JYU DISSERTATIONS 66

Maria Küüsmaa-Schildt

Combined Strength and Endurance Training

Effect of Training Order and Time-of-Day on Adaptations in Neuromuscular and Cardiorespiratory Performance, Muscle Hypertrophy, Serum Hormone Concentrations and Wellbeing

UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF SPORT AND HEALTH SCIENCES

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ABSTRACT

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The aim of this study was to assess the daily fluctuations in strength and endurance performance and to examine chronic adaptations to time-of-day-specific same-session combined strength (S) and endurance (E) training over the 24-week training period in previously untrained men. Participants were assigned to the morning (m) or evening (e) E+S or S+E group (mE+S n=9; mS+E n= 9; eE+S n=12, eS+E n=12) and trained 2-3 $d{\cdot}wk^{-1}$ in the respective time-of-day and training order. In the total group of participants, isometric strength and maximal cycling performance were, respectively, 4.5% and 1.5% higher in the evening compared to the morning. Based on the time-of-day of peak isometric strength performance, high morning performance (n=8), high evening performance (n=19) and neutral types (n=45) were identified. This grouping was valid also for endurance performance. Strength and endurance performance increased in the morning and evening in all training groups over the 24-week training period. Isometric force and EMG activity increased more in S+E compared to E+S, when measured in the evening. Maximal cycling performance increased more in E+S compared to S+E. Muscle hypertrophy was similar between the groups after the first 12 weeks, but evening training led to a larger increase over the weeks 13-24. Serum cortisol and testosterone showed a typical diurnal variation and no changes were observed after 24 weeks of time-of-day-specific training. Motivation to participate to the present training program showed a tendency to decrease after prolonged training in the morning hours. Evening training, however, led to worsened perception of time management. These results suggest that strength as well as endurance performance fluctuates over the day, however, the time for peak performance varies between individuals. In adaptations to combined training, the E+S order may potentially inhibit neural adaptations and thereby hinder adaptations in isometric strength performance, whereas in the S+E order the fatigue from the strength session may inhibit adaptations in endurance performance. Therefore, if combined training is performed over prolonged periods of time, training time and order should be selected based on the personal training goals.

Keywords: concurrent training, testosterone, cortisol, diurnal rhythms, HRQoL, motivation, time-management

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TIIVISTELMÄ

Küüsmaa-Schildt, Maria
Yhdistetty voima- ja kestävyysharjoittelu: harjoittelu järjestyksen ja vuorokaudenajan pitkäaikaisvaikutukset hermolihasjärjestelmään, sydän- ja verenkiertoelimistöön, lihaskasvuun, hormonipitoisuuksiin ja koettuun hyvinvointiin
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Tämän väitöskirjan tarkoituksena oli tutkia sekä voima- ja kestävyyssuorituskyvyn päivittäistä vaihtelua että kroonisia adaptaatioita yhdistettyyn voima- (V) ja kestävyysharjoitteluun (K). Osallistujat jaettiin neljään interventioryhmään, jotka harjoittelivat 24 viikon aikana aamulla tai illalla niin, että saman harjoituskerran aikana kestävyysharjoittelu suoritettiin ennen voimaharjoittelua (K+V) tai päinvastaisessa järjestyksessä (V+K). Tutkittavat harjoittelivat 2-3 kertaa viikossa sinä vuorokauden aikana ja siinä voima- ja kestävyysharjoituksen järjestyksessä, joka heille oli tutkimuksen alussa määritelty. Koko tutkimusryhmässä isometrinen voima ja maksimaalinen kestävyyssuorituskyky olivat illalla ~4.5% ja ~1.5% paremmat kuin aamulla, mutta yksilöiden välillä esiintyi suurta vaihtelua. Voima- ja kestävyyssuorituskyky paranivat sekä aamulla että illalla kaikissa harjoitteluryhmissä 24 viikon aikana. Isometrinen voima ja EMG-aktiivisuus lisääntyivät enemmän V+K ryhmässä verrattuna K+V ryhmään. Maksimaalinen kestävyyssuorituskyky parani puolestaan enemmän K+V ryhmässä verrattuna päinvastaiseen harjoittelujärjestykseen. Iltaharjoittelu johti suurempaan ulomman reisilihaksen kasvuun viikoilla 13-24. Seerumin kortisolin ja testosteronin pitoisuudet osoittivat tyypillistä vuorokausivaihtelua, eikä muutoksia havaittu harjoittelujakson jälkeen. Motivaatio osallistua kyseiseen harjoitteluun näytti hieman laskevan aamuharjoittelijoilla. Iltaharjoittelu puolestaan johti ajankäytön hallinnan heikentymiseen. Nämä tutkimustulokset viittaavat siihen, että voima- ja kestävyyssuorituskyky vaihtelevat vuorokauden ajan myötä, mutta yksilöiden välillä oli suuria eroja. Yhdistetyn voima- ja kestävyysharjoittelun adaptaatiot osoittivat, että kestävyysharjoitus ennen voimaharjoitusta voi mahdollisesti heikentää hermolihasjärjestelmässä tapahtuvaa adaptaatiota, mikä näkyy maksimivoimassa. Voimaharjoittelu ennen kestävyyttä voi puolestaan aiheuttaa akuuttia väsymystä ja heikentää kestävyysominaisuuksien kehittymistä. Näin ollen, jos yhdistettyä voima- ja kestävyysharjoittelua suoritetaan pitkällä aikajaksolla, harjoituksen vuorokauden aika ja järjestys on valittava kulloistenkin tavoitteiden perusteella.

Asiasanat: testosteroni, kortisoli, vuorokausirytmit, elämänlaatu, motivaatio, ajanhallinta

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This thesis has not only been read and evaluated by both of my supervisors but I would like to acknowledge the two leading experts in the field of exercise science, who have put time and effort by giving me their valuable comments, which helped to improve the scientific quality of this dissertation – thank you for reviewing this thesis prof. Eduardo Lusa Cadore and prof. Michael R. Deschenes. In addition, honored opponent prof. Glyn Howatson, thank you for agreeing to critically review this thesis and act as an opponent in the public defense. I would also like to thank all the co-authors. Thank you for sharing the knowledge and experience.

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Jyväskylä, February 2019
Maria Küüsmaa-Schildt

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following original articles, which are referred to in the text by their Roman numerals:

I Küüsmaa M., Sedliak M., Häkkinen K. 2015. "Effects of time-of-day on neuromuscular function in untrained men: Specific responses of high morning performers and high evening performers". Chronobiology International 32(85): 1115-24.

II Küüsmaa M., Schumann M., Sedliak M., Kraemer W.J., Newton R.U., Malinen J-P., Nyman K., Häkkinen A., Häkkinen K. 2016. "Effects of morning versus evening combined strength and endurance training on physical performance, muscle hypertrophy, and serum hormone concentrations". Applied Physiology Nutrition Metabolism 41(12): 1285-1294.

III Küüsmaa-Schildt M., Eklund D., Avela J., Rytkönen T., Newton R.U., Izquierdo M., Häkkinen K. 2017. "Neuromuscular adaptations to combined strength and endurance training: order and time-of-day". International Journal of Sports Medicine 38(9): 707-716.

IV Küüsmaa-Schildt M., Liukkonen J., Vuong M.K., Nyman K., Häkkinen K., Häkkinen A. 2019. "Effects of morning vs. evening combined strength and endurance training on physical performance, sleep and well-being". Submitted for publication.

Additionally, some previously unpublished results are included in this thesis.

CONTENTS

1 INTRODUCTION

Since the beginning of mankind, human history has been shaped by the changes in the light and darkness from the cyclic interaction of the Sun, Moon and Earth. Evolution has adjusted human beings anatomically, physiologically and behaviorally to respond this cyclic environment (Reilly et al. 1997, p. 1). Although in the modern world the use of artificial lighting and darkened bedrooms has given civilized societies considerable independence from natural daylight, human beings are still considered as diurnal creatures – active in the daytime, asleep at night (Reilly et al. 1997, p. 1). It is because biological rhythms are not only produced by the solar clock but also by the social clock from the surrounding environment and by the internal biological clocks from different tissues in human body (Roenneberg et al. 2003). The field of biology that examines periodic (cyclic) phenomena in living organisms and their adaptations to solar- and lunar-related rhythms is called chronobiology (lat. *chrono* = *time*). Out of several different biological rhythms (e.g. annual, monthly, infradian, ultradian), circadian rhythms have a period of "about a day" (lat. *circa* = about and *dies* = a day) and are synchronized to the external 24-hour environment (Roenneberg et al. 2007). Almost all physiological, biochemical and behavioral variables in human beings are circadian rhythmic, including also physical performance (Teo et al. 2011a). Therefore, circadian rhythms have been associated with our functioning and health, whereas the violation of these rhythms may lead to various functional and structural health issues and to a decrease in physical and mental performance (Jančoková et al. 2013, p. 9). The fact that various factors influencing physical performance are peaking at certain time-of-day have led to the assumption that adaptations to training might be time-of-day-specific, however this topic has received limited scientific attention.

In general, regular exercise training has known to have widespread health benefits by positively affecting nearly all organ systems of the body (Gabriel & Zierath 2017). Contemporary scientific research supports this idea by demonstrating that physical exercise can play a significant role in prevention of certain physiological as well as psychological conditions (Garber et al. 2011). At the same time, over the past decades the physical fitness among young Finnish men has decreased and body mass increased (Santtila et al. 2006). In order to counteract a decline in physical fitness, global exercise recommendations suggest participating in endurance as well as strength training. Adaptations to both training modes not only contribute toward potential sporting excellence but, in most instances, contribute toward the delayed onset of age-related diseases (Cartee et al. 2016; Hughes et al. 2018). When both strength and endurance training are simultaneously incorporated into a training regimen, it is called combined or concurrent training. Despite the potential additive benefits when concurrently performing these divergent exercise modes, some research suggests that this approach may lead to attenuated training adaptations (Fyfe et al. 2014). In 1980, Dr. Robert Hickson was the first to examine the effects of combined strength and endurance exercise, when combined into the same training program. He reported that concurrent training with high intensity and frequency interfered with leg strength improvements relative to resistance exercise alone but yielded similar improvements in cardiorespiratory fitness as endurance exercise alone (Hickson 1980). Since then, many research groups have committed to examine how factors such as exercise mode and intensity, muscle groups trained, subject characteristics etc. influence the outcome of combined strength and endurance training. Additionally, the impact of training session sequence on chronic training adaptations, strength training performed prior to endurance training or endurance training prior to strength training, has not found a complete consensus (Murlasits et al. 2018).

Therefore, the aim of the present dissertation was to investigate the effects of prolonged same session combined strength and endurance training with different exercise sequence (strength before endurance or vice versa) on neuromuscular, cardiorespiratory and hormonal adaptations in previously untrained men after a prolonged time-of-day-specific combined training period. In addition, the diurnal fluctuations in strength and endurance performance together with the effects of time-of-day-specific (morning vs. evening) combined training on adaptations in above mentioned training-related parameters as well as sleep, well-being and motivation were in the focus of this doctoral thesis.

2 REVIEW OF THE LITERATURE

2.1 Effects of exercise training on physical fitness

2.1.1 General responses and adaptations to exercise training

Human beings live in a 24-hour world, where the alteration of day and night regulate the sleep/awake cycle. During the daytime, physical activity, which is defined as any bodily movement that is produced by skeletal muscles and results in energy expenditure, is tightly incorporated into everyday life through occupational, sports, conditioning, household, or other activities (Caspersen et al. 1985). The amount of physical activity in a day, however, is largely subject to personal choice and may vary considerably (Caspersen et al. 1985). Exercise, however, is a subset of physical activity that is planned, structured, repetitive and has a purpose (Caspersen et al. 1985). Exercise elicits responses that are specific to the mode of activity performed (e.g. resistance vs. endurance exercise), although some degree of crossover in these mode-specific adaptations exists (Fyfe & Loenneke 2018). The initial responses are likely to occur after each training session and the cumulative effect of repeated bouts of exercise will lead to chronic adaptations (Widegren et al. 2001). The magnitude of these changes depends largely on the intensity and duration of the training sessions, the force or load used in training, and the body's initial level of fitness (Hawley 2009; Manley 1996, p. 61). Regular training for strength and/or endurance has been associated with increased functional abilities and numerous physical and mental health benefits (Garber et al. 2011).

2.1.2 Acute responses and chronic adaptations specific to strength training

Resistance training contains low-repetition performance with near maximal muscular contractions, which has shown to increase maximal contractile force (Gergley 2009). Neural and hypertrophic adaptations have been reported as main factors responsible for improved strength performance (Häkkinen et al.

1985; Deschenes & Kraemer 2002; Aagaard 2003), however, also adaptations in other morphological, biomechanical and metabolic factors influence the strength development (Folland & Williams 2007). The muscles which are exercised will experience adaptive changes, whereas non-exercised muscles will experience little or no training effect (Bottinelli et al. 1999). The magnitude of hypertrophy or strength improvements depends on the volume, intensity and type of the training stimulus (Docherty & Sporer 2000; Hawley 2002) (TABLE 1). Higher loads and lower volume have been associated with enhanced motor recruitment and neural potentiation, while higher volumes and lower loads have been associated with muscular hypertrophy (Häkkinen 2002, p. 21). Nevertheless, adaptations in the neuromuscular system after strength training, not only support improvements in athletic performance, but also improve health-related musculoskeletal function and offset loss of muscle mass and strength (Kraemer et al. 2002).

TABLE 1 Volume, intensity and type of the training stimulus (Plisk & Stone 2003; Häkkinen 2002, p. 21).

Training goal	Mechanisms	Intensity (% of 1RM)	Action speed	Repetitions per set	Rest between sets
Power	Neural	30-60%	Explosive/ maximal	8-10	2-8 min
Maximal strength	↓	80-100%	Slow to explosive	1-3	<8 min
Hypertrophy	↓	60-80%	Slow to explosive	6-12	1-4 min
Strength endurance	Metabolic	30-40%	Brisk/ continuous	20-30	<5 min

1 RM = one repetition maximum

Neuromuscular adaptations

As mentioned above, neural factors play an important role in muscle strength gains, especially in the early stages of strength training (< 8 weeks), when the disproportionately larger increase in muscle strength occur without noticeable hypertrophy in trained muscles (Häkkinen & Komi 1983; Moritani & deVries 1979; Deschenes & Kraemer 2002) (FIGURE 1). It has been proposed that enhanced motor unit activation, which results from a greater number of fibres being recruited, increased firing frequency, decreased co-contraction of agonists, better synchrony of high-threshold motor units and inhibition reflexive mechanisms (e.g. Golgi tendon organ) that normally govern the amount of force that can be generated will lead to increased strength performance (Crewther et al. 2011; Folland & Williams 2007; Sale 1992, p. 281-283).

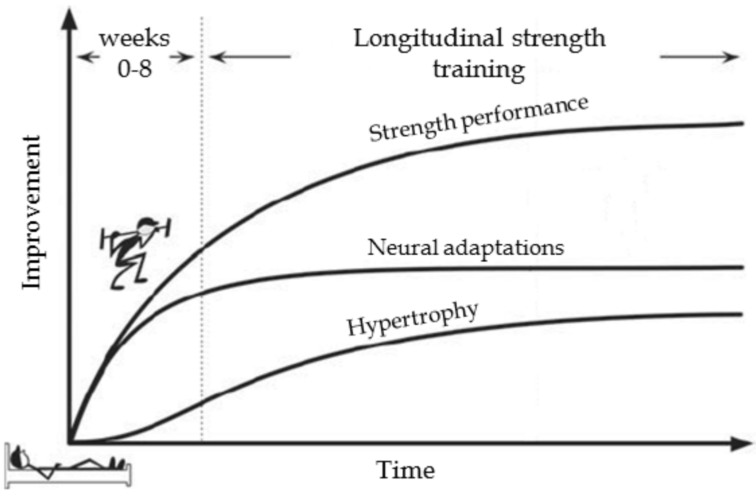

FIGURE 1 Time course of neural and hypertrophic adaptions after regular strength training. Modified from Hughes et al. (2018).

Changes in neuromuscular activity have been assessed by surface electromyographic activity (EMG) (Häkkinen & Komi 1983) or by calculating the voluntary activation level during maximum isometric contraction (Harridge et al. 1999). Increased surface EMG amplitude has been observed already during the initial weeks of resistance training in healthy young subjects (Häkkinen & Komi 1983) and has been interpreted as an increase in neural drive denoting the magnitude of the efferent neural output from the central nervous system to active muscle fibres (Gabriel et al. 2006; Folland & Williams 2007). Interpretations of neural adaptation based solely on EMG amplitude should be made with caution due to methodological constraints and confounding factors (Farina et al. 2014). Nevertheless, studies have shown that untrained individuals cannot fully activate their muscle during maximum voluntary contractions, even when fully motivated (Knight & Kamen 2001; Dudley et al. 1990). Strength training has been suggested to potentially increase the activation level in young as well as older subjects (Knight & Kamen 2001), although not all agree (Ramsay et al. 1990; Harridge et al. 1999).

Adaptations in skeletal muscle

Hypertrophic adaptations occur at slower rates compared to the neuromuscular adaptations since the rate of muscle protein synthesis must exceed degradation for an extended period before accretion of contractile protein occurs (Deschenes & Kraemer 2002). Intracellular regulation of the muscle protein synthesis responses to exercise is mediated through complex mechanisms, however mechanistic target of rapamycin complex 1 (mTORC1) modulation of the phosphorylation states of two downstream target substrates, $p70^{S6K1}$ and 2E-BP1 has been proposed as one possible pathway (Drummond et al. 2009). In addition, it has been proposed that hypertrophy occurs also through the activation of local satellite cells by IGF-I, causing them to become mitotically active and add new nuclei that lead to the synthesis of additional contractile proteins of the myofiber

(Deschenes & Kraemer 2002). However, strength training is primarily anaerobic and results in increased muscle glycolytic enzyme activity and intramuscular adenosine triphosphate (ATP)/phosphocreatine stores, which along with hypertrophy of muscle fibres have been suggested to lead to a possible reduction of muscle mitochondrial and capillary density (Tanaka & Swensen 1998; Costill et al. 1979), however, not all agree (Hellsten & Nyberg 2015).

Cardiorespiratory adaptation

Usually it has been found that changes in maximal oxygen consumption (VO_2max) and capacity to generate ATP via oxidative metabolism are minimal or nonexistent after resistance training (Gergley 2009; Tanaka & Swensen 1998; Glowacki et al. 2004). It has even been proposed that resistance training induced muscle hypertrophy could hinder improvements in aerobic performance (Nelson et al. 1990) through decreases in mitochondrial volume in type IIa fibers (McDonagh & Davies 1984). On the other hand, circuit weight training using lighter resistances, a higher number of repetitions per set, and shorter rest periods may lead to improved endurance performance and increases in VO_2max (Chromiak & Mulvaney 1990). In addition, strength training has been shown to decrease the proportion of the maximal pedal force required during cycling and alter the pattern of fibre recruitment (Sunde et al. 2010). This in turn will lead to improved movement economy, delayed fatigue, improved anaerobic capacity, and enhanced maximal speed (Rønnestad & Mujika 2014). Therefore, stronger individuals can perform aerobic activity at a lower percentage of their relative strength and use fibres with a more oxidative metabolism and more resistance to fatigue (Rønnestad et al. 2010; Mikkola et al. 2007).

2.1.3 Acute responses and chronic adaptations to endurance training

Typically, endurance training has been described to involve dynamic submaximal muscular contractions of large muscle groups, which are performed against a relatively low load over a prolonged period of time (Zory et al. 2010; Hughes et al. 2018). Classically, endurance training is known as moderate intensity continuous training, however, high intensity interval training (HIIT) has gained some popularity. HIIT involves repeated bouts of activity close to maximal intensity (≥80%), interspersed by periods of rest or low intensity exercise (Gibala et al. 2012; MacInnis & Gibala 2017). Interval training can possibly serve as a time-effective alternate to traditional endurance training, inducing similar or even superior changes in exercise performance and cardiovascular system (MacInnis & Gibala 2017; Gillen & Gibala 2014). Nevertheless, adaptations in aerobic capacity after both HIIT and moderate intensity continuous training are achieved through a complex set of central (cardiorespiratory system, e.g. improved VO_2max) and peripheral adaptations in muscular level (e.g. increased ability of skeletal muscle to generate energy via oxidative metabolism) (Chromiak & Mulvaney 1990; Nader 2006). All these mechanisms together support increases in cardiorespiratory fitness and endurance performance capacity (Hughes et al. 2018).

Cardiorespiratory adaptations

The functionally most important adaptation in the cardiorespiratory system is the improvement in maximal cardiac output, which is the result of an enlargement in cardiac dimension, improved contractility, and an increase in blood volume (Hellsten & Nyberg 2015). This all allows greater filling of the ventricles and consequently a larger stroke volume (Hellsten & Nyberg 2015). Enhanced cardiac output, in turn, allows improvements in VO_2max (Holloszy & Coyle 1984) (FIGURE 2).

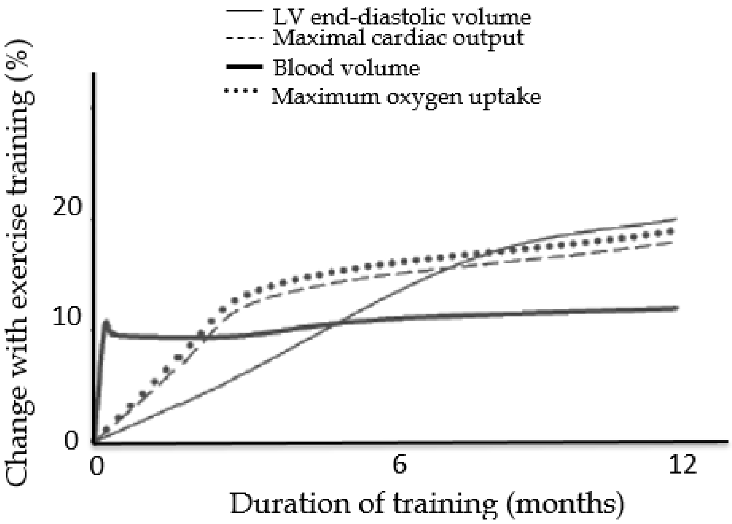

FIGURE 2 Time course of relative cardiovascular changes in response to endurance training. Modified from Hellsten and Nymerg (2015).

Adaptations in skeletal muscle

The local adaptations in skeletal muscle after endurance training, such as increased mitochondrial number and size, improved capillary density, muscle blood flow and activity of oxidative enzymes, has been shown to greatly enhance the oxidative capacity of the endurance-trained muscle (Joyner & Coyle 2008; Manley 1996, p. 69). Endurance training has shown to increase the capacity to synthesis adenosine triphosphate (ATP) aerobically through increased expression of genes that regulates mitochondrial biogenesis and angiogenesis (Egan and Zierath 2013). Metabolic and mechanical stress induced by endurance exercise in skeletal muscle has shown to modulate different signaling pathways including adenosine monophosphate-activated protein kinase (AMPK) (Hardie et al. 2012). Repeated activation of AMPK can, however, enhance skeletal muscle oxidative capacity through the modulation of different transcription factors and co-regulators, such as PGC-1α, which has been shown to be one of the central modulators of mitochondrial biosynthesis (Egan and Zierath 2013). In addition, prolonged endurance training can lead to a transition of fast-twitch IIb fibers to IIa fibers, which have a higher oxidative capacity and that are less fatigable, yet highly capable of producing high contractile power (Bottinelli et al. 1999; Abernethy et al. 1990; Aagaard et al. 2011). No substantive evidence indicates that fast-twitch fibers will convert to slow-twitch fibers under normal training conditions (Manley 1996, p. 69). Combination of that all, aid in the body's ability to transport and use oxygen for energy generation, which will result in a slower rate of utilization of muscle glycogen and blood glucose, a greater reliance on fat oxidation, and less lactate production during submaximal exercise (Hawley 2009) and, therefore, delay the onset of muscle fatigue during prolonged aerobic performance (Joyner & Coyle 2008).

Although the contractile forces generated during steady-state exercise are typically much lower than those observed with resistance exercise, previous studies have demonstrated that prolonged, steady-state exercise does stimulate anabolic intracellular signalling and muscle protein synthesis (Pasiakos 2012). Low- to moderate intensity aerobic activity primarily recruits slow-twitch fibres (Abernethy et al. 1990) and therefore, small increases in cross-sectional area in that fibre type have been observed (Harber et al. 2012). Due to a much slower hypertrophy rate, cycle training requires a longer period to significantly increase muscle size compared to typical resistance training (Ozaki et al. 2015). However, increases in muscle mass after endurance training have been predominantly observed in the quadriceps muscle, when the mode of exercise used has been cycling, frequency and load has been high and the individuals undertaking training have had a limited level of exercise experience and/or sedentary life style (Konopka & Harber 2014).

Neuromuscular adaptations

Furthermore, previous studies have shown that endurance training may also lead to small but significant increases in strength performance, as far as previously untrained subjects are concerned (Izquierdo et al. 2005; Lo et al. 2011; Glowacki et al. 2004). Cycling, with sufficiently high intensity, has been reported to increase muscular strength in lower body (Grandys et al. 2008), whereas, lack of change in muscle strength has been noticed when other modes of exercise program (mostly running) have been applied (Hickson 1980; Kraemer et al. 1995; Dolezal & Potteiger 1998). Cycle training has been proposed to improve muscle function more, as it consists of mainly concentric activity, compared to the running, where repeated eccentric contractions may lead to muscular damage (Millet & Lepers 2004). Strength gains, however, seem to favour older adults, as for younger adults, higher-intensity intermittent cycling may be required to achieve strength gains (Ozaki et al. 2015). Since little force overload is placed on the upper-body musculature during lower-body endurance training, no great improvement in upper-body strength would be anticipated (Glowacki et al. 2004).

2.1.4 Acute responses and chronic adaptations to exercise training in the endocrine system

Exercise as a stressor can disrupt homeostasis and thereby, evoke certain hormonal responses (Phillips et al. 1997), which activate energy production pathways, mobilize energy substrates, help to induce cardiovascular adjustments and facilitate adequate hydration maintenance (Hackney & Lane 2015). The effects of exercise on endocrine system are mostly positive in nature and cause improved functional aspects of tissues and organs, which may possibly result in improved health and/or performance (Hackney & Lane 2015).

To elicit acute hormonal responses, exercise needs to reach certain threshold in terms of intensity, volume and duration to evoke strong enough feedback (Kraemer et al. 2008, p. 321; Viru et al. 1996). The acute responses to exercise training are not just the result of glandular tissue secreting the hormone in response to stimuli, but also influenced by the alterations in vascular fluid content and rate of hormonal clearance (Hackney & Lane 2015; West & Phillips 2010). Therefore, it is possible for the rate of secretory increases and the rate of hormonal metabolic clearance to match one another and mask the changes in the level of hormone concentrations of the target tissue (Crewther et al. 2011; Goodman 2001). In addition, for a hormone to initiate and activate a physiological process there must be adequate numbers of target tissue cells that express functional receptors for the hormone, a high affinity level of the receptors and sufficient post-receptor amplification mechanisms within the cells (Crewther et al. 2011; Viru & Viru 2004; Goodman 2001; Widmaier 1992). Receptors mediate the hormonal effect by either up-regulating (i.e. increase in receptor content and/or binding sensitivity) or down-regulating (i.e. decrease in receptor content and/or binding sensitivity) in response to exercise training (Crewther et al. 2011; Kraemer & Ratamess 2005). Therefore, the effect of the hormone depends not only on the amount of hormone molecules available, but also on the state of receptors (number of binding sites, affinity of binding to hormone) (Viru & Viru 2004). The chronic exercise does not eliminate the acute exercise response but may attenuate the overall effect of the responsiveness (Hackney & Lane 2015; Viru 1992). This literature overview focuses on cortisol (C) and testosterone (T) and to the effects that strength and endurance training have on the concentrations of these two hormones.

Testosterone

Testosterone, a 19-carbon steroid secreted by the Leydig cells in the testis, is considered to be an anabolic hormone (Florini 1970; Mauras et al. 1998). In skeletal muscle, T stimulates myofibrillar protein synthesis (Mauras et al. 1998) and inhibits protein degradation (Demling & Orgill 2000); combined these effects account for the promotion of muscle hypertrophy (Vingren et al. 2010). However, the Leydig cells secrete T only when they are stimulated by luteinizing hormone (LH) from the pituitary gland (Fry & Kraemer 1997), which in turn, is stimulated by a hypothalamic neurohormone, gonadotropin-releasing hormone (GnRH) (Kraemer et al. 2008, p. 319). Most of the T in blood is bound by sex hormone binding globulin (SHBG), whereas, free T is the most biologically active fraction (Vingren et al. 2010) and interacts with androgen receptors (Kraemer & Ratamess 2005). T also has a stimulating effect on the production of other anabolic hormones (Vingren et al. 2010). For example, together with 22-kD growth hormone, T has a synergistic effect on insulin-like growth factor-1 (IGF-I) release (Mauras et al. 2003), which has shown to directly increase anabolic gene transcription (Glass 2005). In addition, T has an effect on the nervous system by interacting with receptors on neurons and increasing the amount of neurotransmitters released, regenerating nerves, increasing cell body size and dendrite length/diameter (Kraemer & Ratamess 2005).

Cortisol

Cortisol has been nominated as the adaptation hormone, which has a wide spectrum of tasks in metabolic control, that are essential for adaptations in stress situation, including activation of catabolic processes and anti-anabolic action (Crewther et al. 2011; Viru & Viru 2004). C is released from the adrenal cortex in response to the stress of exercise and mediated through interaction with glucocorticoid receptors (Kraemer & Ratamess 2005). Catabolism is important in creation of an increased pool of free amino acids as branched chain amino acids can be used as additional substrate for oxidation (Viru & Viru 2004). Moreover, free amino acids are available as "building blocks" for protein synthesis (Viru & Viru 2004). Furthermore, C is involved in maintenance of blood glucose levels by acting upon skeletal muscle and adipose tissue (Wolfe 2001), by stimulating the liver to produce enzymes involved in the gluconeogenic and glycogenetic pathways allowing conversion of amino acids and glycerol into glucose and glycogen (Viru & Viru 2004).

2.1.4.1 Hormonal responses and adaptations to strength training

Several studies have reported pronounced elevations in circulating T levels subsequent to a resistance exercise workout (Ahtiainen et al. 2003; Häkkinen & Pakarinen 1995; Häkkinen et al. 2000; Kraemer et al. 1998a) (FIGURE 3). These elevations have been attributed to plasma volume reductions, adrenergic stimulation, lactate stimulated secretion and potential adaptations in T synthesis and/or secretory capacity of the Leydig cells in the testes (Kraemer & Ratamess 2005; Hansen et al. 2001). In addition, factors such as, the muscle mass involved (Hansen et al. 2001), training intensity and volume (Häkkinen & Pakarinen 1993a; Raastad et al. 2000; McCaulley et al. 2009), nutritional intake (Kraemer et al. 1998a) and training experience (Tremblay et al. 2004) appear to influence the acute serum total T responses to resistance exercise. In addition, resistance training has been shown to modulate androgen receptor content (Willoughby & Taylor 2004), which have shown to support the biological effect of target tissue, by activating the signaling processes that contribute to the long-term steroid action (i.e. gene transcription) (Crewther et al. 2011). However, changes in resting T concentrations to long-term resistance training have been inconsistent (Potteiger et al. 1995). Elevated resting T concentrations have been reported after long-term resistance training programs in some studies (Kraemer et al. 1999; Stokes et al. 2013), whereas several studies have shown no differences (Hickson et al. 1994; Häkkinen et al. 1985; Alén et al. 1988; Ahtiainen et al. 2003; Häkkinen et al. 2000) (FIGURE 3) or reductions (Rankin et al. 2004) in basal concentrations of T.

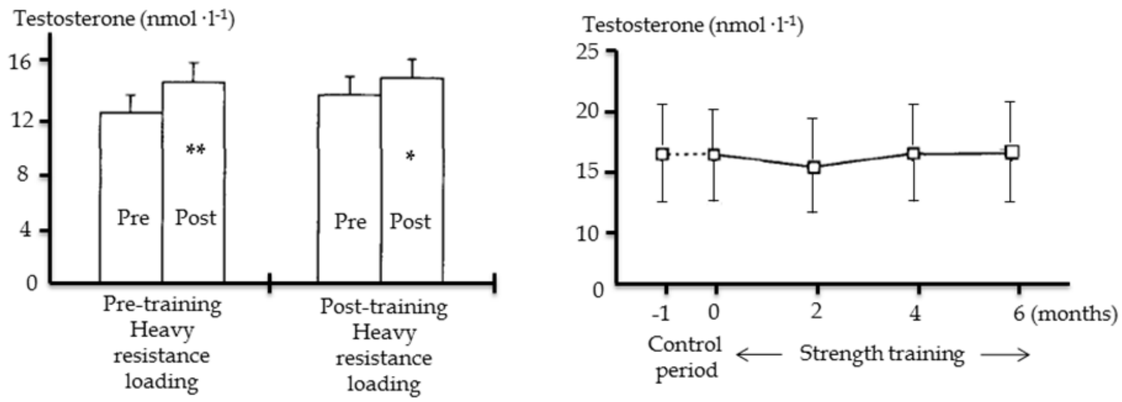

FIGURE 3 Serum testosterone responses to heavy resistance loading before and after the 6-month intervention (on the left) and total testosterone concentrations during the 1-month control period and 6-month strength training period in middle aged men (on the right). Modified from Häkkinen et al. (2000).

Earlier studies have shown that metabolically demanding protocols, high in total work (i.e. high volume, moderate to high intensity with short rest periods) elicit significant acute elevations in C in response to strength training (Kraemer et al. 1999; Häkkinen & Pakarinen 1993b; McCaulley et al. 2009; Smilios et al. 2003), but not after low volume strength training (Miranda et al. 2018; Stokes et al. 2013). To support that, significant correlations between blood lactate and serum C have been reported (Kraemer & Ratamess 2005). Similarly to T, some elevations in C concentrations have been attributed to plasma volume reductions (Kraemer & Ratamess 2005). In addition, glucocorticoid receptor responses to exercise seem to support the biological effect at target tissue similarly to the androgen receptors (Crewther et al. 2011). No consensus exists about the effects of chronic strength training on C secretion, as no change (Häkkinen et al. 1987; Häkkinen et al. 2000; Ahtiainen et al. 2003; Potteiger et al. 1995), reductions (Häkkinen et al. 1985; Alén et al. 1988; Kraemer et al. 1998b; Shakeri et al. 2012) or elevations (Häkkinen & Pakarinen 1991) have been reported by Kraemer and Ratamess (2005).

Some studies have shown changes in the T/C ratio after prolonged strength and power training period (Alén et al. 1988; Häkkinen et al. 1985), whereas, others have not (Ahtiainen et al. 2003). Although, some studies have shown that changes in T/C ratio during strength and power training have been positively related to performance improvements (Alén et al. 1988; Häkkinen et al. 1985), the suggestion of T/C ratio representing the anabolic/catabolic status of skeletal muscle appears to be an oversimplification (Fry & Kraemer 1997). Moreover, it has been suggested that T and C responses to individual training sessions may contribute to the adaptive responses to strength training by regulating long-term muscle performance (Crewther et al. 2011; West et al. 2010, Ahtiainen et al. 2005; Hansen et al. 2001). For example, a correlation between the acute response of T to single training session and the magnitude of the increase in strength and power, and muscle mass resulting from chronic strength training has been shown (Ahtiainen et al. 2003; Ahtiainen et al. 2005; Kraemer &

Ratamess 2005). However, others have suggested that physiologically elevated hormones after exercise do not enhance intracellular anabolic signalling or myofibrillar protein and thereby synthesis of muscle size and strength (Crewther et al. 2011; West et al. 2010; West & Phillips 2010).

2.1.4.2 Hormonal responses and adaptations to endurance training

Higher levels of T have been reported to be desirable for endurance performance (Grandys et al. 2009). T has been shown to stimulate bone marrow erythropoiesis and increase erythrocyte count by enhancing the renal production of erythropoietin (Shahidi 2001; Shahani et al. 2009) and enhance lactate transport across the muscle sarcolemma (Enoki et al. 2006). The beneficial effects of androgens on endurance performance have been supported also through significant correlations between VO_2max and T concentrations (Grandys et al. 2009). T concentration have been shown to increase in response to both continuous and interval type endurance exercise (Hackney & Lane 2015; Wahl et al. 2013; Stokes et al. 2013), showing that T increases in response to exercise depend on exercise intensity or duration (Tremblay et al. 2005; Hackney et al. 2012), with peak concentrations usually occurring at the end of exercise (Gray et al. 1993). Wahl et al. (2013) suggest that especially interval training will promote anabolic processes, which does not necessarily mean muscle hypertrophy, but increased expression of aerobic enzymes or adaptations in other processes (erythropoiesis) as described above. Nevertheless, prolonged endurance training has been shown to decrease basal T concentrations in men when high training work-load has been used (Hackney et al. 2003; Mäestu et al. 2005; Hoogeveen & Zonderland 1996; Hackney & Lane 2018) (FIGURE 4), due to changes in the central as well as in the peripheral components of the hypothalamus-pituitary-gonadal (HPG) axis (Hackney et al. 2003). However, short duration moderate intensity and low volume endurance training may increase T concentrations significantly in young healthy, previously untrained men (Grandys et al. 2009).

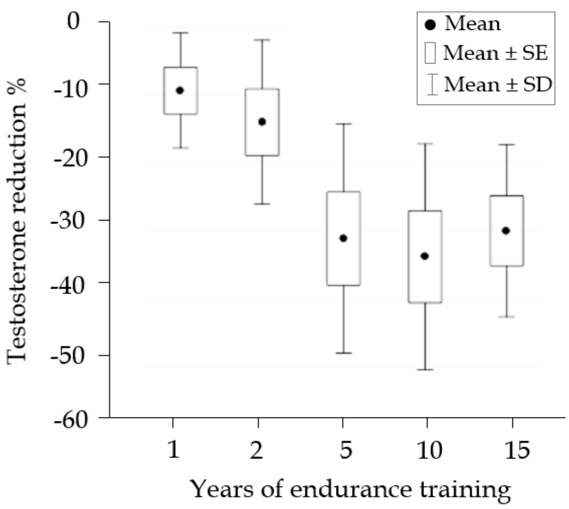

FIGURE 4 Testosterone levels of endurance-trained runners. Modified from Hackney and Lane (2018).

The blood level of C has been shown to increase if the exercise intensity is close to anaerobic threshold (Port 1991). During prolonged exercise, the C response is variable (Viru 1992). The most frequent variant of C dynamics is the initial increase that is substituted by a decrease to a pre-exercise or lower levels after 20-30 min (Viru & Viru 2004). If the exercise continues for longer than two hours, a secondary increase in the blood C concentrations may be observed (Viru & Viru 2004). Kokalas et al. (2004) suggested that continuous exercise causes greater responses in serum C compared to interval training. Nevertheless, post-exercise increases in C have been reported to be essential because of its catabolic action leaving a "pool" to the synthesis of new amino acids (Viru & Viru 2004).

The T/C ratio has been shown not to change (Grandys et al. 2009), decrease (Hoogeveen & Zonderland 1996) or increase (Hug et al. 2003) in response to endurance training. Therefore, the data concerning the influence of endurance training on basal hormone levels are inconsistent (Grandys et al. 2008).

2.2 Effects of combined strength and endurance training on physical fitness

Guidelines for general health and fitness promote the inclusion of both strength and endurance training components to the exercise programme (Garber et al. 2011). The simultaneous combination of strength and endurance training is known as combined or concurrent training (Hickson 1980). The advantage of the concurrent training is to simultaneously maximise the benefits associated with both endurance and resistance training and impact overall health and functional capacity/fitness even when the adaptations occur at somewhat lower magnitude when compared with each training mode alone (Fyfe & Loenneke 2018; Murlasits et al. 2018; Sabag et al. 2018). To save time people have started to combine strength and endurance training into the same training session, as lack of time is one of the major reasons for restraining from regular physical activity in young adults (Stutts 2002). For positive adaptations to occur during concurrent strength and endurance training, body must adapt to both training stimuli simultaneously (Gravelle & Blessing 2000). However, there is debate to which extent the adaptations to strength and endurance training are complementary and when antagonistic (Cadore et al. 2011). Previous combined strength and endurance training studies have proposed divergent results, showing that in previously untrained individuals combined training can lead to similar cardiovascular or musculoskeletal adaptations compared with either training regime alone (Davis et al. 2008), increase endurance performance (Støren et al. 2008; Izquierdo et al. 2001) or a diminished range of musculoskeletal and/or cardiovascular adaptation (Nelson et al. 1990; Chtara et al. 2005; Kraemer et al. 1995).

2.2.1 Possible mechanisms for compromised adaptations with combined training

Under certain circumstances, combining strength and endurance training may interfere with the training response induced by either type of training alone (Glowacki et al. 2004; Hickson 1980). The chronic interference hypothesis suggests that strength and endurance exercise represent the opposite ends of adaptation continuum (Egan & Zierath 2013) and therefore, trained muscles are unable to adapt optimally at the same time to both strength and endurance training (Leveritt et al. 1999; Nader 2006). The mechanisms of the interference effects are likely multifactorial, but neuromuscular, morphological, molecular factors as well as residual fatigue and overtraining have been proposed to lead to not optimal adaptations after combined training (Murach & Bagley 2016; Baar 2014; Fyfe et al. 2014). In addition, a number of methodological factors, such as training status, the length of the training intervention, the measurement tools used to assess key outcome variables, genetic factors, nutrient availability as well as frequency and type of strength and endurance training have been suggested to amplify or reduce the interference effect (Hickson 1980; McCarthy et al. 2002; Fyfe et al. 2014; Baar 2014). The most important factors involved in interference effect are discussed below.

2.2.1.1 Interference in morphological and neuromuscular adaptations

Classical studies by Hickson (1980) and Häkkinen and co-workers (2003) have suggested the sensitivity of maximal and explosive strength performance after combined strength and endurance training in physically active young men compared to the strength training alone (FIGURE 5).

The attenuated muscle strength development after concurrent training has been associated with morphological and neuromuscular adaptations in skeletal muscles. The limited hypertrophy of type I fibres (Kraemer et al. 1995) and greater fast-to-slow MHC-isoform transitions and preferential hypertrophy of IIa fibres (Putman et al. 2004; Chromiak & Mulvaney 1990) with concurrent training has shown to reduce total protein synthesis and change skeletal muscle fiber population (Nader 2006; Fyfe et al. 2014). The functional consequence of such conversion would appear to be lower rate and absolute amount of force development resulting in lower power output (Widrick et al. 2002). Nevertheless, others have suggested that strength interference during concurrent training cannot be wholly attributed to the inhibition of fiber type transformations or fiber hypertrophy, but that alterations in motor unit recruitment may be partly responsible for the inhibition in strength development observed during concurrent training (Leveritt & Abernethy 1999; Chromiak & Mulvaney 1990). The relatively small number of maximal or near maximal contractions involved in strength training favor different patterns of motor unit activation than the continuous low level contractions involved with endurance training (McCarthy et al. 2002). Therefore, concurrent training may hinder organization of efficient motor unit recruitment patterns necessary for forceful muscular contraction at the level of the peripheral or central nervous system (Eddens et al. 2018).

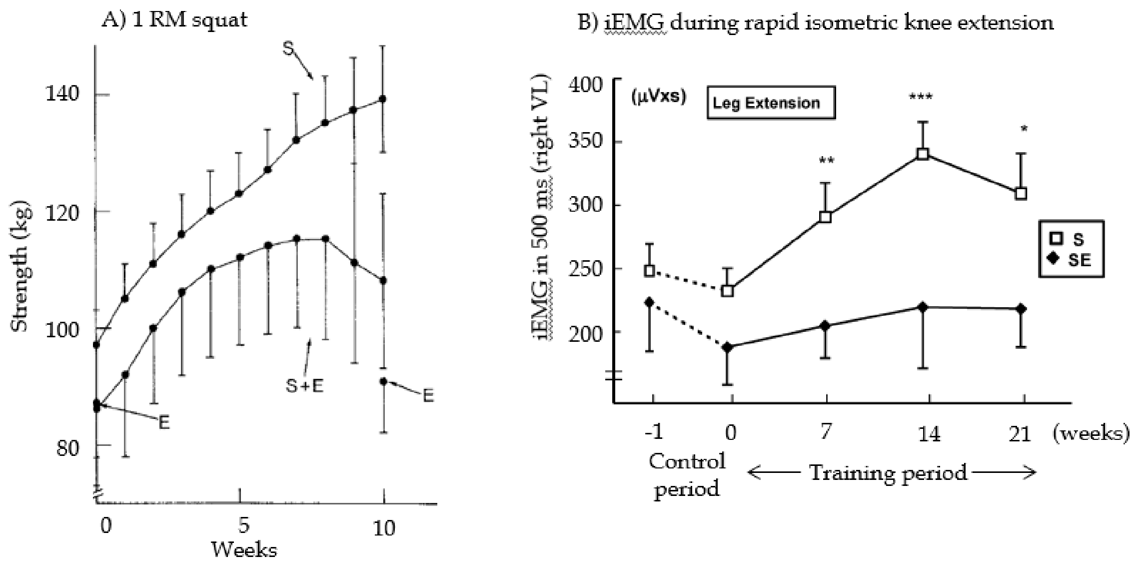

FIGURE 5 Changes in A) dynamic strength and B) averaged integrated electromyographic activity (iEMG) of vastus lateralis (VL) during the first 500 ms in the rapidly produced isometric knee extension after combined strength and endurance training. Modified from (A) Hickson (1980) and (B) Häkkinen et al. (2003).

2.2.1.2 Molecular adaptations

Molecular adaptations have also been often suggested as an explanation for possible difficulties in optimizing performance when training concurrently for strength and endurance (Apró et al. 2013; de Souza et al. 2013). The adaptive responses following resistance and endurance exercise are divergent (Apró et al. 2013) and may, therefore, lead to opposing phenotypes and place skeletal muscle in the situation of conflict (Baar 2006). Adaptations to resistance exercise are facilitated by the mechanistic target of rapamycin complex 1 (mTOR1) by mediating skeletal muscle growth (Goodman et al. 2011). The peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) has been proposed to be the pathway, which, in turn, mediates the characteristic increase in muscle oxidative capacity seen after endurance training (Olesen et al. 2010). Mechanistically, cross talk between these two pathways has been linked to the adenosine monophosphate-activated protein kinase (AMPK), an enzyme activated during energetic stress (Winder 2001). A few investigations in humans have suggested inhibition of skeletal muscle growth processes (Coffey et al. 2009a) or increased protein turnover/breakdown markers at the molecular level with acute concurrent exercise (Pugh et al. 2015). However, other studies with human participants have showed simultaneous increases in AMPK and mTORC1 signalling following endurance (Mascher et al. 2011) and resistance exercise (Dreyer et al. 2006) as well as after concurrent exercise (Apró et al. 2013; Wang et al. 2011). Recent findings of Lundberg et al. (2013) proposed that concurrent exercise elicited greater mTOR and p70S6K phosphorylation compared with the resistance-only group. This indicates that translational capacity was reinforced, rather than compromised, by the combined strength and endurance training (Lundberg et al. 2013).

2.2.1.3 Type of endurance training

It might be that the specific components of endurance training (e.g. modality, intensity, duration) are detrimental to resistance training outcomes (Wilson et al. 2012). For example, endurance training modality has been shown to play a significant role in the possible interference of training adaptations. A meta-analysis, examining interference of aerobic and resistance exercises, has reported significant decrements in hypertrophy and strength after running but not cycling (Wilson et al. 2012). The interference from running has been linked to the eccentric component and consequential muscle damage (Sabag et al. 2018). However, muscle damage, as a mechanism for decreased strength and hypertrophy is debatable, as exercise induced muscle damage is known to decrease after the first exercise session (McHugh 2003). In addition, de Souza et al. (2007) reported a decrease in leg strength endurance after the higher intensity intermittent runs in physically active young men and suggested it to originate from fast twitch motor units, which were activated already during the running exercise. Compared to running, cycling consists of primarily concentric activity (Wilson et al. 2012) and is biomechanically similar to the resistance training activities (Glowacki et al. 2004). The similarity in loading pattern could collaboratively support hypertrophy, despite local energetic challenges (Murach & Bagley 2016). Others, however, have suggested that similar activation patterns in strength and endurance training may lead to antagonistic adaptations in skeletal muscle (Gergley 2009; Sabag et al. 2018). Nevertheless, interference effect has been observed to occur only in the predominate muscle groups used during endurance loading, indicating that peripheral, rather than systemic fatigue causes the observed attenuation of lower body muscular strength (Sabag et al. 2018; Nader 2006).

