhaustion conducted at a competition pace in runners. However, these peripheral physiological benefits were not correlated to a significant improvement in running performance. Similarly, Varela-Sanz et al. (2011) have recently reported a non-significant increase of approximately 13% in time limit running test under CS condition, at a competitive velocity. Although the small number of subjects (n=6) might partly explain the lack of significant differences between CS and non-CS conditions, these investigators showed a cardiac benefit in runners wearing CS, resulting in a significant decrease in relative intensity (i.e. maximal heart rate) sustained during the time limit.

Even if physiological benefits from the wearing of CGs were identified during endurance running performance, it is likely that the exercise duration reported in these recent running studies was not sufficient to highlight any possible benefits of wearing CS on performance responses. Other methodological limitations may be considered in the previous investigations,

including the use of a treadmill that can potentially change the normal running kinematics and the subsequent energetic requirements of high-intensity endurance running (Wank, Frick, & Schmidtbleicher, 1998) but also, the use of a running time to exhaustion as performance indicator, which may modify pacing strategies related to race performance. Finally, no running analysis has been conducted on the relationship between any form of CGs and performance responses throughout off-road exercises, so-called trail-running, including uphill and downhill sections. In contrast with flat road, the muscular contractions induced during trail-running are specific and dictated by the occurrence of a strong concentric modality during uphill section and a dominant eccentric regimen to downhill section. This running exercise might result in higher muscle oscillations and variations in physiological responses, particularly during the repeated downhill sections (Millet et al., 2011).

In contrast with most of laboratory settings, the evaluation of selected metabolic and/or muscular variables remains specific in the outdoor context and requires a serie of measurements conducted before and following exercises (e.g. Easthope et al., 2010; Millet et al., 2011; Sultana et al., 2012). Therefore, the objective of the current study was to examine the effect of a new non-graduated CS (18 mmHg) on physiological responses and performance indicators following prolonged trail running in experienced off-road runners. Considering the findings reported in the CGs running investigations but also, the specificity of our running task, it may be hypothesized that the physiological benefits of wearing CS (e.g. improved muscle oxygenation, decreased HR response) appear more accentuated during trail running (~1.5 hr) at a competition pace, improving thus performance indicators and physiological responses following prolonged exercise.

Methods

Subjects

Eleven male trained runners ([mean ± SD] age: 34.7 ± 9.8 years; height: 178.4 ± 7.0 cm; body mass: 72.3 ± 6.8 kg) participated to this study after medical examination. All subjects had a minimum of 3 consistent years of trail running experience over different race distances (from 20 to 80 km). Run training time ranged from 8 to 12 $h \cdot wk^{-1}$, interspersed with competitive events. All subjects gave their informed written consent to participate in the current study, which has been conducted according to the Declaration of Helsinki. A local ethics committee for the protection of individuals gave approval concerning the project before its initiation.

Experimental design

An overview of the experiment is given in Figure 1. All subjects completed both laboratory and field sessions. At the initial laboratory session, participants performed an incremental exercise test to exhaustion on a motorized treadmill. Pulmonary gas exchanges were collected breath-by-breath and averaged for every 10-s period using a metabolic measurement system (Oxycon Alpha®, Jaeger). The system was calibrated prior to each exercise test according to the manufacturer's instructions. After 6-min of warm-up exercise at 10 $km \cdot h^{-1}$, the treadmill speed was increased by 1 $km \cdot h^{-1}$ every 2 minutes (with a 4% grade). This maximal session did allow to determine mean values in maximal oxygen uptake ($\dot{V}O_2max$), maximal ventilation ($\dot{V}_E max$) but also, maximal heart rate (HRmax). During laboratory and field testing sessions, HR values were monitored using a polar unit (RS800CX, Polar®, Kempele, Finland). During the first visit, particular attention was paid to familiarize participants with the experimental procedures, especially the completion of maximal voluntary contraction (MVC) and counter movement jump (CMJ) to quantify indicators of muscle power.

Subsequently, to familiarize the participants with the experimental off-road sessions, two practice runs were completed on the course. The second run was entirely conducted at a pace closer to the race context. The subjects were habitual users of CS during training and racing, avoiding potential discomfort in the calf area. Likewise, red markers were placed on the ground every 200-m to facilitate the displacement of our runners during the course. These runs were performed between 2 and 4 weeks before the experimental runs. After a standard and controlled warm-up of 10-min, the off-road sessions consisted of completing two maximal (race effort) 15.6-km trail runs, in a random order, on two separate days one week apart, wearing CS or not wearing CS (non-CS). Runners were asked to wear the same shoes and the similar clothing (without thigh compression) for CS and non-CS conditions. For the CS session, subjects wore socks extending from below the knee to the lateral malleolus (constant pressure of 18 mmHg applied to the calf / 94% Polyamide and 6% Lycra). During the first run, subjects consumed carbohydrate (CHO) in the form of gel (25 g, two per runner) and energy drinks (6% CHO / 600 ml of water per runner). Fluid intake was measured by weighing the bottle after the first run on an electronic scale (accurate to 1 g). Subsequently, the quantity of ingested CHO gels and fluid intake was individually replicated during the second run. Finally, the runners were separated to avoid pacing strategies or psychological impact affecting run time. Likewise, the day before each trial, the runners were asked to refrain from strenuous exercise and they were also asked to keep the same nutritional routine before each trial, with the same breakfast at the same time, similar to what they would do before a race.

More precisely, the 15.6-km trail-running consisted of the completion of three 5.2-km loops with a brief rest period of 40-s fixed between the loops for data collection (Figure 1). Each loop

was divided into two sections completed systematically in the following order: uphill (2200 m) and downhill section (3000m) with average gradients of 13% and 9%, respectively. The positive elevation was 275-m for each loop. The profile of trail-running was characterized by the completion of 100% single tracks in the mountain and repeated technical portions with rocky/root filled paths. Weather conditions were stable with ambient temperatures ranging from 20 to 24°C (South of France) during the sessions.

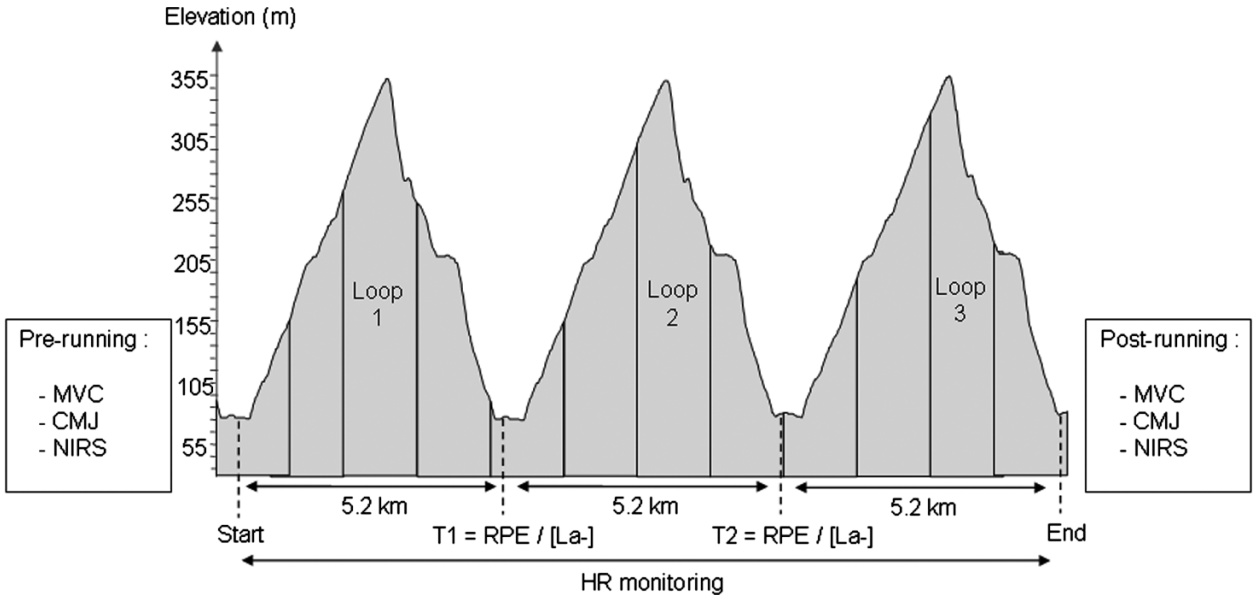

Figure 1: General view of the experimental sessions and run profile.

Measurements during CS and non-CS sessions

During the 40-s rest periods fixed between the loops of CS and non-CS sessions (Figure 1), the ratings of perceived exertion (RPE) scale using the Borg 6-20 was presented to the subjects who was asked to say the number that reflected the perceived exertion for 1) an "overall" or total body rating (RPE_{global}), 2) a central or "heart/lungs" rating ($RPE_{central}$), and 3) a peripheral or "legs" rating ($RPE_{peripheral}$) (Borg, 1998). Moreover, the blood lactate concentration ($[BLa^-]$) was obtained using a Lactate Pro® analyser (Akray, Kyoto, Japan) from 5-µL samples of blood taken from the earlobe during the rest periods of CS and non-CS runs. Athletes were also equipped with a RS800CX G3 (Polar®, Kempele, Finland) including a GPS receiver fixed on the arm for monitoring HR and displacement values.

Measurements before and following CS and non-CS sessions

Near-infrared spectroscopy measurements. Oxygenation profile of the right vastus lateralis (VL) muscle was recorded using a continuous-wave near-infrared spectroscopy (NIRS) system (PortaMon®, Artinis Medical Systems BV, Zetten, Netherlands). This analysis was conducted prior to the warm-up of run sessions and following CS and non-CS bouts (~ 5 min) to monitor concentration changes in oxyhemoglobin (HbO_2), deoxyhemoglobin (HHb) and total hemoglobin (tHb). A probe was attached to the middle part of the VL muscle (15-20 cm above the centre of patella) longitudinally. Pulsed light was emitted from the emission of a three-segment photodiode at two different wavelengths (760 and 850 nm) and was detected, as a function of distance, using a photodiode detection probe that received NIRS signals at 2 Hz. To prevent variations in placement of the NIRS emitter-detector, the angle and location of the probe were held constant using velcro straps. Similarly, the position of the NIRS probe was noted with a marker to ensure identical placement on each subject for all testing sessions. Finally, a light-impermeable cloth covered the probe to reduce room light interaction with the near-infrared signal. Before placement on the VL, the site was shaved and cleaned using alcohol swab. Subjects lay supine in a horizontal position with slightly inclined upper body (15°) keeping their arms at their sides for the duration of the test. In order to determine muscular oxygen uptake ($m\dot{V}O_2$) and blood flow (mBF), two venous occlusions were applied above the belly of the VL (compression of femoral artery), using air inflated to 70 mmHg, each lasting 20 s with a 2 min recovery interval (Ahmadi, Sinclair, & Davis, 2008a; Ahmadi, Sinclair, Foroughi, & Davis, 2008b). The medium time-derivative of HHb, HbO_2 and tHb was determined over a time period of 20 seconds beginning once the pressure of 70 mmHg was reached. Given that the venous outflow was blocked, the initial linear increase in HHb was used to calculate mVO_2 (in $mlO_2 \cdot min^{-1} \cdot 100g$) (Van Beekvelt, Colier, Wevers, & Van Engelen, 2001). Moreover, mBF was measured during venous occlusion by evaluating the linear increase in tHb during the time period of 20 seconds. Given that the venous outflow was blocked, the increase in tHb (HbO_2 + HHb) was directly related to the arterial inflow (in mlO_2.min-1.100g) (Van Beekvelt et al., 2001). During the pre/post bouts, $m\dot{V}O_2$ and mBF were calculated as the average obtained from the two venous occlusions.

Maximal voluntary contraction. Instantaneous isometric torque at the knee joint was recorded using a Biodex® isokinetic dynamometer (Shirley, NY). Subjects were placed in a seated position and were securely strapped into the test chair. Extraneous movement of the upper body was limited by two crossover shoulder harnesses and a belt across the abdomen. All measurements were taken before (after a standard warm-up) and 45-min after the CS and non-CS run sessions from the subject's right leg, with the knee and hip flexed at 90 degrees from full extension.

