STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH
135

Milan Sedliak

Neuromuscular and Hormonal Adaptations to Resistance Training

Special Effects of Time of Day of Training

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston liikunta- ja terveystieteiden tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Liikunnan salissa L304
toukokuun 6. päivänä 2009 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2009

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Editors
Harri Suominen
Department of Health Sciences, University of Jyväskylä
Pekka Olsbo, Marja-Leena Tynkkynen
Publishing Unit, University Library of Jyväskylä

URN:ISBN:978-951-39-3543-6
ISBN 978-951-39-3543-6 (PDF)

ISBN 978-951-39-3548-1 (nid.)
ISSN 0356-1070

Jyväskylä University Printing House, Jyväskylä 2009

ABSTRACT

Sedliak, Milan
Neuromuscular and hormonal adaptations to resistance training: special effects of time of day of training
Jyväskylä: University of Jyväskylä, 2009, 84 p.
(Studies in Sport, Physical Education and Health,
ISSN 0356-1070; 135)
ISBN 978-951-39-3543-6 (PDF), 978-951-39-3548-1 (nid.)

The majority of the processes in human physiology and behaviour exhibit daily variation. Neuromuscular performance has been repeatedly shown to be lower in the morning hours compared to the rest of the day. However, the scientific data available provide somewhat conflicting results on the origin and mechanisms of diurnal variation in maximum strength and power. In addition, very limited scientific evidence exists as to whether this diurnal variation can be diminished by time-of-day-specific resistance training. The present study was designed to obtain more information on the mechanisms behind both diurnal variation in muscle strength and power, and the mechanisms behind adaptations to time-of-day-specific resistance training. The present results on the mechanism of diurnal variation suggest that neither myoelectrical activity nor muscle tone could fully explain the diurnal variation in maximum strength. It seems plausible that processes within the muscle tissue are at least partly responsible for the diurnal variation. Importantly, it was noted that diurnal patterns were affected by test order-related confounding factors. Anticipatory stress prior to the first session, and learning and/or improved muscular coordination due to the frequent testing, seemed to be the major confounding effect regardless of the test design. Anticipatory stress could induce a masking effect during the first morning test session, resulting in transient elevation of both serum cortisol concentrations and temperature levels. The findings on the training-induced adaptations indicate that resistance training performed in the morning, but not in the afternoon, attenuated the diurnal variation in maximum strength and power. However, the absolute increase in maximum voluntary strength and muscle size was similar regardless of the time of day of training. Myoelectrical activity increased in both training groups but with no obvious effect of time of day. Interestingly, time-of-day-specific training adaptations most likely occurred also at the psychological level, by reducing the anticipatory stress, but only in the group of subjects who repeatedly trained in the morning hours. It can be suggested that time-of-day-specific strength training may be beneficial in sports where maximum strength performance needs to peak at a particular time of day, especially in the morning hours.

Keywords: time of day, resistance training, muscle strength, muscle hypertrophy, testosterone, cortisol

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ACKNOWLEDGEMENTS

The work described in this dissertation was carried out by the author in the Department of Biology of Physical Activity, and partly in the Department of Health Sciences, University of Jyväskylä, as part of a larger multi-institutional project.

I express my deepest gratitude to my supervisor and boss, Professor Keijo Häkkinen, PhD., firstly for the opportunity to conduct my doctoral project in Finland. From the very first visit to his office, he provided me with guidance and helped me to keep my feet on the ground. Importantly, he always listened to my ideas, and each decision we made was based on open and thorough discussion and mutual agreement. Secondly, Keijo took good care of me as a boss and made my stay in Finland possible.

Professor Sulin Cheng, PhD., and Docent Taija Finni, PhD., also did a great job as my supervisors. Among other things, both Sulin and Taija gave me freedom and trust when incorporating my doctoral project into the larger project that they, together with Keijo Häkkinen, were responsible for. Their invaluable comments and suggestions as co-authors, as well as their different perspectives on the problems we encountered, taught me a great deal about science and the world of research.

I owe my sincere thanks to my other co-authors: Mr Timo Haikarainen, Mr Markus Lind and Mr Jussi Peltonen are not only acknowledged for their critical comments on our manuscripts, but also for their countless working hours during the measurements, data analysis, and training supervision of subjects. They created a joyful atmosphere, which helped us to keep going from dawn to dusk or even longer. Professor William Kraemer, PhD., is also acknowledged for his expertise and insightful comments.

An incredible amount of work was done by Mrs Sumei Cheng and Mrs Heli Vertamo when dealing with the study participants on an everyday basis. Without medical and dietary supervision, and the laboratory personnel, this work could not have been accomplished. Special thanks go to Professor Markku Alén, PhD., Mrs Arja Lyytikäinen, Mr Risto Purtinen and Mr Markku Ruuskanen. In addition, all other colleagues, students and participants involved in the project are deeply acknowledged.

I want to thank all my other colleagues, especially the other doctoral fellows, with whom I did not only meet during working hours. Numerous department trips, “saunailtas”, “pikkujoulut”, or just nights out in the city made my years in Finland hugely enjoyable, and will always be remembered with a big smile on my face.

Last but not least in our department, I thank our dear secretaries Ms Minna Herpola and Mrs Katja Pylkkänen, for the absolutely indispensible job they have done. Without them, I am pretty sure that I would not be writing these acknowledgements, but rather filling in some of the numerous official reports or unsuccessfully faxing my article proofs to the publishers.

I would like to acknowledge several people outside the Department who played an indirect but important role in accomplishing this doctoral work.

First of all, thanks to Professor Dušan Hamar, PhD., from my former Department of Kinanthropology, Comenius University, Slovakia. It was his idea for me to come to Jyväskylä to do my research, and it was his contacts and letter of support that got the process started. I also wish to express my sincere thanks to my former supervisors at Comenius University, Slovakia: Professor Vojtech Štulrajter, PhD., Docent Jana Lipková, PhD., and Docent Alica Scholzová, PhD., for their patient guidance during my very first steps in the world of science, and also to Professor Michal Zeman, PhD., for always keeping his office door open to me, and for his expert advice on the chronobiological aspects of my thesis.

I am very grateful to Professor Jukka Viitasalo, PhD., and all the staff members of KIHU, where I spent my first year in Finland. Their hospitality and professionalism were among the factors that triggered the decision to transfer my studies to Finland.

Outside the world of science, I extend my thanks to all the great friends I have made in Finland, both of Finnish and non-Finnish origin. There are many, but I feel obligated to mention Mr Béla Pavelka, as well as the Ahola-Olli, Karhunen and Kyppö families. Of course, I must thank my own family for making me who I am; my parents Anna and Milan, my little brother Peter and my grandparents who are no longer with us. Finally, my deepest gratitude goes to my beloved Alexandra for her love and never-ending patience with my numerous deadline extensions for finishing this thesis, and to our dearest son Kubko for teaching me, through his smile, what is important in life.

The research was financially supported by the Finnish Ministry of Education, the Finnish Funding Agency for Technology and Innovation, the MEGA Electronics and HUR companies. I wish to express my sincere thanks to the referees of my thesis, Professor Michael Fry, PhD., and Professor Jim Waterhouse, PhD., for their thorough and fair review and valuable advice for finishing this work. Ms Ritva Taipale, Mr Neil Cronin, and Mr Simon Walker are acknowledged for revision of the English language of this thesis.

Milan Sedliak

Jyväskylä, May 2009

CONTENTS

ORIGINAL PAPERS

This thesis is based on the following original research articles, which will be referred to by their Roman numerals.

I Sedliak M, Finni T, Cheng S, Haikarainen T, Häkkinen K. Diurnal variation in maximal and submaximal strength, power and neural activation of leg extensors in men: multiple sampling across two consecutive days. International Journal of Sports Medicine. 2008 29:217-224.

II Sedliak M, Finni T, Cheng S, Kraemer WJ, Häkkinen K. Effect of time-of-day-specific strength training on serum hormone concentrations and isometric strength in men. Chronobiology International. 2007; 24:1159-1177.

III Sedliak M, Finni T, Peltonen J, Häkkinen K. Effect of time-of-day-specific strength training on maximum strength and EMG activity of the leg extensors in men. Journal of Sports Sciences. 2008; 26:1005-1014.

IV Sedliak M, Finni T, Cheng S, Lind M, Häkkinen K. Effect of time-of-day specific strength training on muscular hypertrophy in men. Journal of Strength and Conditioning Research. Accepted for publication September 2008.

V Sedliak M, Haverinen M, Häkkinen K. Muscle tone, strength and EMG activation in untrained men - interaction effect of time of day and test order-related confounding factors. International Journal of Sports Medicine. Submitted for publication.

ABBREVIATIONS AND DEFINITIONS

BF	Biceps femoris (muscle)
CSA	Cross-sectional area
EMG	Electromyography
GLM	General linear modelling
KE	Knee extensor muscles
MRI	Magnetic resonance imaging
MVC	Maximum voluntary contractions
QF	Quadriceps femoris (muscle)
RF	Rectus femoris (muscle)
RMS	Root mean square
SD	Standard deviation
SJ60	Loaded squat jump with 60% of one repetition maximum
TnS	Non-time-of-day-specific design
TOD	Time of day
TS	Time-of-day-specific design
VL	Vastus lateralis (muscle)
VM	Vastus medialis (muscle)
1 RM	One repetition maximum

1 INTRODUCTION

Life on Earth has evolved in a rhythmic surrounding. Earth, like other planets of the solar system is rotating on its axis and revolving around the Sun. As a result of these movements, various cyclic changes in e.g., sun light intensity, duration and temperature are present on the surface of Earth and have imprinted its timing upon living matter from the earliest days of evolution (Haus and Touitou 1994).

One of the most influential cyclic changes for the organisms has been an approximately 24-hour light-dark cycle caused by a single rotation of Earth around its axis. The vast majority of organisms, including humans have adopted this cycle in a form of numerous endogenous daily rhythms in biochemical, physiological and behavioural processes. These rhythms allow organisms to anticipate and prepare for regularly recurring environmental changes or in other words, it allows for predictive, rather than entirely reactive, homeostatic regulation of function. For example, prior to waking in humans, sympathetic autonomic tone and plasma cortisol concentrations rise, presumably in anticipation of increased energetic demands. It should be kept in mind that daily rhythms are only a part of the biologic time structure including rhythms with longer or shorter periods, as well as growth, development and aging.

Chronobiology (lat. "c*hrono*" = time) is the science of investigating and objectively quantifying phenomena and mechanisms of the biological time structure (Halberg 1986, Haus and Touitou 1994). It is quite a young scientific field and the Cold Spring Harbour symposium in 1960 is generally considered as the founding moment of chronobiology. Beside the basic chronobiology, dealing typically with molecular mechanisms of the biological clock(s) in different uni- and multi-cellular models, various applied branches of chronobiology are slowly but surely gaining importance and recognition. Perhaps the most appealing ones to the general public are chronomedicine and chronopharmacology. For instance, significant improvements in the treatment of cancer and high blood pressure have been recently achieved by its timing according to the stages in the daily sensitivity-resistance cycles of target tissues

and organs (Giacchetti et al. 2006, Hermida et al. 2007, Levi et al. 2007). From sports science and sports medicine perspective, daily variations in limiting factors of physical performance have been the most studied topic to date. It is understandable, since daily variation in psycho-physical performance plays an important role especially in competitive sport and physically demanding occupations. According to Winget et al. (1994) the selection of the best time of day for performance (e.g., late afternoon for muscle strength) can mean as much as a 10% difference in how well athletes or ordinary people may perform as compared to other times of day. Another quite well studied topic has been the "jet lag" syndrome or deteriorating influence of transmeridian air travel on performance (for a review see Waterhouse et al. 2007), as well as health related risk of physical exercise at certain time of day, e.g. early morning exercise in cardiac patients (Atkinson et al. 2006).

Surprisingly, the question of whether training adaptations may depend on the time of day of training has received little scientific attention up to date. The assumption of time-of-day-specific training adaptation is based on the fact that various limiting factors of physical performance are peaking at certain (specific to the limiting factor) times of day. For instance, morning and afternoon hours are significantly different regarding levels of maximum strength which can be voluntarily exerted by an individual and several steroid hormone blood concentrations (e.g. testosterone, cortisol). Indeed, the limited scientific evidence has suggested that time-of-day-specific training period can produce time-of-day-specific adaptations and alter typical daily variation in neuromuscular performance (Souissi et al. 2002). The present work aimed to expand these results by studying time-of-day-specific adaptation to resistance training performed at the minimum (morning) or maximum (afternoon) of daily rhythm not only in maximum strength but also in muscular hypertrophy. In addition, possible mechanisms responsible for time-of-day-specific training adaptation were studied by examining surface electromyography and serum concentrations of selected hormones.

2 REVIEW OF LITERATURE

2.1 Biological rhythms – an overview

Biological rhythms represent ubiquitous regulating mechanisms found in most organisms, including plants, animals, fungi and cyanobacteria. As defined by Haus and Touitou (1994), a biological rhythm is a regularly recurring component in a series of measurements of a biologic variable obtained as a function of time. Apart from the rhythms in physics, biological rhythm does not recur exactly to the same initial level. Biological rhythm can be described by the following rhythm parameters:

Frequency (f) is the number of cycles occurring per time unit

MESOR (C_0, M) stands for Midline Estimating Statistic of Rhythm and it represents the value between the highest and the lowest values of the cosine function best fitting to the data

Amplitude (A) is the measure of one half of the extent of the rhythmic change

Acrophase (Φ) represents the crest time (peak) of the cosine curve best fitting to the data

Phase - the value of a rhythmic biological variable at a certain time

Period (T) - duration of one complete cycle in a rhythmic variation

Based on the period, biological rhythms are typically divided into 3 main groups: daily (circadian), ultradian and infradian. Daily recurring rhythms with a period of approximately 24 hours have so far been the most studied group of rhythms. Since daily rhythmicity of selected variables is the main topic of the present thesis, the structure and mechanisms of daily rhythms will be discussed in detail in the following chapter. The second group includes rhythms with a period shorter than 24 hours. They are termed ultradian and in humans are represented by e.g., the 90-minute cycle of REM sleep stage, the 4-hour nasal cycle, or the 3-hour cycle of growth hormone secretion. The third group of infradian rhythms, by contrast, have a period longer than 24 hours and can be further divided into, among others, circaseptan (a period about 1 week e.g.,

activity of the immune system (Haus and Smolensky 1999), circatrigintan (about 30 days e.g., menstrual cycle) and circannual rhythms (about 1 year e.g., seasonal variation in some serum hormonal levels) (Haus and Halberg 1970). In the text above, examples of various physiological parameters were selected to point out the fact that biological rhythmicity of various periods is present in a wide variety of physiological processes in the human body. However, it must be kept in mind that a single parameter can exhibit rhythms with different periods. For instance, the hormone cortisol (cortisol is used as an example across the review of literature since there exists a large amount of scientific data on cortisol and its concentrations were also assessed in the present thesis) is secreted into the blood stream in ultradian pulses with an interpulse interval of 77 ± 4 min (Veldhuis et al. 1989). The magnitude of these pulses is, however, internally modulated by another self-sustaining oscillating system into a daily rhythm resulting in more than 6-fold higher morning serum levels of cortisol as compared to the late evening concentrations (Veldhuis et al. 1989, Figure 1).

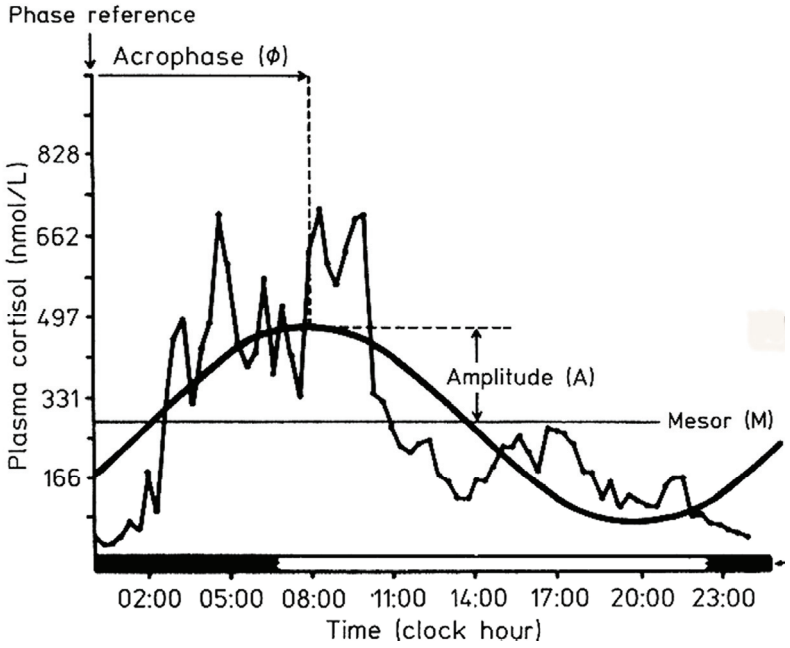

FIGURE 1 Circadian rhythm of plasma cortisol of a human subject. The thin line represents the raw plasma concentrations assessed across 24-hour period with approximately a 20-min sampling frequency. Ultradian pulses can be seen. The thick line represents a fitted cosine curve with the basic rhythm parameters determined. On the x axis, the white part represents a time period illuminated with sun light, the black parts represent nights (modified from House and Touitou 1994).

Daily levels of cortisol (typically assessed in the morning) can be further affected by infradian variation of e.g., a circaseptan period - highest values on Mondays, the lowest on Thursdays (Haus and Touitou 1994), or a circannual period - elevated concentrations during winter (Walker et al. 1997). The mutual relations between mechanisms responsible for these rhythms of various periods are still poorly understood.

2.1.1 Daily rhythms – endogenous versus exogenous origin

In human, a vast majority of physiological, biochemical and behavioural processes exhibit daily fluctuations. The simplest explanation of this phenomenon would be that these daily fluctuations may merely reflect different patterns of behaviour imposed by the cyclical environment, e.g. sunlight-related night sleep versus diurnal (daylight-related) activity patterns resulting in differential metabolic demands. However, it has been repeatedly shown that when humans or experimental animals are held in isolation without direct contact with the solar or social time cues, daily cycles of various processes do not become disorganised. Actually, they continue oscillating, with a period of slightly different from that of 24 hours. For instance, periods for body temperature, plasma melatonin and cortisol have been reported to be very similar in duration of 24 hours and 11 minutes on average when measured under an artificial 28-hour day (Czeisler et al. 1999). The meaning of this result could be explained (again) by the example of serum cortisol. As mentioned above, after several days living under an artificial day-length of 28 hours, daily rhythm of serum cortisol does not reset to a new 28-hour day but begins to oscillate with an approximate 24 h and 11 min period. This clearly implies that daily rhythm of cortisol has an endogenous origin with an intrinsic period being slightly longer than 24 hours. Therefore, a term circadian (from the Latin circa diem = about a day), introduced by Franz Halberg, is widely used to describe daily rhythms of the acknowledged endogenous origin with a period close to 24 hours (e.g., circadian rhythm of core body temperature, melatonin and testosterone (Halberg et al. 1964, Quay 1964, Dray et al. 1965).