In addition, the intensity of endurance training may influence the residual fatigue observed in the neuromuscular system before the strength training. High-intensity (especially >90% VO_2max) endurance training has been shown to recruit from the same motor unit pool as strength training (Nelson et al. 1990; Sale 1987). This will decrease the motor units' neurophysiological capacity to generate force (de Souza et al. 2007) and potentially impede the subjects' ability to perform resistance training (Sabag et al. 2018). In addition, during high-intensity endurance training, energy production is generally anaerobic in nature and thus, the accumulation of metabolic by-products such as lactate, hydrogen ions and inorganic phosphate in the sarcoplasm has been reported to inhibit contractile force (Ament & Verkerke 2009).

2.2.1.4 Training frequency, duration and volume

Interference in maximal strength adaptations in young healthy active men has been observed to occur when training volume is high and frequency more than 3 days per week, especially when the combined training is performed over a prolonged period of time (>7-8 weeks) (Hickson 1980). However, when the training frequency is low (≤3 days per week), interference with strength and endurance adaptations is generally absent (McCarthy et al. 2002; Izquierdo et al. 2004) even, if the concurrent training period has been between 8 and 16 weeks (McCarthy et al. 2002; Izquierdo-Gabarren et al. 2010) or ≥20 weeks (García-

Pallarés et al. 2010; Häkkinen et al. 2003), however, the maximal explosive strength adaptations might be still restricted (Häkkinen et al. 2003)

In addition, it has been suggested that individuals performing concurrent strength and endurance training need to contend with the double of training load, compared with the people engage to strength-only or endurance-only training and may, therefore, become over-trained. However, it has been argued, that if overtraining was a factor causing interference effect during concurrent training, then both strength and endurance measures should be inhibited (Dudley & Djamil 1985). However, this argument presumes that the thresholds for the effects of overtraining on strength and endurance adaptations are similar, which may not be the case.

2.2.1.5 Time between the strength and endurance training sessions

Different time intervals (minutes, hours, and/or days) of rest between endurance and strength exercise can be utilized, when designing a concurrent exercise protocol (Murach & Bagley 2016). However, it has been suggested that the time required to give body sufficient recovery between training sessions may be the limiting factor when attempting to induce simultaneous adaptations to strength and endurance training (Leveritt & Abernethy 1999). Therefore, to maximize the training response, it has been proposed that aerobic and resistance exercise within the concurrent training program should be separated by a minimum of 3 h, but preferably 6-24 h (Murach & Bagley 2016; Sabag et al. 2018). Some studies have reported that improvements in neuromuscular performance are larger after performing strength and endurance training on separate days, compared when performed on the same day among rowing and canoeing athletes (García-Pallars & Izquierdo 2011) as well as in heathy young men (Sale et al. 1990), but not all agree (Eklund et al. 2015). Adaptations in endurance performance, however, have shown to be less influenced by the time period between the strength and endurance training sessions (Sale et al. 1990).

2.2.2 Order of same-session combined strength and endurance training

When strength and endurance trainings are performed in the same day and combined into the same training session, intra-session sequence may significantly influence the adaptations to combined training (TABLE 2), as the lack of recovery between the two training sessions may lead to the situation where the second training mode is always performed in a fatigued state (Murach & Bagley 2016; Eddens et al. 2018; Murlasits et al. 2018; Tan et al. 2014). Both central (e.g. alterations in excitation contractile process) (Leveritt & Abernethy 1999) and peripheral factors (e.g. accumulation of metabolites as inorganic phosphate, lactic acid, ammonia and depletion of energy substrates) (Coffey et al. 2009b; Bell et al. 2000) have been proposed to cause acute fatigue from the first session (Reed et al. 2013). In addition, the residual fatigue from the first component of concurrent training may compromise the ability to develop tension during the second portion of concurrent training (Craig et al. 1991), which has been found to be a critical element in strength adaptations (Atha 1981).

TABLE 2 Training studies investigating the order effect after the prolonged combined strength and endurance training intervention in different populations.

Study	Subjects	Training mode	Neuromuscular performance	Cardiorespiratory performance	Hypertrophy
Schumann et al. 2014a; Eklund et al. 2015	Healthy young men	24 wks; 2-3 x wk; E: 30-50 min at AT and at or >AnT of cycling; S: whole body, 2-5 x 3-20 reps @ 40-95% of 1 RM	1 RM leg press, Isometric leg press E+S ↑ = S+E ↑ VA%, EMG, explosive strength E+S → < S+E ↑	VO_2max, T_{exh}, Wmax E+S ↑ = S+E ↑	Total body and leg lean mass by DXA and CSA of VL by ultra-sound E+S ↑ = S+E ↑
Chtara et al. 2005, 2008	Active young men	12 wks; 2 x wk; E: 5 x run at 100% vVO_2max, duration 50% T_{exh}; S: whole body circuit training for strength endurance and power	1 RM, CMJ E+S ↑ = S+E ↑	VO_2max E+S ↑ > S+E ↑	-
Makhlouf et al. 2015	Male elite soccer players	12 wks; 2 x wk; E: 2 x 10-16 x 15 sec @ 110-120% max running speed; S: whole body, 3 x 5-10 RM	1 RM squat, 1 RM bench press CMJ E+S ↑ = S+E ↑	Yo-Yo Intermittent recovery test E+S ↑ = S+E ↑	-
Eklund et al. 2016	Healthy young women	24 wks; 2-3 x wk; E: 30-50 min at AT and at or >AnT of cycling; S: whole body, 2-5 x 3-20 reps @ 40-95% of 1 RM	1 RM leg press E+S ↑ = S+E ↑	Wmax E+S ↑ = S+E ↑	CSA of VL by ultrasound E+S ↑ = S+E ↑
Davitt et al. 2014	Young inac-tive women	8 wks; 4 x wk; E: 30 min @ 70-80% HRR; S: whole body 3 x 8-12 reps @90-100% of 10RM	10RM leg press 10RM chest press E+S ↑ = S+E ↑	VO_2max E+S ↑ = S+E ↑	Lean mass by BOD POD E+S ↑ = S+E ↑
Gravelle and Blessing 2000	Active women	11 wks; 3 x wk; E: 25-45 min @ 70% VO_2max of rowing; S: lower body 2-4 x 6-10 RM	1RM E+S ↑ = S+E ↑	VO_2max E+S → < S+E ↑	-

MacNeil et al. 2014	Young men and women	6 wks; 3 x wk; E: 22.5 min @65-75% of VO_2peak of cycling; S: lower body 3 x 10 @ 65-80% of 1RM	Isometric KE E+S ↑ = S+E ↑	VO_2peak E+S ↑ = S+E ↑	Leg lean mass by DXA E+S ↑ = S+E ↑
Cadore et al. 2012a, 2013	Healthy elderly men	12 wks; 3 x wk; E: 20-30 min @ 80-100% HR_{VT2}, 6x4min @ 100% HR_{VT2} of cycling; S: whole body, 2 x 20-6 RM	1 RM bilateral KE E+S ↑ < S+E ↑ Force per unit of muscle mass E+S ↑ < S+E ↑ 1RM bilateral elbow flexion E+S ↑ = S+E ↑	VO_2peak and Wmax both E+S ↑ = S+E ↑	Muscle thickness of quadriceps femoris E+S ↑ = S+E ↑
Wilhelm et al. 2014	Healthy elderly men	12 wks; 2 x wk; E: 20-40 min @85-95% ; S: whole body power-type training, 2 x 8-18 RM HR_{VT2} of cycling	1 RM, power @ 60% of 1RM, EMG E+S ↑ = S+E ↑	VO_{2peak}, VT_2, T_{exh} E+S ↑ = S+E ↑	QF thickness by ultrasound: E+S ↑ = S+E ↑
Banitalebi et al. 2015	Elderly women	8 wks; 3 x wk; E: 16-30 min @ 45% of VO_2max on ergometer; S: whole body, 40-75% of 1RM	-	VO_2max E+S ↑ = S+E ↑	-

E: endurance training; S: strength training; RM = repetition maximum; HR_{VT2} = heart rate corresponding to second ventilatory threshold; vVO_2max = velocity associated with VO_2max; T_{exh} = time to exhaustion; CMJ = countermovement jump; EMG = electromyographic activity; HRR = hear rate reserve; AT = aerobic threshold, AnT = anaerobic threshold, Wmax = aerobic power; CSA of VL = cross-sectional area of vastus lateralis; HR_{max} = maximal heart rate; KE = knee extension; QF = quadriceps femoris; VA% = voluntary activation percentage

2.2.2.1 Effects of endurance training on strength training adaptations

Although both exercise orders have been shown to result in enhanced lower-body dynamic strength and quadriceps femoris muscle quality, greater improvements have been sometimes observed when strength training has been performed prior to endurance training (S+E), in comparison to the reversed order (E+S) in young and old previously untrained men (Eddens et al. 2018; Murlasits et al. 2018). After the E+S order, a deficit in maximal strength, explosive strength or in neuromuscular activity has been observed after prolonged combined training program (Cadore et al. 2013; Schumann et al. 2014b; Eklund et al. 2015). Based on that, researchers have proposed that by strategically sequencing training sessions, the interference effect could be avoided (Cadore et al. 2013). The exact underlying mechanisms of this interference remain to be elucidated, although both neuromuscular (central or peripheral in origin) and metabolic processes (e.g. disturbance in homeostasis, due either to accumulation of metabolic waste, depletion of energy supply or combination of both) have been suggested (Murlasits et al. 2018; Eddens et al. 2018). For example, cycling exercise has been shown to recruit quadriceps femoris muscles even at low workloads (Ericson et al. 1985) and, thereby, reduce the isometric force production for several hours. Fatigue-induced acute adjustments in the nervous system following an endurance training session have been reported to alter muscle recruitment (van Dieën et al. 1998), decrease rate of force development (Lepers et al. 2001; Bentley et al. 2000) and reduce neural input to the muscles (Paavolainen et al. 1999). This, however, makes it impossible for resistance training to be performed at intensities that would otherwise be used without prior endurance training (Bentley et al. 2000).

In Eklund et al. (2015) young men in the S+E group demonstrated increased EMG, voluntary activation % and force in isometric actions after 24 weeks of training, while no changes were observed in the E+S group. Furthermore, the highly significant correlation observed between individual changes in VA% and changes of knee-extension strength development during the latter half of the 24-week training period for the E+S group demonstrated that, individuals, who experienced reduced voluntary activation, also had limited strength gains (Eklund et al. 2015) (FIGURE 6). These findings suggest that potential inhibition or interference in the nervous system might occur in the E+S group, when the combined training period is prolonged (Eklund et al. 2015).

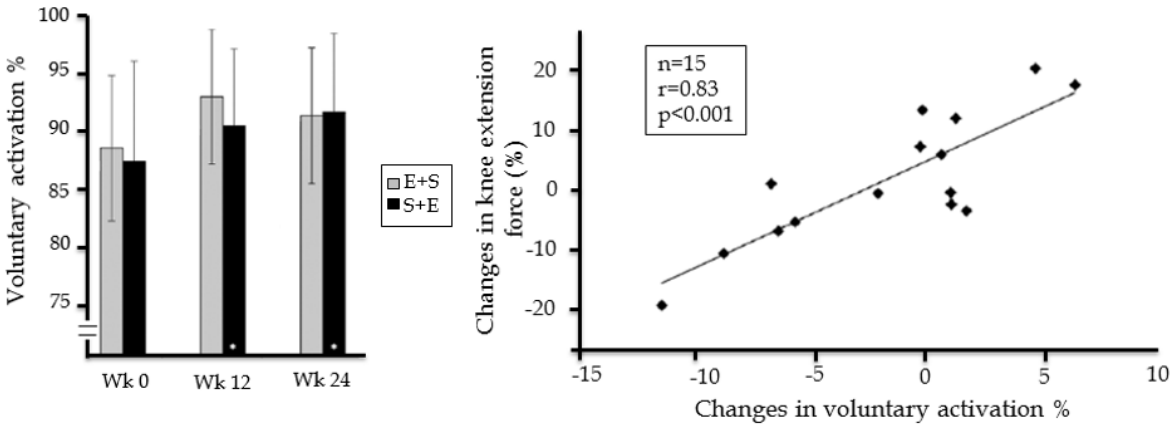

FIGURE 6 Changes in voluntary activation % in E+S and S+E groups after 24-week same-session combined strength and endurance training (on the left) and correlation between the individual changes in the voluntary activation and in maximal knee extension force in the E+S group during weeks 13-24 (on the right). Modified from Eklund et al. (2015).

Similarly, Cadore et al. (2013) have shown in older population that after a prolonged combined training period neuromuscular economy improves more in the S+E training group. However, as between group differences were observed only in rectus femoris but not vastus lateral muscle, the interference of the intra-session exercise order in the neural adaptations as a mechanism to explain the different strength gains needs further investigation (Cadore et al. 2013).

However, others have shown that for the individuals not accustomed to regular resistance or endurance training, the intra-session sequencing of combined training may not influence the magnitude of training-induced adaptations in neuromuscular performance and muscle hypertrophy (Collins & Snow 1993; Chtara et al. 2008; Gravelle & Blessing 2000). The inconsistent findings may be a result of differences in training protocols, including training frequencies and intensities (Wilhelm et al. 2014) and subject populations observed.

2.2.2.2 Effects of strength training on endurance training adaptations

Maximal aerobic capacity has not been usually associated with concurrent training interference and most of the studies have also reported that the order of strength and endurance training does not seem to influence the adaptations in cardiorespiratory performance (Eddens et al. 2018; Murlasits et al. 2018; Collins & Snow 1993; Schumann et al. 2014a; Cadore et al. 2012a; MacNeil et al. 2014; Eklund et al. 2015), however, others disagree (Chtara et al. 2005; Gravelle & Blessing 2000). For example, Gravelle and Blessing (2000) have shown limited increases in VO_2max in physical active women in the E+S group compared with the opposite order, whereas, Chtara et al. (2005) observed that S+E training order results in lower enhancements in maximal endurance performance compared with the inverse order, and suggested it to be a consequence of fatigue resulting from the strength training. Residual fatigue due to inadequate recovery after strength training may impair neural recruitment patterns, reduce movement efficiency due to alteration in kinematics during endurance exercise and increased energy expenditure, increase muscle soreness and reduce muscle glycogen stores (Doma et al. 2017). The association between muscle glycogen content and endurance performance has been most apparent during endurance exercises performed at intensities from 65-85% of VO_2max (Wilson et al. 2007). Training sessions repetitively commencing in a low glycogen state, however, may increase the risk of an overtraining syndrome and as a result, impair training capacity (Petibois et al. 2003).

2.3 Effects of combined training on hormonal profile

Concurrent training influences both anabolic and catabolic endocrine factors (Bell et al. 2000; Kraemer et al. 1995), however, the exact responses of T and C to concurrent training are yet to be fully elucidated (Jones et al. 2017). Cadore et al. (2012b) have shown that regardless of the order of the modalities performed during a concurrent training session, in the older men T and C responses are always higher after the first exercise modality, than they are in the second. Moreover, the magnitude of the total T response in the E+S session was greater than that observed after the S+E session, suggesting that the order of the exercise modalities can influence the acute T response to the training session (Cadore et al. 2012b). Similar results have been confirmed also by Schumann et al. (2014b) in young men. In the second exercise of the training session, strength training has resulted in a greater stimulus for T, when compared with endurance training (Cadore et al. 2012b). It is possible that lactate produced during the strength training session, may have stimulated T production from testes, as demonstrated in the study performed by Lu et al. (1997). Whereas, when endurance training was performed as second in the training session, the lactate accumulated during strength training may have been used as energy substrate, thus, influencing the acute T responses (Gastin 2001). In addition, it is possible that strength training bout stimulated the acute increases in androgen receptor content and that led to changes in the circulating T level (Willoughby & Taylor 2004). Therefore, Cadore et al. (2012b) suggested that the endurance exercise, after a strength training session, may not have been able to further stimulate or maintain T levels. No consensus has been found about the C response to concurrent training as greater responses have been observed after the S+E order (Taipale & Häkkinen 2013) as well as after the E+S order (Jones et al. 2017) in young active men, whereas similar C responses to both loading sequences have been observed in women (Eklund et al. 2016). Jones et al. (2017) proposed that higher C concentrations after the E+S loading may result in acute unfavorable responses to strength training, when strength training is conducted with high loads (Jones et al. 2017). Furthermore, after simultaneous performance of both strength and endurance exercise negative correlations between C level and the magnitude of the T responses have been observed, indicating that those subjects with lower levels of C tend to have higher acute T increases, and, therefore, C may have an inhibitory effect on T production (Cadore et al. 2012b) and may not provide ideal environment for strength development and fiber hypertrophy (Kraemer et al. 1995). Therefore, the results of Cadore et al. (2012b) suggest that, if the objective is to optimize the response of T to a concurrent training session, aerobic exercise before strength training can keep T levels elevated for longer. In addition, hormonal responses to combined loadings depend on the training status of the subjects and the specificity of the combined protocol performed (Cadore et al. 2012b; Schumann et al. 2013; Taipale & Häkkinen 2013). Changes in basal levels after same-session combined strength and endurance training however have received little attention.

2.4 Effects of time-of-day on strength and endurance performance and training adaptations

2.4.1 Circadian rhythms in humans

Over millions of years of evolution, living species have developed strategies to accommodate to the cycle of day and night, as well as to other rhythmic fluctuations in their environments (Schulz & Steimer 2009). Therefore, the physiological and behavioral parameters in humans have been shown to follow sinusoidal rhythms (Waterhouse et al. 1993), which are known as circadian rhythms (Cappaert 1999). The sleep-wake cycle regulation is one of the most conspicuous features of the circadian system (Atkinson & Reilly 1996). Circadian rhythms originate from endogenous pacemaker in the suprachiamatic nucleus (SCN) in the anterior hypothalamus (Schulz & Steimer 2009), close to the optic chiasm (Ralph et al. 1990) and have a period close to 25 hours (Atkinson & Reilly 1996). However, these endogenous rhythms are synchronized or "entrained" to the normal 24-hour environment by *zeitgeber* (German - meaning "time givers"), so that rhythms are adjusted with the environmental cycles. The most important of which, are the light (Roenneberg et al. 2003; Schulz & Steimer 2009) and social interactions (Atkinson & Reilly 1996), however, other external cues such as scheduled feeding, temperature or exercise training may adjust the rhythms to correspond to our 24-hour environment (Camera 2018; Roenneberg et al. 2003).

Body core temperature has been often used as the marker of the circadian rhythms (FIGURE 7), with daily peak-to-trough range around ~1°C, with an acrophase in the late afternoon, close to 18:00 and a minimum at about 05:00 h (Waterhouse et al. 2005; Wright et al. 2002). Like other circadian rhythms, the rhythm of body temperature is mainly endogenous and persists in free-living conditions, without the influence of zeitgebers (Colin et al. 1968). This rhythm reflects the combined effects of the body clock, sleep, and physical and mental activity (Waterhouse et al. 2005).

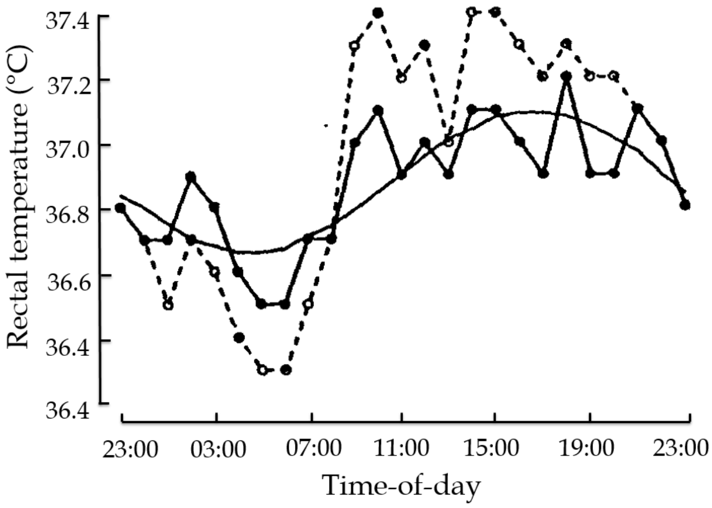

FIGURE 7 Endogenous diurnal fluctuation of rectal temperature. — normative endogenous temperature data; ●—● raw temperature data. Modified from Waterhouse et al. (1993).

Human behavior has shown large inter-individual variation in temporal organization (Kudielka et al. 2006; Roenneberg et al. 2003) and chronotype has been used to describe the behavioral phenotypes. Chronotype reflects one's innate circadian rhythm towards morningness or eveningness and is usually evaluated by using self-assessment questionnaires (Horne & Ostberg 1976). It is assessed subjectively based on the time-of-day at which individuals naturally prefer to be asleep, awake or perform mental or physical tasks (Roenneberg et al. 2003). Chronotype broadly classifies individuals as morning types (i.e larks), neither types and evening types (i.e owls) (Rae et al. 2015). In general, most circadian rhythms appear to peak earlier in the morning chronotypes compared to the evening chronotypes (Kudielka et al. 2006). Also, a person's preferred time-of-day for exercise and force production may be driven in part by his/her innate circadian rhythm (Tamm et al. 2009; Brown et al. 2008; Rae et al. 2015).

2.4.2 Diurnal variation in neuromuscular performance

It has been documented that several components in sports performance, including muscle strength, fluctuate in a sinusoidal manner with time-of-day, with morning nadirs (daily minimum) and afternoon maximum values (Reilly & Waterhouse 2009; Zbidi et al. 2016; Teo et al. 2011a; Atkinson & Reilly 1996; Chtourou et al. 2012 a,b; Souissi et al. 2002). The acrophase of the muscle capacity to develop maximal force has been found to occur in the early evening between 16:00 and 19:30 (Callard et al. 2000; Giacomoni et al. 2006; Guette et al. 2005a; Nicolas et al. 2005; Teo et al. 2011a). Peak-to-trough variation of maximum strength has been reported to range from 5% up to 21% (Coldwells et al. 1994; Guette et al. 2005b; Nicolas et al. 2005) depending on the population and the muscle groups tested as well as on the experimental design (Nicolas et al. 2007). Even if considering only the lower end of 5% to 10%, it is considerably large not only for sports performance but also for any activity requiring maximum/near maximum strength and power (Jančoková et al. 2013, p. 42).

Diurnal fluctuations in force production have been observed in a variety of muscles, including quadriceps femoris (Callard et al. 2000; Guette et al. 2005a; Nicolas et al. 2005; Giacomoni et al. 2006; Racinais et al. 2005; Sedliak et al. 2008a). Although diurnal rhythms in muscle force have been well established during maximal isometric contraction in young healthy untrained men, the results concerning EMG data are more controversial (Sedliak et al. 2008a; Nicolas et al. 2005; Onambele-Pearson & Pearson 2007). The previous studies with rather small numbers of participants have been unable to determine the exact mechanisms behind the diurnal rhythms, although both central and/or peripheral mechanisms have been suggested (Chtourou & Souissi 2012). EMG (Nicolas et al. 2005; Sedliak et al. 2008a) and muscle stimulation (Guette et al. 2005b; Giacomoni et al. 2005) have been widely used to discriminate the involvement of peripheral and central mechanisms in the diurnal variations (Chtourou & Souissi 2012). In TABLE 3 the current knowledge about the diurnal variation in neuromuscular performance and suggested mechanisms behind the morning-to-evening difference in young healthy men are collected.

In addition, it has been proposed that the diurnal variations in muscle performance can be influenced also by fluctuations in core temperature, with evening higher values possibly providing passive warm-up with enhanced metabolic reactions, increased extensibility of connective tissue, reduced muscle viscosity and increased conduction velocity of action potentials (Teo et al. 2011a; Chtourou & Souissi 2012). However, other authors suggest that there are other factors than body temperature, which influence short-term anaerobic performance (Martin et al. 1999), such as warm-up duration, hormone concentration, differences in nutritional status from morning to evening, decreased flexibility in the morning, insufficient time to recover from sleep inertia, preferred exercising time and differences in motivation (Youngstedt & O'Connor 1999; Teo et al. 2011a; Cappaert 1999; Chtourou & Souissi 2012; Gauthier et al. 1996; Souissi et al. 2002). Also, the test repeatability may influence the results as the less accurate the methods, the greater the probability of type II statistical error in the context of diurnal variations (Atkinson & Reilly 1996; Giacomoni et al. 2006).

TABLE 3 Diurnal variations in neuromuscular performance of knee extensors and suggested mechanisms.

Study	Subjects	Measurement time	Action/Muscles tested	Muscle strength	EMG and muscle activation	Suggested mechanisms
Nicolas et al. 2005	12 young active men	06:00 and 18:00	50 max. isokinetic KE at 2.09 rad · s^{-1}; rmsEMG	Isokinetic KE torque M<E	rmsEMG M=E	Greater contractile capacity, higher fatigability in E
Guette et al. 2005	10 young active men	06:00, 10:00, 14:00, 18:00, 22:00	Max. unilateral isokinetic KE torque; Femoral nerve stimulation; rmsEMG	Isokinetic KE torque M<E (18:00)	rmsEMG M=E Muscle activation M=E	Muscular rather than neural mechanisms
Giacomoni et al. 2005	12 young active men	02:00, 06:00, 10:00, 14:00, 18:00, 22:00	Peak isokinetic KE at 1.05 and 3.14 rad · s^{-1}; Max. isometric KE force (muscle stimulation)	Peak isokinetic force (3.14 rad · s^{-1}) M < E Voluntary KE force M=E	KE under muscle stimulation M<E	Masking effect of motivation
Racinais et al. 2005	11 young active men	07:00-09:00 and 17:00-19:00	Max. isometric KE force; rmsEMG of VL (in normal and warm conditions)	In normal conditions M<E In warm conditions M=E	rmsEMG VL M=E	Peripheral mechanisms, partly due to low body t°C in M
Onambele - Pearson & Pearson 2007	12 young healthy men	07:45 and 19:45	Max. isometric KE torque and force; rmsEMG of BF	Isometric KE torque and force M<E	rmsEMG BF M=E	Peripheral and cellular mechanisms
Sedliak et al. 2008a	32 young healthy men	07:00-08:00, 12:00-13:00, 17:00-18:00, 20:30-21:30	Max. unilateral KE force EMG of VL, VM, BF	Max. KE force M<E	EMG M=E	Peripheral rather than central mechanisms
Edwards et al. 2013	10 young active men	07:30 and 17:30	Peak concentric KE torque and power at 1.05 and 4.19 rad · s^{-1};	Peak concentric torque M<E power at 1.05 (M<E)	QF muscle activation % M=E	Peripheral mechanisms and endogenous factors

			Max. isometric KE force; QF muscle activation %	and 4.19 rad $\cdot$ s^{-1} (M=E) Max isometric KE force M<E		
Gonçalves Araujo et al. 2011	8 young active men	02:00, 06:00, 10:00, 14:00, 18:00, 22:00	Peak torque and average power in isokinetic KE; Maximum isometric KE force and torque	Isokinetic performance M<E Isometric performance Force M=E Torque M<E	-	-
Deschenes et al. 1998a	10 young healthy men	08:00, 12:00, 16:00, 20:00	Peak isokinetic KE torque at 1.05, 1.57, 2.09 and 3.14 (50reps) rad $\cdot$ s^{-1}	Peak KE torque at 3.14 rad $\cdot$ s^{-1} M<E	-	Higher body t°C and optimal hormonal levels in E
Teo et al. 2011b	20 young active men	08:00, 12:00, 16:00, 20:00	Max. isometric midthigh pulls; 1RM squat	Max. isometric mid-thigh pulls M<E 1RM squat M=E	-	Time-of-day effect in elasticity of musculotendinous tissues
Pereira et al. 2011	30 young healthy men	07:30-09:30, 13:30-15:30, 19:30-21:30	Isometric KE explosive force; EMG of RF and VL	M<E	M=E	Peripheral rather than neural mechanisms

KE = knee extension; KF = knee flexion rmsEMG = root mean square electromyographic activity; VL = vastus lateralis; M = morning; E = evening; VM = vastus medialis; BF = biceps femoris; QF = quadriceps femoris; 1 RM = one repetition maximum; RFD = rate of force development; t°C = temperature in Celsius

2.4.3 Diurnal variation in cardiorespiratory performance

The literature regarding the diurnal rhythms in endurance performance is far more inconclusive (Chtourou & Souissi 2012). It has been suggested that the diurnal variation of exercise performance dissipates as the exercise duration increases (Racinais 2010; Chtourou & Souissi 2012; Dalton et al. 1997; Deschenes et al. 1998b), whereas others have observed significant diurnal variation in cycling performance (Atkinson & Reilly 1996; Atkinson et al. 2005; Torii et al. 1992). Poorer performance is then usually observed in the early morning, while the best performance late in the afternoon (Drust et al. 2005). The effects of time-of-day on performance are probably not explained by one or two variables but represent the effect of a combination of factors (Bessot et al. 2011). Some of the parameters, which have been linked to the ability to perform in middle- and long-duration events (oxygen consumption, heart rate, ventilator thresholds and performance economy) have been shown to fluctuate during the day (Racinais 2010). However, there are large discrepancies between studies that have focused on circadian maximal oxygen uptake patterns (Bessot et al. 2011). Even though some studies have observed a diurnal variation in VO_2max (Hill 2014; Hill et al. 1988), others have concluded that it is not time-of-day dependent (Deschenes et al. 1998b; Dalton et al. 1997; Torii et al. 1992; Reilly & Brooks 1990; Bessot et al. 2011; Bessot et al. 2006). VO_2 kinetics seem to be time-of-day-specific during moderate-intensity cycling exercise (Brisswalter et al. 2007) but not during high-intensity running (Carter et al. 2002) or cycling exercise (Bessot et al. 2006). Ventilatory threshold has also been suggested not to depend on time-of-day (Racinais 2010; Hill et al. 1989). As there is no consensus, whether prolonged exercise capacity depends on time-of-day or not, the current knowledge about diurnal variation in endurance performance and suggested mechanisms behind the morning-to-evening difference in young men are collected in TABLE 4.

In addition, it has been proposed that the convergent results concerning the effect of time-of-day on long-duration exercise performance could be because of the reluctance of some subjects to exercise to voluntary exhaustion during troughs in arousal (Chtourou & Souissi 2012), as the time-of-day effects on physiological responses to long duration exercise seem to weaken as the exercise intensity is increased towards maximum. In addition, similarly to the neuromuscular performance, significant circadian rhythms may not be observed in maximal physiological capacities, when measurement error approaches the range of the rhythm amplitude (Chtourou & Souissi 2012).

TABLE 4 Diurnal variations in cardiorespiratory performance.

Study	Subjects	Measurement time	Type of exercise performance	Variables with diurnal variation	Variables without diurnal variation	Reasoning
Forsyth and Reilly 2004	11 young healthy men	02:00, 06:00, 10:00, 14:00, 18:00, 22:00	Rowing until exhaustion	VO_2, HR, Th_{lac} (M<E)	Power, RPE, La	Diurnal variation in body t˚C
Hill 2014	20 young healthy men	06:30-09:30 and 17:00-20:00	Cycling until exhaustion	T_{exh} , HR_{max} VO_2max, La (M<E)	Work rate	M<E in VO_2max, anaerobic capacity, VO_2 kinetics conflate to produce M<E in T_{exh}
Reilly and Garrett 1998	7 young healthy men	08:30 and 17:30	Cycling until exhaustion at 70% of VO_{2max}	-	T_{exh}, VO_2, HR	Central (motivational) or local factors influence cycling performance more than body t˚C
Deschenes et al. 1998b	10 young healthy men	08:00, 12:00, 16:00, 20:00	Cycling until exhaustion	La	T_{exh}, VO_2max, HR	Diurnal variations in some variables (La), does not lead to variations in cycling performance
Atkinson et al. 2005	8 young male cyclists	07:30 and 17:30	Cycling 16.1-km time trial	Time trial, La, power (M<E)	HR during time trial	-
Dalton et al. 1997	7 young male cyclists	08:00-10:00, 14:00-16:00, 20:00-22:00	15 min max cycling test	-	Total work, average power, VO_2max, HR	Biological rhythm in body t˚C has no effect on cycling performance
Fernandes et al. 2014	9 young male cyclists	08:00 and 18:00	Cycling 1000-m time trial	Time trial (M<E)	VO_2, HR	Concomitant increase in aerobic and anaerobic contributions in E
Bessot et al. 2006	11 young male cyclists	06:00 and 18:00	Cycling until exhaustion	T_{exh}, La (M<E)	VO_2	Greater anaerobic metabolism and body t˚C in E
Bessot et al. 2011	15 young male cyclists	06:00 and 18:00	Cycling until exhaustion	-	VO_2max, HR_{max}, P_{max},	Fluctuations in max aerobic endurance or gestural efficiency

M=morning; E=evening; Th_{lac} = lactate threshold; La = lactate; body t˚C = body temperature; T_{exh} = time to exhaustion; HRmax = maximal heart rate; P_{max} = peak power output reached during the incremental test

2.4.4 Diurnal variation in the endocrine system with special reference to cortisol and testosterone

Endocrine factors have an integral role in homeostasis, to respond to the fluctuations in demands and stresses over the course of the day (Gamble et al. 2014). Both C and T exhibit circadian rhythmicity with peak concentrations in the morning, around the commencement of the diurnal activity, and reduced concentrations in the evening and over the night (Van Cauter 1990; Hayes et al. 2012; Kraemer et al. 2001; Touitou & Haus 2000) (FIGURE 8). The magnitude of diurnal change in T levels have been shown to be significantly less than in C (Hayes et al. 2010). Hayes et al. (2010) have reported that, on average, in diurnally active persons T concentration declined 42% from 06:00 (awakening) to 23:00, compared with 92% for C during this time-course.

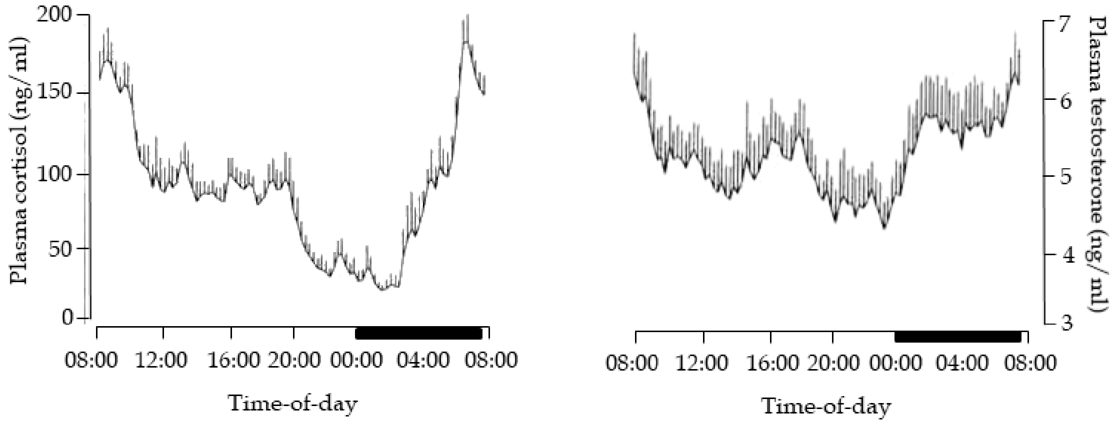

FIGURE 8 Diurnal rhythms (mean + SEM) in plasma cortisol and testosterone. The sleep times are indicated as black bars. Modified from Van Cauter et al (1990).

Not only do circulation levels of various endocrine factors oscillate over the 24 h period in response to behavioral fluctuations associated sleep-wake cycles, but so does the responsiveness of target tissues, which are orchestrated by an intrinsic time keeping mechanisms (i.e. circadian clock). T and C are considered as important biomarkers in exercise science, because it has been suggested that they possibly correlate with athletic performance (Crewther et al. 2009; Giacomoni et al. 2005; Martin et al. 1999). For example, Chtourou et al. (2013) have suggested that, similarly to the peak short-term performance, greater T and C responses can be observed in the afternoon compared with the morning strength training sessions. In addition, higher T/C ratio in the evening may provide a more desirable physiological condition (more anabolic environment with less physiological stress), which prepares participants for the physical performance (Bird & Tarpenning 2004; Hayes et al. 2013). Nevertheless, Teo et al. (2011b) found that although both hormonal responses (T and C) and physical performance (dynamic and isometric force) displayed a circadian pattern, no correlation was found between these two variables. Therefore, the notion that diurnal fluctuations of hormonal profiles can influence daily changes in neuromuscular performance remains doubtful (Teo et al. 2011b).

There is also little evidence in the literature to suggest that exercise, especially strength training, has any influence on the circadian profile of T and C (Teo et al. 2011a). Previous studies have shown that the influence of short-term strength (Deschenes et al. 1998a; Kraemer et al. 2001; Häkkinen et al. 1988) and endurance (Labsy et al. 2013) training protocols seem to be insufficient to alter the circadian profile of T and C. However, not in full agreement with that, Cook et al. (2014) found that strength training in the morning might attenuate the circadian decline in both T and C, when compared to a rested control trial.

2.4.5 Time-of-day-specific adaptations to strength training

Strength performance

Several studies on strength training have suggested an effect of training time on neuromuscular performance adaptations (Chtourou et al. 2012a, b; Sedliak et al. 2007; Souissi et al. 2002). Souissi et al. (2002) was one of the first to show that after weeks of time-of-day-specific resistance training, greater adaptations in short-term performance occur at the time-of-day when the training was regularly performed, than at other times. However, the absolute increases in maximum strength have been found to be similar between the morning and evening strength training groups (Souissi et al. 2002). It has been proposed that, the subjects who train in the morning hours improve their performance in the morning and in the evening, whereas those, who train in the evening hours, improve their performance only at this time-of-day (Chtourou & Souissi 2012; Souissi et al. 2002; Zbidi et al. 2016; Sedliak et al. 2008a; Gueldich et al. 2017). Therefore, training at a specific time-of-day could modify the typical diurnal pattern of short-term maximal performances (Chtourou & Souissi 2012) (FIGURE 9). Training in the morning hours can improve typically poor morning performances to the same or even higher level as their normal daily peak typically observed in the evening, thereby decreasing the amplitude of the diurnal variations in strength performance and blunting the typical diurnal variation (Sedliak et al. 2007, 2008a, 2009; Chtourou & Souissi 2012; Zbidi et al. 2016). However, the amplitude of the diurnal variations of short-term maximal performances has been shown to increase in the evening training group (Sedliak et al. 2007, 2008a, 2009; Chtourou & Souissi 2012). Thus, the choice of morning or evening training time (temporal specificity of training) seems to be crucial when improvements in neuromuscular performance are the desired goal (Zbidi et al. 2016).

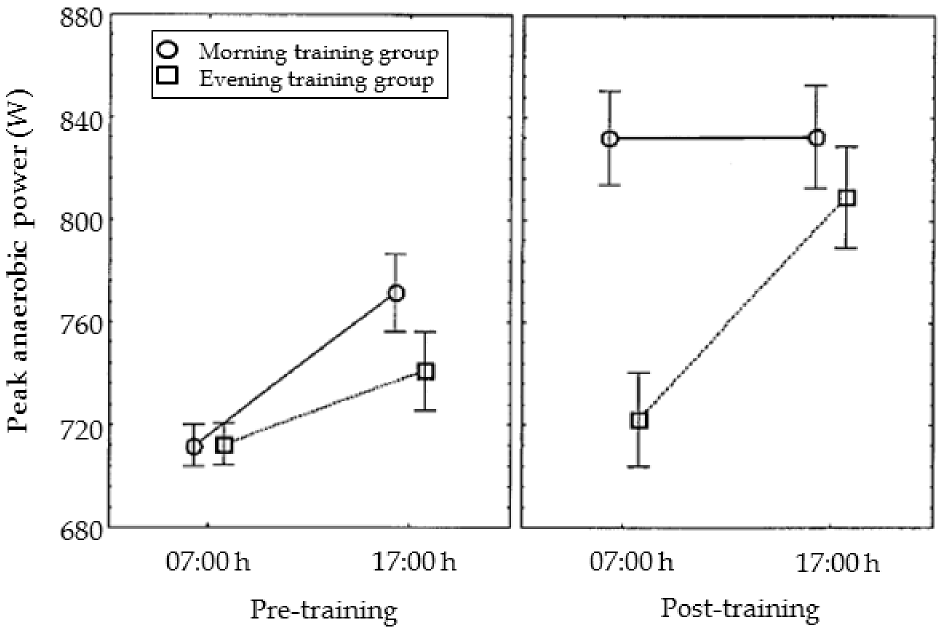

FIGURE 9 Peak anaerobic power of the morning training group and evening training group when measured in the morning and evening before and after the time-of-day specific strength training. Modified from Souissi et al (2002).

The exact mechanisms responsible for time-of-day specific adaptions are still unclear and remain under discussion (Chtourou & Souissi 2012). Sedliak et al. (2008b) has investigated the roles of central and peripheral mechanisms involved in the temporal specificity of resistance training and failed to show any time-of-day-specific adaptation in EMG of knee extensors and, therefore suggested, that peripheral rather than neural adaptations are the main source of temporal specificity in resistance training adaptations. Others, however, have proposed that changes of the neural drive motor unit properties and/or muscle membrane properties rather than adaptations and improvement of the muscle contractile properties could be the main source of temporal specificity to the electrostimulation training (Gueldich et al. 2017). Therefore, no confident conclusions can be drawn.

Muscle hypertrophy

There is limited information upon hypertrophic adaptations to time-of-day-specific resistance training (Sedliak et al. 2017). Although Sedliak and co-workers found no statistically significant differences in the magnitude of muscular hypertrophy after strength training between morning and evening training groups (Sedliak et al. 2009, 2017), a tendency for smaller gains in muscle size were reported, when repeatedly performing strength training in the morning hours (Sedliak et al. 2009). It has been proposed that exercise-induced contraction and its timing may regulate gene expression and protein synthesis related to muscle anabolism and metabolism (Zambon et al. 2003; Schroder & Esser 2013). Early morning resistance loading has been proposed to induce significantly higher between-subject variation in some muscle growth- or metabolism-related signaling pathways compared to the same acute loading later in the day (Sedliak et al. 2017, 2013). Therefore, strength training in the morning may not provide an optimal stimulus for some individuals. It is of course questionable to what extent there is causality between high morning between-subject variability in the acute responses (cell signaling) and long-term adaptations (cell hypertrophy) (Sedliak et al. 2017). In addition, whereas training in the evening hours appears to augment some markers of hypertrophic potential (elevated IGPBP-3, suppressed C and a superior cellular environment), training in the morning may further increase the catabolic environment through the already elevated C concentrations and thereby attenuate the protein synthesis (Burley et al. 2016). Therefore, performing resistance exercise when catabolic hormones are diminished (i.e. in the evening hours) has been suggested to optimize muscle hypertrophy development (Burley et al. 2016).

Because adaptations to resistance training are dependent on several factors of the acute variables of the training program (e.g. training frequencies, number of set, rest period between sets, type of exercise), further research is needed to clarify the issue of the temporal specificity of the strength training (Chtourou & Souissi 2012). Based upon these results presented above, the time-of-day-effect on physical performances is, however, a factor that must be taken into account by coaches, athletes and sport scientists (Chtourou & Souissi 2012).

2.4.6 Time-of-day-specific adaptations to endurance training

The literature about time-of-day-specific adaptations to endurance training is sparse. Hill et al. (1989) studied young healthy male and female college student and suggested that adaptations in the anaerobic threshold were time-of-day-specific, but not in other tested variables such as VO_2max, VCO_2max or respiratory exchange ratio. The authors showed that, after 6 weeks of training, the ventilator anaerobic threshold was higher in the morning than in the evening for the morning training group, and it was higher in the evening for the evening training group (Hill et al. 1989). Torii et al. (1992) found that young healthy sedentary men 4 weeks of endurance training enhanced VO_2max only in the p.m. training group. However, VO_2max testing was performed only in the afternoon, both before and after the training for the a.m., p.m. and evening training groups. Although Torii et al. (1992) suggested that adaptations to training were greater when aerobic training was performed in the afternoon than other times, more testing times would have been relevant. Likewise, Hill et al. (1998) showed a temporal specificity after 5 weeks of high-intensity training designed to increase time to exhaustion during an exhaustive cycling test in female college students. The evening training group had greater work capacity in the evening at their regular training time, however, performance of the morning training group was not training time dependent (Hill et al. 1998). The authors suggested that there was a significant effect of the time of day of training in the adaptation of cycling performance (Hill et al. 1998). However, because both morning and the evening training groups were not evaluated before training, the authors could not compare the improvement from before to after the regular training at a particular time-of-day (Chtourou & Souissi 2012).

2.4.7 Adaptations in the endocrine system after time-of-day-specific training

The effect of time-of-day-specific training on endocrine system has received little attention in the previous research studies. Sedliak and co-workers are the few, who have observed the effects of regular training at a certain time-of-day on hormonal profiles. They showed that C concentrations decreased significantly in subjects, who regularly trained in the morning hours (Sedliak et al. 2007), while resting serum T concentrations remained unchanged in both morning and evening training groups (Sedliak et al. 2007, 2017). However, authors suggested that this reduction in serum C may presumably be because of a decreased anticipatory psychological stress before the morning training sessions, rather than to adaptations induced by a regular training at this time-of-day, since the same phenomenon was present also in the control group (Sedliak et al. 2007). It was not a surprise that resting total T concentrations were not affected by time-of-day-specific training as resistance training with the training frequency of 2-3 sessions per week allows for at least 48 hours of recovery (Sedliak et al. 2017). This, however, leaves sufficient time for photic and social contact factors to reset any possible phase-shifting in hormonal profiles, caused by the exercise training (Duffy et al. 1996; Teo et al. 2011a). Due to the lack of substantial evidence on the effects of exercise on the circadian profile of T and C, more studies are required to see whether exercise can exert a strong enough influence to alter the circadian rhythms of T and C or not (Teo et al. 2011a). In addition, the effects of different training stimulus (i.e. type of exercise, intensity and work/rest ratio) and longer training periods (> 10 weeks) should be monitored. However, the adaptations to time-of-day specific combined strength and endurance training have not received attention in previous literature.

2.5 Effects of exercise training on sleep, quality of life, time management, motivation and self-esteem

Regular physical activity has been associated in addition to beneficial changes in physical performance, also with benefits in mental health (Garber et al. 2011). In addition to regular exercise training, it is well known that appropriate sleep is needed to maintain physiological as well as psychological health (Buxton et al. 2010; Meier-Ewert et al. 2004; Youngstedt 2005; Pilcher & Huffcutt 1996; Spiegel et al. 1999). However, in the modern busy world people may lack of motivation to be engaged to long-term exercise training (Wilson and Brookfield 2009) due to the competing demands on their time from work and family obligations (Teixeira et al. 2012). Therefore, humans frequently alter the timing and duration of sleep in exchange for other activities (Goel et al. 2013).

2.5.1 Effects of exercise training on sleep

In general, it has been proposed that regular aerobic as well as strength training has positive effects on subjective sleep quality in clinical populations as well as in healthy fit individuals (Driver & Taylor 2000; Kubitz et al. 1996; Uchida et al. 2012; Kredlow et al. 2015; Kovacevic et al. 2018), when moderate-intensity exercise at doses that approximate public health guidelines have been used. Others, however, have suggested that, when only young adults were considered, strength or endurance exercise has only minor beneficial effects on total sleep efficiency (Stepanski & Wyatt 2003; Youngstedt 2005; Kovacevic et al. 2018). Previous literature about the effects of combined strength and endurance training on sleep is sparse; however, Kovacevic et al. (2018) have suggested that the addition of resistance exercise to aerobic training may offer no additional benefit for objective sleep quality. However, in general it is not clear how large the benefits from the exercise training are, and to what extent variables such as exercise type and duration affect these benefits (Kredlow et al. 2015).

