NEUROMUSCULAR FATIGUE AFTER SHORT-TERM MAXIMAL RUN IN CHILD, YOUTH, AND ADULT ATHLETES

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Master's thesis
Biomechanics
Spring 2013
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ABSTRACT

Sami Äyrämö (2013). Neuromuscular fatigue after short-term maximal run in child, youth, and adult athletes. Department of Biology of Physical Activity, University of Jyväskylä, Master thesis, Biomechanics, 96 pp.

Introduction and goal. Prior studies have shown that pre-pubertal children experience less fatigue and recover faster after high-intensity exercise than adults. However, maturity-dependent changes in the extent of peripheral and central fatigue remain unclear. In this study, the existing knowledge was extended by investigating both peripheral and central mechanisms of fatigue in a 50 s maximal run for three different age groups. **Methods.** Children (N = 8; 11.9 ± 1.4 years), Youth (N = 8; 14.9 ± 1.1 years), and Adults (N = 8; 21.3 ± 3.3 years) served as subjects. The maximal 300 m (Children), 350 m (Youth), and 400 m (Adults) running tests were performed during the period between the competitive indoor and outdoor seasons on a 200 m indoor track. The blood lactate concentration and the blood pH were determined from the capillary blood sampled from a fingertip in the morning before breakfast, before and after the warm-up, immediately before the maximal 50 s run, and 3, 6, 9, 12, 15, 30, 60 min after the run. The pre- and post-fatigue tests involved measurements of the passive twitch and maximal voluntary contraction (MVC) torques from the plantar flexors. The maximal M-wave, the maximal electromyography (EMG) activity, the H-reflex, and the V/M_{max}-ratio were also analyzed from the soleus muscle. In addition, the Hoffman-reflex (H-reflex) recruitment curve and the H_{max}/M_{max}-ratio were measured before the run. **Results**. The average running speed differed between the groups (Children 5.65 ± 0.54 m/s; Youth 6.57 ± 0.27 m/s; Adults 7.68 ± 0.30 m/s, $p < 0.001$). The running speed decreased from the fastest 100 m to the last 100m distance by 12.2 ± 6.5 % ($p < 0.01$), 9.8 ± 5.1 % ($p < 0.001$), and 12.2 ± 3.1 % ($p < 0.001$) in Children, Youth, and Adults, respectively. The peak values of the post-fatigue blood lactate (BLa) concentration were 10.2 ± 1.1 mmol/l, 13.3 ± 3.7 mmol/l, and 17.4 ± 1.8 mmol/l for Children, Youth, and Adults, respectively. The values differed significantly ($p < 0.001$) from the pre-fatigue values in each group. The peak values of BLa were significantly lower in Children

compared to Youth ($p < 0.05$) and Adults ($p < 0.001$) and lower in Youth compared to Adults ($p < 0.01$). The minimum level of blood pH decreased after the run significantly to 7.18 ± 0.03, 7.14 ± 0.07, and 6.97 ± 0.06 ($p < 0.001$ for each) in Children, Youth, and Adults, respectively. The minimum values of blood pH were significantly lower in Children and Youth compared to Adults ($p < 0.001$ for both). The MVC torque decreased by 16.1 ± 13.0% in Adults ($p < 0.01$) and the relative change differed ($p < 0.01$) from Youth in which no significant change was observed. The passive twitch torque decreased in Youth (-19.2 ± 12.2 %; $p < 0.01$) and Adults (-23.7 ± 13.7 %; $p < 0.01$). In both of these groups, the relative decrement was greater than in Children ($p < 0.05$). Twitch contraction and half-relaxation times decreased by 9.4 ± 5.8 % ($p < 0.01$), 9.4 ± 7.4 % ($p < 0.01$), and 9.8 ± 3.4 % ($p < 0.001$) in Children, Youth, and Adults, respectively, whereas the maximum rate of torque development decreased only in Youth (34.4 ± 30.1 %; $p < 0.05$) and Adults (23.5 ± 23.7 %; $p < 0.05$). The H_{max}/M_{max}-ratio, measured before the run, was lower in Children compared to Youth ($p < 0.05$) and Adults ($p < 0.01$). No fatigue-induced changes were observed in the maximal EMG activity, H-reflex, or V/M_{max}-ratio. **Discussion and conclusion**. Since neural changes were not observed after the run, it seems that the fatigue was mainly caused by peripheral factors in all groups. Both the neuromuscular tests and the post-fatigue levels of metabolic by-products indicate that Children were not able to fatigue themselves to the same extent as Youth and Adults. On the other hand, it is generally known that children need less time to recover from maximal exercise. The degree of the speed deceleration in Children was comparable to that of Youth and Adults and it is likely that neuromuscular system recovered more in Children than Youth and Adults during the 6 min delay between the end of the run and the beginning of the neuromuscular tests.

Key words: central fatigue, high-intensity, maturity

TIIVISTELMÄ

Sami Äyrämö (2013). Hermolihasjärjestelmän väsyminen lyhytkestoisessa maksimaalisessa juoksusuorituksessa lapsilla, nuorilla, ja aikuisilla. Liikuntabiologian laitos, Jyväskylän yliopisto, Biomekaniikan pro gradu -tutkielma. 96 s.

Johdanto ja tutkimuksen tavoite. Aikaisempien tutkimusten perusteella on havaittu että esipuberteetti ikäiset lapset väsyvät vähemmän ja palautuvat nopeammin kuin aikuiset kovatehoisten urheilusuoritusten yhteydessä. Nuoren urheilijan kypsymiseen liittyvät muutokset perifeerisissä ja hermostollisissa väsymysmekanismeissa ovat kuitenkin vielä selvittämättä. Tämän tutkimuksen tavoitteena on tuottaa uutta tietämystä iän ja kypsymisen vaikutuksista perifeeristen ja hermostollisten mekanismien rooliin 50 s maksimaalisessa juoksusuorituksessa. **Menetelmät:** Tutkimukseen osallistui 24 miespuolista koehenkilöä jotka jaettiin kolmeen ikäryhmään: Lapset (N = 8; 11.9 ± 1.4 v), Nuoret (N = 8; 14.9 ± 1.1 v), ja Aikuiset (N = 8; 21.3 ± 3.3 v). Koehenkilöt suorittivat maksimaalisen 300 m (Lapset), 350 m (Nuoret) ja 400 m (Aikuiset) juoksutestin 200 m:n halliradalla sisä- ja ulkoratakauden välisellä ajanjaksolla. Veren laktaattipitoisuus ja pH määritettiin testipäivänä ennen aamiaista, ennen ja jälkeen verryttelyn, välittömästi ennen juoksua, sekä 3, 6, 9, 12, 15, 30, ja 60 min juoksun jälkeen sormen päästä otetuista kapillääriverinäytteistä. Väsymyksen voimakkuutta sekä sen taustalla olevia mekanismeja selvitettiin mittaamalla maksimaalisessa tahdonalaisessa (MVC) ja sähköstimulaatiolla aiheutetussa passiivisessa (pT) plantaarifleksoreiden voimantuotossa tapahtuvia muutoksia ennen ja jälkeen juoksusuorituksen. Tämän lisäksi analysoitiin muutokset soleus lihaksesta mitatussa maksimimaalinen M-aallossa (M_{max}), EMG-aktiivisuudessa, Hoffman-refleksissä (H-refleksi) ja V/M_{max}-suhteessa. H_{max}/M_{max}-suhde analysoitiin ennen verryttelyä mitatusta herkkyyskäyrästä. **Tulokset.** Maksimaalisen 50 s juoksun keskinopeudet erosivat merkitsevästi ryhmien välillä (Lapset 5.65 ± 0.54 m/s; Nuoret 6.57 ± 0.27 m/s; Aikuiset 7.68 ± 0.30 m/s, $p < 0.001$). Keskimääräinen juoksunopeus laski ryhmittäin seuraavasti nopeimman ja viimeisen 100m:n osuuden välillä: Lapset -12.2 ± 6.5 % ($p < 0.01$); Nuoret -9.8 ± 5.1 % ($p < 0.001$); ja Aikuiset -12.2 ± 3.1 % ($p < 0.001$). Juoksun jälkeen mitatut

veren laktaattipitoisuuden maksimiarvot erosivat merkitsevästi juoksua edeltävistä arvoista: Lapset 10.2 ± 1.1 mmol/l ($p < 0.001$); Nuoret 13.3 ± 3.7 mmol/l ($p < 0.001$); ja Aikuiset 17.4 ± 1.8 mmol/l ($p < 0.001$). Veren maksimilaktaattipitoisuuden nousu oli lasten ryhmässä pienempi verrattuna nuorten ($p < 0.05$) ja aikuisten ($p < 0.001$) ryhmiin sekä nuorten ryhmässä pienempi verrattuna aikuisten ryhmään ($p < 0.01$). Veren pH-arvo laski kaikissa ryhmissä merkitsevästi ennen juoksua mitatuista arvoista: Lapset 7.18 ± 0.03; Nuoret 7.14 ± 0.07; ja Aikuiset 6.97 ± 0.06 ($p < 0.001$ pareittain). Aikuisten ryhmässä mitattu matalin veren pH-taso oli juoksun jälkeen alempi verrattuna lasten ($p < 0.001$) ja nuorten ryhmään ($p < 0.001$). Maksimaalisen tahdonalaisen plantaarifleksion aikana tuotettu vääntömomentti laski (-16.1 ± 13.0 %; $p < 0.01$) aikuisten ryhmässä. Suhteellinen muutos juoksua edeltäviin arvoihin erosi merkitsevästi ($p < 0.01$) verrattuna nuorten ryhmään, jonka MVC:ssä ei tapahtunut merkitsevää muutosta. Sähköstimulaatiolla tuotetun passiivisen lihassupistuksen vääntömomentti laski nuorten (-19.2 ± 12.2 %; $p < 0.01$) ja aikuisten (-23.7 ± 13.7 %; $p < 0.01$) ryhmissä. Molemmissa ryhmissä suhteellinen lasku oli suurempi verrattuna lasten ryhmään ($p < 0.05$). Passiivisen lihasnykäyksen supistus- ja puolirelaksaatioaika lyhentyi kaikissa ryhmissä: Lapset -9.4 ± 5.8 % ($p < 0.01$); Nuoret -9.4 ± 7.4 % ($p < 0.01$); ja Aikuiset -9.8 ± 3.4 % ($p < 0.001$). Passiivisen lihasnykäyksen aikana mitattu maksimi voimantuottonopeus puolestaan laski nuorten (-34.4 ± 30.1%; $p < 0.05$) ja aikuisten (-23.5 ± 23.7 %; $p < 0.05$) ryhmissä. Juoksun aiheuttamien muutosten lisäksi ennen verryttelyä mitatun H_{max}/M_{max}-suhteen havaittiin olevan lasten ryhmässä matalampi verrattuna nuorten ($p < 0.05$) ja aikuisten ($p < 0.01$) ryhmiin. Väsytys ei aiheuttanut muutoksia MVC:n aikaisessa EMG:ssa, H-refleksissä tai V/M_{max}-suhteessa. **Pohdinta ja johtopäätökset.** Koska hermostollisissa tekijöissä ei havaittu väsymyksen aiheuttamia muutoksia, voidaan olettaa että maksimaalisen juoksun aiheuttama väsymys johtuu lähinnä perifeerisistä tekijöistä kaikissa tutkituissa ikäryhmissä. Vähäisemmät muutokset niin hermolihasjärjestelmän voiman tuotossa kuin aineenvaihduntatuotteiden tasoissa viittaavat siihen, että lapset eivät kykene kuormittamaan itseään maksimaalisessa anaerobisessa suorituksessa samassa määrin kuin murrosikäiset nuoret ja aikuiset. Toisaalta, koska myös lasten ryhmässä tapahtui merkitsevää keskinopeuden laskua maksimaalisen juoksusuorituksen aikana ja heidän tiedetään palautuvan aikuisia nopeammin

maksimaalisista suorituksista, on todennäköistä, että lasten ryhmässä hermolihasjärjestelmän palautuminen edistyi nuorten ja aikuisten ryhmiin verrattuna pidemmällä juoksusuorituksen ja hermolihasjärjestelmän mittausten välillä olleen kuuden minuutin viiveen aikana.

Avainsanat: hermostollinen väsymys, kovatehoinen, kypsyminen

ABBREVIATIONS

ACh	Acetylcholine
aEMG	Average rectified electromyography
AP	Action potential
aRTD	Average rate of torque development
ATP	Adenosine triphosphate
BLa	Blood lactate concentration
CAR	Central activation ratio
CMEP	Cervicomedullary motor evoked potential
CNS	Central nervous system
CT	Contraction time
DHPR	Dihydropyridine receptor
EMG	Electromyography
EMG_{RMS}	The root mean square of electromyography
HFF	High frequency fatigue
HRT	Half-relaxation time
iEMG	Integrated electromyography
LFF	Low-frequency fatigue
MEP	Motor evoked potential
M-wave	Muscle compound action potential
MRTD	Maximum rate of torque development
MRTR	Maximum rate of torque relaxation
MVC	Maximal voluntary contraction
PCr	Creatine phosphate
P_i	Inorganic phosphate
RPE	Rating of Perceived Exertion
RyR	Ryanodine receptor
SR	Sarcoplasmic reticulum
SSC	Stretch shortening cycle

TES	Transcranial electrical stimulation
TMS	Transcranial magnetic stimulation
VA%	Voluntary activation

CONTENTS

1 INTRODUCTION

The ever increasing competition in the contemporary international sport society has made it necessary for the target-oriented children to start systematic training at a younger age. Among the large assortment of sports, this applies also to the speed endurance sports, such as long sprint running events (200 – 800 m), sprint skiing, ice hockey, and soccer, that demand simultaneously high capacity for explosive generation of force, high power output, and fatigue resistance. Muscle fatigue refers to any exercise-induced decline in the ability of a muscle to produce force or power (Gandevia 2001). It has been conventional for researchers to speak about peripheral and central fatigue depending on the site of impairment (Edwards 1981). Peripheral fatigue denotes that the exercise-induced decline in performance is caused by mechanisms within the muscles themselves (Fitts 2008). Central fatigue means that the nervous system fails to activate the muscles to their extreme limit (Gandevia 2001). Due to the nearly all-out nature of speed endurance efforts, a high ability to resist fatigue and to tolerate pain is one of the main prerequisites for high-level performance, for instance, in the 400 m run (e.g., Schiffer 2008).

The characteristics of exercise-induced muscle fatigue differ between children and adults (Ratel et al. 2006b, 2009). It has been well-established that children are not able to fatigue themselves during exercise to the same degree as adults (Falk & Dotan 2006). Children also recover faster than adults from high-intensity exercise (Hebestreit et al. 1993; Ratel et al. 2006a). These differences have been associated, for example, with the children's greater aerobic capacity and the lower level of anaerobic capacity and neural activation (Ratel et al. 2009).

Much research has focused on studying the maturity-related differences in the rate of recovery during repeated high-intensity efforts (Heberstreit 1993; Ratel et al. 2002; 2006b). However, the maturity-related differences concerning the sites and mechanisms causing fatigue during prolonged sprint running events have not been widely addressed by researchers. Neural contributions to the fatigue observed during lactic maximal sprints

remain also unstudied in children. Perhaps due to ethical limitations the methods of nerve and muscle stimulation have been rarely used in the studies investigating the exercise-induced neuromuscular fatigue in children.

In the present study, maturity-related differences in neuromuscular fatigue caused by a maximal short-term run are assessed between male children, adolescents, and adults. The subjects performed a maximal 50 s run on a 200 m indoor track. Exercise-induced changes in the neuromuscular system were assessed through a set of neuromuscular tests that were accomplished before and after the run. The tests involved the measurement of voluntary and evoked isometric force production in the plantar flexors, the maximal M-wave, H-reflex, V-wave, and the maximal EMG from the soleus. The main hypothesis of the study is that fatigue has mainly peripheral origins in adults, whereas in children and, probably, in youth, neural mechanisms are involved as well.

2 LITERATURE REVIEW

2.1 Exercise-induced neuromuscular fatigue

Exercise-induced neuromuscular fatigue, or muscle fatigue, is defined (Gandevia 2001): ”*Any exercise-induced reduction in the ability of a muscle to generate force or power; it has peripheral and central causes.*” Physiological mechanisms of fatigue are diverse, interdependent and conventionally divided into peripheral and central factors (Edwards 1981). The dichotomy of central and peripheral fatigue has also been criticized, because of the mutual interaction between the peripheral and central mechanisms (Åstrand et al. 2004, 457; Enoka 2007). In other words, metabolic or mechanical stress within the skeletal muscles influences the processes within the central nervous system and the other way around. Despite the wide variety of measurement techniques, exact quantification of central and peripheral factors is difficult. Muscle fatigue is also a “*task dependent*” phenomenon (Enoka 2007). Task dependency means that the mechanisms contributing to the development of fatigue during physical exercise depend on the characteristics of the task being performed (Enoka 2007). For instance, eccentric muscle work induces a greater decline in the ability to generate eccentric than concentric force and vice versa (Linnamo et al. 2000). In this review, peripheral and central factors of exercise-induced neuromuscular fatigue are overviewed.

2.1.1 Motor units firing rates and the “*muscle wisdom*” hypothesis

Motor unit firing rates are dependent on the strength of contraction so that firing rates tend to decrease in the course of MVC effort (Bigland-Ritchie et al. 1983; 1986a; Woods et al. 1987) and increase during submaximal efforts (Bigland-Rithcie et al. 1986b). During submaximal contractions new motor units are also recruited (Bigland-Rithcie et al. 1986b). It is generally accepted that the downward trend of the firing rate during MVC is an

intelligent feature of the nervous system that matches the extent of neural drive to the fatigue-induced decline in the speed of muscle contractions, and hence protects the peripheral processes from overloading and development of complete failure of activation. Thus the longer the duration of the muscle twitch, the lower the required firing rate for tetanus will be. The phenomenon is commonly referred to as "*muscle (or muscular) wisdom hypothesis*" (e.g., Garland & Gossen 2002). Due to the heterogeneous behaviour of motor units, the relevance of the muscle wisdom hypothesis in submaximal or dynamic contractions is less clear compared to the maximal voluntary contractions (Garland & Gossen 2002). Kuchinad et al. (2004) demonstrated that the motor unit firing rates increase during low and decrease during high force submaximal voluntary contractions. Moreover, they showed an inverse correlation between the change of the firing rate and the half relaxation time of the muscle twitch, which is in agreement with the muscle wisdom hypothesis.

