ACUTE INCREMENTAL ENDURANCE PERFORMANCE AND EXPLOSIVE STRENGTH TRAINING PERIOD: MUSCLE ACTIVITY, ACID-BASE BALANCE AND HORMONAL RESPONSES

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ABSTRACT

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Introduction. The acute physiological responses of human body to endurance and strength exercise occur in musculoskeletal, cardiovascular, respiratory, endocrine and immune systems. These responses can impair a performance, and for example, acid-base balance is strictly regulated in order to prevent negative outcomes. Muscle function can be examined by electromyography (EMG) and nowadays it is also possible to use textile electrodes, which allows recording of activity of muscle groups during normal locomotion. The main aims of this study was to study the associations between activity of hamstring and quadriceps muscles and acid-base balance during incremental speed running test, compare differences in muscle activation during cycling and running, and investigate the effects of additional explosive strength training on the physiological determinants of endurance performance in recreationally active persons. Furthermore, another aim was to investigate the acute responses in blood cell counts, acid-base balance and testosterone and cortisol induced by incremental speed running test.

Methods. Sixteen recreationally active persons, nine women (age 22.6 ± 2.0 yr; VO_{2max} 42.8 ± 4.1 ml/kg/min) and seven men (age 28.1 ± 10.7 yr; VO_{2max} 50.9 ± 5.4 ml/kg/min), participated in the study. The study contained pre-, mid- and post-measurements and two training periods, each lasting six weeks. One period included light endurance exercises and the other speed, explosive strength and running technique exercises. During measurement day, the subjects performed an incremental running test (IRT) on an indoor running track, 20 m maximum sprint with a flying start, countermovement jumps (CMJ), and half squats. Blood samples were taken in several different time points (FAST, PRE, POST, POST30' and POST60'). After two resting days, the subjects performed also a bicycle test (ICT). EMG shorts (Myontec Ltd.) were used during all the tests.

Results. The IRT induced a significant increase in blood leukocyte count both in men (66.8%; $p<.05$) and in women (80.8%; $p<.01$) and concentration of testosterone increased significantly in men (18.4%; $p<.05$) during IRT. Blood pH decreased both in men and women ($p<.05$ and $p<.01$, respectively), and the change in HCO_3^- and BE ($p<0.05$ and $p<.01$, respectively) showed significant metabolic acidosis. The change in the activity of hamstring and quadriceps muscles correlated well with the decrease in blood pH ($r=-0.61$, $p<.05$). The results also indicated that the increase in total muscle activity during running is achieved by increasing more the activity of hamstring muscles (92.1 %) than quadriceps muscles (65.2 %). During maximal speed running, the activity of hamstring muscles was dominant (60.3 ± 5.9 %), while during maximal cycling the activity of quadriceps was higher (57.6 ± 4.1 %).

Conclusions. The present study showed that increased electrical activity of hamstring and quadriceps muscles correlated well with the changes in acid-base balance in recreational active persons during the IRT. So, it seems that a greater increase in muscle activity (Δ = maximal load - the first load) is needed for a bigger decrease in blood pH and BE (Δ = POST - PRE), and EMG shorts are a useful method for determining this change. The results indicated also that increased total activity of thigh muscles is achieved mainly by increasing the activity of hamstring muscles during running. Activation of hamstring and quadriceps muscles during maximal running and cycling were significantly different, so this information can be beneficial for those who use both type of exercises in their training programs, such as triathletes. However, the use of special cycling shoes and pedals could have affected the activation of hamstring muscles during the ICT.

Key words: incremental exercise test, EMG shorts, muscle activation, acid-base balance

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Johdanto. Akuutin kestävyys- ja voimaharjoituksen fysiologiset vasteet ilmenevät luurankolihaksissa, hengitys- ja verenkiertoelimistössä, sekä endokriinisessa että immuunijärjestelmässä. Nämä vasteet voivat heikentää suorituskykyä, ja esimerkiksi happo-emästasapaino on tarkoin säädelty negatiivisten seurausten varalta. Lihasten toimintaa voidaan tutkia elektromyografialla (EMG), ja nykyään on myös mahdollista käyttää tekstiilielektrodeja, jotka mahdollistavat lihasryhmien sähköisen aktiivisuuden tallentamisen normaalin liikkumisen aikana. Tämän tutkimuksen tarkoituksena oli tutkia yhteyksiä etu- ja takareisien lihasaktiivisuuksien ja happo-emästasapainon välillä, vertailla lihasaktiivisuuksia pyöräilyn ja juoksun aikana, sekä selvittää nopeusvoimatyyppisen harjoittelun vaikutukset kestävyyssuorituksen fysiologisiin määrittäjiin normaalissa elämässä liikunnallisesti aktiivisilla henkilöillä.

Menetelmät. Kuusitoista liikunnallisesti aktiivista henkilöä, yhdeksän naista (ikä 22.6 ± 2.0 v; VO_{2max} 42.8 ± 4.1 ml/kg/min) ja seitsemän miestä (ikä 28.1 ± 10.7 v; VO_{2max} 50.9 ± 5.4 ml/kg/min) osallistuivat tutkimukseen. Tutkimus sisälsi alku-, väli- ja loppumittaukset, joiden välissä oli kaksi kuuden viikon harjoittelujaksoa. Toinen harjoittelujakso sisälsi kevyttä kestävyysharjoittelua, toinen nopeus-, nopeusvoima- ja juoksutekniikka-harjoituksia. Mittauspäivinä koehenkilöt suorittivat nousevatehoisen juoksutestin (IRT = incremental running test) sisäjuoksuradalla, 20m nopeustestin lentävällä lähdöllä, vertikaalihyppyjä (CMJ = countermovement jump), sekä puolikyykkyjä. Verinäytteitä otettiin useissa eri ajankohdissa (FAST, PRE, POST, POST30’ ja POST60’). Kahden välipäivän jälkeen koehenkilöt suorittivat myös pyörätestin (ICT = incremental bicycle test). EMG shortsit (Myontec Oy) olivat käytössä kaikissa testeissä.

Tulokset. IRT:n seurauksena leukosyyttien kokonaismäärä veressä nousi sekä miehillä (66.8%; $p<.05$) että naisilla (80.8%; $p<.01$), ja testosteronipitoisuus nousi miehillä (18.4%; $p<.05$). Veren pH laski sekä miehillä että naisilla ($p<.05$ ja $p<.01$, vastaavasti), ja muutokset HCO3- and BE konsentraatioissa ($p<0.05$ and $p<.01$, vastaavasti) osoittivat selkeää metabolista asidoosia. Muutos etu- ja takareisien aktiivisuudessa korreloi hyvin alentuneen veren pH:n kanssa. ($r=-0.61$, $p<.05$). Tulokset osoittivat myös, että lihasten kokonaisaktiivisuuden nousu juoksun aikana saavutetaan lisäämällä enemmän takareisien aktiivisuutta (92.1 %) kuin etureisien aktiivisuutta (65.2 %). Maksimaalisen juoksuvauhdin aikana takareisin aktiivisuus oli pääroolissa (60.3 ± 5.9 %), kun taas maksimaalisessa pyöräilyssä etureisien osuus oli suurempi (57.6 ± 4.1 %).

Johtopäätökset. Tämä tutkimus osoitti että kasvanut etu- ja takareisien lihasten sähköinen aktiivisuus IRT:n aikana korreloi hyvin happo-emästasapainossa tapahtuneiden muutosten kanssa liikunnallisesti aktiivisilla henkilöillä. Näyttääkin siltä että suurempiin muutoksiin veren pH:ssa ja BE:ssä (Δ = jälkeen – ennen) vaaditaan suurempaa lisäystä lihasaktiivisuudessa (Δ = viimeinen kuorma – ensimmäinen kuorma), ja EMG shortsit ovat käytännöllinen menetelmä tämän muutoksen määrittämisessä. Tulokset osoittivat myös, että lisääntynyt lihasaktiivisuus juoksun aikana saavutetaan pääasiallisesti lisäämällä takareisien aktiivisuutta. Taka- ja etureisien aktivoinnit olivat erilaisia maksimaalisessa juoksussa ja pyöräilyssä, joten tästä tiedosta voi olla hyötyä niille, jotka käyttävät molempia lajeja omissa harjoitusohjelmissaan, kuten triathlonistit. Kuitenkaan ICT:n aikana käytössä ei ollut pyöräilykenkiä ja lukkopolkimia, joten niiden käyttö olisi voinut vaikuttaa takareisien aktivaatioon.

Avainsanat: Nousevatehoinen kestävyyssuoritus, EMG shortsit, lihasaktivaatio, happo-emästasapaino

ABBREVIATIONS

ACTH	Adrenocorticotropin hormone
ADP	Adenosine diphosphate
AP	Action potential
ATP	Adenosine triphosphate
CNS	Central nervous system
CO_2	Carbon dioxide
EMG	Electromyography
EPO	Erythropoietin
H^+	Hydrogen ion
Hb	Hemoglobin
HCl	Hydrochloric acid
Hct	Hematocrit
H_2CO_3	Carbonic acid
HCO_3^-	Bicarbonate
HPA	Hypothalamic-pituitary-adrenal
IGF	Insulin-like growth factor
MU	Motor unit
$NaHCO_3$	Sodium bicarbonate
O_2	Oxygen
OH^-	Hydroxide ion
PCr	Phosphocreatine
pCO_2	Partial pressure of carbon dioxide
pO_2	Partial pressure of oxygen
RBC	Red blood cell
SHBG	Sex hormone-binding globulin
WBC	White blood cell

CONTENTS

1 INTRODUCTION

Endurance has been described as the capacity to maintain a certain velocity or power output for the longest possible period of time. One major factor affecting endurance performance is the aerobic resynthesis of adenosine triphosphate (ATP). This aerobic ATP resynthesis requires a sufficient delivery of oxygen from air to the mitochondria of muscle cells for the oxidation, without forgetting the adequate supply of energy from carbohydrates and lipids. (Jones & Carter 2000.) Three physiological factors that determine the level of endurance performance - maximal oxygen consumption (VO_{2max}), second lactate threshold (LT_2) and efficiency (economy) – have been found to be superior to other factors. VO_{2max} is the upper limit of aerobic metabolism, while LT_2 is the intensity of exercise where blood lactate increase above resting levels. Exercise economy has been defined as the oxygen uptake required at a given intensity of exercise. (Jones & Carter 2000; Joyner & Coyle 2008.)

The purpose of training is to stress the body's physiological processes or structures to provide a stimulus for adaptation, which is detected as an improved functional capacity. In endurance sports, chronic adaptations can be achieved only if training intensity and duration are sufficient to elicit an adaptive response. The third factor affecting the training load is frequency, which is why elite and sub-elite runners perform approximately 10-14 exercise sessions per week. A typical form of exercise for endurance runners has been long slow distance (LSD) training, while less attention has been given to high-intensity training, although there is scientific evidence (e.g. Helgerud et al. 2007) of its effectiveness. According to present knowledge, strength training can also have a positive impact on endurance performance. Explosive and heavy strength training can potentially improve exercise economy, LT, anaerobic capacity and maximal speed, as well as reduce or delay fatigue. (Midgley et al. 2007; Rønnestad & Mujika 2014.) Focusing on endurance running, Paavolainen and colleagues (1999) developed the following hypothesis: in addition to aerobic power and running economy, endurance running performance is also influenced by "muscle power factor" associated with neuromuscular and anaerobic characteristics. Their finding supported the hypothesis, and this model is presented in Figure 1.

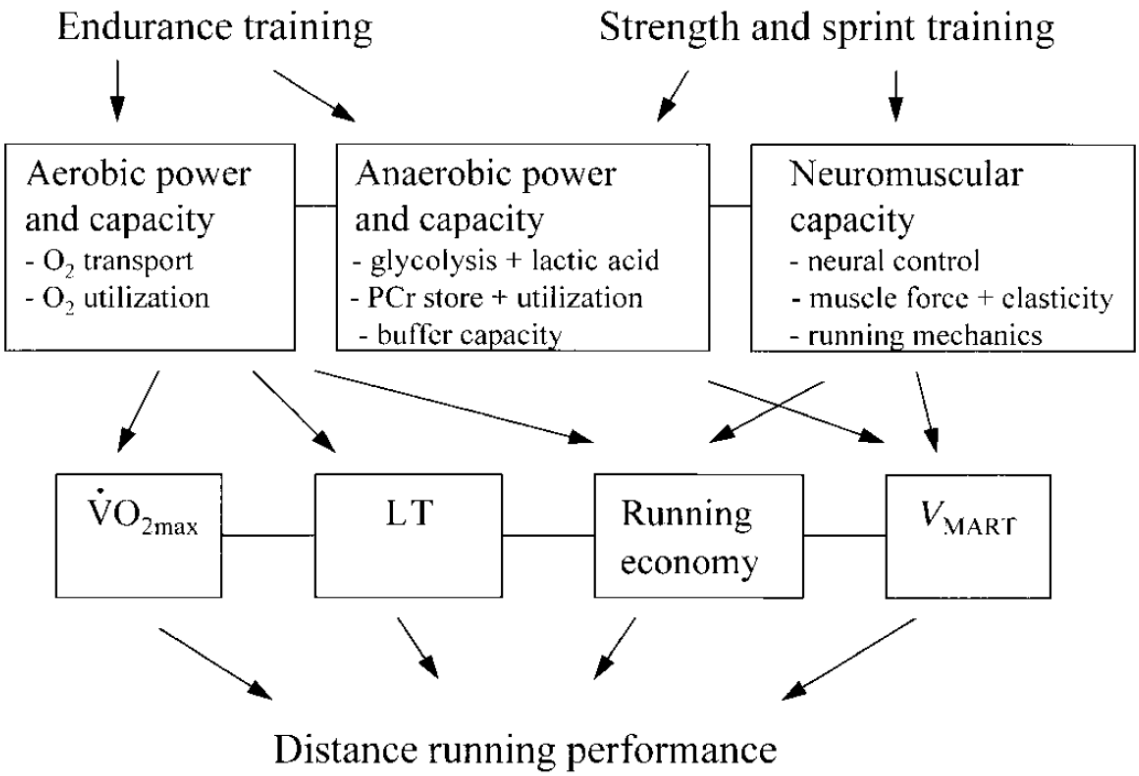

FIGURE 1. Hypothetical model of determinants of distance running performance in well-trained endurance athletes as influenced by endurance and strength training (Paavolainen et al. 1999). **PCr** = phosphocreatine, **VO2max** = maximal oxygen uptake, **LT** = lactate threshold, $\mathbf{V_{MART}}$ = peak velocity in maximal anaerobic running test (MART).

Neuromuscular system can adapt to training and also to more acute challenges, like muscle fatigue. Fatigue has been defined as an acute impairment of performance which include both increase in the effort required to exert a desired force and an eventual inability to produce this force. Muscle function can be examined by electromyography (EMG) and the two most common types of electrodes have been inserted and surface electrodes. However, nowadays it is also possible to use textile electrodes, which allows recording of muscle activity during normal locomotion. Normally, EMG is based on measurement and analysis of individual muscles separately, but in many practical applications it is not very functional because of the system complexity. Textile electrodes allow monitoring the level of muscle activity from a group of agonist and synergistic muscles during training. (Basmajian & De Luca 1985, 1, 22, 50–57; Enoka & Stuart 1992; Finni et al. 2007.)

It is known that the endocrine system is strongly influenced by exercise and this stimulus is dependent on the intensity, duration and mode of exercise, as well the training status of the person. The hormones cortisol and testosterone indicate the balance between body's anabolic and catabolic activity, and their concentration in blood increases after acute exercise. However, endurance training can lead to a reduced androgen levels. (Vervoorn et al. 1992; Zitzmann & Nieschlag 2001; Tremblay et al. 2004; Tremblay et al. 2005; Brownlee et al. 2005.) Exercise also causes changes in acid-base balance and in immune system (e.g. white and red blood cells in the circulation) (Rowbottom & Green 2000; Del Coso et al. 2009; Mairbäurl 2013).

The main aims of this study was to study the associations between activity of hamstring and quadriceps muscles and acid-base balance during incremental speed running test, compare differences in muscle activation during cycling and running, and investigate the effects of additional explosive strength training on the physiological determinants of endurance performance in recreationally active persons. Furthermore, another aim was to investigate the acute responses in blood cell counts, acid-base balance and hormones testosterone and cortisol induced by incremental speed running test.

2 PHYSIOLOGICAL RESPONSES TO ACUTE ENDURANCE EXERCISE

The body's physiological responses to acute endurance and strength exercise occur in musculoskeletal, cardiovascular, respiratory, endocrine and immune systems. The cardiovascular and respiratory systems respond to increased skeletal muscle activity to provide oxygen and nutrients for muscles and also to remove CO_2 and metabolic by-products by increasing oxygen uptake (VO_2) and ventilation. The musculoskeletal system changes its ability to extract oxygen, choose energy sources and remove metabolic by-products. In addition, the endocrine system maintains body's homeostasis both at rest and during exercise, while the immune system provides surveillance against foreign proteins, viruses and bacteria. (HHS 1996.)

2.1 Energy metabolism

Skeletal muscle has a capacity to increase energy turnover by 1000 times from its resting rate to meet demands of maximal contraction during exercise. Because the availability of ATP is limited, it needs to be resynthesized to meet this metabolic demand. The contraction of muscle cell initiates both ATP hydrolysis and resynthesis, and the cell will use different strategies in an attempt to match the resynthesis rate with the rate of hydrolysis. Myosin adenosine triphosphatase (ATPase) represents 70 % of the total ATP turnover in skeletal muscle during the theoretical maximum rate of hydrolysis. Hydrolysis of ATP leads to an increase in the concentration of adenosine diphosphate (ADP), adenosine monophosphate (AMP) and inorganic phosphate (Pi). Phosphocreatine (PCr) is the most immediate substrate source for ATP resynthesis, and also ADP with the production of AMP is used to resynthesize ATP. In addition, skeletal muscle also uses lipids and carbohydrate as substrates for resynthesis of ATP, but the rates at which these substrates can resynthesize ATP are significantly lower than from PCr and ADP. (Ball 2014.)

An important factor in long duration performances is the availability of carbohydrate reserves as a substrate for muscle metabolism and for the central nervous system (CNS). More precisely, important is the ability to preserve glycogen stores at sufficient level during the

entire performance, because depleted glycogen stores are associated with fatigue appearing during a prolonged exercise. It is possible to delay the appearance of fatigue by consuming carbohydrates during exercise, but it is good to note that glycogen stores can potentially be exhausted when the duration of exercise exceeds 90 minutes. In addition, endurance training can increase a capacity to oxidize lipid reserves in the muscles, which reduces the mobilization of glycogen stores at submaximal intensities (60-85 % of VO_{2max}). (Hausswirth & Le Meur 2012.)

2.1.1 Energy transfer during exercise

In short-duration, high-intensity performances, such as the 100 meter sprint, energy is produced from the immediate sources of energy, which are the intramuscular high-energy phosphate ATP and PCr. These compounds are able to provide energy for a 20 to 30 second running at marathon pace. Resynthesis of ATP and PCr must continue at high level if intensive exercise continues. During this kind of exercise, the energy to phosphorylate ADP comes mostly from stored muscle glycogen breakdown via anaerobic glycolysis with resulting lactate formation. In this way it is possible to form ATP quickly without oxygen. (McArdle et al. 2010, 163.)

Because the glycolytic reactions can produce only a little ATP, aerobic metabolism provides almost all of the energy transfer if exercise continues for longer than a few minutes. Oxygen consumption rises at the beginning of exercise for a few minutes exponentially, but stabilizes after this. This steady-state mirrors a balance between energy required by the working muscles and ATP produced by aerobic metabolism. In this state any lactate formed is oxidized or reconverted to glucose. (McArdle et al. 2010, 164–165.) In the study of energy system contributions in middle-distance running events made by Hill (1999), the results showed that for women, the energy is contributed anaerobically in the 400 meter, 800 meter and 1500 meter events on average 62 %, 33 % and 17 %, respectively. The percentages for men were 63 %, 39 % and 20 %, respectively. On this basis it can be said that already in the 800-meter race, energy is produced mainly aerobically.

2.1.2 Energy system contributions during incremental exercise

Bertuzzi et al. (2013) found out that during an incremental exercise test (IET) on a treadmill, aerobic metabolism dominates throughout the IET, and energy system contributions undergo a slow transition from low to high intensity. The aerobic system contribution during the IET was 86–95 % and anaerobic glycolytic contribution was 5–14 %, depending on the person. They did not notice any evidence of the sudden increase in glycolytic system contribution.