In addition, time-of-day when exercise training is performed has been suggested to have an effect on the quality and amount of sleep obtained (Sargent et al. 2014a). For healthy adults, 7-8 hours of sleep per night have been recommended (Chennaoui et al. 2015). Some studies, however, have proposed that early morning training sessions may limit the effectiveness of training because of sleep restriction (Sargent et al. 2014b), whereas others have shown that training in the evening close to bed time may in turn disturb the subsequent night's sleep (Irish et al. 2015). However, newer research suggests that acute exercise within few hours of bedtime may actually be beneficial not detrimental for sleep quality (Kredlow et al. 2015).

2.5.2 Effects of exercise training on health related quality of life

Regular physical exercise has been characterized as a positive health behavior, which has in addition to physiological also psychological benefits (Rosa et al. 2015; Penedo & Dahn 2005). A physically active lifestyle has been shown to enhance feelings of "energy", well-being, quality of life, and cognitive function (Garber et al. 2011). Quality of life or health-related quality of life (HRQoL), as used in the present study, refers to perceived physical, mental and social components (Bize et al. 2007; Häkkinen et al. 2010; Sillanpää et al. 2012). The existing cross-sectional evidence suggests that higher physical activity levels are favorably associated with better scores in various HRQoL dimensions (e.g. physical functioning and vitality) (Bize et al. 2007), especially in populations with chronic conditions (Joos et al. 2004; Oldervoll et al. 2004). Yet there are few studies in the general population suggesting that physical activity with at least moderate intensity is beneficial on HRQoL (Häkkinen et al. 2010; Laforge et al. 1999; Brown et al. 2004). Although the majority of the studies have assessed the relation of leisure time physical activity to HRQoL, then the best results have been reported when using multimodal training programs (e.g. combining strength and endurance) as it simultaneously addresses multiple measures associated with quality of life and well-being (Atlantis et al. 2004; Sillanpää et al. 2012).

2.5.3 Effects of exercise training on time management, intrinsic motivation and self-esteem

People in modern societies know the benefits of physical activity (Crombie et al. 2004), however, the lack of motivation and commitment may disturb the long-term engagement to exercise training (Wilson & Brookfield 2009). Lack of time due to the competing demands on their time from work and family obligation (Teixeira et al. 2012) have been reported as the main reason not to be engaged into regular exercise training. However, literature is sparse whether the time management could be improved by strategically scheduling training session to the morning or evening hours. Due to the inherent satisfaction, intrinsic motivation has been shown to be a critical parameter to start (Pelletier et al. 2013; Rintaugu & Ngetich 2012; Rosa et al. 2015) and be engaged to an exercise training program (Edmunds et al. 2006; Silva et al. 2011). In addition, intrinsic motivation has also been associated with psychological health and improved well-being (Teixeira et al. 2012). Psychological benefits associated with regular training have been proposed to influence one's self-concept such that people develop a higher degree of physical competence and physical acceptance (Alfermann & Stoll 2000). This subsequently leads to heightened perceptions of global self-esteem (Sonstroem 1984). High self-esteem has been found to be related to a range of positive qualities such as life satisfaction, positive social adjustment, independence, adaptability, resilience to stress and a high level of achievement in education and work (Biddle et al. 2000, p. 88). Therefore, self-esteem has been considered as one of the strongest predictors of subjective well-being and an important aspect of quality of life and mental health (Biddle et al. 2000, p. 88).

3 PURPOSE OF THE THESIS

The purpose of the present thesis was twofold. Firstly, to examine the diurnal variation in strength and endurance performance, a cross-sectional design was used to study the differences in morning and evening performance. Secondly, the aim was to examine neuromuscular, cardiorespiratory, hormonal as well as psychological adaptations to time-of-day-specific same-session combined strength and endurance training in men after a 24-week training period. Especially the effect of training time (morning vs. evening) and strength and endurance training order were of interest. The specific aims of the present study were:

1. To investigate the diurnal variations in cardiorespiratory and neuromuscular performance along with electromyographic activity, voluntary activation level and serum hormone concentrations.

2. To investigate the effects of training time and strength and endurance training order on adaptation in neuromuscular performance, muscle hypertrophy and serum hormone concentrations and their relationship to strength and endurance performance after time-of-day-specific combined training.

3. To investigate the effects of training time and strength and endurance training order on adaptations in cardiorespiratory performance after time-of-day-specific combined strength and endurance training.

4. To investigate the effects of time-of-day-specific combined strength and endurance training on sleep, quality of life, perception of time management, motivation and self-esteem.

4 METHODS

4.1 Participants

Seventy-two men who were previously untrained but physically active and had similar backgrounds of both health status and physical condition were recruited for the study. They had no history of systematic strength and/or endurance training at least over the past year. The target group was free of acute and chronic illnesses and reported not to use any medications that could influence on their hormonal, neuromuscular or cardiorespiratory system, circadian rhythms or sleep cycle. In addition, a cardiologist reviewed each participant's health questionnaire and ECG. Participants' morningness and eveningness was assessed according to the Munich Chronotype Questionnaire (Roenneberg et al. 2003) based on which none of the participants belonged to an extreme chronotype. Out of 72 participants, there were 27 neutral types, 5 slight late and 1 moderate late as well as 30 slight early types and 9 moderate early types. None of the selected participants worked in night shifts. After participants were informed of the purpose and risks of the study, they provided written consent before participation. This study complied with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Jyväskylä.

During the 24-week intervention period some of the participants dropped out due to medical issues, lack of motivation or personal reasons, i.e. change of residence. Only the participants who successfully completed at least 90% of the entire 24-week training intervention were included in the longitudinal data analyses (n=52) (articles II-IV).

4.2 Experimental design

The combined strength and endurance training period lasted for 24 weeks and were surrounded by the testing periods which took place before training (pre-measurements), after 12 weeks of training (mid-measurements) and after completing all 24 weeks (post-measurements) of combined training (article II, III, IV). The pre-measurements took place in September/October before the training intervention, mid-measurements were conducted after 12 weeks of training in January/February and post-measurements in May/June after completing 24 weeks of combined training. Data from the pre-measurements was used in the cross-sectional design to investigate diurnal rhythms in strength (article I) and endurance performance (FIGURE 10).

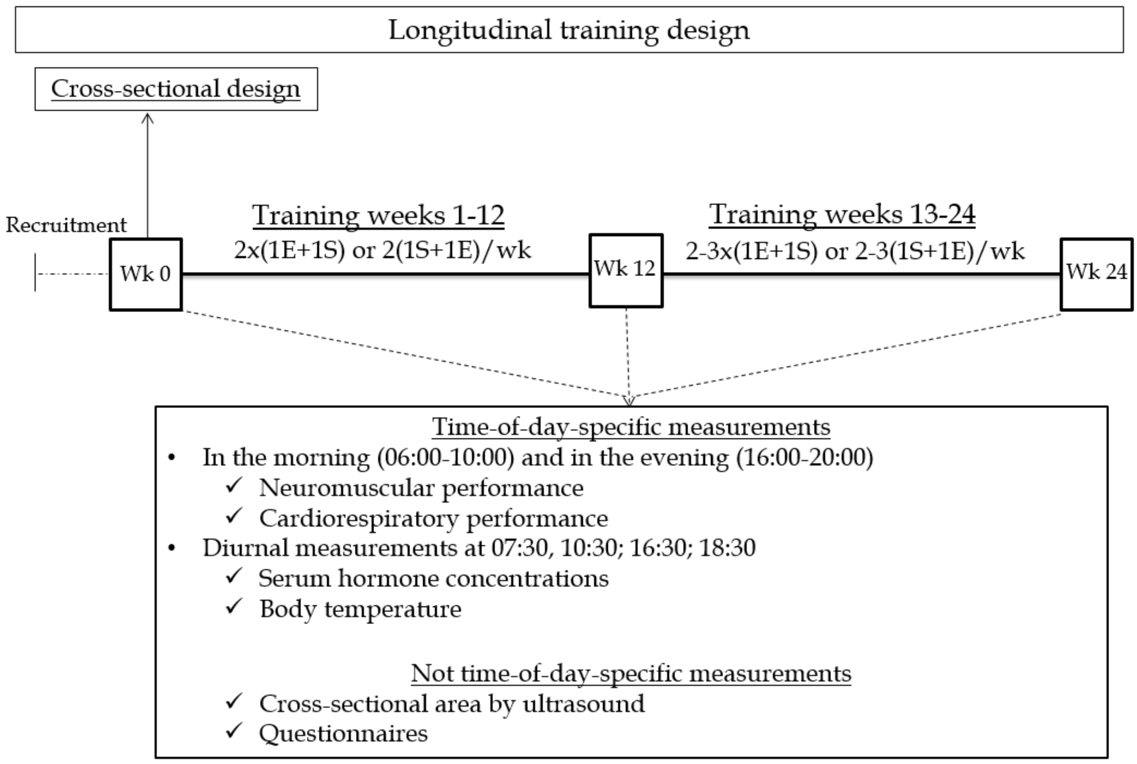

FIGURE 10 Study design and measurements. E+S=endurance training before strength training; S+E=strength training before endurance training.

Following the basal measurements for strength and endurance performance, participants were matched into four training groups based on the anthropometrics (TABLE 5) and strength and endurance performance (TABLE 10, 12): (i) training in the morning (m) and performing endurance (E) training always before the strength (S) training (mE+S, n=9), (ii) training in the morning with strength always preceding endurance training (mS+E, n=9), (iii) training in the evening (e) and performing endurance before strength training (eE+S, n=12), (iv) training in the evening with strength always preceding endurance training (eS+E, n=12). The control group (CG, n=10) was asked to maintain their pre-experimental physical activity level throughout the study.

TABLE 5 Basal anthropometric characteristic (mean ± SD) of the participants.

	Age ± SD (years)	Height ± SD (m)	Weight ± SD (kg)	BMI ± SD ($kg \cdot m^{-2}$)
mE+S (n=9)	36.1 ± 6.5	1.80 ± 0.04	86.1 ± 8.9	26.5 ± 2.1
mS+E (n=9)	30.8 ± 5.0	1.82 ± 0.08	82.0 ± 14.0	24.8 ± 3.8
eE+S (n=12)	31.4 ± 4.6	1.80 ± 0.07	78.0 ± 8.5	24.1 ± 2.5
eS+E (n=12)	31.4 ± 6.5	1.81 ± 0.06	80.0 ± 10.6	24.5 ± 2.7
Controls (n=10)	32.4 ± 4.9	1.81 ± 0.08	79.0 ± 12.8	23.9 ± 2.7

m=training in the morning; e=training in the evening; E+S=endurance before strength training; S+E=strength before endurance

A nutritional information lecture was held for the participants before the start of the study to instruct them to consume nutrients according to the national guidelines. Participants were asked to keep their daily energy intake and habitual physical activity level constant throughout the intervention period and avoid any additional strength and/or endurance training.

4.3 Measurements

All the participants performed strength and endurance measurements twice: once in the morning (between 06:30 ± 00:30 and 09:30 ± 00:30) and once in the evening (between 16:30 ± 00:30 and 19:30 ± 00:30). The morning and evening measurements were carried out in a random order across all participants. Within individuals, the tests were always carried out in the same order and at the same time-of-day (±1h) at pre-, mid- and post-measurements. The time interval between two consecutive strength and/or endurance performance measurements was at least 36 hours and participants were asked to avoid any unnecessary physical activity. The participants received instructions to follow their usual sleeping habits with a minimum of 6 hours of sleep taken on the night preceding each measurement session. They were asked to avoid alcohol for 24 hours and caffeine for 12 hours before the endurance and strength performance tests. They were also requested to choose the least physically demanding option when coming to the measurements (preferably car or public transport). In case it was not possible, they were asked to arrive 15 min earlier and rest before the measurements. The last training session and the first measurement were always separated by a minimum of two and maximum of four days.

4.3.1 Neuromuscular performance measurements

4.3.1.1 Familiarization session

Before the start of the measurements, a familiarization testing session was carried out for all participants on a non-training-specific time-of-day. During the familiarization session participants were familiarized with the neuromuscular testing procedures and the device settings were recorded for the further measurements for each participant. The placement of electromyographic (EMG) electrodes (I, III) was marked with indelible ink tattoos according to the SENIAM guidelines (Hermens et al. 2000) to ensure repeatable electrode positioning (Häkkinen & Komi 1983). A small mark was also tattooed to the mid-point (between the greater trochanter of the femur and lateral joint space) of the lateral side of the right thigh to ensure the repeatability of the ultrasound measurements for muscle cross-sectional area (II) throughout the study. All the force measurements were performed in the same testing session in the following order described below.

4.3.1.2 Bilateral isometric leg press force

Maximal bilateral isometric leg press force (MVC_{LP}) (I; IV) was measured using a horizontal dynamometer (designed and manufactured by the Department of Biology of Physical Activity, University of Jyväskylä, Finland) at the knee angle of 107° (180 ° = knee fully extended) (Häkkinen et al. 1998). All the subjects were allowed to have three warm-up trials for the isometric leg press, which also served as a short warm-up. Participants were instructed to generate maximum force as rapidly as possible against the force plate for a duration of 2-4 seconds. Participants were verbally encouraged to perform their maximal. A minimum of three and maximum of five trials (if the maximal force level continued to increase by more than 5%) were used to determine the maximal isometric leg extension with one minute break separating the trials. Isometric force signals were passed in real-time to an analog-to-digital (AD) converter (Micro 1401, Cambridge Electronic Design, UK). The trial with the highest peak force was selected for further analysis. Force signals were sampled at 2000 Hz and low-pass filtered (20 Hz).

4.3.1.3 Electromyography during isometric leg press

Muscle activity during MVC_{LP} was recorded through surface electromyography (EMG_{LP}) (I) from vastus lateralis (VL) and vastus medialis (VM) muscles of the right leg. The raw EMG signal from the measurement was amplified by 1000 and sampled at 3000 Hz. The signals were passed from a portable transmitter (Telemyo 2400R, Noraxon, Scottsdale, AZ, USA) from which the signal was relayed to a desktop computer via an AD converter (Micro 1401, Cambridge Electronic Design, UK). Analysis of the isometric EMG was performed using a customized script (Signal 4.10, Cambridge Electronic Design Ltd., Cambridge, UK) and converted to integrated EMG. Maximum EMG was determined from the 500-1500 ms time period of contraction representing the peak force phase.

4.3.1.4 Maximal unilateral isometric knee extension force

Maximum voluntary isometric force of knee extensors (MVC_{KE}) (I) was measured using unilateral knee extension of the right leg at the knee angle of 107° (180 ° = knee fully extended) (Häkkinen et al. 1998). The participants were secured to a sitting position in the modified knee extension device (David 200; David Health Solutions Ltd., Helsinki, Finland) with a safety belt in the hip area. The correct position was endured by the adjustable back support, lever arm and ankle pad. Left leg rested in horizontal position on a support. The upper extremities were placed next to the body holding handgrips. Participants were instructed to generate maximum force rapidly against the ankle pad and maintain it for 2-4 seconds. A minimum of three up to five trails (if the maximal force level continued to increase by more than 5%) were used to determine the maximal isometric leg extension with a one-minute resting period separating the trials. The trial with the highest peak force was taken for further analysis. The force signal was low-pass filtered (20 Hz) and analyzed (Signal 4.10, Cambridge Electronic Design Ltd., Cambridge, UK).

4.3.1.5 Maximal unilateral knee extension force and muscle stimulation

Maximal voluntary unilateral isometric knee extension force (MVC_{VA}) (I; III) was measured using the device designed and manufactured in the Department of Biology of Physical Activity (University of Jyväskylä, Finland). The participant was seated with knee angle 107° in the right leg and left leg rested in horizontal position on a chair. Hip and knee angles were firmly secured by a seatbelt at the hip, strapped pad over the right knee and with a Velcro strap above the right ankle. Participants were asked to perform three maximal trials by increasing force gradually over 3 seconds. The trial with the highest force was used for further analysis. The force signal was sampled at 2000 Hz and low-pass filtered (20 Hz). Maximal force was manually analyzed on Signal 4.04 (Cambridge Electronic Design, UK).

To assess the voluntary activation percentage (VA%) (I; III) of the quadriceps femoris muscle, the interpolated twitch technique (Merton 1954) was used to stimulate the right quadriceps muscles during MVC_{VA}. Four galvanically paired self-adhesive electrodes (7 cm PolarTrode; Polar Frost USA; Anaheim, CA; USA) were placed on the proximal and mid-region of the quadriceps muscle belly of the right thigh. The current of single 1-ms rectangular pulses were increased progressively by using a constant-current stimulator (Model DS7AH, Digitimer Ltd, UK) in 5mA steps until a plateau in the passive twitch response was observed. 25% of the stimulation current was added to ensure maximal effect for the knee extension trials. This supramaximal single-pulse stimulation was delivered to the muscle at rest 3 seconds before the voluntary knee extension, during the plateau of voluntary peak knee extension force and 5 seconds after the cessation of contraction. VA% was calculated according to the formula by Bellemare & Bigland-Ritchie (1984):

$$\text{Activation level (\%)} = [1-(P_{ts}/P_t)] \cdot 100,$$

where P_{ts} is the amplitude of the twitch elicited by the electrical stimulation on top of the maximal voluntary contraction and P_t is the amplitude of the twitch delivered to the muscle 5 seconds after the voluntary contraction.

4.3.1.6 Electromyography during isometric unilateral knee extension

Muscle activity during MVC_{VA} was recorded through surface electromyography (EMG_{VA}) (I; III) from VL and VM of the right leg. EMG was collected from the maximum force level over the 500 ms time period, immediately before the superimposed twitch. EMG was amplified by a factor of 1000 (NeuroLog Systems NL844, Digitimer Ltd, UK) and sampled at a frequency of 2000 Hz. The raw EMG signal was band-bass filtered (20-350 Hz) and, due to technical reasons, manually converted to root mean square on Signal 4.04 software (Cambridge Electronic Design, UK).

4.3.1.7 Dynamic bilateral leg press one repetition maximum

Maximal bilateral concentric leg press strength (1RM) (II) was measured by the horizontal leg press (knee angle ~60°) (David 210, David Health Solutions Ltd., Helsinki, Finland). Before commencing maximum efforts, a short warm-up was performed (5x70%; 3x80% and 2x90% of the predicted 1 RM). A minimum of three and maximum of five trials of single repetitions were allowed. Each trial was separated with one minute of rest. The testing continued until the participant was unable to extend legs to a full extension of 180°.

4.3.2 Cross-sectional area of m. vastus lateralis

Cross-sectional area of VL (CSA) (II) was assessed by a B-mode axial-plane ultrasound (SSD-α10, Aloka Co Ltd, Tokyo, Japan) with the extended-field-of-view mode (23-Hz sampling frequency) using a 10 MHz linear array probe. This method has been shown to reliably detect training-induced changes in skeletal muscle, as the ultrasound CSA has been shown to be comparable with the data from the magnetic resonance imaging (Ahtiainen et al. 2010), which is considered as the golden standard in body composition measurements. Three panoramic images were taken at 50% of femur length (marked during the familiarization session). During the measurements participants laid in the supine position, with lower limbs extended and resting in a Styrofoam knee support. A line, representing the mid-point, was drawn across the thigh, perpendicular to the measurement table. The probe was manually moved in an axial plane from the lateral to the medial side of the thigh along the drawn line. Images were analyzed with the ImageJ software (National Institute of Health, USA, version 1.44) by manually tracing along the border of the m. vastus lateralis. The mean of the two closest values was used for further statistical analysis. The measurements always took place in the morning at the same time of the day (±1h). All the measurements and analysis were done by the same technician.

4.3.3 Cardiorespiratory performance measurement

Time to exhaustion (T_{exh}) (II), peak power in Watts (Wpeak) (III), and maximal oxygen uptake (VO_2max) (IV) were measured during the graded maximal aerobic cycling test to volitional exhaustion on a mechanically braked cycle ergometer (Ergomedic 839E, Monark Exercise AB, Sweden). Test protocol started with an intensity of 50 W and was increased by 25 W every two minutes. Pedaling frequency was sustained at 70 $r \cdot min^{-1}$ (rpm) throughout the test. The participants were encouraged by the testing personnel to continue cycling until volitional exhaustion or until the participants failed to keep up the required rpm for longer than 15 seconds. Peak wattage achieved during the cycling test was calculated with the following formula:

$$Wpeak = Wcom + (t/120)*25,$$

where Wcom is the last cycling power completed and is the time in seconds the non-completed power was maintained (Kuipers et al. 1985).

Oxygen uptake was determined continuously breath-by-breath throughout the incremental cycling test by using a gas analyzer (MasterScreen CPX, CareFusion, Hoechberg, Germany). VO_2max used for further analysis was calculated as the highest VO_2 value averaged over 60 seconds.

4.3.4 Serum hormone concentrations

Venous blood samples of testosterone (T) and cortisol (C) were collected during one day at 7:30 ± 00:30, 9:30 ± 00:30, 16:30 ± 00:30 and 18:30 ± 00:30 at pre-, mid- and post-measurements (I, II). Participants were asked to keep two days of rest before the day when the blood samples were drawn. Participants had to arrive to the lab 10-15 min before the scheduled time and then sit quietly on a chair until the blood samples were collected. Physical activity during that day was also asked to be kept as low as possible; however, the subjects were allowed to leave the lab in between the blood samples drawn and have a normal working day. Participants were asked to fast 12 hours before the first blood sample, after which a standardized breakfast was provided. They were instructed to consume another light meal at around 12:00 - 13:00, which had to follow previously given instructions about the relative content of protein (10-20%), fat (25-35%) and carbohydrate (50-60%). Thereafter, participants were asked to avoid food until the last two blood samples (16:30 ± 00:30 and 18:30 ± 00:30) were drawn. For possible correlations between the serum hormone concentration and force production as well as for the between-group comparison the mean of the two morning or two of evening measurements was used (I).

Venous blood samples (~10 ml) were collected by a qualified laboratory technician from an antecubital vein with a vacutainer and test tubes (Vacuette, GEiner Bio-OneGmbH, Kremsmünster, Austria) containing appropriate preservatives. Samples were centrifuged at 3.500 rpm (Heraus Megafuge 1.0 R, Gendro Laboratory Products, Hanau, Germany) for 10 min, plasma harvested and all samples were stored at −80°C until assayed. Analysis of total serum T and C was performed using chemical luminescence techniques (Immunlite 2000, Simens Healthcare, Diagnostics Products Ltd., Llanberies, UK) and hormone specific immunoassay kits (Siemens, New York, USA). The sensitivity for serum hormones was: T 0.5 $nmol \cdot l^{-1}$ and C 5.5 $nmol \cdot l^{-1}$. The intra-assay coefficient of variation was 8.3% and 5.3% for T and C, respectively. The inter-assay coefficient of variation was 9.1% for T and 7.2% for C. In addition, the T/C ratio was calculated.

4.3.5 Body temperature

Intra-aural body temperature was measured 4 times during one day at 07:30 ± 00:30, 09:30 ± 00:30, 16:30 ± 00:30 and 18:30 ± 00:30 (I) during the same session when venous blood samples were collected. Participants had to arrive to the lab 10-15 min before the scheduled time and then sit quietly on a chair until the body temperature was measured by digital thermometer (ThermoScan PRO, BRAUN, Southborough, MA, USA). Two trials were required. The mean of two values were used for further statistical analyses.

4.3.6 Self-reported questionnaires

At each measurement time point participants were asked to fill out questionnaires assessing their sleep, fatigue and general well-being, time management, motivation to participate in exercise training, self-esteem and health related quality of life (IV). The questionnaires were filled in on different days in the laboratory conditions when participants came for the physical performance tests. All questionnaires were respectively filled out at the same time of the day at every measurement point.

4.3.6.1 Pittsburgh Sleep Quality Index

Sleep quality over the previous month was assessed by the Finnish version of Pittsburgh Sleep Quality Index (PQSI) (Buysse et al. 1989). The 19 self-rated questions (items) formed seven sleep quality component scores, including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, sleep medication use, and daytime dysfunction. Each component score ranged from 0 to 3. The seven component scores were summed to yield the total PSQI score, which has a range of 0-21. Higher scores indicated worse sleep quality. Scores higher than 5 indicate poor sleep quality.

4.3.6.2 Time management

Time management behavior was measured with selected and adapted items from the Macan's (1990) 33-item Time Management Behavior Scale. The present self-report questionnaire consisted of 8 questions about time management and exercise training on the five point Likert scale from 1-5, ranging from strongly disagree (1) to strongly agree (5). Negatively worded items were reverse-scored so that a higher score indicated greater time management skills. The total TMB score was calculated as the mean of the scores of the individual questions. Grater score indicated better time management behavior. The list of eight items used in the present study:

1. My training regimen leaves me sufficient time to rest
2. My training regimen leaves me sufficient time for other leisure-time activities
3. My training regimen leaves me sufficient time for my family/friends
4. I am having difficulties combining my training regimen with other aspects of my life *

5. I am able to manage my time
6. I have considered quitting my training regimen due to a lack of time *
7. My training regimen makes me busy *
8. I can usually focus on my training regimen

* indicating a negatively worded items which was reverse-scored for analysis.

4.3.6.3 Intrinsic motivation by Sport Motivation Scale

Intrinsic motivation was assessed by the Finnish version of Sport Motivation Scale (Pelletier et al. 1995). The scale composes of 28 items that are subdivided into subscales assessing intrinsic motivation, extrinsic motivation and amotivation. In the present study only the 12 items of the intrinsic motivation were assessed. Respondents were asked to evaluate each item on a five-point Likert-type scale ranging from strongly agree (5) to strongly disagree (1). Intrinsic motivation was calculated by a composite score from the three intrinsic motivation subscales. A greater score indicated a higher intrinsic motivation.

4.3.6.4 Rosenberg Self-Esteem questionnaire

The Rosenberg Self Esteem Scale (Rosenberg et al. 1995) was used to measure global sense of self-worth. This is a 10-item self-report scale with positively and negatively worded statements answered on a four point Likert scale from strongly agree to strongly disagree. Five of the items comprise positive self-evaluations, such as self-confidence, self-efficacy, and self-worth. The rest of five items comprise negative self-evaluations. Negatively worded items were reverse-scored. A measure of self-esteem was obtained by summing up the scores obtained in the scale's items, then recording five items with the reverse score. The sum of these scores ranges from 10 to 40. Higher scores indicate higher self-esteem, while scores less than 15 are associated with low self-esteem, scores between 15 and 25 with average or normal level self-esteem and scores above 25 high self-esteem.

4.3.6.5 RAND-36-item health survey

Health related quality of life (HRQoL) was assessed by using the Finnish RAND 36-item health survey 1.0 (Aalto et al. 1999). 36 items reflecting functioning and well-being in the RAND-36 are combined into eight domains: physical functioning, role limitations caused by physical health problems (role physical), role limitations caused by an emotional problem (role emotional), vitality, mental health, social functioning, bodily pain and general health perception. These eight health domains were calculated from the 36 questions as instructed by the RAND-36-item health survey (Aalto et al. 1999) based on a two-step protocol. First, pre-coded numeric values were recoded per the scoring key. Each item was scored on the 0 to 100 range with higher scores indicating higher HRQoL, so that the scores represent the percentage of total possible score achieved. In step 2, items in the same scale were averaged together to create the 8 scale score.

4.4 Grouping

4.4.1 Separation to high morning performance types and high evening performance types (I)

To classify the high morning performance types (HMPT), high evening performance types (HEPT) and the neutral types (NT), the mean of MVC_{LP} and MVC_{KE} morning to evening differences (expressed as percentage of morning values) was used (I). The criteria for determining the different types was set based on the whole group mean morning to evening differences, with 4% higher values in the evening, for both, MVC_{LP} and MVC_{KE}. HMPT were expected to show similar 4% morning to evening difference, with higher values observed in the morning. For HEPT the criterion was set so, that double of the morning to evening difference observed in the large group exists (8%), with higher values observed in the evening. Based on that, any participant that demonstrated morning performance (mean of MVC_{LP} and MVC_{KE} morning to evening difference) at least 4% greater than his evening performance was classified as HMPT. Any participant that demonstrated evening performance (mean of MVC_{LP} and MVC_{KE} morning to evening difference) at least 8% greater than his morning performance was classified as HEPT. All the remaining participants were determined as NT. None of the participants included to the HMPT group showed higher evening force values for either MVC_{LP} or MVC_{KE} compared to the morning values and none of the participants in the HEPT group showed higher morning force values for either MVC_{LP} or MVC_{KE} compared to the evening values. Out of all participants, 8 were classified as HMPT, 19 as HEPT and 45 as NT. This grouping was used to analyze morning to evening changes in neuromuscular as well as cardiorespiratory performance.

4.4.2 Grouping based on strength and endurance training order or time-of-day of training (II, III, IV)

When time-of-day of training did not have significant effect to the training adaptations, data from the mS+E and eS+E groups were combined and presented as $S+E_{m+e}$ and the data from mE+S and eE+S presented as $E+S_{m+e}$. Similarly, when order of combined strength and endurance training did not have an effect on the training adaptation, data from mS+E and mE+S was presented for combined morning group (MG) and data from eS+E and eE+S for combined evening group (EG).

4.5 Strength and endurance training programs

The present training program consisted of two 12-week progressive same-session combined strength and endurance training periods, which were performed either in the morning or in the evening. The morning training groups (mE+S and mS+E) performed all training sessions between 06:30-10:00, while the evening training groups (eE+S and eS+E) performed their training sessions between 16:30-20:00. The training programs were identical for the E+S and S+E groups independent of the training time, only the sequence of strength and endurance training was reversed. Endurance and strength training were combined into one training session so that no more than a 5-10 minute break was allowed between two training sections. The duration of the combined endurance and strength training sessions progressively increased from 60 to 120 minutes. During the first 12 weeks (wks 1-12) participants trained two times per week [2x(1S+1E) or 2x(1E+1S)] and during the second 12-week training period (wks 13-24) all participants performed 5 training sessions in two weeks [5x(1S+1E) or 5x (1E+1S)] to allow further progression in training adaptations. All the training sessions were supervised.

Strength training consisted of exercises that aimed at improving both maximal strength and muscle hypertrophy and was planned as a whole body periodized program with the main focus on knee extensors and flexors as well as hip extensors. Each training session consisted of three lower-body exercises: bilateral dynamic leg press, seated dynamic knee extension and flexion. Four to five exercises were performed for other main muscle groups (lateral pull down, standing bilateral triceps push down, bilateral biceps curl, seated military press, or bilateral dumbbell fly, trunk flexors and extensors). Strength training was designed to improve muscular endurance during the first four weeks (circuit training), muscle hypertrophy during the subsequent four weeks (weeks 5-8) and maximal strength as well as muscle hypertrophy during the last four weeks (weeks 9-12) of the 12-week training period in all of the trained muscle groups. TABLE 6 presents a summary of strength training periodization for lower extremities. The similar periodization was repeated over the second 12-week period (weeks 13-24) with adjusted intensities for each subject to match the new strength level.

TABLE 6 Summary of the strength training program for the lower extremities.

	Training wks 1-12			Training wks 13-24		
	wks 1-4	wks 5-8	wks 9-12	wks 13-14	wks 15-20	wks 21-24
Training type	Circuit	Hyper-trophic	Maximal	Circuit	Hyper-trophic	Maximal
Intensity (% of 1RM)	40-70	70-85	75-95	50-75	75-85	80-95
Sets	2-3	3-4	3-5	3-4	3-4	3-5
Repetitions	10-20	10-15	3-8	10-15	10-15	3-8
Rest (min)	no	1.5-2	2-3	no	1.5-2	2-3

1RM = 1 repetition maximum

Endurance training was carried out on cycle ergometers and the training intensity was based on the maximum heart rate (HR_{max}) determined during the training-time-specific graded maximal incremental cycling test. For the first 12 weeks of training the pre-training test results were used. For training weeks 13-24 the training heart rate zones were readjusted according to the results obtained from the mid-measurements. Endurance training sessions averaged from 30-50 minutes. Interval (85-100% of HR_{max}) and continuous (65-80% of HR_{max}) training protocols were performed weekly. Interval training consisted of 4x4 min high-intensity intervals (85-100% of HR_{max}), which were separated by 4 min active resting periods (70% of HR_{max}). During the first training period both interval and continuous training sessions were performed once a week, whereas during the second training period, when the training frequency increased, one additional high-intensity interval training session was added. Participants were instructed to maintain a constant pedaling cadence (around 70 rpm) while the resistance on the cycle ergometer was adjusted in accordance with heart rate (Polar FT7, Polar Electro Oy, Kempele, Finland).

4.6 Statistical analysis

Descriptive data were generated for all variables and expressed as mean ± SD (I-IV) or 95% confidence interval (CI) (IV). Normality of the data was checked and subsequently confirmed using the Shapiro-Wilk test. For normally distributed data, morning to evening differences at week 0, 12 and 24 were checked by using paired samples T-tests. Within-group changes over time in the morning and in the evening were examined by using absolute values with repeated measures general linear model (GLM), where Time, with 3 levels (4 levels for hormonal data analysis) was the only factor. One-way analysis of variance (ANOVA) was used to assess time×group interactions in relative changes over time in more than two groups and Independent Samples T-test when only two groups (e.g. HMPT and HEPT) were analyzed for the between-group differences. Bonferroni post hoc procedures were applied when appropriate. Effects of the time-of-day-specific training on strength and endurance performance as well as on resting serum hormone concentrations and muscle hypertrophy were examined by a two-factor GLM with univariate ANOVA by using absolute changes. Time-of-day of the training and training order were set as fixed factors when appropriate. To analyze between-group differences in morning to evening changes, relative values were used. For non-normally distributed data, morning to evening differences at week 0, 12 and 24 in performance variables were analyzed by using Wilcoxon-Signed Rank Test and Friedman test was performed to observe significances in diurnal rhythms of serum hormone concentrations. For within-group changes over time Friedman test was used. For between-group differences Kruskal-Wallis ANOVA or Mann-Whitney U-test used, depending on the number of groups compared. A Bonferroni adjustment was applied by multiplying the pairwise p-values with the number of comparisons. Between-group analyses were performed with relative values from morning to evening differences.

Pearson correlation coefficients or Spearman correlation coefficients were calculated, respectively for normally and non-normally distributed data to assess relationships between neuromuscular performance, hormonal concentrations and/or cross-sectional are (I, II, III). Area under the curve (AUC) (II) was calculated for T and C concentrations by using the following equation:

$$AUC = [(y_1+y_2)\ /2) \times (t_1-t_2)],$$

where y_1 and y_2 are the consecutive resting T or C concentrations and *t* the time separating the two measurement time-points. AUC was calculated for the total period between 7:30 and 18:30. Effect sizes (es) for both within-group and between-group comparisons are presented as Cohen's d for the normally distributed data and for non-normally distributed data effect sizes are calculated based on the following equation:

$$es = Z/\sqrt{n},$$

where Z is the z-score and n are the number of observations on which Z is based. An effect size of ≥0.20 was considered small, ≥0.50 medium and ≥0.80 large (II, III). Statistical significance was accepted when $p<0.05$, whereas values $p\leq0.07$ were accepted as a significant trend. Analysis was performed using the Statistical Package for Social Sciences (SPSS version 22-24, Chicago, IL).

5 RESULTS

5.1 Diurnal rhythms in body temperature, serum hormone concentrations and in strength and endurance performance

5.1.1 Diurnal rhythms in body temperature and serum hormone concentrations

Body temperature increased significantly from 07:30 ± 00:30 to 16:30 ± 00:30 with the mean morning to evening difference of 0.2 ± 0.4 °C ($p<0.05$) (TABLE 7). Serum T and C as well as the T/C ratio showed diurnal variation, T and C demonstrating the highest concentrations in the morning (07:30 ± 00:30) and decreasing concentrations throughout the day ($p<0.001$), and the T/C ratio lowest values in the morning and increasing concentrations throughout the day ($p<0.001$) (TABLE 7).

5.1.2 Diurnal rhythms in neuromuscular performance

The average testing time in the morning was 07:26 ± 01:03 and in the evening 17:57 ± 01:13. At the whole group level, 4.4 ± 12.9% ($p<0.01$) higher force values were observed in the evening compared to the morning for MVC_{LP}, 4.3 ± 10.6% ($p<0.01$) for MVC_{KE} and a trend to reach the statistical significance level (2.8 ± 10.8%; $p=0.07$) was observed for MVC_{VA} (TABLE 8). No significant morning to evening difference was observed for the absolute EMG_{LP}, EMG_{VA} or VA%.

5.1.3 Diurnal rhythms in cardiorespiratory performance

The average testing time for endurance performance was 07:42 h ± 01:40 in the morning and 17:40 ± 01:40 in the evening. At whole group level, a significant morning to evening difference was observed in Wpeak and T_{exh} with, respectively, 1.3 ± 4.6% ($p<0.05$) and 1.4 ± 5.2% ($p<0.05$) higher values observed in the evening compared to the morning (TABLE 8). VO_2max did not show any significant morning to evening difference.

TABLE 7 Absolute values (mean ± SD) for resting body temperature, serum testosterone, cortisol and T/C ratio (n=72) at baseline.

	7:30±00:30	9:30±00:30	16:30±00:30	18:30±00:30	Diurnal changes
Body temperature (°C)	36.2 ± 0.4	36.3 ± 0.3	36.3 ± 0.4	36.2 ± 0.5	7:30<16:30* 16:30>18:30$
T (nmol/L)	13.8 ± 3.6	11.6 ± 3.7	10.7 ± 3.3	11.0 ± 3.4	7:30>9:30,16:30 & 18:30***
C (nmol/L)	409.7 ± 109.7	258.5 ± 85.3	197.1 ± 96.2	129.5 ± 72.0	7:30>9:30,16:30 &18:30*** 9:30>16:30#&18:30### 16:30>18:30$$$
T/C ratio	0.036 ± 0.014	0.049 ± 0.021	0.067 ± 0.037	0.111 ± 0.075	7:30<9:30,16:30 &18:30*** 9:30<18:30### 16:30<18:30$$$

T = testosterone; C = cortisol; * sign. different from 7:30; *<0.05; ***<0.001; # sign. different from 9:30; #<0.05; ###<0.001; $ sign. different from 16:30; $<0.05; $$$<0.001

TABLE 8 Absolute values (mean ± SD) for the morning and evening measurements of neuromuscular and cardiorespiratory performances at baseline.

	Morning measurement (mean ± SD)	Evening measurement (mean ± SD)
Neuromuscular performance		
MVC_{LP} (N) (n=71)	2692 ± 713	2799 ± 759 **
MVC_{KE} (N) (n=69)	746 ±148	772 ± 142 **
MVC_{VA} (N) (n=66)	584 ± 106	597 ± 106 (*)
Voluntary activation (%) (n=62)	89 ± 6	90 ± 6
VL EMG_{LP} (mV) (n=70)	0.57 ± 0.26	0.56 ± 0.25
VM EMG_{LP} (mV) (n=70)	0.66 ± 0.33	0.66 ± 0.37
VL EMG_{VA} (mV) (n=70)	0.29 ± 0.11	0.28 ± 0.11
VM EMG_{VA} (mV) (n=70)	0.32 ± 0.15	0.31 ± 0.14
Cardiorespiratory performance		
Maximal power output (W) (n=72)	250 ± 37	253 ± 39 *
Time to exhaustion (min:sec) (n=72)	17:49 ± 02:34	18:29 ± 02:36 *
VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$) (n=69)	38.2 ± 6.3	38.1 ± 6.1

* sign. morning/evening difference; (*)<0.07; *<0.05; **<0.01

5.1.4 Diurnal rhythms in body temperature and serum hormone concentrations in the high morning performance (HMPT), high evening performance (HEPT) and neutral types (NT)

No significant increase in body temperature was observed for HMPT (36.0 ± 0.6 °C vs. 36.1 ± 0.6 °C) or for NT (36.3 ± 0.3 °C vs. 36.3 ± 0.4 °C). In HEPT, body temperature significantly increased between 07:30 ± 00:30 and 16:30 ± 00:30 from 36.0 ± 0.5 °C to 36.3 ± 0.6 °C ($p<0.01$). All three groups showed significantly higher serum T and C concentrations in the morning compared to the evening ($p<0.001$) and significantly lower T/C ratio values in the morning compared to the evening values (TABLE 9). A significant difference for morning to evening variation in the T/C ratio was found between HMPT and HEPT 67 ± 57% vs. 168 ± 166% ($p<0.05$), respectively.

TABLE 9 Serum hormone concentrations (mean ± SD) for the high morning performance, high evening performance and neutral types as the mean of two morning (7:30±00:30 and 9:30±00:30) and evening (16:30±00:30 and 18:30±00:30) measurements.

	High morning performance types (n=8)		**High evening performance types (n=19)**		**Neutral types (n=45)**	
	Morning	**Evening**	**Morning**	**Evening**	**Morning**	**Evening**
T (nmol/L)	14 ± 3	11 ± 2 **	12 ± 3	10 ± 3*	13 ± 4	11 ± 3***
C (nmol/L)	364 ± 66	194 ± 56***	335 ± 69	151 ± 72***	328 ± 94	163 ± 73***
T/C ratio	0.041 ± 0.012	0.067 ± 0.026*	0.039 ± 0.014	0.102 ± 0.072***¤	0.044 ± 0.017	0.088 ± 0.045***

* sign. morning/evening difference; T = testosterone; C = cortisol *<0.05; **<0.01; *** <0.001; ¤ sign. difference between high morning performance types and high evening performance types in m/e difference; ¤<0.05

5.1.5 Diurnal rhythms in neuromuscular performance in the high morning performance, high evening performance and neutral types

HMPT showed significantly higher force values in the morning compared to the evening ($p<0.05$) for MVC_{LP} and MVC_{KE} (lower part of FIGURE 11a). HEPT showed significantly higher force values in the evening compared to the morning ($p<0.05$) for MVC_{LP}, MVC_{KE}, MVC_{VA} and for VA% (upper part of FIGURE 11a). NT showed 2.1 ± 6.7% ($p<0.05$) higher force values in the evening compared to the morning for MVC_{LP}.

Pearson correlation did not reveal any significant correlations between the daily variations in maximal force and serum hormone levels in any of the groups.

Between-group comparison

In MVC_{LP} significant between-group differences for the morning to evening changes were observed between HEPT and HMPT (p<0.001), HMPT and NT (p<0.01) and HEPT and NT (p<0.001) (FIGURE 11a). In MVC_{KE} morning to evening changes were significantly different between HMPT and HEPT (p<0.001) (FIGURE 11a) and between HEPT and NT (p<0.001). In VA%, HEPT showed significantly different morning to evening change compared to NT (p<0.05). When the two extreme groups (HMPT vs. HEPT) were compared, then in addition to significant between-group differences in diurnal variations in force production, morning to evening changes in VL EMG_{VA} showed between-group difference (p<0.05) (FIGURE 11a). A trend (p=0.060) for between-group difference in VL EMG_{LP} was observed between HMPT who showed 10.2 ± 13.9% higher values in the morning and HEPT with 8.4 ± 24.8% higher evening values.

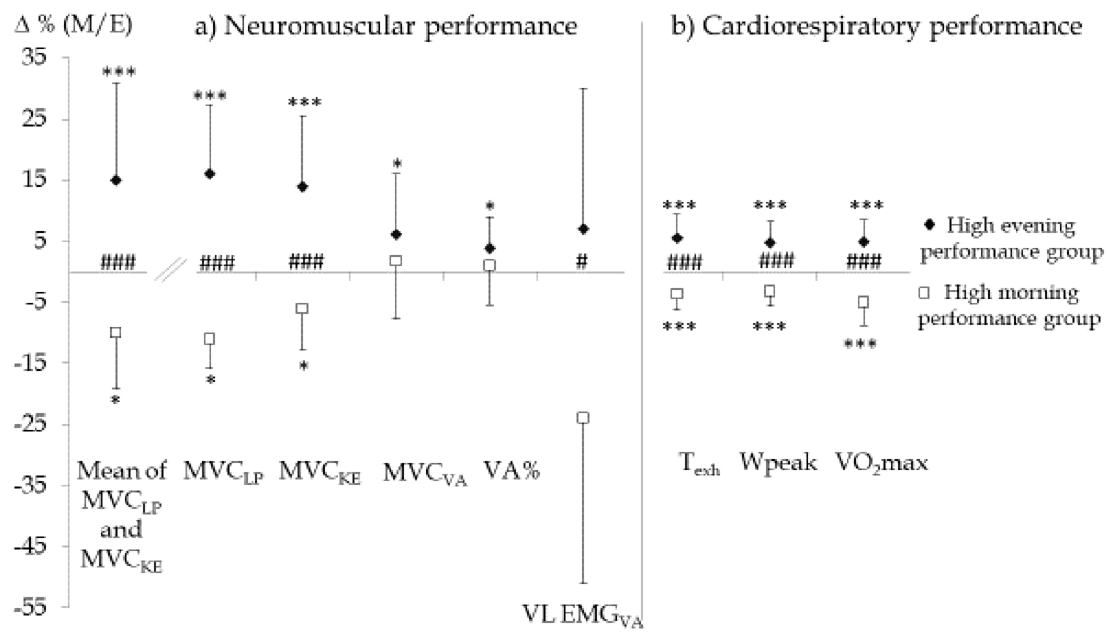

FIGURE 11 Morning/evening differences (mean ± SD) in neuromuscular (a) and cardiorespiratory (b) performance in the high evening performance group and high morning performance group. MVC_{LP}=maximal voluntary force during isometric leg press, MVC_{KE}=maximal voluntary force during isometric knee extension, MVC_{VA}=maximal voluntary force before muscle stimulation; VA%=level of voluntary activation; VL EMG_{VA}=electromyographic activity during MVC_{VA} from vastus lateralis; T_{exh}=time to exhaustion during maximal cycling test; Wpeak=peak wattage achieved during the maximal cycling test; VO_2max=maximal oxygen consumption during the maximal cycling test; * sign. morning/evening difference; *<0.05; ***<0.001; # sign. between-group difference; #<0.05; ###<0.001.

5.1.6 Diurnal rhythms in cardiorespiratory performance in the high morning performance, high evening performance and neutral types

HMPT showed significantly higher values in the morning with significant ($p<0.001$) morning to evening differences observed in Wpeak, T_{exh} and VO_2max. HEPT showed significantly higher endurance performance values in the evening with significant ($p<0.001$) morning to evening differences observed in Wpeak, T_{exh} and VO_2max (FIGURE 11b). NT did not show any significant morning to evening difference in endurance performance variables.

Between-group comparison
Significant between-group differences were observed for the morning to evening changes in T_{exh}, Wpeak and VO_2max for HEPT and HMPT ($p<0.001$) (FIGURE 11b), HMPT and NT ($p<0.01$) as well as for HEPT and NT ($p<0.01$).

5.2 Neuromuscular adaptations to time-of-day-specific combined strength and endurance training

Depending on the variable analyzed, results for the training adaptations are presented by using the grouping, which distinctly expresses the most important findings. The used groupings are: (i) all five groups separately (mE+S, mS+E, eE+S, eS+E and CG), (ii) groups combined based on the morning and evening training time (MG, EG and CG) or (iii) based on the strength and endurance training order ($E+S_{m+e}$, $S+E_{m+e}$ and CG).

5.2.1 Dynamic strength performance (1RM)

In 1RM dynamic leg press absolute values no significant diurnal fluctuation (morning vs. evening) was observed at any time point in any of the groups.

Morning measurements: Increased 1RM dynamic leg press force was observed during the first 12 weeks ($p<0.01$) and after completing 24 weeks of training ($p<0.001$) (TABLE 10). Improvements were independent of the training time and order. Significantly increased maximal dynamic strength performance was observed in CG ($p<0.05$), however, all training groups, except mE+S, increased the morning strength performance more than CG ($p<0.05$).

Evening measurements: 1RM strength increased similarly in all training groups during the first 12 weeks ($p<0.01$) and by the end of the study ($p<0.001$) (TABLE 10). Improvements in all of the training groups were larger than in CG ($p<0.05$), which showed no significant change in 1RM in the evening.

Relative changes in 1RM were similar in the morning and evening in mE+S and mS+E. A trend for statistical significance was observed in eE+S ($p=0.062$) and eS+E ($p=0.061$) for the changes between weeks 1-24 being larger in the evening.