2.2 Peripheral fatigue

Peripheral fatigue refers to the exercise-induced activation and/or contraction failures at or distal to the neuromuscular junction (Gandevia 2001). Mechanically peripheral fatigue manifests itself in a reduced twitch force, shortening velocity, and peak power (Fitts 2008). The extent and cause of peripheral fatigue can be assessed by nerve or muscle stimulation techniques. The stimuli are delivered to the relaxed muscle or to the peripheral motoneurons innervating the muscle. The origin of fatigue is determined by analyzing the characteristics of the evoked force twitches. (Maffiuletti & Bendahan 2009) As a result of peripheral fatigue the evoked twitch force/torque and the rate of tension development decrease, and the contraction and half-relaxation times increase (Fitts 2008). The propagation of electrical impulses along the axons of motoneurons into the interior parts of muscle fibers can be analyzed by recording the EMG response from the target muscle (Maffiuletti & Bendahan 2009). The simultaneous application of nerve stimulation and EMG measurement produce information about conditions of the neuromuscular junction and the surface membrane of the muscle fiber. In addition, a large number of other methods, such as blood tests, muscle

biopsies, the 31P Magnetic Resonance Spectrocopy technique, have been applied in order to determine the origins of peripheral fatigue in various tasks (Maffiuletti & Bendahan 2009).

2.2.1 Failures in neuromuscular transmission

The neuromuscular junction is the interface between the nervous system and a skeletal muscle (e.g., MacIntosh et al. 2006, 32). In fact, Merletti et al. (2004) uses the concept of "*the fatigue of the neuromuscular junction*" when they refer to the fatigue at this site. Nevertheless, it is more common to speak about peripheral fatigue, particularly when the neuromuscular junction is distal to the site of nerve stimuli.

As a result of the neuromuscular transmission an axonal nerve impulse is converted into the muscle fiber action potential. The process begins when an action potential reaches the pre-synaptic motoneuron terminal. Subsequently, neurotransmitters (ACh) are released from the pre-synaptic terminal into the synaptic cleft, where they attach to the specific receptors on the end-plate region of the muscle fiber. This opens ACh-gated ion-channels, which allow the influx of Na^+ and efflux of K^+ ions through the membrane of the muscle fiber. This bidirectional flux of ions causes the depolarization of the end plate region, which subsequently generates an action potential by activating the nearby voltage-gated Na^+ and K^+ channels. (e.g., Enoka 2008, 189)

A failure in the neuromuscular transmission can be due to the axon-branch point failure, the depletion of ACh in the pre-synaptic terminal, or the desensitization of ACh receptors at the end-plate region (Sieck & Prakash 1995). Even though the neuromuscular transmission is not thought to be sensitive for physiological firing rates, an axonal propagation failure has been demonstrated in a rat's nerve both *in vitro* and *in situ* by Krnjevic & Miledi (1959). This early study demonstrated also that the axon branches are sensitive to the adequate supply of oxygen. Consequently, the authors hypothesized that hypoxia in the surroundings

of the intramuscular segments of the motoneuron could be the main cause of the axon-branch point block.

Nevertheless, the neuromuscular transmission has proved to be a highly robust mechanism under physiological conditions (Gandevia 2001; Bigland-Ritchie et al. 1983; Westerblad & Allen 2009). Even though the firing rate in a motor unit could be relatively high immediately after the initiation of maximal contraction, it will decrease within the first few seconds so that the sustained firing rates in humans are lower than 30Hz (MacIntosh et al. 2006, 204; Bigland-Ritchie et al. 1983). Thus it seems that the muscle wisdom protects the neuromuscular junction from detrimental firing rates (Bigland-Ritchie et al. 1983; Garland & Gossen 2002). MacIntosh et al. (2006, 231) suggest that the intact blood supply is capable of providing sufficient metabolic conditions for the impulse propagation in the axon branches.

2.2.2 Excitation-contraction coupling

A muscle fiber action potential is converted into the mechanical force twitch in a process called excitation-contraction coupling (E-C coupling). This process involves the following steps (Allen et al. 2008): 1) the transmission of AP on the sarcolemma; 2) the propagation of AP down the transverse tubules; 3) the reversal of Ca^{2+} conductivity in the sarcoplasmic reticulum; 4) the movement of Ca^{2+} down its concentration gradient into the sarcoplasm; 5) the beginning of the Ca^{2+} re-uptake into the sarcoplasmic reticulum; 6) the Ca^{2+} attachment to the troponin; and 7) actomyosin interaction and the mechanical work produced by the cross-bridges. A failure in the steps 1-6 inhibits the activation of the contractile system, whereas a failure in the step 7 implies that regardless of the Ca^{2+} activity the fatigue occurs within the myofibrillar mechanism itself (Edman 1995).

2.2.3 Sarcolemmal activation failure

A failure of generating an action potential at the muscle fiber surface and/or propagating it down the T-tubules is associated with the so-called high-frequency fatigue (Edwards 1981). In the presence of HFF, the decline in the force generation capacity is greater at high compared to low frequency stimulation (Sejersted & Sjøgaard 2000). Accordingly, HFF can be examined by delivering a short high-frequency train of stimuli to the peripheral nerve and measuring the subsequent force twitch (e.g., Strojnik & Komi, 2000). HFF is also characterized by: 1) rapid recovery of the force during low frequency stimulation; 2) a reduction both in the amplitude and the duration of muscle membrane action potentials, and 3) a decrease in the extracellular [Na^+] and an increase in the extracellular [K^+] aggravate the loss of force output (Sejersted & Sjøgaard 2000).

Because the process of sarcolemmal activation is mainly sensitive to continuous high-frequency stimulation, it is not usually thought to be the site of failure under physiological conditions (e.g., Allen et al. 2008; Åstrand et al. 462). However, eccentric contractions may produce an increase in plasma [K+] and an acute reduction in sarcolemmal excitability (Piitulainen et al. 2008). In most cases, the high-safety factor of the neuromuscular junction along with the muscle wisdom likely protects the muscle fibers from the sarcolemmal impairments (Bigland-Ritchie et al. 1983; Garland & Gossen 2002).

2.2.4 Decrease in Ca^{2+}-transients

As the muscle fiber action potential propagates down the T-tubules, it finally triggers the release of Ca^{2+} from the sarcoplasmic reticulum into the sarcoplasm. This involves activation of the voltage-gated dihydropyridine receptors (DHPR) that subsequently signal the ryanodine receptors (RYRs) for the rapid release of Ca^{2+} at the sarcoplasmic reticulum (MacIntosh et al. 2006, 101). The calsium-ions are central mediators, aka second messengers, in the process of converting action potentials into the cross-bridge force twitch (MacIntosh et al. 2006, 98). Consequently, the activation of the contractile system is highly

sensitive to the Ca^{2+}-transients (Allen et al. 2008). Impaired Ca^{2+}-release is thought to be a consequence the long-lasting low-frequency fatigue (LFF) (e.g., Keeton & Binder-Macleod 2006). A decrease in the amplitude of the Ca^{2+}-transient leads to a decrease in the peak twitch force and, probably, in the peak rate of force development, whereas the delayed Ca^{2+}-uptake slows the muscle relaxation (Fitts 2008). The extent of LFF can be assessed by measuring the force response to a short low frequency (e.g., 20Hz) train of electrical stimuli applied to the peripheral motoneuron before and after a fatiguing workout (e.g., Strojnik & Komi, 2000). Moreover, the ratio of force responses to the low- to high-frequency stimuli expresses the degree of LFF in relation to HFF.

There are several metabolites that may affect the efficiency of the Ca^{2+}-release. First of all, the Ca^{2+} release from SR is dependent on the sarcoplasmic [ATP] (Allen et al. 2008). Although the average level of [ATP] in a muscle is not generally thought to decrease below 60 % of the resting level as a result of an exercise (Allen et al. 2008), it has been demonstrated that [ATP] may decrease down to 20 % of the resting level in individual fast-twitch muscle fibers after a 25 s all-out cycling effort (Karatzaferi et al. 2001). Since there is a high consumption of ATP near the space between SR and T-tubules in particular, the lack of ATP may become significant at this region, and thereby limit the rate of Ca^{2+} release from SR (Allen et al. 2008). Furthermore, the effects of the low [ATP] could be exacerbated by a rise in Mg^{2+}, AMP and adenosine (Allen et al. 2008).

The effects of inorganic phosphate on Ca^{2+} movements are two-fold (Westerblad et al. 2002). At the early stages of repeated tetanic stimulation, sarcoplasmic [Ca^{2+}] may first increase as a result of concurrent facilitation of Ca^{2+}- release and inhibition of Ca^{2+} reuptake by increased P_i (Westerblad et al. 2002). This compensates the simultaneous decline in the myofibrillar Ca^{2+} sensitivity, and hence delays the reduction in the force output indeed (Fitts 2008). If the tetanic stimulation is prolonged, the degradation of PCr causes a net increase in the sarcoplasmic [P_i] and consequently some P_i enters SR. There P_i precipitate with Ca^{2+}-ions, which decreases the amount of releasable Ca^{2+} and subsequently the amplitude of tetanic Ca^{2+}-transients (Allen et al. 2008).

The Ca^{2+} release may also be reduced by P_i through the Mg^{2+}-dependent inhibition of the RYR channels. There is also evidence that the depletion of glycogen may inhibit the release of Ca^{2+} from SR. To what extent the aforementioned mechanisms affect Ca^{2+} transients remain unclear. (Allen et al. 2008)

2.2.5 Myofibrillar failures

The myofibril is an element within the muscle fiber, which produces the mechanical movements of the muscle. It contains two contractile protein myofilaments called actin and myosin. The cross-bridge interaction between the actin and myosin myofilaments is controlled by regulatory proteins known as tropomyosin and troponin. Tropomyosin inhibits the interaction between the myosin head and the actin filament until the sarcoplasmic Ca^{2+} transient turns up. As the Ca^{2+} ions attach to the troponin, the tropomyosin ceases to inhibit the myosin binding site, and thereby allows the cross-bridge interaction, which generates the muscle contraction. (e.g., MacIntosh et al. 2006, 156)

Exercise induces changes in the interior of muscle fiber that directly affect the cross-bridge functions (Fitts 2008; Allen et al. 2008). The level of muscle force, contraction speed, and power depend on the number of strongly bound cross-bridges as well as the force and cycle rate generated by each individual cross-bridge (Fitts 2008). A cross-bridge failure may occur at the early stages of fatigue despite sufficient $[Ca^{2+}]$ in the sarcoplasm. Thus it precedes the depletion of Ca^{2+} (Westerblad et al. 1998). As the troponin molecules desensitize to Ca^{2+} due to prolonged activation, a decrease in the amplitude of $[Ca^{2+}]$ transient further limits the force output and the rate of cross-bridge cycling (Westerblad & Allen 2009; Fitts 2008).

What mechanisms are involved in the cross-bridge fatigue? An increase in sarcoplasmic $[P_i]$ and $[H^+]$ may directly reduce the myofibrillar Ca^{2+} sensitivity and the force output per cross-bridge (Westerblad et al. 2002; Allen et al. 2008; Fitts 2008). Numerous studies have demonstrated an inverse relationship between the intracellular $[P_i]$ and the force production

during fatiguing stimulation (review Allen & Trajanovska 2012). Fitts (2008) hypothesized that the mechanisms, through which P_i and H^+ disturb the cross-bridge function, could be different, which is why their net influence is probably additive. An increase in the sarcoplasmic [P_i] and [H^+] may also decrease the rate of relaxation (Allen et al. 2008). On the other hand, the contribution of acidosis in the peripheral muscle fatigue has been widely questioned recently (e.g., Westerblad et al. 2002; Allen et al. 2008; Allen & Trajanovska 2012).

Impairment in the cross-bridge interaction may also decrease the shortening velocity (Cooke 2007). This is not thought to occur before the isometric force output has first decreased by 10 % (Fitts 1996). Potential causes include an increase in sarcoplasmic [ADP] along with a decrease in [ATP], whereas P_i, H^+ and Ca^{2+} may not have an impact on that parameter at all (Allen et al. 2008, Cooke 2007). According to Cooke (2007), ATP and ADP are not, however, significantly attributed to the mechanical and energetic changes in fatigued muscle fibers (Cooke 2007). Myofibrillar Ca^{2+} sensitivity could also be inhibited by reactive oxygen species, but this remains to be confirmed in the normal physiological conditions (Allen et al. 2008; Fitts 2008).

2.2.6 Limitation in energy expenditure during high-intensity exercise

Even though the main focus of this study is in the neuromuscular issues, a short overview to principles of the energy expenditure will be given herein, because of its seamless interaction with the muscular force/power generation. Several ATP-dependent cellular processes including sarcolemmal Na^+/K^+-pumping, Ca^{2+}-movement, and cross-bridge interaction, are involved in the muscle contraction (e.g., MacIntosh 2006, 208). It is clear that any exercise performance at high-intensities exceeding the maximum level of aerobic capacity is limited by the availability of immediate energy sources ATP and PCr (Sahlin et al. 1998). Because the amount of free ATP is very limited within a skeletal muscle, the regeneration process must be initiated immediately after the onset of exercise (Hirvonen et al. 1987).

The main energy substrates of the ATP regeneration are PCr, glycogen, and free fatty acids (e.g., MacIntosh et al. 2006, 208). Only in very short (< 5 s) maximal efforts, the physical performance is predominantly limited by the rate of ATP utilization instead of its regeneration (Sahlin et al. 1998). Vice versa, this means, that in few seconds, the power output will be limited by the rate of ATP regeneration (Sahlin et al. 1998).

Consequently, in the course of maximal exercise, muscle fatigue sets in and the maximal power output starts to decrease within the first 10 s due to the shortage of PCr (Hirvonen et al. 1987; Sahlin et al.1998). Subsequently, the PCr breakdown is supplemented with the glycolytic ATP regeneration. The slower ATP production through the glycolytic pathways implies an unavoidable decrease in the power output. Even though the size of glycogen stores would suffice to sprint even the 400 m distance at a maximal speed (Lakomy 2000), the decrease in the power output accelerates as the sprinting distance is extended, because in addition to ATP, anaerobic glycolysis produces also metabolic by-products into the muscle and blood, and thereby disturb both neuromuscular and enzymatic functions (Sahlin et al. 1998).

2.3 Central fatigue

Central fatigue is defined (Gandevia et al. 2001): ”*A progressive reduction in voluntary activation of muscle during exercise*”. Thus, central fatigue refers to an inability to maximally exploit the force production capacity of a muscle. Central fatigue can be observed by delivering a supramaximal stimulus to an appropriate point of the neural pathway during maximal voluntary contraction. This is a classic approach known as the interpolated twitch method (Merton 1954). In the presence of central fatigue, the evoked muscle twitch produces an additional increment over the force produced by voluntary activation. The extent of central fatigue is often expressed as the level of voluntary activation, which is based on the ratio of the amplitudes determined from the interpolated and control twitch: VA% = 100 x (1 - interpolated twitch/control twitch) (e.g., Gandevia et al. 1996). Another formula is known as the central activation ratio (Kent-Braun & Le Blanc,

1996), which has the simple form: CAR = MVC/(MVC + interpolated twitch). The extent of voluntary drive can be also estimated from the magnitude of surface EMG activity. The various measures of the total magnitude of muscle activity, such as EMG_{RMS} (e.g., Tomazin et al. 2012), aEMG (e.g., Nummela et al. 1992), and iEMG (e.g., Nummela et al. 1994), provide coarse estimates for the extent of neural activation, since these are affected by several factors (Kamen and Gabriel, 2010). The peripheral factors can be taken into account by normalizing the chosen EMG estimate to the amplitude or the area of maximal M-wave (e.g., Tomazin et al. 2012). The V-wave measurement provides an indirect estimate for the extent of descending drive to motoneurons (Aagaard et al. 2002). The V-wave is measured otherwise similarly with the H-reflex (Palmieri et al. 2004), but it is evoked by supramaximal stimulus during maximal voluntary contraction. The maximal voluntary drive cancels the antidromic action potentials, which enables the evoked reflex impulses to reach the muscle fibers. Even though the exact quantification of central fatigue is not possible, Kent-Braun (1999) has estimated, for example, that central mechanisms accounted for approximately 20 % of the total muscle fatigue in a sustained 4 min dorsiflexion MVC task.

Exercise-induced neural impairment may theoretically occur anywhere in the pathway from the brain to the neuromuscular junction of the activated muscle. These mechanisms are often divided into spinal and supraspinal factors (Gandevia 2001). The potential sites and mechanisms causing central fatigue include, for example, intrinsic properties of the motoneuron itself, recurrent inhibition, reflex inhibition and spindle disfacilitation (e.g., Taylor et al. 2000). Moreover, the activation failure may be located within the brain (e.g., Gandevia et al. 2001).

2.3.1 Intrinsic motoneuron properties

The most distal site of central fatigue is within the peripheral motoneurons, because the excitability of the motoneuron may decrease. Numerous studies have shown that prolonged stimulation of a motoneuron leads to a decrease in its firing rate (for review, Gandevia 1998). For instance, Kernell & Monster (1982) applied a prolonged steady intracellular

current to a cat motoneuron *in situ* and discovered a gradual long-lasting decrease in the firing rates. They also observed that the higher the initial rate of firing or intensity of the stimulus, the greater the decrease in the firing rate will be. Moreover, most of the decrease occurred during the first 30-60 s of the stimulation. This late depression of firing rates is known as "*late adaptation*" (Gandevia 2001). However, intrinsic motoneuron properties do not probably contribute to the development of central fatigue, because the reduced activity in motor units can be counteracted by cortispinal stimulation and tendon vibration (Gandevia 2001). Moreover, Nordstrom et al. (2007) concluded that the available studies do not provide much evidence for that motoneuron adaptation would contribute to the development of central fatigue. It feels even more doubtful that it would affect during dynamic human movements such as running and cycling.

2.3.2 III- and IV-afferent inhibition

Group III- and IV-reflex inhibition has often been associated with exercise-induced fatigue. These afferent nerves inform the CNS about mechanical and metabolic disturbances at the surroundings of free nerve endings in the skeletal muscle (MacIntosh et al. 2006, 49). To be more precise, the free nerve endings are muscle receptors, which are sensitive to various mechanical and chemical changes in muscles involving muscle contraction, pressure, pain, stretch, temperature and metabolic by-products (MacIntosh et al. 2006, 49).

A conventional way to study the inhibitory feedback from group III- and IV-afferents involves the occlusion of the blood flow to the fatigued muscle. This maintains artificially ischemia in the muscles and, thereby, keeps the group III- and IV-afferents active. Using this approach, Bigland-Ritchie et al. (1986a) and Woods et al. (1987) found out that the firing rates do not recover in motor units as long as the fatigued muscles are kept ischemic after contractions. The interpretation was that the group III- and IV-afferents remained active due to the prolonged production of metabolites in the muscles and consequently kept on the motoneuron inhibition. The inhibitory effects of III- and IV-afferents are probably mediated through pre-synaptic inhibition of Ia terminals (Duchateau & Hainaut 1993).