LT_2 (also known as anaerobic threshold, AnT) is defined as the level of work or oxygen consumption just below the point at which metabolic acidosis and the associated changes in gas exchange occur (Wasserman et al. 1973). In other words, the LT_2 is a point where aerobic metabolism alone cannot supply the energy needed for increasing work rate and thus the contribution from anaerobic metabolism becomes significant. Also, lactic acid is released into the circulation as a product of glycolysis. The LT_2 can be determined in an incremental exercise test. During the test, oxygen (O_2) uptake increases in a relation to the rising work rate to fuel aerobic muscle contraction, while the production and excretion of carbon dioxide (CO_2) remains stable. Up to a certain point CO_2 and work rate increases at the same rate, but after that, CO_2 is produced in excess. (Hopker et al. 2011.) Wasserman and colleagues (1973) argued that the increase in the respiratory exchange ratio (RER) caused by the buffering of lactic acid by sodium bicarbonate ($NaHCO_3$) is temporary, and occurs only when lactate concentration is increasing and bicarbonate (HCO_3^-) concentration is decreasing. Hopker and colleagues (2011) state that the LT_2 is commonly described by reference either to a blood lactate change (LT_2) or to ventilatory gas exchange data (ventilatory threshold, VT_2). Because metabolic thresholds (aerobic and anaerobic) reflect the adaptation to training, they can be useful physiological variables to predict endurance performance. High first lactate threshold (LT_1) and LT_2 may reflect the ability to tolerate high velocities during long-duration exercise. (Bertuzzi et al. 2013.)

2.2 Acid-base balance

Acids are substances that release hydrogen ion (H^+) during dissolution in liquid, while bases receive H^+ and form hydroxide ions (OH^-). The hydrogen ion concentration of a liquid

solution can be measured by pH, which means the concentration of protons or H^+. Neutral solution has the same amount of H^+ and OH^-, therefore its pH is 7.0. When H^+ concentration increases, pH decreases (acidosis) and when H^+ concentration decreases, pH increases (alkalosis). So, the acidic solution has a pH of less than 7.0 and the alkaline solution has a pH of more than 7.0. Chemical and physiological mechanisms can regulate changes in H^+ concentration (buffering). (McArdle et al. 2010, 300.)

H^+ concentration in the blood is normally about 40 nEq/L, and there will be no major changes due to accurate regulation. Usually, H^+ concentration is expressed on a logarithmic scale, using pH units:

$$pH = log \frac{1}{[H^+]} = -\log [H^+] .$$

The normal arterial blood pH (40 nEq/L) is

$$pH = -\log(0{,}00000004)$$
$$pH = 7.4 .$$

The pH of venous blood and interstitial fluids is a bit lower (7.35) due to the extra amounts of carbon dioxide (CO_2) released from the tissues to form H_2CO_3 in these fluids. Instead the urine pH can vary more (4.5 – 8.0), depending on the acid-base status of the extracellular fluid. (Guyton & Hall 2012, 379-380.) The tolerable limits for arterial blood pH extend from 6.9 to 7.5, but the maximum and minimum values can be tolerated only for a short period of time (Kenney et al. 2012, 200–202).

According to one theory, three independent variables – the partial pressure of CO_2, relative electrolyte (strong ions) concentrations and total weak acid concentrations (A_{TOT}) – determine pH in blood plasma. Difference between strong ions is known as SID:

$$SID = \left(\sum Strong\ base\ cations\right) - \left(\sum Strong\ acid\ anions\right)$$

A_{TOT} is mainly composed of albumin and phosphate. Also the observed changes in H^+ and bicarbonate (HCO_3^-) can be used to describe an alteration in acid-base status, although this does not imply causation. (Stewart 1981, 32; Kellum 2000.) Base excess can be used to describe the quantity of metabolic acidosis or alkalosis. It is the amount of acid or base that must be added to a sample of blood in vitro for restoring the pH of the sample to 7.40 (pC02 = 40 mmhg). Standard base excess (SBE) is the value calculated for anemic blood (Hb 5g/dl), describing an average content of hemoglobin across the entire extracellular fluid space. SBE quantifies the change in metabolic acid-base balance in vivo. Table 1 shows the limit values of metabolic and respiratory acidosis and alkalosis. (Kellum 2000.)

TABLE 1. Observational acid-base patterns (adapted from Kellum 2000).

Disorder	**HCO_3^- (mmol/l)**	**pCO_2 (mmHg)**	**SBE (mmol/l)**
Metabolic acidosis	< 22	= (1.5 x HCO_3^-) + 8 = 40 + SBE	< -5
Metabolic alkalosis	> 26	= (0.7 x HCO_3^-) + 21 = 40 + (0.6 x SBE)	> +5
Acute respiratory acidosis	= [(pCO2 – 40)/10] + 24	> 45	0
Acute respiratory alkalosis	= [(pCO2 – 40)/5] + 24	< 35	0

HCO_3^- = bicarbonate; **pCO_2** = the partial pressure of carbon dioxide; **SBE** = standard base excess

2.2.1 Buffer systems

There are three different mechanisms for pH regulation: chemical buffers, pulmonary ventilation and renal function. Chemical buffers (bicarbonate, phosphate and protein) provide the rapid first line of defense, while physiologic buffers (the pulmonary and renal systems) present the second line of defense in acid-base regulation. (McArdle et al. 2010, 301.)

Bicarbonate buffer. The bicarbonate buffer system contains carbonic acid (H_2CO_3) and sodium bicarbonate ($NaHCO_3$) in solution. Hydrochloric acid (HCl) converts to the weaker H_2CO_3 by merging with $NaHCO_3$ as follows:

$$HCl + NaHCO_3 \rightarrow NaCl + H_2CO_3 \leftrightarrow H^+ + HCO_3^- .$$

Any additional increase in H^+ concentration from H_2CO_3 dissociation causes the dissociation reaction to move in the opposite direction to release carbon dioxide (CO_2) into solution:

$$H_2O + CO_2 \leftarrow H_2CO_3 \leftarrow H^+ + HCO_3^- .$$

This is the result of acidosis. An increase in plasma CO_2 or H^+ concentration stimulates ventilation to eliminate excess CO_2. In alkalosis, the reaction goes in the opposite direction. (McArdle et al. 2010, 301.)

Phosphate buffer. The phosphate buffer system is important for buffering renal tubular fluid and intracellular fluids and it consist of phosphoric acid (H_3PO_4) and sodium phosphate (McArdle et al. 2010, 301; Guyton & Hall 2012, 383).

Protein buffer. Hemoglobin (Hb) can buffer pH changes significantly more than other plasma proteins. Hemoglobin increases its affinity to bind H^+ by releasing oxygen to the cells. The H^+ generated when H_2CO_3 forms in erythrocyte combines readily with deoxygenated hemoglobin (Hb^-, protein) as follows:

$$H^+ + Hb^- \rightarrow HHb .$$

About 60-70 percent of the total chemical buffering of the body fluids happen inside the cells and most of this results from the intracellular proteins. (McArdle et al. 2010, 301; Guyton & Hall 2012, 383.)

Ventilatory buffer. Increase in the quantity of free H^+ stimulates the respiratory center to increase alveolar ventilation. This reduces alveolar pCO_2 and as a result, CO_2 is removed from the blood. The recombination of H^+ and HCO_3^- accelerate and free H^+ concentration in plasma decreases. (McArdle et al. 2010, 302.) According to Wasserman et al. (2014), below LT_2 arterial H^+ is restrained by ventilating CO_2 produced by aerobic metabolism. Instead,

above LT_2 arterial H^+ is regulated by ventilating CO_2 produced from aerobic metabolism and CO_2 from HCO_3^- buffering of lactic acid. However, the chemical buffers and the respiratory system provide only a short-term solution to neutralize the acute effects of exercise-induced acidosis (Kenney et al. 2012, 200–202).

Renal buffer. The kidneys provide an essential longer-term defense mechanism, which maintain the body's buffer reserve. The kidneys regulate extracellular fluid by three mechanisms: secretion of H^+, reabsorption of filtered HCO_3^-, and production of new HCO_3^-. (McArdle et al. 2010, 302; Guyton & Hall 2012, 385.)

2.2.2 Acid-base balance and exercise

Conventional belief is that lactate production causes metabolic acidosis and increased lactate production (lactic acidosis) is one of the many causes of muscle fatigue induced by exercise. However, this view has been strongly questioned, and it is presented that metabolic acidosis is caused by an increased reliance on nonmitochondrial ATP turnover, while the production of lactate retards acidosis. (Robergs et al. 2004.) Still, increased production of lactate occurs at the same time with cellular acidosis and remains a good indirect marker for metabolic conditions of cell that induce metabolic acidosis. Lactate production increases under anaerobic conditions to prevent pyruvate accumulation and supply the NAD^+ (nicotinamide adenine dinucleotide) needed for glycolysis. If muscle does not produce lactate, metabolic acidosis and thus muscle fatigue will occur more quickly and exercise performance will be severely impaired. (Robergs et al. 2004; Cairns 2006.)

So, according to Robergs et al. (2004) and Robergs et al. (2005), H^+ is not formed during lactate production from pyruvate. Instead, H^+ formation occurs during glycolytic reactions that involve ATP hydrolysis. Increased muscle acidity (decreased pH) can interfere with contractile (myofilament function and excitation-contraction coupling), metabolic and other cellular processes (Cairns 2006). Some of the possible mechanisms for impaired muscle performance are presented in Table 2.

TABLE 2. Proposed mechanisms for impaired muscle performance with intramuscular acidosis (adapted from Cairns 2006).

Mechanism	
Contractile processes	
Myofilament function	↓ Maximum force (↓ maximum cross-bridge cycling)
	↓ Ca^{2+} sensitivity (↓ Ca^{2+} binding to troponin)
	↓ Maximum velocity of shortening (↓ myosin ATPase activity)
Excitation-contraction coupling	↓ Ca^{2+} release by SR (↓ Ca^{2+} release channel activity, ↓ charge movement)
	↓ Ca^{2+} uptake by SR (↓ calcium ATPase activity)
	↓ Free energy from ATP hydrolysis
Metabolic processes	↓ Rate of glycolysis/glycogenolysis (↓ PFK, ↓ GP activities)
	↓ Rate of cAMP production
Other cellular processes	↑ K_{ATP} channel conductance

ATP = adenosine triphosphate, **ATPase** = adenosine triphosphatase, **cAMP** = cyclic adenosine monophosphate, **GP** = glycogen phosphorylase, $\mathbf{K_{ATP}}$ = ATP-dependent K^+ channel, **PFK** = phosphofructokinase, **SR** = sarcoplasmic reticulum

↓ indicates decrease, ↑ indicates increase

According to Del Coso et al. (2009), endurance-trained cyclists do not have a better blood HCO_3^- buffering capacity or respiratory compensation to the acidosis than untrained individuals. However, it may be that training can develop the ability to tolerate low pH, so that muscle power can be maintained at the same level during exercise. The ability to buffer H^+ may be particularly important for maintaining performance during short repeated sprints in team sports, in addition to good physical fitness (Bishop et al. 2004). However, it has been suggested that a drop of 0.2 units in pH has no impact on muscle glycogenolysis, glycolysis and pyruvate oxidation during exercise. Instead, accumulation of interstitial potassium in muscle may be linked to the process of fatigue. (Bangsbo et al. 1996.) Blood and muscle pH return to normal levels within 30-40 minutes after cessation of exercise. This is achieved mainly by bicarbonate and respiratory removal of excess CO_2, and the process can be enhanced by active recovery. (Kenney et al. 2012, 200–202.)

2.3 Hormones cortisol and testosterone

The endocrine system includes a gland (host organ), hormones (chemical messengers) and a receptor organ (target). Endocrine glands secrete hormones directly into extracellular environment around the gland. Then these hormones diffuse into blood for transport to their target. Hormones can be divided into three categories: proteins and polypeptides, derivates of the amino acid tyrosine and steroids. Steroid hormones are secreted by the adrenal cortex, the ovaries, the testes and the placenta. Steroids are usually synthesized from cholesterol after a stimulus, because there is normally very little hormone storage in endocrine cells producing steroids. Steroids are highly lipid soluble, so they can simply diffuse across the cell membrane. (McArdle et al. 2010, 401; Guyton & Hall 2011, 881–882.)

2.3.1 Cortisol

Cortisol is one of the most prominent secretory product of the adrenal cortex. Adrenocorticotropin (ACTH) released from the pituitary stimulates the adrenals to produce and release cortisol, and in turn, ACTH is kept within an optimal range through a feedback action of cortisol. This feedback is transmitted via centrally located glucocorticoid receptors connected with a neural control mechanism, which allows the regulation of the activity of the hypothalamic-pituitary-adrenal (HPA) axis. (De Kloet et al. 1998; Feitosa et al. 2002.) The cortisol secretion has its own circadian rhythm, and its concentration in the blood is at its highest in morning just before awakening and the lowest around the midnight (Guyton & Hall 2011, 933).

External challenges such as endurance training, inactivity, fasting and stress temporarily increase plasma cortisol levels (Feitosa et al. 2002). Therefore, cortisol is known as a catabolic stress hormone and it affects glucose, free fatty acid and protein metabolism by, for example, breaking down the proteins into amino acids and by suppressing the immune system. Even though cortisol concentration in blood increases during exercise, perhaps the majority of the changes and the effects are taking place after exercise. The concentration of cortisol may remain elevated for two hours after cessation of exercise, possibly influencing tissue repair and recovery. The plasma concentrations of cortisol increase more in sedentary

people than in trained individuals, when submaximal exercise if performed at the same absolute intensity. (Brownlee et al. 2005; McArdle et al. 2010, 417, 432.)

According to Viru et al. (1996), the concentration of cortisol rises significantly during a short endurance performance at an intensity of more than 80 % of VO2max, in a 40-minute performance at an intensity of more than 60-70 % of VO2max, or if the performance lasts long enough. Also Tremblay et al. (2005) came to the same conclusion that the duration of the performance is meaningful, because plasma concentrations of cortisol were higher during 120 minutes of running at low intensity than during shorter running tests. The results obtained by Hill et al. (2008) support the results of the other studies. They reported that moderate to high intensity (60 to 80 % of VO_{2max}) exercise provokes increases in circulating cortisol levels due to a combination of hemoconcentration and HPA axis stimulus. Similar results have also been observed in women (Consitt et al. 2002). Both continuous (40 minutes, 1 minute break in the middle) running exercise at velocity of 80 % of VO_{2max} and intermittent (40 minutes; 2 minutes running, 2 minutes walking) at the velocity of 100 % of VO_{2max} can lead to acute serum hormone responses in both middle-distance and marathon runners. However, continuous running exercise seems to result in a lower cortisol response in runners who are adapted to do long-duration continuous running. (Vuorimaa et al. 2008.)

2.3.2 Testosterone

The testes in the male and ovaries in the female are the reproductive glands. These endocrine glands produce hormones that promote sex-specific physical characteristics and initiate and maintain reproductive function. Testosterone is the major androgen secreted by the interstitial cells of the testes. Also the adrenal glands secrete relatively small amounts of testosterone, as do also the ovaries in women. (McArdle et al. 2010, 417; Guyton & Hall 2012, 980.) Plasma testosterone concentration is generally considered as a physiologic marker of anabolic status. Testosterone influences directly on muscle synthesis, and indirectly it has an effect on the protein content of a muscle fiber by promoting growth hormone (GH) release leading to insulin-like growth factors (IGF) synthesis and release from liver. Testosterone has also positive effects on force-production of muscles, because it interacts with neural receptors. (McArdle et al. 2010, 418.)

Testosterone concentrations increase in response to exercise when a certain intensity threshold is reached. Generally, the highest concentrations are found at the end of exercise. Also, a low intensity long-lasting performance can increase testosterone secretion. (Galbo et. all 1977; Wilkerson et al. 1980.) Endurance training results in reduced androgen levels, but the lowering effect may be seen as part of a general response pattern to stress in an individual. In addition, mental stress has a negative impact on testosterone secretion. (Zitzmann & Nieschlag 2001.) Elite athletes seem to react differently to high-intensity exercise compared to less trained athletes. According to Slowinska-Lisowska and Majda (2002), immediately after a 400 meter run the elite athletes showed decreased total and free testosterone concentrations, while for the less trained athletes, the response was opposite. In another context, Vuorimaa et al. (2008) reported that 40-minutes intermittent of running exercise (2 minutes running, 2 minutes walking) at a velocity corresponding to 100 % of VO_{2max} resulted in a higher testosterone response in male runners who were adapted to middle distance compared with marathon runners. In women, testosterone levels rise after a bout of endurance exercise linearly related to both intensity and duration. This acute increase is temporary and the levels return to normal within hours. (Consitt et al. 2002.)

2.3.3 Sex hormone-binding globulin

Sex hormone-binding globulin (SHBG) is a circulating steroid-binding plasma glycoprotein, which specifically binds and transport androgens and estradiol, as well as regulates the bioavailability and metabolic clearance of sex steroids (An et al. 2000). After the secretion of testosterone, it binds loosely with plasma albumin or tighter with SHBG. SHBG circulates in the blood for 30 minutes to several hours, and during this time, the testosterone is either transferred to the tissues or degraded into inactive product. (Guyton & Hall 2011, 980). Because the concentration of SHBG in the blood is primarily determined by free estrogen and testosterone, it is an indirect measure of androgenicity (Oh et al. 2002). Different studies have reported contradictory results related to an acute SHBG response to various modes of exercise (An et al. 2000). In a study by Caballero et al. (1992) it was found that SHBG concentrations decreased in professional race cyclists during the competition period.

2.3.4 Relationship between testosterone and cortisol

In some circumstances, a negative relationship exists between the hormones testosterone and cortisol. Increase in the concentration of cortisol reduces testosterone production, and this has been observed in both rats and humans (men). The reason for this may be the fact that cortisol interferes testicular testosterone production process. (Brownlee et al. 2005.) A balance between anabolic and catabolic activity can be indicated by the ratio between free testosterone and cortisol (testosterone/cortisol ratio). During a short intensive training period this ratio decreases, but during a rest period anabolic activity begins to dominate. (Vervoorn et al. 1992.) A negative relationship between cortisol and total testosterone can be observed after a continuous endurance exercise (60–90 minutes). However, it is important to distinguish the forms of testosterone, because free testosterone has an opposite relationship with cortisol compared to total testosterone. (Brownlee et al. 2005.)

2.4 Blood cells

Three major cellular elements of the blood are red blood cells (erythrocytes), platelets (thrombocytes) and white blood cells (leukocytes). Plasma is largely aqueous fluid in the blood from which the cellular elements can be suspended. (Silverthorn 2007, 538.)

2.4.1 White blood cells

There are different types of white blood cells (WBC), called lymphocytes, monocytes, neutrophils, eosinophils and basophils. The monocytes develop in the tissues into macrophages. WBCs can be divided into phagocytes and granulocytes according to their shape and function. There are three types of lymphocytes: B-lymphocytes, T-lymphocytes and natural killer cells (NK cells). T-lymphocytes develop in the thymus cells and attack and destroy the virus-infected cells or cells that regulate other immune cells. B-lymphocytes develop in the bone marrow to plasma cells, which secrete antibodies (immunoglobulins). (Silverthorn 2007, 538, 787–788, 791.)

The immune system is a cellular and molecular structure with a specialized role in defense against infections. It basically consists of two kinds of response to the invading microbes. The innate (natural) responses occur to the same extent regardless of how many times the person is exposed to the infectious agent, while the acquired (adaptive) response develops from the repeated exposure to the infection. The innate responses use the phagocytic cells (neutrophils, monocytes and macrophages), cells that release mediators that cause inflammatory reactions (basophils, eosinophils and mast cells), and NK cells. The acquired responses are associated with the antigen-specific B- and T-lymphocytes proliferation, which occurs when surface receptors of these cells attach to the antigen. (Delves & Roit 2000.)

WBCs are an important part of the immune system and defense against infections. WBCs indirectly affect athletes' training programs, because infections disturb exercise performance. (Horn et al. 2010.) The total number of WBCs (leukocytes) in circulation increases during and immediately after exercise. This phenomenon is called leukocytosis, and it is affected by exercise intensity and duration. After exercise, the amount of circulating lymphocytes and monocytes tends to decrease, while the number of circulating neutrophils continues to increase. Precise mode of action of leukocytosis is not known, but it is thought that the leukocytes are "flushed" out of marginal pools in regions like the lungs and spleen. Also both catecholamines and cortisol may play a role in this process as mediators. (Rowbottom & Green 2000.) Horn et al. (2010) observed that endurance athletes (cyclists and triathlonists) had considerably lower total white cell and neutrophil counts than athletes competing in team or skill-based sports. They suspected that the lower WBCs originate from an adaptive response rather than an underlying pathology.