TABLE 10 Baseline absolute values and relative changes in neuromuscular performance in the morning and in the evening.

	Morning measurement			Evening measurements		
	wk 0 absolute value	wk 0-12 Δ% ± SD	wk 0-24 Δ% ± SD	wk 0 absolute value	wk 0-12 Δ% ± SD	wk 0-24 Δ% ± SD
1 RM (kg)						
mE+S	161.8 ± 20.4	10.4 ± 4.8***	13.8 ± 5.5***	158.3 ±21.4	14.7 ±6.4***	17.5 ± 7.7***
mS+E	149.1 ± 22.3	13.5 ± 4.8***	16.9 ± 6.2***	146.6± 24.6	15.9 ± 8.0**	20.7 ± 9.0***
eE+S	142.6 ± 24.7	15.1 ± 5.8***	18.1 ± 8.1***	140.4 ± 25.6	16.2 ± 8.4***	20.5 ± 8.9***
eS+E	141.4 ± 26.0	14.1 ± 9.1***	19.3 ± 11.6***	136.9 ± 26.2	18.5 ± 10.6***	23.6 ± 12.9***
Controls	142.1 ± 25.9	5.6 ± 5.1*	5.1 ± 5.7*	144.2 ± 23.2	4.3 ± 4.0	3.2 ± 4.4
Isometric leg press force (N)						
mE+S	3013 ± 668	11.8± 10.7*	13.7 ± 21.6	3159 ± 773	10.3 ± 10.5*	12.6 ± 22.7
mS+E	2673 ± 838	5.8 ± 9.6	5.9 ± 13.9	2792 ± 938	3.6 ± 9.6	7.9 ±14.7
eE+S	2792 ± 814	7.7 ± 7.2	11.8 ± 11.2**	2913 ± 787	8.1 ± 9.1	13.6 ± 8.9*
eS+E	2498 ± 551	12.6 ± 12.7	20.0 ± 19.4**	2744 ± 663	9.9 ± 13.9	14.7 ± 21.7
Controls	2496 ± 825	-3.2 ± 10.8	-6.5 ± 11.3	2446 ± 829	2.5 ± 14.3	3.0 ± 20.1
Isometric knee extension force (N)						
mE+S	683 ± 59	-0.2 ± 10.7	3.1 ± 14.0	670 ± 101	5.9 ± 6.2	2.5 ± 9.0*
mS+E	581 ± 87	9.5 ± 16.9	5.7 ±12.4	567 ± 96	12.0 ± 14.2	14.2 ± 13.1
eE+S	578 ± 95	5.6 ± 14.2	11.1 ± 12.9	627 ± 92	-3.1 ± 13.9	5.3 ± 14.6
eS+E	569 ± 143	7.1 ± 13.3	14.9 ± 21.0	567 ± 152	13.9 ± 16.1(*)	17.2 ± 19.4*
Controls	587 ± 90	6.1 ± 8.8	-2.6 ± 10.9	615 ± 86	-2.6 ± 9.8	-7.1 ± 8.7
Voluntary activation %						
mE+S	92.7± 3.0	-2.1 ± 6.3	-0.7 ± 5.0	91.4 ± 5.7	1.6 ± 5.7	1.2 ± 4.6
mS+E	91.1 ± 5.5	2.6 ± 4.9	0.9 ± 8.1	91.3 ± 4.1	1.0 ± 5.6	2.8 ± 5.6
eE+S	91.1 ± 5.5	-1.5 ± 4.0	0.8 ± 3.0	91.2 ± 5.4	-2.1 ± 4.3	0.6 ± 4.5
eS+E	92.6 ± 2.2	-1.6 ± 6.5	-1.9 ± 3.5	90.8 ± 2.8	-2.2 ± 4.7	1.4 ± 3.2
Controls	92.2 ± 5.2	3.2 ± 5.2	0.8 ± 6.2	95.2 ± 2.3	-1.8 ± 2.4	-2.1 ± 3.5
rmsEMG VL (500ms)						
mE+S	0.25 ± 0.09	22.9 ± 41.0	37.7 ± 45.8	0.27 ± 0.09	8.6 ± 22.8	21.6 ± 50.2
mS+E	0.26 ± 0.08	36.6 ± 36.3	41.1 ± 40.0*	0.26 ± 0.06	49.4 ± 25.8*	40.9 ± 40.2
eE+S	0.24 ± 0.07	14.2 ± 29.2	22.5 ± 27.6	0.28 ± 0.08	7.8 ± 11.6	9.2 ± 22.3
eS+E	0.27 ± 0.08	14.1 ± 37.6	19.1 ± 17.6*	0.23 ± 0.05	25.0 ± 24.7	36.2 ± 25.3*
Controls	0.36 ± 0.18	24.1 ± 11.5	6.4 ± 18.9*	0.40 ± 0.21	3.6 ± 16.9	2.6 ± 40.1

* Significant with-in group change, (*)<0.07, *<0.05, **<0.01, ***<0.001

5.2.2 Isometric strength performance

5.2.2.1 Maximal isometric bilateral leg press force (MVC_{LP})

In MVC_{LP} absolute values significant diurnal variation was observed in eS+E, with higher values observed in the evening at week 0 ($p<0.01$), at week 12 ($p<0.01$) and at week 24 ($p<0.05$), but not in other training groups. When the training groups were combined based on the training time, both MG and EG showed significant morning to evening difference in MVC_{LP} at week 0 (MG: 4.3 ± 7.3%, $p<0.05$; EG: 7.5 ± 8.9%, $p<0.01$) and at week 24 (MG: 4.7 ± 7.2%, $p<0.05$; EG: 5.7 ± 9.0, $p<0.01$), with higher values observed in the evening (FIGURE 12).

Order and time-of-day of the combined strength and endurance training did not influence the adaptation in MVC_{LP} at any time point in any of the groups.

Morning measurements: MVC_{LP} significantly increased in mE+S between weeks 0-12 ($p<0.05$) and between weeks 0-24 in eE+S ($p<0.01$) and in eS+E ($p<0.01$) (TABLE 12). Changes in the training groups were larger ($p<0.05$) compared to CG.

Evening measurements: MVC_{LP} significantly increased in mE+S between weeks 0-12 ($p<0.05$) and in eE+S between weeks 0-24 ($p<0.05$) (TABLE 12). No significant between-group differences were observed in the evening at any time point.

Relative changes in MVC_{LP} were similar in the morning and evening at all time-points, independent of the groups analyzed.

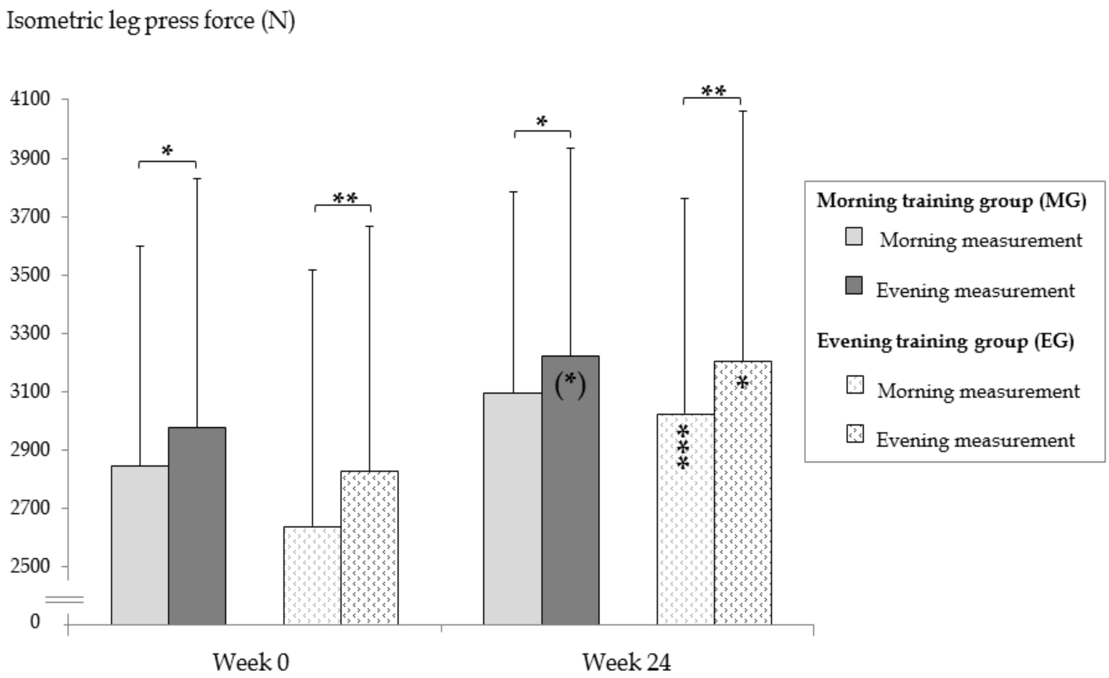

FIGURE 12 Absolute values (mean ± SD) of isometric leg press force in the morning and evening at week 0 and week 24. * changes from week 0 to week 24 or as indicated; (*) $p<0.06$, * $p<0.05$, ** $p<0.01$, $p<0.001$.

5.2.2.2 Maximal isometric knee extension force

In MVC_{VA} absolute values no significant morning to evening fluctuation was observed in any group at any measurement time point. Time-of-day of training did not have significant effect on the training adaptations and therefore, data from the mS+E and eS+E are combined and presented as $S+E_{m+e}$ and data from mE+S and eE+S presented as $E+S_{m+e}$.

Morning measurements: MVC_{VA} increased significantly over the training period in the $S+E_{m+e}$ from 574 ± 119 N to 629 ± 129 N ($p<0.05$) but the increase in $E+S_{m+e}$ from 625 ± 95 N to 669 ± 107 N was not significant (FIGURE 13).

Evening measurements: MVC_{VA} increased in $S+E_{m+e}$ from 567 ± 128 N to 632 ± 120 N ($p<0.01$) during the first 12 weeks and to 646 ± 121 N by week 24 ($p<0.01$). The increases in $S+E_{m+e}$ were significantly larger than the non-significant changes in $E+S_{m+e}$, during weeks 0-12 ($p<0.05$) (eS+E > eE+S; $p<0.05$) and 0-24 ($p<0.05$) (FIGURE 13).

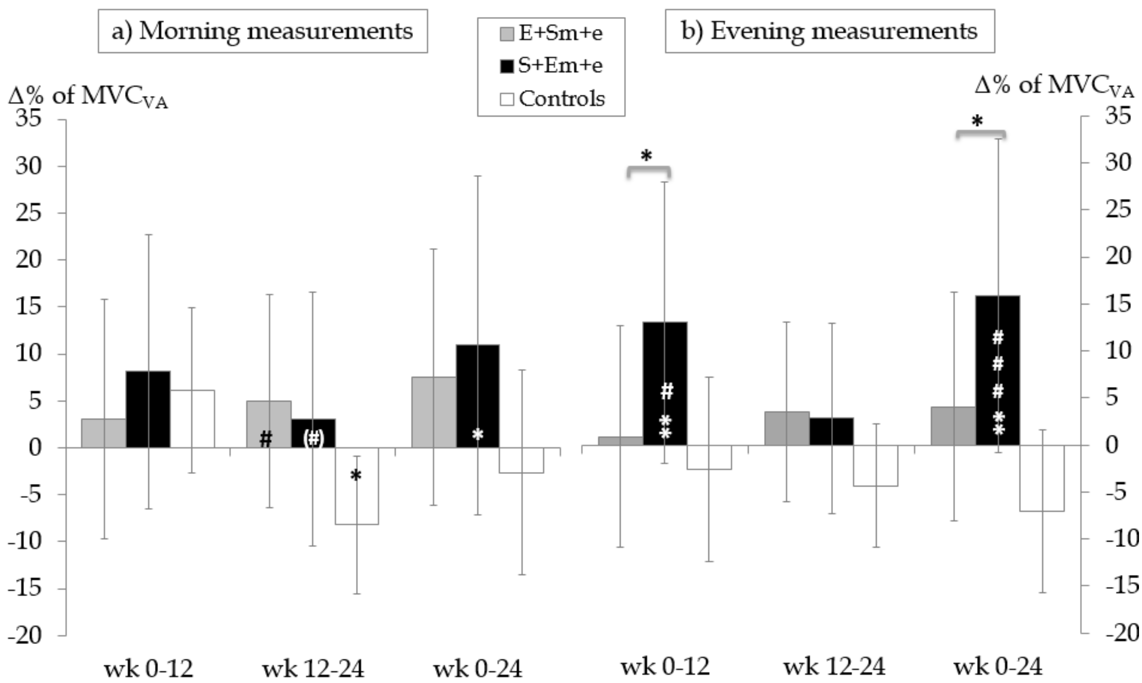

FIGURE 13 Relative changes (mean ± SD) in maximal unilateral knee extension force in the morning and in the evening after 12 and 24 weeks of combined training; *sign. within-group increase or as indicated, *<0.05, **<0.01; # sign. different from the control-group, (#)<0.06, #<0.05, ###<0.001. $E+S_{m+e}$ = combination of morning and evening training groups who performed endurance before strength training; $S+E_{m+e}$ = combination of morning and evening training groups who performed strength before endurance training.

5.2.2.3 Electromyographic activity (EMG) and level of voluntary activation (VA%) during maximal isometric knee extension force

VL rmsEMG during MVC_{VA} and VA% did not show significant diurnal fluctuation in any group in any of the measurements. Time-of-day of training did not have significant effect on changes in EMG or VA% and therefore, data from mE+S, eE+S, mS+E and eS+E are combined and presented, respectively, for $E+S_{m+e}$ and $S+E_{e+m}$.

Morning measurements: Both $S+E_{m+e}$ and $E+S_{m+e}$ significantly increased VL rmsEMG by week 24 (FIGURE 14; TABLE 10). VA% remained statistically unaltered in all groups after 24 weeks of training.

Evening measurements: $S+E_{m+e}$ increased VL rmsEMG activity during weeks 0-12 ($p<0.01$) and 0-24 ($p<0.01$), whereas the changes in $E+S_{m+e}$ were not significant. Significant between-group differences were observed between the $S+E_{m+e}$ and $E+S_{m+e}$ groups after 12 ($p<0.01$) and 24 weeks of training ($p<0.05$) (FIGURE 14). VA% remained statistically unaltered after 24 weeks of training (TABLE 10), however a significant between group difference ($p<0.05$) was observed between the VA% change in $S+E_{m+e}$ (2.1 ± 4.5%) and in CG (-2.1 ± 3.5%).

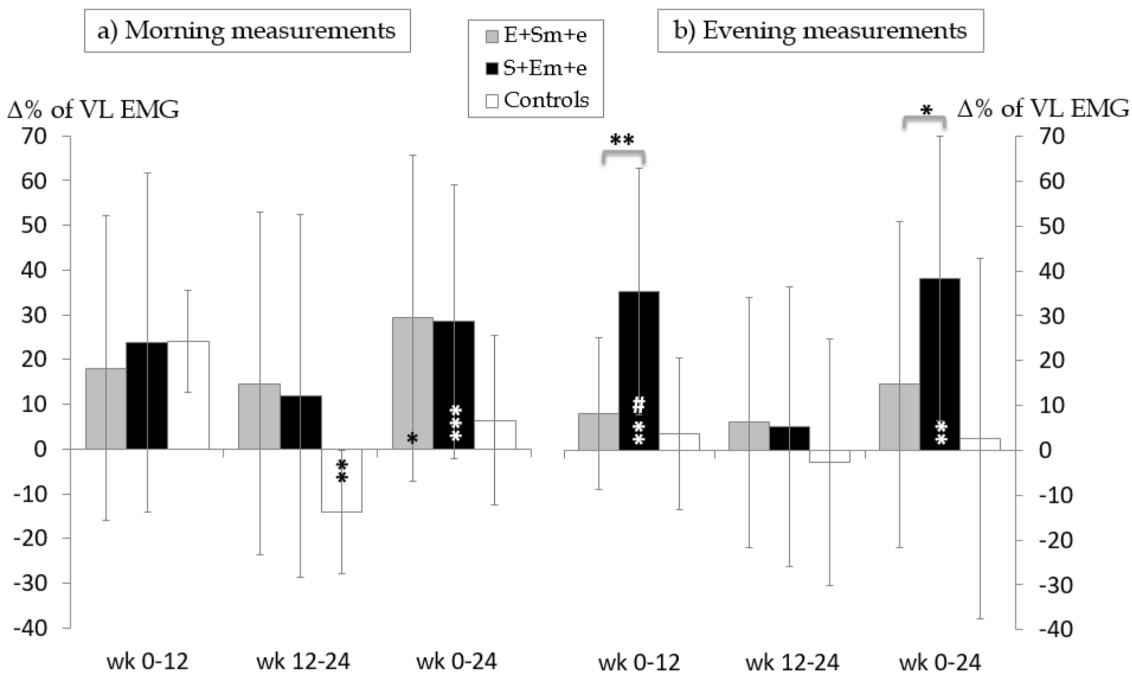

FIGURE 14 Relative changes (mean ± SD) in maximal VL rms EMG during unilateral knee extension in the morning and in the evening after 12 and 24 weeks of combined training; *sign. within-group increase or as indicated, *<0.05, **<0.01, ***<0.001; # sign. different from the control group, #<0.05.

In $S+E_{m+e}$ and $E+S_{m+e}$, a significant correlation between the individual changes in VA% and MVC_{VA} in the morning was found between weeks 0-12 ($S+E_{m+e}$ r=0.625, $p<0.05$ (FIGURE 15); $E+S_{m+e}$ r=0.635, $p<0.01$) and in the $S+E_{m+e}$ group during weeks 0-24 (r=0.521, $p<0.05$). Significant correlations were found in the morning between the individual changes in VA% and VL rmsEMG in $S+E_{m+e}$ during weeks 0-12 (r=0.685, $p<0.01$) and 0-24 (r=0.479, p=0.050). In $S+E_{m+e}$ changes in MVC_{VA} and VL rmsEMG significantly correlated during weeks 0-12 and 0-24 both in the morning (wks 0-12 r=0.509, $p<0.05$; wks 0-24 r=0.479, $p<0.05$) and in the evening (wks 0-12 r=0.462, $p<0.05$; wks 0-24 r=0.481, $p<0.05$).

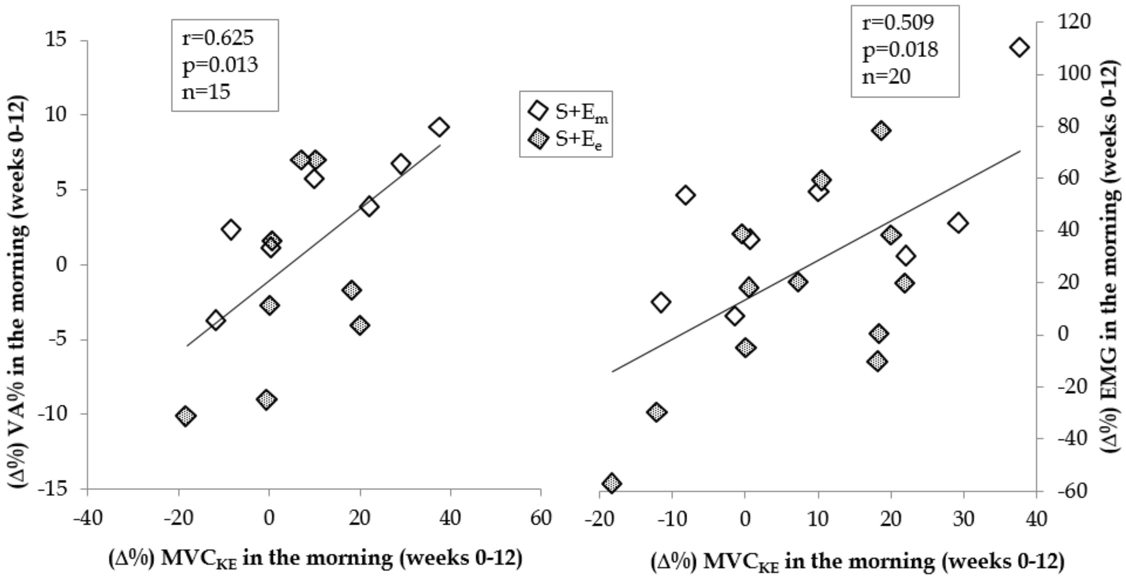

FIGURE 15 Correlations between the individual changes in the voluntary activation % and the relative changes in maximal knee extension force or in the maximal vastus lateral rmsEMG during maximal knee extension force in the SE_{m+e} group during weeks 0-12 when measured in the morning. SE_m= strength training performed before endurance in the morning; SE_e = strength training performed before endurance in the evening.

5.2.3 Adaptations in vastus lateralis cross-sectional area

All training groups similarly increased CSA of VL during the first 12 weeks of training ($p<0.05$) (FIGURE 16; TABLE 11). During the weeks 12-24 only eE+S and eS+E continued to increase CSA (both $p<0.01$), showing a significant time-of-day effect ($p<0.05$). During weeks 0-24 all training groups increased CSA ($p<0.01$), however, a statistical trend for time-of-day effect was observed in favor of the evening training time ($p=0.059$).

In the eS+E group the individual changes in CSA and evening 1 RM dynamic leg press force correlated significantly throughout the 24 weeks of training (weeks 0-12: $r=0.657$, $p<0.05$; weeks 12-24: $r=0.579$, $p<0.05$; week 0-24: $r=0.638$, $p<0.05$).

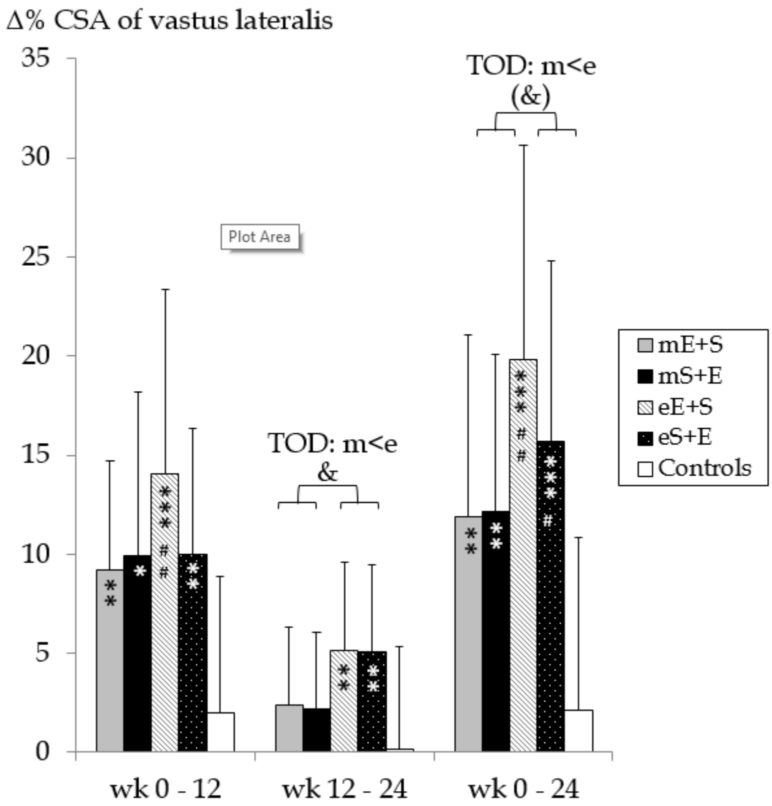

FIGURE 16 Relative changes (mean ± SD) in vastus lateralis cross-sectional area (CSA) after 12 and 24 weeks of combined training. * significant with-in group; # significantly different from the control group & significant time-of-day main effect; TOD = time-of-day main effect; m = morning; e = evening; E+S = endurance before strength training; S+E = strength before endurance training.

TABLE 11 Absolute values (mean ± SD) and relative changes (mean ± SD) in vastus lateralis cross-sectional area.

	wk 0 absolute value	wk 0-12 Δ% ± SD	wk 0-24 Δ% ± SD
mE+S	24.6 ± 3.2	9.2 ± 5.5**	11.9 ± 9.2**
mS+E	20.8 ± 2.5	9.9 ± 8.3*	12.2 ± 7.9**
eE+S	21.2 ± 4.9	14.0 ± 9.3***	19.8 ± 10.8***
eS+E	21.8 ± 3.4	10.0 ± 6.4**	15.7 ± 9.1***
Controls	21.8 ± 3.8	0.2 ± 5.2	2.1 ± 8.7

5.3 Adaptations in endurance performance

5.3.1 Time to exhaustion and maximal power output during cycling

In T_{exh} and Wpeak absolute values no significant morning to evening variation was observed at any time point when all five groups were analyzed separately. When the groups were combined based on the training time, in EG significantly higher values were observed in the evening compared to the morning at week 24 in T_{exh} and Wpeak ($p<0.05$), but not at week 0. In MG T_{exh} and Wpeak showed similar values in the morning and in the evening in all measurement time-points.

Morning measurements: All training groups similarly increased both T_{exh} ($p<0.01$) and Wpeak ($p<0.01$) in the morning during the weeks 0-12 (FIGURE 17; TABLE 12). Only mE+S and eE+S continued to significantly increase T_{exh} ($p<0.01$) and Wpeak ($p<0.01$) during weeks 12-24. By week 24, T_{exh} ($p<0.01$) and Wpeak ($p<0.01$) had increased in all training groups. A significant order effect was found in the changes in the morning T_{exh} ($p<0.05$) and Wpeak ($p<0.05$) in favor of the E+S order. T_{exh} increased more in mE+S ($p=0.051$) and eE+S ($p<0.001$) compared to eS+E and Wpeak improved more in eE+S than in eS+E ($p<0.05$).

Evening measurements: All training groups increased T_{exh} ($p<0.05$) and Wpeak ($p<0.01$) similarly and significantly in the evening during the first 12 weeks (FIGURE 17; TABLE 12). During weeks 12-24 mS+E did not statistically improve T_{exh}, while the significant increases in the other training groups significantly differed from mS+E ($p<0.05$). Wpeak continued to improve in all training groups also during weeks 12-24 ($p<0.05$). During weeks 12-24 a significant order and time-of-day (TOD) effect was observed for T_{exh} ($p<0.05$) and Wpeak ($p<0.05$) in favor of the E+S order and evening training time. The eE+S group improved Wpeak significantly more compared to mS+E ($p<0.05$) over the last 12-week training period. After completing 24 weeks of training all four training groups significantly increased T_{exh} ($p<0.001$) and Wpeak ($p<0.001$) in the evening. In T_{exh} a trend for the order effect ($p=0.051$) was observed in favor of the E+S groups.

Relative changes in T_{exh} and Wpeaks were similar in the morning and evening in all training groups at all time-points.

TABLE 12 Baseline absolute values (mean ± SD) and relative changes (mean ± SD) in cardiorespiratory performance in the morning and in the evening.

	Morning measurement			**Evening measurements**		
	wk 0 absolute value	**wk 0-12 Δ% ± SD**	**wk 0-24 Δ% ± SD**	**wk 0 absolute value**	**wk 0-12 Δ% ± SD**	**wk 0-24 Δ% ± SD**
Time to exhaustion (min:sec)						
mE+S	18:20 ± 02:17	15.6 ± 5.8**	21.9 ± 6.6***	18:47 ± 02:50	11.2 ± 7.0*	19.5 ± 5.9***
mS+E	17:20 ± 03:20	16.0 ± 12.7*	21.3 ± 13.7**	17:28 ± 02:59	15.3 ± 7.1**	19.0 ± 8.4**
eE+S	16:44 ± 02:23	18.4 ± 7.4***	28.1 ± 7.2***	16:57 ± 02:35	16.2 ± 10.4**	28.5 ± 11.1***
eS+E	18:22 ± 03:39	11.2 ±7.9**	15.3 ± 7.5***	18:32 ± 03:45	10.0 ± 8.8*	17.8 ± 8.4***
Controls	19:19 ± 02:48	3.2 ± 3.6	2.7 ± 4.4	19:37 ± 03:14	1.6 ± 5.4	3.4 ± 7.5
Maximal power output (W)						
mE+S	254 ± 29	14.1 ± 5.3**	19.7 ± 6.0***	260 ± 35	10.1 ± 6.3**	17.6 ± 5.3***
mS+E	242 ± 42	14.1 ± 11.0**	18.8 ± 11.7**	243 ± 37	13.6 ± 6.2***	16.9 ± 7.3***
eE+S	236 ± 33	15.5 ± 7.4***	23.6 ± 7.3***	241 ± 35	12.8 ± 9.8**	22.4 ± 11.3***
eS+E	255 ± 46	10.0 ± 6.9**	13.7 ± 6.6***	257± 47	8.9 ± 7.7**	15.9 ± 7.3***
Controls	367 ± 35	2.9 ± 3.3	2.4 ± 4.0	270 ± 40	1.4 ± 4.8	3.0 ± 6.7
Maximal oxygen consumption (ml·kg^{-1}·min^{-1})						
mE+S	36.1 ± 4.4	6.8 ± 5.9*	9.8 ± 7.4**	36.0 ± 3.8	5.2 ± 7.9	12.7 ± 9.6*
mS+E	37.7 ± 8.3	5.0 ± 7.0	7.5 ± 5.2**	36.3 ± 8.3	7.8 ± 8.9	10.0 ± 7.8*
eE+S	35.6 ± 3.7	11.4 ± 8.9**	13.1 ± 8.2**	37.4 ±4.0	5.0 ± 5.5	8.5 ± 6.5**
eS+E	39.3 ± 6.7	2.9 ± 3.4*	4.3 ± 4.2*	39.0 ± 6.6	2.3 ± 6.9	5.4 ± 8.6
Controls	41.0 ± 7.0	2.1 ± 9.7	0.2 ± 9.9	41.0 ± 6.3	1.6 ± 7.2	-1.4 ± 6.4

* Significant with-in group change, *<0.05, **<0.01, ***0.001

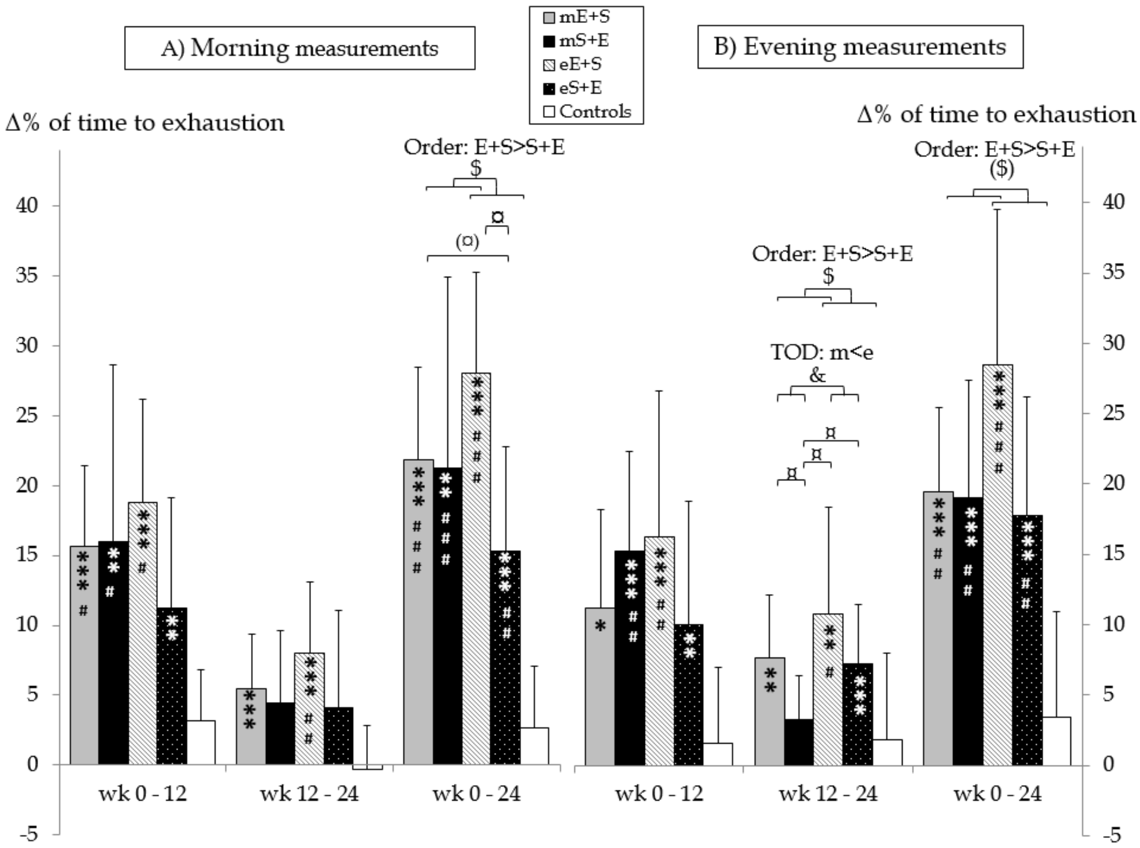

FIGURE 17 Relative changes (mean ± SD) in time to exhaustion (Texh) in the morning and in the evening after 12 and 24 weeks of combined training. Within-group increase; *<0.05, **<0.01; ***<0.001; sign. between-group differences as indicated; (¤)<0.06; ¤<0.05; sign. different from controls; #<0.05, ##<0.01, ###<0.001; sign. order main effect; ($)<0.06; $<0.05; sign. time-of-day (TOD) main effect; &<0.05.

5.3.2 Maximal oxygen consumption

No significant diurnal fluctuation was observed in absolute values of VO_2max at any time point in any of the groups.

Morning measurements: All four training groups significantly improved VO_2max by the end of the study ($p<0.05$) (TABLE 12; FIGURE 18). A significant order effect was observed after the first 12 weeks ($p<0.05$) and after completing the whole 24-week training intervention ($p<0.01$) in favor of the E+S order.

Evening measurements: mE+S, mS+E and eE+S significantly improved VO_2max between weeks 0-24 ($p<0.05$). No order or time-of-day effects were observed in the evening.

Relative changes in VO_2max were significantly different in the morning and in the evening for eE+S during weeks 0-12 (11.4% ± 8.9% vs. 5.0% ± 5.5%; $p<0.05$,) and 0-24 (13.1% ± 8.2% vs. 8.5 ± 6.5%; $p<0.05$), with greater changes observed in the morning. In all other groups, changes in the morning and evening were of similar magnitude.

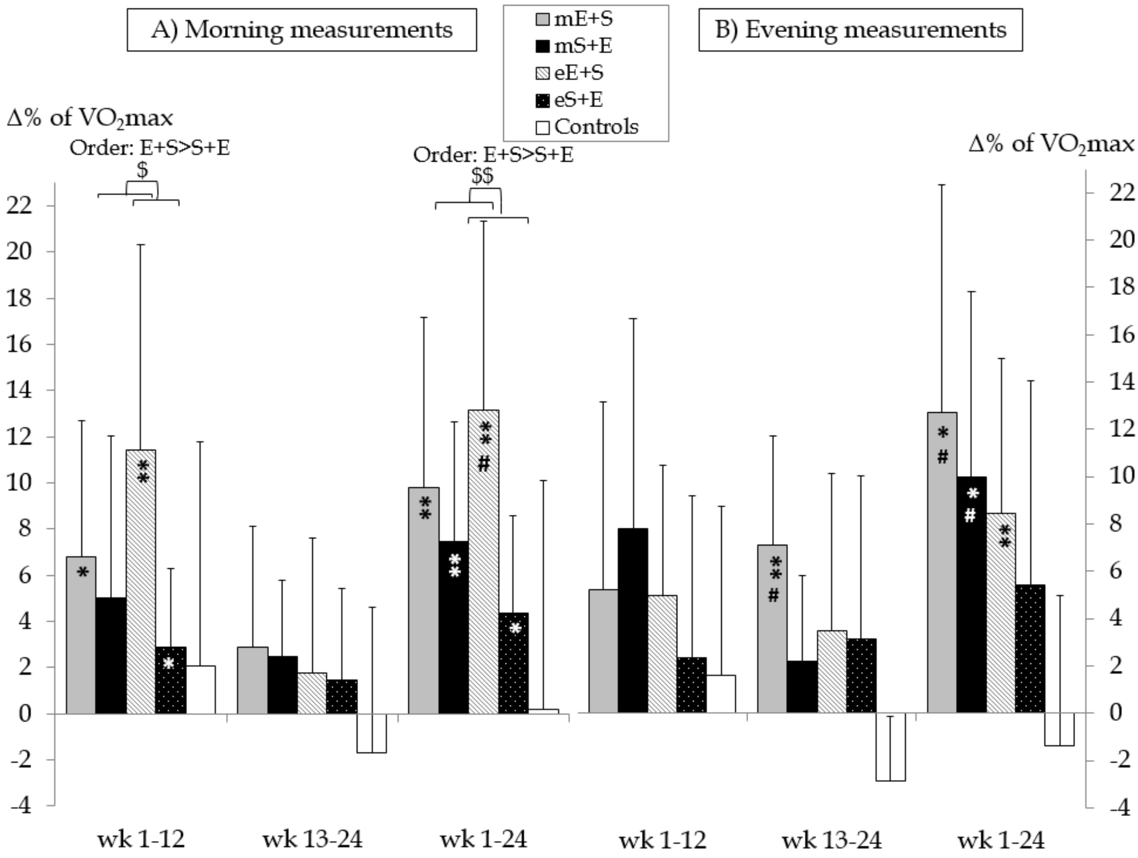

FIGURE 18 Relative changes (mean ± SD) in maximal oxygen consumption (VO_2max) in the morning and in the evening after 12 and 24 weeks of combined training. within-group increase; *<0.05, **<0.01; sign. different from controls; #<0.05; sign. order main effect; $<0.05; $$<0.01.

5.4 Changes in serum testosterone and cortisol diurnal concentrations

No major changes in T or C diurnal concentrations were observed in the training groups when analyzing each group separately. However, when the groups were combined based on the training time, for MG a 17.4 ± 20.6% increase was observed in the basal morning T levels (p<0.05) and a 16.1±13.0% increase in AUC of T (p<0.05) for weeks 13-24 (FIGURE 19A). EG showed an increase of 22.7 ± 29.6% in basal morning T values (p<0.01) for weeks 12-24 and AUC of T increased during the weeks 12-24 (14.1 ± 17.0%; p<0.05) and 0-24 (15.0 ± 16.7%; p<0.01) (FIGURE 19B). When the training groups were combined according to the strength and endurance training order, $E+S_{m+e}$ group increased AUC of T by 18.0 ± 19.0% during weeks 12-24 (p<0.01) and 12.6 ± 15.7% after completing the whole 24-week training (p<0.05). $S+E_{m+e}$ group increased AUC of T by 11.0 ± 11.9% after the second training period (p<0.05). Basal morning T concentrations increased similarly in the E+S_{m+e} and S+E_{m+e} groups after the weeks 12-24 (E+S_{m+e}: 17.4 ± 26.7%; S+E_{m+e}: 23.5 ± 26.6%; p<0.01) and weeks 0-24 (E+S_{m+e}: 22.9 ± 27.9; S+E_{m+e}: 14.4 ± 22.4%, p<0.05). AUC of C remained statistically unchanged by the training time and order interaction. In MG a statistical trend for the increased basal morning (11.9 ± 17.7%; p<0.06) C levels during the weeks 12-24 was observed (FIGURE 19A). Basal morning C concentrations increased by 14.7 ± 26.0% (p<0.01) in EG when the changes from weeks 0-24 were analyzed (FIGURE 19B). In E+S_{m+e} and S+E_{m+e} basal morning C concentrations increased during the weeks 0-24 (E+S: 19.1 ± 32.2%; S+E: 13.0 ± 22.3%; p<0.05).

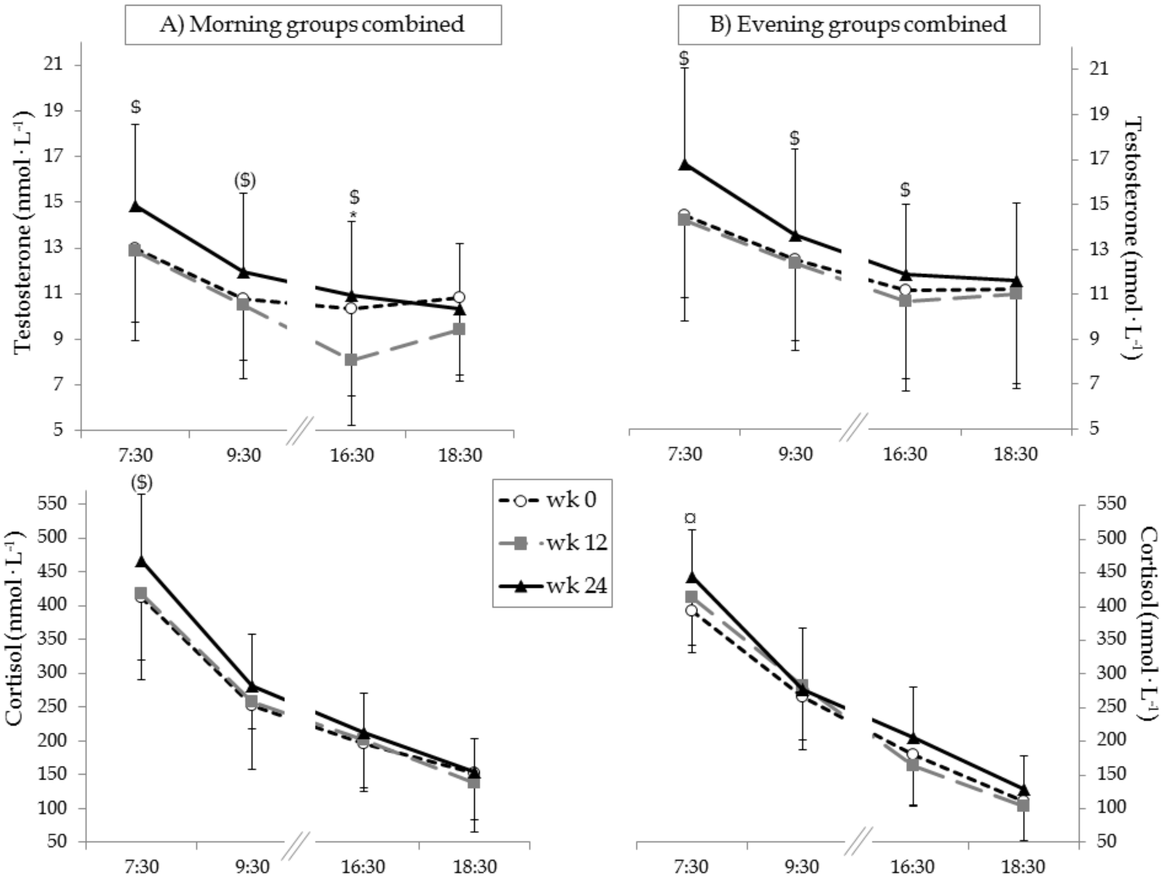

FIGURE 19 Diurnal variations in serum testosterone and cortisol concentrations (mean ± SD) at week 0, week 12 and week 24 in the combined morning group (A) and combined evening group (B). * sign. within-group change for wks 1-12; $ within-group change for wks 13- 24; ¤ sign. within-group change for wks 1-24.

5.5 Adaptations in sleep, quality of life and psychological well-being

5.5.1 Adaptations in sleep behavior

The self-reported sleep duration was similar between MG (07:21 ± 00:40), EG (07:12 ± 00:44) and CG (07:26 ± 00:30) in the beginning of the study and did not significantly change over the intervention period. The average time to go to bed (MG: 23:12 ± 00:50; EG: 22:51 ± 00:34; CG: 23:28 ± 00:42) and average get-up time (MG: 07:20 ± 01:03; EG: 06:54 ± 00:57; CG: 07:17 ± 01:17) were similar between the groups before the study and stayed unchanged also after 24 weeks of training. Based on PSQI sleep quality was good (total score <5) in all three groups before the start of the study and did not change over the intervention period (TABLE 13).

TABLE 13 Changes (mean ± SD) in sleep behaviour based on Pittsburgh Sleep Quality Index.

		Baseline Mean±SD	**wks 0-24 Δ% (95% CI)**
Total score (range 0-21)	MG	4.6±2.3	-0.3 (-1.2 to 0.7)
	EG	4.3±1.9	-0.3 (-1.2 to 0.7)
	CG	3.8±1.5	-0.6 (-1.5 to 0.3)
Subjective sleep quality (range 0-3)	MG	1.1±0.7	-0.2 (-0.4 to 0.1)
	EG	0.6±0.6	0.2 (-0.1 to 0.5)
	CG	1.1±0.3	-0.4 (-0.8 to -0.03)
Sleep latency (range 0-3)	MG	0.8±0.6	-0.6 (-0.3 to 0.2)
	EG	0.9±0.7	-0.3 (-0.5 to -0.1)
	CG	0.6±0.5	0.2 (-0.3 to 0.7)
Sleep duration (range 0-3)	MG	0.4±0.6	0.0 (-0.4 to 0.4)
	EG	0.6±0.6	-0.1 (-0.3 to 0.1)
	CG	0.3±0.5	-0.2 (-0.7 to 0.3)
Habitual sleep efficiency (range 0-3)	MG	0.2±0.5	0.2 (-0.1 to 0.5)
	EG	0.3±0.5	-0.4 (-0.3 to 0.2)
	CG	0.0±0.0	0.1 (-0.1 to 0.3)
Sleep disturbances (range 0-3)	MG	1.1±0.5	-0.1 (-0.3 to 0.2)
	EG	1.0±0.4	0.1 (-0.1 to 0.4)
	CG	0.8±0.4	0.0 (-0.3 to 0.3)
Use of sleeping medication (range 0-3)	MG	0.1±0.2	0.1 (-0.2 to 0.1)
	EG	0.0±0.2	0.0 (-0.1 to 0.1)
	CG	0.0±0.0	0.0 (0.0 to 0.0)
Daytime dysfunction (range 0-3)	MG	1.0±0.6	0.1 (-0.3 to 0.4)
	EG	0.8±0.5	-0.1 (-0.4 to 0.1)
	CG	1.0±0.7	-0.2 (-0.7 to 0.3)

5.5.2 Adaptations in health related quality of life

Health related quality of life

From HRQoL dimensions, the score for bodily pain decreased ($p<0.05$) in MG from weeks 0-24 (TABLE 14). This change in bodily pain dimension in MG was significantly different from the change observed in EG ($p<0.01$). There was a trend for a significant improvement in general health dimension in EG during the weeks 0-12 (77.50 ± 14.14 vs. 84.17±14.35; $p<0.06$), however, no between-group differences were observed. In vitality, a non-significant decrease in MG and a non-significant increase in EG during weeks 0-24 led to a trend for a significant difference between these two groups (MG vs. EG $p<0.07$). No significant changes were observed in the scores of other dimensions.