In contrast, Butler et al. (2003) showed that the recovery of potentials evoked at the level of the corticospinal tract was independent of the muscle ischemia after MVC. Since the evoked potentials in the corticospinal tract are unaffected by presynaptic inhibition (Nielsen & Petersen 1994), the aforementioned observation does not support the assumption that the group III- and IV-afferents directly inhibit the alpha-motoneuron pool. Central fatigue was also shown to recover while the muscle contraction was continued for one minute by electrical stimulation after fatiguing voluntary contraction of plantar flexors (Löscher et al 1996). This provides additional evidence that the central motor drive is not significantly affected by the inhibitory reflex feedback from the small diameter afferents. The inhibitory effects of the group III- and IV-afferents are both task- and muscle-specific and they remain controversial (Taylor & Gandevia 2008)

2.3.3 Ia-afferent disfacilitation

Voluntary activation of the motoneurons is facilitated in the spinal cord by excitatory Ia-afferent feedback from the muscle spindles (e.g., Macefield et al. 1993). The muscle spindle is a mechanoreceptor that monitors the changes in the muscle length and provides facilitatory monosynaptic inputs to the motoneurons innervating the agonist and synergist muscles, and inhibitory disynaptic inputs to antagonist motoneurons (e.g., MacIntosh 2006, 42). Spindle sensitivity is modulated by central input via gamma-motoneurons (e.g., MacIntosh 2006, 43).

Macefield et al. (1993) demonstrated that motoneurons firing rates are higher in the presence than in the absence of Ia-afferent spindle support. Furthermore, they showed that the motor unit firing rates do not decrease in a natural way when the Ia-afferent feedback is blocked. Thus any exercise-induced decrease in the spinde facilitation seems to reduce the voluntary drive to the muscles (Macefield et al. 1991). Ia-afferent disfacilitation can be counteracted by tendon vibration during MVC (Bongiovanni & Hagbarth 1990). The following mechanisms could be related to the spindle disfaciliation (Hagbarth & Macefield 1995): 1) A progressive withdrawal of tonic support via the fusimotor loop; 2) an E-C

coupling or a contractile failure in the intrafusal muscle fiber itself due to the accumulation of metabolites; 3) a decline in the fusimotor output or the adaptation of the spindle receptors. Disfacilitation may also result from a fatigue-induced increase in the compliance of a muscle-tendon complex, which consequently reduces the responsiveness of the muscle spindles to mechanical stimuli (Avela et al. 1999).

2.3.4 Inhibitory interneurons

Both the descending drive and the afferent sensory feedback are modulated at the spinal level by several interneurons including Renshaw cells, Ib inhibitory interneurons, reciprocal Ia inhibitory interneurons, etc. (Windhorst & Boorman, 1995). Recurrent inhibition refers to the modulation of interspike intervals through an inhibitory loop from the alpha-motoneuron back to itself (Windhorst & Boorman, 1995). It has been demonstrated that recurrent inhibition may increase during a sustained maximum isometric contraction (Windhorst & Boorman, 1995) and decrease during sustained submaximal contractions (Löscher et al. 1996a). The functionality of Renshaw cell and its effects on the motoneurons interspike intervals are related to the adaptive adjustment of firing rates to the fatiguing muscles (Windhorst & Boorman, 1995; Löscher et al. 1996a). Both the peripheral and supraspinal mechanisms may regulate the Renshaw cell activity (Löcher et al. 1996a). However, according to Taylor & Gandevia (2008) the role of the recurrent inhibition in fatigue is uncertain. An indirect way to study the recurrent inhibition is the paired H-reflex method, in which the first conditioning stimulus generates the recurrent inhibition in the peripheral nerve, which is then assessed by another consecutive stimulus (e.g., Löscher et al. 1996a). Another interneuron mechanism that may reduce the mechanical force output involves a decline in antagonist interneuron inhibition and the subsequent increase in the agonist-antagonist coactivation (e.g., Weir et al. 1998).

2.3.5 The Hoffman reflex – a measure of net facilitation to the alpha-motoneuron pool

The Hoffmann reflex (H-reflex) is a method for measuring the sensitivity of the monosynaptic Ia-reflex arc (Palmieri et al. 2004). Although it is an integrated measure of inhibitory and facilitatory inputs to the motoneuron pool, and does not in itself represent any individual physiological mechanisms, it is introduced herein because of its central role in the studies concerning the excitability of the motoneuron pool. In contrast to the stretch reflex, the electrically evoked H-reflex response is unaffected by the muscle spindle input. Moreover, while the mechanical stretch of the spindles produces a longer-lasting asynchronous series of action potentials, the electrical stimulation of the axons of Ia afferents elicits a synchronized short-lasting pattern of action potentials (e.g., Voigt et al. 1998). The size of the evoked H-reflex response estimates the number of motoneurons that can be recruited in a given state, provided that external factors, such as the pre-synaptic Ia-inhibition, can be controlled (Palmieri et al. 2004). When the maximal H-reflex is normalized to the maximal M-wave, it expresses the percentage of motoneuron pool that can be recruited. In order to study exercise-induced changes in the H-reflex between subjects, it is produced at a fixed percentage of M_{max} (typically 10 - 25 %) (Palmieri et al. 2004).

Previous studies indicate that explosive training decreases the H_{max}/M_{max}-ratio (Casabona et al. 1990; Maffiuletti et al. 2001), whereas endurance training may increase it (Maffiuletti et al. 2001; Ogawa et al. 2009). These differences could be explained by both genetic and training-induced effects on the number of low- and high-threshold motor units and recruitment order. Avela et al. (2006) discovered a lower H_{max}/M_{max}-ratio in high-jumpers compared to sprinters, which is slightly surprising, because both events involve large amounts of explosive training. Nielsen et al. (1993) observed a low H_{max}/M_{max} ratio in ballet dancers, which was associated with a long-lasting increase in presynaptic Ia-inhibition that may result from high amounts of co-contractions performed during ballet training. Avela et al. (2006) observed also that 10x10 drop-jump exercise induces a greater decrease in the H-

reflex in sprinters than high-jumpers. This was associated with potential training-induced structural adaptation to the impact load in high-jumpers. It has been suggested that H-reflex is affected more by training history than genetics, because it seems to be independent of the aerobic capacity in untrained men (Piscione et al. 2012).

2.3.6 Supraspinal fatigue

Supraspinal fatigue is defined (Gandevia 2001): "*Fatigue produced by a failure to generate output from the motor cortex; a subset of central fatigue"*. Sites and mechanisms of exercise-induced fatigue in the motor cortex are studied by transcranial magnetic stimulation (TMS) (e.g., Taylor & Gandevia 2001). In order to localize the origin of activation failure, both the motor cortex and cervicomedullary stimulations along with the H-reflex measurements can be used in parallel (see, e.g., Methods in Hoffman et al. 2009). The degree of voluntary activation at the supraspinal level is assessed by superimposing magnetic or electrical supramaximal stimulus to the motor cortex during voluntary contraction. An increase in the size of the superimposed twitch indicates that the motor cortex is not driven maximally (Gandevia et al. 1996).

The fatigue-induced decline in cortical excitability was first demonstrated by Brasil-Neto et al. (1993). They showed that repetitive muscle activation may induce a decrease in the amplitudes of the motor evoked potentials to TMS without corresponding changes to transcranial electrical stimulation (TES). However, whether the decrease in the cortical excitability attributed to a decrease in force production or not was not assessed. Later, Gandevia et al. (1996) showed that the descending output from the supraspinal sites is not necessarily optimal, since the size of the cortically evoked superimposed twitches increased in the course of maximal voluntary contraction. Because they measured a concomitant increase in the amplitude of MEPs, the activation failure was attributed to the sites driving the motor cortex. Moreover, an increase in the duration of the silent period, that follows the MEP response, suggested the inhibition of the motor cortex (Taylor et al. 1996). However, the extent to which the aforementioned changes in the cortical excitability contribute to

central fatigue is less clear, since the cortical changes seem to recover while the group III- and IV-reflex feedback is maintained by keeping the muscles ischemic during the post-exercise rest period, whereas both the force output and the activation level remain depressed (Gandevia et al. 1996; Taylor et al. 1996; Taylor et al. 2000). Thus, the III- and IV-afferent inhibition is probably mediated via supraspinal sites upstream of the motor cortex (Taylor & Gandevia 2008). Although the majority of TMS studies have applied isolated single joint contractions, it has more recently been shown that the failure at the supraspinal level may occur in whole-body exercise as well (see review by Gruet et al. 2012).

Several metabolic, thermodynamic, circulatory, and neurohumoral factors have been associated with the supraspinal fatigue (e.g., Nybo and Secher 2004). Besides the potential changes in the cerebral blood circulation, substrate availability, and heat storage, a number of neurotransmitters, such as serotonin, dopamine, glutamate, acetylcholine, adenosine and gamma-aminobutyric-acid, have been of interest to researchers in the field of exercise science (e.g., Nybo & Secher 2004; Meeusen et al. 2006). Furthermore, a set of neuromodulators, such as ammonia (NH3) and interleukins (IL-6), have been attributed to exercise-induced central fatigue (Meeusen et al. 2006). It seems, however, that the available knowledge on the exact mechanisms and their relative contributions to the high-intensity exercise performance remains quite limited.

2.4 High-intensity exercise and muscle fatigue

A positive pacing strategy, in which the highest speed is attained during the early stages of the performance and then followed by a progressive deceleration of speed, is a unifying feature for the most speed endurance sports including the running events from 100-400m (Tucker & Noakes 2009; Hanon & Gajer 2009). In sports with low resistive drag forces, such as cycling and speed skating, the fast start is even more important, since the optimal performance necessitates the maximization of the kinetic energy at very early stages of the sprint (Tucker et al. 2006). Mero et al. (1992) have divided the sprint running distances into three phases: 1) acceleration; 2) constant speed; and 3) deceleration. An aggressive

acceleration and a relatively short constant speed phase are common for both alactic and lactic sprint performances, whereas a more remarkable difference can be observed after the highest speed has been reached. The causes and consequences of fatigue during lactic maximal whole-body exercise and sprint running, in particular, are overviewed.

2.4.1 Fatigue-induced changes in running speed, stride characteristics, and force production during maximal sprint running

Mero et al. (1992) reported that the loss of speed during a world class 100 m race ranges from 0.9 to 7.0 %. Several studies have shown that in a maximal 400 m or 43 - 70 s run the loss of speed ranges from 13 to 39 % (e.g., Nummela et al. 1992; Hirvonen et al. 1992; Nummela et al. 1996; Ferro et al. 2002; Hanon & Gajer 2009; Hobara et al. 2010; Tomazin et al. 2012). For instance, in the men's world record performance on 100 m/9.58 s, the speed deceleration from the fastest 60 - 70 m section to the final 90 - 100 m section was only 1.6 % (Graubner & Nixdorf 2011), but in the 400 m world record run, 43.18 s, the deceleration was 17.3 % from the fastest (50 - 100 m) to the last section (350 - 400 m) (Ferro et al. 2002). This demonstrates the remarkably greater extent of fatigue caused by long lactic sprints. It seems that the fastest athletes are capable of running fast during the first half of sprint distance and, subsequently, they experience relatively a greater loss of speed at the end of the run (Hanon and Gajer, 2009). This suggests that the best speed endurance sprinters possess a greater capacity in speed and higher pain threshold that make them more tolerant of fatigue during the final part of the run. Hence, capacity for the fast and aggressive start seems to be necessary for all running events up to 800 m/110 s, because it seems difficult to profit from the slow start during the final part of the run (Tucker et al. 2006; Saraslanidis et al. 2011).

2.4.2 Early decline of performance

When compared to the short running sprints, in the longer (> 20 s) maximal runs the speed distribution is influenced by a greater number of variables, e.g., the pacing strategy and energy supply (Mero et al. 1992). Consistently, previous experiments and event analyses have indicated that in the long lactic sprints the running speed decelerates non-linearly involving at least two thresholds (Nummela et al. 1996; Hanon & Gajer 2009). The first decline in the running performance appears within the first 10 - 15 s, which is followed by a progressive loss of speed for about next 20 - 30 s until a greater decrease in performance appears (Nummela et al. 1996; Hanon & Gajer 2009).

The initial loss of speed is a consequence of prolonged ground contact times and the lower stride rate, whereas the stride length can be usually maintained (Nummela et al. 1996; Hanon & Gajer 2009; Mero et al. 1992; Ae et al. 1992; Graubner & Nixdorf 2011; Ross et al. 2001). For example, in the world fastest 200 m/19.19 s run, the average stride length was greatest during the final 50 m section (Graubner & Nixdorf 2011). Moreover, changes in vertical leg stiffness and displacement of the vertical center of body mass during the ground contact phase appear at the same time with the initial loss of speed (Hobara et al. 2010). The vertical leg stiffness correlates significantly with the stride rate and the running speed in maximal 400 m runs (Hobara et al. 2010).

The initial changes in the ground contact time and stride rates has been linked with a slower rate of muscle relaxation (Place et al. 2010). As the stride rate starts to decline during the first 10 – 15 s after the start, the runners seem to compensate this by slightly increasing or maintaining the stride length over the next 50 - 100 m distance and, thereby, minimize the loss of speed (Mero & Peltola 1989; Hanon & Gajer 2009; Hobara et al. 2010).

2.4.3 The late decline of performance

In a maximal 400 m run, the second turning-point in the running speed occurs after 30-40 s of running (Hanon & Gajer 2009; Hirvonen et al. 1992; Nummela et al. 1996; Hobara et al. 2010). It is caused by a decline in both the stride length and stride rate so that the latter decreases more especially during the last seconds (Hanon & Gajer 2009). The second turning point in the stride rate is a consequence of the increase in the contact time, while the swing time seems to remain unchanged throughout the whole sprint distance (Nummela et al. 1992; 1996; Hobara et al. 2010; Saraslanidis et al. 2011).

Both the braking and propulsive phases of the ground contact lengthen at the end of the maximal 50 s run (Nummela et al. 1994). The resultant ground reaction forces also decrease both in the braking and propulsive phases at the end of the 400 m sprint (Nummela et al. 1994). The changes in the stride length can be explained by the changes in the ground reaction forces (Mero et al. 1992; Nummela et al. 1994). A significant decline in the drop-jump performance was also shown occur not until after 40 s/300 m in a 53 s/400 m sprint (Nummela et al. 1992). Immediately after the run, the peak vertical force production in the drop jump test was 14 % lower compared to the pre-run test, which refers to the inhibition of explosive stretch-shortening type of force production (Nummela et al. 1992). Hobara et al. (2010) showed also that vertical leg stiffness decreases until the finish line in a 400 m sprint and correlates with the vertical displacement of the center of body mass, running speed, and stride rate.

2.4.4 Limitations in energy expenditure

The amount of free ATP is very limited in the skeletal muscle. The rate of ATP utilization is to a large extent limited by the rate of its regeneration, since the ATP concentration remains quite stable in the skeletal muscle even during extreme muscle activity. Gastin (2001) suggested that 30 - 40 % decrease in [ATP] may occur, whereas Karatzaferi et al. (2001) showed, somewhat controversially, that [ATP] may decrease down to 20 % of the resting

level in type IIX-fibers in a maximal 25 s cycling exercise. In order to avoid an exhaustive depletion of ATP, the rate of PCr breakdown reaches its maximum immediately as the muscle contraction begins, but starts to decline already after 1.3 seconds (Gastin 2001). In well-trained sprinters PCr depletes in about 5 s during a maximal 11 s sprint (Hirvonen et al. 1987), whereas in a maximal 50 s run, the PCr concentration could be reduced by 50 % after 12 s and by 90 % after the run (Hirvonen 1992). The importance of PCr for high-intensity exercise was demonstrated by Bodganis et al. (1995), who showed that the recovery of power output between two consecutive 30 s maximal cycling sprints proceeds in parallel with the restoration of muscle PCr despite low pH values. Sahlin et al. (1998) suggested that the availability of PCr limits the muscle power output even before it is totally depleted, because the glycolytic energy supply, that means a slower rate of ATP regeneration, starts to increase only a few seconds after the onset of activity. The development of PCr depletion seems to be temporally related to the initial decline in performance, such as the initial loss of running speed, the decrease in the stride rate, the increased contact time, and leg stiffness, during sprint runs (Ae et al. 1992; Ferro et al. 2002; Nummela 1992,1996; Hanon & Gajer 2009; Hobara et al. 2010).

In maximal efforts, the anaerobic processes dominate the total energy supply when the overall duration is less than 75 s (Nummela & Rusko 1995; Spencer & Gastin 2001; Zouhal et al. 2010). A classical reference by Newsholme et al. (1992) suggests that anaerobic glycolysis contributes 65, 62.5, and 50 % of ATP generation in maximal 200, 400, and 800 m runs. Other studies have estimated that the overall contribution of anaerobic energy sources in a maximal 50 s run is approximately 60 % (Nummela & Rusko 1995; Spencer & Gastin 2001; Zouhal et al. 2010). Consequently, high levels of BLa (16.1-22.2 mmol/l) have been measured after maximal runs ranging from 25 s to 110 s (Saraslanidis et al. 2010; Hanon et al. 2011; Hanon et al. 2010; Lacour et al. 1990).

In the course of a given maximal run, the aerobic energy supply begins to dominate after 15 - 30 s of running (Spencer & Gastin 2001), albeit this depends, for example, on the athletes training history (Nummela & Rusko 1996). In a 50 s maximal run, aerobic and anaerobic

glycolysis reaches the maximum rate approximately after 25 s (Hirvonen et al. 1992; Hanon et al. 2010). Thereafter, the glycolytic processes will be attenuated, likely, due to the excessive accumulation of metabolic by-products. In the context of sprint running, the slowing rate of the glycolytic energy supply co-occurs with a more remarkable deceleration of the running speed including several mechanical changes, such as decreased stride length and rate, prolonged ground contact, decreased vertical leg stiffness and force production (Hirvonen et al. 1992; Nummela et al. 1992;1994;1996; Hanon & Gajer 2009; Hobara et al. 2010). However, it is not definitely clear whether the attenuation of glycolytic processes is the cause or the consequence of the slowing running speed (see, e.g., discussion Hanon et al. 2010). For instance, the impaired glycolytic energy supply may re-accelerate the utilization of PCr for the ATP regeneration at high levels of blood lactate (Hirvonen et al. 1992). It is anyway clear that the rate of energy supply and the availability of the immediate energy sources, PCr and ATP, limit the force and power generation during maximal sprint runs.