We must bear in mind that a circadian rhythm of serum cortisol itself is only a so-called overt rhythm; a measureable final product of multi-level feed-forward - feedback loop mechanisms with multiple endogenous and exogenous inputs. A current simplified model of general circadian timing mechanism based on both animal and human experiments is presented as follows. A core unit of the circadian timing are individual cells of certain tissues that generate rhythmic gene expression with a periodicity of approximately 24 h (Reppert and Weaver 2001). This individual cell oscillatory "clock", best studied in cells of paired suprachiasmatic nuclei (SCN) of the anterior hypothalamus is composed of interacting positive and negative transcriptional / translational feedback loops clock genes (Lowrey and Takahashi 2004). In the SCN, the positive loop components consist of the transcriptional activators CLOCK and BMAL1 proteins, which, as dimers, promote the transcription of E-box containing cryptochrome (Cry1 and Cry2), period (Per1 and Per2) and Rev-Erb-alfa genes (Okamura 2004). CRY and PER proteins are imported from the cytoplasm into the nucleus to inhibit the activity of CLOCK / BMAL1, thus inhibiting their own transcription. The REV-ERB-alfa protein acts to inhibit Bmal1 transcription and therefore forms an additional negative loop to the cycle. A positive loop works through Per/Cry inhibition of Rev-Erb-alfa transcription which results in lifting the Rev-Erb-alfa inhibition of Bmal1 transcription, thus activating the system (Preitner et al. 2002), as well as clock controlled output

genes. The cell "clock" further coordinates the timing of the expression of hundreds of genes within the cell with specific cellular functions e.g., related to electrical firing rates of neurons or hormonal production. Importantly, these clock-controlled genes and their temporal patterns are different among different tissues (Okamura 2004).

To date, the presence of the cell clock of very similar molecular basis to that of the SCN has been proven in several other organ tissues e.g., skin fibroblasts, liver, kidneys, heart, lungs, testis, adrenal glands and skeletal muscle (Zylka et al. 1998, Balsalobre et al. 2000, Bittman et al. 2003, Fuller et al. 2008). All these tissues/organs cells can act as autonomous circadian oscillators. However, these cell clocks need to be synchronized within the tissue/organ and between tissues/organs in order to provide optimal functioning of the entire organism. The current theory holds that in mammals this synchronisation is provided thanks to hierarchical organisation of the circadian timing. The main regulating structures of the model are the primary oscillators – pacemakers. The SCN has been repeatedly shown to be the master pacemaker in mammals (Reppert and Weaver 2002), although a very recent report has suggested that besides the SCN, the dorsomedial hypothalamus may be another primary oscillator activated by restricted food intake (Fuller et al. 2008). What makes the SCN clock cells, apart from other cell types, the primary oscillator is the ability of the SCN cells to both synchronise and sustain each other via interneuronal, circuit interactions. Under normal light/dark conditions, the SCN receives information about environmental light and dark periods via a monosynaptic neuronal pathway originating in a subset of light sensitive retinal ganglion cells in the eye (Hannibal and Fahrenkrug 2002) ensuring that endogenous oscillations of the SCN cells are daily reset and synchronised to a 24-h period. Hence, light is a prime environmental synchroniser for the circadian timing system in mammals, though not, however, an exclusive one. Several other environmental synchronizers such as food intake or physical activity (exercise) may interact with light signals and affect a part or the entire circadian timing system via the SCN or other primary oscillators (Borer 2003, Fuller et al. 2008). The timing information from the SCN is then further distributed across the organism to autonomous cellular oscillators (secondary oscillators) in the above mentioned tissues/organs – e.g., liver, kidneys, heart, lungs, testis, adrenal glands and skeletal muscle. In other words, the secondary oscillators are synchronised by the SCN-dependent cues and this synchronisation sustains circadian organisation at the level of particular tissues/organs and also ensures appropriate internal synchronisation between different physiological and metabolic systems (Hastings et al. 2007). The SCN-dependent cues may be direct via neural and hormonal pathways or indirect via the SCN control over appetite and locomotor activity (Reppert and Weaver 2002, Cuninkova and Brown 2008). The relative importance of the pathway type is tissue/organ dependent. At the end of the hierarchy are the above mentioned overt circadian rhythms of specific physiological or behavioural processes (e.g., body core temperature, plasma melatonin, plasma cortisol rhythms).

To point out the complexity of the circadian timing system, it should be noted that some of the overt rhythm products are an integral part of the hierarchy for some other overt rhythms - e.g., melatonin and cortisol as signalling molecules for synchronizing various secondary oscillators with the SCN (Balsalobre et al. 2000, von Gall et al. 2002, Cuninkova and Brown 2008). In addition, an overt circadian rhythm may be acutely and/or chronically modified by random or non-random environmental inputs. Depending on the biological strength and/or timing of input(s), the effects can range from acutely affecting certain exogenous rather than endogenous components of circadian rhythms - e.g., transient increase in cortisol as response to psychological stress or high intensity exercise (Mason et al. 1973, Häkkinen and Pakarinen 1993) - to desynchronization of the entire circadian timing which may cause, e.g., temporary decrease in physical performance in the case of jet lag syndrome after transmeridian flight (Waterhouse et al. 2007, Arendt 2009) or, in the case of long-term exposure to desynchronization, may have severe health consequences (e.g., increased cancer risk in night-shift workers, Kolstad 2008).

2.1.2 Daily variation in the endocrine system with special reference to cortisol and testosterone

If endocrine signalling (e.g., melatonin, cortisol) is a key part of the signalling pathways by which the SCN circadian timing cues are transmitted to secondary oscillators, it is not surprising that many circulatory hormones exhibit circadian variation, most likely in order to optimise physiological functioning of the organism. Similarly to cortisol, testosterone is another steroid hormone frequently studied in relation to physical exercise being under a circadian control.

Both serum cortisol and testosterone display a circadian pattern with early morning peaks and evening nadirs (e.g., Veldhuis et al. 1987, Van Cauter et al. 1996). The hierarchical circadian mechanisms controlling serum cortisol (see Figure 2) and testosterone are also similar and under a dual SCN-driven control of endocrine and neural origin. A detailed model of the hierarchical circadian mechanism controlling plasma cortisol levels is presented in Figure 2.

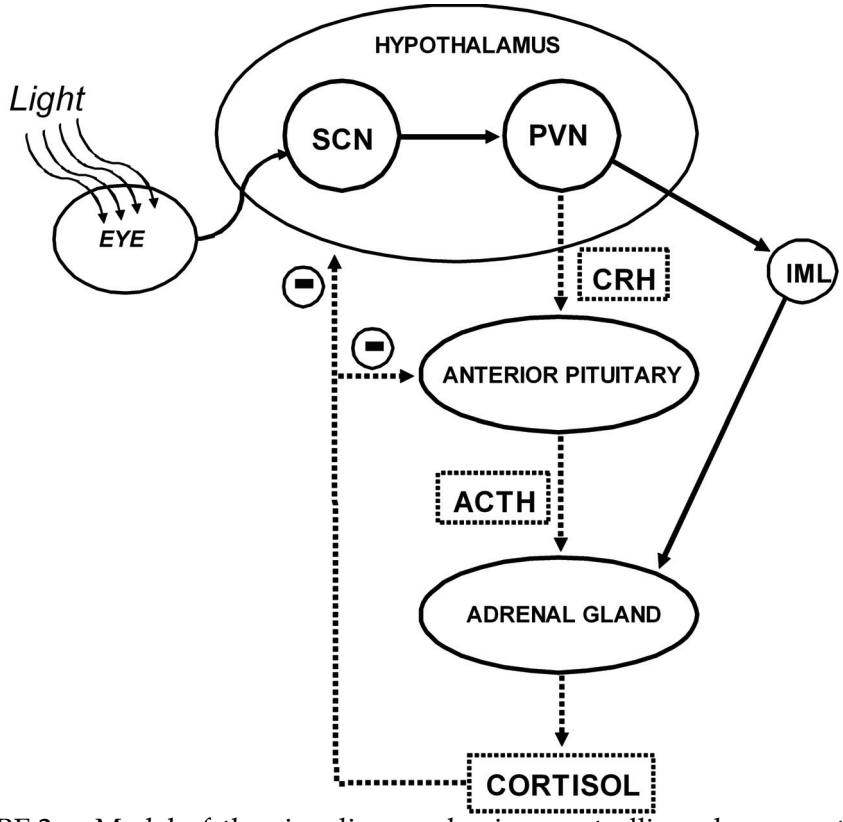

FIGURE 2 Model of the circadian mechanism controlling plasma cortisol in stress-free conditions (modified from Brown et al., 2001). The primary oscillator in this model is the suprachiasmatic nucleus (SCN) synchronized to the external environment light (external synchronizer) via neural connections with eye retina. Paraventricular nucleus (PVN), anterior pituitary and adrenal gland are secondary oscillators. The SCN has been proposed to drive the circadian rhythm of cortisol via two distinct pathways. The first one is via hypothalamic-pituitary-adrenal axis (HPA) modulation. Direct neural connections between the SCN and the paraventricular nucleus (PVN) may be responsible for circadian modulation of PVN release of corticotropin-releasing hormone (CRH) into the portal blood supply of the medial eminence. CRH stimulates anterior pituitary release of ACTH into the systematic circulation, which induces the adrenal gland to synthesize and release cortisol. Cortisol exerts a negative feedback effect at the levels of the hypothalamus and the anterior pituitary. Recently, a second neural pathway has been suggested to adjust timing of the HPA axis. The SCN may directly modulate the circadian changes in sensitivity of the adrenal cortex to ACTH stimulation via sympathetic fibres innervating the adrenal cortex. However, contradictory results have been published with regards how the sympathetic innervations modulate sensitivity of the adrenal cortex (Jasper and Engeland 1997, Ulrich-Lai et al. 2006). It must be noted that the actual functional role of the adrenal cortical clock remains to be clarified but, possibly, the adrenal cortical clock could be involved in rhythmic transcriptional activation of genes associated with hormonal biosynthesis, in gating of the response of the adrenal cortex to external cues or in apoptosis of adrenal cortical cells (Fahrenkrug et al. 2008).
Full circles and ellipses: anatomical structures
Dashed boxes: hormonal signals
Arrows: direction in which neural (full lines) and circulating humoral (dashed lines) signals are propagated.
⊖: inhibitory interactions
IML: intermediolateral column of the spinal cord

For plasma testosterone, the circadian timing mechanism is somewhat less understood and there are distinct gender differences in loci of secretion. In men, the endocrine controlling pathway is via the hypothalamus-pituitary-gonadal (testes) axis (HPG). The SCN-stimulated gonadotropin-releasing hormone (GnRH) secretion drives pituitary gonadotropin (luteinizing hormone - LH) release. LH feeds forward on testicular Leydig cells to stimulate the time-delayed output of testosterone (Urban et al. 1988). Two gonadal hormones, testosterone and inhibin B, negatively feedback on the hypothalamus and anterior pituitary, respectively (Anawalt et al. 1996). The second, neural pathway via autonomic systems is thought to modulate the sensitivity of gonads to LH stimulation. However, very recent work of Walton et al. (2007) has suggested that there is also an endogenous rhythm of testosterone production within the testis, independent of LH. To illustrate the complexity of this matter, testosterone (and its derivatives) can alter the circadian timing system by acting on androgen receptors within the SCN (Karatsoreos et al. 2007).

A number of factors can temporarily alter the circadian (diurnal) rhythm parameters of testosterone and cortisol, particularly if assessed under normal every-day conditions. Viru and Viru (2001) listed seasonal variation, sleep deprivation, nutritional status, and emotional strain among the confounding factors. Moreover, cortisol levels are especially sensitive to exposure to various acute or chronic stressors. For instance, anticipation of an exercise test has been shown to acutely increase cortisol concentration prior to testing (Mason et al. 1973). A single bout of physical loading e.g., resistance exercise of adequate volume, intensity and duration, is another potent stressor eliciting acute elevations in both serum testosterone and cortisol (Häkkinen and Pakarinen 1993, Hickson et al. 1994, Kraemer et al. 1998a, Kraemer et al. 1999, Ahtiainen et al. 2003).

However, it is not only resistance exercise that can mask the circadian variation in serum testosterone and cortisol. Also the time of day might alter the magnitude and duration of acute hormone responses to acute resistance exercise, as well as subsequent circadian patterns during a recovery period. An afternoon bout of resistance exercise blunted total and free testosterone production during the following night in a study of Nindl et al. (2001b) but caused a significant rise in total testosterone from 04:00 to 07:00 h, as reported by McMurray et al. (1995). However, both studies found no effect on total overnight cortisol patterns. An acute bout of resistance exercise performed in the morning did not significantly affect total testosterone diurnal concentrations during the following 16-h waking period, as compared to the control day (Kraemer et al. 2001). In a resistance trained group of elite weightlifters, Häkkinen et al. (1988b) reported a greater testosterone response during the afternoon sessions as compared to the morning sessions during multiple training sessions per day. On the contrary, Bird and Tarpenning (2004) found the acute response of cortisol but not testosterone to be lower in the afternoon as compared to the morning - however, this result was obtained with only one loading session per day. They also reported testosterone to cortisol ratio (T/C

ratio) to be higher in the afternoon hours (both resting and after loading). The T/C ratio had been originally suggested to be an indicator of the anabolic/catabolic status of skeletal muscle during strength training (Häkkinen et al. 1987, Häkkinen and Pakarinen 1994). According to Bird and Tarpenning (2004), it could mean that afternoon hours, with more testosterone relative to cortisol in circulation, may be more favourable for, at least, hypertrophic adaptation of muscle compared to the morning hours. However, this type of interpretation for the T/C ratio may be an oversimplification as it can be only an indirect measure of the actual biological process (for review see Kraemer and Ratamess 2005).

2.1.3 Daily variation in neuromuscular performance – origin and mechanisms

As stated above, the properties of physical exercise as a stressor may be time-of-day dependent. Interestingly, physical performance itself seems to be under the influence of time of day. Diurnal variation in physical performance has been reported for numerous sports tasks (e.g. standing long jump, tennis service, swim time-trials) and for various physiological variables limiting physical performance (Winget et al. 1985, Reilly and Down 1992, Atkinson and Speirs 1998, Kline et al. 2007). It is incorrect to speak of a single performance rhythm since a location of the peak performance seems to be dependent on the task/limiting factor involved. It has been suggested that complex skills tend to peak earlier in the day possibly due to an earlier acrophase in the circadian rhythm in alertness (Atkinson and Speirs 1998, Reilly et al. 2005). Gross motor skills, including for example muscle strength, anaerobic power output, and self-chosen work-rate peak later in the early evening (Drust et al. 2005). The diurnal minimum is almost always found in the early morning. Such a diurnal pattern is typically seen in maximal strength of the knee extensor muscles of untrained individuals, both in maximal voluntary isometric (Coldwells et al. 1994, Callard et al. 2000, Guette et al. 2005a) and isokinetic (Deschenes et al. 1998, Nicolas et al. 2005) conditions. Peak-to-trough variation has been reported to range from 6% (Guette et al. 2005a) to 18% (Coldwells et al. 1994).

However, there has been scientific controversy as to the origin of diurnal variation in muscle strength (and other performance variables). In a review article from 1999, Youngstedt and O'Connor proposed that diurnal variation in physical performance could be caused or affected by across-a-day differences in the following confounding factors: nutritional status, joint stiffness, sleep inertia, time of usual activity, previous rest, environmental and body temperature, motivation and expectancy. These authors also called for more methodologically sound experiments to be able to clearly distinguish between possible endogenous and exogenous factors. On the other hand, Drust et al. (2005) suggested at least some input of endogenous mechanism based on the studies that controlled for some of the above factors. Indeed, a recent study from Youngstedt´s group reported a circadian rhythm in swim performance of 2-4 min duration to be independent of behavioural and environmental confounding factors (Kline et al. 2007a). Swim performance peaked 5 – 7 hours

before the minimum body temperature and was worst within ± 1 hour time window around the body temperature minimum (05:00 h). It was the first and, to date, the only study in human neuromuscular performance using an ultra-short sleep/wake protocol that allows separation (if present) of an endogenous portion of the rhythm (Monk and Welsh 2003). The contradictory views on the nature and mechanisms of diurnal variation in maximal muscle strength and power have not yet been satisfactorily resolved. A possible major candidate for this mechanism appears to be the circadian rhythm in core body temperature. The body temperature peaks and nadirs occur close to the peak and nadir in maximum strength and power (e.g., Reilly and Down 1992). Based on this observation, some earlier studies have suggested that the simultaneous increases in body temperature and anaerobic performance are causally related and that the circadian rhythm of body temperature could exert a passive warm-up effect (Coldwells et al. 1994, Bernard et al. 1998) and, for example, affect Ca^{2+} retention by the sarcoplasmic reticulum (Stein et al. 1982). Other authors have opposed the passive warm-up theory and suggested that parallel diurnal variation in body temperature and maximum strength and power stem from the drive of a common circadian oscillator (Gauthier et al. 1996, Gauthier et al. 2001). Interestingly, recent studies of Racinais and colleagues propose that exposure to a warm (>28 ^{o}C) and humid environment can increase morning muscle strength and diminish its diurnal variation (Racinais et al. 2005, Racinais et al. 2006). The authors concluded that both heat exposure and the diurnal rise in body temperature can cause a passive warm-up effect and improve muscle contractility, but these two factors cannot be combined to improve strength to a greater level. However, the mechanisms behind their findings can be only speculated upon.

To summarise, it is clear that causation between diurnal variation in body temperature and in muscle strength cannot be concluded from the present scientific evidence. The variation in body temperature may not solely explain the diurnal variation in maximal muscle strength and power, and the majority of authors have proposed its multi-factorial origins.

Surface electromyography (EMG) has been used extensively to investigate the mechanisms and anatomical loci (neural system vs. skeletal muscle tissue) of neuromuscular diurnal variation. Again, various studies have yielded conflicting results. The detailed information on reports using different techniques and analyses of EMG and muscle groups is presented in Table 1. Briefly, Gauthier et al. (1996) and Castaingts et al. (2004) reported that both central (neural input to the muscles) and peripheral (contractile state of the muscle) mechanisms may alter across a day. On the contrary, Martin et al. (1999) , Nicolas et al. (2005) and Guette et al. (2005a) suggested that agonist (but not antagonist) muscle tissue and its contractile properties are the source of diurnal changes in peak torque. A similar conclusion was drawn by Giacomoni et al. (2005), reporting a significant diurnal variation only when electrical twitches were superimposed during MVC, but in men only. They proposed that a motivational component could have a masking effect on the diurnal variation in voluntary maximum strength of knee extensors. To add to the complexity of

this matter, Onambele-Pearson and Pearson (2007) showed 20% higher patellar tendon stiffness in the morning as compared to the afternoon. Surprisingly, this had no detrimental effect on the force production as there was on average 16% increase in MVC torque from morning to the afternoon.

TABLE 1 Overview of the scientific studies on diurnal variation in neuromuscular performance using surface EMG. RMS = root mean square.