TABLE 14 Changes (mean ± SD) in health related quality of life dimensions after 24-week time-of-day- specific combined strength and endurance training

		Baseline Mean±SD	**wks 0-24 Δ% (95%CI)**
Physical function	MG	98.6±2.3	0.6 (-0.2 to 1.4)
	EG	99.2±1.9	0.0 (-0.8 to 0.8)
	CG	99.5±1.6	0.0 (-1.7 to 1.7)
Role physical	MG	98.6±5.9	-4.2 (-13.9 to 5.6)
	EG	95.8±15.9	2.1 (-5.5 to 9.7)
	CG	97.5±7.9	2.5 (-3.2 to 8.2)
Role emotional	MG	90.7±19.2	5.6 (-0.8 to 11.9)
	EG	93.1±17.0	-6.9 (-20.7 to 6.8)
	CG	80.0±35.8	10.0 (-6.1 to 26.1)
Vitality	MG	74.2±13.1	-7.2 (-17.5 to 3.1)
	EG	68.8±18.0	7.5 (-0.3 to 145.3)
	CG	69.0±17.1	4.5 (-4.3 to 13.3)
Mental health	MG	81.6±5.5	-0.9 (-11.4 to 9.6)
	EG	78.8±20.0	5.0 (-3.4 to 13.4)
	CG	80.8±12.8	1.2 (-5.9 to 8.3)
Social functioning	MG	94.4±9.8	-1.4 (-6.1 to 3.3)
	EG	94.3±12.2	0.5 (-6.5 to 7.6)
	CG	86.3±23.9	6.3 (-6.6 to 19.1)
Bodily pain	MG	93.2±10.1	-13.2 (-22.4 to -4.1)
	EG	84.2±12.0	4.2 (-1.8 to 10.1)
	CG	90.5±10.9	2.5 (-1.3to 6.3)
General health	MG	80.0±13.0	1.7 (-8.7 to 12.1)
	EG	77.5±14.1	5.2 (0.4 to 10.0)
	CG	81.5±11.1	4.0 (-2.0 to 10.0)

5.5.3 Adaptations in time management, intrinsic motivation and self-esteem

Intrinsic motivation to participate in the current training program tended to decrease in MG, whereas in EG and CG no significant changes were observed over the training period (TABLE 15). The total score for time management decreased in EG over the 24-week training period significantly ($p<0.05$), whereas it stayed unchanged for MG and CG (TABLE 15). In EG significant decreases were observed in the questions "I have enough time for other hobbies when exercising regularly" ($p<0.05$) and "I have enough time for friends and family when exercising regularly" ($p<0.05$). For the question "Exercising makes me busy" a trend for a significant decrease was observed ($p\leq0.07$) in EG. No between-group differences in time management were, however, observed. Global self-esteem improved ($p<0.05$) in both MG and EG over the 24-week combined strength and endurance training period but not in CG (TABLE 15). No between-group differences were observed over the intervention period.

TABLE 15 Changes (mean ± SD) in intrinsic motivation, time management and self-esteem after 24-week time-of-day-specific combined strength and endurance training

		Baseline Mean±SD	**wks 0-24Δ% (95% CI)**
Intrinsic motivation	MG	48.0±4.3	-3.1 (-5.9 to -0.4)
	EG	43.5±8.0	0.7 (-2.0 to 3.4)
	CG	45.1±5.9	0.7 (-2.3 to 3.7)
Time management	MG	34.4±3.2	-0.6 (-3.2 to 2.0)
	EG	33.7±4.2	-3.0 (-5.2 to -0.8)
	CG	33.0±2.0	-1.7 (-3.5 to 0.1)
Self-esteem	MG	24.6±3.8	1.8 (0.6 to 3.1)
	EG	26.0±3.4	1.6 (0.4 to 2.7)
	CG	26.0±2.5	0.8 (-0.9 to 2.5)

6 DISCUSSION

6.1 Diurnal rhythms in parameters related to exercise performance

6.1.1 Diurnal rhythms in body temperature

Body core temperature has been often used as the marker for the circadian rhythms, with peak values observed in the evening and troughs in the early morning hours (Waterhouse et al. 2005), with a peak-to-trough range about ~1 °C (Wright et al. 2002). In the present study the daily fluctuation in intra-aural body temperature was, however, less than 0.5°C. Reilly & Waterhouse (2009) have proposed that intra-aural body temperature may not be reliable due to the temperature changes produced at the measurement site by the airflow passing the recording site. However, also other studies have used intra-aural body temperature measurements (Atkinson et al. 2005; Castaingts et al. 2004) and found the results to be similar to those by aural measurements.

Due to the overlapping peaks and troughs throughout the day, many studies have proposed that diurnal rhythms in body temperature can explain the fluctuation in force production (Coldwells et al. 1994). They have suggested that higher body temperature in the evening may possibly enhance conduction velocity of action potentials, enzymatic activity and extensibility of connective tissue, and reduce muscle viscosity as well as antagonistic co-contraction (Bambaeichi et al. 2004). However, similar to Callard et al. (2000), in the present study the amplitude of the rhythm in body temperature was too low to account for the changes in maximal force production. Therefore, the present data does not suggest the theory that fluctuation in body temperature would be the main driver for diurnal rhythms in strength performance.

However, the present study showed similarly with previous studies that have separated the types based on the chronotype questionnaires, that with regard to body temperature no diurnal difference was obtained for the morning types, while for the evening types the evening values were higher than the morning values (Kerkhof 1985). In the present study, after separation into the performance type groups, HEPT showed significant daily fluctuations in body temperature, whereas NT and HMPT did not show any fluctuation. This indicates that there are inter-individual differences in endogenous time givers, which may among other rhythms influence exercise performance and body temperature.

6.1.2 Diurnal rhythms in hormonal concentrations

Efficacy of hormones is often dependent on the temporal pattern in which the hormones are secreted (Urbanski 2011) and hormonal concentrations have been suggested to account for circadian variations in muscle strength among several other factors (Edwards et al. 2013). In the present study, the analysis of serum hormone concentrations revealed that diurnal variation in serum testosterone (T) and cortisol (C) was similar to those found in previous studies (Hayes et al. 2012), with the highest values observed in the morning for both hormones. However, our results failed to show any significant relationships between the daily changes in maximal voluntary force and concentrations of serum T, C or T/C ratio and, thereby, reconfirmed what has been reported by other studies (Hayes et al. 2013; Teo et al. 2011b). After separation into the performance types, HMPT and HEPT showed significantly different morning to evening change in the T/C ratio, with significantly larger morning to evening fluctuation in the T/C ratio observed in HEPT compared to HMPT. This indicates that HEPT may have been in a more desirable physiological condition for strength performance in the evening compared to the morning (Hayes et al. 2013). However, as the strength performance and hormonal data was collected on different days, conclusions need to be done with great caution.

6.1.3 Diurnal rhythms in neuromuscular performance

Force production

In the total group of participants, the present results showed maximal isometric force to be significantly greater in the evening (~4%) compared to the morning. This is consistent with the previous studies investigating knee extensor muscles (Araujo et al. 2011; Callard et al. 2000; Deschenes et al. 1998a; Guette et al. 2005b; Sedliak et al. 2008, 2011). The uniqueness of the present study, however, lays in the fact that inter-individual differences were observed in isometric force production; HMPT showing significantly higher force values in the morning (~6-11%), NT showing slightly higher evening performance or no morning-to-evening differences and HEPT showing significantly higher force values in the evening (~6-16%). So far, the efforts to determine the circadian phenotype and thereby the time-of-day preferences, are based on the scores obtained from the chronotype questionnaires, rather than in the actual timing of peak performance. This is the first study, where none of the participants who belonged to the extreme morning or evening chronotype, were divided into the morning, evening and neutral types according to their lower limb strength performance.

EMG and voluntary activation level

Diurnal fluctuations in maximal voluntary force have been explained by (i) changes in the central nervous system drive or (ii) changes at the peripheral level (Guette et al. 2006). To identify the cause of morning-to-evening difference in force production concomitant neuromuscular measurements (EMG and VA%) were conducted. In the present study no morning-to-evening difference was observed in EMG of VL and VM in the large group level. This was in line with previous research, showing no fluctuation in EMG recordings over the day in knee extensors (Guette et al. 2005b; Nicolas et al. 2005; Sedliak et al. 2008a). These authors concluded that daily variations in maximal force can be rather explained by changes at the muscle tissue level than by the failure in the central motor command to activate the knee extensor muscles in the morning. However, others (Callard et al. 2000; Sedliak et al. 2011) have reported maximal EMG activity to accompany higher force values in the evening in the knee extensor muscles and proposed that both neural and muscular factors are involved in the circadian rhythms of force production. After separation into the performance types, EMG followed maximal force production as it increased over the day for HEPT and decreased over the day for HMPT, showing that EMG of knee extensors took place on different time-of-day for HMPT (higher EMG values in the morning) and HEPT (higher EMG values in the evening).

In addition, the possible influence of central factors on diurnal fluctuation in maximal strength performance was also evaluated by the VA%. In the present study VA% did not show any significant morning to evening difference in the total group level. This suggests that the capacity to activate the knee extensor muscles was not affected by the time-of-day and that central mechanisms could not explain the diurnal rhythms observed in isometric strength performance as supported also by some previous research (Guette et al. 2005b; Martin et al. 1999; Onambele-Pearson & Pearson 2007). However, after separation into the performance types, HEPT showed significantly higher VA% in the evening compared to the morning, suggesting that the higher evening force values in this sub-group may have been elicited by the increased central nervous system drive to the quadriceps femoris muscle. It is possible that in the morning HEPT may have experienced reduced central motor drive and lower maximal activation of the muscle, which has been suggested to be a result of a failure to recruit all motor units or a reduction in maximal discharge rate (Kent-Braun & Le Blanc 1996). Therefore, the force measured in the morning may not have been a representative of a true maximal force and the simultaneous modifications in the neural and muscular mechanisms in the evening may have led to increased ability to generate force in HEPT. However, HMPT and NT showed similar VA% both in the morning and in the evening and probably peripheral rather than central factors affect diurnal variations as central motor drive did not show significant time of day effect. However, the mechanisms explaining the diurnal fluctuations in maximal isometric force in these groups need further investigation.

6.1.4 Diurnal rhythms in cardiorespiratory performance

The present study was probably the first one to assess the diurnal fluctuations both in strength and endurance performance in the same group of individuals. In the large group of participants endurance performance during the maximal incremental cycling test (time to exhaustion and maximal power output) showed small (~1.5%) but significant morning to evening difference, whereas VO_2max was stable throughout the day. Nevertheless, the amplitude of fluctuations was smaller in the endurance performance compared to the strength performance. This is in line with previous literature suggesting that the longer the duration of exercise performance is, the more the effect of diurnal rhythms seems to dissipate (Chtourou & Souissi 2012). However, our study is in line with previous literature showing that maximal endurance performance fluctuates with time-of-day in young healthy men (Hill 2014; Atkinson et al. 2005; Bessot et al. 2006), although not all agree (Deschenes et al. 1998b; Dalton et al. 1997). The origin of the diurnal variation in performance is unknown but is explained probably by a combination of factors. Aerobic metabolism could be one of the potential influencers of maximal endurance performance, however, even when fluctuations are observed in VO_2max, the amplitudes reported are rather low (<5%) (Hill 2014; Hill et al. 1988) and cannot fully explain the diurnal fluctuations in maximal aerobic performance (Bessot et al. 2011). In the present study variations in the morning and evening endurance performance were observed also in the absence of rhythms in maximal oxygen uptake. Although, similarly to the present study, some earlier research has proposed that VO_2max does not fluctuate with time-of-day (Bessot et al. 2006; Bessot et al. 2011; Deschenes et al. 1998b), both day-to-day biological variability (Reilly & Brooks 1982; Reilly & Brooks 1990) and technical errors in oxygen uptake measurements could mask possible circadian fluctuations in VO_2max (Bessot et al. 2011). Nevertheless, the pattern of muscle activity, force production and kinematics during cycling have been shown to depend on time-of-day and may, therefore, lead to diurnal variation in endurance performance itself (Moussay et al. 2003; Bessot et al. 2007).

After separation into the performance types based on the isometric force production, HMPT showed significantly higher values (3-5%) in the morning for T_{exh} and Wpeak as well as for VO_2max. HEPT, however, showed ~5% higher values in the evening for all three measured variables. NT did not show any significant fluctuations in the measured endurance performance variables. It is interesting to observe that the performance type grouping was valid for strength as well as for endurance performance. This suggests different individual peak times in strength and endurance performance even for the individuals not belonging to the extreme chronotypes. Nevertheless, factors influencing endurance performance should be examined more thoroughly in future studies in order to determine the mechanisms behind the diurnal rhythms.

6.2 Adaptations to time-of-day-specific combined strength and endurance training: special effect on training time and order

6.2.1 Adaptations in neuromuscular performance

Morning vs. evening combined strength and endurance training

After the participants were divided into five groups for the longitudinal intervention design, morning-to-evening difference in maximal dynamic and isometric strength performance seemed to dissipate. Significant morning-to- evening differences were observed only in eS+E for MVC_{LP}. It is possible, that it is the result of the small participant number in each group, which may have led to a type I statistical error and decreased the statistical significance. However, as shown in the cross-sectional design, peak performance was observed at different times of day for HMPT, HEPT and NT. When participants were divided into five intervention groups, different performance type were not taken into consideration and the combination of HMPT, HEPT and NT may have diminished the diurnal fluctuation in neuromuscular performance in the group level.

When the training groups were combined based on the training time (MG and EG), both MG and EG showed significantly higher MVC_{LP} in the evening compared to the morning at week 0, but also at week 24. Therefore, no training time specific adaptations were observed in isometric strength performance. In dynamic strength performance, however, there was a tendency for temporal adaptations, as the improvements were larger in the evening when training was regularly performed in the evening hours, whereas morning training time led no time-of-day-specific adaptations. Previous strength-only training studies with young men have demonstrated that after regular training at a particular time-of-day, the largest increases in strength performance can be found at the training-specific time-of-day (Chtourou & Souissi 2012; Sedliak et al. 2008a). The present results suggest that after prolonged combined strength and endurance training the training time specific adaptations may not be that expressive. It is possible that the measurement error in the dynamic strength performance was similar to the range of the diurnal rhythm amplitude as a significant increase (~5%) in dynamic strength performance was observed in CG in the morning measurement (Chtourou & Souissi 2012) and increase the probability of type II statistical error (Atkinson & Reilly 1996). This lack of diurnal variation in neuromuscular performance in the present study may have in part masked the time-of-day-specific training adaptations in strength performance. Moreover, previous combined training studied have shown that combined training may lead to smaller magnitude adaptations in strength performance compared to the strength-only training (Hickson 1980; Kraemer et al. 1995), due to endurance training possibly interfering with the neuromuscular system's ability to adapt (McCarthy et al. 2002; Eklund et al. 2015). Therefore, the non-optimal adaptations in strength performance may have diminished the time-of-day effect on adaptations after prolonged combined strength and endurance training.

Intra-session order of strength and endurance training

The adaptations in dynamic strength performance (1RM) were independent of the intra-session strength and endurance training sequence. This is in line with the previous not-time-of-day-specific combined training studies that have also shown that gains in maximal strength performance were not related to the order of strength and endurance training sessions in young previously untrained men (Chtara et al. 2008; Schumann et al. 2014a). Nevertheless, adaptations in maximal isometric force production were larger when strength training was regularly performed before endurance training. Similarly, Eklund et al. (2015) and Cadore et al. (2013) have shown, respectively, in young and old men that neuromuscular adaptations might be compromised when endurance training constantly precedes strength training. However, in the present case order effect was observed only when tested in the evening. This possible interference by prior endurance training has been attributed in addition to impeded molecular adaptations (Baar 2014; Coffey et al. 2009b; Hawley 2009) also to acute fatigue developed in the neuromuscular system (Lepers et al. 2001). Consequently, when the neuromuscular system cannot produce an optimal contraction due to previous fatigue, improvements in muscle strength may be possibly reduced (Bentley et al. 2000).

Analogous to isometric force, the morning increases in rmsEMG were similar between the two training orders, whereas the evening changes in rmsEMG were significantly larger in the S+E order compared to the E+S order. The present results are in line with Eklund et al. (2015), showing that in the S+E order the individual changes in maximal isometric knee extension force were positively correlated to the changes in VL rmsEMG. Therefore, the individuals who increased rmsEMG experienced concomitant increases in maximal knee extension force, while the reverse order produced no significant increases. The results from this study and from Eklund et al. (2015) suggest that performing endurance training before strength may potentially inhibit neural adaptations and, thereby, hinder adaptations in neuromuscular performance.

In addition to rmsEMG, neuromuscular activation was measured in the present study by using the twitch interpolation technique to quantify the level of voluntary muscle activation. Previously, Eklund et al. (2015) observed enhanced voluntary activation after combined training only in the group that performed strength before endurance training and suggested that combined training for longer than 12 weeks may potentially inhibit adaptations in the nervous system when endurance is regularly performed before strength training. Although, in the present study no significant within-group changes were observed in VA%, in the evening the S+E order led to significantly larger changes in VA% compared to the control group. The significant correlations between the non-significant improvements in VA% and knee extension force were found in the morning in both $S+E_{m+e}$ and $E+S_{m+e}$ group. The significant correlations observed between individual changes in VA% and changes of rmsEMG over the 24-week training period were observed only by adhering to the S+E order. Nevertheless, it has been suggested that already small increases in VA% represent a physiologically significant improvement in muscle activation (Herbert & Gandevia 1999). Therefore, it is possible that the statistically insignificant changes in the present study still affected strength performance adaptations.

All experimental groups in the present study followed training programs, which were carefully matched for modes, frequencies, intensities and durations of strength and endurance training. Therefore, differences in improvements in neuromuscular performance might be explained by the sequence of training. Significant order effect was, however, observed only in isometric neuromuscular performance, but not in dynamic strength. A recent systematic review has suggested on the contrary that exercise sequence modifies the adaptations in lower body dynamic strength but not static strength (Eddens et al. 2018). Nevertheless, if residual fatigue or neuromuscular mechanisms were responsible alone for the order related differences in strength, it remains to be answered why these factors would influence one mode of lower-body strength but not the other one (Eddens et al. 2018). In addition, between-group differences in neuromuscular performance were found only in the evening testing time. Therefore, it stays to be elucidated why order effect was observed only at the certain time of the day measurements.

6.2.2 Adaptations in muscle hypertrophy

Morning vs. evening combined strength and endurance training

In the present study all training groups similarly and significantly increased CSA of VL during the first 12 weeks of combined training. Alike, Sedliak et al. (2017) have shown that time-of-day-specific strength training for 2-3 months leads to similar gains in the mid-part of quadriceps femoris CSA in the morning (8.8%) and afternoon (11.9%) groups. These results are well in line with the present data from the first 12 weeks, showing that the mean increases in CSA of VL were 9.5% in the morning and 12% in the afternoon group. This refers indirectly that combined training does not impair muscle hypertrophy as suggested also by previous combined training research (Wilson et al. 2012) and allows to compare the results from strength-only training studies with the present combined training study in terms of muscle hypertrophy. However, during the second 12 weeks of combined training the groups training in the evening hours increased CSA of VL significantly more compared to the groups training in the morning. Also Sedliak et al. (2009) proposed the increase in mid-thigh muscle volume, although minor and statistically insignificant, to favor the afternoon strength training group. In addition, the same research group suggested that strength training in the morning may not provide optimal stimulus for some individuals because of large inter-individual variability in protein signaling after morning compared to the evening strength training (Sedliak et al. 2013, 2017). Therefore, when the training period is prolonged (6 months), the evening training time of combined strength and endurance training may be more beneficial for muscle hypertrophy development. However, further studies examining the potential for diurnal alterations to modulate hypertrophy responses are still required.

Effects of intra-session order of strength and endurance training
In agreement with previous reports (Cadore et al. 2013; Schumann et al. 2014a), the increases in CSA of VL were not related to the strength and endurance training order in the present study. It is possible that moderate but repeated force production during cycling action provided an additional stimulus to promote hypertrophy (Mikkola et al. 2012), rather than counteracted with the adaptations elicited by hypertrophic and maximal strength training program. Although some studies have reported the interference with muscle hypertrophy development when the combined strength and endurance training is performed at a high frequency or high intensity (Hickson 1980; Kraemer et al. 1995), no interference by endurance training have been reported when the training frequency remains ≤3 times per week (Häkkinen et al. 2003; McCarthy et al. 2002). In addition, similar adaptations between the two training orders confirm that the order-specific gains in isometric strength performance were origin from neuromuscular adaptations rather than morphological changes.

6.2.3 Changes in serum testosterone and cortisol concentrations

In the present study both serum total T and C exhibited significant diurnal variation in all five groups, well in line with previous studies (e.g. Kraemer et al. 2001). Combined training neither in the morning nor in the evening led to changes in diurnal rhythms in resting serum total T and C concentrations. However, increased morning basal T and C concentrations were observed independent of the strength and endurance training order or training time. This is well in line with previous combined training research showing similar increases in basal T and C values in both S+E and E+S order (Schumann et al. 2014b). No previous studies appear to have dealt with possible phase shifting properties of prolonged combined strength and endurance training on hormonal rhythms. Previous strength training studies, however, have shown that the influence of short-term training protocols seem to be insufficient to alter the circadian profile of T and C, at least in previously resistance-trained men (Häkkinen et al. 1988; Kraemer et al. 2001). However, at the same time, there is lack of longitudinal training studies observing the effect of prolonged exercise training on circadian hormonal profile. Sedliak et al. (2007) showed that several weeks of strength training in the morning hours might decrease the resting C concentrations, whereas the evening training did not have that effect. In the same study, no significant changes were observed in resting T concentrations (Sedliak et al. 2007). The authors also proposed that the changes in C concentrations may have occurred due to the decreased anticipatory psychological stress prior to the morning testing (Sedliak et al. 2007). Therefore, this present study is in line with previous time-of-day-specific strength training studies (Sedliak et al. 2007, 2017) suggesting that the time-of-day-specific training seems not to affect diurnal variation in resting T and C in previously untrained men. However, in the present case the low sampling frequency may have precluded our ability to evaluate the possible training-induced phase shifts properly (Sedliak et al. 2007). In addition, although the overall training volume was large (2-3 E sessions and 2-3 S sessions per week) for previously untrained men, the training frequency of 2-3 double sessions (2-3 E+S or S+E sessions) allowed for at least 48 hours of recovery. This, however, may have provided sufficient time for photic and social *zeitgebers* to reset any possible phase-shifting effect caused by the exercise training (Duffy et al. 1996). It is possible that increases in T and C basal concentrations were caused by the seasonal variation due to sampling at different times of the year (Andersson et al. 2003; Persson et al. 2008).

6.2.4 Adaptations in cardiorespiratory performance

Morning vs. evening combined strength and endurance training

After the participants were divided into five groups for the longitudinal intervention design, no significant diurnal variation in T_{exh}, Wpeak and VO_2max absolute values was observed at any time point. In general, the literature concerning time-of-day effects on endurance performance yields to somewhat inconclusive results (Bessot et al. 2006; Deschenes et al. 1998b). Similar to the present study, Deschenes et al. (1998b) have suggested that maximal aerobic performance remains constant throughout the day, despite the fact that certain important physiological parameters are subject to diurnal variation. In addition, similarly to the neuromuscular adaptation, the small participant number and combination on HMPT, HEPT and NT in each intervention group may have abolished the diurnal fluctuations in maximal cycling performance.

When groups were combined based on the training time, in EG significantly higher values were observed in the evening compared to the morning at week 24 in T_{exh} and Wpeak ($p<0.05$), although no morning-to-evening difference was observed in the beginning of the study. In addition, the latter 12 weeks of training seemed to lead to greater increases in the evening T_{exh} in EG compared to MG. This suggests that the present time-of-day-specific combined training may have led to training time specific adaptation in endurance performance. However, it seems to take longer than 12 weeks in order to observe training-time-specific adaptations. In MG, however, T_{exh} and Wpeak did not show any training time specific adaptations. Previous literature regarding time-of-day-specific endurance training is limited and equivocal, as some of the studies have shown that adaptations to endurance training are time-of-day-specific (Hill et al. 1998; Torii et al. 1992), while others disagree (Hill et al. 1989). Nevertheless, VO_2max increased similarly in all of the groups in the morning and in the evening, independent of the time-of-day when the combined training was performed.

Effects of intra-session order of strength and endurance training

The present time-of-day-specific combined training led to significant improvements in endurance performance in all of the training groups. Improvements in cycling performance were larger compared to the changes in maximal oxygen consumption. It is probably because Wpeak and T_{exh}, are in addition to cardiorespiratory factors also influenced by the neuromuscular component. After the first 12 weeks the increases in cardiorespiratory performance were similar in all training groups. However, when the training period became prolonged, greater improvements were observed in the E+S order for the cycling performance (T_{exh} and Wpeak) both in the morning and in the evening as well as for maximal oxygen uptake in the morning measurements.

Previous studies that have investigated the effects of simultaneous strength and endurance training on endurance performance but have not controlled the time-of-day of training, mostly demonstrate that strength and endurance training sequence does not interfere with the development of endurance performance (Eklund et al. 2015; Schumann et al. 2014a; Cadore et al. 2013; Collins & Snow 1993). However, similar to the present results, Chtara et al. (2005) found larger improvements in endurance performance when endurance training preceded strength training. Strength loading has been shown to impair muscle force generation capacity (Schumann et al. 2013) and therefore, strength training before endurance training may cause difficulty in optimizing physiological adaptations to endurance training (Chtara et al. 2005).

The intensity of endurance training sessions has been proposed to explain the differences between the studies (Wilson et al. 2012). In the present study, both long-duration continuous as well as high-intensity interval cycling were used. Schumann et al. (2014a), who did not observe an order effect in endurance performance, used a similar endurance training program as in the present study, however, a smaller amount of high-intensity interval training sessions was included in the earlier intervention. In addition, high-intensity cycling has been shown to recruit fast-twitch fibres, which are also recruited during resistance exercise (Murach & Bagley 2016). Therefore, fatigue from strength training in close proximity with intensive cycling exercise may cause interference in optimizing physiological adaptations to endurance training (Dolezal & Potteiger 1998; Nelson et al. 1990), especially when performed over a prolonged period. In the present study differences between two training orders were observed after completing the second training period (wks 13-24) during which both training intensity and frequency were increased. Possibly the increased training intensity, the larger amount of interval training sessions as well as increased training frequency during the second training period may have led to suppressed endurance performance adaptations in the groups who started with strength training.

6.3 Training time related adaptations in sleep, quality of life, motivation, time-management and self-esteem

6.3.1 Effects of combined training time on sleep

For optimal physical performance sufficient amount of sleep is needed (Davenne 2009). Throughout the intervention period participants of the present study slept for 7-8 hours per night, which has been suggested to be a good amount of sleep for healthy young adults (Grandner et al. 2010). Moreover, good sleeping habits and moderate physical activity have been suggested to be mutually beneficial (Chennaoui et al. 2015). Previous research about chronic aerobic as well as strength training has shown significant benefits on subjective sleep quality and on total sleep time in clinical populations as well as in fit healthy individuals (Kubitz et al. 1996; Driver & Taylor 2000; Uchida et al. 2012; Kredlow et al. 2015; Kovacevic et al. 2018). However, for young adults exercise training may have only minor beneficial effect on total sleep time and sleep efficiency (Stepanski & Wyatt 2003; Youngstedt 2005; Kovacevic et al. 2018). The present combined strength and endurance training program did not have any effect on participants' sleep duration, latency, disturbance, efficiency or quality. Previous literature about the effects of combined strength and endurance training on sleep is sparse. Kovacevic et al. (2018), however, suggest that the addition of resistance exercise to aerobic training may offer no additional benefit for objective sleep quality. In the present study a ceiling effect may have left little room for improvements in sleep behavior in young healthy physically active men who were good sleepers already before the training intervention.

Timing of the exercise training has also shown to be an important factor to influence the sleep characteristics. Earlier studies have found most positive effects on sleep when exercise took place 4-8 h before bedtime, compared to other times of the day (Kubitz et al. 1996; Youngstedt et al. 1997; Youngstedt 2005), suggesting that exercising too close to bedtime could increase physiological arousal and disrupt subsequent sleep (Irish et al. 2015). In the present study, however, evening combined strength and endurance training a few hours before bedtime did not negatively influence sleep. In addition, the time to go to bed or wake up times remained the same throughout the intervention period. This is in line with studies which have found that exercising before bedtime may not necessarily disturb sleep (Myllymäki et al. 2011; Kredlow et al. 2015) or may even improve it (Flausino et al. 2012), though a rapid decline in the core body temperature which was initially increased by the training (Kräuchi et al. 2000). Interestingly, also early morning training did not change the sleep characteristics in the present study, although Sargent et al. (2014 a,b) have proposed that morning exercise may lead to chronic sleep restriction. Others, however, have suggested that a bout of moderate-intensity aerobic exercise in the morning may induce even greater improvements in sleep quality than training in the afternoon or evening (Fairbrother et al. 2014).

6.3.2 Effects of combined strength and endurance training time on quality of life

Both a sufficient amount of physical activity and sleep is needed to preserve health and well-being. HRQoL captures physical, emotional and social well-being and, therefore, reflects the definition of health as stated by the World Health Organization. Higher HRQoL scores have been observed after being engaged to an exercise training program (Vuillemin et al. 2005; Bize et al. 2007; Häkkinen et al. 2010), especially when strength and endurance training are combined (Goldfield et al. 2017; Sillanpää et al. 2012). The evidence for younger adults, however, is inconclusive (Häkkinen et al. 2010). In the present study, the dimension scores of HRQoL were initially within the expected values for healthy young men (Aalto et al. 1999). However, after 24 weeks of training the ones who trained in the morning reported a significantly increased level of perceived bodily pain. Furthermore, this change was significantly different from the change observed in the evening training group. In addition, the changes to the opposite direction in vitality dimension (the decrease in the MG and the increase in EG) led to the significant between-group difference. These results refer that chronic morning exercising may negatively influence quality of life, at least in certain dimensions. In the dimensions of "physical function", "role physical", "role emotional", "mental health, "social functioning" and "general health", no significant changes were observed during the present combined strength and endurance training intervention. A ceiling effect is the likely explanation for non-significant effects observed for the above listed dimensions, as scores of healthy adults anyway tend to aggregate in the highest categories (Bize et al. 2007), which in the present case were already at baseline higher than the average Finnish norms (Aalto et al. 1999).

6.3.3 Effects of combined strength and endurance training time on intrinsic motivation, time-management and self-esteem

Intrinsic motivation

Training can improve physiological and psychological health only when subjects are motivated enough to exercise regularly over a prolonged period of time. The level of intrinsic motivation has been shown to be a critical parameter to start and be engaged to an exercise training program (Pelletier et al. 2013; Edmunds et al. 2006), as it refers to something that is inherently interesting or enjoyable (Pelletier et al. 2013). There is lack of evidence in previous literature about the effect of time-of-day when the training is performed on intrinsic motivation. Therefore, the present study is unique to assess the differences between the morning and evening training sessions. Motivation to participate in the present exercise training program did not change over the training period in EG, whereas in MG it showed a tendency to decrease over the 24-week training period. Although there was rather a high number of dropouts in both groups, it was similar between the morning and evening training groups and in both groups only one subject reported that they wanted to quit because of finding the training program too demanding. All the other subjects had to quit because of an accident or health problems, which were not related to the present training program. However, it needs to be kept in mind that the present study required participants to perform all their training sessions in the laboratory under supervised conditions. Furthermore, subjects in the present study who were recruited through public advertisement were supposedly highly motivated to participate. As the initial motivational level to start a physical activity program has been shown to be important (Rosa et al. 2015), this may have biased the sample towards subjects that were already motivated. Therefore, it may feel harder to stay motivated to perform trainings in the morning hours over the prolonged period of time and may, therefore, influence the long-term engagement to exercise training.

Time management

The subjects who regularly trained in the evening felt decreased perception of time management after 24 weeks of training. The similar effect was not observed in the morning training group. For many, once the working day is over, the evening is a moment when exercise is incorporated into the daily routine (Chennaoui et al. 2015). However, the participants of the evening training group felt that while participating in the present intervention they did not have enough time for other hobbies, friends or family and that exercising was making them busy. In the modern world, the primary reason for inactivity has been reported the lack of time to exercise (Stutts 2002) due to the competing demands on their time from educational, career and family obligations (Teixeira et al. 2012). The present intervention suggests that the time-of-day when the exercise training is performed may influence the perception of time management. Therefore, flexibility to choose the time-of-day when to exercise would be important in the promotion of time management. Good time management, which leaves enough time for other activities and family/friends, may help a person to obtain feelings of control (Van Eerde 2003) and, therefore, may have a positive effect on long-term engagement to exercise training.

Self-esteem

The participants of the present study reported a high self-esteem based on the RSES already in the beginning of the study. A growing literature suggests that physical activity can improve SE (Sonstroem & Morgan 1989; Zamani Sani et al. 2016). Subjects in the present study were able to further improve global SE, independent of the intervention group they belonged to. The potential mechanisms by which involvement in exercise might promote SE are enhanced mood and body image, enhanced perceived physical competence, enhanced sense of autonomy and personal control over the body and improved sense of belonging (Biddle et al. 2000, p. 95-96). This all, in turn, contributes to their mental wellbeing and presumably through that, also to their quality of life. As it has been suggested that training interventions with higher intensities and longer duration are more likely to produce positive changes (Biddle et al. 2000, p. 112, 115), it seems that the present intervention had high enough intensities and duration to elicit changes in SE.

6.4 Methodological strengths and limitations

Studies where diurnal variations in strength and endurance performance are measured from the same individuals are sparse. In the present study, the morning-to-evening difference both in neuromuscular and cardiorespiratory performance was measured twice a day from all of the study participants. In addition to that, compared to previous research, the present study focused also on diurnal fluctuation in individual level. The rather large number of participants allowed separating the different performance types based on the isometric strength performance, however the grouping was valid also for endurance performance. Our study showed great inter-subject variability at peak performance times and, therefore, emphasizes the importance of individual level analyzes. In addition, several other rhythms showed inter-individual variation, one of them being body temperature. In the present study, however, the diurnal changes in the body temperature were smaller compared to the previous studies (Castaingts et al. 2004; Gauthier et al. 2001), referring that the intra-aural temperature measurement technique may have been not reliable enough to assess the diurnal fluctuations in the body temperature. In addition, two measurement time-points (morning and evening) may not have been optimal to reveal the true diurnal variation in strength and endurance performance and detect significance at the 5% level (Giacomoni et al. 2006). Due to the large number of subjects, the morning (06:00 - 10:00) and evening (16:00 – 20:00) measurements sessions were speared over the four-hour time period, which may have allowed some natural temporal variation in physiological measures related to exercise performance. However, the selected times of the day were chosen to represent the usual troughs and peaks of circadian rhythm in physical performance (Bessot et al. 2011).

Longitudinal training studies assessing the effects of training time on diurnal variations in exercise performance are sparse, especially where strength and endurance performance are measured simultaneously. In addition to time-of-day measurements in physical performance, temporal adaptations to a wide spectrum of variables were included into this doctoral thesis. However, all the neuromuscular measurements focused on the quadriceps muscles, whereas no adaptations in upper body strength or hypertrophy were assessed, although previous combined strength and endurance training studies have shown that the adaptations that take place in the lower body are not always faithfully replicated in the upper body muscles (Sabag et al. 2018). Nevertheless, all the measurements were conducted with great precision by the experienced staff members, with a maximal variation of one hour from the preliminary measurements. The measurements that required precision (e.g. ultrasound, muscle stimulation etc.) were always conducted by the same staff member. The present longitudinal design (24 weeks) provided the long enough time-period to observe prolonged training adaptations, however also brought some methodological difficulties alongside. The technical difficulties of EMG measurements were recognized. To minimize the methodological and physiological errors during the EMG-recordings we standardized the measurement procedure and permanently marked EMG electrode positions subcutaneously. Nevertheless, changes in skin impedance, subcutaneous fat mass as well as changes in muscle morphology are known to influence the ability to reliably detect longitudinal changes in EMG (Folland & Williams 2007), however, it was impossible to eliminate the variations in these parameters. The interpretation of EMG reflecting the neural drive is also considered a simplification (Folland & Williams 2007), and therefore muscle activation level was assessed with the interpolated twitch method to strengthen the neuromuscular findings.

All the training sessions were performed in the laboratory gym and were supervised by study staff members. However, whereas training sessions included of dynamic movements, neuromuscular measurements included both dynamic as well as isometric force measurements. Earlies studies have questioned the validity of isometric measurements to monitor dynamically induced training adaptations (Baker et al. 1994), due to the low relationship between the dynamic and isometric tests (Baker et al. 1994; Wilson & Murphy 1996). Nevertheless, isometric tests have been shown to be highly repeatable and allowed us to include neuromuscular measurements (EMG, VA%) and ensured a greater reliability.

Nevertheless, the large number of participants and longitudinal intervention design provides valid information about exercise related diurnal variations in human performance as well as training prescription. However, all the present results and conclusion are specific to the training protocols used and valid for individuals with similar anthropometrics and physiological characteristics.

7 MAIN FINDINGS AND CONCLUSIONS

Cross-sectional design

The present study showed that isometric strength and maximal cycling endurance performance are higher in the evening compared to the morning. Central factors seem not to be exclusively responsible for the daily variations in maximal strength and the lack of diurnal rhythms in oxygen consumption could not explain the rhythms in cycling endurance performance.

1. Isometric strength performance was higher in the evening compared to the morning. However, in individual level, the maximal strength performance seems to peak at different times. We identified the high morning performance types who showed significantly higher maximal voluntary isometric force levels in the morning and the high evening performance types who showed higher values in the evening. This means that persons not belonging to the extreme chronotypes may still demonstrate variations in time-of-day when the maximal isometric performance peaks. Although in the high evening performance group the diurnal fluctuations in maximal force were associated with the central activation level, the origin of the diurnal fluctuations needs further investigation.
2. Maximal cycling endurance performance showed small but significant morning-to-evening difference, with higher values observed in the evening. Maximal oxygen consumption, however, presented stable rhythms throughout the day. Separation into different performance types was valid also for maximal cardiorespiratory performance, with high evening performance types showing higher cardiorespiratory performance in the evening and high morning performance types in the morning.

Longitudinal combined strength and endurance training intervention

Time-of-day-specific same session combined training led to improved strength and endurance performance as well as muscle size. However, when the training period was prolonged over 12 weeks, the strength and endurance training order and time-of-day of the training started to be important factors to optimize the magnitude of adaptations to time-of-day-specific combined strength and endurance training.

1. The magnitude of adaptation in strength performance after time-of-day-specific combined training were somewhat less training time related than expected. However, the time-of-day of neuromuscular testing influenced the present results. In the evening, improvements in maximal isometric strength performance seemed to be accompanied by increased neuromuscular capacity in the group that performed strength training constantly before endurance training, while the reversed order may not have provided optimal conditions for adaptations in neuromuscular performance. However, the sequence of strength and endurance training influenced adaptations only in isometric, but not in dynamic strength performance.
2. Greater gains in muscle mass were observed after regularly performing combined training sessions (>12 weeks) in the evening hours, compared to the same training program in the morning hours.
3. Serum testosterone and cortisol levels presented typical diurnal rhythms, which stayed uninfluenced by the time-of-day-specific combined training. Increased basal morning testosterone and cortisol values were observed by the end of the 24-week training period, which were not influenced by the strength and endurance training order or training time.
4. Larger improvements in endurance cycling performance were observed when endurance training was performed before strength training sessions preferably at the time-of-day when the improvements are desired, especially after the prolonged training period.
5. The good sleep behavior was not disrupted by the training sessions performed in the morning or in the evening hours. From the time management point of view, morning hours seem to be better for training compared to the evening, as it left time also for family/friends and other activities. However, regular training in the morning might be more challenging to stay motivated to participate in prolonged training programs. Independent of training time, the current combined training program was able to further increase the already good self-esteem in young healthy men.

The present results suggest that exercise performance fluctuates throughout the day; however, the time for peak performance may vary between individuals. In addition, although improvements in physical performance were observed in all training groups, individuals who wish to perform strength and endurance in close proximity to each other over prolonged training periods are advised to choose the training time and order based on the individual training needs and goals.

YHTEENVETO (FINNISH SUMMARY)

Tämän väitöskirjan tarkoituksena oli tutkia sekä voima- ja kestävyyssuorituskyvyn päivittäistä vaihtelua että kroonisia adaptaatioita yhdistettyyn voima- (V) ja kestävyysharjoitteluun (K) nuorilla miehillä, joilla ei ollut aikaisempaa harjoitustaustaa. 24 viikon mittainen interventiojakso koostui kahdesta 12 viikon mittaisesta yhdistelmäharjoitusjaksosta ja mittaukset suoritettiin viikoilla 0, 12 ja 24. Osallistujat jaettiin neljään interventioryhmään niin, että he harjoittelivat 24 viikon interventiojakson aikana aamulla (a) tai illalla (i) suorittaen saman harjoituskerran aikana kestävyysharjoittelun ennen voimaharjoittelua (K+V) tai päinvastaisessa järjestyksessä (V+K) (aK+V, n = 9; aV+K, n = 9; iK+V, n = 12, iV+K, n = 12). Tutkittavat suorittivat 2-3 yhdistelmäharjoituskertaa viikossa [ts. 2-3 x (1K+1V) tai 2-3 x (1V+1K) sinä vuorokauden ajan ja siinä voima- ja kestävyysharjoituksen järjestyksessä, joka heille oli tutkimuksen alussa määritelty. Voimaharjoittelu koostui hypertrofisesta- ja maksimivoimaharjoittelusta ja oli suunniteltu koko vartalon harjoitteluksi keskittyen enemmän alaraajoihin. Kestävyysharjoittelu suoritettiin polkupyöräergometrilla ja se koostui sekä matalan että korkean intensiteetin harjoittelusta.

Koko tutkimusryhmässä isometrinen voima ja maksimaalinen kestävyyssuorituskyky olivat illalla 4.5% ja 1.5% paremmat kuin aamulla. Isometrisen maksimivoiman ajankohdan perusteella tunnistettiin ne koehenkilöt, joilla suorituskyky oli korkeampi aamulla kuin illalla (n = 8), korkeampi suorituskyky illalla verrattuna aamuun (n = 19) ja ne, joilla ei havaittu aamu- ja iltasuorituskyvyn välistä merkitsevää eroa (n = 45). Samanlainen ryhmittely ilmeni myös kestävyyssuorituskyvyssä. Voima- ja kestävyyssuorituskyky paranivat sekä aamulla että illalla kaikissa harjoitteluryhmissä 24 viikon harjoittelujakson aikana. Isometrinen voima ja EMG-aktiivisuus lisääntyivät kuitenkin enemmän V+K ryhmässä verrattuna K+V ryhmään, kun mittaukset suoritettiin illalla. Maksimaalinen kestävyyssuorituskyky parani puolestaan enemmän K+V ryhmässä verrattuna päinvastaiseen harjoittelujärjestykseen. Ulomman reisilihaksen hypertrofia oli samanlainen ryhmien välillä ensimmäisten 12 viikon jälkeen, mutta iltaharjoittelu johti suurempaan kasvuun reisilihaksen poikkipinta-alassa viikoilla 13-24. Seerumin kortisolin ja testosteronin pitoisuudet osoittivat tyypillistä vuorokausivaihtelua, eikä muutoksia havaittu 24 viikon vuorokaudenajan spesifisen harjoitusjakson jälkeen. Motivaatio osallistua yhdistettyyn voima- ja kestävyysharjoitteluun näytti hieman laskevan aamuharjoittelijoilla. Iltaharjoittelu puolestaan johti ajankäytön hallinnan huonontumiseen.

Nämä väitöskirjatutkimuksen tulokset viittaavat siihen, että maksimaalinen isometrinen voima- sekä kestävyyssuorituskyky vaihtelevat vuorokauden ajan mukaan, mutta yksilöiden välillä saattaa esiintyä suuria eroja. Lisäksi tämän tutkimuksen tulokset osoittivat, että suorittamalla kestävyysharjoituksen ennen voimaharjoittelua voi mahdollisesti heikentää adaptaatioita hermolihasjärjestelmässä ja täten adaptaatioita isometrisessä voimantuotossa aiemmin harjoittelemattomilla henkilöillä. Järjestys, jossa voimaharjoittelu suoritetaan ennen kestävyyttä voi puolestaan aiheuttaa akuuttia väsymystä ja heikentää kestävyysominaisuuksien kehittymistä. Näin ollen, jos yhdistettyä voima- ja kestävyysharjoittelua suoritetaan pitkällä aikajaksolla, harjoituksen vuorokauden aika ja järjestys on valittava kulloistenkin tavoitteiden perusteella.

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ORIGINAL PAPERS

I

EFFECTS OF TIME-OF-DAY ON NEUROMUSCULAR FUNCTION IN UNTRAINED MEN: SPECIFIC RESPONSES OF HIGH MORNING PERFORMERS AND HIGH EVENING PERFORMERS

by

Maria Küüsmaa, Milan Sedliak & Keijo Häkkinen, 2015

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Effects of time-of-day on neuromuscular function in untrained men: specific responses of high morning performers and high evening performers

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ABSTRACT

It has been clearly established that maximal force varies during the day in human muscles but the exact mechanisms behind the diurnal rhythms are still not fully clarified. Therefore, the aim of this study was to examine the diurnal rhythms in maximal isometric force production in a large group of participants and also by separating the high morning performance types (n=8) and the high evening performance types (n=19) from the neutral types (n=45) based on their actual maximal isometric force levels. Measurements were performed in the morning (7:26 h ± 63 min) and in the evening (17:57 h ± 74 min) for maximal bilateral isometric leg press force (MVC_{LP}) together with myoelectric activity (EMG_{LP}), maximal unilateral isometric knee extension force (MVC_{KE}) and maximal voluntary activation level (VA%) during maximal unilateral isometric knee extension force (MVC_{VA}) together with myoelectric activity (EMG_{VA}). In addition, venous blood samples were drawn four times a day and serum testosterone and cortisol concentrations were analyzed. None of the participants belonged to the extreme morning or evening chronotype according to the Munich Chronotype Questionnaire. In the total group of participants MVC_{LP} and MVC_{KE} were 4.4 ± 12.9% ($p<0.01$) and 4.3 ± 10.6% ($p<0.01$) higher in the evening compared to the morning. MVC_{VA} and VA% did not show significant diurnal variation. The high morning performance types showed lower force values in the evening compared to the morning for MVC_{LP} (10.8 ± 9.1%; $p<0.05$) and MVC_{KE} (5.7 ± 4.9%; $p<0.05$). No significant diurnal variation was observed for MVC_{VA} and VA%. The high evening performance types showed higher force values in the evening for MVC_{LP} (16.1 ± 15.9%; $p<0.001$), MVC_{KE} (13.5 ± 11.3%; $p<0.001$) and MVC_{VA} (6.2 ± 9.9%; $p<0.05$) with a concomitant higher VA% in the evening ($p<0.05$). The neutral types showed significantly higher evening force values for the MVC_{LP} (2.1 ± 6.7%; $p<0.05$). All the other neuromuscular variables did not show significant diurnal variations. EMG_{LP} and EMG_{VA} did not show significant diurnal fluctuations in any group. Serum testosterone and cortisol concentrations showed normal daily rhythms with higher values observed in the morning in all of the groups ($p<0.001$). Between-group differences were observed for MVC_{LP} ($p<0.001$) and MVC_{KE} ($p<0.001$) between all of the three groups. Diurnal changes in VA% differed between the high evening performance types and the neutral types ($p<0.05$) and the testosterone/cortisol ratio ($p<0.05$) as well as VL EMG_{VA} ($p<0.05$) differed between the high morning and high evening performance types. In conclusion, we were able to identify the high morning performance types, the high evening performance types and the neutral types who showed significantly different diurnal rhythms in force production, irrespective of their actual chronotype. Therefore, the questionnaires designed to determine the chronotype may not always be sensitive enough to determine the "morningness" or "eveningness" in maximal neuromuscular performance. In general, central factors could partially explain the diurnal fluctuations in maximal strength performance, but peripheral mechanisms were also possibly involved.