2.4.5 Accumulation of metabolic by-products

The rapid depletion of PCr during the first seconds of maximal sprint results in the accumulation of P_i ions. For instance, Bodganis et al. (1995) estimated that [P_i] increased from 2.9 to 18.5 mmol/l during a 30 s maximal sprint. There is evidence that an increase in the sarcoplasmic [P_i] has a negative influence on metabolic enzymes, Ca^{2+} availability, and cross-bridge functionality (Allen et al. 2008). Place et al. (2010) suggested that the reducing stride rate could be attributed to the accumulation of P_i due to its negative effects on the relaxation time. On the other hand, elevated muscle P_i did not prevent the recovery of power output in two consecutive maximal 30 s cycling tests (Bodganis et al. 1995).

The muscle ATP decreases slightly but significantly during a maximal 50 s sprint (Hirvonen et al. 1992) and the depletion could be severe in fast-twitch fibers (Karatzaferi et al. 2001). According to Sahlin et al. (1998) a small decrease in [ATP] leads to a relatively large increase in [ADP], which has an inhibitory influence on the muscle power output and

metabolic enzymes. Thus the decline in ATP could affect especially fast-twitch fibers during the late stages of lactic sprints.

Prolonged glycolytic energy supply leads to a gradual accumulation of H^+-ions in the muscles and blood. When blood pH was measured after a 300 m run, that was run using the 400 m pacing strategy, it correlated with the extent of speed deceleration during the last 100 m of the complete 400 m run (Hanon et al. 2010). Even though this would not necessarily imply a causal relationship, it has been suggested that acidosis impairs the performance by inhibiting glycolytic enzymes and, thereby, energy metabolism (Sahlin et al. 1998). It has also been hypothesized that the inhibitory effects of acidosis are centrally mediated (Cairns 2006; see also Bigland-Ritchie et al. 1986a). Nummela et al. (1996) suggested that the abrupt change in the stride contact time after 300 m of running in a 400 m sprint could be explained by the attainment of an individual tolerance of acidosis.

Another potential metabolic cause of central fatigue is ammonia, which is produced as a result of adenosine monophosphate (AMP) degradation which is split into NH_3 and inosine monophosphate (IMP) (Allen et al. 2008). To the best knowledge of the author there is no data available on the effects of ammonia during short maximal efforts, but Nybo et al. (2005) reported a relationship between the ratings of perceived effort and the ammonia concentration in the cerebrospinal fluid in a 3 h cycling test. Tomazin et al. (2012) hypothesized that the post-exercise decline in the level of central activation ratio was caused by an increased concentration of ammonia in the blood and brain. It has also been shown that the plasma $[K^+]$ may double in a one minute all-out sprint (Medbø & Sejersted, 1990). Since an increase in the extracellular $[K^+]$ impairs the excitability in the sarcolemma, its negative effects on sprint running cannot be excluded (MacIntosh et al. 2006, 237). The precise extent, effects, and mechanisms underlying the aforementioned metabolic perturbations are partly unclear in the context of sprint running and out of the scope the present thesis.

2.4.6 Stretch-reflex alterations

Instead of pure concentric, eccentric, or isometric contractions, the skeletal muscles are stretched (eccentric contraction) before the shortening phase (concentric contraction) during natural weight-bearing human locomotion such as running, jumping, and cross-country skiing. This type of muscle work is referred to as the stretch-shortening cycle (SSC; Komi 2000). The pre-stretch of a muscle activates the stretch reflexes that result in an increase in the muscle stiffness, which finally results in an increase in the force generation during the subsequent phase of concentric contraction. A proper pre-activation of the muscle before the ground contact and a short delay between the eccentric and concentric phases are prerequisites for the effective utilization of SSC. (Komi 2000)

The contact time in high-speed sprinting is 80 - 100 ms, and the peak of the ground reaction force occurs 10 - 40 ms after the start of the ground contact (Mero et al. 1992). Consequently, the short-latency stretch-reflex with the delay of 40 ms, approximately, is assumed to make a contribution to the force and power enhancement at the end of the eccentric phase and, probably, during propulsive phase as well (Komi 2000). Thus, a reduction in the stretch-reflex force enhancement mechanisms could contribute to the fatigue developing in the course lactic sprint effort. The neural mechanisms involved in the SSC fatigue include the pre-synaptic inhibition by group III- and IV-afferents and Golgi Tendon organ, and the Ia-disfacilitation of the motoneuron pool (Komi 2000). The net disfacilitation at the alpha-motoneuron pool can be assessed by the H-reflex method (Palmieri et al. 2004). Reduced short-latency stretch-reflexes, H-reflexes, and EMG-activity have been discovered after long duration SSC-tasks, such as marathon running (Avela & Komi 2008). The mechanical changes induced by lactic sprint runs, including prolonged braking and propulsive phases of the ground contact (Nummela et al. 1994; 1996), the decreased braking and propulsive ground reaction forces (Nummela et al. 1994), reduced vertical stiffness (Hobara et al. 2010), a greater knee flexion during the support phase (Saraslanidis et al. 2011), resembles the mechanical changes observed after prolonged SSC tasks (e.g., marathon running). On the other hand, the EMG activity has been shown to

increase during the overall ground contact phase and, especially, during the braking phase, and remain unchanged during the propulsive phase at the end of the 400 m sprint (Nummela et al. 1994, 1996). Minor reductions in EMG occur during the initial phase of the ground contact during a drop-jump test performed after a maximal 400 m run (Nummela et al. 1996). This was hypothetically associated to the reduced spindle support and increased tendon organ inhibition. Repeated running sprints 12 x 40 m had neither any effects on the net alpha-motoneuron pool excitability or soleus EMG activity (Perrey et al. 2010). The small number of studies on lactic sprint runs does not provide much support for stretch-reflex alterations, which suggests that changes in running mechanics are mainly caused by other mechanisms than SSC fatigue. However, the number of studies is limited, which is why the knowledge on genetic, training, gender, and age factors is scarce.

2.4.7 Neuromuscular fatigue in high-intensity exercise

Most of the studies concerning neuromuscular adaptation in high-intensity exercise have applied running or cycling sprints, and drop-jump workouts. Only a few of them have utilized electrical stimulation for investigating the sites and mechanisms of fatigue during or after high-intensity exercise (Lattier et al. 2004; Skof & Strojnik 2006; Perrey et al. 2010; Tomazin et al. 2012). Even though fatigue protocols have varied from maximal 400 m sprints on a treadmill to sprint intervals in uphill and from short to long sprint intervals, all of the previous studies conclude that peripheral low frequency factors are the main contributors in post-exercise fatigue. It seems that the sarcoplasmic reticulum and the cross-bridge structure are the most sensitive sites in maximal short-term running.

In addition to the obvious signs of low frequency fatigue, slight reductions (3 - 6 %) in the voluntary activation were observed both after a maximal 400 m run (Tomazin et al. 2012) and a 12 x 40 m interval session (Perrey et al. 2010). In the former case, voluntary activation was unchanged 30 s after the 400 m run, but then decreased so that after 5 min of recovery it was 6 % below the pre-exercise value. The authors hypothesized that the delayed deficit in the voluntary activation resulted from slow accumulation of ammonia into the

blood and the brain (Tomazin et al. 2012). Quite similarly VA% was reduced by 2.7 % after 10 x 40 m running sprints with 30 s recovery intervals (Perrey et al. 2010). For the running performance, however, the effects of the activation deficit were probably insignificant, because all of the other related parameters, muscle EMG activity, H-reflex, and hand-grip force were unchanged after sprints. The research protocol shows that the delay from the end of the last sprint to the measurement of VA% was about 3 min, which is why the hypothesis of the delayed accumulation of ammonia could be applicable in this case as well. A quite different observation regarding the voluntary activation after an all-out 30 s cycling was made by Fernandez-del-Olmo et al. (2011). They applied transcranial magnetic stimulation (TMS) interpolation technique and reported 34 % reduction in the level of voluntary activation and 16 % and 36 % reductions in MVC force and resting twitch, respectively. Since there were no changes in the cortical EMG responses (the motor evoked potential and the silent period), the authors concluded that major cause of fatigue were the processes driving the motor cortex.

A few studies have demonstrated that the muscle EMG activity increases, particularly in the braking phase, during maximal lactic running sprints suggesting that the central nervous system has a capability to compensate the developed peripheral fatigue (Mero & Peltola 1989, Nummela et al. 1992; 1994). It has been shown that, at the end of 400 m sprint, the decrease in the ground contact forces is minimized by increasing the neural pre-activation immediately before the start of the contact phase (Nummela et al. 1994). Corresponding findings have been made after short repeated cycling sprints with an addition that there was also a downward shift in the EMG-frequency spectrum (Billaut et al. 2006). Changes in the muscle EMG activity, the EMG frequency components and running mechanism (e.g., contact time) have been associated with an increase in the neural drive, modifications in the motor unit recruitment pattern, and a decrease in the muscle fiber conduction velocity (Nummela et al. 1994; Billaut et al. 2006). Controversially, there are two studies at least in which the muscle EMG activity was shown to decrease during maximal repeated cycling sprints (Racinais et al. 2007; Mendez-Villaneuva et al. 2008). Even though these findings are also interesting, any changes in the muscle surface EMG activity should be interpreted

with caution due to the many factors that contribute to the EMG output (Kamen & Gabriel 2010, 119).

Some evidence of exercise-induced changes in the neuromuscular propagation and the sarcolemmal impulse propagation were observed after repeated sprint runs (5 x 300 m and 12 x 40 m), so these processes cannot be completely excluded from the mechanisms underlying the fatigue in maximal short-term runs (Skof & Strojnik 2006; Perrey et al. 2010). A greater degree of high-frequency fatigue was observed after a 62 s drop-jump task by Strojnik & Komi (1998). A common factor between drop-jumping and sprint running is the stretch-shortening cycle (Strojnik & Komi 1998; Ross et al. 2001). However, Tomazin et al. (2012), for instance, did not find evidence in support of HFF after sprint running. There appear to be also other differences in physiological responses to maximal one minute drop-jumping and sprint running efforts. While the maximal 1 min drop-jumping did not cause any changes in the knee extensor MVC (Strojnik & Komi 1998), 14 % reduction was observed after maximal 400 m maximal run (Tomazin et al. 2012). Interestingly, the level of the blood lactate concentration was also low after the aforementioned drop-jump effort when compared to maximal 25 - 60 s runs (Hanon et al. 2010; Saraslanidis et al. 2011). Moreover, both the voluntary activation and the muscle EMG activity were shown to increase as a result of a maximal one minute and long submaximal about 7 min drop-jump efforts (Strojnik & Komi 1998; Strojnik & Komi 2000). Perhaps the mechanical stress of the contact phase in the drop-jump movement and the subsequent neural inhibition from the peripheral mechanoreceptors limit the exercise intensity so that metabolic processes cannot be exploited at the maximal rate during high-intensity jumping workouts. Nummela et al. (1992) demonstrated that the muscle EMG during running stride increased in the course of a maximal 50 s run, but decreased when it was measured by a maximal drop-jump test. It seems that the exercise-induced changes in force production and neural activity converge with long sprint running responses as the period of a drop-jump workout is extended to several minutes or even half an hour (Strojnik & Komi 2000; Kuitunen et al. 2004). Similarly to 400 m run (Tomazin et al. 2012), the low-frequency fatigue was found to be the predominant cause of fatigue after about 7.5 min and 29 min drop-jump efforts at

submaximal intensities (Strojnik & Komi 2000; Kuitunen et al. 2004). It was also found out in the longer drop-jump test that the number of jumps completed by the time of exhaustion correlated with the decrease in the level of voluntary activation (Kuitunen et al. 2004). The role of central factors seems to increase with the duration of the task. For example, Millet et al. (2003) observed that after a maximal 30 km run the level of voluntary activation decreased 11 % from the pre-run values. Moreover, the neuromuscular measurements indicated that high-frequency fatigue was more predominant than low-frequency fatigue after the run.

2.5 Maturity-related factors in neuromuscular fatigue during high-intensity exercise

Significant differences in speed, strength and endurance separate children from adults. In addition, numerous studies have established that both the fatigue resistance and the rate of recovery change during maturation (Ratel et al. 2006b). However, less is known about the underlying mechanisms. For instance, there are only few studies on the maturity-related changes in neuromuscular functions in the context of high-intensity exercise. In this chapter, the current knowledge on the effects of maturation on muscle fatigue is overviewed. The main focus is on the high-intensity exercise. In order to outline the study the high-intensity exercise is defined according to Ratel et al. (2009): "*the intensity where the demand of the ATP turnover exceeds the maximal capacity of the aerobic system*".

2.5.1 Children fatigue less than adults

A number of studies have demonstrated that children recover more quickly and maintain a steadier level of performance during a high-intensity intermittent exercise compared to adults (Ratel et al. 2009). There also exists a piece of evidence that children might be more fatigue resistant than adults during continuous high-intensity whole-body efforts (e.g., Beneke et al. 2005).

Studies on the age-related differences in a 30 s all-out cycling test (Wingate test) have shown that age-related comparisons are sensitive to the indices of power output and fatigue. For example, when the power output was normalized to the body-mass, adults showed a greater fatigue, that is, a greater reduction in the power output than adolescents during Wingate test, whereas normalization to the muscle mass did not produce significant difference between the groups (Beneke et al. 2005). When children and adolescents were compared in the same test, there was no difference in fatigue when the power output was normalized to the body mass, but when the power output was normalized to the muscle mass the children showed somewhat unexpectedly greater fatigue than adolescents (Beneke et al. 2007). The authors speculated that the unexpected difference could be explained by the 18 % higher braking resistance in relation to the body mass in the boys group.

Few studies have addressed maturity-dependent changes in the fatigue resistance in the context of high-intensity whole-body exercise in particular, but a somewhat greater number of experiments have been carried out using single joint isometric and isokinetic movements. These studies have shown that prepubertal and adolescent boys experience less fatigue compared to adults in maximal isometric and isokinetic knee extension tasks (Paraschos et al. 2007; Kanehisa et al. 1994; Armatas et al. 2010) and also in a maximal isometric elbow flexion task (Halin et al. 2003). Controversially age-dependent differences in fatigability were not observed between men, women, boys or girls in a maximal isometric knee extension test that was conducted by Streckis et al. (2009). Similarly, there were no differences in the extent of fatigue between prepubertal, adolescent, and adult men in a isokinetic knee intermittent anaerobic extension test, but the rate of recovery between the sets were shown to be maturity-dependent so that recovery progressed faster in young subjects compared to the adults (Zafeiridis et al. 2005).

When compared to adults, pre-pubertal children showed significantly less fatigue and higher rate of recovery in two consecutive all-out 30 s cycling sprint tests (Hebestreit et al. 1993). A significant difference between adult males and prepubertal boys were found in their capability to recover during the rest periods in the course of maximal 10 x 10s running and

cycling sprint experiments (Ratel et al. 2002, 2006a). Thirty seconds rest periods in cycling and 3 min in running were enough for the prepubertal boys for maintaining their peak power output constant throughout the ten sprint repetitions (Ratel et al. 2002; 2006a). As for the adults, 3 min rest periods were enough for maintaining the running speed throughout the repetitions (Ratel et al. 2006a). It is, however, likely that keeping the running speed constant necessitated some modifications to the running technique by the adults because of the simultaneous decline in the power and force output characteristics (Ratel et al. 2006a). Regardless of age high-intensity intervals by running causes greater fatigue and higher RPE than cycling (Ratel et al. 2004). The authors suggested that the greater fatigue in sprint running compared to cycling is due to additional muscle recruitment and greater accumulation of lactate. It remains to be clarified whether the more consistent sprint interval performance in children extrapolates to the continuous maximal sprints. The lower extent of fatigue in children has been attributed to the limited energy utilization and more efficient recovery during rest (Ratel et al. 2009). While previous studies have addressed mostly male subjects, it is important to be conscious that the development of fatigue resistance may differ between genders due to the different maturation profiles (Dipla et al. 2009).

2.5.2 Lack of motivation

To the knowledge of the author motivational aspects have been barely studied in the present context. Based on their own practical experience Ratel et al. (2006b) suggested that motivation does not affect significantly to the children's performance and fatigue in experimental high-intensity efforts. Hebestreit et al. (1993) also observed that children were able to motivate themselves during two consecutive Wingate tests. A piece of evidence is provided by Zafeiridis et al. (2005) who reported that both children and adults started the maximal intermittent knee extension/flexion test at maximal intensity that was determined by a maximal pre-test and the development of fatigue during the repetitions was not significantly different between the groups. Thus children appear to be well-motivated in short maximal efforts.

2.5.3 Active muscle mass and power capacity

When compared with adults, children are characterized by a smaller size of muscle fibers, smaller cross-section area in the muscles, and lower lean mass of body (Van Praagh & Doré 2000). Moreover, their power generation capacity increases with age until the individual maximum is reached in adulthood (Van Praagh & Doré 2000). Falk & Dotan (2006) came to the conclusion that children have "*less to recover*" at the end of maximal exercise bout, because their power generation capacity is limited by maturity-dependent neuromuscular factors. This is line with findings that adults reach a greater initial force level with respect to muscle size and they also developed a greater extent of muscle fatigue than younger subjects in the course maximal isokinetic and isometric knee extension and elbow flexion tests (Kanehisa et al. 1994; Halin et al. 2003). On the other hand, even though adults experienced a greater percentage reduction in the absolute force production capacity compared to children during an isokinetic fatigue test, the difference was insignificant if the force was normalized to the muscle volume (Kanehisa et al. 1994). Moreover, Zafeiridis et al. (2005) showed that both the total amount of work and the attained peak torque during the first set of intermittent maximal isokinetic knee extension/flexion exercise correlated inversely with the rate of recover during the rest intervals.

According to regression studies age in itself predicts the maximal power generation capacity only modestly during the period of maturation, whereas much more can be explained by the anthropometric characteristics (Doré et al. 2000; Martin et al. 2000). For instance, the lean leg volume explains nearly 90 % of the variation in cycling peak power in boys between years from 7.5 to 18 (Doré et al. 2000). Moreover, the lean thigh volume explains about 76 % of the variation in the cycling peak power across the life span (Martin et al. 2000). Thus the growth of muscle mass during the period of maturation results in an increase in the power generation capacity and, subsequently, in an ability to develop fatigue. Besides the growth of anthropometric dimensions several other qualitative characteristics, such as neural activation, the muscle fiber type distribution, anaerobic enzyme activities, myofibrillar dimensions may enhance the power generation capacity in the course of

maturation (Doré et al. 2000). It seems that the growth of muscle mass involves an increase in the force/power capacity, which is related to the greater extent of fatigue that can be developed during high-intensity efforts.