Subjects were then asked to perform three trials of MVC (4–5 s) with a rest period of 60-s between each MVC. The highest MVC value of trials was used.

Countermovement jump. Participants were instructed to adopt a standing position with hands on hips. This position was held for 3-s before the completion of a maximal vertical jump. Volunteers were instructed to keep their hands on their hips throughout the jump, and their legs straight whilst in the air. Participants stood fully erect, and following a verbal command, initiated a countermovement followed by a maximal vertical jump in one continuous motion. Before and immediately after the run sessions (~ 1 min), CMJ heights (in cm) were recorded from the Bosco test that consists of measuring the flight time with a digital timer (+/- 0.001 s) (Bosco, Luhtanen, & Komi, 1983). The highest CMJ value of three jumps was used.

Statistical analysis

Data are presented as mean ± SD. The Kolmogorov-Smirnov test was applied to ensure a Gaussian distribution of the data. The performance and physiological responses throughout CS and non-CS runs but also, between the pre/post periods of each condition were compared by using paired t-tests. For this analysis, the NIRS data expressed as the delta between the pre/post periods (%) were evaluated by an arcsine transformation. Furthermore, a 2 (condition) x 3 (time) repeated-measures analysis of variance was used to examine the effects of trail-running sessions on dependent variables within the three loops of exercise. A Tukey post hoc test was used to determine significant differences among CS and non-CS conditions. Statistical significance was accepted at $P < 0.05$.

Results

For the incremental run exercise, mean values in $\dot{V}O_2max$, HRmax, $\dot{V}_E max$ were 4.32 ± 0.43 L.min-1 (60.1 ± 6.5 $mL \cdot kg^{-1} \cdot min^{-1}$), 183 ± 10 $beats \cdot min^{-1}$ and 142.4 ± 20.5 $L \cdot min^{-1}$, respectively. The analysis of the three loops indicated no significant change in run times (~2.5%, Figure 2) between the non-CS and CS conditions. The average finishing time of our subjects was 5681.1 ± 503.5 and 5696.7 ± 530.7 s for the non-CS and CS runs, respectively. The evaluation of isolated run indicated that the mean values in run time for the loop #1 were significantly lower as compared to those reported for the loops #2 (-6.10% only for the CS run, $P < 0.05$, Figure 2) and #3 (-9.95% and -8.60%, respectively for the non-CS and CS runs, $P < 0.05$, Figure 2). No significant variation in $[Bla^-]$ and HR values was observed throughout the loops and between

the run sessions (Table 1). Moreover, mean values in $RPE_{globlal}$, $RPE_{central}$ and $RPE_{peripheral}$ were significantly higher during the loop#3 compared to those reported during the loop#1 ($P < 0.05$), without any significant differences between the CS and the non-CS runs. The analysis of the pre/post measurements indicated significant higher values in mV̇O2 and mBF during the post-run, characterized by significant values in $\Delta m\dot{V}O_2$ and ΔmBF (Figure 3, $P < 0.05$) for the two trail-running sessions. No significant variations in MVC and CMJ were observed following run sessions (212 ± 45 vs. 214 ± 55 Nm, 35 ± 6 vs. 32 ± 5 cm, respectively for the non-CS and CS conditions).

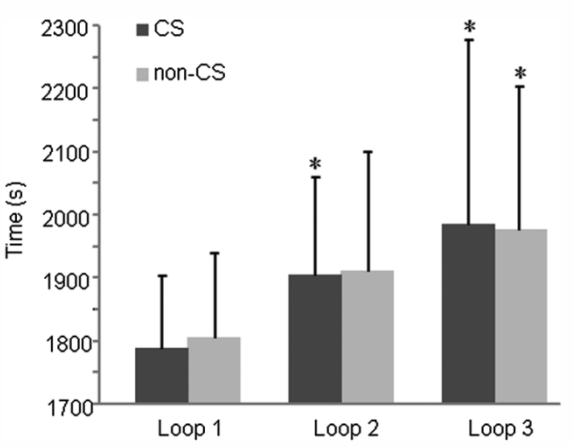

Figure 2- Variations in performance time within the three loops of compression socks (CS) and non-CS runs. *indicates a significant difference to loop 1 ($p<0.05$) for the CS and non-CS runs ($P < 0.05$)

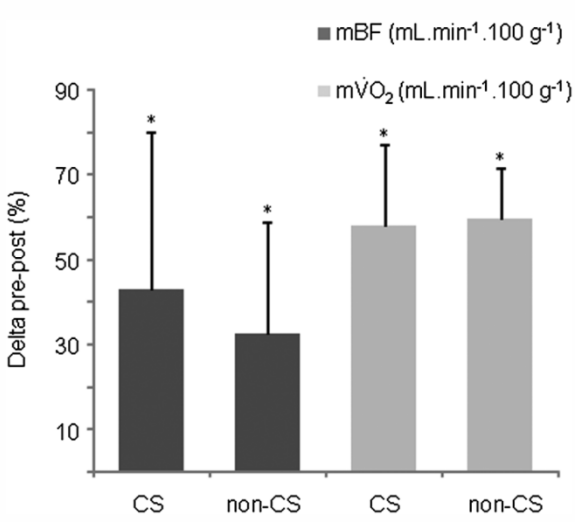

Figure 3- Variations in muscle oxygenation profile following the CS and non-CS runs. *indicates a significant difference in mBF and $m\dot{V}O_2$ between the pre and post-period for the CS and non-CS runs ($P < 0.05$)

Discussion

The originality of our experimental setting was based on a holistic analysis focusing on the use of CS within actual off-road running conditions in trained trail runners ($\dot{V}O_2max$ > 60 $ml \cdot O_2 \cdot min^{-1} \cdot kg^{-1}$). In contrast with our experimental hypothesis, the main finding of this work was that wearing CS did not effect on physiological variables and performance indicators measured during and following prolonged exercise.

The use of CS is increasingly widespread in the domain of trail running (> 50% of engaged racers on average, unpublished data), independent of performance level or race distances. However, the practical interest of wearing CS in the running activity under actual racing conditions has received little supporting scientific evidence. Most of identified investigations focusing on the use of CGs during running performance have selected either outdoor flat exercises (not exceeding 10 km or 45 min) or time to exhaustion exercises as performance indicator (Ali et al. 2011; Dascombe et al. 2011; Goh et al. 2011; Ménétrier et al. 2011; Sperlich et al. 2010; Varela-Sanz et al. 2011). The present investigation is the first to report performance data in relation with the wearing of CGs following prolonged running (> 1-h). However, our findings indicate a lack of significant differences in run times between the CS and non-CS session. Interestingly, our athletes adopted a positive pacing strategy (e.g. Stearns et al. 2009) during which the average speed gradually decreases over the duration of CS and non-CS runs (Figure 2), suggesting that the use of CS has no effect on external factors potentially involved in the pacing strategy. All participants reported a similar effort for each loop of CS and non-CS runs, characterized by a lack of RPE variations between conditions (Table 1). The perceptual scales provide a reflection of subjective intensity and, coupled with the physiological measures such as HR, the relevant information is that our runners have performed trail running exercises (across the loops) at the same and high intensity (i.e. > 90% HRmax). Our results are consistent with recent studies that showed a lack of change in running performance with the use of CS (Ali et al. 2011; Dascombe et al. 2011; Goh et al. 2011; Ménétrier et al. 2011; Sperlich et al. 2010; Varela-Sanz et al. 2011), suggesting that wearing CS (graduated or non-graduated) has no ergogenic effect during various running tasks. Thus, future research is required to analyze the effects of wearing CGs during off-road running exceeding two hours of exercise, especially when fatigue process, muscular damage or muscle oscillations are particularly accentuated (Millet, Martin, Lattier, & Ballay, 2003; Millet et al. 2011).

Table I. Variations in HR, [Bla $^{-}$], $RPE_{peripheral}$, $RPE_{central}$ and $RPE_{globlal}$ values within the three loops of CS and non-CS runs. *indicates a significant difference with the loop # 1 for the CS and non-CS runs ($P<0.05$).

	Loop 1		Loop 2		Loop 3	
	CS	Non-CS	CS	Non-CS	CS	Non-CS
HR (beats mitr $^{-1}$)	166±11	167±9	168±12	166±19	169±12	165±121
Blood lactate [Bla $^{-}$]	4.19±1.39	3.75±1.90	5.47±2.65	4.72±2.24	4.70±1.66	5.07±1.71
RPE peripheral	12.7±2.2	13.1±2.3	14.2±1.8	14.8±2.0	15.8±2.5*	16.1±2.4*
RPE central	13.2±1.9	13.2±2.5	15.3±1.6	14.8±2.0	16.1±2.3*	17.0±1.8*
RPE global	13.9±1.7	13.6±1.6	15.6±1.9	15.5±1.4	17.1±2.5*	17.5±1.6*

During endurance cycling and running exercises, the oxygenation profile has often been investigated from in situ measurements based on the variations in tissue oxygenation index (TOI), HHb or HbO_2 (Dascombe et al. 2010; Scanlan, Dascombe, Reaburn, & Osborne, 2008). These investigations have demonstrated that wearing CGs improved muscle oxygenation without any significant variation in performance response. Alternatively, other NIRS parameters such as $m\dot{V}O_2$ and mBF may be used to quantify the muscle oxygenation responses following exercises (Ahmadi et al. 2008a, 2008b). Based on the principle of venous occlusion, these authors have monitored muscle oxygenation using $m\dot{V}O_2$ and mBF parameters before and following eccentric exercise-induced muscle damage. Considering this recent method, the present study is the first to report on the variations in $m\dot{V}O_2$ and mBF before and following prolonged endurance exercise. The choice of measurement periods (pre/post) was essentially linked to the complexity of analyzing physiological parameters during trail running.

In contrast with earlier studies (Dascombe et al. 2010; Ménétrier et al. 2011; Scanlan et al. 2008), no significant variation in NIRS parameters was identified between CS and non-CS runs (Figure 3). The absence of change in oxygenation profile between run sessions might have been influenced by the position of the NIRS probe on the vastus lateralis whereas the compression level was applied to the calf. This suggests that 1) wearing CS during exercise has no effect on measured systemic blood flow (i.e. effect of CS on blood flow in the vastus lateralis) following exercise and 2) the potential benefits of CS on circulatory responses may be expected locally on the compressed muscle region and thus, depend on the amplitude of the myogenic response generated by external compression (Bochmann et al. 2005). Furthermore, mBF and $m\dot{V}O2$ reported in the present work were significantly higher following both CS and non-CS runs when compared to the resting values. The immediate increments in $m\dot{V}O_2$ after run sessions might result simply in the increased blood flow to the exercise limbs. In fact, during exercise, the vascular portion of actives muscles is considerably increased by the dilatation of local arterioles. This hyperaemic reaction could be observed after all types of exercise and thus, an increase in mBF and $m\dot{V}O_2$ would be an expected finding following any type of exercise (Ahmadi et al. 2008a, 2008b). Alternatively, it has been acknowledged that HR may be used as an indirect measure of venous return and/or circulatory flow, according to the Frank-Starling mechanism (Ali, Caine, & Snow, 2007; Varela-Sanz et al. 2011). In this regard, the lack of change in HR

values across the three loops of CS and non-CS runs suggests that wearing CS does not influence venous return (and oxygenation profile) during exercise and strengthens our finding concerning the NIRS responses obtained immediately after running.