Authors & year of publication	Sample size & test design	Test & muscle(s) tested	Muscle strength	EMG and muscle activation	Neuromuscular efficiency	Suggested mechanism(s) for diurnal variation
Gauthier et al. 1996	7 males, 6 females 06:00, 09:00,12:00,15:00, 18:00, 21:00, 24:00 across the same day	elbow flexion, isometric **MVC at 90º** and at 25, 50, 75% of MVC **elbow flexors**	peak torque **highest at 18.00,** lowest **at 09:00**,	maximum RMS of biceps brachii. **higher at 09:00** than **18:00**	maximum at **18:00**, minimum at 09:00 RMS/torque ratio	both **neural** and **contractile state of muscle**
Martin et al. 1999	12 males, 1 female 07:00 and 18:00, counterbalanced, within 24 h	thumb adduction, isometric **MVC** and at 25, 50, 75% of MVC, adductor pollicis, left arm	peak torque **higher at 18.00** than **at 07:00** (8.9%)	absolute RMS - **no difference**, muscle activation - **no difference** (07:00-97,1%, 18:00-98,5%), tetanus torque **higher at 18:00**	**no difference -** RMS/force	**contractile state of muscle**
Castaingts et al. 2004	11 males, 06:00 and 18:00 within the same day	plantar flexion, drop jumps, isometric **MVC at 90º**, 25% of MVC, **jumping height,** soleus and gastrocnemius muscles	peak torque **higher at 18:00** than **at 06:00** (8,6%), Drop jump height **higher at 18:00** (10.9 %)	mean amplitude during MVC - **no difference**	el.stimulation and drop jump – higher at 18:00, reflex stim.MVC and 25% MVC25 - unchanged	for efficiency: both **peripheral** and **central mechanisms** – test depended!
Nicolas et al. 2005	12 males, 06:00 and 18:00, separated by 36h	isokinetic knee extension, 50 **MVC** repetitions, vastus medialis, lateralis, rectus femoris and biceps femoris, dominant leg	peak torque **higher at 18:00** than at **06:00** (7.7%)	RMS of agonist muscles - **no difference**	fatigue protocol: decrease **higher at 18:00** but during first 20 reps only	for fatigue: **peripheral mechanisms** - higher contractile capacity but lower fatigue resistance at 18:00
Guette et al. **2005**	10 males, 06:00, 10:00, 14:00, 18:00, 22:00, minimum 8h separation	unilat. knee extension, isometric **MVC at 90º,** vastus medialis, lateralis, rectus femoris and semitendinosus, dominant leg	peak torque **highest at 18:00, lowest** at **06:00** (6.6%)	M-wave, muscle activation, RMS/M-wave ratio - **no difference**, rate of torque development - **lower at 06:00** than at 14:00, 18:00 and 22:00	——	modification **at the muscular level**
Guette et al. 2005	12 males, 06:00-08:00, 17:00-19:00 within the same day	unilat. plantar flexion, isometric **MVC at 90º**, el. **twitch torque,** soleus muscle, dominant leg	peak torque and **higher in the morning** than afternoon (4.7%),	Normalised EMG (RMS/M-wave) and H-wave twitch torque **higher in the morning** than afternoon	——	spinal reflex excitability not involved in reduction of force
Guette et al. **2006**	11 males, 06:00-08:00, 17:00-19:00 within the same day or two consecutive days	unilat. plantar flexion, isometric **MVC at 90º**, soleus, gastrocnemius med. and lat., tibialis anterior**,** dominant leg	peak torque and **higher in the morning** than afternoon (7%),	**higher morning** normalised **soleus** EMG (21,6%) and muscle activation (6,8%), single twitch and tetanus torque - **unchanged**	——	**impairment of the central command**
Giacomoni et al. 2006	12 males, 08:00-10:00, 17:00-19:00, at least 36 hours recovery	unilat. knee extension, isometric **MVC at 90º**, vastus lateralis, dominant leg	**no difference** in peak torque MVC	**no difference** in RMS of vastus lateralis	**no difference** in torque/RMS ratio	not reported
Giacomoni et al. 2005	12 males, 8 females, 02:00, 06:00, 10:00,14:00, 18:00, 22:00 randomly over 3-4 days	unilat. knee extension and flexion, **isometric MVC at 90º ,** isokinetic **MVC**	**no significant** peaks at 14:00 or 18:00 for or isometric and isokinetic peak torques during flexions and extensions	**highest** superimposed twitch torque **at 18:00**, **lowest at 06:00** only **in males**	——	not reported, suggested masking effect of motivation
Racinais et al. 2005	11 males, 07:00-09:00, 17:00-19:00, two times both in random order in normal and warm conditions	knee extension, isometric **MVC** and at 25, 50, 75% of MVC, vastus lateralis	peak torque **higher in the afternoon (+8.4%)** but not in hot and humid environment	**no difference** in RMS of vastus lateralis	——	**contractile state of muscle,** partly due to low morning muscle (body) temperature
Onambele-Pearson et al. 2007	12 males, 07:45, 17:45, counterbalanced, within 24 hours	unilat. knee extension, isometric **MVC** between **90 and 30º,** biceps femoris	peak torque **higher in the afternoon** at knee angles from **75 to 90º**	**no difference** in muscle activation (91% and 88% for morning and evening, respectively)	——	**contractile state of muscle**, unknown mechanism proposed to counteract 21% evening decrease in pattelar tendom stiffnes

2.2 Effect of time of day on adaptation to resistance training

2.2.1 General adaptation to resistance training

As mentioned above, a single bout of resistance exercise can act as a stressor. At an appropriate level, this can induce a sequential cascade starting with muscle activation, subsequent acute signalling events due to deformation of muscle fibres, followed by acute hormonal and inflammatory responses. With a lag of several hours, protein synthesis peaks, resulting (if higher than protein degradation) in subsequent muscle fibre hypertrophy (Spiering et al. 2008). The magnitude of these processes can be somewhat modulated by manipulation of resistance exercise variables: type of exercise, load, volume, rest period, exercise order (Kraemer et al. 1996, Fry 2004). For instance, myofibrillar protein synthesis (Kumar et al. 2008) and acute hormonal response (e.g., increases in serum total and free cortisol, testosterone and growth hormone, Crewther et al. 2006) seem to be larger following a hypertrophic type of loading [60-80% of one repetition maximum (1 RM), 6-12 repetitions per set, 3-5 seconds repetition duration, 2-4 sets] as compared to high-load (80-100% of 1 RM, 1-8 repetitions per set, 2-4 sets) and high-speed protocols (40-60% of 1 RM, 5-8 repetitions per set, 2-3 sets) according Kraemer and Häkkinen (2002). If bouts of resistance exercise are repeated regularly over a longer period, of months and years, adaptive responses exhibit another specific time course. In previously untrained individuals, the initial 2-4 weeks of training result in rapid increases in muscle strength, accounted for largely by adaptations in the facilitatory and/or inhibitory neural pathways acting at various levels in the nervous system (Moritani and deVries 1979, Häkkinen and Pakarinen 1994). This period likely involves learning the right pattern of intra- and intermuscular coordination, i.e. properly timed activation of stabilizers, synergists and antagonists (Rutherford and Jones 1986). As resistance training continues, training gains become smaller, ultimately reaching a plateau (Kraemer and Häkkinen 2002). In addition, biological factors such as muscle fibre type distribution, endocrinological profile, macronutrient intake, age and sex have been recognized for their importance in adaption to strength training (Kraemer and Ratamess 2005, Crewther et al. 2006, Folland and Williams 2007, Hulmi et al. 2008). The extent to which muscle strength, power and/or muscular hypertrophy is developed depends largely on the loading protocol used. Typically, three subtypes of adaptations are studied with regards to resistance training - neural, hormonal and morphological - although all subtypes are closely interconnected and this separation is mostly methodology-related. Several comprehensive review articles have been published recently on this area of research (Fry 2004, Kraemer and Ratamess 2005, Crewther et al. 2006, Folland and Williams 2007, Wernbom et al. 2007). The main points will be briefly discussed here:

Neural adaptations

According to Folland and Williams (2007), neural adaptations are essentially changes in coordination and learning that facilitate improved recruitment and activation of the involved muscles during a specific strength task. Based on the surface EMG, numerous studies have observed increased agonist muscle EMG with training, interpreted as an increased neural drive to agonist muscles (Komi et al. 1978, Moritani and deVries 1978, Häkkinen and Komi 1983, Narici et al. 1989, Häkkinen et al. 1996, Aagaard et al. 2002a, Häkkinen et al. 2003) while some other studies found no change in EMG (Garfinkel and Cafarelli 1992, Narici et al. 1996). Some contradictory results have been published also on antagonist co activation (for a review see Folland and Williams 2007). The issue of antagonist co activation is complicated since some levels are necessary to provide joint stability, as well as its dependence on the type of exercise, velocity and range of motion (Karst and Hasan 1987). While the EMG activity of agonist muscle may increase with resistance training, its interpretation as an increased neural drive seems to be an oversimplification. Surface EMG reflects many factors which could be altered by training, e.g. fibre type and size, and their orientation relative to the skin surface, intramuscular ionic concentrations, and thickness of fat layer (Folland and Williams. 2007). It must be kept in mind that surface EMG is also vulnerable to cancellation of motor-unit action potentials (Keenan et al. 2006) and must be interpreted with caution. Besides EMG, transcranial magnetic stimulation has been recently employed to study neural and especially cortical adaptations. Interestingly, decreased corticospinal excitability found after resistance training (Jensen et al. 2005) was contrary to the studies using electrically evoked spinal reflexes (e.g., Aagaard et al. 2002b) suggesting a need for further research on the corticospinal adaptation to resistance training.

Hormonal adaptations

The endocrine system seems to influence and help to mediate other adaptations in the nervous system and muscle fibres (Kraemer et al. 1998b). Several peptide and steroid hormones, typically growth hormone, insulin-like growth factor I (IGF-I), and insulin have been studied in relation to resistance training (Kraemer et al. 1999, Andersen et al. 2003, Ahtiainen et al. 2005). Here, the primary focus is on the two steroid hormones examined in the present work – testosterone and cortisol. Testosterone is considered an anabolic hormone promoting, among others, protein synthesis in muscle tissue (Ferrando et al. 1998), the result being increased muscle mass and strength (Kraemer et al. 1990). Testosterone can increase muscle protein synthesis and slow down muscle protein degradation (Bhasin et al. 2003), increase muscle sensitivity to IGF-I via up-regulation of the IGF-I receptors (Thompson et al. 1989), and increase satellite cell proliferation resulting in muscle fibre hypertrophy (Doumit et al. 1996, Sinha-Hikim et al. 2003). Testosterone can also interact with neurons in the CNS resulting in, e.g., enhancement of acute force production or neural cell regeneration and growth (Nagaya and Herrera 1995, Brooks et al. 1998). Indirect evidence implies that muscle strength development in men may be

positively dependent on chronic total (both free and bounded forms) serum testosterone concentrations (Bhasin et al. 2001, Kvorning et al. 2006) and/or free testosterone levels (Häkkinen et al. 1985). Interestingly, heavy-strength training using hypertrophy, neural, and/or power protocols applied over several weeks or months has been shown to induce some periodic alternations but no chronic changes in resting total and free testosterone and testosterone binding proteins concentrations (for a review, see Kraemer and Ratamess 2005). Rather than changes in resting concentrations, enhancement in acute response to a bout of exercise has been found after a resistance training period in men (Kraemer et al. 1999, Tremblay et al. 2004). Furthermore, resistance training may increase androgen receptor expression in trained muscle cells (Kadi 2000, Willoughby and Taylor 2004) thus providing more binding sites and thus possibly resulting in higher physiological activity of testosterone.

Cortisol is a multi-functional hormone typically considered to have catabolic properties counteracting the effects of testosterone. In skeletal muscle, cortisol is involved in protein degradation and it decreases protein synthesis. It may also suppress the HPG axis by inhibiting GnRH (MacAdams et al. 1986, Breen et al. 2004), stimulate lipolysis in adipose cells and increase gluconeogenesis. Therefore, the role of cortisol in the process of adaptation to strength training may be more complex than purely catabolic, e.g., increasing free amino acid and lipid pool post exercise available for subsequent adaptive protein synthesis (Viru and Viru 2001). A high-volume bout of resistance exercise (moderate-to-high resistance with short rest intervals between sets) induces significant acute elevations in serum cortisol (Häkkinen and Pakarinen 1993, Folland and Williams 2007). This acute response is typically attenuated with long-term resistance training (Staron et al. 1994, Kraemer et al. 1999), perhaps partly due to down-regulation of glucocorticoid receptors (Willoughby et al. 2003). Findings on the chronic adaptations of cortisol to strength training are even more variable than those on testosterone. Besides studies reporting a lack of changes (e.g., Häkkinen et al. 1990, Fry et al. 1994, Häkkinen et al. 2000), some authors have found a reduction in cortisol concentrations after strength training (Häkkinen et al. 1985, Kraemer et al. 1998b, McCall et al. 1999). Differences in the timing of the test and training sessions across the years and/or day may partially contribute to the inconsistency in findings between different studies due to the normal seasonal and circadian fluctuations in testosterone and cortisol. However, it seems that when the overall volume/loading of the strength training (such as 2 or 3 sessions a week) remains within normal physiological range, no systematic changes will occur in the serum concentrations of anabolic and catabolic hormones (Häkkinen et al. 2000, Kraemer and Ratamess 2005). Indirect evidence also suggests that such training loading would not alter circadian patterns of testosterone and cortisol (Cain et al. 2007).

Morphological adaptations

An increase in anatomical cross-sectional area (aCSA) of the exercised muscles/muscle groups over a relatively short period of time (8-12 weeks) is a

typical finding regardless of the assessment technique used (Folland and Williams 2007). However, magnetic resonance imaging (MRI) is considered as a superior method due to its high accuracy, repeatability and non-invasive nature (Walton et al. 1997). Similar to the training-induced gains in strength, the increase in aCSA progressively declines over time (e.g., a year) as an individual approaches his/her genetic potential (Alway et al. 1992). Even though the increase in muscle size and strength are theoretically dependent (bigger size = higher strength), this relation is not linear, however. Typically, the increase in strength is relatively higher than the increase in aCSA, partly as a result of neural adaptations. Some authors have also suggested that aCSA may underestimate gains and used the physiological CSA (pCSA - perpendicular to the fibres) instead. Fukunaga et al. (1996) reported a better correlation of pCSA and muscle volume with strength than using aCSA. On the other hand, Bamman et al. (2000) did not find pCSA to be superior over aCSA. The main mechanisms suggested for skeletal muscle growth are hypertrophy of existing muscle fibres via addition of contractile proteins to the periphery of a myofibril, increase in myofibril number (MacDougall et al. 1982) and activation of satellite cells providing new myonuclei for a muscle cell (Kadi et al. 2005). Hyperplasia of the muscle cells (splitting/branching of a fibre) is highly controversial but a possible supplementary mechanism (for a review, see Folland and Williams 2007). Besides resistance exercise, variables such volume and/or intensity (Fry 2004), biological factors related to fibre type distribution, endocrine profile, macronutrient intake, age, sex and many others have been recognized for their importance in morphological adaptation to resistance training (Kraemer and Ratamess 2005, Crewther et al. 2006, Hulmi et al. 2008).

2.2.2 Time-of-day-specific adaptations

G. G. Luce, in his book published in 1971, has speculated that adaptations to exercise training might be specific to the time of day of training. The theoretical basis for this speculation was circadian variation in human physiology related to performance (Luce 1971). A study published in 1989 by David Hill et al. showed for the first time direct evidence for what they called "circadian specificity in training" in humans (Hill et al. 1989). They reported that, after the 6-week interval training period, subjects who trained in the morning had a relatively higher ventilatory threshold in the morning, while subjects who trained in the afternoon had relatively higher values in the afternoon. In 1992, Torii et al. (1992) demonstrated that aerobic training had a significantly higher effect in the afternoon (15:00 h) compared to morning (09:00 h) and evening (20:00 h) training sessions. However, this was probably due to a methodological bias rather than a real adaptation effect, as all subjects were tested only in the afternoon between 14:00 and 16:00 h. Hence, the afternoon training group had an advantage of being tested at the training specific clock-time as compared to the morning and evening training groups. Indeed, a later study confirmed that greater improvements (in time to exhaustion and oxygen deficit after interval

training) can be expected to occur at the time of day at which training is regularly performed, but the magnitude of adaptations is similar (Hill et al. 1998). A similar pattern of time-of-day-specific training adaptation has been also reported after heavy resistance training by Souissi et al. (2002). It was the only scientific article published using resistance training before initiation of the present work. In the study of Souissi et al., two groups of males performed an identical 6-week progressive high-load type of training either only in the morning (07:00–08:00 h) or only in the evening (17:00–18:00 h). They found that ''adaptation to strength training is greater at the time of day at which training was conducted than at other times''. This was true for both training-specific isokinetic peak torque during knee extension and, interestingly, for non-specific peak anaerobic power during the Wingate test. No significant difference between the two groups was observed in the magnitude of improvement in peak torque and peak anaerobic power, as well as in 1 RM knee extension. These results also suggest that a typical diurnal pattern in maximum strength might be altered in terms of diminished amplitude by time-of-day-specific training. However, the possible mechanisms behind the reported time-of–day-specific adaptations were not directly addressed in any of the above mentioned studies.

3 PURPOSE OF THE STUDY

This work contains data from two independent studies. The first study was a large training study conducted during 2004, which resulted in four original manuscripts (I – IV). Its purpose, methods and results will be discussed in detail below. In addition, a smaller cross-sectional study was conducted in 2006 (manuscript V). Its purpose was to expand on selected findings from the 2004 training study, as well as to retest those findings in a modified experimental design. Therefore, the methodology and results of the 2006 study will be addressed and discussed only briefly in the present work (referred to here as Muscle Tone study). For more detailed information, see manuscript V.

The overall purpose of this study was to examine the effect of time-of-day-specific resistance training on the magnitude of adaptation over a prolonged period in men. More specifically, the following issues have been addressed:

1. Daily variation in maximum strength, power, EMG activity and body temperature across two consecutive days in men prior to the start of the time-of-day-specific resistance training.

2. Effect of this time-of-day-specific resistance training on the magnitude and daily variation patterns of selected neuromuscular (maximum strength, power and EMG activity) and hormonal variables (serum testosterone and cortisol) and body temperature.

3. Effect of this time-of-day-specific resistance training on the magnitude of muscle hypertrophy.

Hypotheses

It was hypothesized that:
1. Before time-of-day-specific resistance training:

Maximal strength and explosive power performance of the leg extensors muscles will follow their typical diurnal pattern with significantly lower morning performance as compared to the rest of the day.

Maximal voluntary activation of the muscles will not show significant diurnal variation.

Serum testosterone and cortisol concentrations and oral body temperature will show their usual diurnal patterns.

2. After time-of-day-specific resistance training:

In the morning, but not afternoon experimental group, the morning performance level will be increased and will not statistically differ from performance levels achieved during the rest of the day. The diurnal patterns will persist in the afternoon experimental group.

The EMG activity will increase compared to the control group but no significant diurnal variation will be present.

Diurnal rhythms of serum testosterone, cortisol and oral body temperature will not be significantly changed by the intervention.

Neuromuscular performance and muscle hypertrophy in both morning and afternoon training groups will be similar and significantly higher as compared to that of the control group with no resistance training.

4 RESEARCH METHODS

4.1 Subjects

The subjects of original publications I-IV were recruited via posting advertisements in local workplaces, institutions, and the Jyväskylä city web-page, as well as by using local e-mail lists. Out of the initial 118 screened volunteers, seventy-five males with similar backgrounds of both health status and physical condition fulfilled the screening requirements. These subjects were subsequently measured at the Pre-test and randomized into three groups (52 subjects in two training groups, 23 subjects in a control group). The groups did not statistically differ anthropometrically (height, weight, body fat) and in selected neuromuscular performance (isometric knee extension torque and counter-movement jump height) parameters. Forty-nine subjects completed the entire experiment. Their background information is presented in Table 2. Sixteen subjects dropped out and the data of ten participants were excluded due to non-compliance with the instructions and/or an incomplete data set. It must be noted that some strength test data from 25 participants (mostly from the control group) was lost due to a recording error discovered during the final analysis phase. Therefore, a smaller sample size, as stated in Table 2, will be presented for the unpaired test results in the results section. In addition, only 24 participants were randomized for the muscle hypertrophy assessment from the entire experimental sample, due to the high cost of MRI scanning.