2.5.4 Muscle fiber composition

Fatigable fast-twitch muscle fibers play an important role in sports that are based on explosive force production and fast movements. It has been well-established that the greater the percentage of type II fast-twitch fibers the more fatigable the muscles are during high intensity exercise (e.g., Thorstensson & Karlsson 1976; Komi & Tesch 1979; Hamada et al. 2003). A lower number of fast twitch fibers in childhood may counteract exercise-induced fatigue during high-intensity bouts by limiting glycolytic activity, anaerobic capacity and power generation capacity. Therefore, it may appear obvious that potential growth-related transformations between the slow fatigue resistant and the fast more fatigable fiber types affect the force production and fatigue characteristics. However, a little evidence is available on the maturity-related fiber transformations (reviews Van Praagh & Doré 2000; Ratel et al. 2006b).

Glenmark et al. (1994) presented that the proportion of slow-twitch fibers may decrease in men from adolescence to adulthood. Kriketos et al. (1997) suggest that the age-dependent transformation occurs only within the fast-twitch fiber types so that a part of the oxidative IIa fibers convert into the glycolytic IIb fibers from infancy to adulthood (Kriketos et al. 1997). An indirect evidence of the maturity-related fiber type transformations was recently obtained by a non-invasive ^{31}P-magnetic resonance spectroscopy technique by Tonson et al. (2010), who showed that children utilize more oxidative metabolism than adults, which indicates that children's muscles consist of higher proportion of oxidative fibers (Tonson et al. 2010). While it appears plausible that the fiber type composition contribute to maturity-related changes in the fatigue profile further studies are still needed.

2.5.5 Muscle metabolism

Exercise-induced accumulation of anaerobic metabolites (La, H^+, P_i etc) is classically attributed to muscle fatigue (Fitts 2008). Several experiments have established that the profiles of anaerobic and aerobic enzymes are not identical in children and adults and glycolytic capacity enhances with maturation (e.g., Ratel et al. 2006b). Consistently, there is quite indisputable evidence available that the blood lactate concentration and acidosis reach the highest levels in adulthood (Falgairette et al. 1991; Kuno et al. 1995; Hebestreit et al. 1996; Beneke et al. 2005; Zafeiridis et al. 2005). However, the higher blood lactate concentration does necessarily imply that the rate of lactate production in the muscle increases with maturation. This has been questioned by Beneke et al. (2005) who estimated that the age-related differences in the blood lactate kinetics are more likely due to the faster elimination of lactate out of blood compartment than the lactate production in the muscles or its invasion to the blood.

For example, Ratel et al. (2002, 2006a) showed that adults produce a higher level of blood lactate than children after maximal intermittent treadmill running and cycling efforts. LeClair et al. (2011) reported that that the children show lower anaerobic capacity than adults in terms of maximal accumulated oxygen deficit in an exhaustive cycling exercise at 110 % intensity of the maximal aerobic power. Interestingly, the intensities below the maximal aerobic power did not produce differences in the time to exhaustion between the children and adults. Higher concentrations of metabolic by-products may also explain the finding that adults express a higher rate of perceived exertion than children during maximal running sprints (Ratel et al. 2006a).

In general the lower rate of accumulation of metabolites has been associated with lower anaerobic enzymes activity. Kazcor et al. (2005) showed that activity of a glycolytic enzyme, lactate dehydrogenase, is lower in children compared with adults. On the other hand, Beneke et al. (2005) did not attribute the low lactate levels in children to the lower anaerobic profile in the muscles compared to adolescents and adults, but instead to the

lower relative muscle mass, the higher total lactate water space relative to the body mass, and more appropriate conditions for aerobic metabolism. ^{31}P magnetic resonance spectroscopy studies have anyway indicated that oxidative capacity of muscles is higher in children than adults (Taylor et al. 1997; Tonson et al. 2010). Children also experience a faster muscle oxygen uptake response than adults at the onset of exercise (Armstrong & Barker 2009). However, according to Armstrong & Barker (2009), knowledge on mechanisms underlying the differences in the oxygen uptake response remains narrow, but it could be related to the feedback control of the rate of oxidative metabolism, distribution of muscle fiber types, or motor unit recruitment strategy. It has also been speculated that the increased accumulation of blood lactate could be associated with the maturity-related changes in the size of fast twitch type II fibers and testosterone levels (Mero 1988; Falgairette et al. 1991).

In general, the maturation-related effects of metabolic by-products should be interpreted with caution, because the findings are not supported invariably (review Ratel et al. 2006b) and even the inhibitory effects of the by-products remain under debate (e.g., Westerblad et al. 2002; Allen et al. 2008).

2.5.6 Neural factors

The development of peripheral fatigue during repeated maximal voluntary isometric plantar flexions is influenced by the level of voluntary activation and force production at the beginning of the task (Nordlund et al. 2004). In addition, Falk & Dotan (2006) proposed that children need less time to recover after high-intensity exercise because their power generation capacity is limited by their inability to recruit higher hierarchy motor units maximally. However, a little evidence is available on the maturity-dependent differences in the neural activation deficit. In the following two studies the maturity-related differences were assessed through the twitch interpolation technique (Merton 1954). Belanger & McComas (1989) studied the age-related differences in the level of activation during plantar flexor MVC, but did not find any significant differences for children and adolescents. More

recently it was shown that the level of voluntary activation in plantar flexors is lower in 7 years old compared to 10 - 11 years old children and lower in children compared to adults (Grosset et al. 2008).

It has also been shown that the level of activation and the agonist EMG decreases less in children compared to adults during maximal isokinetic and isometric contractions (Paraschos et al. 2007; Armatas et al. 2010). This indicates that maximal exercise results in greater inhibition or disfacilitation of the descending motor pathways in adults compared to children (Paraschos et al. 2007). It has also been shown for maximal intermittent and sustained voluntary contractions that the pre-fatigue levels of the mean power frequency and muscle fiber conduction velocity are higher in adults than children and that both of these parameters decline more in adults compared to children during the contractions (Halin et al. 2003; Armatas et al. 2010). These findings indicate that the proportion of high-threshold fast-twitch muscle fibers was greater in the adults, and/or that the adults were capable of recruiting a greater percentage of fast-twitch fibers at the onset of the contractions with the result that some of them dropped off later due to fatigue (Halin et al. 2003; Armatas et al. 2010).

On the other hand, children were shown to experience a lower decline in force production but a greater reduction in voluntary activation compared to adults during a sustained maximal contraction of knee extensors (Streckis et al. 2009). In other words, there was less peripheral but more central fatigue in children than adults. This suggests that the greater central fatigue in the children was not because of metabolite-induced group III- and IV-inhibition, but rather it was caused by a decline in the descending voluntary motor drive.

Maturity-related differences in force/power production and fatigue could be also attributed to agonist-antagonist co-activation. Grosset et al. (2008) showed that co-activation of the tibialis anterior (antagonist) and triceps surae (agonist) muscles in isometric plantar flexion decreases with age from prepuberty to adulthood. Antagonist activation was also higher in

children compared to adults during 25 maximal isokinetic knee extensor contractions (Parachos et al. 2007). However, the adults showed a greater decrease in force production.

3 THE PURPOSE OF THE STUDY

Prior studies have shown that the speed endurance type of maximal short-term exercise sessions and competitive performances induce remarkable muscle fatigue. This fatigue manifests itself in a significant decrease in the speed or power generation as well as accumulation of metabolic by-products during the final stages of the performance. It is generally accepted that exercise-induced muscle fatigue may have both central and peripheral origins. It is also generally accepted that the contributions of various mechanisms depend on the task performed. Numerous studies have established that in sprint running fatigue occurs mainly due to peripheral origins. It seems clear that the so-called low-frequency fatigue plays a major role in the loss of force and power production capacity during maximal short-term run. LFF can further be associated with several metabolic changes, such as the accumulation of inorganic phosphate and hydrogen ions and the depletion of the creatine phosphate and ATP stores. While this evidence appears indisputable, it nevertheless concerns mainly male adults. Moreover, only a few studies have applied neuromuscular stimulation methods to investigate neural adjustments in the context of lactic sprint running.

Thus, the main purpose of this study is to extend the knowledge on the mechanisms underlying neuromuscular fatigue during lactic maximal running sprints to the pre-pubertal and pubertal children. Furthermore, the aim is also to clarify the role of neural adjustments in the muscle fatigue induced by a short-term maximal run in child, adolescent, and adult athletes. The main hypothesis of the study is that, in adults, the fatigue is mainly caused by peripheral factors during a maximal short-term run, even though neural adjustments due to the inhibitory reflex feedback may occur. In children, the neural factors play a greater role in the muscle fatigue compared to adults.

4 METHODS

4.1 Subjects

Twenty four male volunteers participated in the study. Based on age and the previous performances in the long-sprint events they were divided into three groups (TABLE 1): Children (N = 8; 11 - 14 years), Youth (N = 8; 14 - 16 years), and Adults (N = 8; 18 - 24 years). Adults represented good national level athletes in the 400 m flat and 400-m hurdles events (the group mean of the 400 m record was 49.5 ± 1.8s), whereas the subjects in the two other groups were active although less specialized young runners training and racing on a more diverse set of distances. All participants signed an informed consent and were aware of the protocol, benefits, and possible risks of the study. They were also advised of their right to retire from the study at any time. The study was performed according to the declaration of Helsinki, and was approved by the Ethical Committee of the University of Jyväskylä, Finland.

TABLE 1. Group and subject characteristics.

Group	N	Age	Height	Weight
Children	8	11.9 ± 1.4	161.4 ± 9.6	47.9 ± 7.1
Youth	8	14.9 ± 1.1	179.6 ± 2.8	63.9 ± 4.2
Adults	8	21.3 ± 3.3	182.0 ± 6.7	73.2 ± 7.0

4.2 Protocol

The study was undertaken between the end of competitive indoor season and the beginning of the outdoor season (March-June) on a 200 m indoor track. The subjects were advised to

rest or exercise lightly the day before the test and have a fasting period of ten hours before the blood sampling in the morning of the test day. After the blood sampling, electrodes were fixed, each subject was familiarized with the measurement equipment and the protocol.

After the initial preparations were finished, the recruitment curve of the H-reflex was collected and the resting blood sample taken. Thereafter, each subject performed 60 min warm-up session. The normal individual racing warm-up without long maximal sprints was recommended. In order to activate the energy production systems, each subject ran a short maximal 80-100 m sprint after 45 min of warm-up. During the last 15 min of the warm-up session three maximal counter-movement jumps were performed on a contact mat.

As the warm-up session was completed, a facemask for the portable gas analyzer was positioned, a blood sample was taken and the right foot, the right thigh, and torso were attached with belts to the seat and pedal of the ankle dynamometer. When a subject was ready, the pre-fatigue neuromuscular tests were started. The pre-fatigue measurements included the H/M-ratio, the supramaximal passive twitch, isometric MVC effort, and superimposed twitch during isometric MVC contraction. After the neuromuscular tests, the gas analyzer and the heart rate monitor were fixed and the subjects moved on to the indoor track. The third blood sample was taken 2-4 min and the gas analyzer was turned on 2 min before the maximal 300/350/400 m/50 s run started. Intermediate times were recorded after each 100 m interval for Children and Adults, and after the 50, 150, 250, 350 m splits for Youth.

In order to minimize the recovery from the run before the neuromuscular tests, the subjects were moved back to the laboratory as quickly as possible on a wheeled table immediately after finishing the run and the first post-fatigue blood sample was taken. Six minutes after the finish of the run, the post-fatigue neuromuscular test set was started. The test set was identical with the pre-fatigue set, except that it was started with two supramaximal stimuli for measuring the maximal M-wave at the beginning and the two post-fatigue blood samples

were taken 5 and 8 min after the run. The neuromuscular tests were completed 11 min after the finish of the run. Thereafter, the collection of breathing gas was continued until 15 min had elapsed from the finish of the run. 16-18 min after the run, three countermovement jumps were performed. The blood samples were further drawn 11, 14, 30, and 60 min after the run. The countermovement jumps were not included in the data analysis due to the malfunction in the contact mat.

4.3 Neuromuscular measurements

4.3.1 Measurement equipment

Torque measurements. An ankle dynamometer (University of Jyväskylä, Finland) was used to measure the exercise-induced changes in the plantar flexor torque. During the familiarization phase the seat position of the ankle dynamometer was adjusted to the following joint angles: ankle 90 ± 3°, knee 180 ± 3° and hip 120 ± 3°. The subjects were firmly attached to the seat by belts at the shoulders and thighs. The foot was firmly attached to the pedal of the force transducer. All the force, EMG, stimulation experiments were performed in this seated position. The torque signal was amplified (x100, Kistler Charge Amplifier, Type 5011) and supplied to the A/D-converter (Power1401, CED Ltd., Cambridge, UK). The torque signals were analyzed with Spike 5.14 software (CED Ltd., Cambridge, UK).

Electromyography. Bipolar circular SEMG electrodes (interelectrode distance 2 cm) were placed over the soleus and medial gastrocnemius muscles according to the recommendations by SENIAM (Hermens et al., 1999). The ground electrode was placed over the tibia. In order to ensure good skin-electrode contact, common skin preparation techniques (shaving, abrasion with sandpaper, cleaning with alcohol) were applied before the placement and fixation of the electrodes. Whenever the impedance of the fixed electrodes exceeded 10 kΩ the preparation procedure was repeated. The fixation of the

sensor electrodes was ensured with taping and thigh straps. The data were recorded and filtered (sampling rate 1500 Hz, bandwidth 10-500 Hz, gain x 2) using a wireless EMG transmitter/receiver system (Noraxon 2400T/R, Scottsdale, USA). The SEMG electrodes were connected to the transmitter device with preamplified leads (gain x500). The amplified and filtered signal was digitized with an A/D converter (Power1401, CED Ltd., Cambridge, UK). The EMG signals were analysed with Spike 5.14 software (CED Ltd., Cambridge, UK). Only the data from the soleus were analyzed.

Electrical motoneuron stimulations. In order to evoke H-reflex, M-wave, V-wave, and force twitches the posterior tibial nerve was stimulated with a single rectangular (200 μs) pulse (Digitimer DS7®, Hertfordshire, UK). The self-adhesive cathode (1.5 x 1.5 cm) was placed and fixed in the popliteal fossa. The anode, which was a large oval-shaped (5 x 8 cm) self-adhesive electrode, was placed over the upper edge of the patella tendon. The placement of the fixed cathode was determined in the standing position by a handheld cathode electrode. The stimulation site was determined as the place where the greatest M-wave response from the soleus was obtained. The stimulus site was marked to the skin and the fixation of the stimulation electrode was ensured by taping and thigh straps.

4.3.2 H/M-recruitment curve

The recruitment curve of the H-reflex was measured from the soleus before the warm-up. During the data collection the subjects sat muscles relaxed with the stimulated leg in an extended position in the ankle dynamometer bench. The belts were not yet tightened. The stimulation intensity was increased from 5-10 mA by 1-5mA steps (pulse duration 200 μs, stimulus interval 10 s) until the H-reflex disappeared. Thereafter, the stimulus intensity was further increased in 10mA steps until either the increase in the M-wave levelled off or the subject wanted to retire. The former condition was satisfied with all the subjects. The H_{max}/M_{max}-ratio was analyzed from the recruitment curve.

4.3.3 Maximal M-wave

The maximal peak-to-peak amplitude of the M-wave was analyzed from the soleus before the warm-up (as the largest M-wave amplitude observed in the H/M-recruitment curve), between the warm-up and the run (as the mean of two maximal M-wave responses observed during the supramaximal passive twitch stimulation), and twice after the run. The first post-fatigue measurements of the maximal M-wave was performed immediately at the beginning of the post-fatigue neuromuscular tests 6 min after the run. The stimulus was delivered using 150 % and 180 % of the intensity that produced the plateau in the H/M-recruitment curve in the pre-fatigue tests. The amplitude of the post-fatigue maximal M-wave was represented by the mean of the two responses. If the greater stimulus intensity produced a greater M-wave response then the first measurement was discarded from the further analyses. Another post-fatigue estimate for the amplitude of the maximal M-wave was recorded during the stimulation of two passive supramaximal twitches approximately 8.5 minute after the run.

4.3.4 H-reflex

H-reflex was measured before and after the running effort from the soleus. The subjects were positioned in the ankle dynamometer bench while the belts were not tightened. The stimulus intensity was adjusted so that the peak-to-peak amplitude of the M-wave response was within the range 10 – 30 % of M_{max}. In order to maintain the target level, the size of M-wave was continuously controlled from the display of the Spike software. The stimulus intensity was manually adjusted. During the pre-fatigue measurements, the procedure was continued until four responses within the pre-determinated range of the M-wave amplitude were successfully produced. In order to keep the recovery time from the end of the run to the subsequent measurements as short as possible at most 12 trials were allowed during the post-fatigue tests. The means of the peak-to-peak amplitudes of the H-reflex were analyzed.

4.3.5 aEMG

The maximal EMG-activity during the MVC effort and the three MVC efforts with twitch interpolation were analyzed for the soleus before and after the run. Hence, the average rectified amplitude of the soleus EMG was computed over 200 ms period, before the supramaximal stimulus was delivered, starting from the point where the torque reached a plateau. Electromyographic activity is reported as a normalized value of aEMG/M_{p2p} and also as the ratio of the maximal aEMG and the maximal MVC torque (e.g., Strojnik & Komi 1998). The former is obtained by normalizing the aEMG estimate to the peak-to-peak amplitude of the maximal M-wave obtained during the pre- and post-fatigue measurements of the passive twitch.

4.3.6 V/M_{max}-ratio

In order to estimate the magnitude of the efferent motoneuronal output during voluntary muscle activation, supramaximal stimulus was delivered to the posterior tibial nerve during the last three MVC efforts (see Section 4.3.8). The amplitude of the V-wave response was measured and normalized by the amplitude of the maximal M-wave. The mean of three V/M_{max}-ratios were analyzed. Voluntary activation could not be determined from the superimposed twitches due to the unsteadiness of MVC output and mistiming of the stimuli.

4.3.7 Supramaximal passive twitch

The measurement of the supramaximal passive twitch was performed in the ankle dynamometer during the pre- and post-fatigue tests. The stimulus intensity was determined during the collection of the H/M-recuitment curve data so that it corresponded 150 % and 180 % of the prefatigue M_{max} intensity. Peak torque, contraction time, and half relaxation time were analyzed from the largest twitch. The maximum rate of torque development and the maximum rate of torque relaxation are represented by the mean values of the two

twitches. Thirty seconds and 10 s recovery intervals were used in pre- and post-fatigue tests, respectively. The smaller interval was used in the post-fatigue tests for minimizing the time to recover from the run before the following measurements.

4.3.8 MVC and superimposed twitch

The MVC torque was measured before and after the run through four maximal voluntary contractions, each lasting 2-3 s with 60 s (pre-fatigue) and 20 s (post-fatigue) rest intervals in-between. The short rest interval between the post-fatigue efforts was used for minimizing the time to recover from the run. The delay from the end of the run to the first and fourth post-fatigue effort was 10 min and 11 min, respectively. The best of the MVC efforts was selected for further analysis. Due to the imprecision in timing the interpolated stimuli over the MVCs the superimposed twitch torque could not be analyzed. The subjects were verbally instructed and encouraged to produce an all-out effort.