2.4.2 Red blood cells

The majority of the blood cells are erythrocytes: about 5 million per microliter. The corresponding value for WBCs is only 4 000–11 000 per microliter and for platelets from 200 000 to 500 000 per microliter. The glycoprotein ertythropoietin (EPO) control red blood cell production (erythropoiesis) and is mainly produced in the kidneys of an adult. A low oxygen level in the tissues (hypoxia) stimulates EPO synthesis. Red blood cells act as transporters of O_2 from the lungs to the cells and of CO_2 from cells to the lungs. O_2 is transported in the blood either dissolved in plasma or bound to hemoglobin (Hb). Hematocrit

(Hct) indicates the ratio of red blood cells to plasma and it is expressed as a percentage of total blood volume. (Silverthorn 2007, 541–542, 593.)

An increased need for oxygen during exercise is compensated by increasing muscle blood flow and improving O_2 unloading from Hb by decreasing Hb-O_2 affinity. For evaluation of O_2 transport capacity, Hb concentration (cHb) and Hct in blood, as well as total Hb mass (tHb) and total red blood cell volume (tEV) in circulation is needed. Hct increases during exercise due to a fluid loss, a shift of plasma water into the extracellular space and filtration. It has been shown that Hct is lower in athletes than in sedentary people. This phenomenon has been appointed as "sports anemia" and it has been attributed to increased red blood cell destruction during exercise. Plasma volume (PV) is susceptible to acute changes, whereas changes in total red blood cell mass are slow due to a slow rate of erythropoiesis. Because of this, total hemoglobin and red blood cell volume need to be measured in order to achieve a reliable measure of O_2 transport capacity. These values develop slowly by endurance training, and it may require several years of training. (Mairbäurl 2013.)

3 NEUROMUSCULAR SYSTEM AND ENDURANCE EXERCISE

3.1 Muscle structure and function in endurance sports

Human skeletal muscle contains two main types of fibers, slow-twitch (ST) fibers and fast-twitch (FT) fibers, which differ from each other in the type of ATP production mechanism, motor neuron innervation and myosin heavy chain expressed. ST fibers have a low myosin ATPase activity, slow calcium handling ability and slow shortening speed, less well-developed glycolytic capacity than FT fibers, as well as large and numerous mitochondria. Instead, FT fibers have a high capability for electrochemical transmission of action potentials, high myosin ATPase activity, rapid Ca^{2+} release and uptake by an efficient sarcoplasmic reticulum, and also a high rate of cross-bridge turnover. (McArdle et al. 2010, 371–374.) Endurance athletes' trained muscles typically have a higher percentage of ST fibers and the oxidative capacity of these fibers is higher than in muscles of untrained persons (Gollnick et al. 1972).

3.2 Fatigue

Adaptability is one of the most significant features of the neuromuscular system. Neuromuscular system can adapt to the altered demands of chronic usage, for example training, aging and immobilization. Likewise, it has an ability to adapt to more acute challenges, like those associated with sustained activity. One of the well known acute adaptations is called muscle fatigue. Fatigue has been defined as an acute impairment of performance which includes both increase in the effort required to exert a desired force and an eventual inability to produce this force. (Enoka & Stuart 1992.) In other words, fatigue is "an inability of a muscle or group of muscles to sustain the required or expected force". This definition has led to the idea that the onset of fatigue is delayed and is initiated only after a prolonged period of exercise. For instance, maximal force declines progressively with time during submaximal effort. The fatigue threshold is the level of exercise which cannot be sustained indefinitely. (Bigland-Richie & Woods 1984.)

Central factors of muscle fatigue are the physiological processes that appear within the central nervous system (CNS) (Enoka 1995). According to Davis and Bailey (1997), the CNS fatigue is a form of fatigue related to the specific alterations in the CNS function that cannot be reasonably accounted for by peripheral dysfunction within the muscle itself. Central factors comprise the ability to provoke an adequate and proper central command to the task, reliable transmission of the command to the involved motor neuron pools and sustained activation of the muscle by the motor neurons. Motivation, central command and motor unit behavior exhibit a significant role for central factors in muscle fatigue. (Enoka 1995).

Peripheral fatigue occurs on the distal point of nerve stimulation, whereas central fatigue means a failure to activate the muscle voluntarily during exercise. Supraspinal fatigue is a part of central fatigue, referring to a failure to generate output from the motor cortex. (Gandevia 2001). Peripheral fatigue can include impairments in neuromuscular transmission and propagation down the sarcolemma, dysfunction within the sarcoplasmic reticulum involving calcium release and uptake, actin-myosin cross-bridge interactions, accumulation of metabolites and availability of metabolic substrates. (Enoka 1995.) In case of intensive exercise, ATP production is unable to meet ATP utilization and reductions in ATP occur in connection with accumulation of metabolic by-products, for example H+, inorganic phosphate, AMP and ADP. Some of the by-products are supposed to interfere with NA^{+}/K^{+} balance, Ca^{2+} cycling and actomyosin interaction, which leads to fatigue. This is known as metabolic fatigue. Force is recovered quickly after the exercise ends and cellular energy potential is normalized. (Green 1997.)

Eccentric and concentric actions in human locomotion form a natural type of muscle function, which is known as the stretch-shortening cycle (SSC). It can be divided into two phases: lengthening and shortening (Figure 2). In the lengthening phase the muscle is acting eccentrically, followed by a concentric action. (Komi 2000.) Exercise-induced muscle damage, such as disruption of intracellular muscle structure, sarcolemma and extracellular matrix, may affect neuromuscular function. It can be assessed, for example by a vertical jumping performance. After a muscle-damaging exercise, reductions are immediate and long lasting. However, it seems that the jump performances with SSC (countermovement jump and drop jump) are not affected as much as the jumps without SSC (squat jump). So it is possible that the SSC potentially reduces the impact of muscle damage. (Byrne & Eston 2002; Byrne

et al. 2004.) Similar results regarding strength loss have been obtained in MVC after prolonged running performance (Millet et al. 2002; Millet et al. 2003), but instead, Vuorimaa et al. (2006) reported that intensive 20 - 40 minutes running exercise can lead to an acute improvement in the vertical power performance (CMJ and half squat) in long-distance runners, although the muscle activity (EMG) of the leg extensor muscles decreased. This may be due to the fact that the use of a different coordination strategy counteracts strength loss and even enhances power of legs.

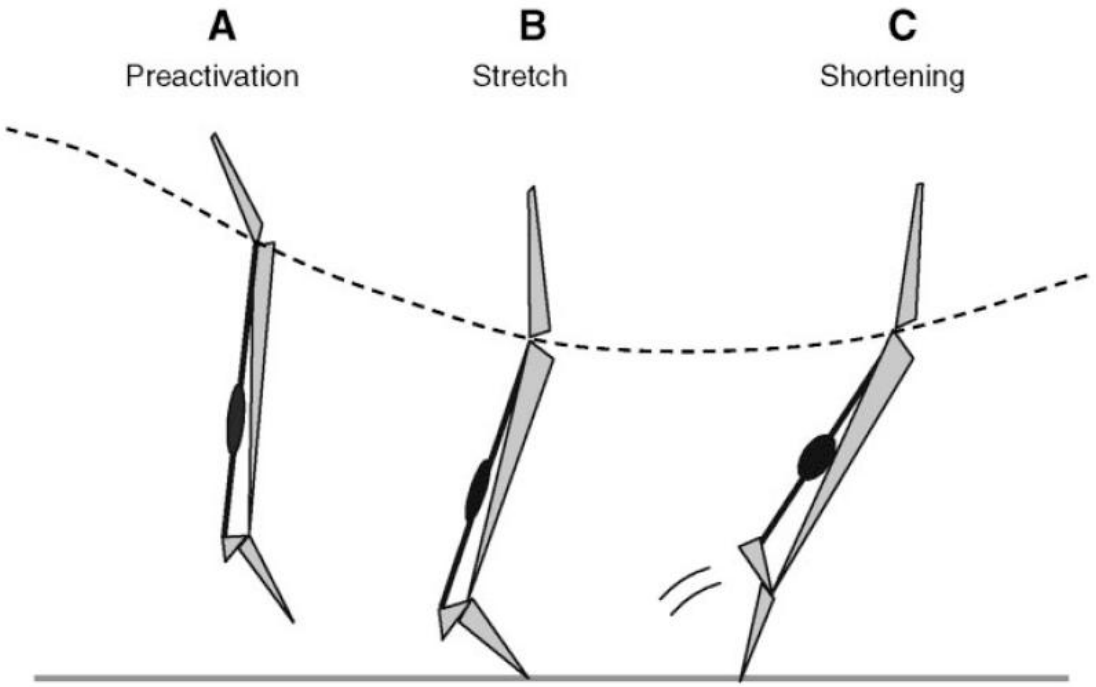

FIGURE 2. A = Preactivation before the ground contact by lower limb extensor muscles, B = Active breaking phase (stretch) and C = Concentric action (shortening). (Komi 2000.)

3.3 Electromyography

Electromyography refers to the examination of muscle function, based on the electrical signals transmitted by the muscles. In these studies, electrodes which are able to detect the current generated by the ionic movement are used. The two of the most common types of electrodes are surface electrodes and inserted (wire and needle) electrodes. However, prior to the signal can be observed, it must be amplified. After that, the amplified signal can be saved to a computer for further analysis. (Basmajian & De Luca 1985, 1, 22, 50–57).

3.3.1 EMG signal generation

Resting membrane potential (RMP) is a term for the potential differences that occurs across a cell membrane with the lack of stimulus-gated activation of ion channels and it is caused by the uneven distribution of ions across the membrane. Potassium (K^+) and organic anions (A^-) contain a greater extent in the cytoplasm, whereas sodium (Na^+) and chloride (Cl^-) are more prevalent in the extracellular fluid. Concentration gradient, from high to low, is induced by a difference in the concentration of two spaces. The Na^+-K^+-pump is large protein that spans the membrane and pumps Na^+ out of cells, while pumping K^+ into cells. The central nervous system can transmit a rapid activation signal (action potential, AP) to the contractile properties in muscle and AP evokes a temporary reversal of the potential differences across the cell membrane. (Enoka 2008, 179–182).

The motor unit (MU) includes α-motoneuron (α-MN) in the spinal cord and muscle fibers it innervates. The α-MN is the final point of summation for all descending and reflex input, so the net membrane current induced to this MN determines the activity of MU. In voluntary contraction, produced force depends on the number of recruited MUs and their discharge frequency. (Merletti & Parker 2004, 2–7). When a number of synapses are activated simultaneously and the input depolarizes the membrane potential sufficiently, AP is generated. AP propagation along the membrane can be divided into four phases, which are depolarization, overshoot, repolarization and afterhyperpolarization (Figure 3). (Enoka 2008, 186–188).

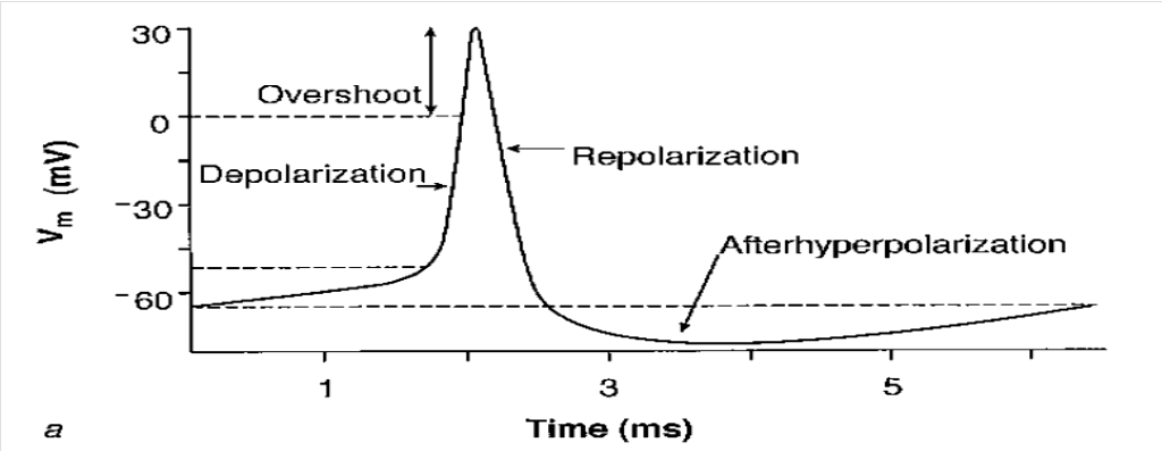

FIGURE 3. Action potential propagation along the membrane. The four phases are depolarization, overshoot, repolarization and afterhyperpolarization. (Enoka 2008, 186).

Electrical activity in muscle can be recorded with electrodes placed over the skin. The resulting surface electromyogram (sEMG) is the sum of the action potentials generated by the MUs and filtered by the volume conductor (Farina et al. 2010). The number and anatomical characteristics of the innervated muscle, without ignoring the properties of the recording electrodes, determine the shape of each motor unit action potential (Farina et al. 2002). EMG activity increases progressively as a function of force generated, which means that it reflects the recruitment of additional MUs and MU firing rate modulation to compensate for the deficit in contractility resulting from impairment of fatigued MUs (Figure 4). (Merletti & Parker 2004, 2–7).

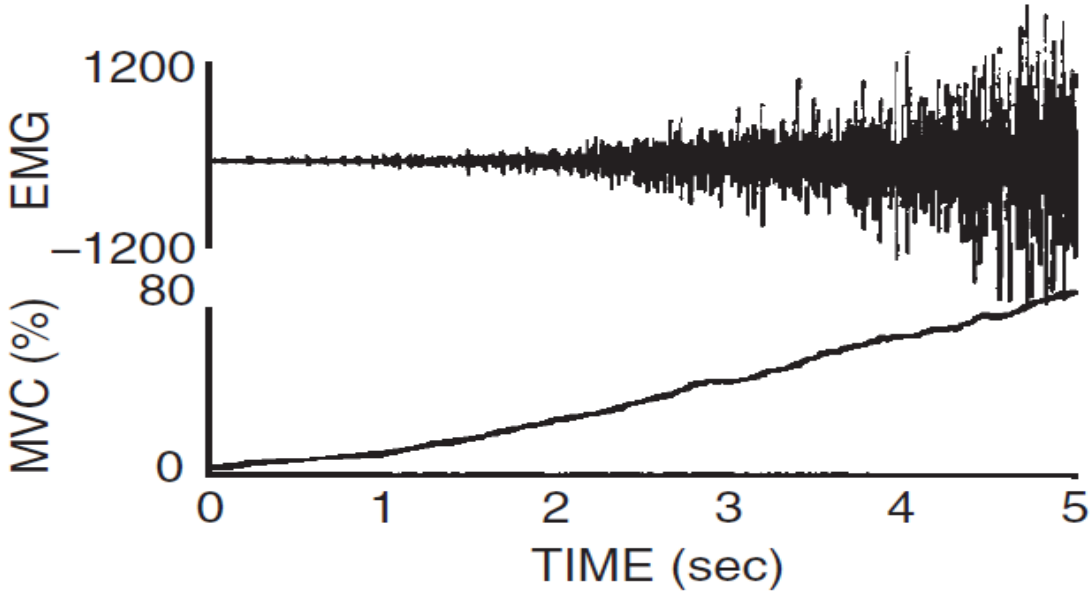

FIGURE 4. Raw EMG signal and generated force (Merletti & Parker 2004, 8).

3.3.2 EMG processing and normalization

There are a number of methods of EMG processing. The mean value of the rectified EMG over a time interval is defined as averaged rectified value (ARV) or mean amplitude value (MAV). It is computed as the integral of the rectified EMG over a time interval divided by the time. Another way to provide amplitude information is the root mean square (RMS), which is defined as the square root of the mean square value. In the same manner as ARV, this quantity is defined for a specific time interval. ARV or RMS values are voltages and are measured in Volt (V). Integrated EMG (iEMG) is sometimes reported but in this case, the unfiltered signal is integrated over a time interval, so it is the area under a voltage curve and is measured in

V/s. Nowadays most of the EMG recording systems are digital, and use MAV, ARV and RMS indicators (Merletti & Parker 2004, 140). Power Density Spectra presentation should include time epoch used for each spectral estimate, type of window used prior to taking the Fourier Transform, algorithm used (for example Fast Fourier Transformation), zero padding applied, the resultant frequency resolution and equations used to calculate, for example, the median frequency (MDF). (Merletti 1999.)

The normalization method used is chosen according to the purpose of the study. Normalization of an EMG signal includes rescaling the data to a percent of a selected reference value, (task EMG / reference EMG) x 100. Normalization is important for EMG studies because it allows between muscle, study and subject comparisons. It also improves the intraindividual and interindividual variability of the unnormalized EMG signal. (Ball & Scurr 2013). Nowadays, the development of EMG shorts has enabled signal normalization for dynamic exercise performances, in real world conditions, such as running and cycling. For example, the subject can perform maximum sprinting, which reflects the maximal muscle activity in running. After this, fatigue can be determined from this maximum value of EMG activity. Albertus-Kajee and colleagues (2011) recommend sprint method (fastest running speed for 30 seconds) to normalize EMG for investigating the maximal muscle activity during running, since it provides a reliable method that is similar to a normal running performance.

3.3.3 EMG shorts / Textile electrodes

Surface EMG is a widely used method in measuring muscle activity in athletes or patients, even though the measurements are difficult to implement in out-of-laboratory settings. Despite the fact that wireless transmission of EMG signals has become a standard, the method still includes a lot of variable factors. However, development of textile electrodes has made it possible to manufacture clothing equipped with these electrodes that can record muscle activity during normal locomotion without skin preparation and problems with hanging wires. Normally, EMG is based on measurement and analysis of individual muscles separately, but in many practical applications it is not very functional because of the system complexity. Textile electrodes allow, among other things, monitoring of the level of muscle activity from a group of agonist and synergistic muscles during exercise. (Lintu et al. 2005; Finni et al. 2007.) Two Finnish companies started the development of a prototype of the EMG garments

in 2001. These prototypes looked the same as tight sports clothing, even though they contained textile electrodes and signal transmission wires for surface EMG measurements. (Sipilä et al. 2007).

The shorts are knitted fabric, and to obtain the EMG signal, are equipped with conductive electrodes and wires integrated into the fabric. The wires transfer the EMG signal from the electrodes to the electronics module attachment point. The electronics module is attached in the waist area of shorts during the measurements. The textile electrodes are placed so that the bipolar electrode pair lies on the distal and the reference electrode longitudinally at the lateral side of quadriceps muscles. The conductive area of electrodes is 42 or 39 cm^2, depending on their size. The conductive electrodes consist of conductive yarns including silver fibers (electrical resistance 10 ohm / 10 cm) and non-conductive synthetic yarns woven together to form a fabric band. Electrodes are sewed onto the internal surface of the shorts. The transferring wires are made of steel fibers with short piece of silver coated fiber at the end to enable reliable soldering to the connector pins. Between the connector and electronic module is rubber sealing, which keeps moisture out of the gold plated electronics contact. The electronics module contains a signal amplifier, microprocessor with embedded software, data memory, interface to computer and wireless transmitter-receiver for storage and online monitoring with a wrist top computer. (Finni et al. 2007; Tikkanen et al. 2012).

Finni et al. (2007) concluded that the shorts with embedded textile electrodes are a promising method to measure ARV of EMG and those provide similar or even better reproducibility as the traditional bipolar surface electrodes. Tikkanen et al. (2014) showed that EMG shorts can be used for energy expenditure estimations and thigh muscle EMG provides more accurate energy expenditure estimations than accelometer or heart rate at low levels of physical activity, if individual calibrations are made. A couple of years earlier, Tikkanen et al. (2012) also demonstrated that it is possible to detect LT_2 during incremental treadmill running by EMG shorts. Commercially available EMG shorts can be used for the analysis of muscle balance (left and right leg), muscle load and quadriceps/hamstrings ratio (Myontec 2014).