Moreover, the non-significant modification in [Bla^-] values among the three loops of CS and non-CS runs (Table 1) does not support the claim purported by many CS manufacturers about the improved removal of blood lactate during exercise. The present study also demonstrates that wearing CS during off-road running exercise does not alter selected indicators of muscle power such as CMJ and MVC. For instance, mean CMJ values were well maintained in the CS and non-CS post-runs and may have been due to the "warm-up" effect. This is in agreement with previous running studies indicating no changes in CMJ values between CS and non-CS conditions (Ali et al. 2007, 2011, Jakeman, Byrne, & Eston, 2010). Similarly, muscle power characterized by MVC results indicated no significant strength loss when analyzing the pre and post periods of CS and non-CS runs. Muscle fatigue is often defined as a reduction in the maximum force (i.e. MVC) that a muscle can exert (Millet et al. 2003). Considering this statement, we suggest that despite the specificity of trail running, the exercise duration may be not long enough to induce muscle fatigue in our trained runners. Recently, Ross, Goodall, Stevens, & Harris, (2010) have shown the occurrence of knee extensor MVC decrement only during the final 5-km of a high-intensity 20-km self-paced run, corresponding approximately to the finishing times (~90-min) observed in our study. However, these authors have reported that the MVC was not significantly different from preexercise values after 20 or 40 min of rest following running exercise. Based on this issue, the lack of strength loss in our study may also be attributed to the time at which the MVC was evaluated (45-min after the end of exercise).

In conclusion, this is the first investigation that examines the effects of wearing non-graduated CS on performance indicators and selected physiological variables following a prolonged trail running exercise. However, it was demonstrated that competitive runners did not gain any physiological benefits and ergogenic aid from wearing CS during off-road running conducted at a race intensity. Although our results confirm a number of scientific data related to the absence of ergogenic aid under any form of wearing CGs during short running distances (< 90-min), a further topic would be relevant to analyze the impact that different level of exercise duration (> 2-h), possibly inducing specific muscle fatigue and associated damage, may have on running performance and physiological responses.

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Declaration

The authors declare that the experiments comply with the current laws of our country in which they were performed. Furthermore, the authors declare that they have no conflict of interest.

References

Ahmadi, S, Sinclair, P.J., & Davis, G.M. (2008a). Muscle oxygenation after downhill walking-induced muscle damage. Clinical Physiology and Functional Imaging, 28, 55-63.

Ahmadi, S, Sinclair, P.J., Foroughi, N, & Davis, G.M. (2008b). Monitoring muscle oxygenation after eccentric exercise-induced muscle damage using near-infrared spectroscopy. Applied Physiology, Nutrition and Metabolism, 33, 743-752.

Ali, A, Caine, M.P., & Snow B.G. (2007). Graduated compression stockings: physiological and perceptual responses during and after exercise. Journal of Sports Sciences, 25, 413-419.

Ali, A, Creasy, R.H., Edge, J.A. (2011). The effect of graduated compression stockings on running performance. Journal of Strength and Conditioning Research, 25, 1385-1392.

Benkö, T, Cooke, E.A., McNally, M.A., & Mollan, R.A.B. (2001). Graduated compression stockings: Knee length or thigh length. Clinical Orthopaedics and Related Research, 383, 197-203.

Bochmann, R.P., Seibel, W, Haase, E., Hietschold, V, Rödel, H, & Deussen, A (2005). External compression increases forearm perfusion. Journal of Applied Physiology, 99, 2337-2344.

Borg, G (1998). Borg's perceived exertion and pain scales. Champaign, IL: Human Kinetics.

Bosco, C, Luhtanen, P, & Komi, P.V. (1983). A simple method for measurement of mechanical power in jumping. European Journal of Applied Physiology, 50: 273-282.

Dascombe, B.J., Hoare, T.K., Sear, J.A., Reaburn, P.R., & Scanlan, A.T. (2011). The effects of wearing undersized lower-body compression garments on endurance running performance. International Journal of Sports Physiology and Performance, 6, 160-173.

Easthope C.S., Hausswirth, C, Louis, J, Lepers, R, Vercruyssen, F, & Brisswalter, J (2010). Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes. European Journal of Applied Physiology, 110, 1107-116.

Goh, S, Laursen, P.B., Dascombe, B, & Nosaka, K (2011). Effect of lower body compression garments on submaximal and maximal running performance in cold (10°C) and hot (32°C) environments. European Journal of Applied Physiology, 111: 819-826.

Jakeman, J.R., Byrne, C, & Eston, R.G. (2010). Lower limb compression garment improves recovery from exercise-induced muscle damage in young, active females. European Journal of Applied Physiology, 109, 1137-1144.

Ménétrier, A, Mourot, L, Bouhaddi, M, Regnard, J, & Tordi, N (2011). Compression sleeves increase tissue oxygen saturation but not running performance. International Journal of Sports Medicine, 32, 864-868.

Millet, G.Y., Martin, V, Lattier, G, & Ballay, Y (2003). Mechanisms contributing to knee extensor strength loss after prolonged running exercise. Journal of Applied Physiology, 94, 193-198.

Millet, G.Y., Tomazin, K, Verges, S, Vincent, C, Bonnefoy, R, Boisson, R.C., Gergelé, L, Féasson, L, & Martin, V (2011). Neuromuscular consequences of an extreme mountain ultra-marathon. PLoS One 6:e17059.

Ross, E.Z., Goodall, S, Stevens, A, & Harris, I (2010). Time course of neuromuscular changes during running in well-trained subjects. Medicine and Science in Sports and Exercise, 42, 1184-1190.

Scanlan, A.T., Dascombe, B.J., Reaburn, P.R., & Osborne, M (2008). The effects of wearing lower-body compression garments during endurance cycling. International Journal of Sports Physiology and Performance, 3: 424-438.

Sperlich, B, Haegele, M, Achtzehn, S, Linville, J, Holmberg, H.C., & Mester, J (2010). Different types of compression clothing do not increase sub-maximal and maximal endurance performance in well-trained athletes. Journal of Sports Sciences, 28, 609-614.

Sperlich, B, Haegele, M, Krüger, M, Schiffer, T, Holmberg, H.C., & Mester, J (2011) Cardio-respiratory and metabolic responses to different levels of compression during submaximal exercise. Phlebology, 26: 102-106.

Stearns, R.L., Casa, D.J., Lopez, R.M., McDermott, B.P., Ganio, M.S., Decher, N.R., Scruggs, I.C., West, A.E., Armstrong, L.E., & Maresh, C.M. (2009). Influence of hydration status on pacing during trail running in the heat. Journal of Strength and Conditioning Research 23: 2533-2541.

Sultana, F, Abbiss, C.R., Louis, J, Bernard, T, Hausswirth, C, & Brisswalter, J (2012). Age-related changes in cardio-respiratory responses and muscular performance following an Olympic triathlon in well-trained triathletes. European Journal of Applied Physiology, 112, 1549-1556.

Van Beekvelt, M.C., Colier, W.N., Wevers, R.A., & Van Engelen, B.G. (2001). Performance of near-infrared spectroscopy in measuring local O2 consumption and blood flow in skeletal muscle. Journal of Applied Physiology, 90, 511-519.

Varela-Sanz, A, Espana, J, Carr, N, Boullosa, D.A., & Esteve-Lanao, J (2011). Effects of gradual-elastic compression stockings on running economy, kinematics, and performance in runners. Journal of Strength and Conditioning Research 25: 2902-2910.

Wank, V, Frick, U, & Schmidtbleicher, D (1998). Kinematics and electromyography of lower limb muscles in overground and treadmill running. International Journal of Sports Medicine, 19,455-461.

Chapter 7

Intervention study 2

7.1. INTRODUCTION

After investigating an applied model with high applicability, it was concluded that a more mechanistic and sensitive laboratory-based approach might be more fruitful in investigating smaller effects. Therefore, in order to elaborate on the high fatigue values encountered in the initial study, a study was conceived that aimed at reducing eccentric muscle damage. During the descent phase of the exercise, trail runners are subject to a large amount of eccentric contractions that have the tendency to cause structural disruption. While numerous pre-exercise treating modalities exist, the connection between heat exposure and potential reduction of eccentric damage is of particular interest to be further investigated. This coupling is related to small chaperone proteins of the heat shock protein (HSP) family. It is known that HSP are ubiquitous proteins and are relatively stress-independent in their function. HSP expression has been shown to increase with heat exposure, but also in the wake of mechanical stress, such as eccentric contractions. Increased HSP leads to a protector effect that increases cell viability. The experiment entailed a controlled trail in which the experimental group was subject to passive heating before eccentric muscle damage was induced by running downhill for 30 minutes. The control group on the other hand had no prior intervention. To extend the potential findings, a second eccentric session was completed by both groups 3 to 5 weeks following initiation. Before testing and in the 2 days following neuromuscular properties, neuromuscular activation patterns and voluntary activation were assessed. Results indicate that passive heating 48 hours pre-exercise can reduce functional consequences of eccentric-induced muscle damage (EIMD). Force depression is similar, but recuperation is enhanced while activation parameters stay comparable. In the second downhill session, these effects become more flagrant and initial depression is dampened. While this is an agreeable circumstance following a 30-minute run, it has the potential to extensively ameliorate the performance and decrease injury liability of ultra-trail runners, who are affected in-run by the development of muscle damage. This is all the more applicable to multi-day running races. As this study was only recently terminated, the article is still in draft form, soon to be submitted.

7.2. THE EFFECTS OF PRIOR PASSIVE HEATING ON FUNCTIONAL CONSEQUENCES OF EXERCISE-INDUCED MUSCLE DAMAGE

The effects of prior passive heating on functional consequences of exercise-induced muscle damage

Easthope CS, Burdon C, Fiatarone-Singh MA, Brisswalter J, Caillaud C

Abstract

It has recently been proposed that heat shock protein (HSP) dynamics may be one of the underpinning mechanisms of the repeated bout effect. While HSP can be induced through exercise, a similar response can be procured through passive heating. Therefore the effect of passive heating on force reduction through eccentric exercise was compared to the effects of prior exercise and rest. In the intervention group, HSP27 and HSP72 were induced (3-fold increase) through immersion of the lower limbs for 75 minutes in warm water (T_{water} = 41.5 °C, T_{rec} = 39.5 °C). Eccentric damage was then induced 48 hours later through downhill running for 30 minutes at -15% on a treadmill and force, muscle activation and voluntary activation were recorded at pre, post, post 24 and post 48 hours. Three to five weeks later both groups repeated the exercise and force measurements. Prior heating showed beneficial effects on the force profile in both bouts, recuperation being accelerated and initial reduction decreased in bout 2 (+10%). The positive effects were magnified in the second repetition for the heated group. From the results it is viable to propose prior passive heating as a strategy to mitigate functional impairment to eccentric damage. This also indicates that HSP is in some capacity involved in the repeated bout effect.

Introduction

Exercise-induced muscle damage (EIMD) is a well-described phenomenon observed primarily following unusual physical exercise, and particularly in response to eccentric (lengthening) muscle contractions. EIMD also involves fully-reversible muscle soreness and functional impairment that can last several weeks. Since the first description of EIMD in the early 20th century[1], a large body of literature has been developed leading to a well founded, if not complete, understanding of the processes involved (for reviews please consult references[2–9]). The aetiology of EIMD is generally considered bimodal[10–12] and can be divided into a number of intertwined responses following the mechanical induction: disturbance of the Ca^{2+} balance, inflammatory response and stress protein signalling. The sum of these responses leads to protein degradation and subsequent restructuring of the damaged tissue. Eccentric contractions evoke a smaller amount of motor units to create a higher force, therefore concentrating the force generation on a small amount of fibers[13]. The sarcomere popping theory proposes that mechanical disruption is created by the "popping" of sarcomeres that are overstretched due to non-linear elongation throughout the fiber[14–17]. Mechanical induction has been identified mainly by histochemical analysis of muscle tissue[18–22]. Analysis of muscle biopsies only give insight into a very small region of the muscle (5-20 mg)[23,24] and inherently induced a certain amount of damage in the tissue [25]. However,

biopsies have showed that disruption is mainly seen around the z-disk region (Z-disk streaming)[18,22] and through the loss of desmin[26–28], a protein composing the ultra-structural integrity of the fiber. After an intense bout of eccentric exercise, a disruption of up to 50% of the muscle volume has been reported[19,22]. The nature of EIMD allows differentiation into two distinct phases, a mechanical disruption phase[27,29–31] and an ensuing inflammation phase[28,32,33]. Subsequently, muscle remodelling is stimulated leading to repair of the damaged fibers[2,34–37] and to the restoration of pre-exercise muscle force-generating capacity[9]. Functionally, EIMD is characterized by a prolonged loss of maximal force generation capacity, a delayed and prolonged increase of perceived muscle soreness, a decrease in neuromuscular efficiency, a reduction in the range of motion and increase of muscle circumference and a subsequent increase in optimal contraction length. This is accompanied by increases in bulk proxy damage markers such as creatine kinase (CK), lactate dehydrogenase (LDH) and plasma interleukin-6 (Il-6)[9,38].