The subjects had no long-term lower extremity strength training experience and had performed no regular physical activity more than once a week during the 3 years prior to the experiment. All subjects were considered healthy and had no medical contraindications (e.g., cardiac or sleep related diseases) that would affect the results of this study. Shift workers were excluded. The Circadian Type Questionnaire (Folkard et al. 1979) was used to estimate morningness vs. eveningness, ability to overcome drowsiness, and flexibility of sleeping habits (Table 2). Additionally, self-reported stress load, length of sleep, daily activity and food intake information were collected one

day before, and throughout, the selected testing days. This study was approved by the Ethics Committee of the Central Hospital of Central Finland and an informed consent form was signed by subjects prior to the investigation.

TABLE 2 Anthropometrical data and frequency of extreme types in the three experimental groups. Factor 1 = morning types vs. evening types, Factor 2 = high ability to overcome drowsiness vs. low ability to overcome drowsiness, Factor 3 = rigid sleeping types vs. flexible sleeping type. Some subjects belonged to two different extreme types

Group	Age (years)	Weight (kg)	Height (cm)	Factor 1 Extreme types	Factor 2 Extreme types	Factor 3 Extreme types	Neutral types in all 3 factors
Control (n=11)	34 ± 8	81,4 ± 10,2	179 ± 8	2 vs. 1	1 vs. 1	2 vs. 1	5
Morning (n=20)	32 ± 7	82,6 ± 8,5	180 ± 5	1 vs. 4	1 vs. 0	3 vs. 0	12
Afternoon (n=18)	33 ± 7	79,8 ± 12,5	181 ± 5	4 vs. 1	3 vs. 0	3 vs. 0	10

4.2 Training protocol

The subjects randomized into the training group first underwent a 10-week preparatory training period (training week 1 – week 10). The training sessions were carried out between 17:00 h and 19:00 h. The preparatory training period was applied to familiarise the training groups with resistance training before the actual time-of-day-specific training, and also to "synchronize" activity patterns of both training groups by training exclusively in the afternoon. Thereafter, the subjects were matched based on their improvements in one-repetition maximum (1 RM) half squat after the preparatory training period and randomized either to the morning or afternoon training group. The morning training group performed all training sessions between 07:00-09:00 h, while the afternoon training group did so between 17:00-19:00 h. Both the preparatory and time-of-day-specific training periods were planned as whole body periodised programs with the main focus on knee extensor muscles. Half squats (~90° angle of the knee joints), loaded squat jumps, leg presses and knee extensions were the primary exercises. For details of the preparatory training period and time-of-day-specific training period see Table 3. 1 RM values for all exercises, except for half-squats, were obtained during the first training sessions in training weeks 1, 11, and 16, according to McDonagh and Davies (1984). All training sessions were supervised. Subjects assigned to the control group did not train, but were instructed to maintain their pre-experimental physical activity.

TABLE 3 Basic variables of the preparatory and time-of-day-specific training. * load expressed as percentage of individual one repetition maximum measured at training weeks 1, 11, and 16. # minimum the last set, maximum last two consecutive sets performed until concentric failure.

Preparatory training	1st - 4th week	Percentage of total volume	5th - 10th week	Percentage of total volume
sessions per week	2		2, 3, 2, 3, 2	
High-load protocol*	40-70%	100 %	60-90%	91 %
sets/reps	2-4 / 6-15		2-5 / 3-12	
Hypertrophy*	.....	.....	.....	.....
sets/reps				
Explosive*	.....	.....	25-60%	9 %
sets/reps			1 / 6-10	
Time-of-day-specific training	11th – 15th week	Percentage of total volume	16th – 20th week	Percentage of total volume
sessions per week	2, 3, 3, 3, 2		3, 3, 3, 3, 2	
High-load protocol*	70-85%	36 %	80-100%	38 %
sets/reps	2-4 / 3-8		2-4 / 1-5	
Hypertrophy*	60-70%	49 %	60-80%	40 %
sets/reps	2-3 / 8-15#		3-4 / 8-12	
Explosive*	40-55%	15 %	50-60%	22 %
sets/reps	2 / 7-8		1-3 / 5-8	

4.3 Experimental protocol

This study was a part of a larger project and the subjects were evaluated with several other physical tests which are not included in this report. Nevertheless, the additional testing should not have had any adverse effect on the present results. The reasons for this are, first, the tests were equal for all subjects and second, they were performed either on the separate test occasions (VO_2max cycling test) at the end of the test protocols used here (5x10 repetitions fatigue protocol) or put relatively low physical strain on the subjects (loaded squat jump with 30% 1 RM).

4.3.1 Familiarization session (Pre-test)

The Pre-test was also used as a procedure to familiarise the subjects with the measurement apparatus the week preceding the experiment. After the initial adjustment of the testing devices and several submaximal trials, the subjects were tested for maximal isometric strength (MVC) during knee extension and flexion (knee angle 120°) as well as jumping height during counter-movement

jumps. Subjects were also familiarized with the half-squat technique on the squatting apparatus (Kraftwerk, Finland).

4.3.2 Actual test sessions

All subjects were tested in two different test designs to address both general training adaptations and those specific to time of day. Additionally, magnetic resonance imaging was taken on two separate occasions. The chronology of the entire experiment is presented in Figure 3.

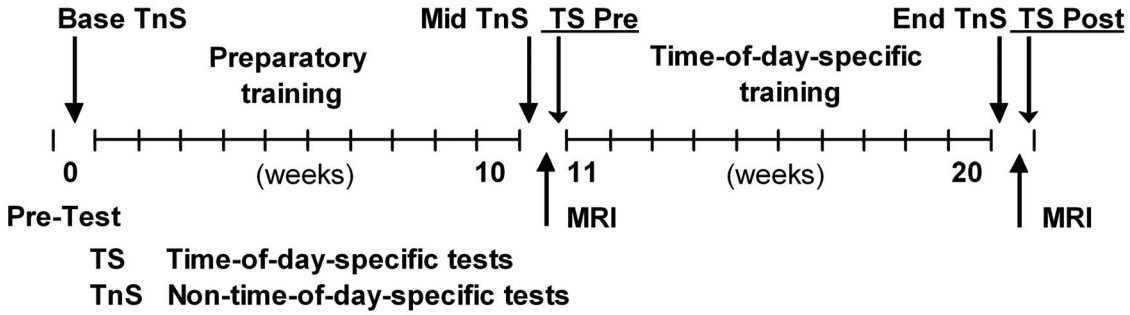

FIGURE 3 Chronology of the experiment. A distance between two consecutive vertical bars is equal to one week. Weeks connected with horizontal line are the training weeks, weeks with no fill represents the test weeks. MRI – magnetic resonance imaging.

Non-time-of-day-specific design (TnS): The tests were administered at three time-points – baseline (week 0), midline (between training week 10 and 11), and endline (after training week 20) (Base, Mid, and End, respectively) – starting at a randomly given clock time (between 09:00 – 16:00 h). These times were identical for each individual but differed between the subjects. For the purpose of this report, unilateral isometric knee extension peak torque of the right leg at the knee angle of 120° (MVC), and 1 RM half-squat were selected from the variables measured.

Time-of-day-specific design (TS): The time-of-day-specific design was used on two separate occasions, before (between training week 10 and 11) and after the time-of-day specific training period (after training week 20) (Pre and Post, respectively). Both Pre and Post tests consisted of the same set of strength measurements performed repeatedly at four different time points throughout two consecutive days (Day 1 & Day 2). The morning of Day 1 was the first session for all participants. The time points were as follows: morning (07:00-08:00 h), noon (12:00-13:00 h), afternoon (17:00-18:00 h), and evening (20:30-21:30 h), (Figure 4).

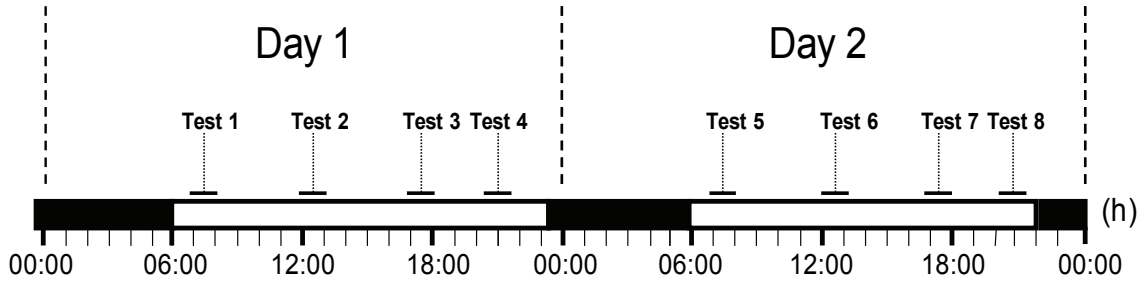

FIGURE 4 Two-day laboratory protocol. Testing sessions and their placement throughout days are labelled with numbers meaning the order as they followed. One unit separated by two vertical lines on the timeline is equal to one hour, values 00:00 represent the midnight of the local time. Sleep episodes are shown as bars with black filling.

The subjects were scheduled to report to the laboratory in fixed 15 minutes intervals (first one at 06:45 h, second at 07:00 h etc.) so four subjects could be measured within an hour. Each test session lasted 30 minutes per subject and consisted of 15 minutes of resting quietly in a supine position. Within the last 5 minutes of supine resting, blood sampling and oral temperature sampling was performed by a laboratory technician. Blood samples were drawn at each session from the antecubital vein using a needle and syringe for the determination of serum total testosterone and cortisol concentrations. Sublingual oral temperature was collected by digital thermometer (Microlife, Heerbrugg, Switzerland). Two trials were required; if the values differed more than 0.1 °C, additional trials were taken until two successive values with the difference of 0.1 °C or less were obtained. The highest value of all trials within a session was taken for further statistical analysis. The following 15 minutes included warm up and the actual test procedure. The tests consisted of recording mechanical power output during the concentric phase of the squat jumps with the extra load of 60% of 1 RM - (SJ60) and maximal isometric knee extension (MVC) force of the right leg at the knee angle of 120°. EMG activity from the vastus lateralis (VL), rectus femoris (RF), vastus medialis (VM) and biceps femoris (BF) muscles was recorded during MVC. A three-minute rest period was allowed between the tests. The subjects were allowed to leave the laboratory between the test sessions and requested to closely adhere to their habitual daily routine.

4.3.3 Methodological adjustments

Several adjustments were applied to assure better control over possible confounding factors. For both test designs, subjects were requested to refrain from alcohol and sexual and strenuous physical activity one day prior to, and throughout, the entire testing procedure. In the case of the training groups, the last training session was separated by at least 48 h from the subsequent test session. Before each session, all subjects performed a standardised warm-up consisting of ten technically performed half-squat repetitions with the 25 kg load, one minute stretching exercise for knee extensors, flexors and back

extensors in total and two all-out repetitions of unloaded squats and countermovement jumps. In addition, 10-min light cycling on the ergometer (~110 bpm hear rate) was included in the non-time-of-day-specific design.

Caffeine

We also encouraged the subjects to avoid caffeine in any form. However, strong habitual coffee consumers detected by the questionnaire were asked not to completely refrain, but rather to minimize their daily dose of caffeine drinks, and not to ingest them within 3 hours prior to a measurement session. The reasoning for this was to avoid the "caffeine withdrawal syndrome" which may have a negative impact on vigilance and physical performance during the testing (Lane and Phillips-Bute 1998).

Sleep and stress

The subjects spent the nights before and during the experiment at their homes. They were instructed to adhere as closely as possible to their usual length and time of sleep. During the time-of-day-specific testing, information on actual sleep length and stress levels was collected by a questionnaire on both test days. The subjects were asked to record a rating of perceived stress-load on a scale from level 1 (a stress-free day), to level 6 (maximum stress). Also, a sleep length shorter than 75% of the typical value for the individual (e.g., shorter than 6h in case of typical 8h sleep length) was recorded as a possible confounding influence. Data from the subjects with high stress load (from level 4 – rather heavy stress – to level 6) and sleep durations that were too short on either of the test days were checked and considered for exclusion.

Diet

Diet was controlled for during time-of-day-specific testing. The diet during the two testing days was similar for all subjects and designed according to the Finnish Nutrition Recommendations (National Nutrition Council 1999). The overall daily energy intake goal was set at 10.5 MJ (2500 kcal) per day. All meals - breakfast, lunch, dinner and light supper - were served within 10 minutes after every measurement session was completed. The amount of food was identical for all subjects; however, they were allowed to consume more or less of basic foods (e.g. bread, pasta, rice and potatoes) and milk or fruit juice, within a limited range, to match their individual dietary needs. Water and non-caffeinated beverages could be consumed ad libitum at meals and between meals. Since the subjects came after an overnight fast, approximately 250 ml of orange or apple juice was ingested 5 min before all morning test sessions, to decrease the possible risk of relative or acute hypoglycaemia during the measurements.

4.3.4 Data collection

4.3.4.1 One repetition maximum half-squats

Maximal dynamic strength of the lower limbs was measured using 1 RM half-squat test. The protocol consisted of several submaximal trials of half-squat separated by 3 minutes rest periods. The starting load was estimated to be approximately 75% of 1 RM and was progressively increased by 5 to 10% at each trial. A barbell with weights, inbuilt in a Smith machine (Kraftwerk, Finland), was held on the shoulders. A trial started from the standing position, squatting slowly down to the 90° knee angle (announced by an audio cue) followed by extension of the knees and hips back to the standing position. The last successful trial performed with the correct technique was taken for further analyses.

4.3.4.2 Loaded squat jumps

Maximal dynamic explosive strength of the lower limbs was measured using the loaded barbell squat jump with 60% of 1 RM (SJ60). The 1 RM values were obtained 3 to 5 days prior to the experiment. The protocol consisted of three trials separated by a one-minute resting period. A barbell with weights, inbuilt in a Smith machine, was held on the shoulders. A sliding sensor arm based on infrared optics (ErgoPower, Ergotest Technology A.S., Langesund, Norway) was placed on the end of the bar, and mean power during the concentric phase of the jump was calculated instantly. The subjects started the trial from the standing position, squatting slowly down to the 90° knee angle which was announced by a sound signal. After reaching a stable squat position with no signs of obvious movement in any part of the subject's body, an assistant gave a loud verbal command to jump. The subjects were requested to move the load as fast and as high as possible. The trial with the highest mean power output in the concentric phase of the movement performed with the correct technique was taken for further analyses. In this variable, only data from sixteen subjects could be utilized for statistical analysis due to a technical error affecting data obtained from the second half of the participants.

4.3.4.3 Maximum voluntary contractions

Maximum voluntary isometric force of the knee extensors was tested using unilateral knee extensions of the right leg at 120° knee angle (180° knee fully flexed). The right leg was selected for the measurements exclusively in order to provide identical testing conditions. Laterality of the lower limb was not expected to have a significant impact on diurnal patterns of their performance, as has been recently shown by (Guette et al. 2005a). The subjects were secured in a sitting position in a knee extension device (Leg Ext/Curl Research, Hur Oy, Kokkola, Finland). The torso was fixed with two horizontal safety belts in the chest and waist area, and the upper extremities were placed next to the body-

holding handgrips. Both thighs were strapped in a position with cushioned straps placed about the knee joint. Similar inter-individual positions of body segments were ensured by the adjustable back support and lever arm. A force transducer was attached to the lever arm and placed at ankle level. The subjects were asked to produce maximal force rapidly and to maintain it for three seconds. Force produced on a transducer attached to the lever arm was amplified, and the analogue signal was collected by a biomonitor ME6000T8 (MEGA Electronics Ltd, Kuopio, Finland). The signal was telemetrically transmitted and stored in the MegaWin software. The sampling frequency was 1000 Hz. Two to three trials (if the second trial was more than 5% higher than the first one) were performed with a rest period of 1 minute. The trial with the highest peak force was saved for further analyses.

4.3.4.4 Electromyography – EMG

In the time-of-day-specific design, surface EMG from VL, RF, VM, and BF of the right leg was recorded during MVC actions. The SENIAM recommendations (Hermens et al. 1999) were followed when placing silver/silver chloride pre-gelled electrode pairs with a sensor diameter of 13 mm and reference electrodes (Blue Sensor M, AMBU, Ballerup, Denmark). The location of the electrodes was marked prior to the first test session with intradermal ink dots to ensure reliable positioning over the sessions. On time-of-day-specific test days, the same pairs of electrodes were kept in place during the entire testing day. Regarding the EMG signal detection characteristics, the common mode rejection ratio was >110 dB, gain was 305 and input resistance was <10 kΩ. The EMG signal, amplified by a factor 1000, was recorded and processed in the identical way to the MVC force mentioned above.

4.3.4.5 Magnetic resonance imaging

Twenty four volunteers were chosen randomly from the original groups for this part of the study: the control group (n=8) and two training groups (morning and afternoon group, n=9 and n=7, respectively). The entire length of quadriceps femoris (QF) was scanned by MRI at the end of week 10 and week 20 (different days when compared to the strength tests). Typical morphological images were acquired in the axial planes by using the proton density fast spin echo (FSE) sequence. The axial image slice spacing was 8.5 mm. From the images, the thigh length was measured from the lateral epicondyle of femur to the lateral tip of the greater trochanter.

4.4 Data analysis

4.4.1 Muscle strength and EMG activity

In MVC, the force signal and EMG activity were analysed by SPIKE 2 software (Cambridge Electronic Design Ltd, Cambridge, England). The force signal was low-pass filtered and peak torque (MVC) was calculated. Raw EMG signal was rectified, band-pass filtered (10 – 500 Hz) and expressed as root mean square (RMS) of the ±100 ms time window around the peak torque during MVC. Subsequently, RMS of VL, RF and VM during MVC were averaged in order to provide overall activity of surface knee extensor muscles - KE RMS, according to Guette et al. (2005a). Antagonist muscle co-activation was obtained by the RMS of BF when it acted as an antagonist (MVC) divided by the RMS of BF during isometric unilateral knee flexion at the knee angle 120° (MVC flex). MVC flexion had been measured 14-10 days prior to the testing. Peak torque, power output and KE RMS of MVC were normalised with respect to the highest value found in each individual and expressed as percentage of this maximum value. The RMS to torque ratio (EMG/torque) was calculated from the normalised MVC (Guette et al. 2005a).

4.4.2 Blood analyses

Blood samples were centrifuged (3500 rpm and 4°C for 10 min) and frozen at -80 °C until assayed. Total testosterone and cortisol serum concentrations were measured by automated chemiluminescent immunoassays using the IMMULITE 1000 system (Diagnostic Products Corporation, Los Angeles, USA). The sensitivity of the assay was 0.5 nmol/L and 5.5 nmol/L for testosterone and cortisol, respectively. The intra-assay coefficient of variation was 5.8% and 4.8%, respectively.

4.4.3 Magnetic resonance imaging

The cross-sectional areas (CSA) of RF and the vasti muscle group were analyzed from the FSE images (Osiris 4.0 software). Because the thigh length varied between the subjects, the results were normalized to 15 samples for the vasti muscle group and 12 for rectus femoris. The reproducibility of drawing the areas was high with a correlation of 0.99 and mean error of 0.36 ± 0.26% when analyzed by the same person. Muscle volume (cm^3) was calculated by multiplying the CSA of each axial slice by the distance between slices, and summed across slices. The statistical analyses yielded similar results for both the RF and vasti muscle group. Therefore, in order to simplify the MRI data presentation, the cross-sectional areas and volume of both RF and vasti muscles were merged (except for the analyses of site-specific hypertrophy) and are presented as quadriceps femoris (QF) CSA and volume. The site-specific hypertrophy was examined by calculating the percentage difference in

respective CSA before and after the time-of-day-specific training separately for RF and the vasti muscle group. Percentage differences in CSA were compared along the examined muscles.

4.5 Statistical analysis

Statistical significance was set at $p<0.05$. Standard descriptive statistics (mean ± standard deviation (SD)) were calculated.