3.4 Hamstring/Quadriceps strength ratio

Bilateral muscle asymmetry and muscle imbalances in the thighs have been claimed to cause many injuries, such as hamstring strains. Research evidence is deficient, but the most commonly reported conventional strength ratio of the knee muscles has been the concentric hamstring-quadriceps ratio (Hcon/Qcon). Knee extension muscle force expressed as a flexor-extensor ratio has been reported to range from 0.43 to 0.90, but the value of 0.6 is considered as a normative value. Another way to express this ratio is a more functional H/Q ratio. During knee extension the quadriceps contract concentrically (Qcon) and the hamstrings contract eccentrically (Hecc). And similarly, when the hamstrings contract concentrically (Hcon), the quadriceps contract eccentrically (Qecc). Thus, the H/Q ratio can be presented by either Hecc/Qcon (knee extension) or Hcon/Qecc (knee flexion). (Coombs & Garbutt 2002.)

Hayes et al. (2004) demonstrated that there are strong negative correlations between the local muscular endurance of various muscle groups and the kinematic changes (for example change in stride length) that occur during the final stages of a run to exhaustion. Their conclusion was that especially during eccentric work, the local muscular endurance of hip extensors and knee flexors is important in preventing or delaying these kinematic changes associated with fatigue during high-intensity endurance running. Still, there is not much research regarding the muscle ratios to sport specific tasks, although hamstrings strength has been found to correlate with distance running performance. Identifying the connection between muscular strength distribution, flexibility and aerobic capacity could promote training methods and hence improve running performance. (Thomas et al. 1983; Sundby & Gorelick 2014.)

Sundby and Gorelick (2014) found that highly trained female runners have significantly higher functional H/Q strength ratios at angular velocities approaching those that occur during running (in dynamometer), compared with the recreational runners. Interestingly, the leg strength of highly trained runners was lower, despite the higher H/Q ratios. The authors suggested that, at least at some level, distance running performance (RE) may be related to greater hamstring muscle strength relative to quadriceps muscle strength and not to absolute muscle strength.

3.5 EMG and energy system contributions during exercise

A more powerful force production with increasing running speed requires considerably increased EMG activity of the two-joint muscles (biceps femoris, rectus femoris and gastrocnemius) during the entire running cycle. In addition, EMG activity of leg extensor muscles during isometric MVC does not reach the same level as with running, so the isometric MVC is not necessarily a good indicator of the full activation potential of human skeletal muscle. (Kyröläinen et al. 2005.)

Already in 1980s, for example, Viitasalo and colleagues (1985) noticed non-linear increase in the integrated EMG (iEMG) at the AT during cycling test. As stated previously, this increase in EMG activity has been shown to reflect the recruitment of additional MUs and an increase MU rate coding as the strength of a muscle contraction increases (Taylor & Bronks 1994). After that, several studies have shown that there is a non-linear increase in EMG during the aerobic-anaerobic transition phase in ergometer cycling. An EMG threshold (EMG_T) represents the point where an increased contribution from fast twitch MU occurs to maintain the required energy supply for muscle contraction. (Lucia et al. 1999.) Some studies have reported that two EMG breakpoints may exist during incremental cycling, the first (EMG_{T1}) near the LT_1 and the second EMG threshold (EMG_{T2}) near the LT_2. (Lucia et al. 1999; Hug et al. 2003; Jurimäe et al. 2007.) As stated previously, Tikkanen et al. (2012) demonstrated that it is possible to detect LT_2 during incremental treadmill running by EMG shorts.

EMG_T have been detected in many muscles, such as vastus lateralis (VL), vastus medialis (VM) and rectus femoris (RF) of healthy and not highly trained subjects during incremental cycling test (Lucia et al. 1999). Jurimäe et al. (2007) reported that EMG_T of four different muscles (VL, VM, biceps femoris and gastrocnemius) were not different from the LT_2 during incremental cycling test. In addition, their subjects were from different sports (non-sport, cyclists, kayakers, handballers and power-lifters) and there were no differences between the groups. So the EMG_T compared to LT_2 was not affected by sport specificity. Candotti et al (2008) concluded in their study that the RF and VL muscles show similar behavior during maximal incremental cycling and the EMG_T and LT power outputs are equivalent for both muscles. In their view, the validity of using EMG to measure the power output corresponding to the LT_2 in recreational cyclists was confirmed based on these results.

4 ENDURANCE AND STRENGTH TRAINING

As already mentioned, endurance performance is generally defined as the ability to maintain the maximal power production in a specific competition distance and the energy cost in a specific competition speed. VO_{2max}, LT_2 and economy are considered to be the physiological factors that affect endurance performance. Anaerobic capacity and maximal speed can also have an effect on performance in shorter events. Strength training can support the performance by improving the economy of movement and anaerobic capacity, delaying fatigue and enhancing maximal speed. (Joyner & Coyle 2008; Ronnestad & Mujika 2014.)

4.1 Characteristics of endurance performance

Maximal oxygen uptake. VO_{2max} reflects maximal rate of aerobic energy expenditure and it can be associated with success in endurance sports (Ronnestad & Mujika 2014). In sports like running and cycling, a limiting factor for VO_{2max} is the rate at which oxygen can be supplied to the muscles, and thus VO_{2max} is strongly related to the maximal cardiac output (Q_{max}). The greater Q_{max} together with an increased extraction of oxygen by exercising muscle during exercise leads to a greater VO_{2max}. The optimal volume and duration for developing VO_{2max} is not exactly known, but the high-intensity training (approximately 80-100 % of VO_{2max}) has been found to be important. (Jones & Carter 2000.) For example, Helgerud et al. (2007) reported that high intensity aerobic training is much more powerful in improving VO_{2max} compared to performing same work at LT or 70 % of the maximum heart rate. They also found that the changes in VO_{2max} corresponded with changes in stroke volume (SV). According to Jones and Carter (2000), this increase in SV leads to a reduced heart rate during a submaximal exercise, and it results from increases in left ventricular size, myocardial contractility and end-diastolic volume, along with a decreased sensitivity to catecholamines.

Economy. Ronnestad and Mujika (2014) point out that probably any improvements in exercise economy will be associated with improved endurance performance. Exercise economy reflects the required oxygen uptake at certain intensity, and the variability in the oxygen cost of submaximal exercise is huge between individuals (Jones & Carter 2000). For

example, running economy (RE) can vary up to 30 % in equal level runners, and in elite or near elite athletes it is a better predictor of performance than VO_{2max} (Saunders et al. 2004). RE can be expressed as steady state VO_2 for a given running velocity (ml/kg/min), the volume of oxygen per distance covered (ml/kg/m) or the distance covered per volume of oxygen consumed (m/ml/kg) (Larisova 2014). Interval training at 93–106 of VO_{2max} and continuous running at LT_2 have been shown to be effective training methods to improve RE (Midgley et al. 2007). Figure 5 shows the wide range of factors affecting running economy.

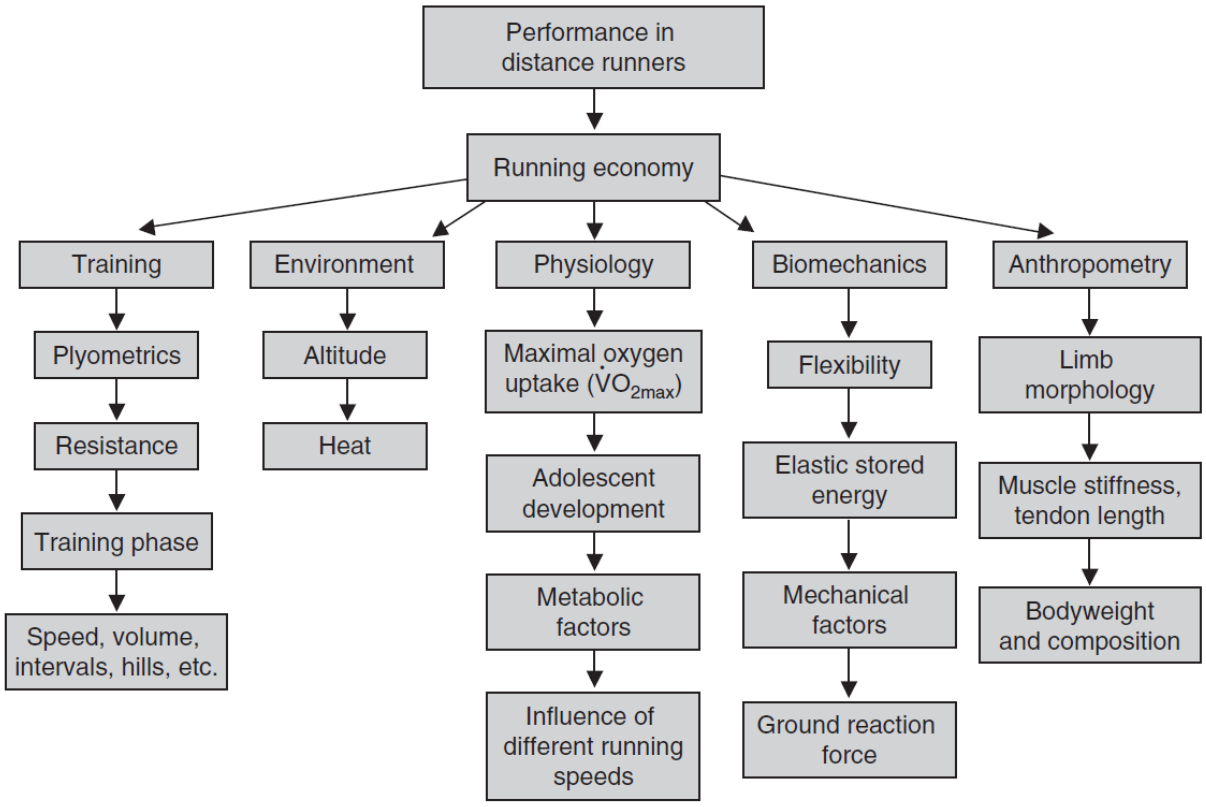

FIGURE 5. Factors affecting running economy (Saunders et al. 2004).

Second lactate threshold. When intensity of exercise increases, lactate production and accumulation accelerate. This happens because the muscle cells cannot meet the additional energy demands aerobically and oxidize lactate at its rate of formation. This threshold for lactate buildup is called the LT_2 and it describes an estimation of a breakpoint on the blood lactate [La^-] curve as a function of exercise intensity. Any shift to the right of the LT_2 on the La^- curve to a higher power output or speed is a sign of a successful training. (Tokmakidis et al. 1998; Jones & Carter 2000; McArdle et al. 2010, 164.) Exercise above the LT_2 causes a

nonlinear increase in metabolic, respiratory and perceptual stress. In addition, it results in more rapid fatigue, whether caused by the effects of metabolic acidosis on contractile function or an accelerated depletion of glycogen. (Jones & Carter 2000.)

4.2 Strength training in endurance athletes

Endurance runners must be able to sustain a high running velocity during competitions, which highlights the importance of neuromuscular characteristics, muscle force and elasticity, running mechanics and the anaerobic capacities. Strength training is one way to try to improve RE, as it may affect the ability to produce high lactate and the production of short contact times and fast forces. (Jones & Carter 2000.) Also one probable mechanism for improved endurance performance after concurrent strength and endurance training is changed muscle fiber type recruitment pattern. This means that strength training may increase the maximum strength of type I muscle fibers and delay their time to exhaustion, and thus postpone the activation of type II muscle fibers. Due to the increased maximum force, peak force or muscle fiber tension at the same exercise intensity decrease to a lower rate of maximal value. (Rønnestad & Mujika 2014.) However, the effects of strength training on endurance performance are still unclear, because there are published studies both for and against strength training. For example, combined endurance and strength training have been reported to impair cardiovascular and musculoskeletal adaptation, although more recent studies have indicated that concurrent endurance and strength training may enhance endurance performance, when compared to endurance training alone. (Aagaard & Raastaad 2012.) More potential strength training-induced mechanisms for improved endurance performance in trained endurance athletes are presented in Figure 6.

Mikkola et al. (2011) reported that eight weeks of combined endurance and heavy or explosive strength training improved endurance and neuromuscular performance in recreational runners. The neuromuscular and/or anaerobic improvements seemed to affect the endurance performance, even though no changes were observed in VO_{2max}. According to Taipale et al. (2010), maximal or explosive strength training is more powerful in improving strength and neuromuscular performance, velocity at VO_{2max} and RE than circuit training in recreational endurance runners. Rønnestad and Mujika (2014) presented that explosive and strength training with maximal velocity during the concentric phase seems to have a

beneficial effect on endurance running performance. Consequently, cyclists should perform only heavy strength training with maximal velocity during concentric phase.

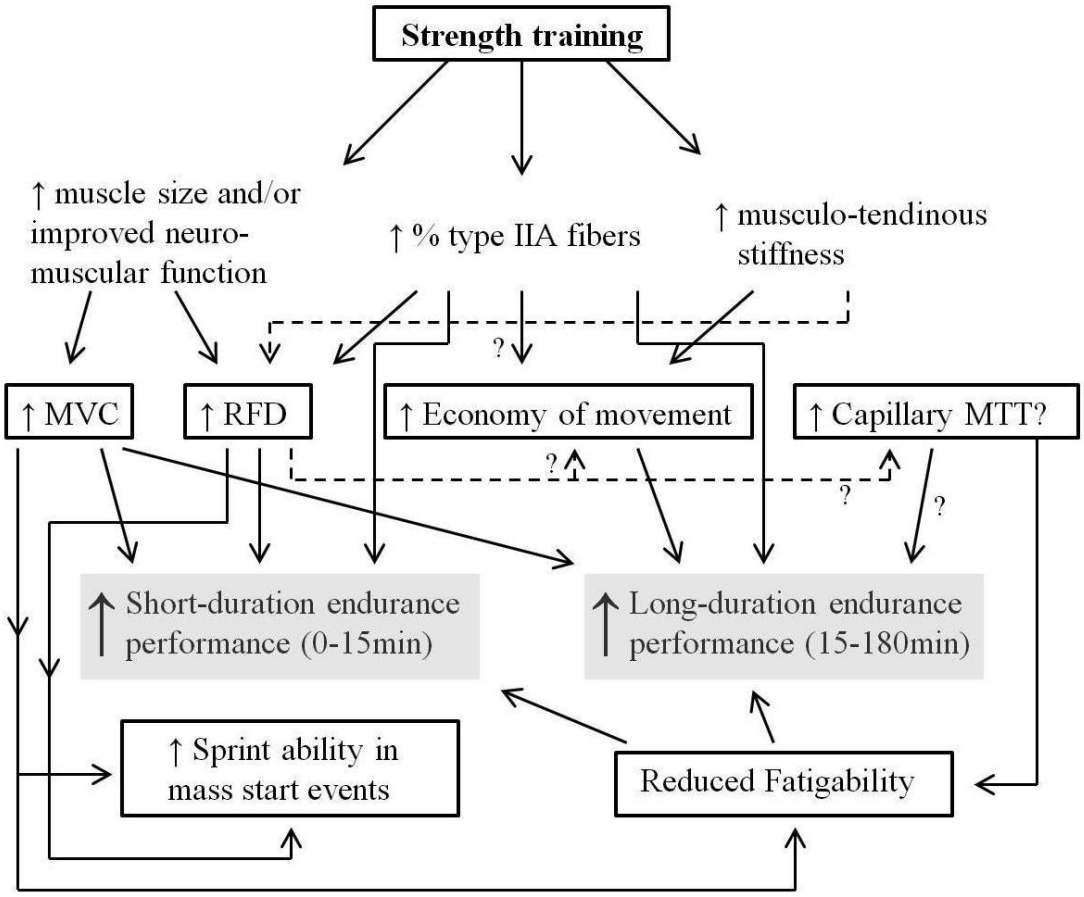

FIGURE 6. Proposed mechanisms by which short-duration and long-duration endurance performance can be increased in well-trained to highly trained endurance athletes by the addition of strength training to ongoing endurance training (Adapted from Aagaard & Raastad 2012). **MVC** = maximal voluntary contraction, **RFD** = rate of force development, **MTT** = mean transit time. Full lines indicate a stimulatory effect; dotted lines indicate proposed potential interactions that await experimental verification with concurrent strength and endurance training. Question marks indicate potential effects that await general experimental verification.

4.3 Speed training

Speed training refers to maximal short-duration (2-10 seconds) exercise bouts followed by long recovery periods (50-100 seconds) (Iaia & Bangsbo 2010). During repeated sprint bouts,

phosphocreatine (PCr) resynthesis is incomplete and glycolytic supply becomes more important. In addition, these sprint efforts also induce the accumulation of H+ ions and a fall in muscle pH. These changes may inhibit the rate of PCr resynthesis after exercise, because the fast phase of PCr resynthesis is an oxygen-dependent process, while the later slow phase may be limited by intramuscular pH changes. (Dawson et al. 1997.) The reason for this type of training is to induce disturbance to the muscle metabolic milieu and ion homeostasis, which evoke beneficial adaptations to the ATP-PCr and lactate systems (Saraslanidis et al. 2011).

Paavolainen et al. (1999) found that simultaneous explosive strength training, including sprinting and endurance training, produced a significant improvement in 5 kilometer running performance in well-trained endurance athletes. There were no changes in VO_{2max} or other aerobic power variables, and the authors suggest that improvements resulted from enhanced neuromuscular characteristics. Training (9 weeks) included various sprints (5 - 10 x 20 - 100 meter) and jumping exercises without additional weight or with the barbell and leg-press and knee extensor-flexor exercises with low loads but high velocities. Improvements were observed in the 5-km running time, RE, maximal anaerobic running test (MART), maximal 20-m speed and 5-jump test. Their hypothesis was that endurance performance and peak treadmill running performance is influenced by the “muscle power factor”, which is related to neuromuscular and anaerobic characteristics, and the results of the study supported this hypothesis.

5 RESEARCH QUESTIONS AND HYPOTHESES

Question 1. Are there changes in the different performances, running/cycling economy or physiological variables after the training period?

Hypothesis 1: Training enhances performance and has a positive impact on the physiological variables.

Reasoning. Strength training can support the performance by improving the economy of movement and anaerobic capacity, delaying fatigue and enhancing maximal speed (Ronnestad & Mujika 2014). Training can affect the acid-base balance and EMG activity by improving the tolerance of decreased pH and muscle fatigue during maximal exercise (Del Coso 2009).

Question 2. Are the acid-base balance, endocrine system and blood cells affected in response to an acute incremental endurance performance?

Hypothesis 2: The cardiovascular and respiratory systems respond to increased skeletal muscle activity to provide oxygen and nutrients for muscles and also to remove CO_2 and metabolic by-products by increasing oxygen uptake (VO_2) and ventilation.

Reasoning. The musculoskeletal system changes its ability to extract oxygen, choose energy sources and remove metabolic by-products. The endocrine system maintains body's homeostasis both at rest and during exercise, while the immune system provides surveillance against foreign proteins, viruses and bacteria. (HHS 1996.)

Question 3. Are there associations between EMG activity and acid-base balance in response to an acute incremental endurance performance?

Hypothesis 3: A more powerful force production with increasing running speed requires considerably increased EMG activity of the two-joint muscles during the entire running cycle

(Kyröläinen et al. 2005) and increased muscle acidity (decreased pH) induced by exercise can interfere with contractile (myofilament function and excitation-contraction coupling), metabolic and other cellular processes (Cairns 2006).

Question 4. How are the hamstring and quadriceps muscles activated during cycling and running determined by EMG shorts?

Hypothesis 4: There is not much research regarding this subject, although the hamstrings strength has been found to correlate with distance running performance. Normally, EMG is based on measurement and analysis of individual muscles separately, but in many practical applications it is not very functional because of the system complexity. Textile electrodes allow monitoring of the level of muscle activity from a group of agonist and synergistic muscles during training.

Reasoning. Identifying the connection between muscular strength distribution, flexibility and aerobic capacity could promote training methods and hence improve running performance. (Sundby & Gorelick 2014).

Question 5. Is the neuromuscular system affected in response to an acute incremental endurance performance?

Hypothesis 5: Intensive 20 - 40 minutes running exercise can lead to an acute improvement in the vertical power performance (CMJ and half squat) in long-distance runners, although the muscle activity (EMG) of the leg extensor muscles decreases.

Reasoning. This may be due to the fact that the use of a different coordination strategy counteracts strength loss and even enhances power of legs. (Vuorimaa et al. 2006).

6 METHODS

6.1 Subjects

Sixteen physically active students (nine women, seven men), from Haaga-Helia (University of Applied Sciences, Vierumäki) volunteered to participate in the study (Table 3). Prior to participation in the study, all subjects had to pass a health questionnaire. The University of Jyväskylä Ethical Committee approved the study in May 2014.

TABLE 3. Characteristics of the subjects before the study.