Both phases of the EIMD engender heat shock protein (HSP) activity, a group of highly conserved ubiquitous stress response proteins which are classified into families by their molecular weight. Especially HSP 27 and HSP72, proteins associated with chaperoning and the prevention of protein agglomeration, have been reported to increase activity following muscle damage induction[21,39–41]. HSP responds to a number of environmental stimuli including heat stress[42], mechanical stretch[43], metabolic stress, oxidative stress, ischemia, hypoxia, intra-cellular calcium and energy depletion[44], hypoxia and acidosis[45]. Not only does an induction of a stressor confer subsequent protection against the same stressor[52], but also against others (cross-tolerance)[53]. For instance, Goto et al.[54] reported that mechanical stress increased cell viability against heat shock and Horowitz et al.[55] observed that heat acclimatization can decrease necrosis in ischemic-reperfusion injury.

The cyto-protection conferred through a single bout of HSP induction has been proposed to play a central role in the attenuation of EIMD through repeated bouts, termed the repeated bout effect (RBe)[3,46,47]. In a second bout of eccentric exercise an attenuation of indirect functional markers and direct indicators such as tissue histograms, tagged neutrophil invasion[48] and HSP activity[21] has frequently been reported. The origin of this attenuation is unclear, but certain cornerstones have been identified: the RBe lasts from 2 weeks to 6 months but is most effective at around 6 to 8 weeks[49], reflex amplitude is increased, and mild initial damage already confers a protective effect for a more strenuous following session[50,51]. HSP activity was analysed after multiple bouts by Thompson et al.[52], who observed a marked response that was blunted in the second bout along with the typical attenuation of functional parameters and serum CK response. This was accompanied by a decrease in baseline HSP expression, suggesting that lower basal HSP levels may be beneficial to HSP action by creating a larger relative response[52]. Paulsen et al.[21] observed a marked increase in HSP27 and HSP72 activity following a second bout of eccentric exercise and also demonstrated a shift in localisation of the small HSPs to the areas of myofibrillar disruption. Following a second bout of moderate EIMD (-15% maximal torque), Vissing et al.[53] confirmed the global increase of HSP response but did not observe the shift in localisation, suggesting that this may be dose dependent.

Two studies have investigated the effect of prior HSP induction through heat shock on a subsequent bout of eccentric exercise. Touchberry et al.[54] recently demonstrated that heat shock induction 48 hours

before a bout of downhill running resulted in a greater HSP72 response and reduced CK activity and immune cell infiltration in Wistar rats. Nosaka et al.[55] used short (20 minutes) heat shock 24 hours before severe eccentric exercise in human biceps muscles and observed accelerated recovery of maximal biceps torque compared to a control group. In a second bout the heated group demonstrated even further accelerated recovery. These observations suggest that prior HSP induction does indeed have a beneficial effect on eccentric damage reduction.

Expanding on these previous studies, the presented experiment was conducted on humans and targeted the main locomotor muscles using a commonly available form of heating while quantifying the heat induced HSP increase. It was hypothesized that passive heat exposure 48 hours prior to a bout of downhill running would reduce functional impairment and decrease recovery time both in this initial bout and in a follow-up bout.

Methods

Participants

Twenty-seven young and healthy sedentary subjects of both genders volunteered to participate in the experiment. Subjects were recruited from the staff and students of the health science faculty via printed advertisements. Inclusion criteria dictated that subjects had not been exposed to any form of prior heat conditioning or eccentric or resistance training experience. Additionally, any medium-term history of musculoskeletal injury in the lower limb or any intake of regular medication led to exclusion. All subjects, after explanation of the protocol, signed an informed consent form that was approved by the University of Sydney Ethics committee (Project number: 13971).

Study design

A controlled randomized study design was conducted. The protocol consisted of a preliminary session in which subjects were introduced to the testing apparatus and baseline values, twitch intensity and $\dot{V}O_2max$ were determined. After 1 week rest, they returned for either passive heating or passive sitting (randomized), followed by the first eccentric session (B1) 48 hours later. A subset of subjects returned 3 to 5 weeks later for a second eccentric session (B2). Muscle function evaluation tests were performed before, immediately post, 24 hours and 48 hours after each eccentric session. During the course of the study subjects completed daily activity logs and also specifically refrained from any strenuous activity for 48 hrs preceding any of the sessions. Caffeine intake was prohibited in the 6 hours before each session and testing hours were respected to avoid diurnal effects. The protocol is graphically presented in Figure 1.

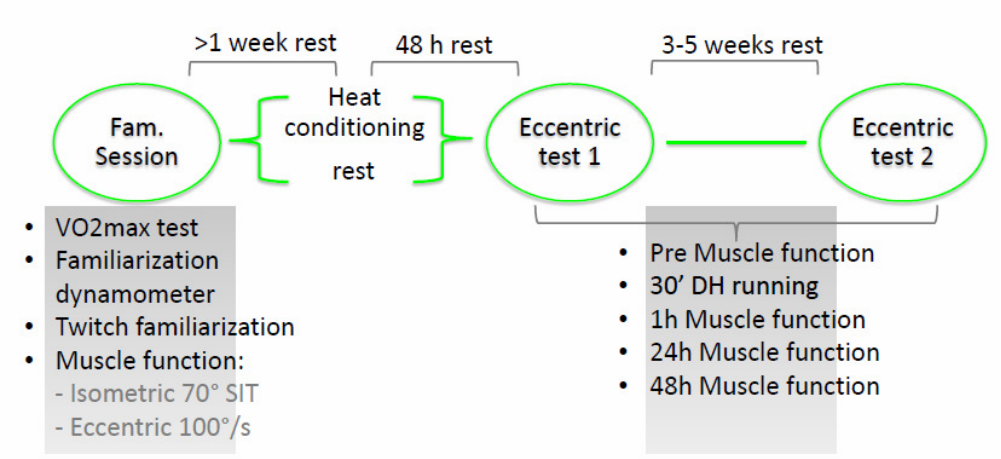

Figure 1: Testing protocol

Maximal oxygen uptake test

In order to standardize the metabolic load of the eccentric sessions, a ramp running protocol to exhaustion was completed while oxygen uptake ($\dot{V}O_2$), ventilation volume ($\dot{V}_e$), heart rate (HR) and rate of perceived exertion (RPE) were monitored. Respiratory measures were recorded using a breath-by-breath metabolic cart (Medgraphics Ultima, Norfolk, UK) which was calibrated using a 3 L syringe and calibration gas before each subject. Heart rate was captured using a telemetric heart rate monitor (Polar S410, Kemple, Finnland) throughout the complete exercise. A Borg scale was used to determine RPE at the end of every minute during exercise. The ramp protocol was completed on a treadmill at 1% inclination and consisted of a 5-minute warm up at 9 $km \cdot h^{-1}$ followed by an increase of 1 $km \cdot h^{-1}$ per minute until exhaustion. $\dot{V}O_2max$ was subsequently determined as the maximal $\dot{V}O_2$ over 15 seconds after classical plateau criteria had been fulfilled. The highest speed at 70% of $\dot{V}O_2max$ (v70) during the ramp was defined as running speed for the eccentric sessions.

Initial twitch calibration

Subjects were securely strapped to a dynamometer with real time force output and two oval rubber carbon electrodes (8x13cm) were placed proximally and distally over the quadriceps femoris. These were connected to a pulse generator (Digitimer DS7A, Welwyn Garden City, England; 200 µs, 400 V, custom amperage). After briefing the subject, double pulses (100Hz) at increasing amperage were given until 70% of MVIC was reached or the subject reached their pain threshold. Stimulation started at an intensity of 50 mA and 10 seconds relaxation time was guarded between pulses. The maximal achieved twitch intensity was recorded and used for all other sessions.

Muscle function evaluation

Subjects were equipped with a 4-channel electromyogram (EMG) recording unit (Noraxon TeleMyo 2400T, Scottsdale, USA) on the three superficial quadriceps femoris (QF) muscles and the short head of the biceps femoris (BF). EMG was recorded in bipolar configuration from sites determined using SENIAM guidelines[56,57] and skin was prepared using a combination of dry shaving, alcohol and abrasive

cream to ensure a resistance of <5 kΩ. Signals were pre-amplified and bandwidth filtered (6 - 500 Hz) and synchronized with the force and angle signals. Electrode placement was meticulously delimitated used indelible marker to ensure congruent placement throughout all sessions. Additionally, two rubber carbon electrodes were placed proximally and distally over the QF and connected to the pulse generator in order to facilitate percutaneous twitch elicitation.

Subjects were subsequently strapped securely to a Biodex dynamometer (Biodex System 3, Shirley, USA) in order to determine maximal voluntary isometric quadriceps force (MVIC) and voluntary activation (%VA). A standardized warm-up consisting of 20 isometric contractions (70° flexion) at 0.5 Hz and 50% MVIC was completed followed by two ramping contractions to 70% MVIC over 3 seconds interspersed with 30 s rest. MVIC testing was completed at 70° flexion using a standard position and strong verbal encouragement. Three MVICs were performed with 1 minute rest between. Each MVIC was accompanied by 5 doublets (100 Hz) at different timepoints: 5 seconds before contraction initiation, 1 second after contraction initiation, and 5, 10 and 15 seconds after contraction cession. EMG, torque and stimulation triggers were recorded for each contraction.

Post-exercise, EMG data was delineated to exclude the twitch artefacts. A 50 Hz notch filter was applied followed by a 3rd order Butterworth filter (6 - 100 Hz). The signal was then rectified and RMS was calculated for each contraction. Voluntary activation was calculated using the interpolated twitch technique (ITT) by expressing superimposed twitch amplitude as a percentile ration of rest twitch amplitude[58]:

$$VA\ [\%] = \left[1 - \left({Superimposed\ twitch}/{Control\ twitch}\right)\right] x\ 100$$

Passive heating

Passive heating of the lower extremities was conducted through full lower-body immersion in warm water for 75 minutes. Water temperature was constantly regulated at 41.5 °C and the subject adopted a sitting position with immersion up to the superior iliac crest, ensuring that the main propulsive muscles were under water. To reduce heat elimination ambient conditions were controlled to 27 °C and 60% RH. Subjects self inserted a rectal thermometer to monitor core temperature throughout the immersion and HR were recorded. Blood pressure and RPE were verified at 5 minute intervals as a precaution against excessive vasodilation.

Eccentric exercise

In order to elicit eccentric muscle damage subjects completed a 30 minute downhill running protocol at a speed corresponding to their 70% $\dot{V}O_2$max (v70). Inclination was set to -15% and subjects were equipped with a HR monitor and the same respiratory apparatus as during the initial ramp protocol. Ambient climate was regulated to 25 °C and 40% RH.