To evaluate diurnal variation in SJ60, MVC, EMG, EMG/torque ratio and oral temperature across two consecutive days, a 2-factor (time of day – TOD, and DAY) general linear model (GLM) with repeated measures was performed across the eight selected time points. A GROUP was included as a between-subject factor in both models. When repeated measures GLM revealed significant F-ratios, pair-wise comparisons with Ryan-Holm-Bonferroni adjustment were employed to localize significant differences (Atkinson 2002). The effects of the preparatory and time-of-day-specific training on non-time-of-day specific MVC and 1 RM were examined by one factor (TRAINING) GLM with repeated measures. Also, a GROUP was included as a between-subject factor and the paired-samples t-test was applied to examine changes within the groups in the non-time-of-day specific MVC and 1RM. The effect of time-of-day-specific training on diurnal variation in MVC, SJ60, EMG, EMG/torque and oral temperature was tested by two factor (TOD and Pre-to-Post) GLM with repeated measures. A GROUP was included as a between-subject factor in both models. In addition, the two-factor GLM repeated measures model was applied separately for each group and the paired-samples t-test was applied to examine Pre-to-Post changes within the groups in the time-of-day-specific MVC. When GLM revealed significant F-ratios, the pair-wise comparisons with Ryan-Holm-Bonferroni adjustment were used similarly to the cross-sectional analyses. Both absolute and normalized values of 1 RM, MVC, and SJ60 were analysed.

All hormonal data at each clock test time were analyzed for normality using the Shapiro-Wilk test. Since only the data of 8 time-points for total testosterone and 10 time-points of cortisol out of the total of 16 (Pre-to-Post, Day 1-to-Day 2) showed some violation of normality, no transformation of the data was done to reduce the skew. A three factor (TOD, DAY and Pre-to-Post) GLM with repeated measures, with the GROUP as a between-subject factor, was used for hormonal and oral temperature data.

The effect of the time-of-day-specific training on quadriceps femoris volume was examined with a one-factor (Pre-to-Post) GLM with repeated measures and GROUP as a between-subject factor using absolute values. Site-specific hypertrophy was studied by a one-factor (cross-sectional areas, CSA) GLM with both simple and repeated contrasts. In addition, one-way ANOVA was used to test the differences between the groups in relative percentage changes from week 10 to week 20 in muscle volume and CSA. Post hoc tests

with the Bonferroni adjustment were performed when ANOVA yielded significant F-ratios. Pearson's bivariate correlation coefficients were calculated between some muscle strength and muscle hypertrophy variables for each group separately as well as for merged groups. Hierarchical clustering was applied in trying to identify possible chronotype, sleep deprivation, or high psychological stress subgroups in diurnal patterns of selected variables. All the analyses were performed by means of SPSS 14.0. (SPSS Inc, Chicago, IL, USA).

4.6 Muscle tone study – methods

Seventeen untrained male volunteers (mean ± SD, age 27 ± 3 years, height 179 ± 6 cm, body mass 82.7 ± 10.7 kg) were divided into four groups and measured at 08:00, 12:00, 16:00 and 20:00 h in a counter-balanced design. The morning group started at 08:00 h and performed all four tests on the same day. The noon, afternoon and evening groups started at 12:00, 16:00 and 20:00 h, respectively, on the first test day and completed the remaining test(s) the following day. For the night prior to, and between, the test days, all subjects were instructed to go to bed at approximately 23:00 h and wake up at 06:00 h and to have a light breakfast within 15 minutes. Other meals were scheduled an hour prior to the tests. Each measurement session started with 15 min supine rest with eyes open. During the last 5 minutes, muscle tone of VL and BF muscles was measured using a computerized muscle tonometer (Medirehabook Ltd, Muurame, Finland). The thicknesses of skin and subcutaneous fat tissue, including the vastus lateralis and biceps femoris muscles, were measured by ultrasound (Aloka Ltd, Japan) after the muscle tone assessment. Total (mJ) and relative (mJ/cm) work done by the probe was calculated by the software from the distance travelled by the probe when pressing the tissues until 10 N pressure force was reached and from tissue thickness. Subsequently, a brief stretching and warm-up was performed followed by 3 to 4 trials (2 min recovery) of three test actions (3 min recovery in between) in the following order: maximum isometric bilateral leg press (MVC, knee angle of 107^{o}, peak force measured), maximum dynamic bilateral leg press (load of 50% of 1RM leg press, DAVID-210 dynamometer, David Fitness and Medical Ltd., Vantaa, Finland, mean power output measured) and unilateral knee flexion MVC (at knee angle of 107^{o}). Bipolar surface EMG (Telemyo 2400, Noraxon Inc, Arizona, USA) of VL, VM and BF muscles was measured and root mean square calculated during the isometric MVC leg press actions, in line with the above-mentioned methodology. During unilateral knee flexion MVC, only EMG of BF was recorded and used to calculate relative co-activation of BF during leg press MVC. The highest peak force and mean power output from a session was taken for further analyses. A familiarization session and 1RM test for dynamic leg press took place one week prior to the actual test day. For statistical analyses, the data were organized in two ways: according to the testing clock time

starting with the morning session (TOD main effect) or according to the session order regardless of testing clock time (TestOrder main effect). A one-factor GLM repeated measures model with a GROUP between-subject factor was used for muscle tone, normalised relative strength, power output and EMG data. A paired-samples t-test was used to compare force and power mean values achieved at 08:00 h versus the highest individual force and power, respectively, from all four test sessions. Bivariate correlations were calculated for MVC force and EMG values.

5 RESULTS

5.1 Daily variation in maximum isometric and explosive strength, EMG and oral temperature across two consecutive days

For this part of the results, data measured after the preparatory training (during the TS Pre test) were used. Since there were no statistically significant differences between the two training groups after the intervention in the two tests used during the experiment (isometric knee extension and loaded jump with 60% of one repetition maximum), both training groups were merged for the purpose of this result section (preparatory training group).

The control group was not included in the statistical analyses of MVC and EMG due to the low sample size (n=4). However, absolute values of the control group MVC are presented in Table 4.

TABLE 4 Absolute values of muscle strength, power and oral temperature at the Pre test in the merged training group and the control group

		Variables (mean±SD)					
		Peak torque MVC (Nm, n=32)		Power output SJ60 (W, n=16)		Oral temperature (°C, n=32)	
	Time of Day	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
PREPARATORY	7:00	264.9 ± 41.9	257.4 ± 43.2	637.7 ± 124.2	649.4 ± 128.4	36.15 ± 0.3§	36.05 ± 0.3*
TRAINING	12:00	270.9 ± 40.3	276.8 ± 44.0	673.7 ± 145.7	681.3 ± 126.2	36.33 ± 0.3#	36.34 ± 0.4#
GROUP	17:00	271.3 ± 45.6	279.9 ± 46.2	667.1 ± 132.9	674.5 ± 135.0	36.33 ± 0.3&	36.43 ± 0.3&
	20:30	271.1 ± 41.9	274.0 ± 47.8	668.2 ± 120.5	676.9 ± 115.4	36.49 ± 0.2+	36.56 ± 0.3
	Time of Day	Peak torque MVC (Nm, n=4)		Power output SJ60 (W, n=11)		Oral temperature (°C, n=11)	
	7:00	242.7 ± 38.1	216.2 ± 30.0	540.1 ± 89.2	546.7 ± 92.7	36.26 ± 0.5	36.04 ± 0.3
CONTROL	12:00	235.4 ± 26.4	228.6 ± 38.2	586.7 ± 101.1	593.8 ± 87.6	36.40 ± 0.4	36.28 ± 0.3
GROUP	17:00	227.8 ± 52.9	237.0 ± 31.6	584.0 ± 104.7	597.3 ± 106.3	36.23 ± 0.3	36.37 ± 0.2
	20:30	227.1 ± 38.7	233.5 ± 43.1	583.4 ± 108.1	599.7 ± 115.2	36.32 ± 0.3	36.31 ± 0.3

5.1.1 Maximum voluntary contractions – MVC

When analysed from the normalized relative values, the peak torque displayed a significant main effect of TOD ($p<0.001$) and interaction of TOD and DAY ($p<0.01$). Post-hoc comparisons revealed that significant diurnal variation was present on Day 2 only with 07:00h values significantly lower when compared to the rest of day ($p<0.001$) (Figure 5).

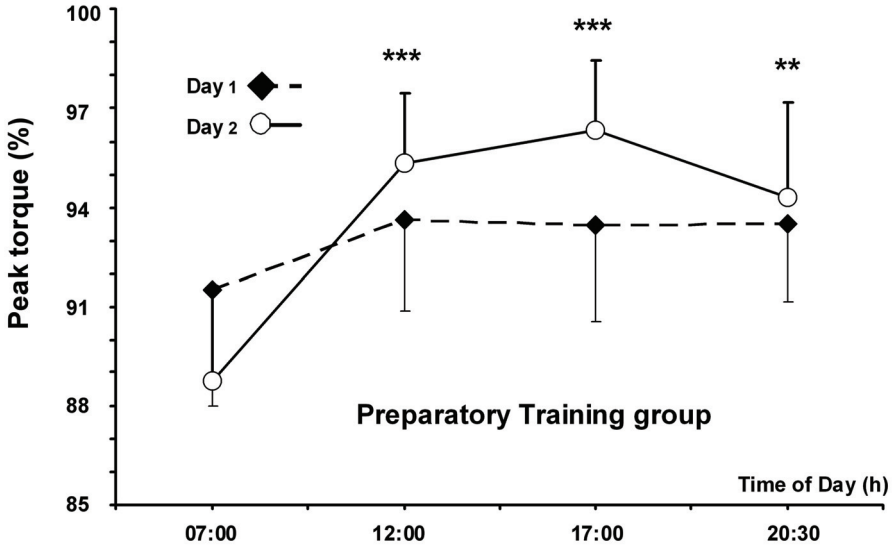

FIGURE 5 Diurnal variation in MVC peak torque of knee extensor muscles (mean and SD) measured throughout two consecutive days in the preparatory training group (n = 32). Mean values expressed as a percentage of the maximum torque of each subject obtained during the two days, SD presented as half of the range. Statistical significance of pair-wise comparisons is shown for both days separately. ** $p < 0.01$, *** $p < 0.001$, relative mean peak torque values significantly higher than at 07:00 h.

5.1.2 EMG activity

KE RMS of the knee extensor during MVC did not significantly alter between time points. Regarding the day-to-day repeatability of diurnal patterns, Day 1 mean KE RMS did not statistically differ from Day 2 (DAY main effect $p=0.089$). No significant main effects or interactions were found in BF co-activation during MVC knee extensions.

5.1.3 EMG/torque ratio

As for the neuromuscular efficiency estimation, TOD main effect was not significant in KE RMS_{norm}/peak $torque_{norm}$ ratio during MVC. In contrast, significant main effect of DAY was present ($p<0.05$), with KE RMS_{norm}/peak $torque_{norm}$ ratio being lower on Day 2.

5.1.4 Loaded squat jumps

A significant TOD main effect in normalized mechanical power output was found in a 2-factor GLM (TOD, $p<0.001$) (Figure 6). No significant main effect of DAY or interactions (TOD x DAY and TOD x DAY x GROUP) were present in this variable.

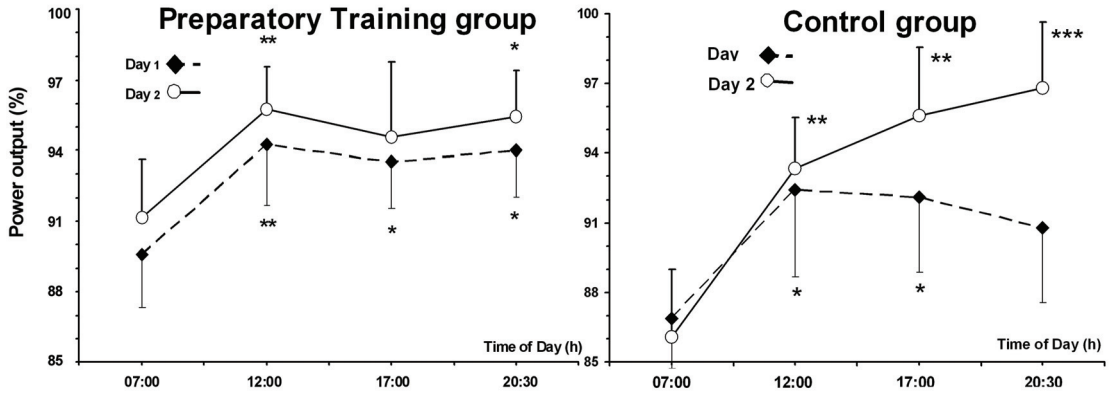

FIGURE 6 Diurnal variation in power output during the concentric phase of loaded squat jump with 60% of one repetition maximum (mean and SD) measured across two consecutive days in the preparatory training (n = 16) and control groups (n=11). Mean values expressed as a percentage of the maximum power output of each subject obtained during the two days, SD presented as half of the range. Statistical significance of pair-wise comparisons is shown for both days separately. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ relative mean power output values significantly higher than at 07:00 h.

5.1.5 Oral temperature

In the preparatory training group, oral temperature exhibited a significant diurnal pattern with the lowest values observed at 07:00 h session and the peaks were located at 20:30 h across both test days (TOD $p<0.001$). However, the 07:00 h temperature on Day 1 was somewhat higher compared to 07:00 h on Day 2 and lower at 17:00h and 20:30 h on Day 2 compared to Day 1. This trend was even more pronounced in the control group. The control group showed significant diurnal variation only on Day 2 ($p<0.01$). On Day 1 oral temperature of the control group at 07:00 and 12:00 h was elevated compared to the Day 2 or to the preparatory training group, resulting in significant TOD x GROUP interaction ($p<0.01$) and TOD x DAY interaction ($p<0.001$). In addition, the TOD x DAY interaction ($p<0.001$) was partly caused by the above mentioned day-to-day differences within the preparatory training group (Table 4).

5.2 Daily variation in muscle tone, maximum isometric and explosive strength and EMG of knee extensors – counter-balanced starting time of testing

For this separate cross-sectional muscle-tone study, only the results of statistical analyses and selected absolute values are presented. For the absolute values of all parameters measured, see Article V. When the data of the four test groups were merged (n=17) and analyzed by GLM repeated measures, both total and relative work during muscle tone measurements were relatively stable and did not significantly differ between the time of day or due to the test order. Isometric force during MVC did not quite achieve a conventional level of statistical significance (TOD main effect, $p=0.081$) and there was no significant difference between the groups, despite differences in diurnal profiles between the groups (Figure 7). However, peak force was significantly higher during the first session compared to the third and fourth session (TestOrder main effect, $p<0.05$) with no between-group differences.

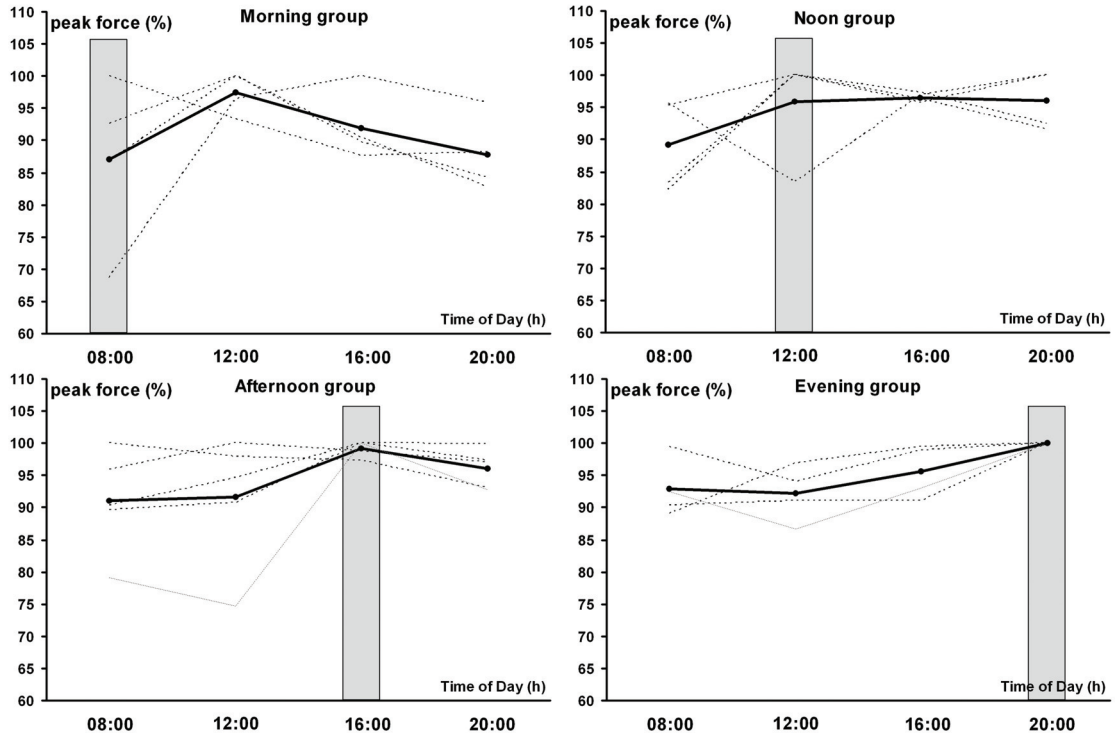

FIGURE 7 Diurnal variation in relative peak force during MVC in four groups with counter-balanced start of testing protocol. Starting time is marked with a grey bar. Thick line represents mean of the group, thin lines represent individual diurnal profiles.

The relative EMGs of VL (Figure 8) and VL+VM were significantly lower at 08:00 h compared to 20:00 h (TOD main effect $p<0.05$). There were also significant between-group differences (between-subject factor GROUP, $p<0.05$). Regarding the test order, relative EMG of VM, but not VL, decreased significantly from session to session (TestOrder main effect, $p<0.001$) with no between-group differences.

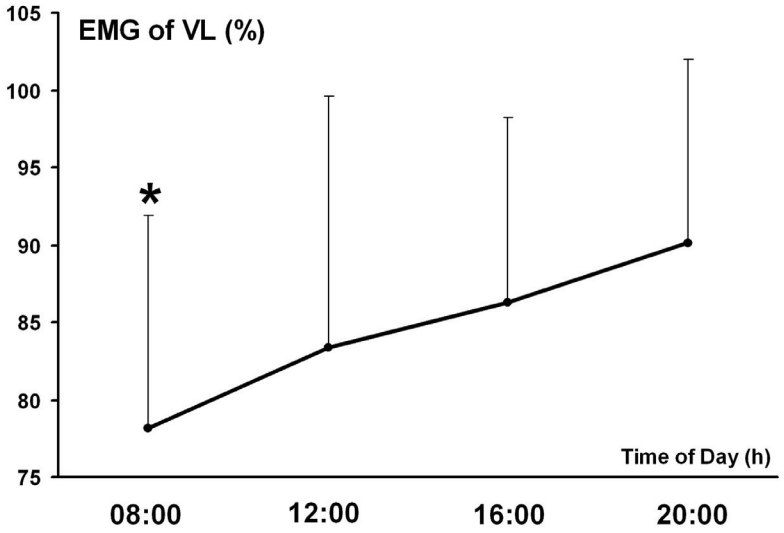

FIGURE 8 Relative EMG of vastus lateralis of the four groups with counter-balanced starting time of testing (mean ± SD). *, 08:00 h values significantly lower than 20:00 h values, $p<0.05$.

In addition, a significant Pearson correlation between relative MVC and relative EMG of VL was observed ($p<0.001$, $r = 0.42$) with the groups´ diurnal patterns similar to the MVC diurnal patterns (Figure 7 and Figure 9).