	All (n=16)	**Men (n=7)**	**Women (n=9)**
Age (y)	25.0 ± 7.5	28.1 ± 10.7	22.6 ± 2.0
Heigth (cm)	175.6 ± 7.3	181.4 ± 5.3	171.1 ± 5.1
Weight (kg)	71.6 ± 11.8	80.9 ± 10.6	64.4 ± 6.3
Lean mass (kg)	58.7 ± 11.3	69.8 ± 4.5	50.0 ± 5.5
Fat%	17.1 ± 7.3	13.0 ± 7.3	20.3 ± 5.8
BMI (kg/m^2)	23.1 ± 2.7	24.6 ± 3.3	22.0 ± 1.5
VO_{2max} running (ml/kg/min)	46.6 ± 6.2	50.9 ± 5.4	42.8 ± 4.1
VO_{2max} bicycle (ml/kg/min)	40.7 ± 6.3	43.9 ± 6.8	39.0 ± 5.6

Fat% = percentage of body fat mass, **BMI** = body mass index, **VO_{2max}** = maximal oxygen uptake

6.2 Study protocol

The study contained pre-, mid- and post-measurements and two training periods, each lasting 6 weeks (Figure 7). The subjects were randomly divided into two groups, which were Group 1 and Group 2. During the first six-week period, Group 1 performed a normal endurance running training, while Group 2 carried out a speed-, running technique- and explosive strength-type training. For the second period, the groups changed the type of training between each other (Figure 8). Between the performance measurements and training periods, there was approximately a week-long break at each point. The subjects performed two to three exercises per week and the exercise duration in both groups was similar. The subjects were also allowed to carry out other physical activities during the study, which they normally

would perform. They were also instructed for filling a food diary before the study and they filled the diaries for 3-5 days at the beginning of the both training periods.

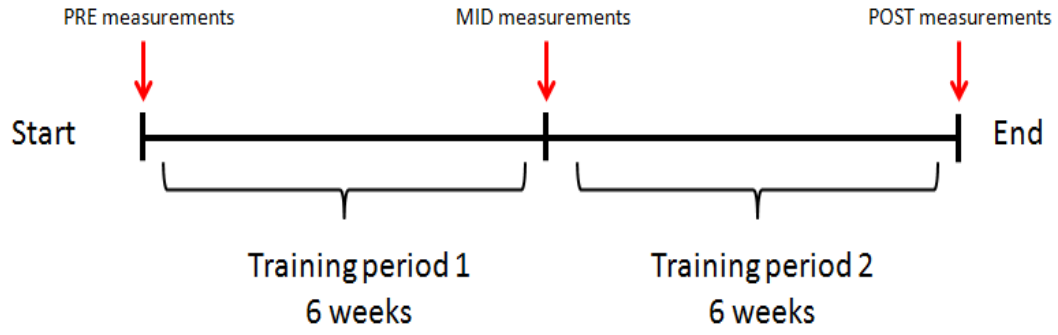

FIGURE 7. Study protocol.

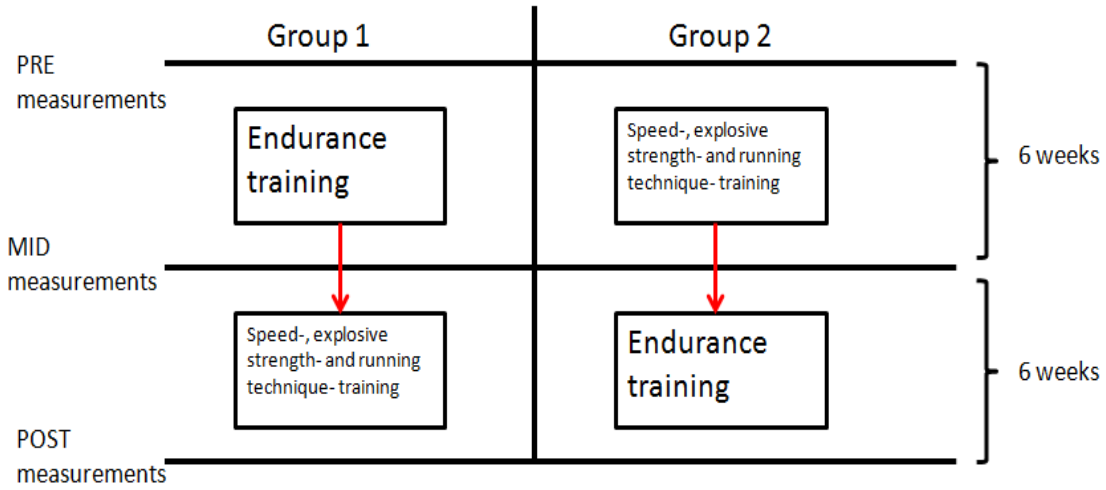

FIGURE 8. Grouping.

6.3 Training period

All subjects performed two six-week training period separated by a break of two weeks, one of which contained a light endurance exercises and the other contained speed, explosive strength and running technique exercises. The endurance training period was considered as a control training period. The intensity of exercises increased progressively during the periods and the planned total duration of training was same in both periods. The subjects used EMG-shorts (Myontec Ltd., Kuopio, Finland) at different phases of the study for six weeks (Figure

9). In addition, the participants filled an Internet-based training diary (Movescount) throughout the study. They also reported all other exercises, which they performed during the study period.

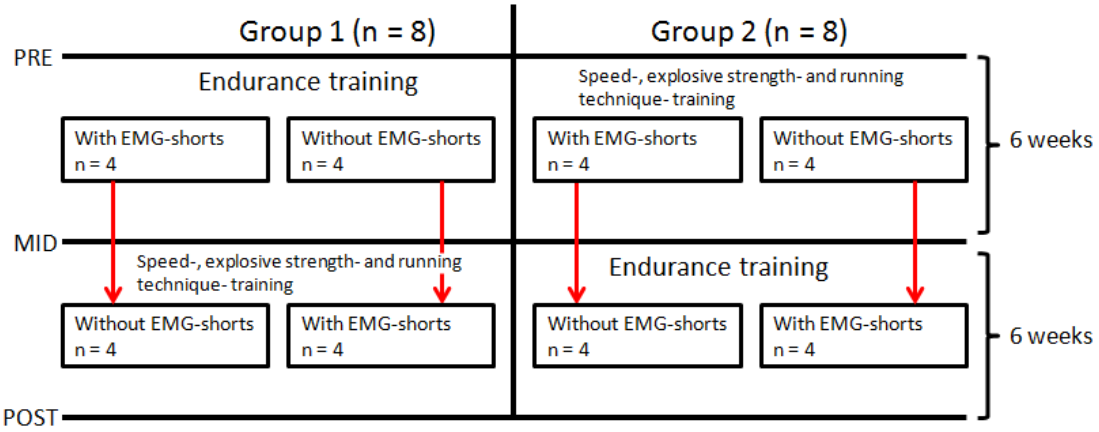

FIGURE 9. EMG-shorts.

Endurance training. The normal endurance training included a light running and walking at the intensity below the aerobic threshold and single training sessions lasted for one hour. The training program is shown in Table 4.

TABLE 4. Endurance training program.

Week	Number of exercises	Exercise
1	3	15 min running + 5 min walking + 15 min running + 5 min walking + 20 min running
2	3	18 min running + 3 min walking + 18 min running + 3 min walking + 18 min running
3	2	18 min running + 3 min walking + 18 min running + 3 min walking + 18 min running
4	3	18 min running + 3 min walking + 18 min running + 3 min walking + 18 min running
5	3	20 min running + 3 min walking + 20min running + 2 min walking + 15 min running
6	2	29 min running + 2 min walking + 29 min running

Speed-, explosive strength- and running technique- training. One exercise consisted of a 15-minute warm-up (running), a 10-minute running technique-section, a 10-15 minute speed-part, 10 minutes explosive strength-section, and finally a 15-minute cool down (running). The

total duration of one exercise was approximately one hour. The training program is presented in Table 5.

TABLE 5. Speed-, explosive strength- and running technique-training program.

Week	**1**	**2**	**3**	**4**	**5**	**6**
Number of exercises:	3	3	2	3	3	2
Warm-up Duration	15 min	15 min	15 min	15 min	15 min	15 min
Running technique Sets x drills x distance	3 x 4 x 30 m	3 x 4 x 30 m	3 x 4 x 30 m	3 x 4 x 30 m	3 x 4 x 30 m	3 x 4 x 30 m
Speed Sets x repetitions, recovery	5 x 20 m, 2min	5 x 40 m, 2 min	5 x 30 m, 2 min	5 x 50 m, 3 min	5 x 60 m, 3 min	5 x 50 m, 3 min
Explosive strength Sets x repetitions	2 x (3 x 6)	3 x (3 x 6)	2 x (3 x 6)	2 x (3 x 6) + 3 x 1	3 x (4 x 6)	2 x (3 x 6) + 3 x 1
Cool down Duration	15 min	15 min	15 min	15 min	15 min	15 min

6.4 Data collection and analysis

All measurements were performed at the Sports Institute of Finland in Vierumäki. The participants used the EMG shorts (Myontec Ltd., Kuopio, Finland) during all tests. The subjects were asked to avoid strenuous physical activity two days before the tests.

6.4.1 Performance measurements

Performance measurements included endurance, speed, maximum strength and explosive strength tests. The subjects completed the endurance test by running at the indoor running track, as well as by bicycle ergometer. The speed test was 20 m maximum sprint with flying start, explosive strength test was countermovement jump (CMJ), and the maximum force of the leg extensors was measured by half squat. All the tests, excluding the bicycle test, were performed on the same day. Between the running test day and the bike test day, the subjects had at least two days of rest. In addition, body composition was determined by using a bioimpedance method (BIA). The measurement protocol is presented in Table 6.

TABLE 6. The measurement protocol of the measurement day 1.

Measurement	**Explanation**
Fasting blood sample	FAST
Body compostition	BIA
A standard breakfast	Recovery 60 min
Speed test	20 m with flying start
Explosive strength test	CMJ
Maximum strength test	Squat in Smith machine
Blood sample 1	PRE
Endurance running test	On an indoor running track until exhaustion
Explosive strength test	CMJ
Blood sample 2	POST
Maximum strength test	Squat in Smith machine
Blood sample 3 (30 min after the running test)	POST30’
Blood sample 4 (60 min after the running test)	POST60’

BIA = bioimpedance, **CMJ** = countermovement jump

Endurance running test. Endurance running test was performed on 200-meter indoor running track. During the run, breath by breath gas exchange data was collected by Jaeger Oxygon Mobile (Viasys Healthcare GmbH, Hoechberg, Germany), heart rate was monitored using wrist-top computer and heart rate belt (Polar S610, Polar Electro, Kempele, Finland) and blood samples from a fingertip were taken for the determination of lactate concentration. The initial speed of the test was 2.3 m/s (8.3 km/h) and the speed was increased by 0.1 m/s every 200 m and by 0.2 m/s every second 200 m. The appropriate running speed was indicated by visual signs which were located on the edge of the track. Every 400-meter the test was stopped in order to take blood samples from a fingertip and write the current heart rate to the log sheet. The test was terminated when the subject was unable to maintain the required speed or wanted to quit voluntarily. Blood lactate concentration and heart rate were also measured before the test (resting values).

Calibrations of the gas analyzer were made once or twice during the measurement day, depending on the number of people tested. Each subject used the same mask size during every test and the sampling unit and the transmitter unit of the analyzer were attached to the upper back of the subjects. The average breath by breath values of the last 60 seconds of every load were considered as the subjects' values for the load. Ventilatory Thresholds (VT_1 and VT_2) were determined using methods commonly used in Finland (Nummela 2010).

Bicycle ergometer test. Bicycle test was carried out by bicycle ergometer (Ergometrics 800, ergoline, Bitz, Germany) in laboratory conditions. Every subject's toes were tied to the pedals with straps, which hold the foot in place. For collecting gas exchange data, monitoring heart rate and taking blood samples from a fingertip, the same equipments was used as in the running test. Prior to the test, the subjects performed a five-minute warm-up (50 W load). The starting load was also 50 W, the power was increased by 25 W every 2 minutes and the cadence was 50-70 RPM. Before the load was increased, the blood samples were taken and heart rate (15 s average) was logged. The test was terminated when the subject was unable to maintain the required power or wanted to quit voluntarily. Blood lactate concentrations and heart rate were also measured before, immediately after, 3 minutes after, 7 minutes after and 10 minutes after the test.

Speed test. The speed test was 20-meter maximum sprint performance with 10-meter flying start on the indoor running track and the running time was measured by photoelectric cells. The subjects completed one to three sprints of which the best time was taken into account. The running times were measured in 0.01 second accuracy.

Explosive strength test. The CMJ was used to evaluate the explosive power production. The subjects performed one to three jumps before the running test and the best result was taken into account. To determine the jump height, the photoelectric cells was used (LUX jump & speed analyzer). In addition, the subjects performed one CMJ after the running test to determine fatigue from the running test. The jump heights were measured in 0.1 cm accuracy.

Maximum strength test. The maximum strength of the leg extensors was measured by half squat in Smith Machine (Smith Press, Cybex International, Owatonna, Minnesota, US). The

maximum strength was that weight which the subject was able to lift up purely from a knee angle of 90 degrees. The test was repeated after the running test, in order to determine the fatigue.

Body composition. Body composition was measured in a fasted state at 8 am using bioimpedance method (InBody 720, Biospace Co. Ltd, Seoul, Korea). In addition, the subjects' heights were measured to the nearest 1 cm by using a measuring tape.

6.4.2 Blood samples

Venous blood samples were taken on the endurance running test day at five different time points (Table 7). The subjects arrived at the test place at 8 am for fasting blood sample (FAST) and for the determination of body composition. Then they ate a standard breakfast, after which began the performance tests. The rest of the blood samples were taken before (PRE), immediately after (POST), 30 minutes after (POST 30') and 60 minutes after (POST 60') the incremental running test. The analyzed variables were testosterone, cortisol, SHBG, pH, pCO_2 and pO_2, Na, K, ionized calcium (Ca), ionized Ca (pH 7.4), HCO_3^-, standard HCO_3^- (HCO_3^-st), total CO_2, BE (extracellular fluid), BE (blood) and oxygen saturation (sO_2). In addition, a complete blood count was analyzed from the FAST, PRE and POST samples, and the variables were leukocytes, lymphocytes, neutrophils, mixed cells (MXD; include monocytes, eosinophils and basophils), erythrocytes, Hb, Hct, thrombocytes (platelets), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).

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TABLE 7. Time points of blood samples during the measurement day 1.

Blood sample	Point	
1	Fasting sample	At 8 am
2	PRE	Before running
3	POST	Immediately after running
4	POST 30'	30 minutes after running
5	POST 60'	60 minutes after running

Complete blood count. Venous blood samples (2 ml) were collected into EDTA tubes and whole blood analyses were performed by automated hematology analyzer (pocH-100i, Sysmex Corporation, Kobe, Japan) using Roche reagents (Roche Diagnostics, Basel, Switzerland). The complete blood count includes the following variables: Leukocytes, erythrocytes, Hb, Hct, MCV, thrombocytes, lymphocytes, MXD and neutrophils.

Hormones. Venous blood samples (4.0 ml) were collected into serum tubes, centrifuged and analyzed by chemiluminometric immunoassay system (Immulite 2000, Siemens Healthcare, Erlangen, Germany), and reagents were also from the same manufacturer. Analyzed hormones were cortisol, testosterone and SHBG. For testosterone, intra-assay coefficient of variation (CV%) was 9.5 (18 nmol/l) and inter-assay CV% 10.9 (11 nmol/l), measuring range 0.7 – 55.0 nmol(l and analytical sensitivity 0.5 nmol/l. For cortisol, intra-assay CV% was 7.0 (437 nmol/l) and inter assay CV% 5.9 (845 nmol/l), range from 28 to 1380 nmol/l, and analytical sensitivity 5.5 nmol/l. For SHBG intra-assay CV% was 26.0 (41 nmol/l) and inter assay CV% 7.2 (106 nmol/l), range from 0.2 to 180 nmol/l, and analytical sensitivity 0.2 nmol/l

Blood gases. Capillary blood samples (200 ul) were collected into capillary tubes and analyzed by blood gas analyzer (GEM Premier 3000, Instrumentation Laboratory, Milan, Italy). Manufacturer of reagents was ILS Laboratories (Mediq Suomi Oy, Espoo, Finland). Analyzed variables were pH, pCO_2, pO_2, Na, K, ionized calcium, ionized calcium (pH 7.4), bicarbonate, total CO_2, BE (extracellular fluid), BE (blood) and sO_2.

Lactate. All samples were analyzed by Biosen S-line lactate analyzer (EKF-diagnostic GmbH, Magdeburg, Germany). Measuring range for lactate is 0.5 – 40 mmol/l and the intra-assay coefficient of variation was < 1.5 %.

6.4.3 EMG measurements

EMG was measured with shorts with an embedded textile electrodes sewed into its inner surface. Bipolar electrodes are located on the distal part of the quadriceps and hamstring muscles, while reference electrodes are located over the tractus iliotibialis on both sides. The

structure and function of the EMG shorts are described in section 3.3.3 (Finni et al. 2007). Due to the different body sizes, four different sizes of EMG shorts (S, M, L and XL) were used. The operation of the equipment was tested for each subject before the start of the tests by using Mbody Live computer software (Myontec Ltd., Kuopio, Finland), which allows real-time monitoring of muscle activity. During the tests, the EMG data was stored in the electronics module and later transferred (via Bluetooth) to a computer for further processing. The data (.asc and .hex file format) was analyzed using Muscle Monitor computer software (Myontec Ltd., Kuopio, Finland), where it is automatically processed (rectified) and expressed as uV units (Figure 10). EMG shorts can be used for the analysis of muscle balance (left and right leg), muscle load and H/Q ratio. Despite the preliminary testing of the equipment, the data of some performances had poor quality; therefore these performances were excluded from the study.

FIGURE 10. A screenshot of Muscle Monitor computer software (Myontec Ltd. Kuopio, Finland). Muscle Monitor was used for data analysis.

In the IRT and ICT, analyzed variables were total muscle loading and activity of hamstring and quadriceps muscles separately during the last 30 seconds of each load. In other tests, the highest muscle activity in one second was determined during each performance.

6.5 Statistical analysis

Data was processed and graphed using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, US) and statistical analysis was done using IBM SPSS Statistics version 22 (IBM Corporation, Armonk, New York, US). All results are presented as the mean ± standard deviation (SD). Due to a small sample size, Wilcoxon signed rank test was used for the analysis of the differences in different measurement points and differences between genders were analyzed with Mann-Whitney U-test. The relationship between EMG and acid-base balance were analyzed using Pearson correlation coefficient (normally distributed data) and Spearman's rank correlation (not normally distributed data). Significance level was set at * $p < 0.05$. The correlations were examined comparing the change in muscle activity (ΔEMG; first load and maximal load) and the change in blood values (PRE and POST) during the IRT

7 RESULTS

Results of this study focus only on acute responses because of some setbacks during the training periods. Although more than half of the subjects performed successfully the entire study period, the effects of training are excluded from the study due to the lack of the amount of reliable data. The results include the acute changes of blood variables in response to the incremental running test, relationships of muscle activity with acid-base balance and blood lactate, contributions of muscle activity of hamstring and quadriceps muscles during running and cycling, likewise the muscle fatigue induced by the incremental running test.

7.1 Acute responses induced by the incremental running test

TABLE 8. The results of the incremental running test (IRT) until exhaustion and other performance tests. Values are means ± SD.