Tissue acquisition

Muscle biopsy samples were obtained from 5 volunteers 10 days before and 48 hrs after passive heating. Collection of samples from the vastus lateralis muscle was completed under local anesthesia using pre-incision and 2-3 passes of a 6 mm Bergstrom needle with suction enhancement. Samples were immediately frozen in liquid nitrogen. For western blot analysis, tissues were homogenized in a 10:1 (volume-to-weight) ratio of ice-cold extraction buffer containing 10 mM Tris-HCl (pH 7.4); 100 mM NaCl; 1 mM each of EDTA, EGTA, NaF, and phenylmethylsulfonyl fluoride; 2 mM Na_3VO_4; 20 mM $Na_4P_2O_7$; 1% Triton X-100; 10% glycerol; 0.1% SDS; 0.5% deoxycholate; and 10 ul/ml protease inhibitor cocktail. Extracted samples were prepared in Laemli buffer (MilliQ water 3.8 ml, 0.5 M Tris pH6.8 1.0 ml, glycerol 0.8 ml, 10% SDS 1.6 ml, 2-mercaptoethanol 0.4 ml, 1% bromophenol blue 0.4 ml) and heated 5 min at 95 ^{o}C. Proteins (20 µg) were separated on a SDS-PAGE (8–10% gel, 200 V, 1 h) followed by a wet transfer to a nitrocellulose membrane for 90 min (200 mA). The nitrocellulose was blocked using 5% non-fat milk in TBS and washed with TBS plus 0.1% Tween-20 then incubated overnight at 4 °C with primary antibody in 1% milk (HSP72, 1/2000). Membranes were then washed and incubated HRP-conjuged secondary anti bodies (mouse for HSP72 and goat anti-rabbit for HSP27). Results were visualised with ECL reagent (Millipore) and visualized using the ChemiDoc XRS+ and image Lab software (Bio-Rad).

Statistics

Data are expressed as means ± SD. Normality and sphericity were verified for all variables. HSP results were treated using a simple paired t-test after verifying normal distribution. Results for the both eccentric sessions were computed using a two-way repeated measures ANOVA (group{2} x time period {4}) followed by a Newman-Keuls posthoc test to identify and between-means differences. The same method was applied on the percentile difference between sessions following a greenhouse-geisser correction in both groups. Statistical significance was globally set at $p = 0.05$.

Results

Population

From 30 recruited participants, 27 completed the first eccentric exercise and follow-up and 12 completed the full protocol. Therefore, the reported results concerning tissue samples are for 5 subjects, concerning bout 1 are for 27 subjects and concerning both bouts are for 12 subjects (Table 1).

	Heat	Control
Population	13	14
Age [years]	27.1 ± 3.9	27 ± 3.9
Weight [kg]	69.4 ± 9.5	68.9 ± 12.6
Height [cm]	174.9 ± 8.1	173.3 ± 8.1
$\dot{V}O_2max$ [$mL \cdot min^{-1} \cdot kg^{-1}$]	51.3 ± 8.6	46.3 ± 8.7

Table 1: Physiological characteristics of subjects in Heat and Control groups

Passive heating

All 27 subjects described a similar kinetic of core temperature increase. At around 60 minutes core temperature reached 39 °C and remained at this level until the end of the measurement period at 90 minutes. Heart rate increased to 121 ± 14 and RPE increased up to 18 ± 2 at the end of the 75 minutes immersion time.

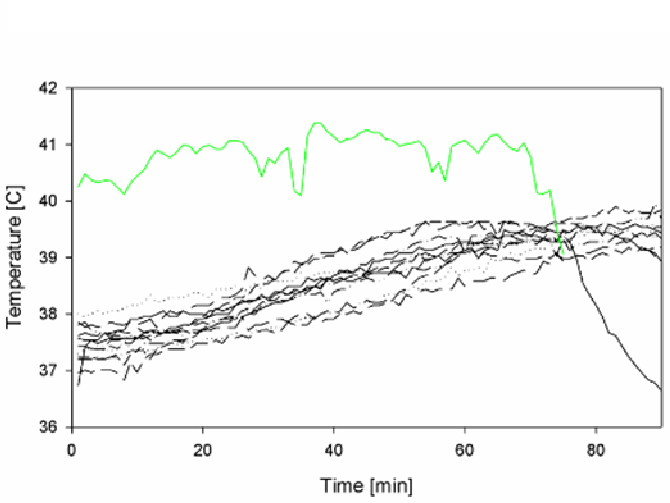

Figure 2: Core temperature during passive heating. Core temperatures continue to drift after immersion is terminated. Solid green line – mean immersion medium temperature, broken lines – individual core temperatures.

Tissue samples

On average 146.2 ± 78mg of muscle tissue was recuperated from the 5 subjects per collection. Following the passive heating intervention, HSP72 and HSP27 expression increased significantly to 3.7 ± 1.9 fold and 3.4 ± 0.4 fold compared to baseline respectively (Figure 3).

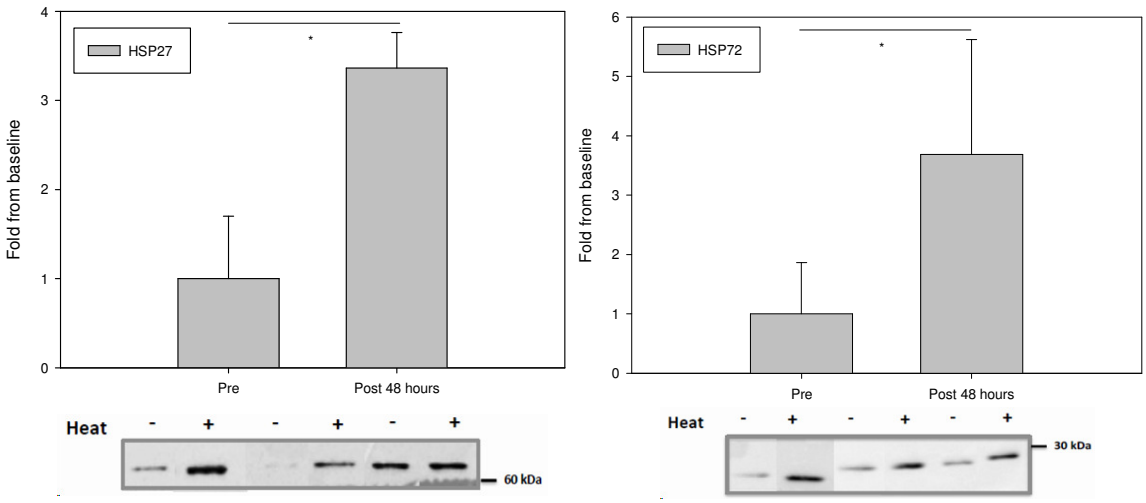

Figure 3: HSP72 and HSP27 before and 48 hours after passive heating (n = 5). * indicates a significant difference from pre to post 48 hours.

Muscle function testing

Downhill running was completed at 11 ± 2 $km \cdot h^{-1}$ which corresponded to 58 ± 6% of $\dot{V}O_2max$. Following downhill running, a strong main effect of within-session repetition on MVIC was found ($p < 0.01$) with no effect of group ($p = 0.48$). Posthoc analysis indicates that no group returned to baseline force ($p < 0.01$) in the first eccentric session, although a non-significant trend to decreased force depression and accelerated recovery in the heat group was observed (Fig 4). In the second eccentric session the non-significant trend was reinforced, heat subjects returning to baseline force by p48 ($p = 0.09$) while control subjects did not ($p < 0.01$, Fig 4).

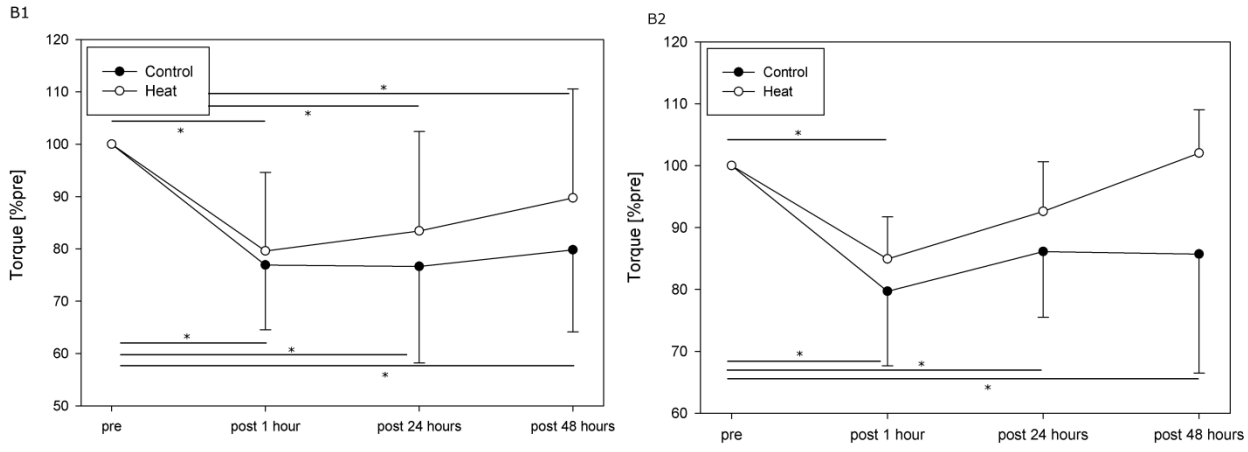

Figure 4: Changes in maximal voluntary isometric torque in the first (B1, n = 27) and second (B2, n = 12) eccentric sessions. * denotes a significant change over time.

The percentile differences between eccentric sessions indicate a main effect for both group ($p = 0.01$, $\eta = 0.55$) and repetition ($p = 0.02$, $\eta = 0.37$). The heat group suffered 10% less initial force depression than in the first bout and were 25% stronger at post 48 hours. The control group on the other hand showed no beneficial effects until post 48, where they were 10% stronger than in the first bout (Fig 5).

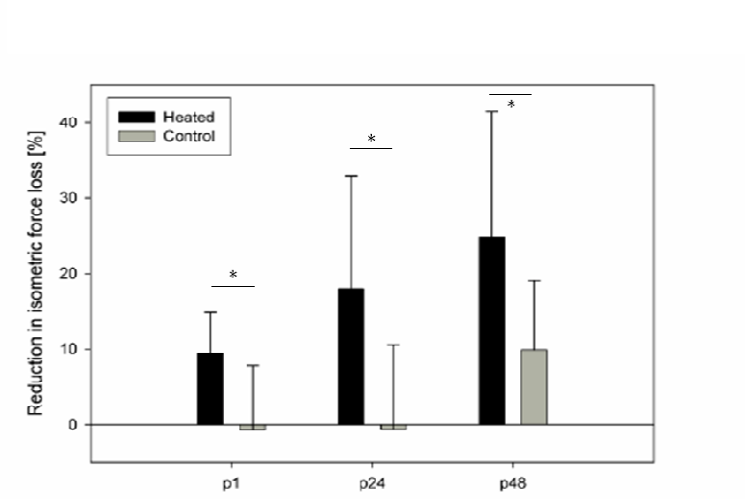

Figure 5: Percentile differences between changes in MVIC in bouts 1 and 2 (n = 12).

No significant differences were evident in the electromyographic data in any of the recorded muscles independent of group, session or time point, values ranging from 83% to 130% of pre-testing RMS. Equally resting twitch amplitude remained unchanged in both groups and sessions ($p = 0.3 - 0.6$). Voluntary activation (Fig 6) remained stable in bout 1 for the heat group ($p = 0.09 - 0.79$), while declining significantly in the control group ($p = 0.00 - 0.03$). In bout 2, both groups' voluntary activation initially remained stable ($p = 0.1 - 0.2$), but the control group suffered decline at p48 ($p = 0.04$) which led to a significant difference between groups ($p = 0.02$).

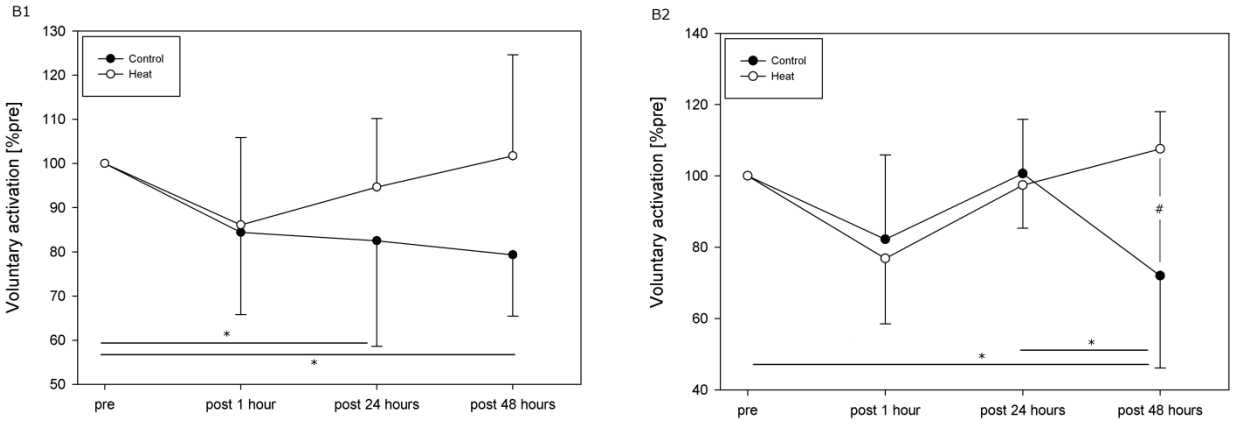

Figure 6: Changes in voluntary activation in the first (B1, n = 27) and second (B2, n = 12) eccentric session. * denotes significant changes between timepoints. # shows differences between groups.