Key words: diurnal variation; maximal isometric strength; neuromuscular performance; knee extensor muscles; electromyography; voluntary activation level; testosterone; cortisol

INTRODUCTION

It has been clearly established that functional capacities during maximal isometric voluntary contraction (MVC) of human muscle varies during the day. Diurnal rhythms in MVC production have been shown for a variety of muscles including quadriceps femoris (Callard et al., 2000; Giacomoni et al., 2006; Guette et al., 2005a; Nicolas et al., 2005; Onambele-Pearson & Pearson, 2007; Racinais et al., 2005; Sedliak et al., 2008). The acrophase of the muscle capacity to develop maximal force has been found to occur in the early evening between 16:00 and 19:30 (Callard et al., 2000; Giacomoni et al., 2005; Guette et al., 2005a; Nicolas et al., 2005; Teo et al., 2011). Peak-to-trough variation of maximum strength has been reported to range from 5% up to 21% (Coldwells et al., 1994; Guette et al., 2005b), depending on the population and the muscle groups tested as well as on the experimental design (Nicolas et al., 2007). However, the previous literature has been unable to determine the exact mechanisms behind the diurnal rhythms. The ability to generate force depends on both peripheral and central mechanisms. Peripheral mechanisms include muscle contractility and metabolism, morphology of muscle fibers and local muscle temperature (Araujo et al., 2011; Edwards et al., 2013; Reilly and Waterhouse, 2009). Central mechanisms include central nervous system command, alertness and motivation (Castaingts et al., 2004; Giacomoni et al., 2005; Racinais et al., 2005; Tamm et al., 2009). Peripheral (Edwards et al., 2013; Guette et al., 2005a; Martin et al., 1999; Racinais et al., 2005; Sedliak et al., 2008;), central (Tamm et al., 2009), or combination of both mechanisms (Castaingts et al., 2004; Gauthier et al., 1996; Sedliak et al., 2011) have been proposed to explain the diurnal rhythms in force production. Electromyography (EMG) and twitch interpolation technique have been widely used to discriminate the involvement of peripheral and central mechanisms in the diurnal variations (Chtourou & Souissi, 2012). Though diurnal rhythm in muscle force has been well established during maximal isometric contraction (Callard et al., 2000; Gauthier et al., 1996; Martin et al., 1999), the results concerning EMG data are more controversial. Previous studies have shown no significant daily changes in EMG activity (Giacomoni et al., 2006; Guette et al., 2005a; Martin et al., 1999; Nicolas et al., 2005; Onambele-Pearson & Pearson, 2007; Racinais et al., 2005; Sedliak et al., 2008), higher EMG in the morning

(Gauthier et al., 1996; Guette et al., 2005b) or higher in the evening (Callard et al., 2000; Sedliak et al., 2011) when compared to the rest of the day. These controversial results can be partly explained by the fact that EMG data as such can be affected by several methodological factors and by the muscle groups examined, as calf muscles seem to have higher EMG in the morning (Guette et al., 2005b; Guette et al 2006).

Moreover, it has been suggested that diurnal fluctuations in physical performance may be partly controlled by the day-time level of hormonal fluctuations of some hormones (Giacomoni et al., 2005; Martin et al., 1999). Both testosterone (T) and cortisol (C) exhibit circadian rhythmicity with morning peaks and evening nadirs (Hayes et al., 2012; Kraemer et al., 2001). T and C are considered as important biomarkers in exercise science, because in addition to circadian rhythmicity they are known to correlate with athletic performance (Crewther et al., 2009). The T/C ratio indicates the anabolic/catabolic environment of an organism due to their roles in protein synthesis and degradation. Higher T/C ratio in the evening may provide a more desirable physiological condition (more anabolic environment with less physiological stress), which prepares participants for the physical performance (Hayes et al., 2013).

In addition to that, person’s chronotype may be a confounding variable in diurnal rhythms of force production (Tamm et al., 2009). Individual circadian rhythm differences in activity, termed “morningness-eveningness”, may modify performance at particular times of day (Brown et al., 2008). Only few studies have examined the temporal fluctuation of performance while considering the individual’s circadian preference. Performance tests administered at optimal times of day are more efficient compared to the tests at non-optimal time of day, as inferred for each subject by the score obtained at morningness-eveningness questionnaire (Schmidt et al., 2007).

The identification of exact mechanism is difficult, because there are many underlying factors that occur at the same time and are thereby contributing to diurnal rhythms in physical performance. The purpose of the present study was twofold. Firstly, to examine the diurnal rhythms of maximal isometric force of the lower limbs along with surface EMG and maximal voluntary activation level as well as with serum hormone concentrations. Secondly, the large number of participants in this study gave us the opportunity to separate the high morning performance types and high evening performance types from the neutral types based on their maximal isometric strength performance of the lower limbs. This allowed us to investigate the neuromuscular mechanisms that underlie diurnal patterns in isometric knee extension force production and determine whether they are different for high morning performers, high evening performers and the neutral types.

METHODS

Participants

72 men volunteered to participate in the study. Their mean ± SD age, height and weight were 32 ± 6 years, 1.8 ± 0.1 m and 80.9 ± 10.8 kg, respectively. These individuals were previously untrained but physically active men with similar backgrounds of both health status and physical condition. The target group was free of acute and chronic illnesses. Participants reported to be free of any medications that could influence on their hormonal and neuromuscular systems. In addition, a cardiologist reviewed each participant's health questionnaire and ECG. Participants' morningness and eveningness was assessed. According to Munich Chronotype Questionnaire (Roenneberg et al., 2003), participants did not belong to an extreme chronotype. Neutral types

(n=27), slight late (n=5) and moderate late (n=1) as well as slight early types (n=30) and moderate early types (n=9) were selected. Shift and night workers were excluded. After participants were informed of the purpose and risks of the study, the participants provided written consent before participation. This study was conducted in accordance with the ethical standards of the journal (Portaluppi et al., 2010), complied with the Declaration of Helsinki and was approved by the Ethics Committee in the University of Jyväskylä.

Experimental design

All the participants performed the present strength measurements twice: once in the morning and once in the evening. The strength measurements lasted about 45 min and were carried out in the morning between 6:00-10:00 h and in the evening between 16:00-20:00 h. The average testing time in the morning was 7:26 h ± 63 min and in the evening 17:57 h ± 73 min. Test times did not statistically differ between the groups. The morning and evening tests were carried out in a randomized order. At least 24 h separated the morning and evening tests and participants were asked to keep two days of rest before the testing and follow their normal sleep rhythm. They were also requested to choose the least physically demanding option when coming to the measurements (preferably car or public transport). In the case it was not possible, they were asked to arrive 15 min earlier and rest before the measurements. Participants were asked to refrain from alcohol for 24 h and from coffee for 12 h before the measurements.

Venous blood samples and intra-aural body temperature were measured on a separate day from the strength measurements and were collected 4 times per day at 7:30 h ± 30 min, 9:30 h ± 30 min, 16:30 h ± 30 min and 18:30 h ± 30 min. Participants had to arrive to the lab 10-15 min before the scheduled time and then sit quietly on a chair until the blood samples and body temperature were collected. Participants were asked to fast 12 hours before the first blood sample, after which everybody were provided with a standardized breakfast. They were asked to eat lunch which had to follow previously given instructions about the relative content of protein (10-20%), fat (25-35%) and carbohydrate (50-60%) at around 12:00-13:00 h. After that, participants were asked to avoid food until the last two blood samples (16:30 h ± 30 min and 18:30 h ± 30 min) were drawn. For possible correlations between the serum hormone concentration or body temperature and force production as well as for the between-group comparison the mean of the two morning or two of evening measurements was used for the serum hormone concentration or body temperature values.

Force measurements

A familiarization session was carried out before the true strength measurement on a non-testing-specific time of day. All the force measurements were performed in the same testing session in the following order described below. All the subjects were allowed to have three warm-up trials for the isometric leg press, which also served as a short warm-up.

Bilateral isometric leg press force (MVC_{LP}): Maximal bilateral isometric strength (N) was measured using a horizontal dynamometer (designed and manufactured by the Department of Biology of Physical Activity, University of Jyväskylä, Finland) at the knee angle of 107° (180 ˚ = knee fully extended) (Häkkinen et al., 1998). Participants were instructed to generate maximum force as rapidly as possible against the force plate for a duration of 2-4 sec. Participants were verbally encouraged to perform their maximal. A minimum of three up to five trails were used to determine the maximal isometric leg extension with one minute break separating the trials. Isometric force signals were passed in real-time to an analog-to-digital (AD) converter (Micro

1401, Cambridge Electronic Design, UK). The trial with the highest peak force was selected for further analysis. Force signals were sampled at 2000Hz and low-pass filtered (20 Hz). Muscle activity was recorded through surface electromyography (EMG_{LP}) during MVC_{LP} from vastus lateralis (VL) and vastus medialis (VM) muscles of the right leg. During a familiarization session the VL and VM motor points of the right leg were measured according to Seniam guidelines, and marked with indelible ink tattoos to endure electrode position. The raw EMG signal from the measurement was amplified by 1000 and sampled at 3000 Hz. The signals were passed from a portable transmitter (Telemyo 2400R, Noraxon, Scottsdale, AZ, USA) from which the signal was relayed to a desktop computer via an AD converter (Micro 1401, Cambridge Electronic Design, UK). Analysis of the isometric EMG was performed using a customized script (Signal 4.10, Cambridge Electronic Design Ltd., Cambridge, UK) and converted to integrated EMG. Maximum EMG was determined from the 500-1500 ms time period of contraction representing the peak force phase.

Unilateral isometric knee extension force (MVC_{KE}): Maximum voluntary isometric force of knee extensors was tested using unilateral knee extension of the right leg at the knee angle of 107° (180˚= knee fully extended). The participants were secured to a sitting position in the modified knee extension device (David 200; David Health Solutions Ltd., Helsinki, Finland) with a safety belt in the hip area. Correct position was endured by the adjustable back support, lever arm and ankle pad. Left leg rested in horizontal position on a support. The upper extremities were placed next to the body holding handgrips. Participants were instructed to generate maximum force rapidly against the ankle pad and maintain it for 2-4 sec. A minimum of three up to five trails were used to determine the maximal isometric leg extension with a one-minute resting period separating the trials. The trial with the highest peak force was taken for further analysis. The force signal was low-pass filtered (20 Hz) and analyzed (Signal 4.10, Cambridge Electronic Design Ltd., Cambridge, UK).

Maximal voluntary muscle activation level (VA%): Maximal unilateral knee extension force (MVC_{VA}) was measured using a device designed and manufactured in the Department of Biology of Physical Activity (University of Jyväskylä, Finland). During the knee extension action muscle stimulation was performed to the right quadriceps femoris muscles. The participant was seated with knee angle 107˚ in the right leg and left leg rested in horizontal position on a chair. Hip and knee angles were firmly secured by a seatbelt at the hip, strapped pad over the right knee and with a Velcro strap above the right ankle. Participants were asked to perform three maximal trials by increasing force gradually over 3 seconds. The trial with the highest force was used for further analysis. The force signal was sampled at 2000 Hz and low-pass filtered (20 Hz). Maximal force was manually analyzed on Signal 4.04 (Cambridge Electronic Design, UK).

To assess the maximal voluntary activation level (%) of quadriceps femoris muscle interpolated twitch technique was used to give a supramaximal stimulus during the isometric knee extension action. Four self-adhesive electrodes (7 cm PolarTrode; Polar Frost USA; Anaheim, CA; USA) were placed on the proximal and mid-region of the quadriceps muscle belly of the right thigh. The current of single 1-ms rectangular pulses were increased progressively by a constant-current stimulator (Model DS7AH, Digitimer Ltd, UK) in 5mA steps until a plateau was observed. 25% of the stimulation current was added to ensure maximal effect for the knee extension trials. This supramaximal single-pulse stimulation was delivered during the plateau of peak knee extension force and 5 seconds after the cessation of contraction (Merton, 1954). Voluntary activation level was calculated according to the formula by Bellemare & Bigland-Ritchie (1984):

$$\text{Activation level (\%)} = [1-(P_{ts}/P_t)] \cdot 100,$$

where P_{ts} is the amplitude of the twitch elicited by the electrical stimulation on top of the maximal voluntary contraction and P_t is the amplitude of the twitch delivered to the muscle 5 seconds after the voluntary contraction. EMG_{VA} was collected from VL and VM from the maximum force level over the 500 ms time period, immediately before the superimposed twitch. EMG was multiplied by 1000 by a preamplifier (NeuroLog Systems NL844, Digitimer Ltd, UK) and sampled at a frequency of 2000 Hz. The raw EMG signal was band-bass filtered (20-350 Hz) and, due to technical reasons, converted to root mean square manually on Signal 4.04 software (Cambridge Electronic Design, UK).

Serum hormone concentrations and body temperature

Venous blood samples and body temperature were measured with the participant in a sitting position. Venous blood samples (~10 ml) for the determination of serum hormone concentration were collected by a qualified laboratory technician from an antecubital vein with a vacutainer and test tubes (Vacuette, GEiner Bio-OneGmbH, Kremsmünster, Austria) containing appropriate preservatives. Samples were centrifuged at 3.500 rpm (Heraus Megafuge 1.0 R, Gendro Laboratory Products, Hanau, Germany) for 10 min, plasma harvested and all samples were stored at −80°C until assayed. Analysis of total serum testosterone (T) and cortisol (C) was performed using chemical luminescence techniques (Immunlite 2000, Simens Healthcare, Diagnostics Products Ltd., Llanberies, UK) and hormone specific immunoassay kits (Siemens, New York, USA). The sensitivity for serum hormones was: T 0.5 $nmol/l^{-1}$ and C 5.5 $nmol/l^{-1}$. The intra-assay coefficient of variation was 8.3% and 5.3% for T and C, respectively. The inter-assay coefficient of variation was 9.1% for T and 7.2% for C. In addition, T/C ratio was calculated.

Intra-aural temperature was collected by a qualified laboratory technician by digital thermometer (ThermoScan PRO, BRAUN, Southborough, MA, USA). Two trials were required. The mean of two values were used for further statistical analyses.

Separation to high morning performance types and high evening performance types

The mean of MVC_{LP} and MVC_{KE} morning to evening differences (expressed as percentage of morning values) was used to classify the high morning performance types, high evening performance types and the neutral types. The criteria for determining the different types was set based on the whole group mean morning to evening differences, which was observed to be 4% for both, MVC_{LP} and MVC_{KE}. Morning types were expected to show similar 4% morning to evening difference, with higher values observed in the morning. For the high evening performance types the criterion was set so, that double of the morning to evening difference observed in the large group exists (8%), with higher values observed in the evening. Based on that, any participant that demonstrated morning performance (mean of MVC_{LP} and MVC_{KE} morning to evening difference) at least 4% greater than his evening performance was classified as “high morning performance type”. Any participant that demonstrated evening performance (mean of MVC_{LP} and MVC_{KE} morning to evening difference) at least 8% greater than his morning performance was classified as “high evening performance type”. All the remaining participants were determined as neutral types. None of the participants included to the high morning performance group showed higher evening force values for either MVC_{LP} or MVC_{KE} compared to the morning values and none of the participants in the high evening performance group showed higher morning force values for either MVC_{LP} or MVC_{KE} compared to the evening values. Out of all of the participants, 8 were classified as high morning performance types, 19 as high evening performance types and 45 as neutral types.

Statistical analysis

Descriptive data were generated for all variables and expressed as mean ± SD. Normality of the data was checked and subsequently confirmed using the Shapiro-Wilk test. For normally distributed data Paired-Samples T-Test was used to compare the values from the morning and evening tests. EMG, body temperature and hormonal data was not normally distributed and for that reason the non-parametric tests were used. For morning and evening comparison Wilcoxon-Signed Rank Test was used for the not normally distributed data. For diurnal rhythms of testosterone, cortisol and T/C ratio Friedman test was performed. Between-group analyses were performed with relative values from morning to evening differences. For normally distributed data One-Way ANOVA was used when all three groups were compared and Independent Samples T-test when only extreme groups (high morning performance group vs. high evening performance group) were analyzed for the between-group differences. Mann-Whitney U-test or Kruskal-Wallis one-way analysis of variance was performed, respectively, for not normally distributed data. For correlations between daily relative changes in strength performance and serum hormone concentration Pearson correlation coefficients were calculated. Statistical significance was accepted when $p<0.05$, whereas values $p\leq0.07$ were accepted as a significant trend. Analysis was performed using the Statistical Package for Social Sciences (SPSS verion 22, Chicago, IL).

RESULTS

Body temperature and serum hormone concentrations

Body temperature significantly increased between 7:30 h ± 30 min and 16:30 h ± 30 min with a mean morning to evening difference of 0.2 ± 0.4℃ ($p<0.05$). Serum T and C as well as T/C ratio showed a regular diurnal variation. T and C demonstrated the highest concentrations in the morning (7:30 h ± 30 min) and decreasing concentrations throughout the day ($p<0.001$), whereas T/C ratio showed lowest values in the morning and increasing concentrations throughout the day ($p<0.001$) (Table 1).

Neuromuscular performance

MVC_{LP} was significantly higher when tested in the evening as compared with the morning. The mean difference between the morning and evening measurements was 4.4 ± 12.9% ($p<0.01$). EMG_{LP} measured from MVC_{LP} did not show any significant morning to evening difference. In MVC_{KE} evening values were also 4.3 ± 10.6% ($p<0.01$) higher compared to the morning values. MVC_{VA} was higher in the evening compared to the morning, although just a trend to reach the statistical significance level was observed (2.8 ± 10.8%; $p=0.07$). No significant morning to evening difference was observed for the absolute EMG_{VA} or VA%. Absolute values (mean ± SD) for the morning and evening neuromuscular performance are presented in Table 2.

High morning performance types

The high morning performance types showed 10.8 ± 9.1% lower force values in the evening compared to morning for MVC_{LP} (2329 ± 474 N vs. 2068 ± 728 N; $p<0.05$) and 5.7 ± 4.9% for

MVC_{KE} (730 ± 171 vs. 682 ± 129 N; $p<0.05$). The corresponding differences in the relative scale (Δ%) are presented in the lower part of Figure 1. No significant morning to evening difference was observed for the MVC_{VA} (532 ± 73 vs. 538 ± 65 N). EMG_{LP}, EMG_{VA} and VA% did not show any significant morning to evening difference.

High evening performance types

The high evening performance types showed 16.1 ± 15.9% higher force values in the evening compared to the morning for MVC_{LP} (2587 ± 680 vs. 2968 ± 728 N; $p<0.001$), 13.5 ± 11.3% for MVC_{KE} (682 ± 144 vs. 770 ± 154 N; $p<0.001$) and 6.2 ± 9.9% for MVC_{VA} (569 ± 110 vs. 599 ± 101 N; $p<0.05$). This difference in MVC_{VA} was accompanied by neural changes so that VA% was significantly higher in the evening (90 ± 6 %) compared to the morning (87 ± 6%) ($p<0.05$). The corresponding differences in the relative scale (Δ%) are presented in the upper part of Figure 1. No significant morning to evening differences were observed for EMG_{LP} and EMG_{VA} in the high evening performance group.

Neutral types

Neutral types showed 2.1 ± 6.7% higher force values in the evening compared to the morning for MVC_{LP} (2804 ± 734 vs. 2859 ± 751 N; $p<0.05$). No significant morning to evening difference was observed for the MVC_{KE} (777 ± 138 vs. 790 ± 135 N) or for MVC_{VA} (599 ± 108 vs. 607 ± 113 N). EMG_{LP}, EMG_{VA} and VA% did not show any significant morning to evening difference.

No significant increase in body temperature was observed for the high morning performance types (36.0 ± 0.6℃ vs. 36.1 ± 0.6℃) or for the neutral types (36.3 ± 0.3℃ vs. 36.3 ± 0.4℃). Whereas in the high evening performance group, body temperature significantly increased between 7:30 h ± 30 min and 16:30 h ± 30 min from 36.0 ± 0.5℃ to 36.3 ± 0.6℃ ($p<0.01$). All three groups showed significantly higher serum testosterone and cortisol concentrations in the morning compared to the evening ($p<0.001$) and significantly lower T/C ratio values in the morning compared to the evening values (Table 3). Pearson correlation did not reveal any significant correlations between the daily variations in maximal force and serum hormone levels in any of the groups.

Between-group differences

Significant between-group differences were observed for morning to evening changes in MVC_{LP} for the high evening performance types and the high morning performance types ($p<0.001$), the high morning performance types and the neutral types ($p<0.01$) as well as for the high evening performance types and the neutral types ($p<0.001$). Morning to evening changes in MVC_{KE} were significantly different between the high morning performance types and the high evening performance types ($p<0.001$) and between the high evening performance types and the neutral types ($p<0.001$) with the morning types showing significantly higher morning values and the evening and neutral types higher evening values (Figure 1). High evening performance types showed significantly different morning to evening change in VA% compared to the neutral types ($p<0.05$), while high performance evening types showed significantly higher values in the evening and neutral type group did not show any significant diurnal variations in VA%. However, when the two extreme groups (high morning performance group vs. high evening performance group) were compared, then in addition to significant between-group differences in diurnal variations in force production, morning to evening changes in VL EMG_{VA} reached a significant between-group difference with higher morning values in the high morning performance types by 24 ± 27% and higher evening values in the high evening performance types by 7 ± 23% ($p<0.05$). There was a trend for statistical significance between the high evening performance group and the high morning performance group in the morning to evening differences in VL EMG_{LP} (10.2 ± 13.9% vs. 8.4 ± 24.8%; $p=0.060$), in the high morning performance types with higher morning values and in the high evening performance type with higher evening values, respectively. Morning to evening variation in T/C ratio showed a significant difference between the high morning performance and high evening performance types (67 ± 57% vs. 168 ± 166%; $p<0.05$), respectively (Table 3).

DISCUSSION

The present study was designed to investigate the effects of the time of day on the neuromuscular performance of thigh muscles and possible mechanisms behind the inter-individual differences in the diurnal fluctuations in strength performance. In the total group of participants, so far the largest experiment sample in this area of research, our results were consistent with the previous studies with smaller sample sizes investigating knee extensor muscles (Araujo et al., 2011; Callard et al., 2000; Deschenes et al., 1998; Guette et al., 2005a; Nicolas et al., 2005; Onambele-Pearson & Pearson, 2007; Racinais et al., 2005; Sedliak et al., 2008; Sedliak et al., 2011; Teo et al., 2011), since maximal force was significantly greater (~4%) in the evening compared to the morning. The uniqueness of the present study including a large group of participants, however, lays in the fact that the high morning performance and high evening performance types were separated from the neutral types and compared with each other. To the best of our knowledge, this is the first study to divide the morning types, evening types and neutral types into the groups according to their lower limb strength performance, since based on the chronotype questionnaire (Roenneberg et al., 2003) none of our participants belonged to the extreme morning or evening chronotype. We were able to allocate the high morning performance types who showed significantly higher force values in the morning (~6-11%), neutral types who showed slightly higher evening performance or no morning to evening differences in maximal isometric force production and the high evening performance types who showed high morning to evening fluctuations (~6-16%), with significantly higher force values observed in the evening. Moreover, the higher evening force values observed in the high evening performance group could at least partly be explained by the increased central nervous system drive to the thigh muscles since VA% was concomitantly higher in the evening.

Diurnal fluctuations in maximal voluntary force have been explained by (i) changes in the central nervous system drive or (ii) changes at the peripheral level (Guette et al., 2006). In the present study myoelectrical activity of VL and VM muscles did not significantly differ between the morning and the evening hours in the large group of participants. This was in line with previous research results studying the diurnal rhythms in knee extensors (Guette et al., 2005a; Nicolas et al., 2005; Sedliak et al., 2008), showing no fluctuation in EMG recordings over the day. These authors have concluded that daily variations in maximal force can be explained by changes at the muscle tissue level rather than by the failure in the central motor command to activate the knee extensor muscles in the morning. Castaingts et al. (2004) have suggested that the diurnal changes in force are influenced by the changes in the contractile and elastic properties of the muscle, which seem to favor the evening hours. However, others (Callard et al., 2000; Sedliak et al., 2011) have reported maximal EMG activity accompanying with higher force values in the evening for knee extensor muscles and proposed that both neural and muscular factors are involved in the circadian rhythms of MVC. In the present study, EMG mirrored maximal force production as it increased over the day for the high evening performance types and decreased over the day for the high morning performance types, although did not reach the significance level due to large inter-individual differences. Moreover, a significant between-group difference was found for VL $EMG_{VA,}$ showing that the maximal activation of knee extensors took place on different times of the day for the high morning performance types and the high evening performance types.

The possible influence of central factors on diurnal fluctuation in maximal strength performance can also be evaluated by the maximal voluntary activation level (VA%). In the present study maximal VA% did not show any significant difference between the morning and evening in the total group of participants. This suggests that the capacity to activate the knee extensor muscles was not affected by the time of day and that central mechanisms could not explain the diurnal rhythms observed in MVC as supported also by previous research results (Guette et al., 2005a; Martin et al., 1999; Onambele-Pearson & Pearson, 2007). However, the present high evening performance group showed significantly higher VA% in the evening compared to the morning. These results suggest that the higher evening force values in the high evening performance group might be also due to the increased central nervous system drive to the quadriceps femoris muscles. To express this in other words, our evening types may have experienced reduced central motor drive in the morning associated with lower maximal activation of the muscle (Guette et al., 2006). This can be a result of a failure to recruit all motor units or a reduction in maximal discharge rate (Kent-Braun & Le Blanc, 1996). Therefore, the force measured in the morning may not have been a representative of maximal force in the evening type group (Kent-Braun & Le Blanc, 1996). However, this was not the case for the high morning performance types and in the neutral types, who showed similar VA% both in the morning and in the evening. However, the between-group comparisons showed that daily fluctuation in VA% significantly differed between the high evening performance types and neutral types. Although both groups showed higher force values in the evening, the diurnal fluctuations in the high evening performers seem to result from the diurnal fluctuations in the central mechanisms. It is possible that the type II error, caused by a small number of participants in the high morning performance group may have led to false negative results and restricted to find significant between-group differences.

One recent study (Tamm et al., 2009) have investigated differences in strength performance in the morning types and evening types by grouping the participants based on the morningness-eveningness questionnaire (Horne & Ostberg, 1976). However, when Tamm et al. (2009) studied the extreme morning and evening chronotypes, only the evening types showed significant diurnal rhythm in force production. EMG recording was mimicking force showing higher values in the evening for the evening types and no morning to evening differences for the morning types. Based on the EMG data, they proposed that the increased central nervous system drive to the triceps surae could explain the higher force values in the evening recorded in the evening type group. No diurnal variations were observed in VA% for the morning or evening types. This difference between our study and the investigation by Tamm et al. (2009) may come from the fact that small muscle groups such as triceps surae may be more easily maximally activated than larger muscle groups such as the quadriceps (Behm et al., 2002). In addition to that it needs to be kept in mind that in the study by Tamm et al. (2009) the morning and evening types were selected based on the chronotype questionnaire. In addition, it is possible that the calf muscles which are frequently used during daily activities may show dissimilar diurnal patterns compared to the knee extensors muscles (Guette et al., 2006). We propose that in the present study, simultaneous modifications in the neural and muscular mechanisms in the evening led to increased ability to generate force during MVC in the high evening performance group. It seems that in the high morning performance types and neutral types peripheral rather than central factors affect diurnal variations as central motor drive did not show significant time of day effect. However, the mechanisms explaining the diurnal fluctuations in maximal isometric force in these groups need further investigation.

In addition to central mechanisms, many studies have proposed that diurnal rhythms in body temperature can explain the fluctuation in force (Coldwells et al., 1994; Teo et al., 2011), since the acrophases of these two rhythms are simultaneous (Racinais et al., 2005). As listed in Bambaeichi et al. (2004) higher body temperature enhances conduction velocity of action potentials, enzymatic activity and extensibility of connective tissue but reduces muscle viscosity and antagonistic co-contraction. Although others have found positive correlations between muscle torque and body temperature, it cannot be concluded that these two variables depend on a common synchronizer or influence each other (Callard et al., 2000). Similar to Callard et al. (2000), the amplitudes of the rhythm in body temperature were too low in this study to account for the changes in maximal force production. Reilly & Waterhouse (2009) proposed that intra-aural body temperature may not be reliable because of temperature changes produced at the measurement site by air flow past the recording site. However, also other studies have used intra-aural body temperature measurements (Atkinson et al., 2005; Castaingts et al., 2004) and found the results to be similar to those by aural measurements. In the present study the diurnal changes in the body temperature were smaller compared to the previous studies (Castaingts et al., 2004; Gauthier et al., 2001). This may refer that the intra-aural temperature measurement technique used in the present study may have been not reliable enough to assess the diurnal fluctuations in the body temperature.

In addition to changes in body temperature, the central nervous system arousal associated with the sleep-wake cycle may influence force production over a day (Birch & Reilly, 2002). The amount of sleep on the night before the measurements and the time between awakening and tests was not documented in the present study. It has been previously shown that the sleep deprivation might temporally change performance patterns, however, Bambaeichi et al. (2004) found that the diurnal fluctuations in muscle strength did not change after partial sleep loss. Moreover, the time awake (associated with fatigue) and sleepiness has been shown to influence the time-of-day-specific performance (Araujo et al., 2011; Carrier and Monk., 2000; Edwards et al., 2007), as performance efficiency on a specific task may decrease over the day because of the amount of hours since awakening (Carrier and Monk, 2000).

Also Edwards et al. (2013) have suggested that several factors may account for circadian variations in muscle strength, including effects of motivation, subjective arousal and sleepiness, hormones (thyroid hormones, T/C ratio) and ionic changes. In the present study, the analysis of serum hormone concentrations revealed that diurnal variation in serum testosterone and cortisol was similar to those found in previous studies (Hayes et al., 2012), with the highest values observed in the morning for both hormones. However, our results failed to show any significant relationships between the daily changes in peak force and concentrations of serum testosterone, cortisol or T/C ratio and, thereby, reconfirmed what has been reported by other studies (Hayes et al., 2013; Teo et al., 2011). Our high morning performance group and high evening performance group showed significantly different morning to evening change in the T/C ratio. The high evening performance types showed significantly larger morning to evening fluctuation in the T/C ratio compared to the high morning performance types. This indicates that the high evening performance types may have been in a more desirable physiological condition for strength performance in the evening compared to the morning (Hayes et al., 2013). However, as the strength performance and hormonal data was collected on different days, we have to be careful with the conclusions.

This is the first study observing the inter-individual differences in maximal voluntary force production in the condition were extreme chronotypes were excluded from the sample. So far the efforts to determine the circadian phenotype and thereby the time-of-day preferences, are based on the scores obtained from the chronotype questionnaires, rather than in actual timing (phase of entrainment). These questionnaires assess the self-reported preferences to perform certain activities and should provide the data about when physical function, hormone levels, body temperature, cognitive faculties and eating and sleeping patterns are active (Levandovski et al 2013). In the present study, the high morning and high evening performance types showed in addition to significant differences in force production, also distinct rhythms in myoelectric activity and serum T/C. This means that persons not belonging to the extreme chronotypes may still demonstrate either high morning or high evening strength performance. Therefore, the questionnaires designed to determine the chronotype may not always be sensitive enough to determine the morningness or eveningness in maximal neuromuscular performance. Our results indicate that the ability to produce maximal voluntary force is not related to the morningness-eveningness determined by the chronotype questionnaire.

In summary, diurnal variations in strength performance are likely to be multi-factorial and the mechanisms behind the diurnal rhythms seem to vary between the individuals as suggested also by the previous studies (Chtourou & Souissi, 2012; Giacomoni et al., 2005). The present results from the total group of participants are in accordance with previous studies, showing that isometric knee extension force is higher in the evening when compared to the morning (Guette et al., 2005a; Nicolas et al., 2005; Sedliak et al., 2008). However, we were able to identify the high morning performance types who showed significantly higher voluntary maximal isometric force levels in the morning and the high evening performance types who showed large morning to evening fluctuations in knee extensor force with higher values observed in the evening. As to the mechanisms, central factors seem not to be exclusively responsible for these daily variations but possibly peripheral mechanisms were also affecting the diurnal fluctuation in maximal strength performance. Nevertheless, in the high evening performance group the diurnal fluctuations in maximal force were associated with accompanying diurnal variations in the central activation. The mechanisms behind high morning performance in the high morning performance types, however, need further investigation. This means that persons not belonging to the extreme chronotypes may still have the ability to demonstrate either high morning or high evening strength performance and chronotype questionnaires may not be sensitive enough to predict the best time for physical performance. In the future also training-induced adaptations should be studied for the persons showing high morning or evening strength performance.

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Resting variables	7:30h±30min	9:30h±30min	16:30h±30min	18:30h±30min	Diurnal changes
Body temperature (˚C)	36.2 ± 0.4	36.3 ± 0.3	36.3 ± 0.4	36.2 ± 0.5	7:30<16:30* 16:30>18:30$^{\$}$
Testosterone (nmol/L)	13.8 ± 3.6	11.6 ± 3.7	10.7 ± 3.3	11.0 ± 3.4	7:30>9:30,16:30 & 18:30***
Cortisol (nmol/L)	409.7 ± 109.7	258.5 ± 85.3	197.1 ± 96.2	129.5 ± 72.0	7:30>9:30,16:30&18:30*** 9:30>16:30$^{\#}$&18:30$^{\#\#\#}$ 16:30>18:30$^{\$\$\$}$
T/C ratio	0.036 ± 0.014	0.049 ± 0.021	0.067 ± 0.037	0.111 ± 0.075	7:30<9:30,16:30&18:30*** 9:30<18:30$^{\#\#\#}$ 16:30<18:30$^{\$\$\$}$

Table 1. Absolute values (mean ± SD) for resting body temperature, serum testosterone (T), cortisol (C) and T/C ratio (n=72). * Sign. different from 7:30; * < 0.05; *** < 0.001; # Sign. different from 9:30; # < 0.05; ### < 0.001; $ Sign. different from 16:30; $ < 0.05; $$$ < 0.001.

Table 2. Absolute values for morning and evening neuromuscular performance (mean ± SD).

Neuromuscular performance	Morning	Evening
MVC_{LP} (N) (n=71)	2692 ± 713	2799 ± 759 **
MVC_{KE} (N) (n=69)	746 ±148	772 ± 142 **
VL EMG_{LP} (mV) (n=70)	0.57 ± 0.26	0.56 ± 0.25
VM EMG_{LP} (mV) (n=70)	0.66 ± 0.33	0.66 ± 0.37
MVC_{VA} (N) (n=66)	584 ± 106	597 ± 106 (*)
Voluntary activation (%) (n=62)	89 ± 6	90 ± 6
VL EMG_{VA} (mV) (n=70)	0.29 ± 0.11	0.28 ± 0.11
VM EMG_{VA} (mV) (n=70)	0.32 ± 0.15	0.31 ± 0.14

* sign. morning/evening difference; (*) < 0.07; ** < 0.01.

	High morning performance types (n=8)		High evening performance types (n=19)		Neutral types (n=45)	
	Morning	Evening	Morning	Evening	Morning	Evening
Testosterone (nmol/L)	14 ± 3	11 ± 2 **	12 ± 3	10 ± 3*	13 ± 4	11 ± 3***
Cortisol (nmol/L)	364 ± 66	194 ± 56***	335 ± 69	151 ± 72***	328 ± 94	163 ± 73***
T/C ratio	0.041 ± 0.012	0.067 ± 0.026*¤	0.039 ± 0.014	0.102 ± 0.072***¤	0.044 ± 0.017	0.088 ±0.045***

Table 3. Morning (mean of the two morning sapling) and evening (mean of the two evening sampling) serum hormone concentrations (mean ± SD) for the high morning performance types, high evening performance types and the neutral types . * sign. morning/evening difference; * < 0.05; ** < 0.01; *** < 0.001; ¤ sign. difference between high morning performance types and high evening performance types in m/e difference; ¤ < 0.05.

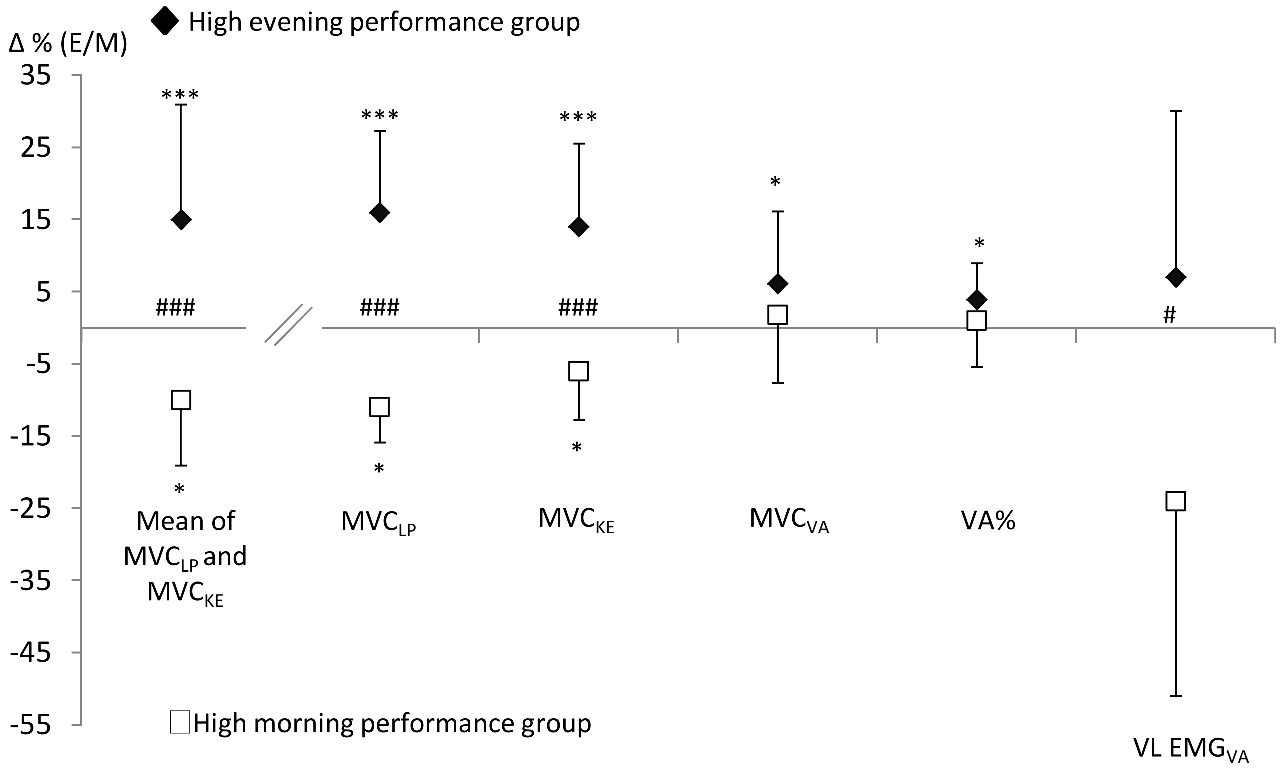

Figure 1. Morning/evening differences [Δ% (E/M)] in neuromuscular performance in the high evening performance group and high morning performance group. * sign. morning/evening difference; * < 0.05; *** < 0.001; # sign. between-group difference; # < 0.05; ### < 0.001.

II

EFFECTS OF MORNING VERSUS EVENING COMBINED STRENGTH AND ENDURANCE TRAINING ON PHYSICAL PERFORMANCE, MUSCLE HYPERTROPHY, AND SERUM HORMONE CONCENTRATIONS

by

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Effects of morning vs. evening combined strength and endurance training on physical performance, muscle hypertrophy and serum hormone concentrations

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ABSTRACT

This study investigated the effects of 24 weeks of morning vs. evening same-session combined strength (S) and endurance (E) training on physical performance, muscle hypertrophy and resting serum testosterone and cortisol diurnal concentrations. Forty-two young men were matched and assigned to a morning (m) or evening (e) E+S or S+E group (mE+S n=9, mS+E n=9, eE+S n=12 and eS+E n=12). Participants were tested for dynamic leg press 1 repetition maximum (1RM) and time to exhaustion (T_{exh}) during an incremental cycle ergometer test both in the morning and evening, cross-sectional area (CSA) of vastus lateralis and diurnal serum testosterone and cortisol concentrations (7:30h; 9:30h; 16:30h; 18:30h). All groups similarly increased 1RM in the morning (14-19%; $p<0.001$) and evening (18-24%; $p<0.001$). CSA increased in all groups by week 24 (12-20%, $p<0.01$), however, during the training weeks 13-24 the evening groups gained more muscle mass (time-of-day main effect; $p<0.05$). T_{exh} increased in all groups in the morning (16-28%; $p<0.01$) and evening (18-27%; $p<0.001$), however, a main effect for the exercise order, in favor of E+S, was observed on both testing times ($p<0.051$). Diurnal rhythms in testosterone and cortisol remained statistically unaltered by the training order or time. The present results indicate that combined strength and endurance training in the evening may lead to larger gains in muscle mass, while the E+S training order might be more beneficial for endurance performance development. However, training order and time seem to influence the magnitude of adaptations only when the training period exceeded 12 weeks.

Keywords: time-of-day, testosterone, cortisol, concurrent training, order effect, muscle cross-sectional area

INTRODUCTION

Previous studies have shown that when performed simultaneously, adaptations to strength and endurance training might be compromised (Cadore et al. 2013, Chtara et al. 2005, Gravelle and Blessing 2000, Hickson 1980). However, both training types are necessary for general health and fitness and for time saving purposes these training modes are sometimes performed during the same training session. When combined into the same session, the intra-session sequence of strength and endurance training may be one factor influencing the training adaptations (Cadore et al. 2013; Chtara et al. 2005; Gravelle & Blessing 2000). It has been proposed that the first mode of exercise may cause fatigue and, thereby, negatively influence the quality and quantity of the second mode of exercise (Leveritt & Abernethy 1999), and/or hamper the acute molecular responses (Fyfe et al. 2014). It has been demonstrated that strength performance (Cadore et al. 2013) or neural adaptations (Eklund et al. 2015) might be interfered, when endurance training constantly precedes strength training, especially when the training period is prolonged. Others have observed compromised gains in endurance performance due to the intra-session sequence of strength and endurance training (Chtara et al. 2005; Gravelle & Blessing 2000). However, a number of studies have suggested that the intra-session sequences of strength and endurance training may not influence the training adaptations in strength and endurance performance or in muscle mass gains (Chtara et al. 2008; Schumann et al. 2014).

In addition to the training mode specificity, adaptations in strength and endurance performance may also be dependent on the time-of-day when the training is performed. Previous studies have shown that greater improvements in maximal strength performance occur at the time-of-day at which resistance training was regularly performed (Chtourou et al. 2012, Sedliak et al. 2008, Souissi et al. 2002). The time-of-day-effect on training-induced hypertrophic adaptations has received less attention. Although Sedliak et al. (2009) found no statistically significant differences in the magnitude of muscular hypertrophy after strength training between morning and evening training, a tendency for smaller gains in muscle size were reported when repeatedly performing strength training in the morning. The literature about endurance performance, on the other hand, has led to inconclusive results. Although some studies have suggested that the adaptations to endurance performance are time-of-day-specific (Hill et al. 1998; Torii et al. 1992), others disagree (Hill et al. 1989).

Testosterone (T) and cortisol (C) are considered as important biomarkers in exercise science. One of the main functions of T is to maintain anabolism, in addition to other tissues, also within the muscular system by promoting protein synthesis. C, however, has been shown to have catabolic function that promotes protein breakdown (Kraemer and Ratamess 2005). Therefore, some studies have suggested T and C to be potent hormones contributing to long-term changes in performance and muscle growth (Ahtiainen et al. 2003; Häkkinen et al. 1985), while others may disagree (West and Phillips 2012). The effects of both prolonged strength as well as endurance training on basal T and C concentrations, however, have shown to be inconsistent or non-existent (Hackney et al. 2003; Kraemer et al. 1995; Kraemer & Ratamess 2005), whereas the data about the effects of combined strength and endurance training are limited. In addition, both T and C exhibit circadian rhythmicity with morning peaks and evening nadirs (Kraemer et al. 2001). Greater T and C responses have been observed in afternoon compared with morning strength training sessions (Chtourou et al. 2013). Nevertheless, the influence of short term strength training protocols have shown to be insufficient to alter the circadian profile of T and C (Häkkinen et al. 1988, Kraemer et al. 2001). Sedliak et al. (2007) have observed decreased serum C concentrations after prolonged time-of-day-specific strength training in the morning. However, they suggested that decreased anticipatory psychological stress before the morning test sessions rather than adaptations in the adrenal cortex induced by regular training in the morning caused these adaptations. Neither morning nor evening training time showed any influence on the rhythms in T concentrations (Sedliak et al. 2007). Therefore, further examination is needed to confirm whether exercise training can exert a strong enough influence to alter the circadian rhythms of T and C (Teo et al. 2011).

The purpose of the present study was to examine how the strength and endurance training sequence and time-of-day (i.e. morning vs. evening) affect the adaptations in muscle strength and hypertrophy as well as endurance performance after 24 weeks of time-of-day-specific same-session combined strength and endurance training. In addition, we wanted to determine whether such training regimens performed in the morning or evening have an effect on diurnal patterns of resting serum T and C concentrations. Since strength and endurance training are often performed concurrently, it was important to understand whether the morning or evening training time or specific strength and endurance training order can optimize the adaptations to combined training.

METHODS

Participants

Seventy-two men who had no history of previous strength or endurance training over the past year were recruited for this study. The participants were considered healthy and had no medical contraindications or musculoskeletal restrictions that could affect the results of this study. In addition, a cardiologist checked each participant's health questionnaire and ECG. According to the Munich Chronotype Questionnaire (Roenneberg et al. 2003) none of the participants belonged to an extreme morning or evening chronotype. Shift and night workers were excluded. After receiving a thorough explanation of the purpose and risks of the study, the participants provided written consent before participation. This study complied with the Declaration of Helsinki and was approved by the Ethics Committee in the University of Jyväskylä.

Some dropouts occurred during the 24-week intervention period due to medical issues, motivation or personal reasons, i.e. change of residence. Thus, only the participants who successfully completed at least 90% of the entire 24-week training intervention were included in the analyses (n=52).

Study design and measurements

The summary graph with the study design and measurements are presented on Figure 1. The 24-week combined strength (S) and endurance (E) training consisted of two 12-week periods which were separated with the pre-, mid- and post-measurements performed during a non-training week. Pre-measurements took place in September/October before the trainings had started, mid-measurements was conducted after 12 weeks of training in January/February and post-measurements in May/June after completing 24 weeks of combined training. During the first part of the intervention period (wk 1-12) participants trained two times per week [2x(1E+1S) or 2x(1S+1E)] and during the second part of the intervention (wk 13-24) an additional session was added every two weeks so that all participants performed 5 training sessions in a 2-week period [5x(1E+1S) or 5x (1S+1E)] to allow further progression in training adaptations. Following the basal measurements for strength and endurance performance, participants were matched into four training groups based on the anthropometrics (Table 1) and strength and endurance performance at baseline (Table 2): (i) training in the morning (m) and performing endurance training always before the strength training (mE+S, n=9), (ii) training in the morning with strength always preceding endurance training (mS+E, n=9), (iii) training in the evening (e) and performing endurance before strength training (eE+S, n=12), (iv) training in the evening with strength always preceding endurance training (eS+E, n=12). The controls (n=10) were asked to maintain their pre-experimental physical activity level throughout the study. A nutritional information lecture was held for the participants before the start of the study to instruct them to consume nutrients according to the national guidelines. Participants were asked to keep their daily energy intake and habitual physical activities constant throughout the intervention period and avoid any additional strength and/or endurance training.

Strength and endurance measurements were conducted both in the morning (between 06:30 h ± 30 min and 9:30 h ± 30 min) and in the evening (between 16:30 h ± 30 min and 19:30 h ± 30 min) independent of the group assignment. The morning and evening tests were carried out in a random order across all participants. Within individuals, the tests were always carried out in the same order and at the same time-of-day (±1h) at all three measurement points. The time interval between two consecutive strength or endurance performance tests was at least 36 hours and participants were asked to avoid any unnecessary physical activity. The last training session and the first measurement were always separated by a minimum of two and maximum of four days. The participants received instructions to follow their usual sleeping habits with a minimum of 6 hours of sleep taken on the night preceding each testing session. They were asked to avoid alcohol for 24 hours and caffeine for 12 hours before the endurance and strength performance tests. A familiarization session was carried out for all participants before the start of the 24-week intervention on a non-training-specific time-of-day. This session included familiarization to the dynamic leg press testing procedures and measuring the starting knee angle for the dynamic leg press (~60°). After the familiarization session the device settings were stored for further measurements to ensure the correct device set ups for each subject throughout the study. In addition, a small mark was tattooed to the mid-point (between the greater trochanter of the femur and lateral joint space) of the lateral side of the right thigh to ensure the repeatability of the ultrasound measurements for muscle cross-sectional area throughout the study.

One repetition maximum (1 RM): Maximal bilateral concentric leg press strength was measured by a horizontal leg press (knee angle ~60°) (David 210, David Health Solutions Ltd., Helsinki, Finland). Before commencing maximum efforts a short warm-up was performed (5x70%; 3x80% and 2x90% of the predicted 1 RM). A minimum of three and maximum of five trials of single repetitions (1 RM) were allowed. Each trial was separated with one minute of rest. The testing continued until the participant was unable to extend legs to a full extension of 180̊.