	All (n=16)	Men (n=7)	Women (n=9)
Distance (m)	3163 ± 697	3571 ± 678	2844 ± 555[a]
V_{MAX} (km/h)	16.1 ± 1.9	17.2 ± 1.9	15.3 ± 1.6[a]
V_{MAX} (m/s)	4.5 ± 0.5	4.8 ± 0.5	4.2 ± 0.4[a]
VO_{2max} (ml/kg/min)	46.6 ± 6.2	50.9 ± 5.4	42.8 ± 4.1[b]
La_{MAX} (mmol/l)	10.3 ± 2.6	11.8 ± 2.6	9.2 ± 2.0
HR_{MAX} (bpm)	190 ± 8	187 ± 8	193 ± 7
20m (s)	2.67 ± 0.31	2.42 ± 0.24	2.87 ± 0.18[b]
CMJ_{PRE} (cm)	31.9 ± 9.2	38.7 ± 8.6	26.7 ± 5.7[a]
CMJ_{POST} (cm)	32.0 ± 8.1	38.5 ± 6.6	26.9 ± 5.1[b]
$Squat_{PRE}$ (kg)	128.7 ± 40.2	167.5 ± 25.2	98.5 ± 14.4[c]
$Squat_{POST}$ (kg)	134.9 ± 39.0	171.8 ± 25.0	106.2 ± 16.3[c]

[a] = $p < 0.05$, [b] = $p < 0.01$, [c] = $p < 0.001$ (significant difference between groups)

Distance = covered distance in the running test, $\mathbf{V_{MAX}}$ = maximal running speed, $\mathbf{VO_{2max}}$ = maximal oxygen uptake, $\mathbf{La_{MAX}}$ = peak value of concentration of lactate, $\mathbf{HR_{MAX}}$ = maximum heart rate, **20m** = 20 meter running test with flying start, $\mathbf{CMJ_{PRE}}$ = countermovement jump before the running test, $\mathbf{CMJ_{PRE}}$ = countermovement jump after the running test, $\mathbf{Squat_{PRE}}$ = half squat before the running test, S$\mathbf{quat_{POST}}$ = half squat after the running test.

Incremental running test. The results of the measurement day 1 (PRE measurements) are shown in Table 8. The most significant differences ($p < 0.001$) between women and men were observed in the maximum strength test (half squat in the smith machine) both before and after the IRT. Significant differences were also found in covered distance ($p < 0.05$), maximal running speed ($p < 0.05$), VO_{2max} ($p < 0.01$), 20 meter running test with flying start ($p < 0.01$) as well as in CMJ both before and after the running test ($p < 0.05$ and $p < 0.01$, respectively) Only the maximum heart rate and peak value of concentration of lactate did not differ between men and women.

Complete blood count. In women, leukocyte count increased 80.8 % ($p < 0.01$), neutrophil count increased 44.6 % ($p < 0.01$), lymphocyte count increased 143.0 % ($p < 0.01$) and mixed cell count increased 72.4 % ($p < 0.01$) in response to the incremental running test. Corresponding values for men were leukocytes 66.8 % ($p < 0.05$), neutrophils 53.5 % ($p < 0.05$), lymphocytes 92.3 % ($p < 0.05$) and mixed cells 68.4 % ($p < 0.05$). The absolute values are shown in Table 9. There were no differences between men and women in PRE or POST values, but lymphocyte count increased statistically significantly ($p < 0.05$) in women compared to men. The results indicate that the IRT caused a remarkable leukocytosis both in men and women.

TABLE 9. Blood leukocyte count of men, women and both men and women (All) measured before (PRE) and after (POST) the incremental running test (IRT). Values are means ± SD.

	Leukocytes (E9/l)	**Neutrophils** (E9/l)	**Lymphocytes** (E9/l)	**Mixed cells** (E9/l)
Women (n = 9)				
PRE	6.7 ± 2.0	3.8 ± 1.6	2.3 ± 0.8	0.6 ± 0.2
POST	12.1 ± 2.7**	5.5 ± 1.8**	5.5 ± 1.8**	1.0 ± 0.6**
Men (n = 7)				
PRE	7.5 ± 2.4	4.6 ± 2.1	2.3 ± 0.6	0.6 ± 0.1
POST	12.5 ± 2.8*	7.0 ± 3.1*	4.5 ± 0.6*	0.1 ± 0.2*
All (n = 16)				
PRE	7.0 ± 2.2	4.2 ± 1.8	2.3 ± 0.7	0.6 ± 0.2
POST	12.2 ± 2.7***	6.2 ± 2.5***	5.1 ± 1.5***	1.0 ± 0.5***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$(significant difference from PRE value)

Blood Hb concentration increased in women 2.8 % ($p < 0.05$) from 136 ± 10 g/l to 140 ± 8 g/l, and in men 3.3 % ($p < 0.05$) from 152 ± 11 g/l to 157 ± 10 g/l. Hct increased 4.0 % ($p < 0.05$) from 40 ± 2 % to 42 ± 2 %, and in men 2.5 % ($p < 0.05$) from 45 ± 3 % to 46 ± 2 %. Both before and after the IRT the mean values of Hb and Hct were different between men and women ($p < 0.05$), but the change induced by the IRT was not statistically significant. Erythrocyte count increased 3.1 % ($p < 0.05$) in women and 2.7 % ($p < 0.05$) in men, as well as thrombocyte count 15.8 % ($p < 0.01$) and 15.4 % ($p < 0.05$), respectively. In women, erythrocyte count was lower PRE and POST ($p < 0.01$) but the change was not statistically significant. Again, all absolute values are presented in Table 10.

TABLE 10. Erythrocytes, hemoglobin, hematocrit and thrombocytes of men, women and both men and women (All) measured before (PRE) and after (POST) the incremental running test (IRT). Values are means ± SD.

	Erythrocytes (E12/l)	**Hemoglobin** (g/l)	**Hematocrit** (%)	**Thrombocytes** (E9/l)
Women (n = 9)				
PRE	4.55 ± 0.29[b]	136 ± 10[c]	40 ± 2[b]	251 ± 57
POST	4.69 ± 0.30*[b]	140 ± 8*[b]	42 ± 2*[b]	291 ± 48**
Men (n = 7)				
PRE	5.12 ± 0.39	152 ± 11	45 ± 3	232 ± 64
POST	5.26 ± 0.38*	157 ± 10*	46 ± 2*	268 ± 60*
All (n = 16)				
PRE	4.80 ± 0.44	143 ± 12	42 ± 3	243 ± 59
POST	4.94 ± 0.44**	147 ± 12**	44 ± 3**	281 ± 52***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$(significant difference from PRE value)

[a] = $p < 0.05$, [b] = $p < 0.01$ (significant difference between groups)

Hormones. In women, changes in concentration of testosterone were moderate in absolute values, but still 89.2 % increase at POST 60' (0.70 ± 0.34 nmol/l) from baseline (0.37 ± 0.26 nmol/l) was statistically significant ($p < 0.05$). In men, the highest concentrations of testosterone were observed immediately after the IRT, and the increase was 18.4 % from 13.21 ± 3.89 nmol/l to 15.64 ± 4.75 nmol/l ($p < 0.05$). After the peak value, concentrations began to decrease and the levels fall below the baseline 60 minutes after the IRT. At each measurement point were clear differences in the testosterone levels between men and women

(Figure 11 and Table 11). In addition, increase from PRE to POST in men was significantly higher than in women ($p < 0.05$).

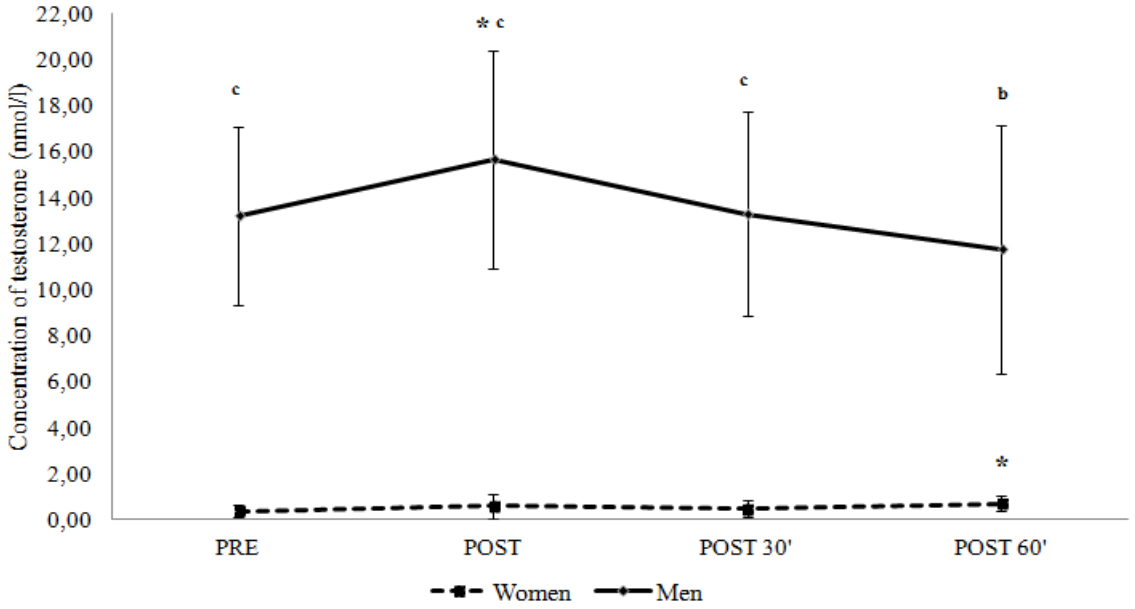

FIGURE 11. Changes in concentration of testosterone in men and women measured before (PRE), immediately after (POST), 30 minutes after (POST 30') and 60 minutes after (POST 60') the incremental running test (IRT). ^{c} = $p < 0.001$ significant difference between men and women. Values are means ± SD, n = 9 (women) and n = 7 (men).

The peak values of cortisol were observed 30 minutes after the IRT both in men and women (Figure 12 and Table 11). Increase in the cortisol levels in women was 10.9 % from PRE to POST, 30.0 % ($p < 0.05$) from PRE to POST30' and 22.4 % ($p < 0.05$) from PRE to POST60'. In men, changes were 8.0 %, 25.7 % and -3.1 %, respectively. Only in women the increase from the baseline was significant (POST30') and 60 minutes (POST60') was not enough to return the levels to the PRE values. In addition, there were no changes in T/C-ratio, but the difference between men and women was very clear ($p < 0.01$). Also in concentrations of SHBG were clear differences between men and women ($p < 0.01$). In women, the SHBG levels slightly increased from PRE to POST (1.5 %) but after that the levels decreased below the baseline. Changes were -4.3 % ($p < 0.05$) and -17.9 % ($p < 0.05$). In men, the SHBG concentrations remained more stable, only at POST30' (-4.3 %, $p < 0.05$) they decreased below the baseline levels (Figure 13 and Table 11).

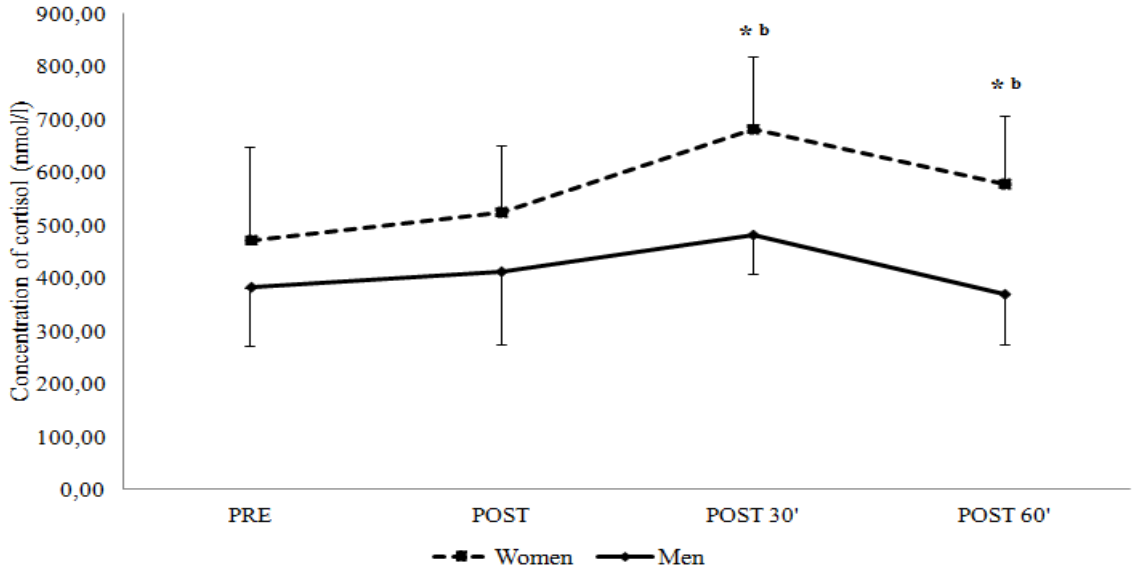

FIGURE 12. Changes in concentration of cortisol in men and women measured before (PRE), immediately after (POST), 30 minutes after (POST 30') and 60 minutes after (POST 60') the incremental running test (IRT). * $p < 0.05$, ** $p < 0.01$ significant difference from pre value; [b] = $p < 0.01$ significant difference between men and women. Values are means ± SD, n = 9 (women) and n = 7 (men).

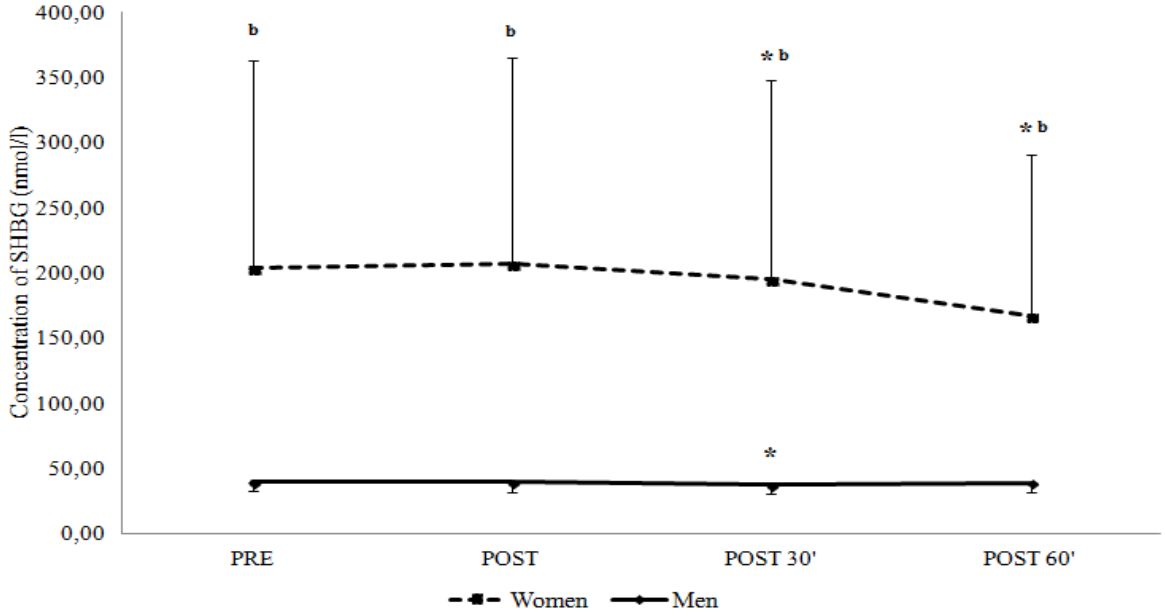

FIGURE 13. Changes in concentration of sex hormone-binding globulin (SHBG) in men and women measured before (PRE), immediately after (POST), 30 minutes after (POST 30') and 60 minutes after (POST 60') the incremental running test (IRT). * $p < 0.05$ significant difference from pre value; [a] = $p < 0.05$ significant difference between men and women. Values are means ± SD, n = 9 (women) and n = 7 (men).

TABLE 11. Concentrations of testosterone, cortisol, sex hormone-binding globulin (SHBG) and also testosterone-cortisol ratio (T/C ratio) in women and men measured before (PRE), immediately after (POST), 30 minutes after (POST 30') and 60 minutes after (POST 60') the incremental running test (IRT). Values are means ± SD.

	Testosterone (nmol/l)	**Cortisol** (nmol/l)	**SHBG** (nmol/l)	**T/C ratio**
Women (n = 9)				
PRE	0.37 ± 0.26^{c}	472 ± 176	203.3 ± 159.5^{b}	$0.1 \cdot 10^{-3}$ ± $0.8 \cdot 10^{-3}$ ^{c}
POST	0.59 ± 0.53^{c}	523 ± 128	206.4 ± 158.8^{b}	$1.2 \cdot 10^{-3}$ ± $1.0 \cdot 10^{-3}$ ^{c}
POST30'	0.48 ± 0.37^{c}	680 ± 138*^{b}	194.6 ± 153.5*^{b}	$0.7 \cdot 10^{-3}$ ± $0.5 \cdot 10^{-3}$ ^{c}
POST 60'	0.70 ± 0.34*^{b}	577 ± 130*^{b}	166.8 ± 123.6*^{b}	$1.3 \cdot 10^{-3}$ ± $0.8 \cdot 10^{-3}$ ^{b}
Men (n = 7)				
PRE	13.21 ± 3.89	382 ± 111	39.0 ± 6.4	$37.4 \cdot 10^{-3}$ ± $15.2 \cdot 10^{-3}$
POST	15.64 ± 4.75*	413 ± 138	39.1 ± 7.4	$38.9 \cdot 10^{-3}$ ± $16.1 \cdot 10^{-3}$
POST30'	13.29 ± 4.44	481 ± 73	37.3 ± 6.7*	$28.1 \cdot 10^{-3}$ ± $9.3 \cdot 10^{-3}$
POST60'	11.74 ± 5.42	370 ± 96	38.9 ± 7.3	$33.9 \cdot 10^{-3}$ ± $18.2 \cdot 10^{-3}$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$(significant difference from pre value)

^{a} = $p < 0.05$, ^{b} = $p < 0.01$, ^{c} = $p < 0.001$ (significant difference between groups)

Acid-base balance. Both in women (from 7.43 ± 0.02 to 7.24 ± 0.05, $p < 0.01$) and men (from 7.42 ± 0.03 to 7.22 ± 0.04, $p < 0.05$), pH decreased significantly during the IRT, but it returned to the baseline levels within 30 minutes (Table 12). In women, both HCO_3^- and HCO_3^-st decreased during the IRT ($p < 0.01$) and remained lowered 30 minutes ($p < 0.01$ and $p < 0.05$, respectively). The same was also observed in the case of pCO2. It decreased during the IRT ($p < 0.05$) and stayed below the baseline at 30 minutes ($p < 0.05$). In men, HCO_3^-, HCO_3^-st and pCO2 decreased during the IRT ($p < 0.05$), but these variables returned to normal level within 30 minutes. In some measurement points, there were differences between men and women in HCO_3^- (POST30' and POST60', $p < 0.05$), HCO_3^-st (POST60', $p < 0.05$) and pCO2 (PRE, $p < 0.05$; POST30' and POST60', $p < 0.01$). Changes were similar in both men and women, and there were no statistically significant differences. In addition, in Table 13 are shown the changes in concentrations of Na, K and ionized calcium (pH 7.4).

TABLE 12. pH, concentrations of actual bicarbonate (HCO_3^-) and standard bicarbonate (HCO_3^-st) and the partial pressure of carbon dioxide (pCO2) measured before (PRE), immediately after (POST), 30 minutes after (POST 30') and 60 minutes after (POST 60') the incremental running test (IRT). Values are means ± SD.

	pH	**HCO_3^-** (mmol/l)	**HCO_3^-st** (mmol/l)	**pCO_2** kPa
Women (n = 9)				
PRE	7.43 ± 0.02	24.7 ± 1.9	25.2 ± 1.3	5.0 ± 0.4[a]
POST	7.24 ± 0.05**	13.6 ± 2.2**	16.2 ± 4.2**	4.2 ± 0.6*
POST30'	7.42 ± 0.03	22.6 ± 0.6**[a]	23.8 ± 0.6*	4.7 ± 0.3*[b]
POST 60'	7.42 ± 0.02	24.5 ± 1.2[a]	24.4 ± 0.9[a]	4.8 ± 0.3[b]
Men (n = 7)				
PRE	7.42 ± 0.03	25.9 ± 2.5	25.8 ± 1.8	5.4 ± 0.4
POST	7.22 ± 0.04*	13.8 ± 1.9*	16.3 ± 3.7*	4.4 ± 0.4*
POST30'	7.41 ± 0.04	24.7 ± 2.7	24.9 ± 2.3	5.2 ± 0.2
POST 60'	7.42 ± 0.03	26.7 ± 2.3	26.3 ± 1.8	5.5 ± 0.3
All (n =16)				
PRE	7.42 ± 0.02	25.2 ± 2.2	25.5 ± 1.5	5.1 ± 0.4
POST	7.23 ± 0.04***	13.7 ± 2.0***	16.3 ± 3.9**	4.3 ± 0.5**
POST30'	7.41 ± 0.03	23.5 ± 2.0**	24.3 ± 1.6**	4.9 ± 0.4*
POST 60'	7.42 ± 0.02	25.0 ± 2.4	25.3 ± 1.6	5.1 ± 0.4

* p <0 .05, ** p <0 .01, *** p < 0.001(significant difference from pre value)

[a] = p < 0.05, [b] = p < 0.01 (significant difference between groups)

TABLE 13. Concentrations of sodium (Na), potassium (K) and ionized calcium (Ca (ph 7.4)) measured before (PRE), immediately after (POST), 30 minutes after (POST 30') and 60 minutes after the incremental running test (POST 60'). Values are means ± SD.