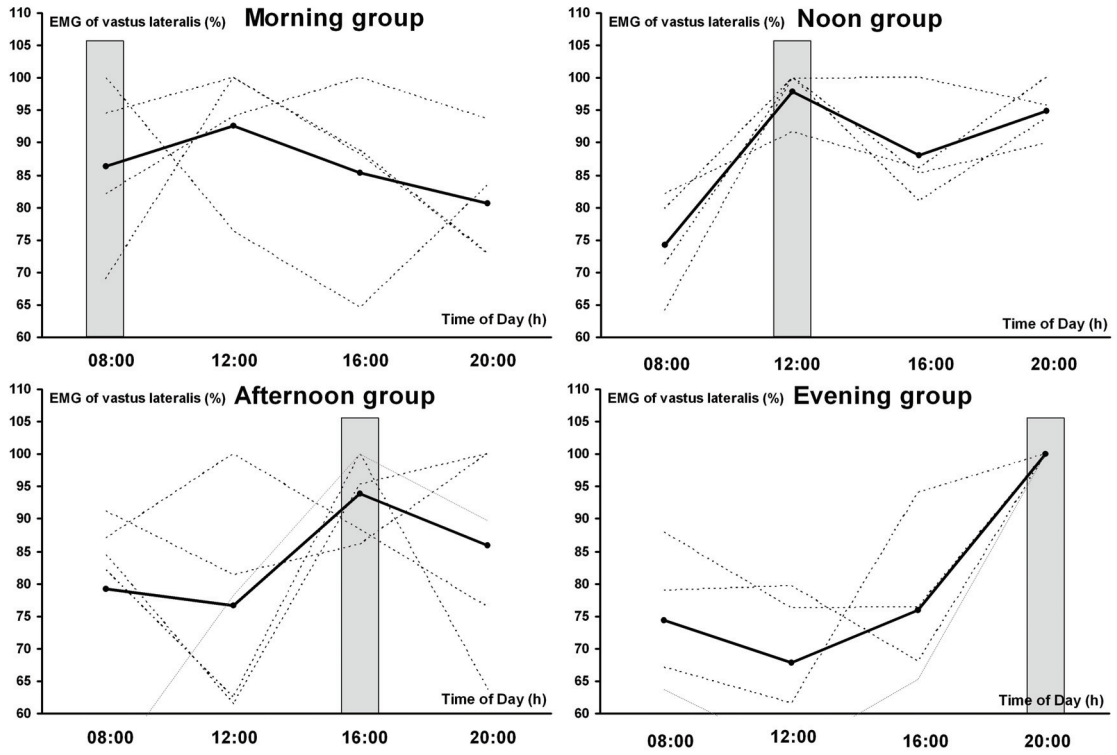

FIGURE 9 Diurnal variation in relative EMG of vastus lateralis during MVC in four groups with counter-balanced start of testing protocol. Starting time is marked with a grey bar. Thick line represents mean of the group, thin lines represent individual diurnal profiles.

Co-activation of BF remained statistically unaltered across the day ranging from 20% (noon) to 23% (evening).

Similar to MVC, a non-significant trend for the lowest performance at 08:00 h was observed in power output during the explosive leg press (TOD main effect, $p=0.098$). Apart from MVC peak force, the group differences in power output were statistically significant in the GLM repeated measures (between-subject factor GROUP, $p<0.05$). When examining for the effect of test order, power output was significantly lower during the first session compared to the third and fourth sessions (TestOrder main effect, $p<0.05$), with no between-group differences (Figure 10).

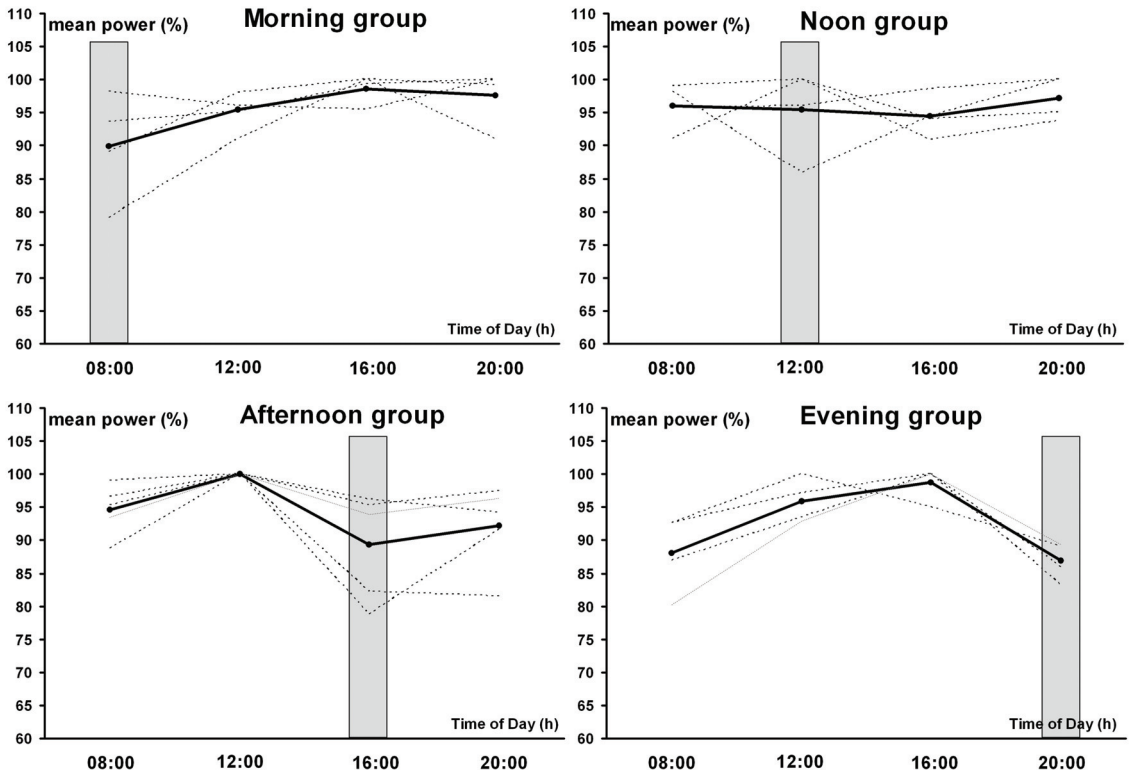

FIGURE 10 Diurnal variation in relative mean power output during explosive leg press in four groups with a counter-balanced start of the testing protocol. Starting time is marked with a grey bar. Thick line represents mean of the group, thin lines represent individual diurnal profiles.

When tested by paired t-tests, significant differences ($p<0.001$) were observed when 08:00 h absolute isometric peak force and mean power output were compared with the respective individual highest absolute peak force (3439 ± 602 N vs. 3774 ± 657 N) and power output (2293 ± 299 W vs. 2490 ± 338 W) found from all the four tested clock times (Figure 11).

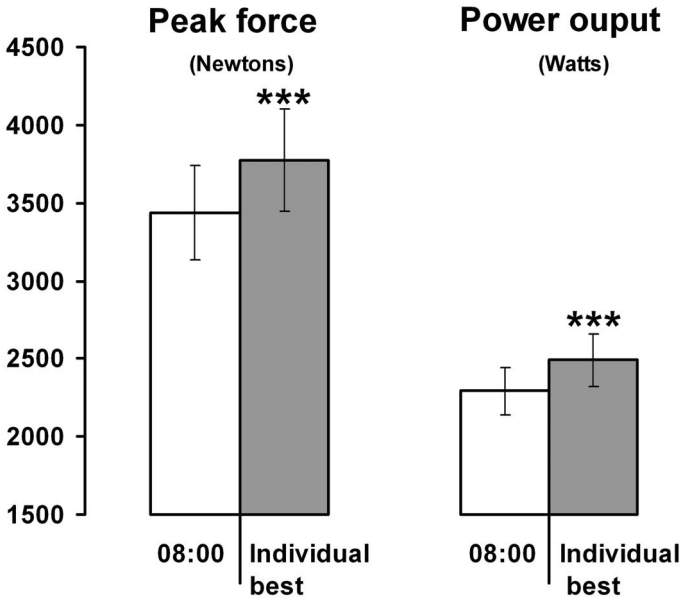

FIGURE 11 Absolute values of peak force and power output from all subject (mean ± SD) reached at 08:00 h (white column) and the respective individual highest values reached at any test time (individual best – grey column). *** significantly higher as compared to 08:00 h values, $p<0.001$.

5.3 Training adaptation to preparatory and time-of-day-specific resistance training

5.3.1 Non-time-of-day-specific design

5.3.1.1 Strength performance

Performance at 1 RM half-squat increased significantly in all three groups during the first 10 weeks of the preparatory training ($p<0.01$, TRAINING main effect). However, the increase was more pronounced in the morning and afternoon training groups than in the control group. The 1RM increase was 28% in the morning group and 27% in the afternoon group when expressed as percentage difference between the Base and Mid tests. Strength gains were significantly higher ($p<0.05$, between-subject factor GROUP) compared to 10% gain in the control group. A similar trend was observed for peak torque – 10%, 9%, and less than 1% in the morning, afternoon, and control groups, respectively.

The second 10-week period of time-of-day-specific training did not result in significant improvements of non-time-of-day-specific strength when tested by GLM repeated measures. The 1 RM half-squat increased by approximately 7% ($p<0.05$, paired-samples t-test), 6% (n.s.), and 3% (n.s.) in the morning, afternoon, and control groups, respectively. Peak torque changed by 1%, 7%, and 3%, respectively (all n.s.). The absolute values and the respective statistics are shown in Table 5.

TABLE 5 Absolute performance values measured in both non-time-of-day-specific (Baseline, Midline, Endline) and time-of-day-specific (Pre at 07:00 and 17:00 h, Post at 07:00 and 17:00 h) designs. All tests are presented in chronological order. Statistical significance marked if p<.05. *, significantly higher than Baseline; §, significantly higher than Midline; ¤, significantly higher than 07:00; #, significantly higher than the same time point in Pre.

Test	Group	Baseline	Midline	Pre 07:00	Pre 17:00	Endline	Post 07:00	Post 17:00
1 RM half-squat	Morning (n=14)	141 ± 20	181 ± 25*	-	-	193 ± 29*§	-	-
(kg)	Afternoon (n=12)	127 ± 16	158 ± 13*	-	-	168 ± 16*	-	-
	Control (n=7)	140 ± 24	153 ± 24*	-	-	157 ± 21*	-	-
Peak torque MVC	Morning (n=14)	256 ± 57	274 ± 38	256 ± 31	282 ± 40¤	276 ± 51	287 ± 35#	295 ± 55
(N·m)	Afternoon (n=12)	248 ± 21	270 ± 29	259 ± 48	281 ± 47¤	288 ± 40*	283 ± 43#	308 ± 53¤#
	Control (n=7)	205 ± 50	205 ± 46	194 ± 46	221 ± 42¤	210 ± 41	215 ± 52	236 ± 39¤
Power output SJ60	Morning (n=14)	-	-	616 ± 95	618 ± 148	-	636 ± 98	643 ± 113
(W)	Afternoon (n=12)	-	-	665 ± 96	693 ± 118¤	-	688 ± 107	731 ± 117¤
	Control (n=7)	-	-	580 ± 131	613 ± 121¤	-	639 ± 118#	665 ± 156

5.3.2 Time-of-day-specific design

For the purposes of this thesis, only the 07:00 h and 17:00 h time points (training-specific) on Day 2 were selected for further analyses of MVC and, EMG during MVC and SJ60. There were two main reasons for this decision. The first was to present data of the specific training times during the day with typical and significant diurnal rhythms in peak torque. Data on Day 1 were more affected with the test design itself than those from Day 2 (for further details, see the results section: "Daily variation in selected neuromuscular parameters and oral temperature across two consecutive days" and manuscript III). The second reason was that the selection of the two time points increased sample size, particularly in the control group (from n=3 to n=7). For detailed information on MVC peak torque values measured across all time points at the Post-test, see manuscript II. The absolute values from MVC and SJ60 are shown in Table 5, and the statistically analysed relative values are presented below.

5.3.2.1 MVC - two-factor GLM repeated measures model with GROUP as a between-subject factor

Significant TOD and Pre-to-Post main effects were found in both absolute and relative peak torque values after performing repeated measures GLM ($P<0.001$). The interaction TOD x GROUP in the relative values reached $P=0.061$. Other interactions (Pre-to-Post x GROUP, TOD x Pre-to-Post, TOD x Pre-to-Post x GROUP) were not significant (Figure 12).

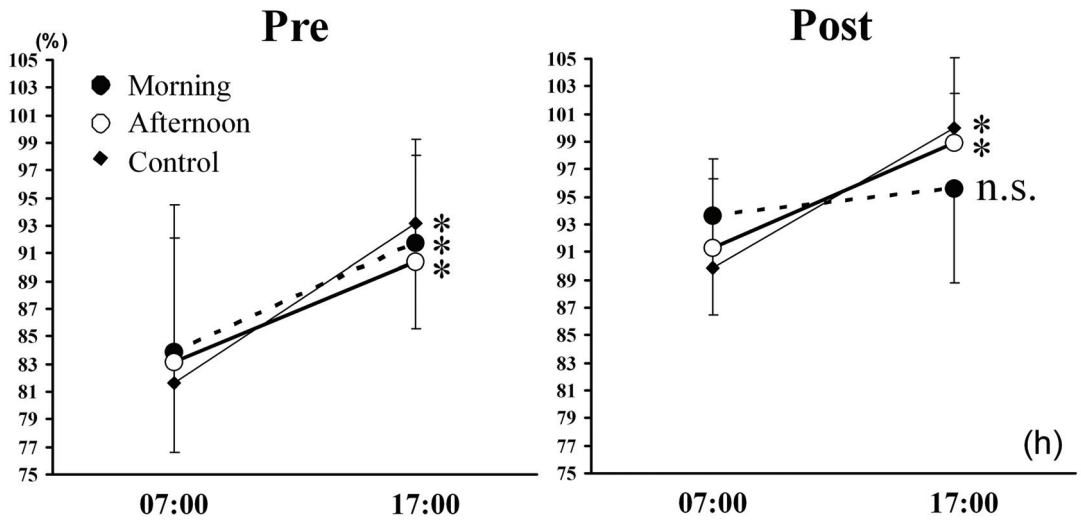

FIGURE 12 Normalized MVC peak torque at 07:00 h and 17:00 h before and after 10-week time-of-day-specific training. 100% = individuals' highest peak torque from all four time points. *, 07:00 h significantly lower than 17:00 h within the same day ($p<0.05$)

In order to better estimate the biological importance of the TOD x GROUP interaction found ($p=0.061$) in the two-factor GLM, further statistical analyses were conducted within each group separately to study this phenomenon.

5.3.2.2 MVC - Group-by-group two-factor GLM repeated measures model

When examining the groups separately, TOD and Pre-to-Post main effects were significant in all three groups ($p<0.001$ - control and afternoon group, $p<0.01$ – morning group and $p<0.05$ - all groups) (Table 5). Interaction of TOD x Pre-to-Post reached significance in the morning group only ($p<0.05$).

5.3.2.3 MVC - paired-samples t-tests

When comparing the percentage changes from Pre07:00 to Post07:00 against percentage changes from Pre17:00 to Post17:00, only the morning group showed significant differences (8%, $p<0.05$). In this group, the increase in peak torque was higher at 07:00 h (13%) than at 17:00 h (5%). The above-mentioned percentage differences were less than 1% ($p=0.882$) and 4% ($p=0.445$) in the afternoon and control groups, respectively. The afternoon group could exert 10% higher peak torques at both at 07:00 h and 17.00 h. The respective values were 12% and 8% in the control group (Figure 13). Similar statistical results ($p<0.05$ in the morning and n.s. in the afternoon and control groups) were obtained from comparisons of Pre07:00 to Pre17:00 percentage changes vs. Post07:00 to Post17:00 percentage changes by paired-samples t-tests.

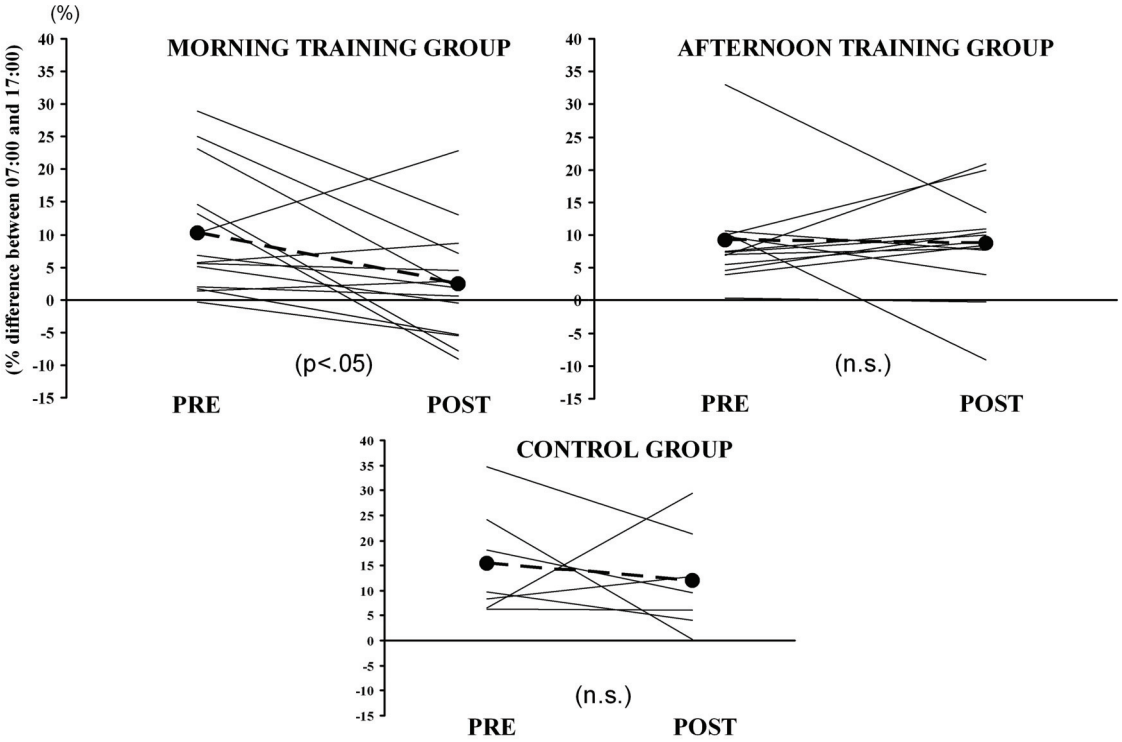

FIGURE 13 Peak torque in the morning and afternoon training groups and the control group expressed as the % difference between 07:00 h and 17:00 h before (PRE) and after (POST) 10 weeks of time-of-day-specific training. Thin lines represent individual values, mean values are in bold dash lines.

5.3.2.4 EMG activity

Both absolute and normalized EMG were higher after training in morning and afternoon training groups when compared to the controls (Pre-to-Post main effect, $p<0.001$, Figure 14). Percentage changes from Pre to Post were as follows: at 07:00 h by 20%, 14%, and -1%; at 17:00 h by 17%, 8%, and 3% in the morning, afternoon, and control groups, respectively. However, no significant main effects of TOD or interactions were found. The relative difference between 07:00 and 17:00 h was less than 2% for all groups in the Pre test. In the Post test, the respective values were -2% -5%, and 8% (n.s.) in the morning, afternoon, and control groups, respectively.