4.4 Blood samples

The blood lactate concentration (Biosen C-Sport analyser, EKF industrie) and blood pH (GEM 3000, Instrumental Laboratory) were analyzed from 20 μl and 180 μl samples, respectively, of capillary blood obtained from a fingertip. The blood samples were taken in the morning before breakfast, before and after the warm-up, immediately before the maximal 50 s run, and 3, 6, 9, 12, 15, 30, 60 min after the run. In addition to the capillary blood samples, venous blood samples were also taken from the ulnar vein in the morning, immediately before the run and 3min after the run. In this study, only the capillary blood samples taken immediately before the run and those taken 3 to 30 min after the run were analyzed. Metabolic variables will be reported more precisely in an accompanying report.

4.5 Statistical analyses

All the statistical analyses were performed on IBM SPSS Statistics Version 19 software. The results are presented by the group-wise sample means and standard deviations. Differences between the pre- and post-fatigue measurements were analyzed using the paired-samples t-tests. The between-group differences in the relative differences in the pre- and post-values were analyzed with one-way ANOVA method with the LSD post-hoc analysis. Normality and honomogeneity of the distributions were checked with the Shapiro-Wilk and the Levene's tests, respectively. In case of non-normality, independent samples Kruskall-Wallis and repeated samples Wilcoxon sign-rank test were applied. Linear relationships between metabolic variables and percentage changes in the neuromuscular measurements were analyzed using the Pearson product moment correlation coefficients. The significance level was set at $p \leq 0.05$.

5 RESULTS

Running performance. The running time did not differ significantly between the groups (TABLE 2), whereas the average running speed in Adults was 35.9 % and 16.9 % higher ($p < 0.001$) compared to Youth and Children, respectively, and 16.3 % higher in Youth compared to Children ($p < 0.001$). In all the groups the running speed decreased significantly between each successive pair of 100 m intervals ($p < 0.05$). The running speed decreased from the fastest to the last 100 m interval by 12.2 ± 6.5 % ($p < 0.01$), 9.8 ± 5.1 % ($p < 0.001$), and 12.2 ± 3.1 % ($p < 0.001$) in Children, Youth, and Adults, respectively. The between-group comparisons were not analyzed for the speed deceleration due to the differences in the running distances and the segmentation of the split times.

TABLE 2. Average running speed differed significantly (*** $p < 0.001$) between the groups.

Group	N	Distance (m)	Time (s)	Speed (m/s)
Children	8	300	53.6 ± 5.7	5.65 ± 0.54
Youth	8	350	53.5 ± 2.3	6.57 ± 0.27
Adults	8	400	52.1 ± 2.1	7.68 ± 0.30

H_{max}/M_{max}-ratio. Before the run Children and Youth exhibited 46 % ($p < 0.01$) and 34 % ($p < 0.05$), respectively, higher H_{max}/M_{max}-ratio than Adults (FIGURE 1). Three examples of the most representative recruitment curves from each group are presented in FIGURE 2.

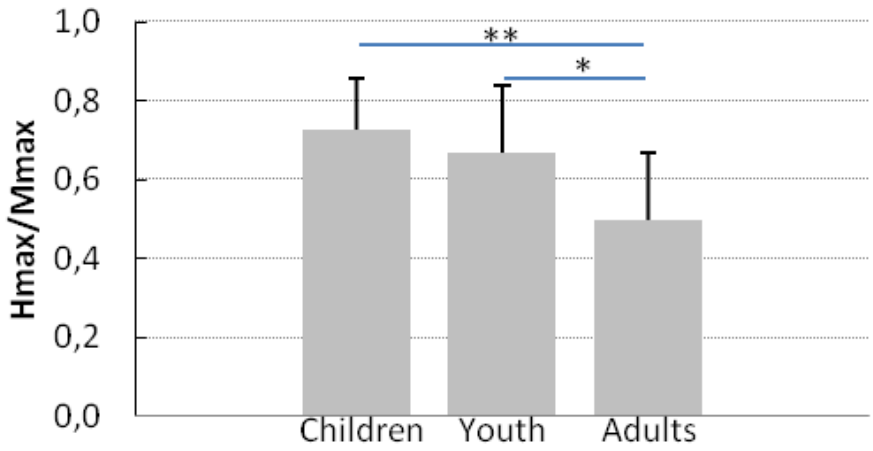

FIGURE 1. Hmax/Mmax-ratio before the run (* $p < 0.05$ and ** $p < 0.01$ between groups).

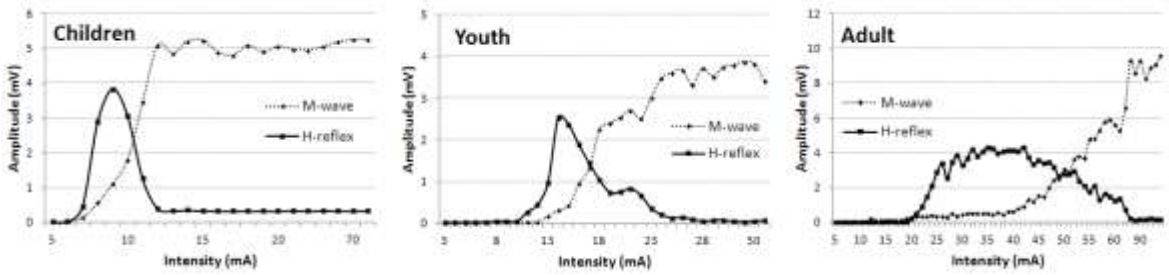

FIGURE 2. Representative H/M-recruitment curves for Children, Youth, and Adults.

Maximal voluntary contraction. The MVC torque in the plantar flexors decreased by 16.1 ± 13.0 % ($p < 0.01$) in Adults, whereas no significant changes were observed in Children and Youth (FIGURE 3). The relative decrease in the MVC torque was significantly greater in Adults compared to Youth (+6.3 ± 15.4 %; n.s.), but interestingly no difference was discovered for Adults and Children.

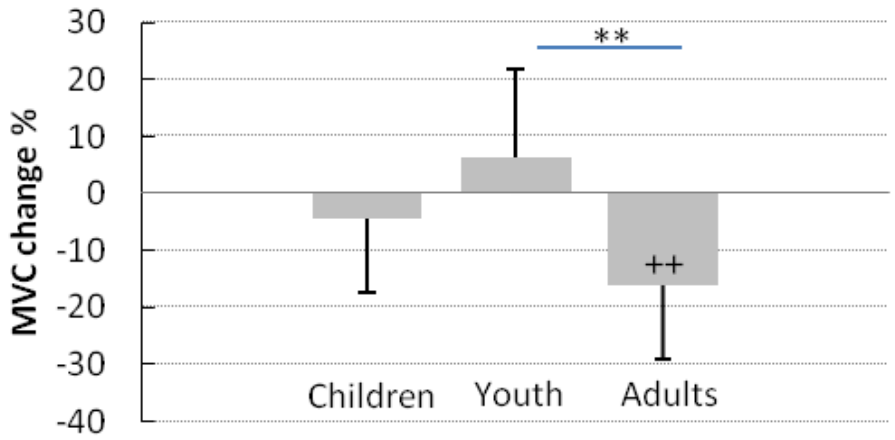

FIGURE 3. Relative changes in plantar flexion MVC torque between pre- and post-run measurements (** $p < 0.01$ between groups; ++ $p < 0.01$ absolute change within group).

Passive twitch characteristics. The passive twitch torque decreased from the pre- to post-measurement by 19.2 ± 12.2 % and 23.7 ± 13.7 % ($p < 0.01$) in Youth and Adults, respectively, and in the both groups the relative decrease was significantly greater compared to Children ($p < 0.05$) (FIGURE 4). In all the groups the twitch contraction and half-relaxation time decreased significantly without differences in the relative change between the groups (TABLE 3). In contrast, the mean rate of torque development decreased significantly only in Adults and the decrement was significantly greater than in Children ($p < 0.05$) (FIGURE 5). Moreover, a decrease in the maximum rate of torque development was observed in Youth and Adults (FIGURE 5, left). There was also a significant difference between Adults and Children in the maximum rate of torque relaxation (FIGURE 5, right).

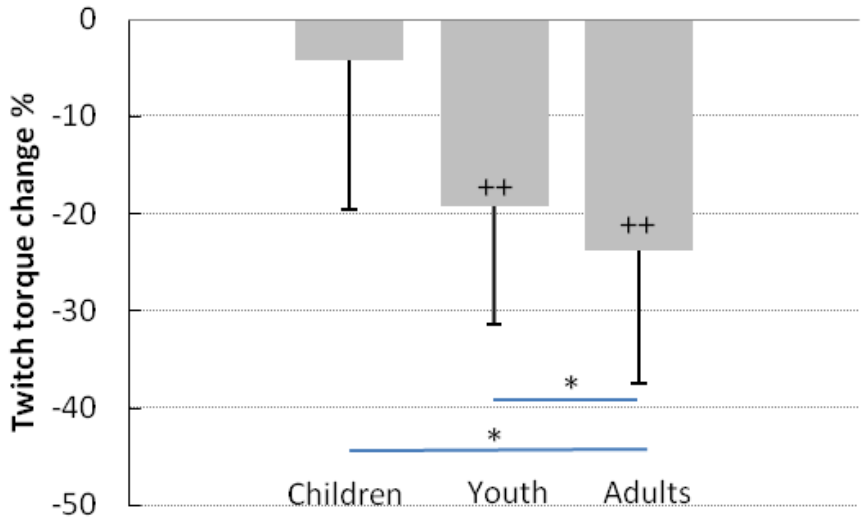

FIGURE 4. Relative changes in passive twitch torque between pre- and post run measurements. (* $p < 0.05$ between groups; ++ $p < 0.01$ the absolute change within a group).

TABLE 3. Parameters of the passive twitch contraction before and after fatigue. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ absolute change within-group.

	Group	Pre	Post	diff (%)
CT (ms)	Children	106 ± 9	95 ± 6**	-9.4 ± 5.8
	Youth	110 ± 8	99 ± 9**	-9.4 ± 7.4
	Adult	105 ± 4	95 ± 3***	-9.8 ± 3.4
HRT(s)	Children	68 ± 8	58 ± 6**	-13.2 ± 8.4
	Youth	66 ± 7	58 ± 6**	-12.3 ± 5.9
	Adult	71 ± 9	62 ± 5*	-11.3 ± 9.3

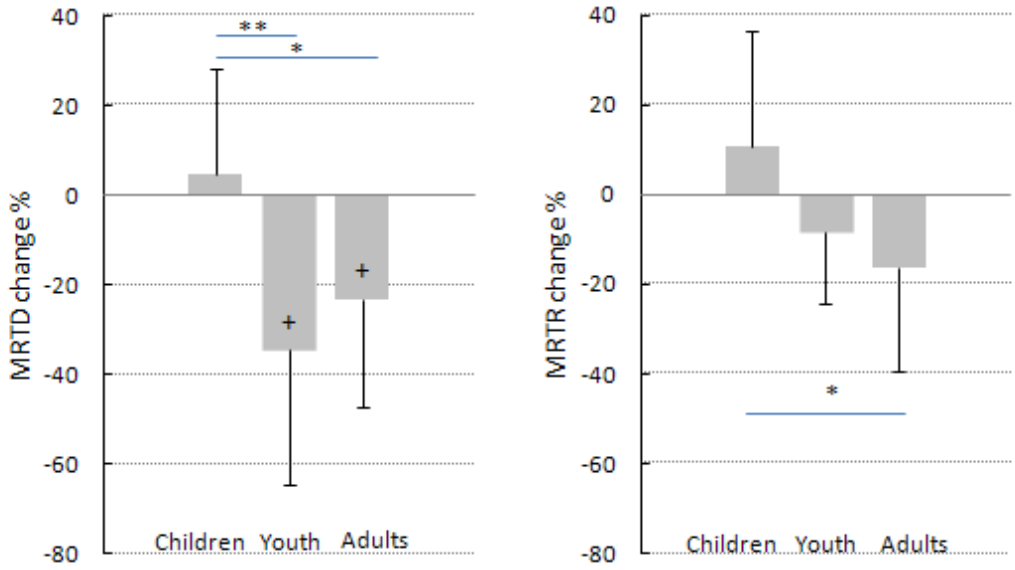

FIGURE 5. Relative changes in the maximal rate of torque development (left) and relaxation (right). (* $p < 0.05$ between groups; ** $p < 0.01$ between groups; + $p < 0.05$ the absolute change within a group).

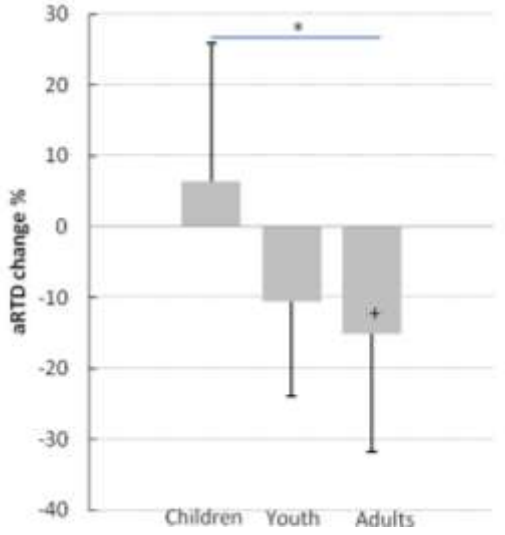

FIGURE 6**.** Relative changes in the mean rate of torque development. (* $p < 0.05$ between groups; + $p < 0.05$ the absolute change within a group.

aEMG. Neither within-group changes nor between-group differences in the relative pre- to post-fatigue changes were observed in aEMG, aEMG/MVC-ratio and aEMG/M_{p2p} (TABLE 4).

TABLE 4. Pre- and post-fatigue values and relative changes in the maximal EMG. No significant within- or between group differences were observed.

	Group	Pre	Post	diff (%)
aEMG (µV)	Children	64 ± 22	65 ± 21	+5.4 ± 29.0
	Youth	62 ± 33	73 ± 38	+19.1 ± 38.6
	Adult	83 ± 32	74 ± 20	+1.0 ± 10.4
aEMG/M_{p2p} (10^{-3})	Children	17 ± 5	18 ± 7	+4.3 ± 23.1
	Youth	21 ± 13	25 ± 22	+19.7 ± 46.1
	Adult	19 ± 7	19 ± 10	+0.3 ± 21.1
aEMG/MVC (10^{-4})	Children	3.03 ± 1.16	3.24 ± 1.13	+8.9 ± 16.0
	Youth	1.88 ± 0.62	2.10 ± 0.83	+9.9 ± 17.7
	Adult	2.00 ± 0.77	2.10 ± 0.68	+19.1 ± 22.1

H-reflex, V-wave, and M-wave.. No significant within-group changes or between-group differences were observed in H-reflex, V/M_{max}-ratio, or peak-to-peak-amplitude of the maximal M-wave (

TABLE 5).

TABLE 5. Pre- and post-fatigue values and relative changes in the evoked potentials. $\%M_{2p2}$ represents the group-wise percentages of the averaged peak-to-peak amplitudes of the M-wave responses recorded during the H/M-measurement in relation to the peak-to-peak to amplitude of the maximal M-wave. No significant within- or between group differences were observed.

	Group	Pre	Post	diff (%)
M_{p2p} (mv)	Children	3.95 ± 1.52	3.56 ± 1.33	-3.4 ± 13.0
	Youth	3.42 ± 1.10	3.22 ± 1.01	-5.0 ± 12.0
	Adult	5.57 ± 1.93	4.78 ± 1.01	-10.7 ± 13.5
$\%M_{p2p}$	Children	18.31 ± 3.87	20.68 ± 6.70	+17.5 ± 42.1
	Youth	18.78 ± 4.19	21.94 ± 6.12	+17.7 ± 25.8
	Adult	19.61 ± 4.93	17.70 ± 5.08	-6.0 ± 33.9
H/M	Children	0.51 ± 0.13	0.60 ± 0.22	+20.7 ± 9.1
	Youth	0.39 ± 0.21	0.42 ± 0.24	+8.8 ± 36.5
	Adult	0.37 ± 0.20	0.36 ± 0.11	+5.7 ± 35.3
V/M	Children	0.18 ± 0.10	0.28 ± 0.13	+131.7 ± 207.6
	Youth	0.23 ± 0.17	0.26 ± 0.12	+57.5 ± 131.9
	Adult	0.19 ± 0.11	0.24 ± 0.31	+9.6 ± 63.5

Blood lactate concentration and pH. In all groups, the blood lactate concentration and blood pH were significantly different from the resting levels after the run. The peak values of BLa after the run were 10.2 ± 1.1, 13.3 ± 3.7, and 17.4 ± 1.8 mmol/l for Children, Youth, and Adults, respectively (FIGURE 7). The peak of BLa was significantly lower in Children compared to Youth ($p < 0.05$) and Adults ($p < 0.001$) and as well lower in Youth compared to Adults ($p < 0.01$).

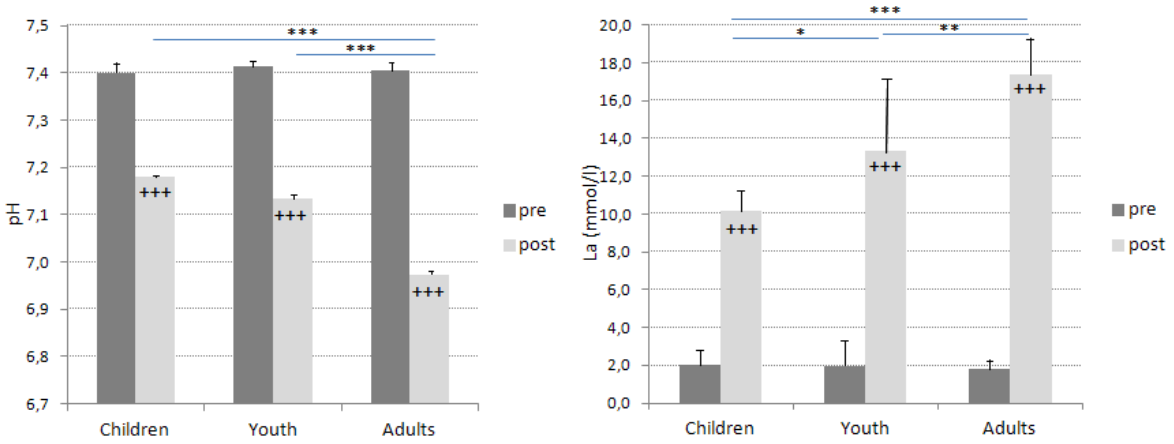

FIGURE 7. The minimum of blood pH and maximum of the blood lactate estimated from the pooled post-fatigue values for each group. The peak values of both blood pH and lactate concentration differed significantly from the pre-fatigue values (+++ $p < 0.01$ between pre- and post-fatigue values). The minimum of post-fatigue blood pH was lower in Adults compared to Youth and Children (*** $p < 0.001$). The maximum level of blood lactate was higher in Adults compared to Youth (** $p < 0.01$) and Children (*** $p < 0.001$), and higher in Youth compared to Children (* $p < 0.05$). BLa

In all the groups the blood lactate concentrations remained above the pre-fatigue levels during the 30 min recovery period and it was during the whole period higher in Adults than Youth and Children (FIGURE 8). Significant differences in BLa between Children and Youth were observed 9 min ($p < 0.05$), 12 min ($p < 0.01$), and 30 min ($p < 0.05$) after the run. The mean estimates of the BLa curves showed peak values 6 min after the run for Children and Youth, and 9 min after the run for Adults (FIGURE 8).