	Na (mmol/l)	**K** (mmol/l)	**Ca (pH 7.4)** (mmol/l)
Women (n = 9)			
PRE	140 ± 2	4.3 ± 0.4	1.17 ± 0.04
POST	143 ± 2*	4.3 ± 0.4	1.13 ± 0.02*
POST 30'	141 ± 2	4.2 ± 0.5	1.16 ± 0.02
POST 60'	139 ± 2	4.2 ± 0.2	1.17 ± 0.02
Men (n = 7)			
PRE	142 ± 2	4.4 ± 0.5	1.19 ± 0.04
POST	144 ± 3*	4.3 ± 0.4	1.11 ± 0.04*
POST 30'	143 ± 3	4.1 ± 0.3	1.16 ± 0.04
POST 60'	142 ± 4	4.3 ± 0.4	1.19 ± 0.02
All (n =16)			
PRE	141 ± 2	4.3 ± 0.4	1.18 ± 0.04
POST	143 ± 3**	4.3 ± 0.4	1.12 ± 0.03**
POST 30'	142 ± 3	4.2 ± 0.4	1.16 ± 0.03
POST 60'	141 ± 3	4.2 ± 0.3	1.18 ± 0.02

* $p < 0.05$, ** $p < 0.01$ (significant difference from pre value)

Changes in BEb and BEecf are shown in Table 14. In women, both BEb and BEecf decreased significantly from PRE to POST ($p < 0.01$) and remained below the baseline values 30 minutes ($p < 0.05$). In men, BEb and BEecf decreased significantly ($p < 0.05$) during the IRT, but only BEb did not return to the baseline in 30 minutes ($p < 0.05$). There were differences between men and women in both variables only 60 minutes after the running ($p < 0.05$).

TABLE 14. Changes in base excess in blood (BE b) and base excess in extracellular fluid (BE ecf) measured before (PRE), immediately after (POST), 30 minutes after (POST 30') and 60 minutes after the incremental running test (POST 60'). Values are means ± SD.

	BE b (mmol/l)	**BE ecf** (mmol/l)
Women (n = 9)		
PRE	0.6 ± 1.7	0.3 ± 2.0
POST	-12.5 ± 2.4**	-13.8 ± 2.6**
POST 30'	-1.3 ± 0.8*	-2.0 ± 0.7*
POST 60'	-0.6 ± 1.1[a]	-0.9 ± 1.3[a]
Men (n = 7)		
PRE	1.3 ± 2.3	1.4 ± 2.7
POST	-12.7 ± 2.3*	-13.9 ± 2.4*
POST 30'	0.3 ± 3.0*	0.1 ± 3.2
POST 60'	1.9 ± 2.3	2.2 ± 2.6
All (n =16)		
PRE	0.9 ± 1.9	0.8 ± 2.3
POST	-12.6 ± 2.3***	-13.8 ± 2.5***
POST 30'	-0.7 ± 2.0**	-1.2 ± 2.3**
POST 60'	0.6 ± 2.1	0.5 ± 2.5

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$(significant difference from pre value)

[a] = $p < 0.05$ (significant difference between groups)

7.2 Associations between muscle activity and blood variables

Associations between muscle activity and blood variables are presented by correlation. The correlations were examined comparing the change in muscle activity (ΔEMG; first load and maximal load) and the change in blood values (PRE and POST) during the IRT (Table 15). There was a high correlation ($r = 0.86$; $p < 0.001$) between ΔEMG and the change in lactate

concentration (ΔLa). Also, a clear negative correlations were observed between ΔEMG and ΔpH ($r = -0.61$; $p < 0.05$), ΔEMG and ΔBEecf ($r = -0.58$; $p < 0.05$) and ΔEMG and ΔBEb ($r = -0.61$; $p < 0.05$). On this basis it can be said that the more the ΔEMG increased the more the ΔpH, ΔBEb and ΔBEecf decreased during the IRT (Table 15 and Table 16). However, there were no positive or negative associations between ΔEMG and performance in the IRT, CMJ or half squat.

TABLE 15. Changes (Δ) in pH, base excess in blood (BE b) and base excess in extracellular fluid (BE ecf) measured before (PRE) and immediately after (POST) incremental running test. Mucle activity (Normalized EMG) is normalized to a maximal value (1 s) of each subject in the 20 meter running test and change (Δ) is calculated from values of first load (PRE) and maximal load (POST) in the incremental running test (IRT). Values are means ± SD.

	Pre (n=12)	**Post (n=12)**	**Δ (n=12)**
pH	7.43 ± 0.02	7.24 ± 0.04	-0.19 ± 0.04
BE ecf (mmol/l)	1.2 ± 2.5	-13.4 ± 2.5	-13.6 ± 3.3
BE b (mmol/l)	1.2 ± 2.1	-12.2 ± 2.1	-13.4 ± 2.9
La (mmol/l)	1.8 ± 0.4	10.3 ± 2.6	8.5 ± 2.7
Normalized EMG	4.7 ± 1.2	8.5 ± 1.1	3.8 ± 1.0

TABLE 16. Correlation coefficients of change in muscle activity (Normalized EMG) and in pH, base excess in blood (BE b) and base excess in extracellular fluid (BE ecf) in response to the incremental running test (IRT).

	ΔEMG (n=12)	
	r	*p*-value
ΔpH	-.605	0.037
ΔBE ecf	-.581	0.048
ΔBE b	-.613	0.034

r = Pearson's correlation coefficient, ***p*-value** = significance (2-tailed)

7.3 Muscle activity in running and cycling

During the last load of the ICT (the last 30 seconds), the absolute range of total muscle loading was 3617-7965 uVs. In the IRT, the same values were 4356-10786 uVs. When comparing each subject's own maximum value in the ICT and IRT, the higher values were observed in the IRT (456 ± 1864 uVs). However, it must be taken into account that the individual variations were very large. Figure 14 illustrates the change in the total muscle activity and also separately the activities of hamstring and quadriceps muscles during the IRT. The total activity increased 80.4 % ($p < 0.01$) from the first load to the maximal load. Respectively, the activity of hamstring muscles increased 92.1 % ($p < 0.01$) and the activity of quadriceps muscle increased 65.2 % ($p < 0.01$). The absolute values are shown in Table 17.

TABLE 17. Total muscle activity (Total EMG), contribution of hamstring and quadriceps muscles and differences between hamstring and quadriceps muscles (Difference) normalized to maximal value (1 s) of each subject in 20 meter running test in first load (1), second load (2), second last load (2^{nd} last) and maximal load (MAX) in the incremental running test (IRT). Values are means ± SD and n = 12.

Load	Total EMG	Hamstring	Quadriceps	Difference
1	4.7 ± 1.2	2.7 ± 0.7	2.04 ± 0.7	0.6 ± 0.7
2	5.0 ± 1.2**	2.8 ± 0.7*	2.16 ± 0.7**	0.6 ± 0.7
2^{nd} last	7.6 ± 1.1**	4.5 ± 0.7**	3.12 ± 0.7**	1.4 ± 0.9*
Max	8.5 ± 1.1**	5.1 ± 0.8**	3.37 ± 0.7**	1.7 ± 1.0*

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$(significant difference from 1)

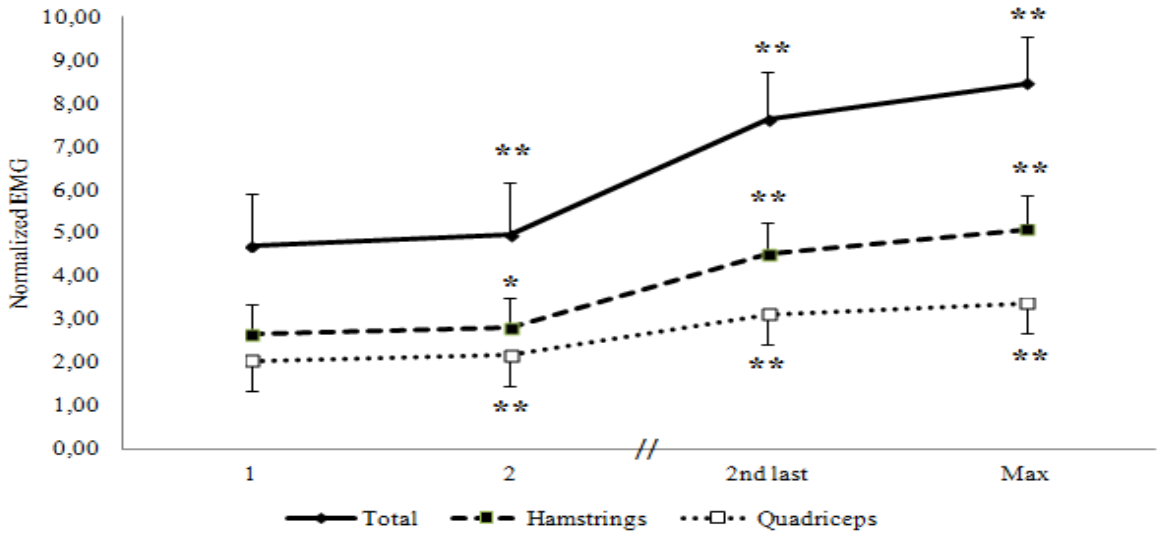

FIGURE 14. Total muscle activity (Total EMG) and contribution of hamstring and quadriceps muscles normalized to maximal value (1 s) of each subject in 20 meter running test in first load (1), second load (2), second last load (2nd last) and maximal load (MAX) in the incremental running test (IRT). Values are means ± SD and n = 12. * $p < 0.05$, ** $p < 0.01$ (significant difference from 1).

In the IRT, contribution of quadriceps muscles decreased 3.5 percentage points ($p < 0.05$) from 43.2 ± 6.2 % (first load) to 39.7 ± 5.9 % (maximal load). Respectively, contribution of hamstring muscles increased 3.5 percentage points ($p < 0.05$) from 56.8 ± 6.2 % to 60.3 ± 5.9 % (Table 18). So the difference between hamstrings and quadriceps changed from 13.6 % to 20.6 % and H/Q-ratio increased from 1.37 ± 0.37 to 1.57 ± 0.35. Instead in the ICT, contribution of quadriceps muscles increased 4.9 percentage points from 52.7 ± 7.7 % to 57.6 ± 4.1, and contribution of hamstring muscles decreased 4.9 percentage points from 47.3 ± 7.7 % to 42.4 ± 4.1 % (Table 19). Thus, the difference increased from 5.4 % to 15.2 %, and H/Q-ratio decreased from 0.94 ± 0.28 to 0.74 ± 0.13. This decline of H/Q-ratio was statistically significant ($p < 0.05$) and Figure 15 illustrates the change in H/Q-ratio both in the IRT and in the ICT.

TABLE 18. Contributions of quadriceps and hamstring muscles shown as a percentage of the total contribution (100 %) and hamstring-quadriceps ratio (H-Q-Ratio; calculated by dividing activity of hamstring muscles by activity of quadriceps muscles) in the incremental running test (IRT). Values are means ± SD and n = 12.

Load	**Quadriceps (%)**	**Hamstring (%)**	**H/Q-Ratio**
1	43.2 ± 6.2	56.8 ± 6.2	1.37 ± 0.37
2	43.2 ± 5.9	56.8 ± 5.9	1.36 ± 0.35
2nd last	40.7 ± 5.6	59.3 ± 5.6	1.49 ± 0.32
Max	39.7 ± 5.9*	60.3 ± 5.9*	1.57 ± 0.35

* $p < 0.05$ (significant difference from 1)

TABLE 19. Contributions of quadriceps and hamstring muscles shown as a percentage of the total contribution (100 %) and hamstring-quadriceps ratio (H-Q-Ratio; calculated by dividing activity of hamstring muscles by activity of quadriceps muscle) in the incremental cycling test (ICT). Values are means ± SD and n = 14.

Load	**Quadriceps (%)**	**Hamstring (%)**	**H/Q-Ratio**
1	52.7 ± 7.7	47.3 ± 7.7	0.94 ± 0.28
2	54.4 ± 6.5	45.6 ± 6.5	0.86 ± 0.22
2nd last	56.7 ± 3.4*	43.3 ± 3.4*	0.77 ± 0.11*
Max	57.6 ± 4.1*	42.4 ± 4.1*	0.74 ± 0.13*

* $p < 0.05$ (significant difference from 1)

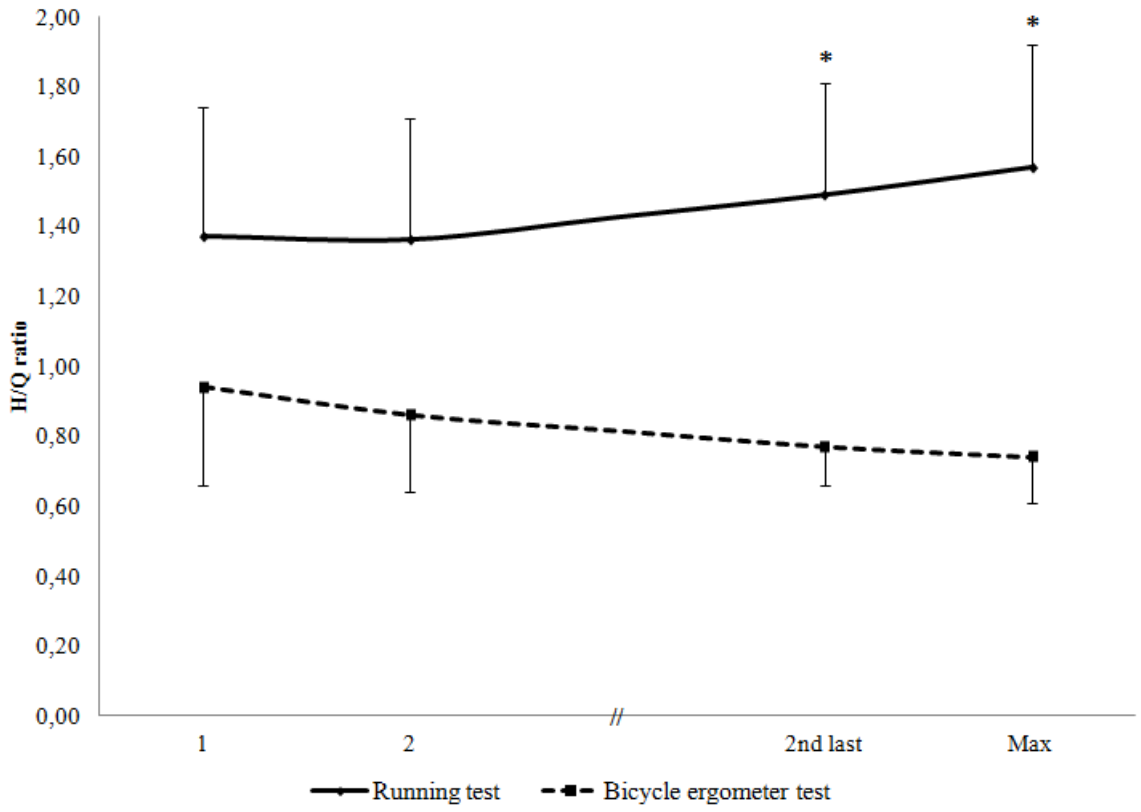

FIGURE 15. Comparison of H/Q-ratio in the incremental running test (IRT) and bicycle ergometer test (ICT). Values are means ± SD, n = 12 (Running test) and n = 14 (Bicycle ergometer test). * $p < 0.05$ significant difference between running and cycling.

7.4 Neuromuscular fatigue

In CMJ, changes in jump heights and muscle activities (CMJ_{PRE} versus CMJ_{POST}) varied a lot between individuals (Figure 16a and 16b). Approximately half of the subjects (n = 7) improved their performance while the rest of the subjects (n = 6) were not able to achieve the same jump height as before the IRT. However, there were clear trends in the muscle activities. Muscle activities were lower in CMJ_{POST} in all subjects, except one individual. On the average, jump heights changed -0.6 ± 2.3 cm and activities -141 ± 108 uV. Detailed results of the performances have already been shown in Table 8. There were not any associations between changes in CMJ, blood variables or the IRT, except a weak correlation between ΔCMJ and ΔpH ($r = 0.51$; $p = 0.051$).

Changes in muscle activities in half squat were also very variable (Figure 17b). It was a remarkable that all subjects (n = 16) were able to achieve the same result as $Squat_{PRE}$ and more than half (n = 9) even improved their performance (Figure 17a). For the entire group the changes in $Squat_{POST}$ were 6.3 ± 7.2 kg and -39 ± 161 uV. In the same way as in CMJ, there were no connections to other variables.

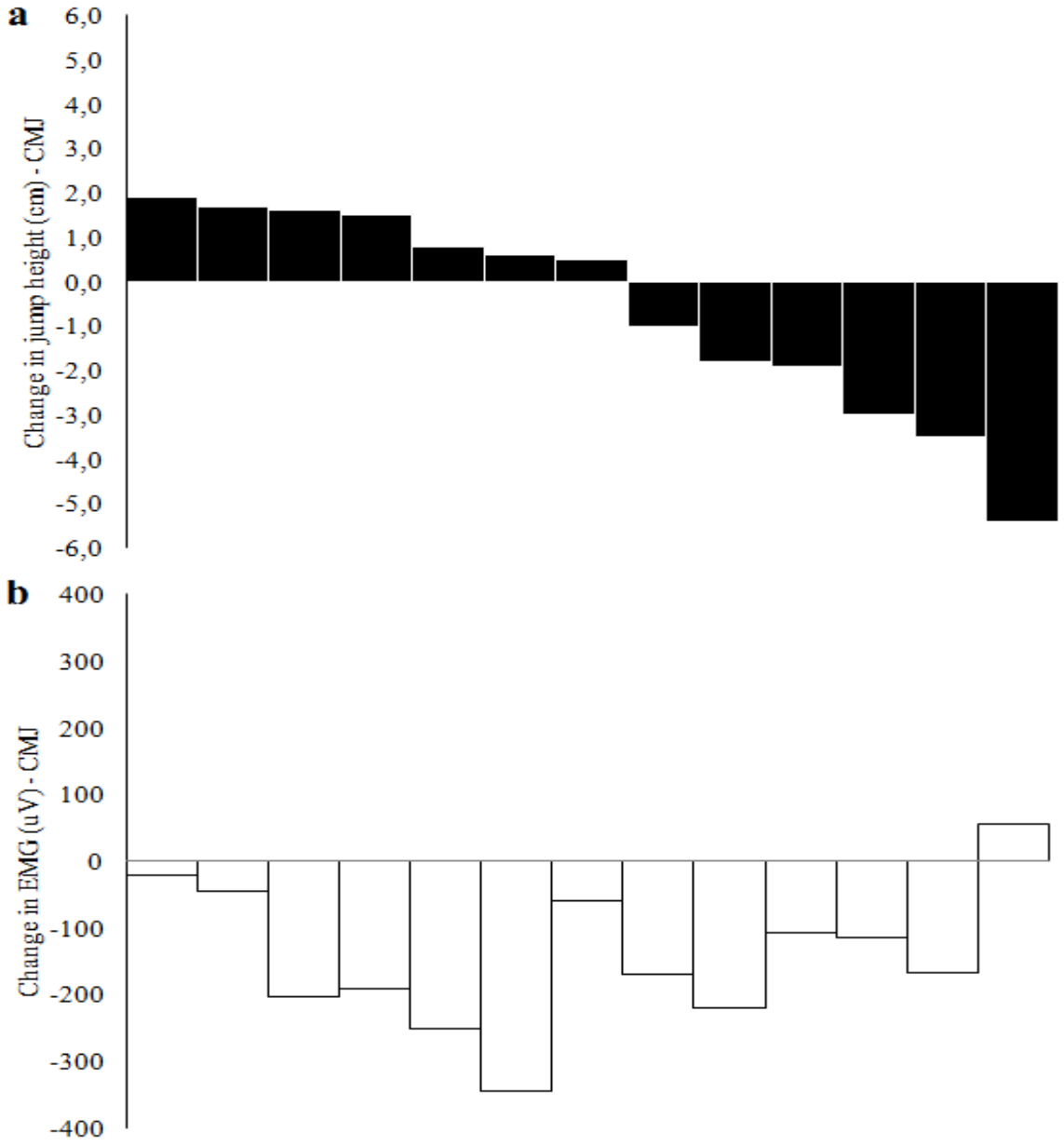

FIGURE 16. Changes in jump height (a) and muscle activity (b) in countermovement jump (CMJ) in response to the incremental running test (IRT). Bars represent individuals and the bars are in both graphs in the same order.