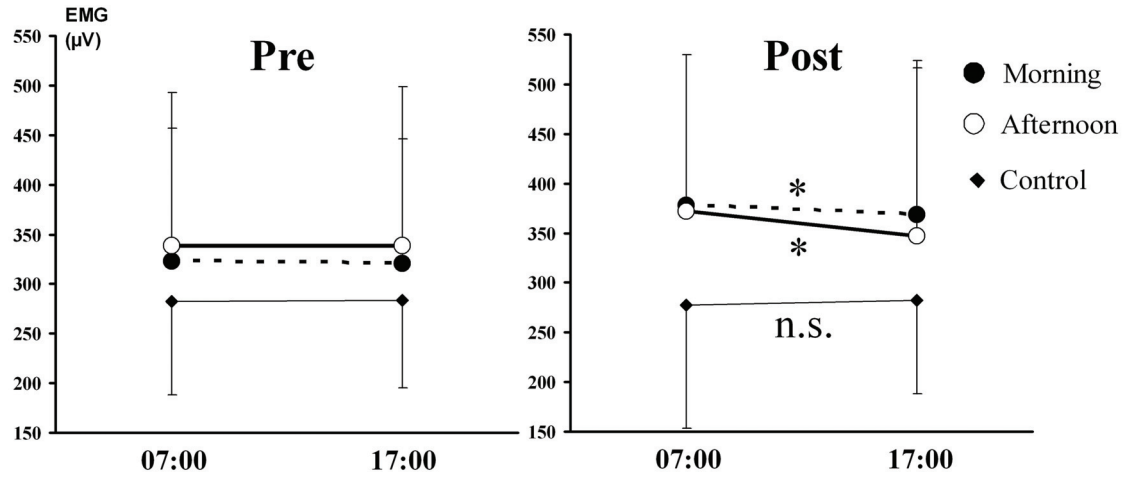

FIGURE 14 Absolute EMG (root mean square of knee extension muscles) at 07:00 h and 17:00 h before and after 10 weeks of time-of-day-specific training in the morning, afternoon training group and control group *, Post mean values significantly higher than Pre mean ($p<0.05$).

5.3.2.5 EMG/torque ratio

Significant TOD main effects were observed in the normalised EMG/peak torque ratios in all three groups and both Pre and Post ($p<0.001$), the ratios being higher at 07:00 than at 17:00 h. There was also a non-significant trend for the effect of training (Pre-to-Post main effect $p=0.603$).

A trend was observed also for an increase in BF co-activation from Pre to Post (8% vs. 12%, $p=0.058$). This could be attributed mainly to the training groups as the BF co-activation of the controls remained similar (8% vs. 9%). TOD main effects or interactions were not significant.

5.3.2.6 Loaded squat jumps

Power output was significantly improved from Pre to Post in all three groups, by 8%, 2% and 4% in the control, morning and afternoon groups, respectively (Pre-to-Post main effect, $p<0.05$). There was also a non-significant trend for the main effect of time of day ($p=0.054$). Between-group differences and interactions were well above p values of 0.05.

5.3.2.7 Hormonal concentrations

Serum total testosterone concentrations exhibited a significant diurnal variation (TOD main effect, $p<0.001$) during all testing days, with gradual decreases from 07:00 h until 20:30 h. Values were significantly different from each other with time of day (pairwise comparisons; $p<0.001$, Table 6). No significant main effects of DAY, Pre-to-Post, or interactions with GROUP were observed. However, a significant DAY x TOD interaction was located between 12:00 and 17:00 h ($p<0.05$) in the contrast test data.

TABLE 6 Absolute values of hormonal concentrations before (Pre) and after (Post) 10 weeks of time-of-day-specific training.

Pre test		Day 1				Day 2			
		7:00	12:00	17:00	20:30	7:00	12:00	17:00	20:30
	Control (n=11)	562±119	312±89	235±111	77±29	496±128	329±92	216±83	86±34
Cortisol	Morning (n=20)	601±122	372±137	300±153	150±115	554±125	359±132	281±100	143±102
(nmol/L)	Afternoon (n=18)	625±139	332±95	270±102	113±57	555±94	307±59	254±90	114±59
	Control (n=11)	19.3±5.8	17.5±4.6	16.2±3.9	12.1±3.9	19.3±5.9	17.2±4.6	15.3±4.4	13.3±3.8
Testosterone	Morning (n=20)	20.9±5.9	17.8±5.5	16.9±5.5	13.2±4.7	20.7±5.9	18.3±6.2	16.2±5.9	12.8±5.3
(nmol/L)	Afternoon (n=18)	19.8±6.1	16.5±3.8	14.9±4.7	12.1±2.9	18.6±5.8	15.7±4.3	14.7±4.7	11.7±4.2

Post test		Day 1				Day 2			
		7:00	12:00	17:00	20:30	7:00	12:00	17:00	20:30
	Control (n=11)	518±122	305±97	219±98	102±47	474±143	263±70	244±124	83±37
Cortisol	Morning (n=20)	519±129	339±119	216±122	122±74	516±99	351±138	225±105	117±60
(nmol/L)	Afternoon (n=18)	603±150	309±88	220±101	109±58	551±155	348±119	255±114	110±49
	Control (n=11)	19.5±6.6	16.8±5.9	16±5.8	14.2±3.9	20.6±5.6	18.1±5.4	16.9±4	14.3±4.1
Testosterone	Morning (n=20)	20±5.5	16.3±6.2	16.4±6.9	12.6±6.2	20.5±5.7	17.5±5.2	15.2±6.5	12.4±5.2
(nmol/L)	Afternoon (n=18)	17.3±4.1	15.1±3.9	14.9±3.9	11.6±3.1	17.9±4.2	14.4±3.5	13.1±3.8	10.8±3.9

When the total serum cortisol concentrations were tested by three-factor GLM, both TOD and Pre-to-Post main effect were significant ($p<0.001$ and $p<0.05$, respectively). In addition, a DAY x TOD interaction was significant at $p<0.01$. The TOD main effect was observed at both Pre and Post, with each time-point within a test day being significantly different from each other (all pair-wise comparisons in Table 6; $p<0.001$). The highest cortisol concentration was consistently found at 07:00 h, while the lowest concentration was found at 20:30 h. The Pre-to-Post main effect showed that the mean cortisol concentration at Pre (319 nmol/L) was significantly higher than at Post (295 nmol/L). Based on the contrast test, the statistical significance of the DAY x TOD interaction was localized between 07:00 h and the rest of the day. A significant TOD x GROUP interaction ($p<0.05$) was present between 07:00 h and 12:00 h, mainly due to the decrease in the 07:00 h cortisol concentrations of the morning group from Pre to Post (Figure 15).

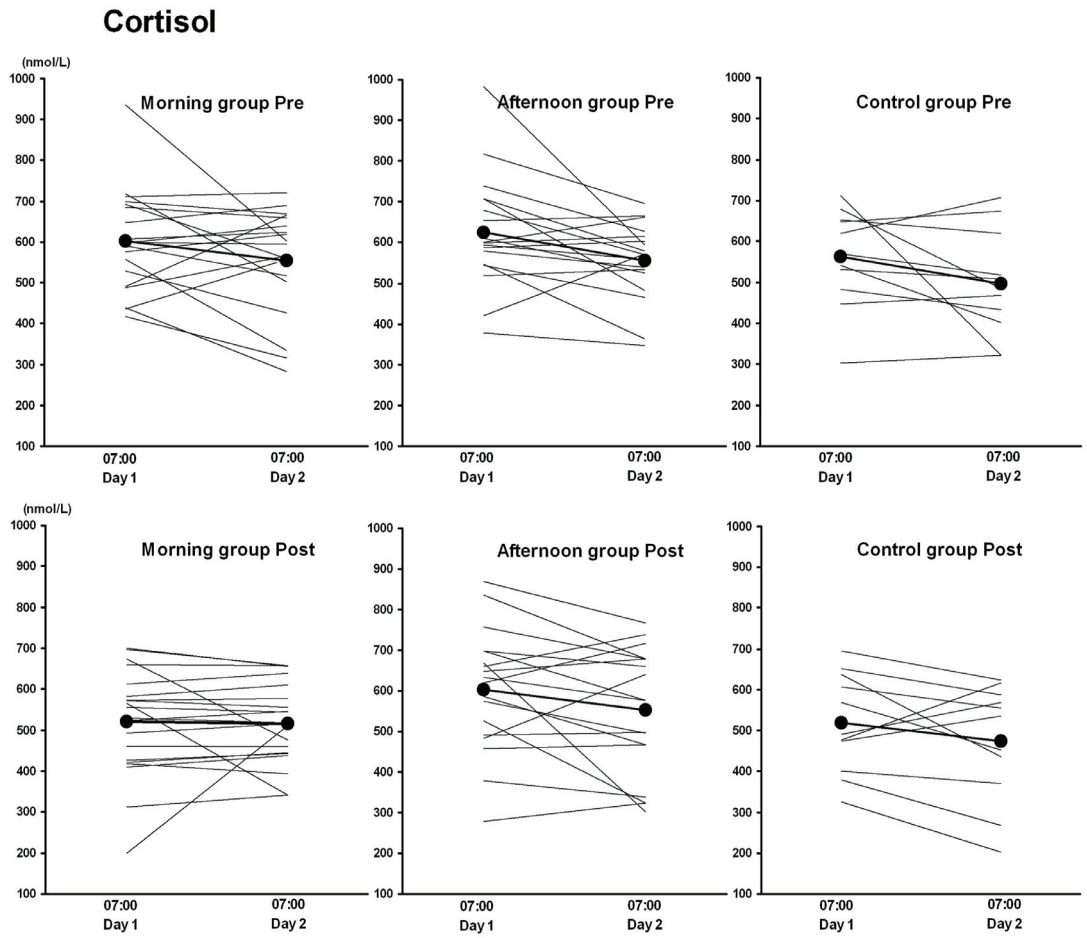

FIGURE 15 Morning serum cortisol concentrations during two consecutive days (Day 1 & Day 2) measured before (Pre – upper panel) and after (Post – lower panel) time-of-day-specific training. Thin lines represent individual changes from 07:00 h Day 1 to 07:00 Day 2; thick lines with circular ending represent means of the groups.

5.3.2.8 Oral temperature

At Post, a significant diurnal variation of a similar pattern was present in all three groups (TOD $p<0.001$) with 07:00 h values significantly lower than the rest of the day. The values of the control groups were slightly, but non-significantly, elevated when compared to the training groups (Table 7).

TABLE 7 Diurnal variation in oral temperature (°C) after the time-of-day-specific training period (Post) measured throughout two consecutive days (Day 1 and Day 2). Values are expressed as mean ± SD.

	Control group		Morning group		Afternoon group	
Time of Day (h)	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
7:00	36.20 ± 0.2	36.16 ± 0.1	36.01 ± 0.4	35.99 ± 0.4	36.16 ± 0.3	36.05 ± 0.3
12:00	36.37 ± 0.4	36.43 ± 0.5	36.34 ± 0.4	36.39 ± 0.3	36.30 ± 0.3	36.32 ± 0.4
17:00	36.50 ± 0.2	36.53 ± 0.3	36.35 ± 0.3	36.32 ± 0.3	36.29 ± 0.3	36.29 ± 0.3
20:30	36.47 ± 0.3	36.51 ± 0.3	36.32 ± 0.4	36.34 ± 0.3	36.45 ± 0.3	36.43 ± 0.2

5.3.2.9 Muscle hypertrophy

Quadriceps femoris (QF) volume was increased significantly ($p<0.001$, Training main effect) in both training groups after the time-of-day-specific training period (from 2180 ± 340 cm^3 to 2237 ± 342 cm^3, an increase of 2.7%, and from 2118 ± 217 cm^3 to 2192 ± 220 cm^3, an increase of 3.5%, in the morning and afternoon group, respectively). The results are shown in Figure 16. The 0.8% difference between the training groups was not significant ($p=0.188$). Both training groups increased their QF volume significantly when compared to the control group (from 2161 ± 191 cm^3 to 2166 ± 193 cm^3, an increase of 0.2%) ($p<0.001$ in both the Pre-to-Post x GROUP GLM interaction and Post Hoc One-way ANOVA).

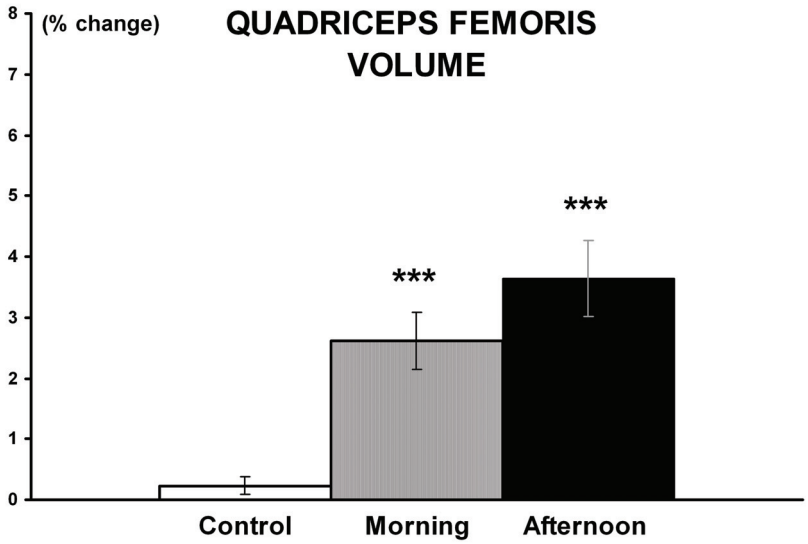

FIGURE 16 Relative changes in quadriceps femoris volume after the time-of-day-specific training. ***, significantly higher as compared to the control group, $p<0.001$.

Similarly to the QF volume, CSA of QF in all 14 slices, except the most distal slice, was increased significantly in both the morning and afternoon groups when compared to the control group but without significant difference between the training groups (Table 8). No significant effect of site-specific hypertrophy was found in the vasti muscle group. In both training groups, the CSA of vasti muscles increased to similar extents across their entire lengths. The percentage increase in the distal rectus femoris CSA was non-significantly greater than in the proximal part of the muscle in both morning and afternoon training groups ($p=0.101$ and 0.141, respectively).

TABLE 8 Relative changes in cross sectional areas of quadriceps femoris (QF) muscle during the time-of-day-specific strength training (week 10 to week 20) in the morning training group (n=9), afternoon training group (n=7) and control group (n=8). CSA 1 represents the most distal and CSA 15 the most proximal slice. *, **, *** - significantly larger CSA compared to the control group at $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

QF CSA	MORNING	AFTERNOON	CONTROL
Δ (%)	Mean (SD)	Mean (SD)	Mean (SD)
CSA 1	3.6 (1.9)***	2.7 (1.9)*	0.0 (0.6)
CSA 2	4.7 (1.5)***	4.7 (2.0)***	0.8 (0.9)
CSA 3	3.5 (1.1)***	4.7 (1.1)***	0.4 (0.7)
CSA 4	2.8 (1.4)***	3.1 (1.2)***	-0.1 (0.5)
CSA 5	2.6 (1.3)**	3.5 (2.0)***	0.1 (0.8)
CSA 6	2.4 (1.4)*	3.6 (2.1)**	0.2 (0.6)
CSA 7	2.3 (1.3)*	3.5 (1.5)***	0.5 (0.5)
CSA 8	2.3 (0.8)*	3.3 (2.2)**	0.5 (0.7)
CSA 9	2.5 (1.5)*	3.3 (1.7)**	0.5 (0.8)
CSA 10	2.1 (1.3)*	3.6 (2.0)***	0.3 (0.5)
CSA 11	2.3 (1.2)**	3.5 (1.6)***	-0.1 (0.8)
CSA 12	2.3 (1.1)***	3.5 (1.7)***	-0.3 (0.6)
CSA 13	2.6 (1.2)***	3.4 (1.4)***	0.2 (0.5)
CSA 14	2.8 (1.2)***	2.8 (1.4)***	0.1 (0.5)
CSA 15	1.9 (1.4)	3.3 (2.3)**	0.0 (0.6)
All CSA	2.7 (0.7)***	3.5 (0.6)***	0.2 (0.3)

Bivariate correlations were performed (with the subjects selected for MRI) to study the relationships between the individual increases in muscle strength and muscle size, and to study the potential effect of the initial muscle strength and muscle size status (at week 10) on the 10-week time-of-day-specific adaptation process. None of the correlations yielded significant p values with r^2 values ranging between 0.03 and 0.37 in both positive and negative directions.

5.4 Background variables

None of the selected background variables assessed before and/or during time-of-day-specific tests had a significant confounding effect on the data measured. In detail, the results were as follows:

5.4.1 Chronotype

Twenty seven subjects scored as a "neutral" or "intermediate" type in all three factors examined by the Circadian Type Questionnaire (Folkard et al. 1979). For the details on the distribution of extreme types, see Table 2. There was no significant difference between the extreme types compared with each other or with neutral types in the diurnal pattern of any strength and hormonal variable measured at either Pre or Post, or in the magnitude of muscle hypertrophy.

5.4.2 Sleep

Information on sleep collected one night prior to and during the experiment showed that an average habitual length of sleep was 7.5 ± 0.9 h and 7.4 ± 0.8 h as reported at Pre and Post, respectively. The waking hours ranged between 05:45 – 06:45 h in the present study, but the habitual waking hours were not recorded. At Pre, the average sleeping times during the night prior to Day 1 and the night between Day 1 and Day 2 were 6.9 ± 0.8 h and 6.9 ± 0.9 h, respectively. Four subjects reported their sleep length prior to or during experiment to be less than 75% of their individual average sleep length. At Post, the average sleeping times during the night prior to Day 1 and the night between Day 1 and Day 2 were 6.9 ± 1.0 h and 7.0 ± 0.9 h, respectively. Two subjects reported their sleep length shorter (1 case) or longer (1 case) than 75% of their individual average sleep length. However, for both Pre and Post, participants with altered sleep did not differ from the rest of the group with regard to the means of oral temperature, neuromuscular performance or hormonal diurnal patterns.

5.4.3 Self-reported stress level

None of the subjects reported a very high stress level, 5 or 6. At Pre, three subjects reported a stress level of 4 either on the first or second day. Mean values for the whole group were 2.3 ± 0.6 and 2.2 ± 0.7 on Day 1 and Day 2, respectively. At Post, five subjects reported a stress level of 4 during the first test day with group means of 2.3 ± 0.9 and 2.3 ± 0.8 on Day 1 and Day 2, respectively. However, those participants reporting stress did not differ from the rest of the group in oral temperature, neuromuscular performance or hormonal diurnal patterns. In addition, there were no significant differences in average stress levels between the test days, Pre and Post and groups.

6 DISCUSSION

The focus of the present thesis was two-fold: The first focus was on diurnal variation in selected neuromuscular and physiological variables when tested with two different test designs. The second focus was on effect of time-of-day-specific resistance training on maximum neuromuscular performance, the diurnal patterns of selected physiological variables, and the magnitude of muscle hypertrophy.