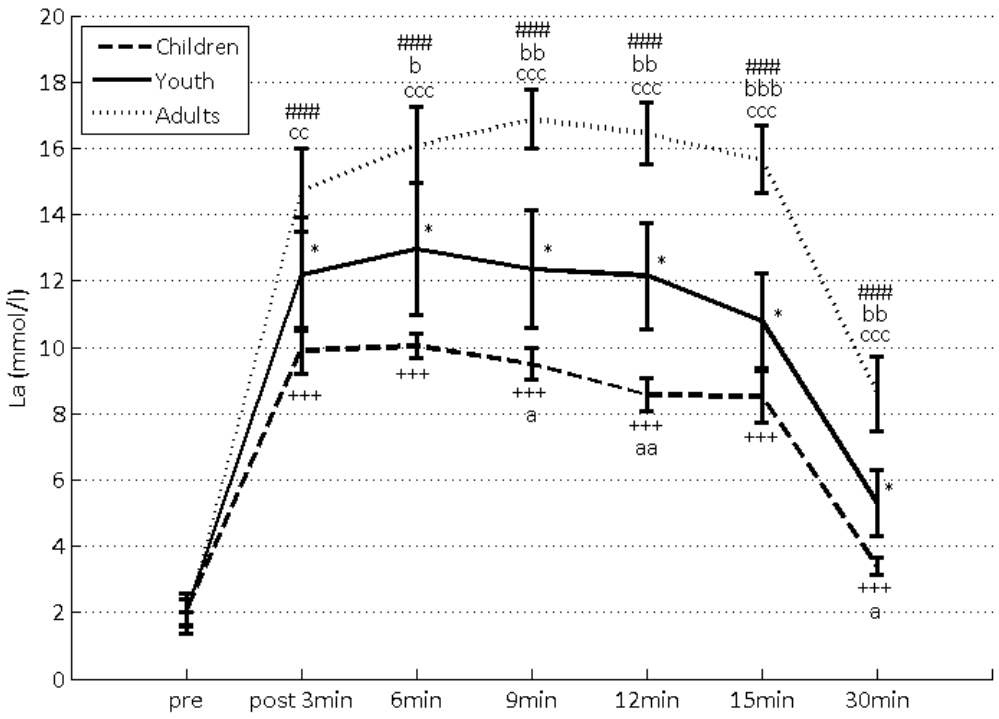

FIGURE 8. The group means, standard deviations, the between-group differences for the level of blood lactate concentration before and after the run, and the within-group differences between the pre- and post-fatigue value for the level of blood lactate concentration before and after the run ([a] $p < 0.05$ between Children and Youth; [aa] $p < 0.01$ between Children and Youth; [b] $p < 0.05$ between Youth and Adult; [bb] $p < 0.01$ between Youth and Adult; [bbb] $p < 0.001$ between Youth and Adult; [cc] $p < 0.01$ between Children and Adult; [ccc] $p < 0.001$ between Children and Adult; +++ $p < 0.001$ between a post-fatigue and pre-fatigue measurement for Children; *** $p < 0.001$ between a post-fatigue and pre-fatigue measurement for Youth; ### $p < 0.001$ between a post-fatigue and pre-fatigue measurement for Adults).

The minimum values of pH after the run were 7.18 ± 0.03, 7.14 ± 0.07, and 6.97 ± 0.06 for Children, Youth, and Adults, respectively (FIGURE 7). The within-group changes between pre- and post-fatigue tests were significant ($p < 0.001$) and the minimum values of blood pH

were significantly lower in Children and Youth ($p < 0.001$ for both) compared to Adults (FIGURE 7).

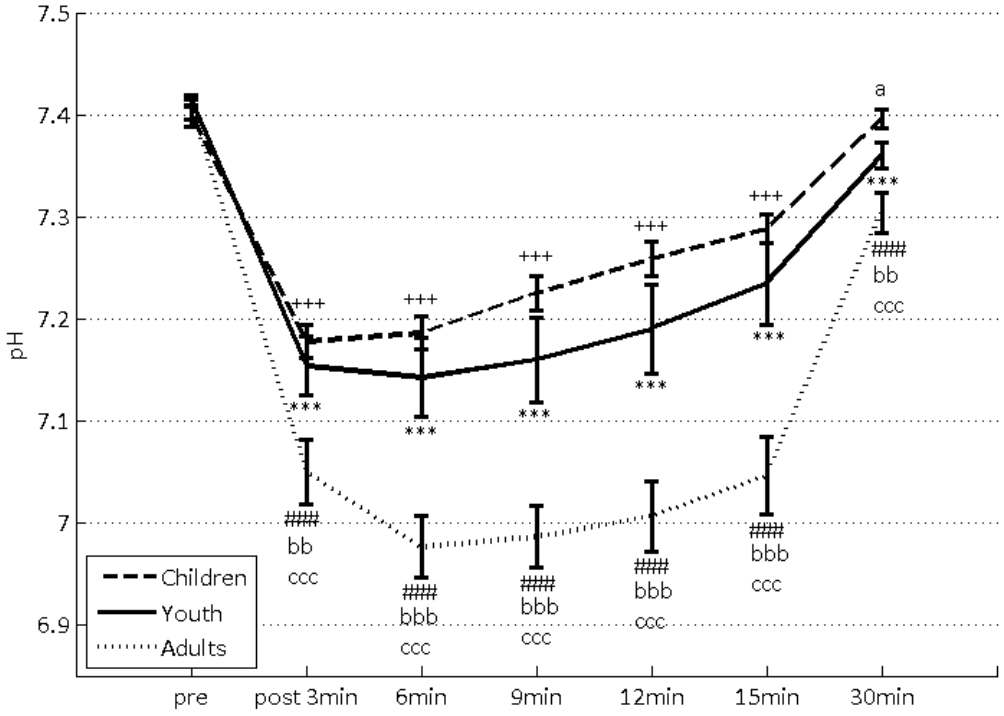

FIGURE 9. The sample means and standard deviations for the groups, the between-group differences for the level of blood pH before and after the run, and the within-group differences between the pre- and post-fatigue value for the level of blood pH before and after the run (^{a} $p < 0.05$ between Children and Youth; ^{bb} $p < 0.01$ between Youth and Adult; ^{bbb} $p < 0.001$ between Youth and Adult; ^{ccc} $p < 0.001$ between Children and Adult; +++ $p < 0.001$ between a post-fatigue and pre-fatigue measurement for Children; *** $p < 0.001$ between a post-fatigue and pre-fatigue measurement for Youth; ### $p < 0.001$ between a post-fatigue and pre-fatigue measurement for Adults).

Blood pH remained significantly below the pre-fatigue levels in Youth and Adults during the whole 30 min recovery period (FIGURE 9), whereas in Children 30 min of recovery was adequate to return the blood pH to the pre-fatigue level (FIGURE 9). Blood pH was significantly lower in Adults compared to Children and Youth during the 30 min period after the run (FIGURE 9). No significant between group differences were observed for Children and Youth during the first 15 min after the run (FIGURE 9). The mean estimates of the pH curves showed turning points 3 min after the run for Children and Youth and 6 min after the run for Adults (FIGURE 9).

Correlation coeffiecients were calculated for the metabolic and significantly changed neuromuscular variables in order to determine the metabolic causes underlying the reductions observed in the neuromuscular tests. The peak values of BLa and blood pH correlated in Children ($r = -0.86$, $p < 0.01$) and Youth ($r = -0.83$, $p < 0.05$). In Adults the linear correlation ($r = -0.67$) between the peak values of BLa and blood pH was not significant. The significant relative change in the peak twitch force observed in Youth (see FIGURE 4) was found to correlate with each of the blood pH values measured during the first 15 min of recovery (TABLE 6). The linear relationship between the individual minimum levels of post-exercise blood pH and the relative change in the peak twitch force in Youth is illustrated by the scatter plot in FIGURE 10. Similarly, the relative change in the peak twitch force inversely correlates with the peak and post-6min, post-9min, and post-12min Bla values in Youth (TABLE 6). The significant relative change observed in the maximum rate of torque development in Youth (see FIGURE 5) does not correlate with any of the metabolic variables (TABLE 6). In Adults, only the significant relative change in the MVC torque (see FIGURE 3) inversely correlated with BLa measured 12 min after the run (TABLE 7). The linear relationship is shown by the scatter plot in FIGURE 11.

TABLE 6. The Pearson correlation coefficients between the blood pH and lactate concentrations and the percentage pre- to post-fatigue changes in the peak twitch torque (pTw) and the maximum rate of torque development (MRTD) in Youth (* $p < 0.05$).

	Post blood pH						Post blood lactate					
	min	**3min**	**6min**	**9min**	**12min**	**15min**	**max**	**3min**	**6min**	**9min**	**12min**	**15min**
pTw	0.80*	0.80*	0.78*	0.77*	0.73*	0.76*	-0,78*	-0.70	-0.77*	-0.71*	-0.78*	-0.69
MRTD	0.49	0.47	0.37	0.36	0.36	0.28	-0.25	-0.30	-0.24	-0.26	-0.43	-0.23

TABLE 7. The Pearson correlation coefficients between the blood pH and lactate concentrations and the percentage pre- to post-fatigue changes in MVC torque, peak twitch torque (pTw), maximum and average rates of torque development (MRTD and aRTD) in Adults (* $p < 0.05$).

	Post blood pH						Post blood lactate					
	min	**3min**	**6min**	**9min**	**12min**	**15min**	**max**	**3min**	**6min**	**9min**	**12min**	**15min**
MVC	0.61	0.18	0.60	0.61	0.64	0.64	-0.44	-0.19	-0.49	-0.39	-0.72*	-0.64
pTw	-0.13	-0.31	-0.14	-0.15	-0.11	-0.07	-0.12	0.17	-0.20	0.06	-0.25	-0.11
MRTD	-0.17	-0.44	-0.25	-0.12	-0.02	0.04	-0.11	0.58	0.19	0.17	-0.33	-0.13
aRTD	-0.17	-0.39	-0.18	-0.19	-0.16	-0.08	-0.15	0.19	-0.21	-0.01	-0.28	-0.18

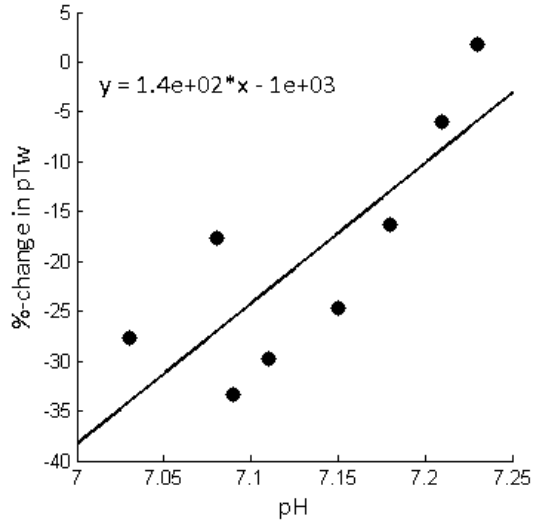

FIGURE 10. A significant correlation (r = 0.80; $p < 0.05$) in Youth between the minimum values of the post-fatigue blood pH and the percentage change in the passive twitch force.

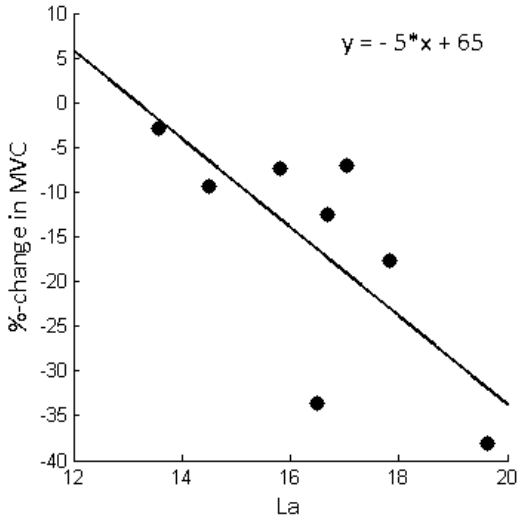

FIGURE 11. A significant correlation (r = -0.72; $p < 0.05$) in Adults between the blood lactate concentration sampled 12 min after the end of the run and the percentage change in the plantar flexor MVC torque.

6 DISCUSSION

Prior studies have demonstrated that children experience less fatigue than adults in maximal isometric and isokinetic knee extension (Kanehisa et al. 1995; Paraschos et al. 2007; Armatas et al. 2010) and isometric maximal elbow flexion tasks (Halin et al. 2005). Moreover, Ratel et al. (2002; 2006a) have also shown that, compared to adults, children are able to maintain more constant speed and power output during repeated running and cycling sprints and recover at a faster rate afterwards. A number of studies have associated E-C coupling failures with muscle fatigue in the context of short high-intensity running and cycling (Lattier et al. 2004; Skof & Strojnik 2006a; Perrey et al. 2010; Tomazin et al. 2012). On the other hand, less evidence is available on the role of neural adjustments in short-term lactic whole-body workouts (Strojnik & Komi 1998; Perrey et al. 2010; Tomazin et al. 2012). To the best knowledge of the author, this is the first study to investigate maturity-related differences in central fatigue in the context of lactic short-term running. In contrast to the hypothesis of this thesis, no evidence of central fatigue was observed in children or adolescents. The main finding is that, regardless of the stage of maturation, the predominant cause of fatigue resides within the muscles themselves, and central fatigue has a little, if any, role. In addition, the present results indicate that the size of the maximal H-reflex in the motoneuron pool innervating the soleus could be also maturity-dependent.

6.1 Running performance and fatigue

Deceleration. In each of the three age groups, the subjects experienced significant fatigue during the maximal 50 s run. This was observed through the consistent slowdown of running speed after the acceleration phase. The decrement of running speed from the fastest to slowest 100 m segment was 12.2 % in Adults and there were only minor differences to Children (12.2 %) and Youth (9.8 %). These between-group differences should be however interpreted with caution because of the differences in the running distances and split intervals. Deceleration of 12.2 % observed in Adults is slightly lower than 15.5 % that was

reported in a study where 100 m segmentation was used for split timing in experimental runs on an indoor track at the end of competitive season (Hirvonen et al. 1992). In an outdoor racing situation the decrease in running speed has been analyzed for 400 m distance using 50 m intervals, which showed deceleration of 13.9, 14.4, and 23.0 % for the regional, national, and world-class athletes, respectively (Hanon & Gajer 2009). However, it should be noticed that shortening of the measurement segment tends to increase the variation in the estimates of the average running speed, which, thereby, leads to a greater difference between the most extreme values of running speed. Hence, the changes in running speed observed in this study indicate that the subjects in each of the age-groups were able to use the aggressive pacing strategy that is characteristics to the competitive runs in speed endurance events, and, hence, fatigue themselves significantly.

The post-fatigue levels of blood lactate concentration and blood acidosis are also comparable with the previous studies on adults (e.g., Hirvonen et al. 1992; Saraslanidis et al. 2001), which also indicate that the runners were able to perform the test run in an all-out manner. The lesser accumulation and earlier peaking of blood lactate and pH in Children and Youth compared to Adults can be explained by several maturity-related factors, such as lower glycolytic enzyme activity (Kazcor et al. 2005) and faster elimination of glycolytic by-products in children (Beneke et al. 2005).

Maximal voluntary contraction. The MVC torque provides an integrated estimate for the adjustments in the entire neuromuscular pathway. In spite of the fatigue observed in all groups during the run, a significant decrease (-16.1 %) in the plantar flexor MVC torque was observed only in Adults. The relative change in MVC differed significantly between Youth and Adults, while, somewhat surprisingly, no difference was observed between Children and Adults. Previous studies on adults have demonstrated quite similar decrements in the knee extensor MVC (post 30 s -13.8 % and post 5 min 10.7 %) after a maximal 400 m treadmill sprint (Tomazin et al. 2012) and in the plantar flexor MVC (-11 %) about 3 min after 12 x 40 min maximal running sprints (Perrey et al. 2010). Thus the degree of muscle fatigue, when quantified through the plantar flexor MVC torque, was consistent with the

previous findings in adults. The increased levels of BLa and acidosis during the post-fatigue MVC tests and the significant correlation between the relative decrease in MVC and post-fatigue BLa measured 12 min after the run indicate that the glycolytic by-products may have contributed to the loss of force production capacity in Adults despite the delay from the end of the run to the neuromuscular tests.

Neither Children nor Youth experienced a decrease in the MVC torque indicating the absence of muscle fatigue after the run. Since the both groups showed a loss of running speed and the significant changes in the blood pH and BLa levels, it is justifiable to discuss the unchanged post-fatigue MVCs with more details. One can speculate about the role of maturity-related activation deficit during the pre-run MVC task, and the time lag from the end of the run to the beginning of neuromuscular tests, which may have allowed full recovery in the younger subjects. Whether the prior practice or familiarization with the MVC task was adequate for the children and youth should also be considered.

In Youth, the unchanged MVC torque could be attributed to the suboptimal activation of the plantar flexors in the pre-fatigue MVC test, since both the peak twitch torque and the maximum rate of twitch torque development were depressed, and hence, peripheral fatigue existed after the run. On the other hand, previous studies do not provide much support for maturity-dependent characteristics of the muscle activation deficit. Belanger & McComas (1989) demonstrated that pubertal children possess a high capacity for voluntary activation of plantar flexors. Furthermore, Pääsuke et al. (2000) did not find any difference in the ratio of isometric twitch force to MVC force in the plantar flexors between post-pubertal (16 years) boys and men. Assuming that the pubertal stage of the adolescent subjects in this study (14.9 years) is comparable with the subjects involved in the aforementioned studies, it is difficult to explain the potential suboptimal activation in the MVC tests by any other factor than insufficient pre-test familiarization of the subjects. In this study, the pubertal stage was not exactly determined for Youth, but their levels of serum testosterone (reported in a separate paper) were comparable to that of Adults indicating that they had reached an advanced stage of puberty.