Cross-sectional area of vastus lateralis (CSA) was assessed by a B-mode axial-plane ultrasound (SSD-α10, Aloka Co Ltd, Tokyo, Japan) with the extended-field-of-view mode (23-Hz sampling frequency) using a 10 MHz linear array probe (Ahtiainen et al. 2010). Three panoramic images were taken at 50% of femur length (marked during the familiarization session). During the measurements participants laid in the supine position, with lower limbs extended and resting in a styrofoam knee support. A line, representing the mid-point, was drawn across the thigh, perpendicular to the measurement table. The probe was manually moved in an axial plane from the lateral to the medial side of the thigh along the drawn line. Images were manually analyzed with the ImageJ software (National Institute of Health, USA, version 1.44) by manually tracing along the border of the m. vastus lateralis. The mean of the two closest values was used for further statistical analysis. The measurements always took place in the morning at the same time of the day (±1h). All the measurements and analysis were done by the same technician, who was aware of the subject's ID but the ID did not reveal the group to which the subject belonged to.

Time to exhaustion (T_{exh}): The graded maximal aerobic cycling test to volitional exhaustion was performed on a mechanically braked bicycle ergometer (Ergomedic 839E, Monark Exercise AB, Sweden). The exercise intensity was increased by 25 W every two minutes starting with 50W. Pedaling frequency was required to be maintained at 70 rpm throughout the test. The participants were verbally encouraged to continue cycling until exhaustion. The test was stopped when the participant was unable to keep up the required pedaling frequency.

Serum hormone concentrations

Venous blood samples were collected during one day at 7:30 h ± 30 min, 9:30 h ± 30 min, 16:30 h ± 30 min and 18:30 h ± 30 min at pre-, mid- and post-measurements. Participants were asked to keep two days of rest before the day when the blood samples were drawn. Physical activity during that day was also asked to be kept as low as possible, however, the subjects were allowed to leave the lab in between the blood samples drawn and have a normal working day. Participants were asked to fast 12 hours before the first blood sample, after which a standardized breakfast was provided. They were instructed to consume another light meal at around 12:00 - 13:00 h. Thereafter, participants were asked to avoid food until the last two blood samples (16:30 h ± 30 min and 18:30 h ± 30 min) were drawn.

Venous blood samples (~10 ml) for the determination of serum total T and C concentration were collected by a qualified laboratory technician from an antecubital vein with a vacutainer and test tubes (Vacuette, Greiner Bio-OneGmbH, Kremsmünster, Austria) containing appropriate preservatives. Samples were centrifuged at 3500 rpm (Heraus Megafuge 1.0 R, Gendro

Laboratory Products, Hanau, Germany) for 10 min, plasma harvested and all samples were stored at −80°C until assayed. Analysis of total T and C was performed using chemical luminescence techniques (Immunlite 2000, Simens Healthcare, Diagnostics Products Ltd., Llanberies, UK) and hormone specific immunoassay kits (Siemens, New York City, NY,, USA). The sensitivities for serum T and C were: 0.5 $nmol \cdot L^{-1}$ and 5.5 $nmol \cdot L^{-1}$, respectively. The intra-assay coefficients of variation were 8.3% and 5.3% for T and C, respectively. The inter-assay coefficients of variation were 9.1% for T and 7.2% for C.

Exercise training programs

Training consisted of two 12-week progressive same-session combined strength and endurance training periods either in the morning or in the evening. The morning training groups (mE+S and mS+E) performed all training sessions between 6:30-10:00h, while the evening training groups (eE+S and eS+E) performed their training sessions between 16:30-20:00h. The training programs were identical for the E+S and S+E group independent of the training time, only the sequence of strength and endurance training was reversed. Endurance and strength training were combined into the one training session so that no more than a 5-10 minute break was allowed during the two training sections. The duration of the combined endurance and strength training sessions progressively increased from 60 to 120 minutes. All the training sessions were supervised.

Strength training consisted of exercises aimed at improving both maximal strength and muscle hypertrophy and was planned as a whole body periodized program with the main focus on knee extensors and flexors as well as hip extensors. Each training session consisted of three lower-body exercises: bilateral dynamic leg press, seated dynamic knee extension and flexion. Four to five exercises were performed for other main muscle groups (lateral pull down, standing bilateral triceps push down, bilateral biceps curl, seated military press, or bilateral dumbbell fly, trunk flexors and extensors). Strength training was designed to improve muscular endurance in the first 4 weeks, which was performed as circuit training (intensity 40-70% of 1 RM). The subsequent 4 weeks (weeks 5-8) were designed to produce muscle hypertrophy (intensity 70-85% of 1 RM) and followed by 4 weeks (weeks 9-12) of mixed hypertrophic and maximal strength training (intensity 75-95% of 1 RM) (Table 3). A similar strength training program with slightly higher intensities was carried out also during the second 12 weeks of training.

Endurance training was carried out on cycle ergometers and the training intensity was based on the maximum heart rate (HR_{max}) determined during the training-time-specific graded maximal incremental cycling test. For the first 12 weeks of training the pre-training test results were used. For training weeks 13-24 the training heart rate zones were readjusted according to the results obtained from the mid-measurements. Endurance training sessions averaged from 30-50 minutes. Interval (85-100% of HR_{max}) and continuous (65-80% of HR_{max}) training protocols were performed weekly. Interval training consisted of 4x4 min high-intensity intervals (85-100% of HR_{max}), which were separated by 4 min active resting periods (70% of HR_{max}). During the first training period both interval and continuous training sessions were performed once a week, whereas during the second training period, when the training frequency increased, one additional high-intensity interval training session was added. Participants were instructed to maintain a constant pedaling cadence (around 70 rpm) while the resistance on the cycle ergometer was adjusted in accordance with heart rate (Polar FT7, Polar Electro Oy, Kempele, Finland).

Statistical analyses

Data are presented as mean ± SD. Normality of the data was checked and subsequently confirmed using the Shapiro-Wilk test. Effects of the time-of-day-specific training on strength and endurance performance as well as on resting serum hormone concentrations and muscle hypertrophy were examined by a two-factor general linear model (GLM) with univariate ANOVA by using absolute changes. Time-of-day of the training and training order were set as fixed factors when appropriate. Within-group changes over time in the morning and in the evening were examined by using absolute values with repeated measures GLM, where Time, with 3 levels (4 levels for hormonal data analysis) was the only factor. Morning and evening differences at pre-, mid- and post-measurements in performance variables were checked by using paired samples T-tests. In addition, to analyse associations between strength performance and muscle mass Pearson correlation coefficients were calculated. Area under the curve (AUC) was calculated for T and C concentrations by using the following equation:

$$AUC = [(y_1+y_2)/2) \times (t_1-t_2)],$$

where y_1 and y_2 are the consecutive resting T or C concentrations and t the time separating the two measurement time-points. AUC was calculated for the total period between 7:30 and 18:30. Statistical significance was accepted at a criterion alpha level of $p<0.05$, whereas values $p\leq0.06$ were accepted as a significant trend. Effect size (d) for pairwise comparisons is reported as Cohen's d with an effect size of ≥0.20 being considered as small, ≥0.50 as medium and ≥0.80 as large.

RESULTS

At baseline no between-group differences were found in strength and endurance performance or in anthropometrics. Absolute values of strength or endurance performance variables did not show significant morning to evening differences in any group at any measurement time point.

Dynamic strength performance

Morning measurements: 1 RM dynamic leg press force increased in the morning during the first 12 weeks and after completing 24 weeks of training the improvements were independent of the training time and order (mE+S: 13.8±5.5%; mS+E: 16.9±6.2%; eE+S: 18.1±8.1%; eS+E: 19.3±11.6%, all $p<0.001$) (Table 2). The control group increased maximal dynamic strength performance by 5.1±5.7% ($p<0.05$), however, all training groups, except mE+S, increased the morning strength performance significantly more ($p<0.05$) than controls.

Evening measurements: 1 RM strength increased in the evening in all training groups during the first 12 weeks and by the end of the study all training groups had similarly increased 1 RM (mE+S: 17.5±7.7%; mS+E: 20.7±9.0%; eE+S: 20.5±8.9%; eS+E: 23.6±12.9%, all $p<0.001$) (Table 2). Improvements in all training groups were larger than in the control group ($p<0.05$), which showed no significant change in maximal dynamic strength performance in the evening.

Cross sectional area

All training groups similarly increased CSA of vastus lateralis during the first 12 weeks of training (mE+S: 9.2±5.6%; mS+E: 9.9±8.3%; eE+S: 14.0±9.3%; eS+E: 10.0±6.4%; all $p<0.05$) (Figure 2; Table 2). During the second training period (wks 13-24) only eE+S and eS+E groups continued to increase CSA (eE+S: 5.1±4.5%; eS+E: 5.1±4.7%; $p<0.01$), which led to a significant time-of-day main effect ($p<0.05$; d=0.685). During weeks 1-24 all training groups had increased CSA (mE+S: 11.9±9.2%; mS+E: 12.1±7.9%; eE+S: 19.8±10.8%; eS+E: 15.7±9.1%; all $p<0.01$) (Table 2), however a statistical trend for time-of-day main effect was observed in favor of the evening training time ($p=0.059$; d=0.623). In the control group CSA remained unchanged.

Only in the eS+E group the individual changes in CSA of vastus lateralis and evening 1 RM dynamic leg press force correlated significantly throughout the 24 weeks of training (week 1-24: r=0.638, $p<0.05$; weeks 1-12: r=0.657, $p<0.05$; weeks 13-24: r=0.579, $p<0.05$).

Endurance performance

Morning measurements: All training groups increased T_{exh} (mE+S: 16.2±5.4%; mS+E: 16.1±12.9%; eE+S: 18.9±7.5%; eS+E: 11.5±7.0%; all $p<0.01$) in the morning during the first 12 weeks of training independent of the training time and order (Figure 3; Table 2). Only mE+S and eE+S continued to significantly increase during weeks 13-24 (5.5±3.9% and 8.1±5.1%, respectively; $p<0.001$). By the end of the study, T_{exh} had increased in all of training groups (mE+S: 22.5±6.4%, mS+E 21.1±13.7%, eE+S 28.3±7.2% and eS+E 15.7±7.5%; $p<0.01$) and the increases were larger than that observed in the control group ($p<0.01$). A statistical main effect for the exercise order ($p<0.05$; d=0.860) was found in changes in the morning T_{exh} with mE+S ($p=0.051$; d=0.982) and eE+S ($p<0.001$; d=1.711) increasing more than eS+E.

Evening measurements: All training groups increased T_{exh} similarly and significantly (mE+S: 11.2±7.4%; mS+E: 15.7±7.6%; eE+S: 15.8±10.5%; eS+E: 10.7±9.1%, all $p<0.05$) in the evening during the first 12 weeks of training (Figure 3; Table 2). During the second 12 weeks the mS+E group did not statistically improve T_{exh}, while the significant increases in other training groups were larger ($p<0.05$) than in mS+E during weeks 13-24. A significant main effect for exercise order ($p<0.05$; 0.722) and time-of-day ($p<0.05$; d=0.493) was observed during the second 12 weeks of training in favor of the evening training time and the E+S order. After completing 24 weeks of training all four training groups significantly increased T_{exh} in the evening (mE+S: 19.7±6.7%; mS+E: 19.4±8.5%; eE+S: 26.9±13.0%; eS+E: 18.2±8.4%, all $p<0.001$), while the increases were statistically larger than in the control group ($p<0.01$). A statistical trend for a main effect for exercise order was observed ($p=0.051$; d=0.617) in favor of the E+S groups.

Hormonal concentrations

Both serum T and C concentrations exhibited significant decreases ($p<0.05$) from the morning (at 7:30 h ± 30 min) to evening (18:30 h ± 30 min) measurements in the beginning (T: 10.8-26.5%; C:57.8-73.9%), after 12 (T: 19.1-359%; C:64.8-77.4%) and after 24 weeks of training (T: 24.7-33.9%; C: 64.5-71.7%). No major changes in T or C concentrations were observed in the training groups when analyzing each group separately. However, when the morning training groups were combined (mE+S and mS+E), a 17.4±20.6% increase was observed in the basal morning T levels ($p<0.05$) and a 16.1±13.0% increase in AUC of T ($p<0.05$) after the second training period (wk 13-24) (Figure 4). The combined evening training groups (eE+S and eS+E) showed an increase of 22.7±29.6% in basal morning T values ($p<0.01$) after the second 12 weeks of training and AUC of T increased after the second 12 weeks of training (14.1±17.0%; $p<0.05$) as well as after the whole 24 weeks (15.0±16.7%; $p<0.01$). When the training groups were combined according to the strength and endurance training order, the combined E+S group (mE+S and eE+S) increased AUC of T by 18.0±19.0% after the second training period ($p<0.01$) and 12.6±15.7% after completing the whole 24-week training ($p<0.05$). The combined S+E group (mS+E and eS+E) increased AUC of T by 11.0±11.9% after the second training period

($p<0.05$). Basal morning T concentrations increased similarly in the combined E+S and S+E groups after the weeks 13-24 (E+S: 17.4±26.7%; S+E: 23.5±26.6%; $p<0.01$) and weeks 1-24 (E+S: 22.9±27.9; S+E 14.4±22.4%; $p<0.05$). AUC of C remained statistically unchanged by the training time and order interaction (Figure 4). In the combined morning group a statistical trend for the increased basal morning (11.9±17.7%; $p<0.06$) cortisol levels during the weeks 13-24 was observed. Basal morning C concentrations increased by 14.7±26.0% ($p<0.01$) in the evening combined group when the changes over the entire 24 weeks of training were analyzed. In the combined E+S and S+E groups basal morning C concentrations similarly increased during the weeks 1-24 (E+S: 19.1±32.2%; S+E: 13.0±22.3%; $p<0.05$).

DISCUSSION

The main results of the present study were that whereas the S and E training order and time-of-day of the training did not influence the magnitude of adaptations in maximal dynamic strength performance, the present combined training intervention induced larger gains in muscle cross-sectional area in the evening training groups compared to the morning groups, irrespective of the exercise order. Endurance performance development seemed to be related to the sequence of S and E training, favoring the E+S order both in the morning and in the evening. Furthermore, prolonged combined training may cause training-time-specific adaptations as training in the evening seemed to lead to greater evening endurance performance adaptations compared to the morning training. Diurnal rhythms in testosterone and cortisol remained statistically unaltered by the training order or time.

Strength performance

After 24 weeks of time-of-day-specific same-session combined S and E training all training groups similarly increased dynamic 1 RM strength performance. The increases in 1 RM were independent of the training time (morning vs. evening) and order. Some of the previous not-time-of-day-specific combined training studies have suggested that endurance exercise at the beginning of an exercise session may interfere with neural adaptations in young (Eklund et al. 2015) and strength development in old men (Cadore et al. 2013). However, in line with the present results, others have found that gains in maximal strength performance were not related to the sequence of S and E training sessions (Chtara et al. 2008; Schumann et al. 2014). The present results might be specific to the type of endurance training, because the eccentric component of endurance running has been proposed to cause muscle damage (Wilson et al. 2012), whereas cycling has been shown to consist of primarily concentric activity which is biomechanically similar to strength training (Gregor et al. 1991). Consequently, endurance cycling has shown to cause small but statistically significant increases in leg muscle strength in previously untrained men (Häkkinen et al. 2003; Mikkola et al. 2012). It is possible that the cycling activity in the present training program led to synergistic adaptations with strength training, as suggested also by Schumann et al. (2014).

Previous strength training studies have demonstrated that after regular strength training at a particular time-of-day, strength performance increased at the training specific time-of-day (Chtourou et al. 2012, Sedliak et al. 2008). However, similar to our results with combined strength and endurance training, Blonc et al. (2010) showed that strength training displayed no temporal specificity and that training adaptations were not influenced by the time-of-day. The assumption that adaptations to resistance training may vary between different training times is based on the fact that various factors influencing maximal strength performance (e.g. body temperature, contractile state of the muscle and/or neural input to the muscle) are highest at a certain time-of-day (Chtourou and Souissi 2012). Therefore, it is possible that lack of diurnal variation in the present maximal dynamic strength performance may have possibly hidden also the diurnal rhythms in maximal strength adaptations. Therefore, future research, where statistically significant diurnal rhythms (morning to evening differences) in maximal dynamic strength performance can be observed, should verify the present results.

Hypertrophy

In agreement with previous reports, the increase in cross-sectional area of vastus lateralis in the present study was not related to the S and E training order (Cadore et al. 2013; Schumann et al. 2014). It is possible that moderate but repeated force production during cycling action provided an additional stimulus to promote hypertrophy (Mikkola et al. 2012), rather than counteracted with the adaptations elicited by hypertrophic and maximal strength training program. Although some studies have reported the interference with muscle hypertrophy development when the combined S and E training is performed at a high frequency or high intensity (Hickson 1980; Kraemer et al. 1995), no interference by endurance training have been reported when the training frequency remains moderate or low (Häkkinen et al. 2003; McCarthy et al. 2002). Thereby, our study is in agreement that S and E training (as cycling) order does not influence the magnitude of adaptations in muscle CSA, especially when the combined training frequency does not exceed 2-3/week.

In the present study all training groups similarly and significantly increased CSA of vastus lateralis during the first 12 weeks. However, a significant main effect for the training time-of-day was found for the changes in CSA of vastus lateralis after the second 12 weeks of combined training, showing that groups training in the evening hours increased CSA significantly more compared to groups training in the morning. Sedliak et al. (2009) have shown that time-of-day-specific strength training for 2-3 months in the morning and afternoon hours is similarly effective when aiming for muscle hypertrophy. However, the increase in mid-thigh muscle volume, although minor and statistically insignificant, favored the afternoon strength training group (Sedliak et al. 2009). Our results are similar with those of Sedliak et al. (2009) for the first training period (3 months), demonstrating that training time of combined strength and endurance training did not significantly influence the magnitude of adaptations in muscle mass. However, when the training period is prolonged (6 months), the evening training time of combined strength and endurance training may be more beneficial for muscle hypertrophy development. In the present study, training programs were carefully matched for all training groups for training frequencies, intensities and duration and therefore, differences in muscle mass gains might be explained by the training time. In addition, Sedliak et al. (2013) have suggested that strength training in the morning may not provide optimal stimulus for some individuals because of large inter-individual variability in protein signaling after morning compared to the evening strength training. In addition, the significant correlations observed between individual changes in CSA of vastus lateralis and strength of the lower extremities demonstrated that only in the eS+E group the individuals who experienced larger gains in muscle mass also increased more their training-time-specific (evening) strength performance. However, it is possible that the present study was underpowered when all four training groups were analyzed separately. Consequently, it is possible that small sample size may have led to insufficient power and type 2 error. Although the present study suggests that regular combined S and E training in the evening hours may be more optimal for muscle hypertrophy, further research is needed to confirm our results.

Endurance performance

Time to exhaustion improved in all training groups over the 24-week training period. After the first 12 weeks the increases were similar in all training groups, however, a significant order main effect was observed in favor of the E+S order after the second training period both in the morning and in the evening. Several studies have shown that the S and E training order does not influence the magnitude of adaptations in endurance performance (Cadore et al. 2013; Chtara et al. 2008; Schumann et al. 2014) or that combined training limited the increase in VO_2max when women performed E before S training (Gravelle and Blessing 2000). However, similar to our present results, Chtara et al. (2005) also found larger improvements in endurance performance when E training preceded S training. Although Dolezal and Potteiger (1998) and Nelson et al. (1990) did not compare different exercise order groups, they did indicate that their combined group that always performed S training first showed disrupted aerobic development. In concordance with our current findings, Nelson et al. (1990) also noted suppressed adaptation in endurance performance only during the second half of a 20-week training program. Given that the strength loading has been shown to impair muscle force generation capacity (Schumann et al. 2013), S training in close proximity with E training may cause difficulty in optimizing physiological adaptations to E training (Chtara et al. 2005). The E training intensity might be one factor to explain why some studies have not found any order effect in endurance performance adaptations. In the previous study by our team (Schumann et al. 2014) a similar E training program as in the present study was used, however, a smaller amount of high intensity interval training sessions were included in the earlier intervention. In addition, the main effect for exercise order was observed after completing the second training period during which both training intensity and frequency were increased. Possibly the increased training intensity, the larger amount of interval training sessions as well as increased training frequency during the second training period may have led to suppressed endurance performance adaptations in the groups who started with S training.

In general, the literature concerning time-of-day effects on endurance performance yields to somewhat inconclusive results (Bessot et al. 2006; Deschenes et al. 1998). Similar to the present study, Deschenes et al. (1998) have suggested that maximal aerobic performance remains constant throughout the day, despite the fact that certain important physiological parameters are subject to diurnal variation. The present results of time-of-day-specific adaptations from the morning measurements are in accordance with Hill et al. (1989) suggesting that the magnitude of the endurance training induced increases in maximal endurance performance were not related to the training time. However, the endurance performance improvements in the evening seemed to be influenced by training time during the latter 12 weeks of training, as evening training seemed to lead to greater increases in time to exhaustion compared to morning training. This suggests that a prolonged training period might be necessary to be able to observe training-time-specific adaptations during combined S and E training.

Diurnal variation in serum testosterone and cortisol

In the present study both total serum T and C exhibited significant diurnal rhythms in all five groups well in line with previous studies (e.g. Kraemer et al. 2001). Neither the morning nor the evening combined training programs led to any systematic changes in typical diurnal variations in resting serum total T and C concentrations. Limited results of acute hormonal response studies have shown temporary phase shifting effects of strength (Nindl et al. 2001) and endurance (Buxton et al. 2003) exercise. However, others have suggested that heavy resistance exercise has no significant effect on the circadian rhythm of salivary testosterone in previously strength-trained men (Häkkinen et al. 1988; Kraemer et al. 2001). Therefore, the influences of short-term training protocols seem to be insufficient to alter the circadian profile of T and C (Teo et al. 2011). Sedliak et al. (2007) showed that several weeks of strength training in the morning hours may decrease the resting C concentrations, whereas the evening training did not have that effect. In the same study, no significant changes were observed in resting T concentrations (Sedliak et al. 2007). The authors also proposed that the changes in C concentrations may have occurred due to the decreased anticipatory psychological stress prior to the morning testing (Sedliak et al. 2007). No previous studies appear to have dealt with possible phase shifting properties of prolonged combined S and E training on hormonal rhythms. However, the low sampling frequency may have precluded our ability to evaluate the possible training-induced phase shifts properly (Sedliak et al. 2007). In addition, although the overall training volume was large (2-3 E sessions and 2-3 S sessions per week) for previously untrained men, the training frequency of 2-3 double sessions (2-3 E+S or S+E sessions) allowed for at least 48 hours of recovery which may have provided sufficient time for photic and social contact factors (Duffy et al. 1996) to reset any possible phase-shifting effect caused by the exercise training. Although, increased morning basal serum T and C concentrations were observed after regular combined S and E training for 24 weeks, these changes did not seem to be related to the training order or the training time. It is possible that increases in T and C concentrations were caused by the seasonal variation due to sampling at different times of the year (Andersson et al. 2003; Persson et al. 2008). Therefore, it remains to be examined whether exercise can exert a sufficiently strong influence to alter the circadian rhythm of T and C.

Conclusions

This study showed that whereas no major between-group differences were observed over the first 12 weeks, the strength and endurance training order and time-of-day of the training may be important factors to optimize the magnitude of adaptations to combined strength and endurance training, when the training period is extended beyond 12 weeks. The present combined training program in the evening led to larger gains in muscle mass compared to the same training program in the morning hours. The mechanisms for these dissimilar gains after morning and evening combined training, however, are unclear. The improvements in strength performance did not seem to be related to the strength and endurance training time or order, while performing strength training regularly before an endurance session may interfere with the quality of the endurance training adaptations. Therefore, when improvements in endurance performance are sought, it is advisable to perform an endurance session before a strength training session preferably at the time-of-day when the improvements are desired, if the training period is prolonged. To improve strength performance, strength and endurance training sessions can be performed in the desired order in the morning or evening based on the personal preferences. However, gains in muscle mass might be larger, when regularly performing combined strength and endurance training in the evening.

Conflict of interest: The authors state that there is no conflict of interest.

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Tables

Table 1. Basal anthropometric characteristics ± SD of the participants.

	Age (yrs) ± SD	Height (m) ± SD	Weight (kg) ± SD	BMI (kg/m^2) ± SD
mE+S (n=9)	36.1 ± 6.5	1.80 ± 0.04	86.1 ± 8.9	26.5 ± 2.1
mS+E (n=9)	30.8 ± 5.0	1.82 ± 0.08	82.0 ± 14.0	24.8 ± 3.8
eE+S (n=12)	31.4 ± 4.6	1.80 ± 0.07	78.0 ± 8.5	24.1 ± 2.5
eS+E (n=12)	31.4 ± 6.5	1.81 ± 0.06	80.0 ± 10.6	24.5 ± 2.7
Controls (n=10)	32.4 ± 4.9	1.81 ± 0.08	79.0 ± 12.8	23.9 ± 2.7

m=training in the morning; e=training in the evening; E+S=endurance before strength training; S+E=strength before endurance.

Table 2. Absolute values ± SD of 1 repetition maximal (1RM) strength in leg press, time to exhaustion and muscle cross-sectional area of m. vastus lateralis at pre-, mid- and post measurements in the morning and in the evening.

	Pre-measurements		Mid-measurements		Post-measurements	
	Morning	Evening	Morning	Evening	Morning	Evening
1 RM (kg) ± SD						
mE+S (n=9)	161.8 ± 20.4	158.3 ± 21.4	178.3 ± 20.5	180.6 ± 17.8	183.5 ± 18.7	185.0 ± 19.7
mS+E (n=9)	149.1 ± 22.3	146.6 ± 24.6	170.0 ± 27.5	170.5 ± 29.3	174.2 ± 26.8	176.7 ± 30.8
eE+S (n=12)	142.6 ± 24.7	140.4 ± 25.6	163.0 ± 21.7	161.6 ± 21.6	166.8 ± 19.5	167.4 ± 20.0
eS+E (n=12)	141.4 ± 26.0	136.9 ± 26.2	160.1 ± 25.1	161.7 ± 24.8	166.6 ± 21.3	166.8 ± 21.5
Controls (n=10)	142.1 ± 25.9	144.2 ± 23.2	149.4 ± 25.1	151.0 ± 26.3	148.6 ± 23.7	148.5 ± 23.6
Time to exhaustion (min:sec) ± SD						
mE+S (n=9)	18:20 ± 02:17	18:47 ± 02:50	21:16 ± 03:25	20:49 ± 02:55	22:23 ± 03:21	22:25 ± 03:21
mS+E (n=9)	17:20 ± 03:20	17:28 ± 02:59	19:47 ± 02:18	20:02 ± 02:51	20:39 ± 02:09	20:37 ± 02:26
eE+S (n=12)	16:44 ± 02:23	16:57 ± 02:35	19:48 ± 02:23	19:38 ± 02:51	21:20 ± 02:25	21:36 ± 02:24
eS+E (n=12)	18:22 ± 03:39	18:32 ± 03:45	20:13 ± 03:01	20:08 ± 02:51	21:03 ± 03:36	21:34 ± 03:00
Controls (n=10)	19:19 ± 02:48	19:37 ± 03:14	19:53 ± 02:34	19:50 ± 02:45	19:47 ± 02:43	20:08 ± 02:31
Cross-sectional area (cm^2) ± SD						
mE+S (n=9)	24.56 ± 3.16	-	26.77 ± 3.25	-	27.38 ± 3.34	-
mS+E (n=9)	20.79 ± 2.51	-	22.80 ± 2.56	-	23.24 ± 2.33	-
eE+S (n=12)	21.22 ± 4.94	-	23.94 ± 4.76	-	25.15 ± 4.86	-
eS+E (n=12)	21.75 ± 3.43	-	23.88 ± 3.76	-	25.05 ± 3.76	-
Controls (n=10)	21.38 ± 3.32	-	21.82 ± 3.81	-	21.79 ± 3.46	-

Table 3. Summary of the strength training program for lower extremities.

	Training weeks 1-12			Training weeks 13-24		
Week	1-4	5-8	9-12	13-14	15-20	21-24
Training type	Circuit	Hypertrophic	Maximal	Circuit	Hypertrophic	Maximal
Intensity (% of 1 RM)	40-70	70-85	75-95	50-75	75-85	80-95
Sets	2-3	3-4	3-5	3-4	3-4	3-5
Repetitions	10-20	10-15	3-8	10-15	10-15	3-8
Rest (min)	no	1.5-2	2-3	no	1.5-2	2-3

Figure legend

Figure 1. Study design and measurements. 1 RM = one repetition maximum in the dynamic leg press; T_{exh} = time to exhaustion during the incremental cycling test; CSA = cross sectional area; m = morning; e = evening

Figure 2. Changes in vastus lateralis cross-sectional area after 12 and 24 weeks of combined training; *sign. ($p<0.05$) within-group increase; # sign. different from controls; & sign. time-of-day main (TOD) effect. Detailed levels of significance are presented in the results section. m = morning; e = evening; E+S = endurance before strength; S+E = strength before endurance

Figure 3. Changes in time to exhaustion (T_{exh}) in the morning (A) and in the evening (B) after 12 and 24 weeks of combined training; *sign. ($p<0.05$) within-group increase; ¤ sign. between-group differences as indicated; # sign. different from controls; $ sign. order main effect; & sign. time-of-day (TOD) main effect. Detailed levels of significance are presented in the results section; m = morning; e = evening; E+S = endurance before strength; S+E = strength before endurance

Figure 4. Diurnal variations in serum testosterone and cortisol concentrations at pre-, mid- and post-measurements in the combined morning group (A) and combined evening group (B). *sign. ($p<0.05$) within-group change from wk 0 to wk 12; $ sign. within-group change from wk 12 to wk 24; ¤ sign. within-group change from wk 0 to wk 24. Detailed levels of significance are presented in the results section

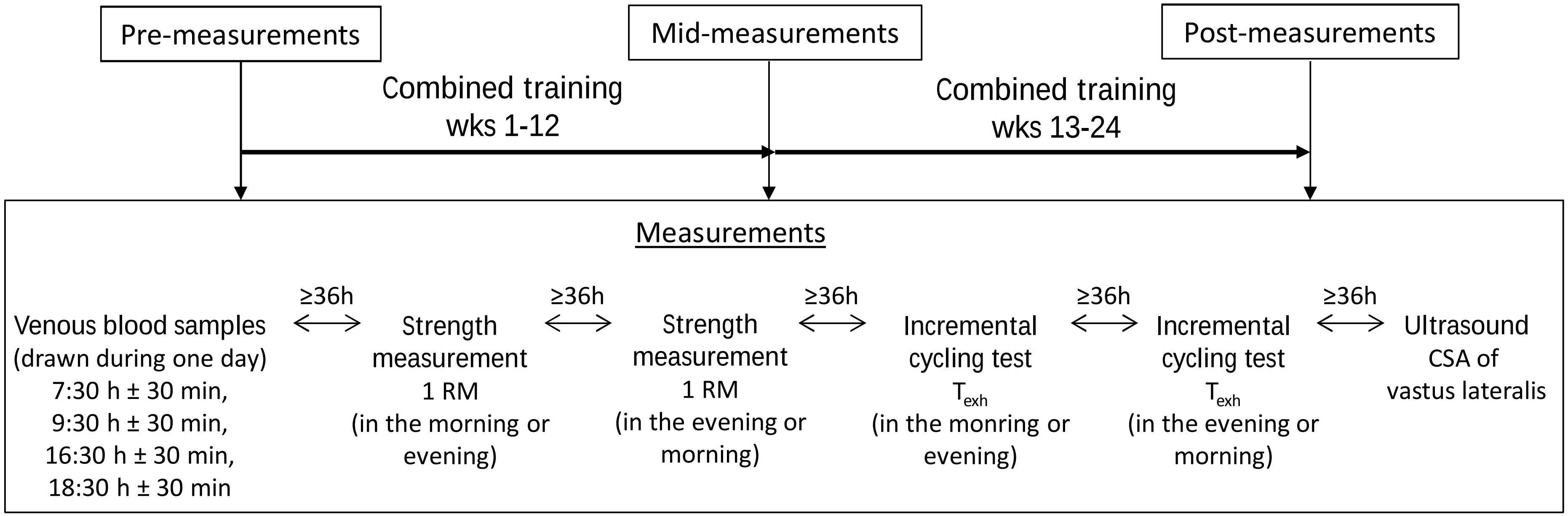
Pre-measurements
Mid-measurements
Post-measurements
Combined training wks 1-12
Combined training wks 13-24
Measurements
≥36h
≥36h
≥36h
≥36h
≥36h
Venous blood samples (drawn during one day) 7:30 h ± 30 min, 9:30 h ± 30 min, 16:30 h ± 30 min, 18:30 h ± 30 min
Strength measurement 1 RM (in the morning or evening)
Strength measurement 1 RM (in the evening or morning)
Incremental cycling test T_exh (in the monring or evening)
Incremental cycling test T_exh (in the evening or morning)
Ultrasound CSA of vastus lateralis

Figure 1.

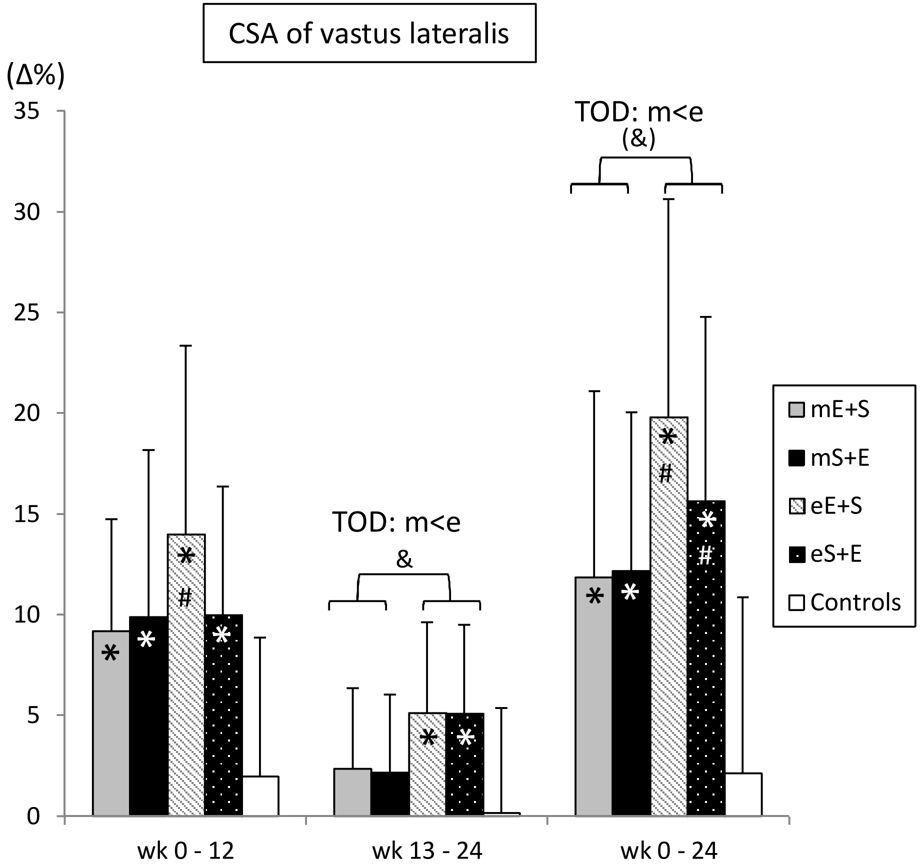
CSA of vastus lateralis
(Δ%)
35
30
25
20
15
10
5
0
TOD: m<e
(&)
TOD: m<e
&
*
#
mE+S
mS+E
eE+S
eS+E
Controls
wk 0 - 12
wk 13 - 24
wk 0 - 24

Figure 2.

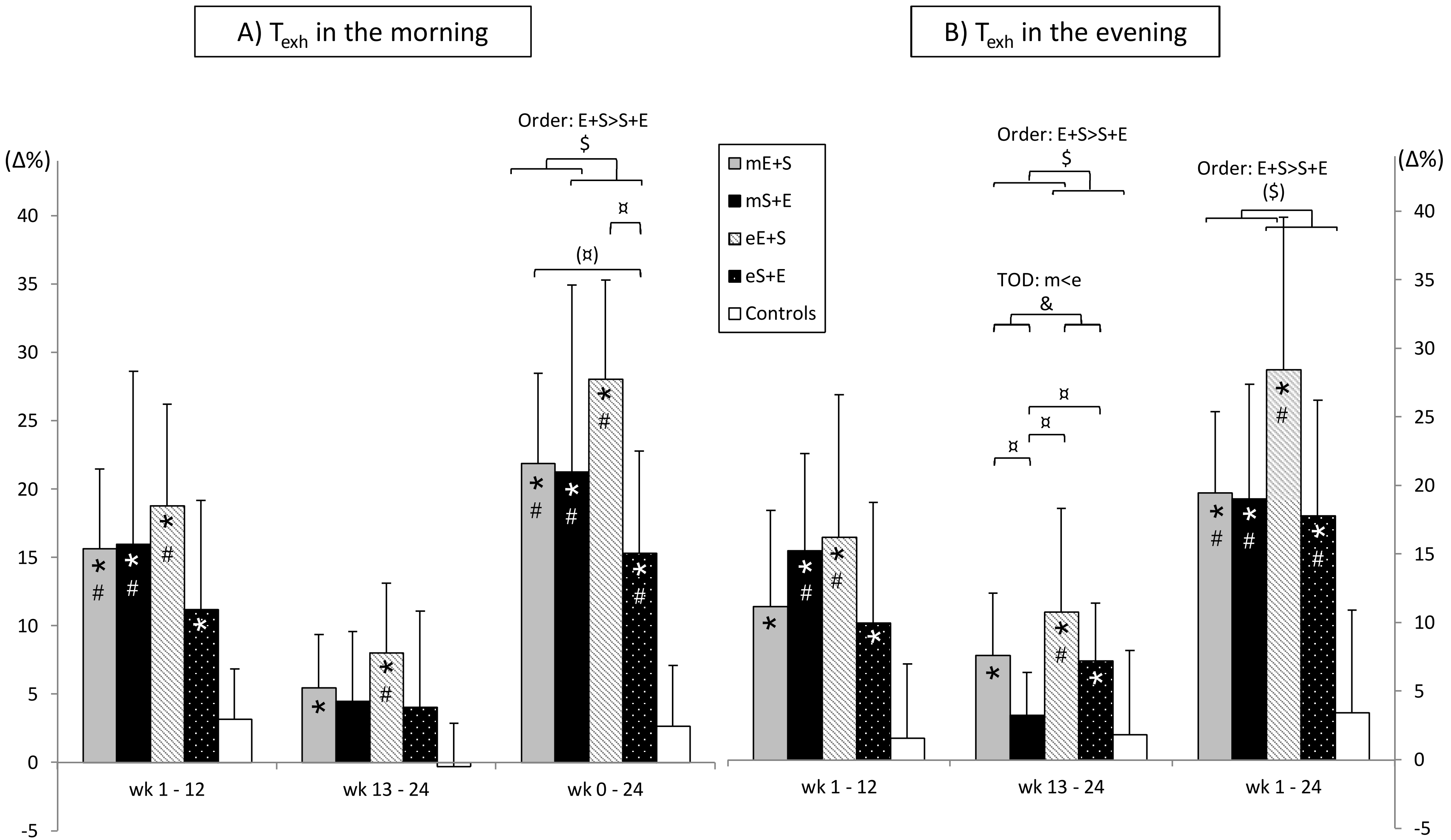
A) T_{exh} in the morning
B) T_{exh} in the evening
(Δ%)
Order: E+S>S+E
$
¤
(¤)
TOD: m<e
&
($)
mE+S
mS+E
eE+S
eS+E
Controls
wk 1 - 12
wk 13 - 24
wk 0 - 24
wk 1 - 24

Figure 3.

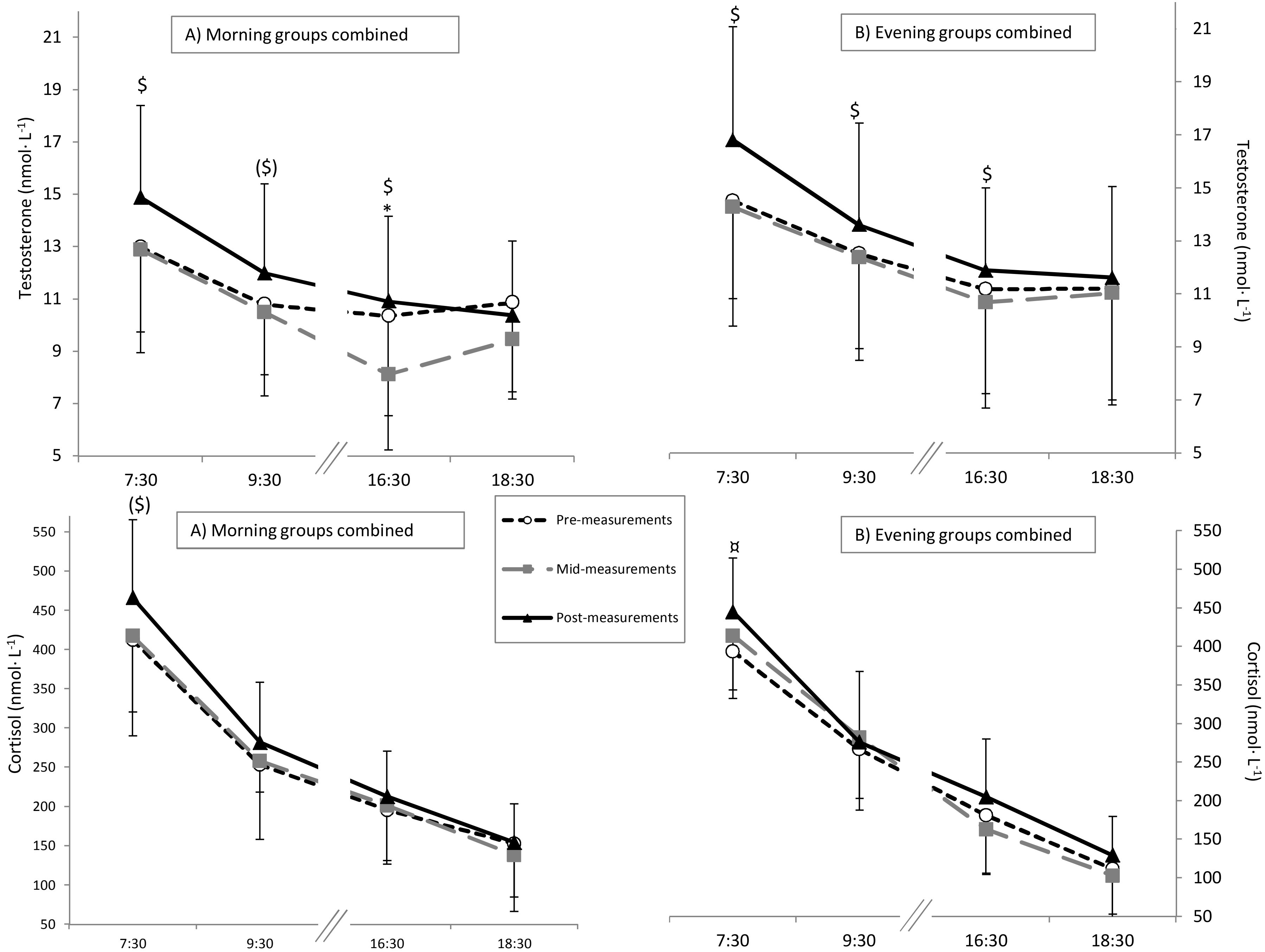
A) Morning groups combined
Testosterone (nmol· L-1)
$
($)
$
*
7:30
9:30
16:30
18:30
B) Evening groups combined
Testosterone (nmol· L-1)
A) Morning groups combined
Cortisol (nmol· L-1)
Pre-measurements
Mid-measurements
Post-measurements
B) Evening groups combined
Cortisol (nmol· L-1)
¤

Figure 4.

III

NEUROMUSCULAR ADAPTATIONS TO COMBINED STRENGTH AND ENDURANCE TRAINING: ORDER AND TIME-OF-DAY

by

Maria Küüsmaa-Schildt, Daniela Eklund, Janne Avela, Tuomas Rytkönen, Robert U. Newton, Mikel Izquierdo & Keijo Häkkinen 2017

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Neuromuscular adaptations to combined strength and endurance training: order and time-of-day

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Abstract

The present study examined the effects of 24 weeks of morning vs. evening same-session combined strength (S) and endurance (E) training on neuromuscular and endurance performance. Fifty-one men were assigned to the morning (m) or evening (e) training group where S preceded E or vice versa (SE_m, ES_m, SE_e and ES_e) or to the control group. Isometric force, voluntary activation, EMG and peak wattage during the maximal cycling test were measured. Training time did not significantly affect the adaptations. Therefore, data are presented for SE_{m+e} (SE_m+SE_e) and ES_{m+e} (ES_m+ES_e). In the morning no order specific gains were observed in neuromuscular performance. In the evening, the changes in isometric force (SE_{m+e} 15.9±16.7%, $p=0.001$; ES_{m+e} 4.1±12.2%, $p=0.615$) and EMG (SE_{m+e} 38.3±31.7%, $p=0.001$; ES_{m+e} 14.67±36.44%, $p=0.486$) were larger ($p=0.014$) in SE_{m+e} than in ES_{m+e} and in voluntary activation larger ($p=0.026$) in SE_{m+e} compared to controls. Peak wattage increased in the morning (SE_{m+e} 15.9±9.2%, ES_{m+e} 22.0±7.0%; $p<0.001$) and evening (SE_{m+e} 16.3±7.2%, ES_{m+e} 21.0±9.0%; $p<0.001$) but were larger ($p<0.05$) in ES_{m+e}. The current training program led to greater neuromuscular adaptations when SE-training was performed in the evening, whereas the ES-training provided more optimal conditions for endurance performance adaptations both in the morning and evening.

Keywords: diurnal rhythms; EMG; voluntary activation; concurrent training; muscle force

Introduction

Maximal neuromuscular performance has been shown to fluctuate with time-of-day, with 5-15% higher strength values observed in the evening [8,13,24,36,46] compared to the morning. However, in the case of endurance performance, the effect of diurnal rhythms seems to dissipate [11,15,17], although some studies have demonstrated that tolerance of high-intensity endurance exercise (e.g. performed as cycling) is higher in the evening [2,6]. It has been proposed that these fluctuations in strength and endurance performance may also affect the chronic adaptations to exercise training [11]. Previous strength training interventions have found that changes in maximal strength performance might be largest at the time-of-day when the training is regularly performed [10,45,47]. Therefore, it has been suggested that strength training in the morning hours may blunt the typical diurnal fluctuations [45,47]. However, the absolute increases in maximum strength have been found to be similar between the morning and evening strength training groups [47]. Literature regarding the time-of-day-effect on endurance training adaptations has not been equally consistent. While some studies have suggested that similarly to strength training, adaptations to endurance training are time-of-day-specific [31], other studies do not demonstrate this interaction [30].

American College of Sports Medicine guidelines for general health and fitness [21] suggest engaging in both endurance and strength exercise. However, combining these two exercise modes within the same training program may lead to an “interference effect” [29,49] due to a divergent influence of the two training regimes on the neural and muscular adaptations [48]. Although some recent studies have suggested that the interference effect can be avoided when more than eight hours separate strength and endurance training [22], performing these two training modes in close proximity may possibly interfere with the training adaptations. Lepers et al. [37] have suggested that strength training adaptations may possibly be interfered by prior endurance training, due to the acute residual fatigue developed in the neuromuscular system. Therefore, one possible factor responsible for the interference effect is the intra-session sequence of strength and endurance exercises [38]. E.g., in elderly men, same-session combined training has been shown to lead to greater improvements in strength performance in the group which always started the session with strength training (order-effect) [7]. However, age-induced functional and physiological changes in the neuromuscular system [42] may have influenced the training adaptations. In previously untrained young participants, the intra-session exercise sequence does not seem to influence the strength improvements [9,44], although neural adaptations have shown indications of being compromised and highly individual when endurance training constantly precedes strength training over a period of several months [19]. Maximal endurance performance development has been shown mostly not to be impaired by the order of performing strength and endurance training [14,16,19].