Discussion

The results indicate that passive heating increases HSP expression in the heated muscles and provides cytoprotection against functional muscle decrements when applied 48 hours before eccentric exercise. The changes in force in combination with unchanged activation and co-activation parameters indicate that the intervention effectively induced muscle damage. A trend to reduced torque depression in the heat group was observed in bout 1, yet the main impact manifested itself in the second bout. There was a significant effect of heating on the percentile changes of force depression from bout 1 to bout 2, indicating that heated subjects gained an advantage over the control group in terms of functional impairment. This was the case for both initial force depression and especially for recovery of baseline force. Voluntary activation data on the other hand expressed a beneficial effect of heating in both bouts. Prior heating therefore is an effective strategy for decreasing functional impairment following EIMD.

As is well-known, HSPs are readily induced by increased tissue temperature. Testing in animal and cell models has indicated that a tissue temperature of > 41.5 °C is necessary for induction. Initially we endeavoured to control muscle temperature during the passive heating protocol, yet this proved complicated due to the submerged position of the tissue and may have been non-indicative of induction[59]. Therefore a subgroup of 5 subjects was recruited to enable quantification of HSP induction by the heating protocol. The results are in line with other heat induction results reported in the literature. Oishi et al.[60] for example found an increase in HSP72 of 2.5 to 6.8 fold in rat soleus 48 hours after heating to 42 °C. Eccentric exercise has been found to increase HSP27 and HSP72 to 2 fold and 10 fold respectively at post 48 hours after severe eccentric exercise in human muscle[39]. Therefore, while the HSP27 results are similar to what would be induced by a bout of eccentric exercise, HSP72 induction is far below.

Voluntary activation and muscle activation parameters were originally only assessed as control parameters to ensure that there are no significant changes in activation patterns or motivation. It has been proposed that the repeated bout effect stems mainly from changes in activation patterns and strategies[13], but in this study no such changes were evident using surface EMG. Interestingly, voluntary activation evolved differently after EIMD in the two groups – the heat group maintaining normal activation ratios while the non-heated group manifested exercise-induced decline. This holds true from both the first and second bout of exercise. A decline in voluntary activation following EIMD has been frequently reported[9] and is presumed to be related to increased group III and IV afference firing due to peripheral nociceptor and pressure sensor activation which reduce voluntary drive[61,62]. Attenuated in the heated group may occur either through reductions in swelling or through reduced perceived pain.

Maximal voluntary isometric force reduction was considered the main outcome variable, and has recently also been proposed as the best indicator of EIMD[2]. As presented in the results, participants suffered an acute force loss of around -20% in the first bout and the exercise can therefore be classified as mild eccentric exercise and is comparable to other protocols investigating downhill running[63]. In bout 1 there is a prevailing trend to faster restoration of baseline force in the heat group while initial induction is similar to control. Although HSP expression was elevated in the heat group at the beginning of the exercise, there was no impact on acute damage development. This was quite surprising, as HSP

are highly implicated in cytoprotection processes and were expected to limit damage development through actively managing debris removal and thus reducing the subsequent inflammation phase[54]. Similar to the observations of Nosaka et al.[55], our results indicate that elevated HSP expression during exercise has no mitigating effect on acute damage development. This may be due to the rapidity of damage development[27] which might surpass the translocation delay of HSP into the critical regions[21,64]. In any case, elevated HSP during exercise seems to be beneficial for the recuperation process. This is not surprising, as HSP72 in particular is associated with debris clearing and chaperoning of damaged protein segments[45]. Effectively it may be that the only benefit of pre-inducing HSP is an elimination of the HSP expression phase, which generally takes from 24 to 48 hours to reach its peak. In the pre-induced muscles, HSP is already plays an active role in the clearance and restructuring, thus diminishing inflammation and subsequent ROS stress. This could explain the enhanced recovery of force.

In the second bout of eccentric exercise, the heated group show comparatively less initial damage and further enhanced recovery. Earlier studies on HSP expression during a repeated bout effect report inconclusive results concerning changes of basal HSP expression[21,52,53]. Nonetheless the results indicated either a relative increase in HSP response[52,53], or an increase in the cytoskeletal fraction[21]. Without having procured tissue samples from the respective subjects, it is difficult to extrapolate how the HSP adaptation will have changed from a single peak (Control) to a double-peaked (Heat) initial invocation. From muscle function alone, we can surmise that a double peaked profile is somewhat beneficial in limiting both initial insult and in accelerating recovery. These results expand on the observations of Nosaka et al.[55], who observed an increased beneficial effect of prior heat exposure on recovery in a second bout of eccentric exercise 2-4 weeks after the initial bout.

There was significant variance in the results, suggesting that subjects either showed different degrees of response to the eccentric stimulus, or adapted running strategies to mitigate EIMD development. Downhill running was chosen as an eccentric modality because of its applicability and pertinence in everyday life. The findings in this study would have been strengthened by the collection of tissue samples before and after both eccentric bouts. Recruitment for this type of protocol proved difficult, as no compensation could be offered due to funding limitations. Additionally, force depression could have been more precisely quantified by using evoked contractions of the knee extensors instead of voluntary. The use of transcranial magnetic stimulation (TMS) to determine the origin of voluntary activation differences in groups would also have been interesting[65]. In a future study we would consider using a more precisely defined eccentric protocol modelled after the one employed by Nosaka et al.[55] for the lower limb, assessing evoked contractions, and using TMS to elucidate the origin of changes in voluntary activation. Furthermore, it could be interesting to quantify changes in conduction velocity of the muscle membrane using high density EMG arrays.

Conclusion

Passive heating has a beneficial effect on force reduction induced by a mild eccentric damage protocol conducted 48 hours later. A trend to attenuation of initial force depression and acceleration of the recovery of baseline is apparent in the heated group. In a second bout conducted 3 to 5 weeks later, the beneficial effects were amplified in comparison to the control group, who only completed an exercise

bout. Initial force depression is limited and baseline force is recovered by 24 hours post-exercise. This can be related to an increased HSP expression in the heated muscle, indicating that the expression of HSP27 and HSP72 is implicated in the repeated bout effect.

Bibliography

1. Hough T. Ergographic studies in muscular soreness. *Am J Physiol*. 1902; 7(1):76–92.

2. Paulsen G, Mikkelsen UR, Raastad T et al. Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exerc Immunol Rev*. 2012; 18(1):42–97.

3. Morton JP, Kayani AC, McArdle A et al. The exercise-induced stress response of skeletal muscle, with specific emphasis on humans. *Sports Med Auckl Nz*. 2009; 39(8):643–662.

4. Clarkson PM, Hubal MJ. Exercise-induced muscle damage in humans. *Am J Phys Med Rehabil*. 2002; 81(11):52–S69.

5. Clarkson PM, Sayers SP. Etiology of Exercise-Induced Muscle Damage. *Can J Appl Physiol*. 1999; 24(3):234–248.

6. Fridén J, Lieber RL. Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components. *Acta Physiol Scand*. 2001; 171(3):321–326.

7. Proske U, Morgan DL. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *J Physiol*. 2001; 537(Pt 2):333–345.

8. Stupka N, Tarnopolsky MA, Yardley NJ et al. Cellular adaptation to repeated eccentric exercise-induced muscle damage. *J Appl Physiol*. 2001; 91(4):1669–1678.

9. Byrne C, Twist C, Eston R. Neuromuscular Function After Exercise-Induced Muscle Damage. *Sports Med*. 2004; 34(1):49–69.

10. Ingalls CP, Warren GL, Williams JH et al. E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. *J Appl Physiol*. 1998; 85(1):58–67.

11. Faulkner JA, Jones DA, Round JM. Injury to skeletal muscles of mice by forced lengthening during contractions. *Q J Exp Physiol Camb Engl*. 1989; 74(5):661–670.

12. MacIntyre DL, Reid WD, Lyster DM et al. Presence of WBC, decreased strength, and delayed soreness in muscle after eccentric exercise. *J Appl Physiol*. 1996; 80(3):1006–1013.

13. Enoka RM. Eccentric contractions require unique activation strategies by the nervous system. *J Appl Physiol*. 1996; 81(6):2339–2346.

14. Morgan DL. New insights into the behavior of muscle during active lengthening. *Biophys J*. 1990; 57(2):209–221.

15. Talbot JA, Morgan DL. Quantitative analysis of sarcomere non-uniformities in active muscle following a stretch. *J Muscle Res Cell Motil*. 1996; 17(2):261–268.

16. Talbot JA, Morgan DL. The effects of stretch parameters on eccentric exercise-induced damage to toad skeletal muscle. *J Muscle Res Cell Motil*. 1998; 19(3):237–245.

17. Telley IA, Stehle R, Ranatunga K et al. Dynamic behaviour of half-sarcomeres during and after stretch in activated rabbit psoas myofibrils: sarcomere asymmetry but no "sarcomere popping." *J Physiol*. 2006; 573(1):173–185.

18. Fridén J, Sjöström M, Ekblom B. A morphological study of delayed muscle soreness. *Experientia*. 1981; 37(5):506–507.

19. Fridén J, Sjöström M, Ekblom B. Myofibrillar Damage Following Intense Eccentric Exercise in Man. *Int J Sports Med*. 1983; 04(03):170–176.

20. Newham DJ, McPhail G, Mills KR et al. Ultrastructural changes after concentric and eccentric contractions of human muscle. *J Neurol Sci*. 1983; 61(1):109–122.

21. Paulsen G, Lauritzen F, Bayer ML et al. Subcellular movement and expression of HSP27, alphaB-crystallin, and HSP70 after two bouts of eccentric exercise in humans. *J Appl Physiol*. 2009; 107(2):570–582.

22. Lauritzen F, Paulsen G, Raastad T et al. Gross ultrastructural changes and necrotic fiber segments in elbow flexor muscles after maximal voluntary eccentric action in humans. *J Appl Physiol*. 2009; 107(6):1923 –1934.

23. Warren GL, Lowe DA, Armstrong RB. Measurement tools used in the study of eccentric contraction-induced injury. *Sports Med*. 1999; 27(1):43–59.

24. Beaton LJ, Tarnopolsky MA, Phillips SM. Variability in estimating eccentric contraction-induced muscle damage and inflammation in humans. *Can J Appl Physiol Rev*. 2002; 27(5):516–526.

25. Malm C, Nyberg P, Engström M et al. Immunological changes in human skeletal muscle and blood after eccentric exercise and multiple biopsies. *J Physiol*. 2000; 529(Pt 1):243–262.

26. Barash IA, Peters D, Fridén J et al. Desmin cytoskeletal modifications after a bout of eccentric exercise in the rat. *Am J Physiol - Regul Integr Comp Physiol*. 2002; 283(4):958 –963.

27. Lieber RL, Thornell LE, Fridén J. Muscle cytoskeletal disruption occurs within the first 15 min of cyclic eccentric contraction. *J Appl Physiol*. 1996; 80(1):278–284.

28. Belcastro AN, Parkhouse W, Dobson G et al. Influence of exercise on cardiac and skeletal muscle myofibrillar proteins. *Mol Cell Biochem*. 1988; 83(1):27–36.