It can be also speculated that the maturity-related differences in the MVC torque could be also related to the age-related differences in muscle activation patterns at high running speeds. Most sprinters apply the forefoot striking technique, which means that they make the initial contact on the ball of the foot (Mero et al. 1992). Although the running technique was not monitored in this study, maturity-related differences in the running technique and muscle coordination during the sprint cannot be excluded. These might have an influence to the extent of fatigue that develop in individual muscles during a maximal fatiguing run. For instance, a fatigue-induced shift from the forefoot to heel striking technique may alter the load between triceps surae, quadriceps, and hamstring muscles. Consequently, the degree of fatigue may vary between the muscle groups after the run. However, these issues require more studies for the more through discussions.

In Children, not only the MVC test, but indeed most of the neuromuscular variables remained at the pre-fatigue level after the run. Therefore, the capacity to fatigue themselves might have been lower in Children compared to the other groups. It should be noted that, similarly to Adults, a significant decrease in the running speed and increases in the concentrations of glycolytic by-products appeared also in Children. Grosset et al. (2008) have shown that, compared to adults, children (7-11 years) are less capable of activating their triceps surae muscles. Pre-pubertal boys have also shown a lower ratio of the isometric twitch force to the MVC force for the plantar flexors compared to post-pubertal boys and men (Pääsuke et al. 2000). The immature muscle activation in children has been attributed to the incomplete recruitment of high-threshold type II motor units in particular (Dotan et al. 2012). Thus it is possible that Children were unable to activate the motor units innervating the plantar flexors maximally during the pre-fatigue MVC tests and, consequently, the MVC torque was underestimated under the non-fatigued conditions. In that case the potential exercise-induced decrease in the MVC test probably remained unidentified, particularly if the high-threshold motor units were fatigued to the greatest degree during the run. There are also other factors for which age-related differences have previously been found, such as antagonist coactivation (e.g., Paraschos et al. 2007), and which thereby may have influenced to the running performance and the level of voluntary contractions in this study.

Another explanation could be that Children recovered before the post-fatigue measurements. It has been previously shown for pre-pubertal boys that 1 min and 2 min rest periods are adequate for nearly complete recovery of peak power and total mechanical work, respectively, between two consecutive all-out 30 s cycling tasks (Hebestreit et al. 1993). In the same study, the recovery of peak power required 10 min rest intervals in Adults, which was though still insufficient for the recovery of total mechanical work (Hebestreit et al. 1993). Moreover, when compared to adults, pre-pubertal children have been shown to need significantly shorter rest intervals between maximal sprints (Ratel et al. 2002: 2006a) and intermittent dynamic knee extension repetitions (Zafeiridis et al. 2005) for maintaining constant performance. The faster rate of recovery in children has been explained by many factors. In accordance with Falk & Dotan (2006), who suggested that children have "*less to recover*" at the end of maximal efforts, it has been shown that they fatigue less than adults during isometric and isokinetic knee extension tasks (Paraschos et al. 2007; Kanehisa et al. 1994; Armatas et al. 2010). The immature short-term power generation capacity and anaerobic performance in children may result from several interdependent factors, such as smaller muscle volume (Dore et al. 2000), the lower percentage of glycolytic type II muscle fibers (Glenmark et al. 1994; Kriketos et al. 1997), and the lower levels of anaerobic capacity (LeClair et al.2011), glycolytic enzyme activity (Kazcor et al. 2005), and testosterone concentration (Falgairette et al. 1991). In this study, the lower accumulation of blood lactate and the higher blood pH after the run indicate that children and adolescents have a lower capacity of anaerobic glycolysis compared to adults. Moreover, the low level of serum testosterone observed in Children (more details will be presented in another report) has been previously associated to the immature force generation capacity (Van Praagh & Dore, 2002). On the other hand, the higher rate of recovery in children compared to adults have been attributed to their greater oxidative capacity, faster regeneration of muscle PCr, and faster efflux of H^+ from the muscle (Taylor et al. 1997; Tonson et al. 2010). The faster and lower peaking of blood lactate and pH in Children compared to Adults in this study are well in line with these considerations. On the other hand, intracellular muscle pH may not have reached the level that is required to impair the contractile system (Fitts 2008). Moreover, Cairns (2006) have stated that the acidosis alone

cannot explain the muscle fatigue, which may partly explain the absence of neuromuscular changes in the presence of elevated blood pH. Since neither the plantar flexor MVC torque nor any other neuromuscular variable were depressed after the run, it seems likely that the 6 min delay before the post-fatigue neuromuscular tests enabled the complete recovery of neuromuscular functions in Children.

6.2 Central factors

In this study, no indications of neural impairments were observed. The evoked V-waves, H-reflexes as well as the aEMG, and aEMG/M_{max} estimates were unchanged after the run. Superimposed twitches were also measured, but an index of voluntary activation could not be analyzed due to unsteadiness in the MVC outputs and mistiming in the delivery of electrical stimuli. Nevertheless, to the knowledge of the author, this is the first time when neural adjustments were studied in children using electrical stimulation after high-intensity running exercise. The unchanged V/M_{max}-ratio after the run suggests that no fatigue-induced reductions occurred in the efferent motoneuronal activity (Aagaard et al. 2002). The unchanged V/M_{max}-ratio in parallel with the unchanged H-reflex response provides indirect evidence that the voluntary central drive remained unchanged after the run in each of the groups. However, this interpretation must be considered with caution due to the imprecise timing of the interpolated stimuli during the MVC efforts. Furthermore, the unchanged EMG activity in the soleus provides further evidence that no central fatigue developed. In the case of adults, the results are in agreement with the previous studies on short-term high-intensity exercise, which have shown a little or no evidence for central fatigue after high-intensity running or jumping sessions (Strojnik & Komi 1998; Lattier et al. 2004; Perrey et al. 2010; Tomazin et al. 2012). Thus the present study supports the previous impression that exercise-induced central adjustments in the spinal excitability and voluntary motor drive are more inherent in prolonged runs upwards of 5 km (e.g., Millet et al. 2003; Racinais et al. 2007; Girard et al. 2012) and become more important with the increasing duration of an SSC task (Avela et al. 1999; Kuitunen et al. 2004).

Due to the relatively long delay before the neuromuscular measurements were undertaken after the run, it is nevertheless necessary to consider the possibility of recovery in the neural processes. It has been shown that central fatigue recovers in one minute after fatiguing intermittent maximal contractions (Taylor et al. 2000) and to a great extent in one or a few minutes after the end of prolonged sustained submaximal contractions (Löscher et al. 1996b; Søgaard et al. 2006). On this account, the typical delay of 2-8 min might be too long for the reliable assessment of neural changes after short maximal runs. On the other hand, Tomazin et al. (2012) observed a significant reduction both in the voluntary activation and the muscle activity of knee extensors 5 min after the end of the maximal 400m run although no changes were observed immediately (30 s) after the run. The authors speculated that the postponed central fatigue could be caused by delayed accumulation of metabolites (e.g., NH_3, H^+) into the blood and the brain. Bodganis et al. (1995) have demonstrated a significant increase in the plasma ammonia concentration in the course of a 6min recovery period following a maximal 30 s cycling sprint. However, although the concentrations of the glycolytic by-products were significantly elevated in the blood after the run, no signs of delayed central modulations were observed in the present study. Although it is not possible to definitely exclude the occurrence of central fatigue due to the relatively long recovery period before the neuromuscular tests, the results of the present study are also consistent with the previous studies that have suggested that fatigue has mainly peripheral origins in short high-intensity running and cycling efforts (Mero & Peltola 1989, Nummela et al. 1992; 1994; Billaut et al. 2006). Moreover no maturity-related differences in central factors could be shown by the present experiments.

6.3 Spinal level factors

Before the warm-up session the H-reflex recruitment curve and the H_{max}/M_{max}-ratio were measured. No exercise-induced changes were observed in the H-reflex after the run, which indicate that there were no net changes in the pre-synaptic inhibition and disfacilitation of the alpha-motoneuron pool. This in line with the previous findings made after repeated sprints by Perrey et al. (2010).

However, the pre-fatigue measurements showed that, compared to Adults, the H_{max}/M_{max}-ratio was 46 % and 34 % higher in Children and Youth, respectively. Previous studies have associated the low H_{max}/M_{max}-ratio with the explosive and anaerobic training history (Casabona et al. 1990; Maffiuletti et al. 2001) and the high H_{max}/M_{max}-ratio with endurance training (Maffiuletti et al. 2001). However, since the prior studies have mainly focused on mature subjects, the present study extends the existing knowledge with its maturity-dependent aspects. The training history may also partly explain the age-related differences found in this study, since in the present study the adult athletes had several years of event-specific sprint training behind, whereas the training history in the groups of younger subjects is likely more diverse. The age-related differences in the maximal H-reflex may also be attributed to the maturity-related changes in muscle fiber distribution. To be more precise, the H_{max}/M_{max}-ratio can be interpreted as the proportion of the entire motoneuron pool that can be recruited by Ia-afferent inputs (Palmieri et al. 2004). Given that the motor units innervating the soleus are recruited by Ia afferent inputs in an orderly fashion according to the ‘‘size principle” (Zehr 2002), the greater size of the maximal H-reflex response could partly be attributed to the greater proportion of small and low-threshold slow-twitch muscle fibers in children (Glenmark et al. 1994). Probably the genetic variation in Children and Youth was also broader in this study, because Adults represented a qualified group of national top sprinters, or, the other way around, the less talented sprinters possessing greater percentages of low-threshold slow twitch motor units have been already eliminated from the group of competitive sprinter before adulthood.

6.4 Peripheral factors

The general impression of the findings concerning the MVC, H-reflex, and V-wave measurements suggests that in all groups the fatigue was mainly caused by peripheral factors. In order to determine the mechanisms underlying the peripheral fatigue, electrical stimuli were delivered to the peripheral nerve of the relaxed calf muscles before and after the run. In Adults and Youth, the passive twitch torque, the maximum rate of tension development, and the contraction and half-relaxation times decreased significantly. In

Children, only the contraction and half-relaxation times decreased. Since there occurred no changes in the M-wave responses, the twitch characteristics indicate that the fatigue was related to the Ca^{2+} movements between the sarcoplasmic reticulum and contracting proteins, and to the functionality of the contracting proteins themselves. The decline in the peak twitch force correlated with the post-fatigue blood pH in Youth, which indicate that acidosis likely contribute to the peripheral impairments. In Adults, despite the highest level of post-fatigue blood pH levels, no significant linear relationships between the blood pH values and neuromuscular tests were observed. This may indicate that also other factors contribute to the peripheral fatigue in Adults (e.g., Allen et al. 2008).

In line with the present findings, there exist a number of studies on high-intensity running and jumping exercise that have also reported similar changes in the twitch characteristics. A decline in the plantar flexor twitch torque has been previously reported after repeated short 12 x 40 m running sprints (-13 %, Perrey et al. 2010) and 5 km maximal run (-16 %, Girard et al. 2012). Furthermore, a decline in the knee extensor twitch torque was reported after 100, 200, and 400 m maximal sprints (~8-35 %, Tomazin et al. 2012), maximal short-term SSC jumping exercise (-9 %, Strojnik & Komi 1998), anaerobic long interval (5 x 300 m) session (28 %, Skof & Strojnik 2006a), and continuous run at the anaerobic threshold (-14 %, Skof & Strojnik 2006b). Thus, the -19 % and -24 % changes observed in Youth and Adults, respectively, are in good agreement with those previous studies. The decrease in the plantar flexor twitch torque observed in this study is probably associated with a depression of propulsive force and simultaneously increased EMG that was observed after a maximal 400 m run by Nummela et al. (1994). As the maximal twitch torque has been shown to recover and potentiate over the pre-exercise values in 10 min after intensive runs (Skof & Strojnik 2006a; 2006b), the impairments observed in the present study may have been even greater if the measurements could have been accomplished with a shorter delay after the run. In a study Tomazin et al. (2012), for example, the twitch force recovered partially only in 5 min after a maximal 400 m run (Tomazin et al. 2012). In our study, the delay from the completion of the run to the twitch measurement was 8-9 min, which likely allowed some recovery. In addition, there is some evidence that dynamic whole-body exercise, such as

skiing and running, may lead to a post-activation potentiation of twitch force and thereby counteract its fatigue-induced reduction (Place et al. 2010).

The extent of muscle fiber force output depends on the total number and force of the strongly bound cross bridges (Fitts 2008). In the absence of changes in the M-wave response, the reduced peak twitch torque indicates changes in Ca^{2+}-release from SR, myofibrillar Ca^{2+} sensitivity, and the force production in active cross-bridges (Allen et al. 2008). Fitts (2008) suggests that the depressed twitch force mainly reflects the decline in Ca^{2+} -transients (Fitts 2008). Accumulation of metabolic by-products, such as inorganic phosphate, hydrogen, and ADP, inhibits Ca^{2+}-kinetics and cross-bridge force production and consequently reduces the twitch force (Allen et al. 2008, Fitts 2008). It has been demonstrated, for instance, that muscle pH remains significantly below the resting level and the muscle $[P_i]$ is more than two times greater than the resting value, 6 min after a maximal 30 s exercise (Bodganis et al. 1995). Therefore, the more prolonged elevation of metabolites may have contributed to the greater reductions in the passive twitch responses in Youth and Adults compared to Children.

Another indicator of the E-C coupling failure in Youth and Adults was observed as the decrease in the maximum rate of torque development, which is also thought to be sensitive to changes in the Ca^{2+}-transient (Fitts 1994). Similar findings have been made by earlier studies after maximal short sprint repetitions (Perrey et al. 2010) and 5 km running (Girard et al. 2012). The decrease in MRTD may probably contribute to some extent to the prolonged force production times during the propulsive phase of the ground contact and increased contact times that have been observed at the end of the maximal 400m run (Nummela et al. 1994).

Although both the twitch contraction and the relaxation times are generally expected to increase with fatigue (Fitts 2008), the present and several other studies on short- or long-term high-intensity running and jumping have reported opposite findings (Strojnik & Komi 1998; Skof & Strojnik 2006b; Perrey et al. 2010; Girard et al. 2012; Tomazin et al. 2012).

The contraction and relaxation times are influenced by the amplitude and the duration of the Ca^{2+} -transient (Fitts 2008), but Strojnik & Komi (1998) suggested that shorter Ca^{2+}-transients could also explain a decline in the twitch peak force. Because both the average rate of torque development and the maximum rate of torque development and relaxation decreased in this study, the reduction in the twitch duration may also simply be a consequence of a shorter Ca^{2+}-transient and subsequent decrease in the peak twitch amplitude. The reduced aRTD, MRTD, and MRTR indicate that the rate of force production decreased in Youth and Adults despite the decreases in the contraction and half-relaxation times. The shortening of muscle twitch has also been associated with an increase in muscle temperature (Strojnik & Komi 1998; Skof and Strojnik 2006a; Skof and Strojnik 2006b). Perrey et al. (2010) hypothesized that a decrease in the amplitude of the twitch force and the contraction time could be also attributed to SSC-induced mechanical changes in the serial elastic components. Potential contributions of the abovementioned factors cannot however be determined by the methods used in the present study.

The lack of changes in the twitch characteristics also supports the aforementioned assumption that in Children the fatigue was vanished by recovery processes before the neuromuscular tests were started. The earlier peaks of the blood pH and lactate also indicate lower accumulation and faster clearance of metabolic by-products. Skof & Strojnik (2006b) associated the restoration of post-fatigue twitch force with a rapid restoration of muscle PCr stores. In fact, recovery of the power output after a maximal 30 s cycling sprint has been dissociated from changes in the concentrations of metabolic by-products, including H^{+}- and P_i -ions, while an association was established between the recovery rate of the power output and PCr stores (Bogdanis et al 1996). Moreover, it has been suggested that in small muscle groups, such as calves, the rate of post-exercise PCr regeneration is related with the muscles oxidative capacity (Bodganis et al. 1996). Recently, Tonson et al. (2010) reported that the rate of PCr regeneration is nearly two times higher in children than adults. Therefore, it might be that in this study the Children's greater aerobic capacity allowed their peripheral processes to recover so quickly that the muscle fatigue developed during the maximal run did not anymore appear in the neuromuscular tests.

6.5 Conclusions

The main finding of this study is that, in contrast to its main hypothesis, no maturity-related differences occur in the fatigue mechanisms after a maximal short-term run. The results suggest that the fatigue has mainly peripheral origins in all the examined age groups. The predominant sites of fatigue were found to be within the muscle fibers themselves. The underlying mechanisms are most likely related to the movement of calsium-ions between the sarcoplasmic reticulum and the myofibrillar contractile structures. Furthermore, these changes are likely affected by several metabolic changes including muscle acidosis and depletion of PCr. Although the neural factors seem to play a minor role in the present experiments, definitive conclusions on their contribution to the fatigue in short-term maximal running exercise should not be made on this basis due to the long delay before the neuromuscular post-fatigue measurements took place. Overall the present results are good in agreement with the previous studies that have examined whole body exercise modes during high-intensity tasks. The maturity-related differences are more likely related to the capacity to exhaust the muscles than the various mechanisms causing fatigue. Due to the lesser degree of fatigue and the higher aerobic capacity, children also recover faster than adults. Therefore, in the case of young subjects the assessment of fatigue is quite sensitive to the delay between the fatiguing exercise and neuromuscular experiments. The underlying physiological characteristics explaining the maturity-related differences may involve a number of factors, such as the higher percentage of slow-twitch fibers, the greater oxidative capacity, the lower anaerobic capacity, hormonal differences, and the limited neural activation capacity.

In this study, the six minute delay from the end of the run to the initiation of the post-fatigue measurements likely disguised part of the exercise-induced neuromuscular changes. Perhaps a more instant measurement of neuromuscular changes including the use of nerve stimulation methods, online recording of muscle EMG, and contact forces could provide further insights to the maturity-related differences in the mechanisms of muscle fatigue in the context of speed endurance sports.

6.6 Practical applications

This study suggests that maturity-dependent differences in speed endurance sports are more related to the power production capacity than fatigue resistance. The recent studies indicate that the best speed endurance runners reach higher peak velocities and experience relatively a greater degree of fatigue towards the end of the run compared to less successful runners. Therefore, given that the long-term aim is to become a top athlete in speed endurance sports, it seems to be of highest importance to ensure the development of both speed and strength capacity during the late stages of maturation. During the early stages of maturation, it is important to learn the motor skills of fast and efficient running. On the other hand, the children's greater ability to recover from exercise enables shorter rest periods during speed and strength training. Nevertheless, when planning training programs for young athletes, the aspects of mental and mechanical load on the immature body should not be left out of considerations.

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