To the best of our knowledge, time-of-day-specific adaptations to prolonged combined strength and endurance training have not been studied. The purpose of the present study was to examine how the strength and endurance training order and time-of-day (morning vs. evening) affect the adaptations in neuromuscular and endurance performance after 24 weeks of time-of-day-specific same-session combined strength and endurance training. To investigate the time-of-day and order specific adaptation, we hypothesized that performing endurance training regularly before strength training would limit neuromuscular adaptations, whereas the intra-session order of strength and endurance training would not influence the adaptations in endurance performance. In addition, we hypothesized that the adaptations in strength and endurance performance would show some time-of-day dependency.

Methods

Participants

Fifty-one recreationally physically active, healthy men (age 32.3±5.6 years, 1.81±0.06 m, 80.8±10.9 kg) participated in the study. Participants had no history of previous strength or endurance training over the past year. They had no medical contraindications or musculoskeletal issues that could put them at risk during testing or training or compromise their ability to adapt. Before involvement in the study, each participant was screened via a health questionnaire and resting ECG by a physician. Participants' chronotype was assessed before the study based on the Munich Chronotype Questionnaire [43]. None of the participants belonged to an extreme morning or evening chronotype or were involved in shift or night work. None of the participants reported the use of medications that would affect the diurnal rhythms or sleep cycle. All participants were informed of the procedures, risks and benefits of the study, and they provided written consent before participation. The study was conducted in accordance with the ethical standards of the journal [25], complied with the Declaration of Helsinki and was approved by the Ethics Committee in the University of Jyväskylä.

Participants were divided into four training groups matched for anthropometrics and physical performance following baseline testing [35]: (i) training in the morning (m) and performing endurance (E) training always before strength (S) training (ES_m, n=9), (ii) training in the morning with strength always preceding endurance training (SE_m, n=9), (iii) training in the evening (e) and performing endurance before strength training (ES_e, n=11), (iv) training in the evening with strength always preceding endurance training (SE_e, n=12). The controls (n=10) were asked to maintain their pre-experimental physical activity level throughout the study. All participants were instructed to continue their normal dietary intake and habitual physical activities throughout the intervention period but to avoid any additional strength and/or endurance training.

Study design and measurements

The study design is described more in detail in Küüsmaa et al. [35]. The 24-week combined strength and endurance training period consisted of two 12-week periods and the measurements were carried out before (Pre), during (Mid) and after (Post) the intervention. Strength and endurance measurements took place both in the morning (between 6:30 ± 30 min and 9:30 ± 30 min) and in the evening (between 16:30 ± 30 min and 19:30 ± 30 min) independent of the group assignment. Within individuals, the tests were always carried out in the same order and at the same time-of-day (±1h) at all three measurement points with 36 hours separating the performance tests. For the measurements after 12 and 24 weeks of training, the last training session and the first measurement were always separated by a minimum of two and maximum of four days. The participants were asked to follow their usual sleeping habits on the night preceding each testing session and to refrain from exercise training for two days before the testing. They were asked to avoid alcohol for 24 hours and caffeine for 12 hours before the physical performance tests.

Neuromuscular performance

Before the start of the measurements a familiarization testing session was carried out for all participants on a non-training-specific time-of-day. During the familiarization session participants were familiarized with the testing procedures and set-up for the equipment were recorded for each participant. Also the placement of electromyographic (EMG) electrodes was marked with indelible ink tattoos according to the SENIAM guidelines [28] to ensure repeatable electrode positioning [32].

Maximal unilateral isometric knee extension force (MVC_{KE}) was measured using a device designed and manufactured by the Department of Biology of Physical Activity (University of Jyväskylä, Finland). The participant was seated in the device with a knee angle of 107° for the right leg and the left leg rested in the horizontal position on a chair [33]. Hip and knee angles were firmly secured by a seatbelt at the hip, pad strapped over the right knee and an adhesive fabric strap above the right ankle. Participants were asked to perform three maximal trials by increasing force gradually over 3 seconds. The trial with the highest force was used for further analysis. The force signal was sampled at 2000 Hz and low-pass filtered (20 Hz). Maximal force was manually analyzed using Signal 4.04 (Cambridge Electronic Design, UK).

To assess the voluntary activation percentage (VA%) of the quadriceps femoris muscle, the interpolated twitch technique [39] was used to stimulate the right quadriceps muscles during the isometric knee extension action. Four galvanically paired self-adhesive electrodes (7 cm PolarTrode; Polar Frost USA; Anaheim, CA; USA) were placed on the proximal and mid-regions of the quadriceps muscle belly of the right leg. The current of single 1-ms rectangular pulses were increased progressively using a constant-current stimulator (Model DS7AH, Digitimer Ltd, UK) in 5mA steps until a plateau in the passive twitch response was observed. To ensure maximal effect for the knee extension trials, 25% of the stimulation current was added. This supramaximal single-pulse stimulation was delivered to the muscle at rest 3 seconds before the voluntary knee extension, during the plateau of voluntary peak knee extension force and 5 seconds after the cessation of contraction. VA% was calculated according to the formula by Bellemare & Bigland-Ritchie [4]:

$$VA\% = [1-(P_{ts}/P_t)] \cdot 100,$$

where P_{ts} is the amplitude of the twitch elicited by the electrical stimulation on top of the maximal voluntary contraction and P_t is the amplitude of the twitch delivered to the passive muscle 5 seconds after the voluntary contraction.

Muscle activity was recorded through surface electromyography (EMG) during MVC_{KE} from the vastus lateralis (VL) muscle of the right leg. EMG was collected from the maximum force level over the 500 ms time period, immediately before the superimposed twitch. EMG was amplified by a factor of 1000 (NeuroLog Systems NL844, Digitimer Ltd, UK) and sampled at a frequency of 2000 Hz. The raw EMG signal was band-bass filtered (20-350 Hz) and converted to root mean square (rmsEMG) on Signal 4.04 software (Cambridge Electronic Design, UK).

Endurance performance

Peaks wattage (Wpeak) was measured during the graded maximal aerobic cycling test to volitional exhaustion on a mechanically braked cycle ergometer (Ergomedic 839E, Monark Exercise AB, Sweden). The exercise intensity was increased by 25 W every two minutes starting with 50 W. Pedaling frequency was sustained at 70 rpm throughout the test. The participants were encouraged by the testing personnel to continue cycling until volitional exhaustion. Peak wattage achieved during the cycling test was calculated with the following formula:

$$Wpeak = Wcom + (t/120)*25,$$

where Wcom is the last cycling power completed and is the time in seconds the non-completed power was maintained [34].

Training programs

The training program has been described in detail previously [35]. To summarize, training during the intervention consisted of two 12-week progressive same-session combined strength and endurance training periods either in the morning or in the evening. During the first 12 weeks (wks 1-12) participants trained two times per week [2x(1S+1E) or 2x(1E+1S)] and during the second 12-week training period (wks 13-24) all participants performed 5 training sessions in 2 weeks [5x(1S+1E) or 5x (1E+1S)]. The morning training groups (SE_m and ES_m) performed all training sessions between 6:30-10:00. The evening training groups (SE_e and ES_e) performed all training sessions between 16:30-20:00. Strength and endurance training was always performed in a row with a maximum of 5-10 min break in between the two training modes. The training programs were identical for the SE and ES group independent of the training time, only differing in the sequence of training modes. All training sessions were supervised.

Strength training. Strength training consisted of hypertrophic and maximal strength exercises for the whole body with the main focus being on the knee extensors and flexors as well as hip extensors. Strength training was periodized to improve muscular endurance in the first 4 weeks, which was performed as circuit training (intensity of 40-70% of 1RM, 2-3 sets, 10-20 repetitions). The subsequent 4 weeks (weeks 5-8) were designed to produce muscle hypertrophy (intensity of 70-85% of 1 RM, 3-4 sets, 10-15 repetitions and 1.5-2 min of rest), followed by 4 weeks (weeks 9-12) of mixed hypertrophic and maximal strength training (intensity of 75-95% of 1 RM, 3-5 sets, 3-8 repetitions and 2-3 min of rest). The same periodization was repeated during the second 12 weeks of training with intensities adjusted for each subject to match the current strength level.

Endurance training. Endurance training was carried out on cycle ergometers. Training intensities were based on the maximum heart rate (HR_{max}) determined during the graded, training-time-specific, maximal, incremental cycling test. During the first 12 weeks interval training session, which consisted of 4x4 min high-intensity intervals (85-100% of HR_{max}) and separated by 4-min active recovery periods (70% of HR_{max}) as well as continuous (65-80% of HR_{max}) training session were performed once a week, respectively. During the second 12 weeks (wks 13-24), when the training frequency increased, one additional high-intensity interval training session was added.

Statistical analyses

Results are presented as means ± standard deviation. Statistical analyzes were performed using the Statistical Package for Social Sciences (SPSS version 22, Chicago, IL). Normality of the data was checked using the Shapiro-Wilk test. EMG and VA% data were log transformed but remained non-normally distributed even after log transformation. Morning and evening differences at wk 0, 12 and 24 performance variables were checked by using paired samples T-tests. Within-group changes over time in the morning and in the evening were examined with repeated measures general lineal models, where Time, with 3 levels (wk 0, wk 12, wk 14) was set as the only factor. One-way analysis of variance (ANOVA) was used to assess time×group interactions in relative changes over time. Bonferroni post hoc procedures were applied when appropriate. For the non-normally distributed data the paired-samples Wilcoxon signed rank test, Friedman test and Kruskal-Wallis ANOVA were used respectively for within-group and between-group differences. A Bonferroni adjustment was applied by multiplying the pairwise p values with the number of comparisons. To analyze associations between different variables in neuromuscular performance, Spearman correlation coefficients (r) were calculated. Statistical significance was accepted a criterion alpha of $p<0.05$. P-values ≤ 0.06 were accepted as a trend. Effect sizes (es) for both within-group and between-group comparisons are presented as Cohen's d for the normally distributed data and for non-normally distributed data effect sizes are calculated based on the following equation:

$$es = Z/\sqrt{n},$$

where Z is the z-score and n is the number of observations on which Z is based.

Results

No between-group differences were found in any variables at the baseline. None of the neuromuscular or endurance performance variables showed significant morning to evening differences in any group at any measurement time point. Time-of-day of training did not have significant effect to the training adaptations and therefore, most of the data from the SE_m and SE_e groups are combined and presented as SE_{m+e} and data from ES_m and ES_e presented as ES_{m+e}.

Maximal unilateral isometric knee extension force

In the morning isometric MVC_{KE} increased significantly in the SE_{m+e} (p=0.028; es=0.439) but not in ES_{m+e} (p=0.104; es=0.430) (Fig 1a; Table 1). There were no statistically significant between-group differences in changes for the experimental groups during the intervention in the morning. In the evening MVC_{KE} increased in SE_{m+e} during the first 12 weeks (p=0.002; es=0.525) and by week 24 (p=0.001; es=0.636), but not in ES_{m+e} (p=0.615; es=0.235). The increases in SE_{m+e} were significantly larger than the changes in ES_{m+e}, during weeks 0-12 (p=0.017; es=0.904) ($SE_e > ES_e$; p=0.039) and 0-24 (p=0.033; es=0.806) (Fig 1b). Changes in

SE_{m+e} were larger than in C during first 12 weeks (p=0.024) and after 24 weeks of training (p=0.004).

EMG and voluntary activation

In the morning both SE_{m+e} and ES_{m+e} increased VL rmsEMG by week 24 (SE_{m+e}: p<0.001, es=0.590; ES_{m+e}: p=0.037, es=0.461) (Fig 2a; Table 1). In the evening only SE_{m+e} significantly increased VL rmsEMG activity during weeks 0-12 (p=0.002; es=0.584) and 0-24 (p=0.001; es=0.602), whereas the changes is ES_{m+e} were not significant (p=0.486; es=0.258). These increases in the evening were significantly larger in SE_{m+e} compared to insignificant changes in the ES_{m+e} group during the first 12 weeks (p=0.004; es=0.512) and after 24 weeks of training (p=0.014; es=0.473) (Fig 2b).

VA% remained statistically unaltered in the SE_{m+e} and ES_{m+e} group after 24 weeks of training in the morning (SE_{m+e}: p=0.093, es=0.052; ES_{m+e}: p=0.801, es=0.084) and in the evening (SE_{m+e}: p=0.444, es=0.394; ES_{m+e}: p=0.846, es=0.076) (Table 1). In the evening, at week 24, the 2.1±4.5% increase in VA% in the SE_{m+e} was significantly larger (p=0.026; es=0.535) than the -2.1±3.5% (es=-0.035) change in the control group (Fig 3).

In the SE_{m+e} and ES_{m+e} groups, a significant correlation between the individual changes in VA% and changes in MVC_{KE} in the morning was found between weeks 0-12 (SE_{m+e} r=0.625, p=0.013 (Fig 4); ES_{m+e} r=0.635, p=0.005) and in the SE_{m+e} group during weeks 0-24 (r=0.521, p=0.046). Individual changes in the morning in VA% and changes in VL rmsEMG were correlated in the SE_{m+e} group during weeks 0-12 (r=0.685, p=0.003) and during weeks 0-24 (r=0.479, p=0.050). In SE_{m+e} changes in MVC_{KE} and VL rmsEMG were correlated during weeks 0-12 and 0-24 both in the morning (wks 0-12 r=0.509, p=0.018 (Fig 5); wks 0-24 r=0.479, p=0.028) and in the evening (wks 0-12 r=0.462, p=0.035; wks 0-24 r=0.481, p=0.027).

Maximal power output during cycling

Wpeak during the cycle ergometer test increased in SE_{m+e} and ES_{m+e} throughout the 24-week training period in the morning (SE_{m+e}: p<0.001, es=0.910; ES_{m+e}: p<0.001, es=1.560) and in the evening (SE_{m+e}: p<0.001, es=0.997; ES_{m+e}: p<0.001, es=1.406) (Table 1). In the morning the increase of 22.0±7.0% in ES_{m+e} was significantly larger compared to 15.9±9.2% in SE_{m+e} during weeks 0-24 (p=0.022; es=0.746) (ES_e > SE_e; p=0.020) (Fig 6). In the evening the increase of 8.5%±5.7 in ES_{m+e} was significantly larger compared to the 5.0±3.8% in SE_{m+e} during weeks 13-24 (p=0.027; es=0.723).

Discussion

The main results of the present study suggest that the order of strength and endurance training may influence the magnitude of adaptations in neuromuscular and endurance performance (order effect), whereas time-of-day of the training does not seem to affect the results. Larger gains in neuromuscular performance were observed in the evening, when strength training was performed before endurance. Endurance performance development seemed to favor the order of endurance training constantly preceding strength, both in the morning and in the evening.

Neuromuscular performance

In the present study no order effect in maximal isometric force development was observed during the training period in the morning. However, in the evening maximal isometric knee extension force increased significantly more in the SE order compared to the ES. Previous combined training studies, which have not controlled the time-of-day-effect have shown that intra-session exercise sequence does not seem to influence maximal strength performance development in young previously untrained participants [9,44].Eklund et al. [19] have, however, shown that neural adaptations might be compromised when endurance training constantly precedes strength training. In elderly men, same-session combined training has been shown to lead to greater improvements in strength performance when the combined training session always started with strength training [7]. This possible interference by prior endurance training has been attributed both to impeded molecular adaptations [3,12,26] and to acute fatigue developed in the neuromuscular system [37]. Failure in force production has been associated with changes in contractile as well as neural properties of working muscles [37]. Consequently, when the neuromuscular system cannot produce an optimal contraction due to previous fatigue, improvements in muscle strength may be possibly reduced [5]. This could be a possible mechanism why isometric strength performance was compromised in the group which started with endurance training.

Analogous to isometric force, the morning increases in rmsEMG were similar between the two orders, whereas the evening changes in rmsEMG were significantly larger in the SE_{m+e} group compared to the ones in ES_{m+e}. The present correlations revealed that in the SE_{m+e} group individual changes in maximal isometric knee extension force development were positively related to the changes in VL rmsEMG, demonstrating that the individuals who increased rmsEMG experienced concomitant increases in maximal knee extension force. Although Eklund et al. [19] did not observe any between-group differences, participants who constantly performed strength before endurance training demonstrated increased force and EMG activity during isometric actions by the end of the 24 weeks of same-session combined training, while the reverse order produced no significant increases. Similarly to the present study, these results are suggesting that performing endurance training before strength may potentially inhibit neural adaptations and, thereby, hinder adaptations in neuromuscular performance such as maximal isometric force. However, it is worth of pointing out that the EMG data was not normalized to maximum M-wave. Although, this is a limitation of the present study, we took great care to minimize the methodological and physiological errors during the EMG-recordings by standardizing the measurement procedure and permanently marking EMG electrode positions subcutaneously.

In addition to rmsEMG, neuromuscular activation in the present study was measured by using the twitch interpolation technique to quantify the level of voluntary muscle activation. Although no significant within-group changes were observed in VA% in the evening, the SE order led to significantly larger changes in VA% compared to the control group. Previously, Eklund et al. [19] observed enhanced voluntary activation after combined training only in the group which performed strength before endurance training. Whereas no significant correlations were observed in the evening, a significant correlation between the improvements in voluntary activation level and knee extension force was found in the morning in both SE_{m+e} and ES_{m+e} groups. However, the level of adaptations varied widely among individuals in both orders, as demonstrated by large standard deviations. The significant correlations observed between individual changes in VA% and changes of rmsEMG over the 24-week training period were observed only by adhering to the SE order. Previously, Eklund et al. [19] have shown that combined training for longer than 12 weeks may potentially inhibit adaptations in the nervous system when endurance is regularly performed before strength training. It has been suggested that already small increases in VA%

represent a physiologically significant improvement in muscle activation [27], therefore, it is possible the statistically insignificant changes in the present study still affected strength performance adaptations.

Not only neuromuscular adaptations but also muscle hypertrophy contributes to training-induced increases in maximal contractile force. However, in concordance with previous studies [40], the previous report by our research team [35] showed no significant differences between SE and ES orders in hypertrophy development. Therefore, it is likely that in the present study neuromuscular adaptations rather than morphological changes were responsible for the order-specific gains in isometric strength performance. In addition, all experimental groups in the present study followed training programmes which were carefully matched for modes, frequencies, intensities and durations of strength and endurance training. Therefore, differences in improvements in neuromuscular performance might be explained by the sequence of training. However, when interpreting the results it needs to be remembered that the present training program consisted of dynamic exercises and that dynamic tests may be more suitable than isometric ones to evaluate the training adaptations [1]. This may help to partly explain the differences between the present study and previous study by our research group [35] which did not find order effect in dynamic strength performance.

The present results suggested that, unlike after strength training only [45,47], prolonged combined strength and endurance training in the morning or in the evening do not lead to time-of-day-specific adaptations in neuromuscular performance. The assumption that adaptations to exercise training depend on the training time-of-day is based on the fact that various physiological variables (e.g. body temperature, contractile state of the muscle, neural input) have been shown to fluctuate relative to the time-of-day [11]. In the present study neuromuscular performance did not show any morning to evening fluctuation at any time point. Although this finding is in contrast to most of the previous time-of-day-specific studies [46], it is possible that the lack of diurnal variation in neuromuscular performance in the present study may have in part masked the time-of-day specific training adaptations in strength performance.

Although the time-of-day of the training did not influence the training adaptations, between-group differences in neuromuscular performance were found only in the evening testing time. Therefore, it is possible that in previous combined training studies testing along the day may have masked the presence of the order effect. The design and results of the present study allows us to suggest that in addition to the training mode, duration and frequency [22], also the time-of-day when the measurements are performed may be an important factor influencing the order effect.

Endurance performance

Peak wattage increased in all training groups over the 24-week combined training period. During the first 12 weeks the increases were similar in both training orders, after which greater improvements were observed in the ES group, compared to the opposite order. With respect to peak wattage, previous studies that have investigated the effects of simultaneous strength and aerobic training on endurance performance, mostly demonstrate that strength and endurance training order does not interfere with the development of endurance performance [7,14,19]. The endurance training intensity might be one factor to explain the differences between the studies. In the previous study by our laboratory [44], which did not observe any order effect in endurance performance, a similar endurance training program as in the present study was used, except a smaller amount of high intensity interval training sessions were included. In addition, in the present study the between-group differences were observed only after the training intensity and frequency were increased during the second training period (wks 13-24). This is supported by Nelson et al. [41] who also noted suppressed adaptation in endurance performance only after the training period was prolonged over 11 weeks. Cycling has been shown to be biomechanically similar to many strength exercises [23], therefore, fatigue from strength training in close proximity with intensive cycling exercise may cause interference in optimizing physiological adaptations to endurance training [18,41], especially when performed over a prolonged period of time. Therefore, it is possible that the increased training intensity, the larger amount of interval training sessions as well as the increased training frequency and total training volume during the second training period may have led to suppressed endurance performance adaptations, when constantly performing strength before endurance training. However, it needs to be remembered that, although, Wpeak is a commonly used measure which has been shown to accurately predict cycling performance [20], it has, in addition to cardiorespiratory factors also a neuromuscular component. However, the physiological mechanisms behind cardiorespiratory adaptations were out of scope of the present report.

The present study suggest that time-of-day-specific combined strength and endurance training will not lead to time-of-day-specific training adaptation in endurance performance when measured as peak wattage produced during the maximal cycling test. Previous literature regarding time-specific endurance training is limited and equivocal, as some of the studies have shown that adaptations to endurance training are time-of-day-specific [31], while others disagree [30]. Similarly to strength performance, in the present study endurance performance did not vary with the time-of-day. This is in accordance with Deschenes et al. [17], who showed that although some physiological variables such as blood pressure, plasma lactate and rectal temperature may fluctuate with time-of-day, while other important variables such as oxygen uptake and pulmonary ventilation fail to demonstrate significant diurnal fluctuation. It is possible that the effects of time-of-day on endurance performance are not explained just by one or two variables but represent the effect of a combination of factors and, therefore, the lack of diurnal variation in endurance performance may have in part masked the time-of-day specific adaptations in the present study.

Conclusions

The present same-session combined training protocol led to adaptations specific to the strength and endurance training order. The magnitude of adaptations in physical performance was similar after morning and evening combined training, however, the time-of-day of neuromuscular testing influenced the present results. In the evening, improvements in maximal strength performance seemed to be accompanied by increased neuromuscular activity in the group that performed strength training constantly before endurance training, while the reversed order may not be optimal conditions for neuromuscular performance adaptations. On the other hand, performing endurance training (by cycling) regularly before strength training may help to avoid possible fatigue caused by strength training and, thereby, lead to greater endurance performance adaptations both in the morning and in the evening, especially when the training period is prolonged and the training intensity and/or frequency increased. Therefore, individuals who wish to perform strength and endurance in close proximity to each other over prolonged training periods are advised to choose the training order based on individual goals.

Conflict of interest: The authors state that there is no conflict of interest.

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Figure legend

Figure 1. Relative changes in maximal unilateral knee extension force after 12 and 24 weeks of combined training; *sign. within-group increase or as indicated, *<0.05, **<0.01; # sign. different from the control-group, (#)<0.06, #<0.05, ###<0.001. ES_{m+e}= combined morning and evening endurance before strength training group; SE_{m+e}= combined morning and evening strength before endurance training group

Figure 2. Relative changes in maximal VL rms EMG during unilateral knee extension after 12 and 24 weeks of combined training; *sign. within-group increase or as indicated, *<0.05, **<0.01, ***<0.001; # sign. different from the control-group, #<0.05. ESm+e= combined morning and evening endurance before strength training group; SEm+e= combined morning and evening strength before endurance training group

Figure 3. Relative changes in maximal voluntary activation during unilateral knee extension after 12 and 24 weeks of combined training; # sign. different from the control-group, #<0.05. ES_{m+e}= combined morning and evening endurance before strength training group; SE_{m+e}= combined morning and evening strength before endurance training group

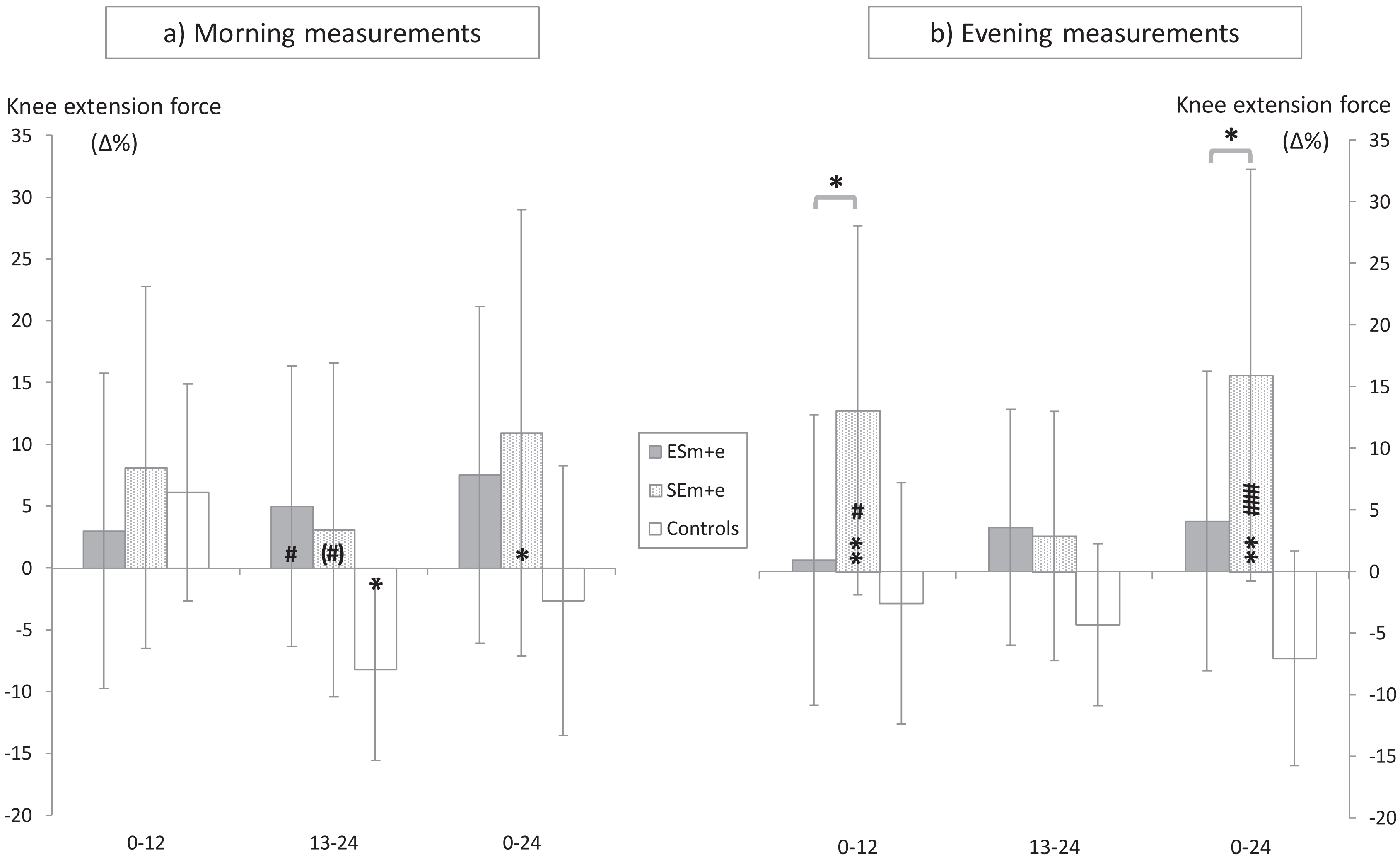
Figure 4. Correlations between the individual change in the voluntary activation % and the relative changes in maximal knee extension force in the morning in SE_{m+e} group during weeks 0-12. SE_m = morning strength before endurance training group. SE_e = evening strength before endurance training group

Figure 5. Correlations between the relative change in the maximal VL rmsEMG during maximal knee extension and the relative changes in maximal knee extension force in the morning in SE_{m+e} group during weeks 0-12. SE_m= morning strength before endurance training group. SE_e = evening strength before endurance training group

Figure 6. Relative changes in maximal power output during cycling after 12 and 24 weeks of combined training; *sign. within-group increase or as indicated, *<0.05,, ***<0.001; # sign. different from the control-group, (#)<0.06, #<0.05, ##<0.01, ###<0.001. ES_{m+e}= combined morning and evening endurance before strength training group; SE_{m+e}= combined morning and evening strength before endurance training group

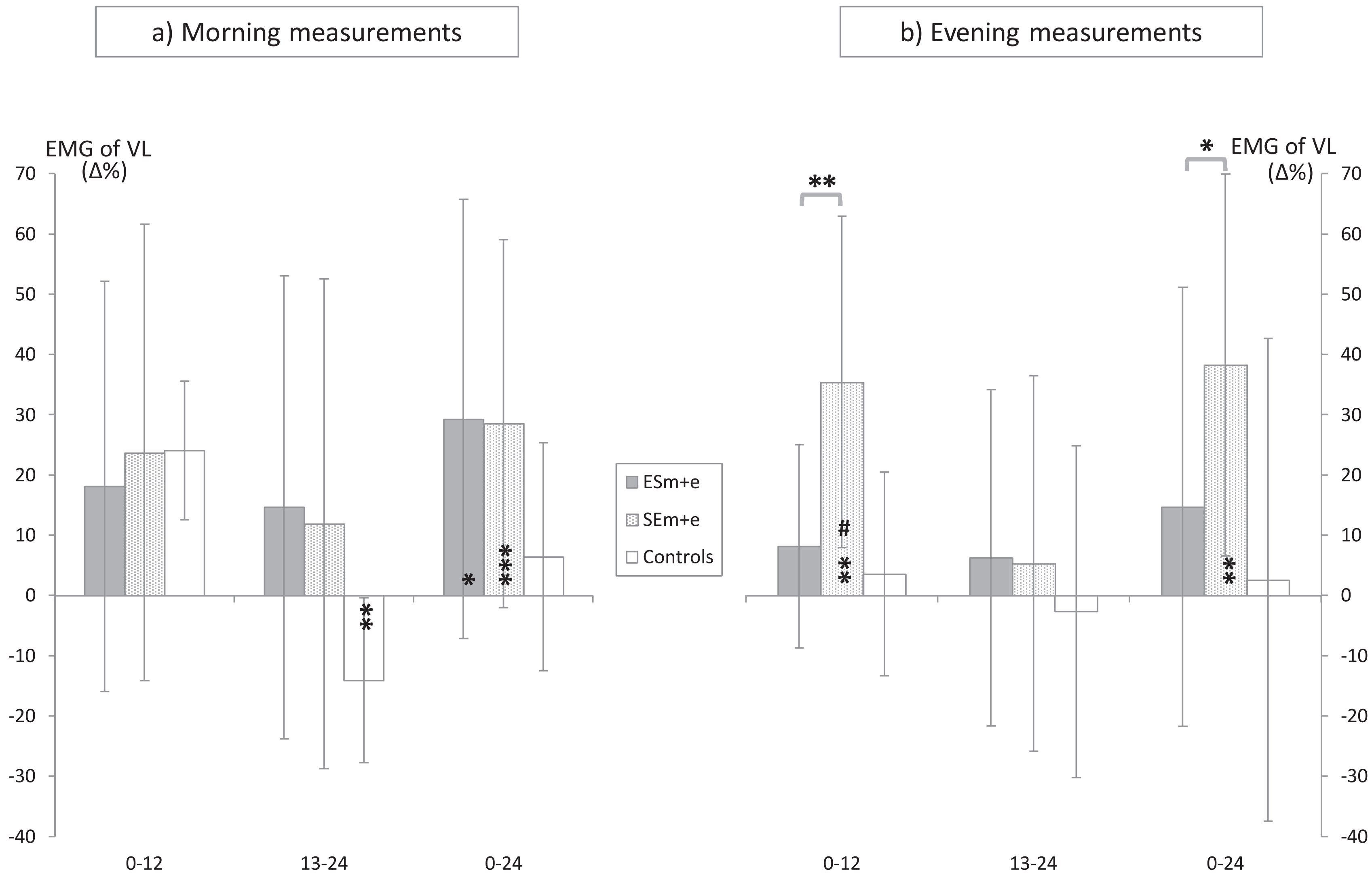
Table legend

Table 1. Absolute values ± SD of isometric knee extension force (MVCKE), rmsEMG of vastus lateralis, vouluntary activation % (VA%) and peak wattage (Wpeak) at pre-, mid- and post-measurements in the morning and in the evening.



a) Morning measurements
b) Evening measurements
Knee extension force
(Δ%)
Knee extension force
(Δ%)
ESm+e
SEm+e
Controls
35
30
25
20
15
10
5
0
-5
-10
-15
-20
0-12
13-24
0-24
#
(#)
*
**

Fig. 1



a) Morning measurements
b) Evening measurements
EMG of VL
(Δ%)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
ESm+e
SEm+e
Controls
0-12
13-24
0-24
**
*
#

Fig. 2

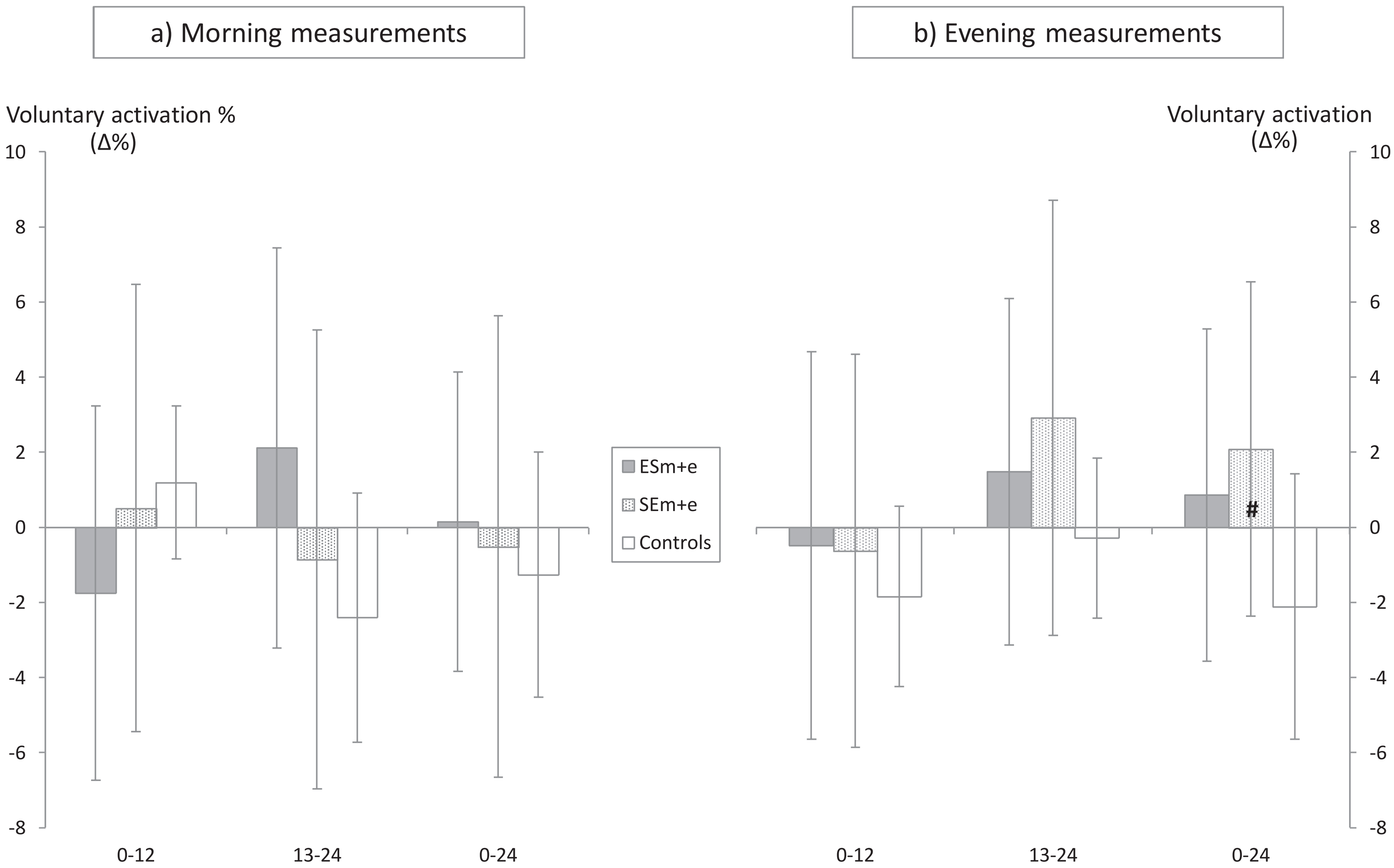

a) Morning measurements
b) Evening measurements
Voluntary activation %
(Δ%)
Voluntary activation
(Δ%)
10
8
6
4
2
0
-2
-4
-6
-8
ESm+e
SEm+e
Controls
#
0-12
13-24
0-24
0-12
13-24
0-24

Fig. 3

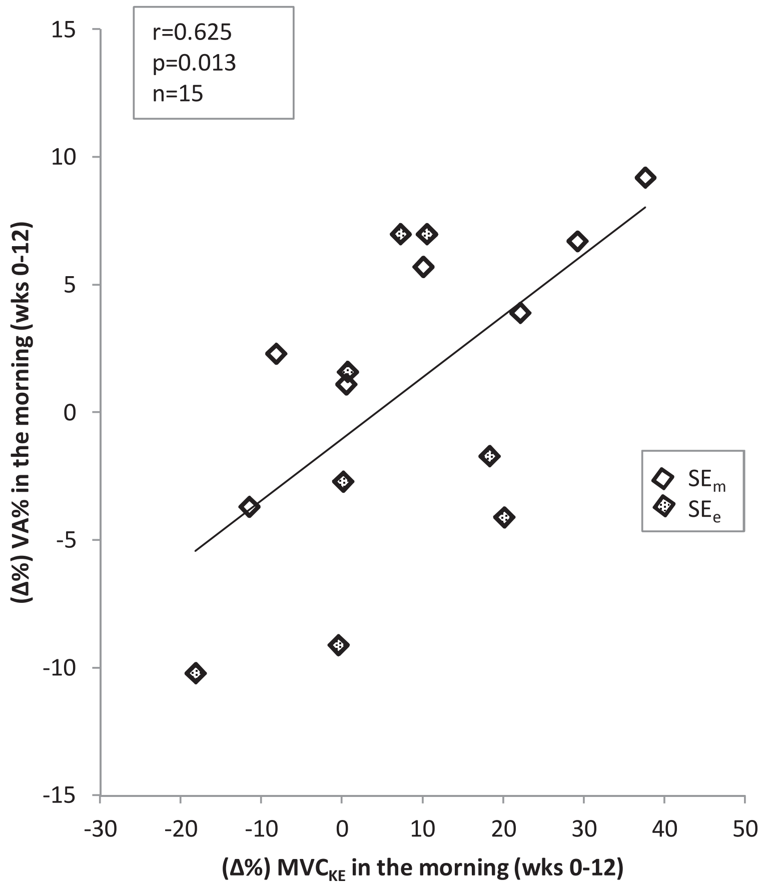
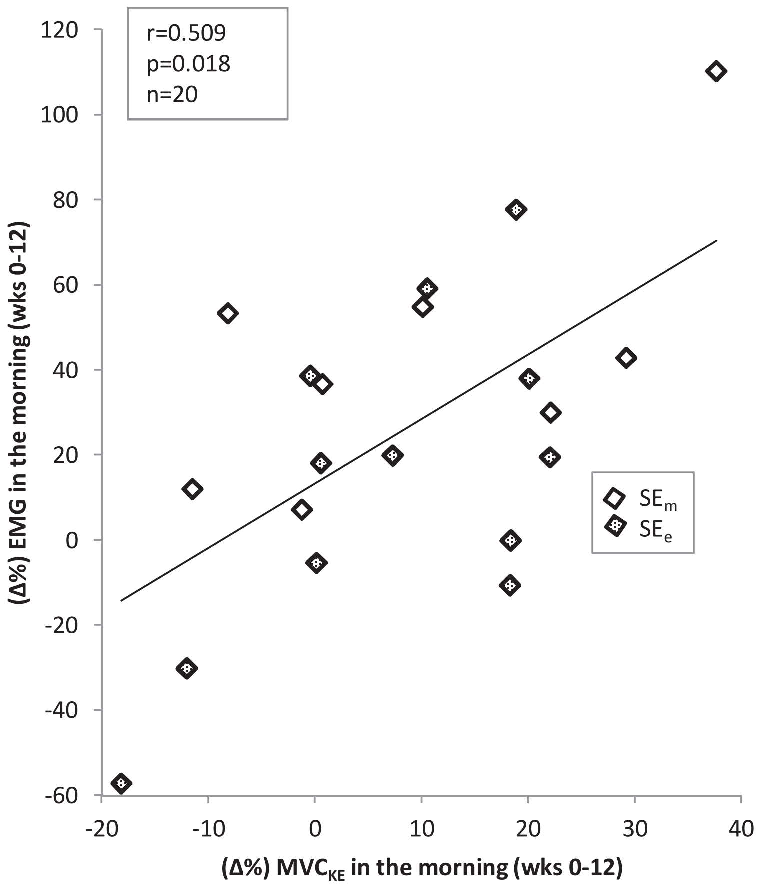

r=0.625
p=0.013
n=15
SE$_m$
SE$_e$
(Δ%) VA% in the morning (wks 0-12)
(Δ%) MVC$_{KE}$ in the morning (wks 0-12)
15
10
5
0
-5
-10
-15
-30
-20
-10
0
10
20
30
40
50

Fig. 4



r=0.509
p=0.018
n=20
SE$_m$
SE$_e$
(Δ%) EMG in the morning (wks 0-12)
(Δ%) MVC$_{KE}$ in the morning (wks 0-12)
120
100
80
60
40
20
0
-20
-40
-60
-20
-10
0
10
20
30
40

Fig. 5

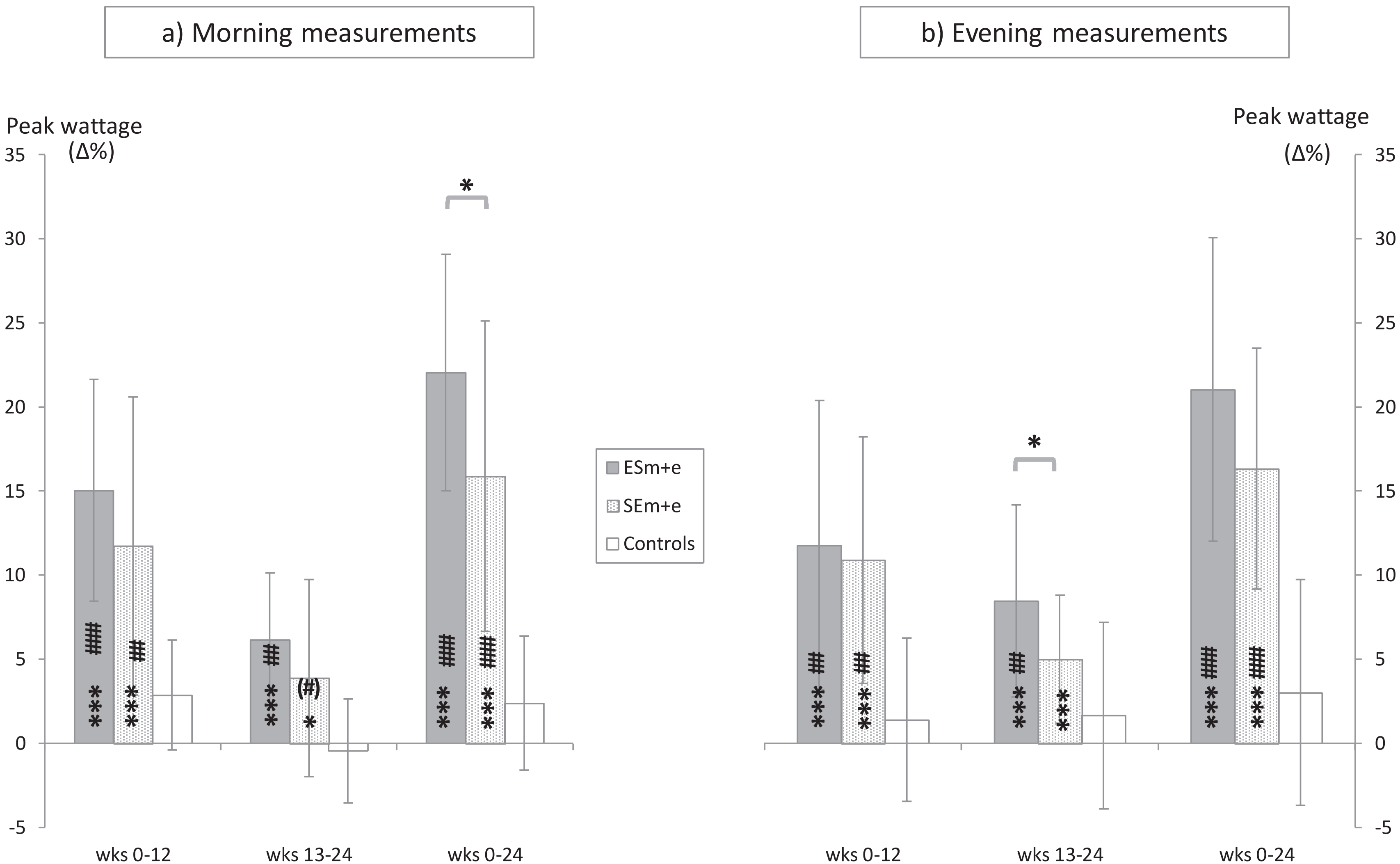

a) Morning measurements
b) Evening measurements
Peak wattage (Δ%)
35
30
25
20
15
10
5
0
-5
wks 0-12
wks 13-24
wks 0-24
ESm+e
SEm+e
Controls
(#)

Fig. 6

Table 1. Absolute values ± SD of isometric knee extension force (MVC_{KE}), rmsEMG of vastus lateralis, vouluntary activation % (VA%) and peak wattage (Wpeak)at pre-, mid- and post-measurements in the morning and in the evening.

	Pre		**Mid**		**Post**	
	Morning	**Evening**	**Morning**	**Evening**	**Morning**	**Evening**
MVC_{KE} (N) ± SD						
SE_{m+e}	574±119	567±128	615±113	632±120*,§	628.9±129*	646±121*,§
ES_{m+e}	625±95	646±96	643±110	652±116	668.6±107	670±105
Controls	584±90	615±86	619±82*	599±95	566.45±7	571±86
rmsEMG ± SD						
SE_{m+e}	0.26±0.08	0.24±0.05	0.32±0.16	0.33±0.11*,§	0.34±0.14*	0.34±0.14*,§
ES_{m+e}	0.24±0.08	0.28±0.08	0.28±0.09	0.29±0.07	0.30±0.08*	0.32±0.13
Controls	0.36±0.18	0.40±0.21	0.44±0.23	0.39±0.15	0.36±0.12#	0.36±0.08
VA% ± SD						
SE_{m+e}	91.8±4.1	91.0±3.4	92.2±4.6	90.4±4.9	91.2±4.5	92.8±3.1
ES_{m+e}	92.4±4.2	91.3±5.4	90.7±5.4	90.7±5.5	92.5±4.0	92.0±5.3
Controls	92.2±5.2	95.2±2.3	95.0±2.3	93.5±2.9	92.7±3.2	93.2±3.8
Wpeak (W) ± SD						
SE_{m+e}	249±43	251±43	275±33*	276±34*	286±38#,*	290±34#,*
ES_{m+e}	245±32	249±37	281±36*	277±36*,§	298±36#,*,§	300±35#,*
Controls	267±35	270±40	274±32*	273±34*	272±34#,*	277±31#,*

SE_{m+e} = morning and evening training groups who performed strength before endurance training; ES_{m+e} = morning and evening groups who performed endurance before strength; * significant change from Pre; # significant change from Mid; §significant difference between changes in SE_{m+e} and ES_{m+e} at time point. Detailed levels of significance are presented in the results section.

IV

EFFECTS OF MORNING VS. EVENING COMBINED STRENGTH AND ENDURANCE TRAINING ON PHYSICAL PERFORMANCE, SLEEP AND WELL-BEING

by

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Submitted to Chronobiology International.

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