29. Duan C, Delp MD, Hayes DA et al. Rat skeletal muscle mitochondrial [Ca2+] and injury from downhill walking. *J Appl Physiol*. 1990; 68(3):1241–1251.

30. Lowe DA, Warren GL, Hayes DA et al. Eccentric contraction-induced injury of mouse soleus muscle: effect of varying [Ca2+]. *J Appl Physiol*. 1994; 76(4):1445–1453.

31. Yasuda T, Sakamoto K, Nosaka K et al. Loss of sarcoplasmic reticulum membrane integrity after eccentric contractions. *Acta Physiol*. 1997; 161(4):581–582.

32. Belcastro AN. Skeletal muscle calcium-activated neutral protease (calpain) with exercise. *J Appl Physiol*. 1993; 74(3):1381–1386.

33. Belcastro AN, Shewchuk LD, Raj DA. Exercise-induced muscle injury: a calpain hypothesis. *Mol Cell Biochem*. 1998; 179(1-2):135–145.

34. Shen W, Li Y, Zhu J et al. Interaction between macrophages, TGF-β1, and the COX-2 pathway during the inflammatory phase of skeletal muscle healing after injury. *J Cell Physiol*. 2008; 214(2):405–412.

35. Bondesen BA, Mills ST, Kegley KM et al. The COX-2 pathway is essential during early stages of skeletal muscle regeneration. *Am J Physiol - Cell Physiol*. 2004; 287(2):475–483.

36. Bondesen BA, Mills ST, Pavlath GK. The COX-2 pathway regulates growth of atrophied muscle via multiple mechanisms. *Am J Physiol - Cell Physiol*. 2006; 290(6):1651–1659.

37. Yu J-G, Fürst DO, Thornell L-E. The mode of myofibril remodelling in human skeletal muscle affected by DOMS induced by eccentric contractions. *Histochem Cell Biol*. 2003; 119(5):383–393.

38. Nosaka K, Clarkson PM, McGuiggin ME et al. Time course of muscle adaptation after high force eccentric exercise. *Eur J Appl Physiol*. 1991; 63:70–76.

39. Thompson HS, Scordilis SP, Clarkson PM et al. A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. *Acta Physiol Scand*. 2001; 171(2):187–193.

40. Thompson HS, Maynard EB, Morales ER et al. Exercise-induced HSP27, HSP70 and MAPK responses in human skeletal muscle. *Acta Physiol Scand*. 2003; 178(1):61–72.

41. Paulsen G, Vissing K, Kalhovde JM et al. Maximal eccentric exercise induces a rapid accumulation of small heat shock proteins on myofibrils and a delayed HSP70 response in humans. *Am J Physiol - Regul Integr Comp Physiol*. 2007; 293(2):844 –853.

42. Ritossa F. A new puffing pattern induced by heat shock and DNP in Drosophila. *Experientia*. 1962; 18(12):571–573.

43. Yoo SY, Chung C, Kim JK et al. Perinuclear translocation of hsp 27 in shear stress exposed human endothelial cells. *Biotechnol Lett*. 2005; 27(6):443–448.

44. Kabakov AE, Gabai VL. *Heat Shock Proteins and Cytoprotection: ATP-Deprived Mammalian Cells*. Springer; 1997.

45. Welch WJ. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and disease. *Physiol Rev*. 1992; 72(4):1063–1081.

46. Koh TJ. Do small heat shock proteins protect skeletal muscle from injury? *Exerc Sport Sci Rev*. 2002; 30(3):117–121.

47. Koh TJ, Escobedo J. Cytoskeletal disruption and small heat shock protein translocation immediately after lengthening contractions. *Am J Physiol - Cell Physiol*. 2004; 286(3):713–722.

48. Pizza FX, Davis BH, Henrickson SD et al. Adaptation to eccentric exercise: effect on CD64 and CD11b/CD18 expression. *J Appl Physiol*. 1996; 80(1):47–55.

49. Nosaka K, Sakamoto K, Newton M et al. How long does the protective effect on eccentric exercise-induced muscle damage last? *Med Sci Sports Exerc*. 2001; 33(9):1490–1495.

50. Hortobágyi T, Houmard J, Fraser D et al. Normal forces and myofibrillar disruption after repeated eccentric exercise. *J Appl Physiol*. 1998; 84(2):492–498.

51. Clarkson PM, Tremblay I. Exercise-induced muscle damage, repair, and adaptation in humans. *J Appl Physiol*. 1988; 65(1):1–6.

52. Thompson HS, Clarkson PM, Scordilis SP. The repeated bout effect and heat shock proteins: intramuscular HSP27 and HSP70 expression following two bouts of eccentric exercise in humans. *Acta Physiol Scand*. 2002; 174(1):47–56.

53. Vissing K, Bayer ML, Overgaard K et al. Heat shock protein translocation and expression response is attenuated in response to repeated eccentric exercise. *Acta Physiol*. 2009; 196(3):283–293.

54. Touchberry C, Gupte A, Bomhoff G et al. Acute heat stress prior to downhill running may enhance skeletal muscle remodeling. *Cell Stress Chaperon*.2012; 17(6): 693-705.

55. Nosaka K, Muthalib M, Lavender A et al. Attenuation of muscle damage by preconditioning with muscle hyperthermia 1-day prior to eccentric exercise. *Eur J Appl Physiol*. 2006; 99:183–192.

56. Hermens HJ, Freriks B, Disselhorst-Klug C et al. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol*. 2000; 10(5):361-374.

57. Hermens HJ, Freriks B. The state of the art on sensors and sensor placement procedures for surface electromyography: a proposal for sensor placement procedures. *Deliv Seniam Proj*. 1997.

58. Shield A, Zhou S. Assessing voluntary muscle activation with the twitch interpolation technique. *Sports Med Auckl Nz*. 2004; 34(4):253–267.

59. Morton JP, Maclaren DPM, Cable NT et al. Elevated core and muscle temperature to levels comparable to exercise do not increase heat shock protein content of skeletal muscle of physically active men. *Acta Physiol Oxf Engl*. 2007; 190(4):319–327.

60. Oishi Y, Taniguchi K, Matsumoto H et al. Muscle type-specific response of HSP60, HSP72, and HSC73 during recovery after elevation of muscle temperature. *J Appl Physiol*. 2002; 92(3):1097 –1103.

61. Le Pera D, Graven-Nielsen T, Valeriani M et al. Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clin Neurophysiol*. 2001; 112(9):1633–1641.

62. Gandevia SC, Allen GM, Butler JE et al. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol*. 1996; 490(Pt 2):529–536.

63. Braun WA, Dutto DJ. The effects of a single bout of downhill running and ensuing delayed onset of muscle soreness on running economy performed 48 h later. *Eur J Appl Physiol*. 2003; 90(1-2):29–34.

64. Morton JP, MacLaren DPM, Cable NT et al. Time course and differential responses of the major heat shock protein families in human skeletal muscle following acute nondamaging treadmill exercise. *J Appl Physiol*. 2006; 101(1):176 –182.

65. Millet GY, Bachasson D, Temesi J et al. Potential interests and limits of magnetic and electrical stimulation techniques to assess neuromuscular fatigue. *Neuromuscul Disord*. 2012; 22, Supplement 3(0):181–186.

Chapter 8
Discussion

8.1 Synopsis of results

In the four presented studies, there is a distinct evolution of orientation from a descriptive basis over a validation stage to intervention-based investigations. The studies build upon each other, as the descriptive studies give the design basis for a validation study which then subsequently serves as a model for the intervention studies. In synopsis, the research conducted during this thesis has included the development and validation of a trail running model to assess fatigue and the evaluation of two intervention strategies. In the first study, classical markers of fatigue were assessed in populations of different age groups. This allowed a preliminary quantitative assessment of fatigue-based changes that would be procured in a typical trial race using classical methodologies. Additionally, differences between age groups were determined and this proved valuable in determining the target population for later studies. In the second study, the reproducibility of classical fatigue markers was verified throughout four repetitions of a short trail run in a young and well-trained population. Results indicate that the first and fourth repetitions lead to deviant performance and fatigue when compared to the other repetitions. This was attributed to learning effects and adaptations related to the repeated bout effect. The third study examined potential benefits of wearing compression garments (CG) during a short trail running exercise using a randomised cross-over design with ample familiarization. Effectively, no differences were found in performance or fatigue between CG and non-CG trials, thus discounting this intervention strategy for this specific type of running course. In the final intervention study, a randomised control trial was used to determine the effects of pre-exercise heating on the functional consequences of two bouts of downhill treadmill running. Prior heating was found to have a beneficial functional effect following both the first and second running intervention. These results support the hypothesis of an HSP-driven repeated bout effect and represent the first recording of muscle damage in HSP-elevated humans. Rather than inherently solving a research paradigm of their own standing, the effectuated investigations and procured results represent part of a collaborative effort of the scientific community to achieve new perspectives and insights on an old research question.

8.2. Integration into the existing literature framework

As described in the individual introductions and discussions, there is a growing amount of research being conducted on trail running. While this can be attributed to the growing importance of trail running as an industry and a social economy, there is also a growing scientific interest in trail running as a model of fatigue. As with many other phenomena, there is often knowledge to be gained by investigating “special cases”. Trail running may be seen as just that: a “special case” of fatigue induction. In trail running, through the substantially greater eccentric strain coupled with prolonged exercise at a low muscular intensity, a number of

stimuli are superimposed. Interestingly and contrary to what may be expected through a simple summation of stimuli, the resulting fatigue expressed as a decrease in voluntary isometric knee extensor torque remains comparable to values reported in "flat" running[1,2]. When comparing the results of maximal voluntary isometric force reduction obtained in the present studies as a function of distance, values of around -8 % in 15 km[3] and -30 % in 55 km[4], these values are coherent with the relationship between distance and force decline presented in Chapter 2 in the section on the flush model (Section 2.4.5; Fig. 2.12). The reduction in maximal voluntary force generating capacity must, in this context, be seen as the sum of a fatigue-induced reduction (central and peripheral) and a damage-induced reduction (peripheral). The same congruent results have been remarked by Millet et al.[1] when comparing their results from the UTMB with the results obtained by Martin et al.[2] during a flat treadmill run. This leads to the question as to how the force depression can remain unchanged, although there is an additional damage stimulus present compared to flat running. The only possible explanation is that fatigue must be less potent, compensating for the additional depression. A similar "critical fatigue threshold" has been proposed by Amman et al.[5] who observed an increased peripheral fatigue in ergorecepter-blocked subjects. In both cases, central drive was supposedly modulated to compensate for the level of afferently indicated peripheral fatigue[5,6]. Before indulging in pure conjecture though, it should be verified that the additional muscle damage did actually occur. In the light that the trail runners were well-trained, a sufficient cytoprotective effect may have already been evoked which shielded the subjects from excessive muscle damage. CK values retrieved from the runners indicate differently though. In average, 24 hours post-trail subjects had a 2-fold increase in CK during the 15 km trail, and a 10-fold increase during the 55 km trail. While the CK values are not irrevocable proof that muscle damage has occurred, they nonetheless give a strong indication that some form of myofibrillar leakage did occur[7]. Additionally, changes in twitch and M-wave properties recorded after the 55 km trail run indicate that some form of peripheral alteration has taken place. To accurately verify the extent and form of muscle damage, muscle biopsies would need to be taken post trail, but no such study has been published to date. To recapitulate, the results obtained indicate similar fatigue compared to flat runs, yet elevated muscle damage which involves a substantial decrease in contractile function.