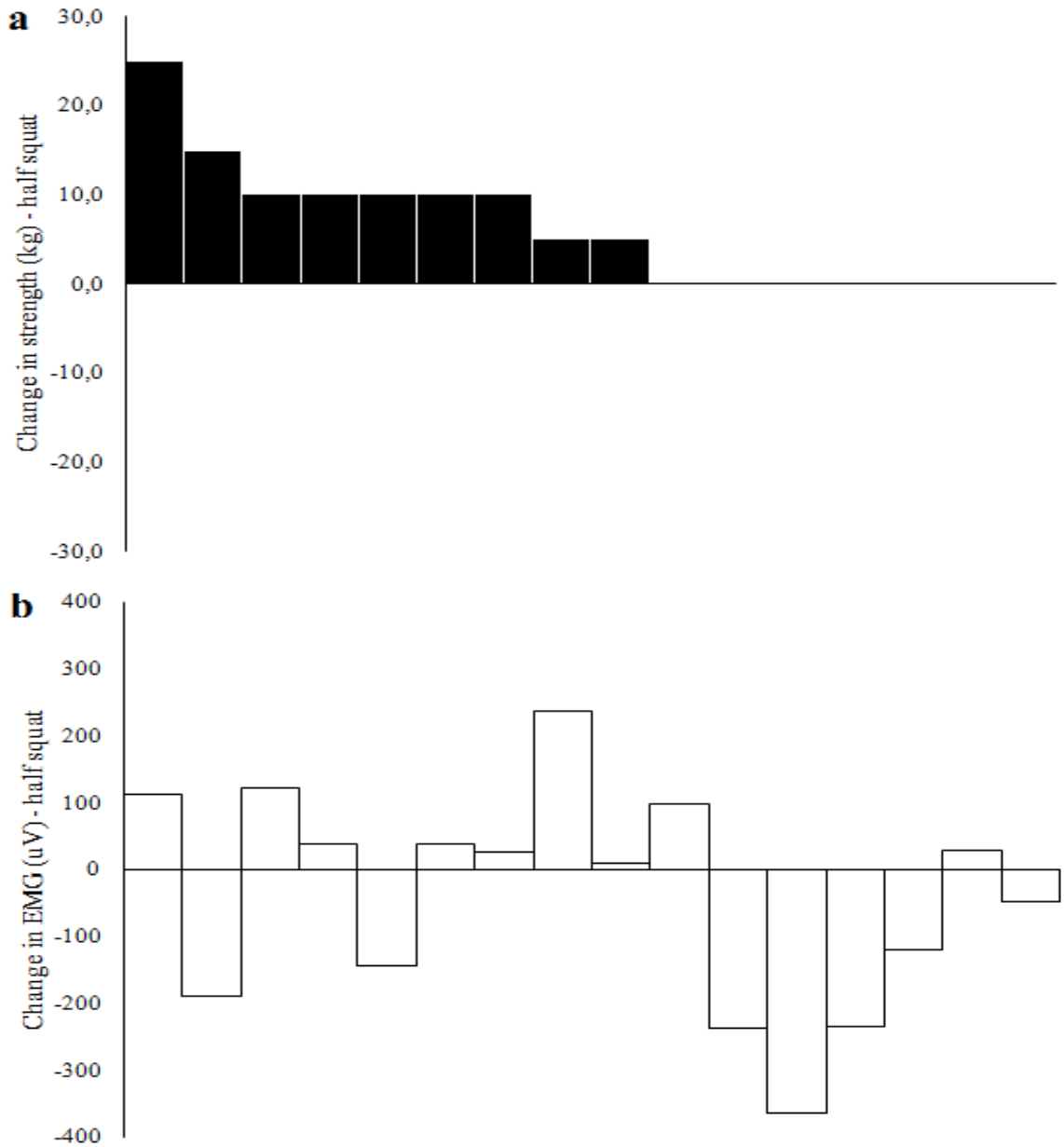

FIGURE 17. Changes in strength (a) and muscle activity (b) in half squat (CMJ) in response to the incremental running test (IRT). Bars represent individuals and the bars are in both graphs in the same order.

8 DISCUSSION

The main findings of this study were that increased activity of quadriceps and hamstring muscles during the IRT can be associated with decrease in blood pH and increase in blood lactate concentration. The results also indicate that rising muscle activity of thigh muscles during incremental running is achieved by increasing more the activity of hamstring muscles compared to quadriceps muscles. In incremental cycling, situation is reversed: the activity of quadriceps muscles increase more than the activity of hamstring muscles. In addition, the IRT induced a lot of acute physiological responses in the body, such as leukocytosis, increased endocrine activity and metabolic acidosis.

Complete blood count. The IRT caused significant increases in blood total leukocyte, neutrophil, lymphocyte and mixed cell (monophil, eosinophil and basophil) counts, which is in accordance with previous literature (Pedersen & Hoffman-Goetz 2000; Rowbottom & Green 2000). It is commonly known that exercise has a positive impact on health, but on the other hand, intensive exercise can also cause negative effects. Leukocytosis is a sign of infection or inflammatory reaction often used in clinical settings (Simpson 2013), and after the IRT all values were above normal clinical ranges. Because WBCs are an important part of the immune system and defense against infections, so for example in athletes, WBCs indirectly affect their training programs, because infections disturb exercise performance (Horn et al. 2010). Different studies have reported that many different types of exercises (mode, duration and intensity) can induce general leukocytosis. Intensity has been found to be more important in some studies (Robson et al. 1999), while others have emphasized the duration of exercise (Gimenez et al. 1986). In this study, the IRT was not very long-lasting, so it is likely that intensity of the IRT had a greater impact on WBC counts. We did not do post-exercise follow ups for WBC counts, but it has been observed that WBC counts return to baseline values within 6-24 hours (Simpson 2013). As expected, RBC count, blood Hb concentration and Hct increased both in men and women in response to the IRT. Hct increases during exercise mainly due to a fluid loss, a shift of plasma into the extracellular space and filtration (Mairbäurl 2013).

Hormones. It has been observed that concentration of cortisol rises significantly during a short endurance performance if intensity is more than 80 % of VO_{2max} (Viru et al. 1996) and the concentration of cortisol may remain elevated for two hours after cessation of exercise, possibly having an influence on tissue repair and recovery (McArdle et al. 2010, 417). But in this study, there were no significant increases immediately after the IRT. However, in women the concentrations of cortisol increased significantly 30 minutes after the IRT and 60 minutes was not enough to return it to the baseline values. Exactly the same behavior of cortisol was not observed in men 30 minutes after the IRT, because the increase was not statistically significant. In addition, there was even slight decrease 60 minutes after the IRT compared to the baseline values. The last loads of the IRT were performed at intensity over 80 % of VO_{2max}, and it affected in women's cortisol levels significantly, but in men, the increase was not significant. The increase has been suggested to be caused by a combination of hemoconcentration and HPA axis stimulus (Hill et al. 2008).

Concentrations of testosterone increase linearly in response to an exercise when a certain intensity threshold is reached and usually the highest concentrations are found at the end of exercise (Wilkerson et al. 1980). This is in line with the findings of this study, because the testosterone levels of men showed a significant increase (18.4 %) immediately after the IRT. The levels decreased to the baseline in 30 minutes and continued to fall below it, although the decrease was not statistically significant. In women, behavior of testosterone levels is somewhat different. It has been shown that the increase in testosterone levels after a bout of endurance exercise is linearly related to both intensity and duration (Consitt et al. 2002). Increases were also observed in the current study, but the highest concentrations were measured 60 minutes after the IRT. The increase was 89.2 % from the baseline. However, this acute increase is temporary and the levels should return to normal within hours (Consitt et al. 2002). SHBG levels of women were considerably higher compared to men, but there were high variations between individuals. Different studies have reported contradictory results related to an acute SHBG response to various modes of exercise (An et al. 2000). In this study, it was observed that in women the SHBG levels decreased significantly after exercise, while in men the levels stayed more stable. 60 minutes after the IRT, T/C-ratio was decreased in men and increased in women. As is known, testosterone level is a marker of body's anabolic status and cortisol level reflects the catabolic state, but it must be taken into account that the relative change in women was very small.

Acid-base balance. Changes in blood pH, HCO_3^- concentration and pCO_2 indicated that the IRT induced both metabolic and respiratory acidosis. Both in men and women, ph decreased significantly during the IRT, but returned to baseline within 30 minutes. However, increase in HCO_3^- concentration was more notable in women compared to men and took longer to return it to the baseline values. Decrease in pCO_2 was also significant both in men and women, but men were able to recover more quickly. HCO_3^- decreasing below 22.0 mmol/l is a sign of metabolic acidosis (Kellum 2000). Concentrations of HCO_3^- after the IRT were 13.77 ± 1.89 mmol/l and 13.59 ± 2.18 mmol/l in men and women, respectively, so metabolic acidosis was substantial. Another determinant of metabolic acidosis is changed BE. BE in extracellular fluid after the IRT were -13.91 ± 2.43 mmol/l in men and -13.79 ± 2.62 mmo/l in women. These values clearly exceed the limit of metabolic acidosis, which is < -5 mmol/l (Kellum 2000). Still, the results showed that recovery of acid-base balance was quite quick (30 – 60 minutes), and occurs mainly via bicarbonate and respiratory removal of excess CO_2 (Kenney et al. 2012, 200–202). Blood pH decreased or in other words, concentration of H+ increased, significantly, but it has been said that 0.2 units decrease in blood and muscle pH probably have no effect on muscle glycogenolysis, glycolysis and pyruvate oxidation during exercise (Bangsbo 1996). In this study, men were more able to restore the body's homeostasis than women, so this could be beneficial if high intensity exercises with short recovery time would be made several times on the same day.

Muscle activity in running and cycling. Muscle activity during the first, second, second last and maximal loads were selected for analysis because the IRT and ICT were performed until exhaustion. This allowed the comparison of different subjects, regardless of the number of loads completed. The IRT and ICT were not fully comparable due to the fact that the power of the initial loads was not matched together. However, the last loads in both tests were performed maximally, so it gives comparable information about the contributions of the activity of hamstring and quadriceps muscles during maximal effort in different performances. At maximal speed in the IRT, the hamstring muscles were dominant. Already in the first load the activity of the hamstring muscles (56.8 ± 6.2 %) was 13.6 percentage points higher than the activity of the quadriceps muscles (43.2 ± 6.2 %). As the speed increased, the difference increased more, reaching 20.6 percentage points at the maximal load. The activity of the hamstring muscles was 60.3 % ± 5.9 of total activity and on this basis it can be said that during the maximal running speed a remarkable larger proportion of the

thighs power is produced by the hamstring muscles. The increase in the proportion of hamstring muscles activity also indicates that increase in total activity is achieved by increasing more the activity of the hamstring muscles rather than the quadriceps muscles. In the IRT, the activity of the hamstring muscles increased 92.1 % from the first load to the maximal load, while the activity of the quadriceps muscles increased only 65.2 %. One factor which can possibly affect these results is stride rate and length during running, as it is well known that running velocity is the product of these two. Increasing step rate by 110 % for a certain running speed (2.4 – 3.8 m/s) has been found to lead to an increase in hip flexor, hamstring and hip extensor loading in the swing phase, while peak force and work of several hip muscles during stance phase, such as the gluteal muscles and piriformis, reduced (Lenhart et al. 2014). However, those results can be generalized only to the above-mentioned speeds, and in the present study the maximal running speed during the IRT varied from 3.9 m/s to 5.4 m/s. Still, Nummela et al. (2007) reported that in well-trained endurance athletes speed increases during running were achieved by increasing both stride lengths and frequencies at the speeds below 7 m/s.

In the ICT, the activity of quadriceps muscles was higher than the activity of hamstring muscles at every load. The difference in contribution of total activity was 5.4 % in the first load and it increased to 15.2 % in the maximal load. At maximal load, the activity of the quadriceps muscles was 57.6 ± 4.1 %, so the contribution was almost identically opposite that of the IRT. The subjects were not allowed to stand up while cycling, so the cycling technique should not have influenced on the results. The contributions of hamstring and quadriceps muscles during exercise can be also expressed as H/Q ratio. During the maximal load in the IRT, H/Q ratio was 1.57 ± 0.35 when during the ICT it was 0.74 ± 0.13. This indicates that the maximal running and cycling performances are very different with regard to the muscular activity of thigh muscles. On the other hand, it is possible that special cycling shoes and pedals could have affected the cycling performance in such a way that the activity of hamstring muscles would have increased more. Thus, the muscle activity in cycling performance would have been more similar to the running performance. This of course requires that the cyclist is accustomed to using these pedals. In this study, H/Q strength ratio was not investigated, but Sundby & Gorelick (2014) reported that the functional H/Q strength ratio of highly trained female runners was higher compared to recreational runners, despite the lower leg strength, and − at least at some level − endurance running performance may be

related to greater hamstring muscle strength relative to quadriceps muscle strength and not to absolute muscle strength.

Other big difference between running and cycling is that delta (Δ) efficiency (the ratio of an increment in the external mechanical power output to the increase in metabolic power required to produce it) is significantly higher during running (45.5 %) than that during cycling (25.7 %) (Bijker et al. 2001). Later, Bijker et al. (2002) showed that a observed difference between running and cycling in the relationship between the mean EMG activity of leg muscles and the load applied can be used to explain the difference in Δ efficiency. They also suggested that a change in the relative contributions of concentric and eccentric muscle actions, combined with the large difference in metabolic cost between both muscle actions can explain the high Δ efficiency during running.

Associations between the muscle activity and acid-base balance. The most interesting findings of this study were that the increase in the hamstring and quadriceps muscles activity correlated well with the decrease in blood pH. Low pH (acidosis) can cause many contractile, metabolic and other cellular processes that may impair a performance (Cairns 2006). Intramuscular acidosis may affect the myofilament function by a decreased maximum cross-bridge cycling, Ca^{2+} binding to troponin and myosin ATPase activity leading to weakened maximum force, Ca^{2+} sensitivity and maximum velocity of myofilament shortening. Excitation-contraction coupling can be disturbed because of decreased Ca^{2+} release channel activity, charge movement and calcium ATPase activity. This may occur in a decreased Ca^{2+} release and uptake by sarcoplasmic reticulum. Also free energy from ATP hydrolysis may be reduced. Other processes which can be impaired are rate of glycolysis and glycogenolysis due to a decreased PFK and glycogen phosphorylase activities, decreased rate of cAMP production and increased ATP-dependent K^+ channel conductance. This study did not provide evidences that the decreased pH would have affected performance. As said previously, the decrease in pH was only approximately 0.2 units, which probably would have no impact on muscle glycogenolysis, glycolysis and pyruvate oxidation during exercise (Bangsbo 1996). The IRT affected on the concentrations of blood Na^+ and Ca^{2+}, so it seems that there were some disturbances in Na^+ and Ca^{2+} activity. In addition, it has been presented that accumulation of interstitial potassium in muscle may be linked to the process of fatigue

(Bangsbo et al. 1996). The concentration of K^+ in blood did not change significantly during the IRT, but a small decline was observed. So this theory may not be rejected on the basis of this study. Higher intensity and shorter durations are needed for bigger changes in acid-base balance (e.g. Medbø & Sejersted 1986), but this study focused on endurance performance. However, the results indicated that in endurance performance bigger changes in muscle activity is needed for decreased pH. This is not necessarily a good thing during a competition, but because of one purpose of the training is to disturb the body's homeostasis, it may be useful to monitor muscle activities during the exercises.

Also the increase in blood lactate concentration correlated very well with the increase in the activity of hamstring and quadriceps muscles. It can be speculated that those subjects who were able to produce more lactate are more capable of increasing muscle activity, although there were no associations between the change in lactate concentration and performance. Even though, the view that the lactate production causes metabolic acidosis and increased lactate production (lactic acidosis) is one of the many causes of muscle fatigue induced by exercise has been questioned (Robergs et al. 2004), the results of this study support the statement that increased production of lactate is a good indirect marker for metabolic conditions of cell that induce metabolic acidosis.

Neuromuscular fatigue. Neuromuscular fatigue induced by the IRT was determined with participants performing CMJ and half squat both before and after the IRT. In this study, CMJs can be considered as preferred indicator of fatigue, because CMJ_{POST} was performed immediately after (under 30 seconds) a cessation of the IRT. Also knee angle during jump was more easily controlled compared to half squat. The average change in heights of the jumps was negligible (-0.6 ± 2.3), but the subjects seemed to divide into two groups, one of which the jump heights improved, while in the other jump heights weakened. Conversely, muscle activities were lower in CMJ_{POST} in all subjects, except one individual. This indicates that the IRT induced neuromuscular fatigue and in some subjects it manifested as weakened performance in CMJ. This difference could be due to, for example, subjects' different kinds of sports background. Vuorimaa et al. (2006) compared long-distance and middle-distance male runners in their study, and reported that intensive 20 - 40 minutes running exercise can lead to an acute improvement in the vertical power performance in long-distance runners, although

the muscle activity of the leg extensor muscles decreases. They suspected that the use of a different coordination strategy counteracts strength loss and even enhances power of legs. In the $Squat_{POST}$, all subjects were able to achieve the same result as $Squat_{PRE}$ and more than half even improved their performance. These results do not necessarily reflect the fatigue induced by the IRT, because time between a cessation of the IRT and half squat performance was delayed several times more than 10 minutes due to a many reasons. Also it remained unclear could the subjects carry out their maximum performance before the IRT, as they were not fully familiar with the half squat in the smith machine. Change in the muscle activity varied a lot between individuals, and it is difficult to make larger conclusions about those.

Limitations of the study. The biggest limitation of this study was that we were not able to investigate the effects of training, i.e. training adaptations. The number of subjects was quite small at the beginning of the study and various reasons reduced the number of subjects who completed the study. With this amount of subjects it was not possible to obtain reliable results about the training adaptations. It was hypothesized that current training methods would have affected more RE than VO_{2max}, and would increase a tolerance of decreased blood pH and muscle fatigue during the IRT. Another limitation was related to the EMG shorts, as in a few performances (in the IRT) parts of the data contained clear errors. We suspect that those errors are mainly due to a deficient contact between the electrodes and the skin. The electrodes were moistened with water, but apparently the electrodes were not wet enough when the actual performance began. This enabled them to move against the skin, which resulted in errors in the data in the beginning of the IRT. In addition, as mentioned before, the half squat performance in smith machine was not the best solution to measure maximum strength. A better option would have been the measurement of isometric MVC of the leg extensor muscles, but it was not possible due to practical reasons. Furthermore, it would have been informative to measure maximum strength of hamstring muscles, but it was also not possible because of a lack of equipment. This would have allowed the calculations of functional and conventional H/Q strength ratios.

Conclusions. The present study showed that increased electrical activity of hamstring and quadriceps muscles correlated well with changes in acid-base balance in recreational active persons during incremental running test. It seems that a greater increase in muscle activity is

needed for bigger changes in blood pH and BE, and EMG shorts are a useful method for determining this change. The results indicated also that increased total activity of thigh muscles is achieved mainly by increasing the activity of hamstring muscles during running. Activation of hamstring and quadriceps muscles during maximal running and cycling were significantly different, so this information can be beneficial for those who use both type of exercises in their training programs, such as triathletes. However, the use of special cycling shoes and pedals could have affected the activation of hamstring muscles during the ICT. With EMG shorts it is possible to determine muscle activity distributions between hamstring and quadriceps muscles, and thereby optimize the activation of these muscles during different types of exercise. In addition, an exercise with incremental speed until exhaustion is an effective method to stimulate the body's homeostasis, as the IRT induced significant leukocytosis and acidosis.

Practical applications. Experiences of the present study showed that the EMG shorts are easy and practical tool for measuring activity of hamstring and quadriceps muscles during running and cycling. Information obtained by this method can be beneficial for both recreational active persons and athletes, as well as for their coaches. It looks like that greater increases in the activity of hamstring muscles are needed compared with activity of quadriceps muscles when approaching the maximum speed during running. So, it can be useful to determine individual muscle activation patterns in order to make locomotion more efficient. For example, if it is found that a person's hamstring muscles activity is low during exercise, training can be targeted to develop hamstring muscles strength.

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