While the data collected from the present experiments do not allow a direct conclusion, the results can be elucidated by applying some of the theoretical concepts introduced in Chapter 2. Using the logic which underlies integrated models, the peripheral alterations induced through fatigue and muscle damage not only result in a diminished contractile function, but also in an increased firing rate of the afferences during the exercise. Additionally, there is extensive stress signaling originating from the calcium leakage which may be centrally registered. If we apply the Flush model in this case, the "filling rate" of the tank increases, accelerating the rise of RPE

into the security reserve. In order to prevent the infringement of the security reserve before exercise is completed, motor drive is decreased in the same measure as the "filling rate" is increased, restoring the rate of RPE increase to its original level. This has been observed by Baron et al.[8], manifesting as a change in pacing during repeated eccentric sprints. An alternative explanation could be that the original planification of exercise already takes into account the eccentric-induced damage and therefore compensates it by an adapted planned pacing strategy before exercise is commenced. If this is the case, the planned exercise strategy is very probably dependent on experience and course knowledge[9–11]. This is supported by the evolution of performance and fatigue over four repetitions of the same trail run[12]. In the initial run, performance was decreased and fatigue increased, indicating that the exercise strategy was not optimal. After learning the course, the subjects managed to perform better while experiencing less fatigue, hence a more optimal exercise strategy. Various studies have been conducted using missing or false distance or duration feedback, which has been observed to have a significant impact on the subjects' regulation strategies[13,14] further strengthening this concept. A final explanation can also be found using the teleoanticipatory approach, namely that the tasks "continued running" and "MVC" are inherently different in nature and have different prolonged consequences for the physiological system[9]. If the teleoanticipatory prediction also takes into account the regeneration phase, perhaps linked through a motivational factor, MVC may be completed at a different level of drive that is related, but not inherently linked to the central fatigue experienced during running. Unfortunately, identification of this type of process is - for the moment - outside the range of the existing measurement tools and rests conjecture.

To summarise the ideas presented above: fatigue may be indifferent between "flat" and "trail" courses as it is regulated to a "critical threshold" level through teleoanticipatory prediction and inhibitory afferent feedback. Indeed, the results observed in the presented studies conform to this idea and present a conclusive case from this perspective.

Strategies to enhance trail running performance, applying the same theoretical framework, must hence alter one of the variables in the flush system. Both EIMD reduction and compression attempt to modify the "filling rate" to a more or less successful degree. Additionally, there is a certain effect of belief and perception which may affect the interpretation of the "filling rate" that is not negotiable, as the modalities engender a sensory component. Nonetheless, compression garments fail to enhance performance, although extensive familiarization with the course was completed to allow optimization of the exercise strategy. This indicates that in a short trail run, compression garments are probably superfluous to performance. No extrapolation can be made to longer trails, as the effects might become more apparent as muscle damage becomes more pertinent. The induction of Heat Shock Proteins on the other hand appears to lead to an attenuation of force depression caused by EIMD in untrained subjects. A second bout of exercise demonstrates a potentiation of the

beneficial effect over the usual repeated bout effect. Presently, these results are not transferable to trail races, due to the differences in population training status and exercise duration.

Concluding this section, the reported results are coherent with existing data and models and provide indirect support of teleoanticipation and negative afferent feedback regulation of exercise intensity. For individual discussion of the results please refer to the respective article discussion sections.

8.3. CRITICAL ASSESSMENT OF METHODS

The methods employed in the presented experiments are not without limits and weaknesses. Generally, it would have been preferable to conduct all experiments as blinded trials, but this was rendered difficult by the physical nature of the tasks. Hence there may be an element of belief that is active and that may have modified outcomes to a modest degree. Pains were taken to keep the participants in the belief that all conditions were equally beneficial, but in certain cases (compression garments) the preconceptions shaped were undoubtedly stronger. Furthermore, creatine kinase is historically considered a marker with high inter-individual variability[7,15,16]. It was used nonetheless in the presented experiments, as it is by far the most common marker reported and enabled inter-study comparison. In the specific studies, limitations are generally elaborated upon in the discussion section, but the main limitations will be recapitulated here for convenience.

In the initial descriptive study, it would have been better to use a treadmill rather than an ergocycle to determine running efficiency. The ergocycle was nonetheless used, as the participants were so fatigued after the effort, that they were incapable of running. It also mitigated the effect of age on morphological parameters such as tendon stiffness and stretch potentiation response.

Furthermore, in retrospect of the reproducibility study, it would have been advisable to use a longer course in the trail running model. The amount of muscle damage induced is rather minimal due to the training status of the participants in relationship to the running distance. There was an effect shown on the depression of force, so the course was sufficient, yet a longer course might have shown stronger effects. Alternatively, less trained runners could have been recruited.

Weaknesses in the intervention study using compression garments include the short running course and the choice of a single measurement time point 45 minutes following exercise. It would have been beneficial to record MVIC at multiple time points directly following the

exercise to better understand the evolution and discretely identify the point of maximal force depression. Using evoked force through TMS of PNS would have been a more reliable indicator and would have additionally allowed conclusions on the origin of fatigue.

The final intervention study on passive heating and eccentric-induced muscle damage would have benefitted from testing a greater population. The main limitation of the proposed technique though, is that the passive heating phase was regarded as extremely uncomfortable by most of the participants. Additionally, it was challenging to keep the participants' heat loss to a minimum and there was quite pronounced inter-individual variation in regard to thermal stress-coping strategies. If the heating technique is to be used more frequently, a modification to reduce subject discomfort would need to be developed.

8.4. Implications for future research and outlook

So how can trail running research continue to evolve? There are several potentially interesting perspectives uncovered in the presented studies that warrant exploration. While there is little published trail-specific knowledge and therefore ample opportunity, there are two main axes of research that seem especially interesting. For one, the development of practical intervention strategies and training strategies is important not only to increase trail running performance, but also to invigorate the trail running industry and advance scientifically backed innovation. Functional advances in engineered products serve as pioneering concepts that can subsequently be adapted for general use. Practical intervention strategies, such as pre-heating, may also find application in boosting the efficiency of training programs during rehabilitation or to prevent age-related decline of muscle function. In a different light, examining trail running as a specific form of simultaneous induction of muscle damage and fatigue can contribute to answering the age-old research question, "What causes fatigue?"

Using prior heating to decrease functional impediment after EIMD has proven effective as a proof of concept in the experiments presented here. To determine the suitability and effects of this intervention on a trail race, a similar protocol would need to be applied to a trail running population using a trail race as an intervention. Collection of tissue samples would allow exact determination of HSP levels, contributing to a better understanding of HSP regulation and its contribution to EIMD reduction. Additionally, this would allow for a better characterization of the EIMD magnitude invoked through a trail.

Furthermore, it would be especially interesting to determine precisely whether or not a trail run induces the same amount of fatigue as a flat run. When assessing this question, it is difficult to determine the precise equivalent. As the races are run at different speeds due to their characteristics, it is not straightforward to determine what length of flat course corresponds to

which length of trail. In a two-pronged study design, this conundrum could be solved while at the same time providing additional information on running economy and the biomechanics of movement. By instrumenting trail running shoes with an array of interior pressure sensors, a relatively precise quantification of the completed work load can be calculated, along with stride frequency and push-off force and angle. This data can prove useful in determining equivalent flat and trail courses. If subjects were additionally equipped with a portable spirometry unit, running economy could be calculated not only during exercise, but also more precisely than byusing delta efficiency or gross efficiency.

One of the more pertinent research questions in fatigue is the contribution of supraspinal factors to central fatigue. Using voluntary, PNS-evoked and TMS-evoked contractions following trail running, it becomes possible to clarify the impact of prolonged, muscle-damaging exercise on supraspinal modulation of motor drive. This could then be the basis for comparing similar protocols in non-damaging exercise, which would indicate whether muscle damage is implicated in limiting central motor drive. Furthermore, it would be very interesting to monitor potential changes in conduction velocity of the membranes before and after fatigue. Using an HDEMG it becomes possible to trace the translocation of the potential along the electrode array. This form of analysis is not common following prolonged exercise due to the relative novelty of the technique and the high computational cost. However, the results from such an experimental approach would be enlightening, especially as trail running involves not only prolonged exercise but also ultra-structural damage which may negatively affect propagation velocity.

Finally, trail running can be seen as inducing a different type of attention strategy to other forms of prolonged exercise. As the terrain is varied, the runners' attention must stay focused on the task and terrain, involving continuous concentration. Disassociation strategies and external focus that are common in flat races[17] may not be applicable in the same form. This therefore provides an interesting model to investigate cognitive performance following prolonged exercise coupled with sustained attentional focus. It would be especially interesting to prime subjects with different paradigms and track both their pacing strategies using global positioning systems (GPS) and the resulting fatigue using a combination of voluntary contractions, PNS and TMS. This could shed some light on the effect of priming on the calibration of the teleoanticipatory system before effort initiation.

8.5. Conclusion

A quote often attributed to Socrates states, "To know, is to know that you know nothing"[18]. Much so, the work of this thesis, while having contributed to the general knowledge on trail running and heat shock protein interaction with eccentric muscle damage, raises more

questions than it answers. In the presented experiments, differences between young and master athletes have been demonstrated and the alterations induced by a 55 km distance trail described. Some of the basic footwork allowing the use of intervention studies has been provided and a useful model has been developed that may be replicated in other studies. A contribution to the growing body of literature on compression garments has been made which will hopefully help to arrive at a final conclusion of the discussion in the near future. Finally, a proof of concept has been provided that HSP cross-tolerance is possible in humans between heating and mechanical and inflammatory stress. The field of trail running research remains a rather sparsely painted canvas, but hopefully this thesis has helped fill in some of the blank areas.

8.6. Chapter 4 Bibliography

1. Millet GY, Tomazin K, Verges S et al. Neuromuscular Consequences of an Extreme Mountain Ultra-Marathon. *Plos One*. 2011; 6(2):e17059.

2. Martin V, Kerhervé H, Messonnier LA et al. Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run. *J Appl Physiol*. 2010; 108(5):1224–1233.

3. Vercruyssen F, Easthope C, Bernard T et al. The influence of wearing compression stockings on performance indicators and physiological responses following a prolonged trail running exercise. *Eur J Sport Sci*. 2013; [ePub]:1–7.

4. Easthope CS, Hausswirth C, Louis J et al. Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes. *Eur J Appl Physiol*. 2010; 6(110):1107–1116.

5. Amann M, Blain GM, Proctor LT et al. Implications of group III and IV muscle afferents for high intensity endurance exercise performance in humans. *J Physiol*. 2011; 589(21):5299–5309.

6. Amann M, Proctor LT, Sebranek JJ et al. Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol*. 2009; 587(1):271–283.

7. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. *Br Med Bull*. 2007; 81-82:209–230.

8. Baron B, Deruelle F, Moullan F et al. The eccentric muscle loading influences the pacing strategies during repeated downhill sprint intervals. *Eur J Appl Physiol*. 2009; 105(5):749–757.

9. Tucker R, Noakes T. The physiological regulation of pacing strategy during exercise: a critical review. *Br J Sports Med*. 2009; 43(6):e1–e9.

10. St Clair Gibson A, Lambert EV, Rauch LHG et al. The role of information processing between the brain and peripheral physiological systems in pacing and perception of effort. *Sports Med Auckl Nz*. 2006; 36(8):705–722.

11. Wegner MS, Whaley MH, Glass SC et al. Effects of a learning trial on self-regulation of exercise. *Int J Sports Med*. 2007; 28(8):685–690.

12. Easthope C, Nosaka K, Caillaud C et al. Reproducibility of running performance and fatigue in trail running. *J Sci Med Sport*. 2013; (In press).

13. Faulkner J, Arnold T, Eston R. Effect of accurate and inaccurate distance feedback on performance markers and pacing strategies during running. *Scand J Med Sci Sports*. 2011:e176–e183.

14. Albertus Y, Tucker R, St Clair Gibson A et al. Effect of distance feedback on pacing strategy and perceived exertion during cycling. *Med Sci Sports Exerc*. 2005; 37(3):461–468.

15. Clarkson PM, Ebbeling C. Investigation of serum creatine kinase variability after muscle-damaging exercise. *Clin Sci Lond Engl 1979*. 1988; 75(3):257–261.

16. Nosaka K, Clarkson PM. Variability in serum creatine kinase response after eccentric exercise of the elbow flexors. *Int J Sports Med*. 1996; 17(2):120–127.

17. Raglin JS. The psychology of the marathoner. *Sports Med*. 2007; 37(4):404–407.

18. Fine G. Does Socrates Claim to Know That He Knows Nothing? *Oxf Stud Anc Philos*. 2008; 35:49–88.