6.1 Daily variation in maximum strength, power, EMG activity and selected physiological variables

For the purpose of studying diurnal variation in selected neuromuscular and physiological variables, we compared the control group (n=11, healthy males with no resistance training for at least 3 years prior to the investigation) to the preparatory training group (n=38, similar health and training background as the controls) which underwent a 10-week progressive periodised preparatory resistance training period between 17:00 h and 19:00 h. This preparatory training period was applied to familiarise the training groups with resistance training before the actual time-of-day-specific training and to "synchronise" activity patterns of the preparatory training group by training exclusively in the afternoon when the diurnal peak in maximum strength typically occurs (for review, see Drust et al. 2005). Such timing of training should also prevent disturbance of the diurnal timing in peak performance in maximum strength (Souissi et al. 2002). The preparatory training period induced the expected increase in maximum strength of the preparatory training group. Both non-time-of-day-specific isometric MVC and 1 RM half-squat increased significantly from the baseline to the midline. Nevertheless, even the control group significantly increased their 1 RM half-squat, but not their MVC. These findings indicate that in all three groups the learning effect could be a substantial part of the improvements, especially in 1 RM half-squat. The most likely explanation

for differences between the two measures of performance is the difference in skill demands and this suggests that more than one or two familiarization sessions may be needed for the tests such as dynamic 1 RM half-squat in untrained subjects or subjects unaccustomed to the rigorous testing procedure.

Regarding the diurnal variation in strength and power, the power output exhibited significant and comparable diurnal variation on both test days. In the preparatory training group, performance was significantly lower in the morning (on average by 5% and 4%) compared to the rest of both Day 1 and Day 2. Reilly and Down (1992) showed a similar diurnal increase in explosive dynamic strength of the lower limbs and they reported an improvement of 5% in relative power output during a stair run and 6% in standing broad jump length. A carry-over effect of cumulative fatigue from Day 1 to Day 2 can not be disregarded as a potential confounding factor in the present study. However, its impact seemed to be minor since the subjects exerted higher (>1%) power outputs on Day 2 at every session. It must be noted that the loaded half-squat was part of the 10-week preparatory training in half of the participants (eight subjects statistically analyzed). The other half (another eight participants) trained with a pneumatic leg press device but undertook a total of twelve maximum familiarization trials - eleven weeks (6 trials) and one week (6 trials) prior to the actual experiment. Even though these two subgroups of subjects did not differ statistically, examination of the plotted data showed somewhat lower average minimum-to-maximum differences (~ 4% versus 8%) and slight day-to-day difference in the diurnal pattern between the subgroups (for further details see manuscript I). This trend was pronounced in the control group in which the minimum-to-maximum difference was more than 10% on Day 2. In addition, the last test session yielded the highest mean power output. It implies a possible influence of different specific skill levels (technique) and/or practice effect between these two groups. It could be hypothesised that changes in technique may induce a masking effect on the diurnal variation in power output.

Indeed, the role of improved technique was confirmed in a later study (muscle tone study) with untrained subjects, when the time of the first session was counter-balanced and randomized. During the explosive leg press with a load of 50% of 1RM (skill-wise less demanding compared to the loaded squat jump), a significant 8% mean difference was found between morning performance and the individual highest value in mean power output. It is of scientific and practical importance to note that none of the participants could achieve their individual best power output in the morning. However, the highest power output was typically exerted either during the third session or the last fourth test session (14 cases out of 17) and the lowest in the morning (8 cases) or during the first session (10 cases, including 3 cases in the group starting the first session at 08:00 h). It is probable that learning, improved inter/intra-muscular coordination, training status, and skill level could be important confounding factors in explosive dynamic movements during the repeated testing. This suggests that in untrained individuals or individuals unaccustomed to testing, technically demanding multi-joint dynamic actions

may require short-term task-specific conditioning training in order to fully adapt to the test demands.

Interestingly, peak torque during isometric MVC showed an even less repeatable diurnal pattern across two consecutive days than did power output in the loaded squat jump. In the preparatory training group, Day 2 data were in accordance with previous studies, showing lower morning values and more than 8% improved performance during the rest of the day (Gauthier et al. 1996, Gauthier et al. 2001, Guette et al. 2005a, Nicolas et al. 2005). By contrast, the minimum-to-maximum peak torque difference was less that 3% on Day 1, resulting in statistically non-significant MVC diurnal variation. Moreover, Day 1 peak torque values at 07:00 h were significantly higher, and 17:00 h values significantly lower, compared to the same time points on Day 2. This characteristic was even more evident in five subjects of the control group, when four of them performed their best peak torque during the first session (07:00 Day1). It is rather difficult to interpret these day-to-day differences and further research is needed. Fatigue due to repeated testing could play some role in the control group, but not in the training group, which exerted the highest overall peak torque typically on Day 2 and their minimum-to-maximum difference was comparable with other studies using the so-called "Latin square" design (Deschenes et al. 1998, Gauthier et al. 2001, Guette et al. 2005a). Drust et al. (2005) stated that such a design, when subjects perform the first session at a different time of day in a counter-balanced order, removes the influence of any learning/fatigue effect. Similarly, specific training status and familiarization processes could not explain the above mentioned day-to-day variation in MVC. All subjects were equally familiarized with measurements because they underwent 6 to 7 maximum trials on three separate occasions (13, 11, and 1 week prior to the experiment). A partial explanation for higher peak torques during first session may stem from different (higher) psychological arousal, motivation and/or anticipatory stress before and during the first morning test session compared to the rest of the experiment. Bambaeichi et al. (2004) and Giacomoni et al. (2005) reported that motivation could have a masking effect on diurnal variation in voluntary MVC both in men and women. They found typical diurnal variation only when superimposed electrical twitches were added upon the MVC peak torque in order to offset the motivational component. In the present study, higher anticipatory stress prior to the first morning test session compared to the other times could be another possible confounding factor. Anticipatory stress most likely induces higher arousal due to elevated catecholamine concentrations which, in turn, can improve strength production (French et al. 2007). Anticipation of physical exercise was also shown to increase cortisol levels an hour prior to unfamiliar exercise, but the anticipatory stress responses disappeared after the first test session (Mason et al. 1973). Indeed, the presence of higher stress on Day 1 could be concluded from the 07:00 h cortisol concentrations, as they were close to the upper limit in the range of reference values in the Finnish population (650 nmol/L). The decreased morning serum cortisol concentration on Day 2 may be a result of habituation to the repeated stressor.

The present findings on oral temperature also indirectly suggest the presence of the anticipatory stress. In the control group, the Day 1 diurnal rise in temperature was blunted and non-significant when compared to the training group and previously reported values (Ilmarinen et al. 1980, Reilly and Down 1992, Gauthier et al. 1996, Nicolas et al. 2005). A similar rise in body temperature was present in the preparatory training group, but it was smaller in magnitude. The 07:00 h Day 1 temperature was more than 0.2 °C and 0.1 °C higher compared to 07:00 h on Day 2 in the control and preparatory training groups, respectively. Also, average morning temperatures at 07:00 h in both the control and preparatory training groups were observed to be more than 0.3 °C higher than usually reported for oral temperature (Gauthier et al. 1996, Nicolas et al. 2005). A similar rise in body temperature induced by an anticipatory stress was observed by Marazziti et al. (1992). They reported that mean axillary temperature was 0.6°C higher during a first test than when measured in relaxed circumstances several weeks later. Additional evidence for anticipatory stress comes from the muscle tone study. In this population of untrained subjects, significant diurnal variation in isometric bilateral leg press force could not be detected statistically. However, when testing the specific hypothesis that individual morning values would be lower than the highest peak force for each participant, there was a highly significant mean difference of 9%. The lowest force was observed in the morning hours for 9 cases out of 17. Interestingly, the highest peak force was typically achieved during the first session (11 cases out of 17) except for the group starting at 08:00 h (1 case out of 4). This may suggest the presence of anticipatory stress prior the first session but its effect was less pronounced in the morning hours, perhaps due to inherently lower morning performance levels. Despite the strong evidence of anticipatory stress effect in the present study, further experiments of more advanced designs are required with better control over other possible effects – e.g., motivation, physical and mental fatigue, skill required and skill acquisition.

EMG activity of the measured knee extensors muscles (VL, RF, and VM) during MVC could not explain any of the above mentioned diurnal and day-to-day differences in peak torque. EMG did not significantly differ between time-points and was only non-significantly lower on Day 2 ($p=0.089$) suggesting that the neural input to agonist muscles was not statistically altered by time of day or day-to-day carry-over effects of testing. This result is in line with the studies of Nicolas et al. (2005), Guette et al. (2005b), Giacomoni et al. (2005) and Racinais et al. (2005), where variation existing at the muscle tissue level, rather then changes in the neural input to knee extensors muscles, was concluded. In contrast to these findings, Gauthier et al. (1996) reported significantly increased EMG activity in the morning hours compared to the afternoon and attributed the diurnal variation in MVC torque both to changes in the central nervous command and contractile state of the muscle. In addition, EMG data from the present muscle tone study showed an opposite trend, with significantly lower EMG activity compared to the rest of day and a decrease from session to session. These conflicting results could be, in part, accounted for by one or more of: differences in the test design and/or the test mode, muscle groups tested

(upper versus lower extremities), training background and test-specific skill level of the subject. Another important issue is the methodological differences in the surface bipolar EMG data recording and analysis procedures. Despite thorough control of the measurement procedures, EMG is always subject to various sources of error (Keenan et al. 2006). Thus the interpretation of the EMG data and their possible diurnal variation should be treated with great caution.

Antagonist co-activation during MVC could be excluded from causing day-to-day and Day 2 diurnal differences in the present study. BF co-activation remained unchanged and ranged from 8% to 10% of the BF EMG when acting as agonist during knee flexion. The level of co-activation was comparable with the values reported previously: 9 – 14% (Grabiner et al. 1989) and 9% - 12% (Guette et al. 2005a). BF co-activation was also stable in the present muscle tone study but the levels were higher (> 20%). Higher co-activation level could be attributed to the test action (leg press), which is known to involve BF muscle more than the knee extension used in the present training study and in the above-mentioned reports. Subjects were also untrained which could be a partial reason for higher antagonist co-activation (Häkkinen et al. 1998).

The EMG_{norm}/peak $torque_{norm}$ ratio during MVC did not significantly differ throughout the day in spite of its tendency to decline from morning towards afternoon on both days. Similar results during MVC were obtained by Giacomoni et al. (2006) for the vastus lateralis muscle. In contrast, Gauthier et al. (1996) and Nicolas et al. (2005) found the ratio of knee extensor and elbow flexors to be significantly lower at 18:00 h compared to 06:00 h. Interestingly, the EMG/peak torque ratio declined significantly from Day 1 to Day 2. Improved intra- and inter-muscular coordination could be one possible explanation. However, the methodological issues related to surface EMG recording and muscle group dependence (Guette et al. 2006), which were mentioned above, may affect detailed interpretation of this variable.

In addition to EMG, muscle tone was examined as a potential co-factor behind diurnal variation in strength and power. Muscle tone (stiffness, compliance, hardness) has been defined as the ratio: [change in force]/[change in length] along the long axis of the muscle (Leonard et al. 2004). This can be significantly affected, for example, by training background (higher tone in sprinters as compared to untrained individuals, Haverinen 2005). Muscle tone has been proposed to indirectly estimate a) the state (and changes) of the viscoelastic and mechanical properties of the joint–tendon–muscle complex e.g., the amount of actin and myosin cross bridge contacts in muscle filaments (Watkins 1999) and, in case of some pathological conditions, it also reflects the state (and changes) of the central nervous system-related activity, such as activity of alpha motoneurons and/or activity of muscle spindles or gamma motoneurons. In the present study, however, muscle tone was shown to be quite stable throughout the day and could therefore be disregarded as a possible (co)-factor contributing to diurnal variation in muscle strength and power.

In addition, none of the factors that was controlled because they might act as confounding influences - length of sleep and its timing, self-reported stress

levels and chronotype of the subjects – had a statistically significant effect on the data. However, in the case of chronotype, infrequent sampling and unequal distribution of the extreme chronotypes in the present study did not allow for evaluation of possible differences in the diurnal pattern of neuromuscular performance and physiological variables. For instance, Kerkhof and Dongen (1996) reported that the difference of the circadian phases of body temperature rhythm between morning-type and evening-type subjects was only 2.1 h. The non-significant relationships between self-perceived stress levels of the subjects and elevated Day 1 morning cortisol concentrations are also not necessarily contradictory since the subjects were asked to rate their perception of stress for the test day as a whole rather than specifically for the 07:00 h session.

To summarise, the present data on diurnal variation in studied variables under normal conditions, found that morning levels of maximum voluntary isometric and dynamic strength were typically lower than during the rest of the day. However, the diurnal patterns were affected by confounding factors such as anticipatory stress and learning and/or improved inter-/intra-muscular coordination - despite a typical familiarization procedure prior to the experiment. EMG activity could not fully explain diurnal variations in strength, and so processes within the muscle tissue might be involved. The design of the present study does not allow for separating endogenous and exogenous components in diurnal variation in strength. Studying the cause of the observed rhythms remains a big challenge for future research. The use of special chronobiological testing protocols is needed, but special attention must be paid to control of cumulative fatigue. Ultra-short sleep-wake cycle protocols, constant routine protocols and forced resynchronisation protocols are among the potential laboratory approaches in the future. Also, the selection of test tasks that induce minimal muscle fatigue and damage, whilst providing high external validity to complex physical/sports performance is of major importance.

6.2 Training adaptation to time-of-day-specific resistance training

In the morning and afternoon training groups, the second 10-week period of time-of-day-specific training resulted in less pronounced strength gains in 1 RM half-squat and MVC when tested at the non-specific training times. The preceding 10-week preparatory training period probably led to attenuation in training adaptation during the second 10-week period, a result which is usually observed after 6 – 8 weeks of training in previously untrained men (Häkkinen 1989). It is important to note, however, that the absolute magnitude of the maximum strength improvements measured either at specific (Souissi et al. 2002) or non-specific training times (as in the present study) seems to be rather similar, regardless of the time of day when training took place. The control group did not significantly improve their 1 RM or MVC when measured in the

non-time-of-day-specific design. However, there was a significant difference between the control group's non-time-of-day-specific MVC at Mid and End compared to 17:00 h, Day 2 time-of-day-specific MVC at Pre and Post (Table 5). The reason for this increase is unknown and it can only be speculated that this was partly a result of the testing sessions being separated on average by 5 days. Prevost et al. (1999) showed that, after only 2 days of strength training in male subjects unaccustomed to maximum strength production, peak torque may increase as much as 22%. Therefore, both midline and end tests could, in part, act as training sessions in untrained subjects of the control group. Also, the repeated testing during the Pre and Post test may additionally improve MVC peak torque via skill learning. However, unchanged EMG in the controls from Pre to Post suggests that, if present, the training effects were of short duration and/or that skill learning was a substantial reason for higher time-of-day-specific MVC.

When tested in the time-of-day-specific design (Post), both the afternoon and control groups persisted significantly lower Day 2 peak torque values at 07:00 h as compared to 17:00 h when examined by a T test. On the contrary, peak torques measured in the morning group at 07:00 h and at 17:00 h did not differ significantly from each other. The relative increase at the training-specific time (07:00 h) was 12% compared to 5% at 17:00 h in the morning group. Similar statistically significant differences between the morning and afternoon groups in the time-of-day-specific training adaptation were also found when comparing peak torque variation at four time points across two consecutive days from Pre to Post (using the full data set). These results have been discussed in detail in manuscript II. The present results are in agreement with those in the report of Souissi et al. (2002). However, the time-of-day-specific adaptation of the morning group in the present study was somewhat less pronounced that in the study of Souissi and co-workers. The possible explanations for the less pronounced adaptation in the present study may stem (at least partly) from different training history of the participants and their more heterogenic chronotype distribution compared to participants in the study of Souissi and co-workers. This issue has been discussed in detail in manuscript III but further experiments are needed to provide stronger scientific proof for this case.

The present study showed that the surface EMG activity of the trained muscles could not explain the trend of the morning group to increase performance more at the time of day of training (07:00 h) than at the other time (17:00 h). The second 10-week time-of-day-specific training period resulted in further significant increases in EMG activity in both training groups compared to the controls, but no significant diurnal variation was present in either group. The biceps femoris co-activation also remained unaltered throughout the day after the time-of-day-specific training period. A tendency for increased antagonist co-activation for an average of 9% at Pre to 13% at Post was seen in both training groups (calculated from all tests). This was in contrast to previous findings showing a decrease (Häkkinen et al. 1998) or no change (Reeves et al. 2005). Since knee flexion was part of the training in the present study, increased

BF EMG activation during knee extension testing from Pre to Post could alter the ratio. An increased antagonist co-activation may have also had a protective function on the knee joint, since higher knee extension forces could be produced by knee extensors at Post. Based on the present findings, adaptations in neural drive, motor unit properties and/or muscle membrane properties and/or antagonist co-activation seemed to have a minor effect in time-of-day-specific adaptation in maximum strength. The EMG/peak torque ratio, considered by some authors as a measure of neuromuscular efficiency (Milner-Brown et al. 1986), reflected the EMG and peak torque behaviour throughout the day and showed no significant day-to-day difference at the Pre test (data not shown). In the present study, neuromuscular efficiency improved significantly from 07:00 h to 17:00 h. Gauthier et al. (1996) also showed significantly higher neuromuscular efficiency of the biceps brachii muscle in the afternoon compared to the morning. However, their findings were the result of combined effects of a significant increase in peak torque and a significant decrease in EMG from the morning to the afternoon hours. In addition to the above-mentioned methodological issues related to the EMG/peak torque ratio, the actual physiological meaning of this ratio has recently been challenged by some authors (for a review, see Folland and Williams 2007).

Power output, when analysed at 07:00 h and 17:00 h on Day 2, showed a rather unexpected increase in the control group which was relatively larger than in both training groups. Even in absolute values, the power output was similar in all three groups after time-of-day-specific training. It is difficult to interpret these finding. Most likely, higher loads used during the loaded squat jumps in both training groups in comparison to the controls (due to a higher training-induced increase in 1 RM in the training groups) could be a possible reason, especially in the morning group. If 1 RM improved at Mid or End, the load during the actual tests at Pre and Post was higher than loads experienced during the training sessions. With a relatively short training background, technique, and consequently power output, could be impaired in some of the subjects. Nevertheless, a similar time-of-day-specific training adaptation as found in MVC was observed. The control and afternoon group performed relatively better in the afternoon while the morning group could exert higher power outputs at 07:00 h. It must be mentioned that this feature was, however, present already at Pre in the morning group, probably due to lower values at 17:00 h compared to 12:00 and 20:30 h values rather than high 07:00 h performance.

Another important finding in the present study was the significant attenuation of 07:00 h cortisol concentrations after the time-of-day-specific training in the morning group but not in the afternoon and control groups during Day 1. Total serum cortisol concentrations exhibited a typical diurnal decrease (Veldhuis et al. 1989, Van Cauter et al. 1996, Deschenes et al. 1998) at both Pre and Post. However, after the time-specific-training, both the afternoon and control groups showed persistently higher Day 1 morning cortisol levels, while the morning group showed a significant decrease in Day 1 at this time. Moreover, the Post 07:00 h cortisol remained stable from Day 1 to Day 2 in most

of the subjects in the morning group. The mechanisms presumably involve psychological adaptations (involving stress and so on) rather than any chronic changes of the diurnal cortisol pattern, but this hypothesis requires further research. The early morning testing was perhaps perceived to be much less stressful by subjects of the morning training group who were accustomed to early morning waking and performance. It must be noted that some attenuation in anticipatory stress levels was also likely present in the afternoon and control groups, since oral temperature at 07:00 h was lower compared to Pre conditions. In summary, repetitive testing across two consecutive days decreased the morning total serum cortisol from the first to the second test day in each group, presumably by gradually attenuating the anticipatory stress response prior to the tests. However, in order to maintain this adaptation over a period of weeks, it seems that the exercise stimuli must be applied repeatedly and the stimuli must be time-of-day-specific (i.e., applied in the morning). Also the familiarization procedure should be, among other requirements, time-of-day-specific. Alternative explanations for the present findings (e.g., differences in the activity of glucocorticoid receptors, CRH, ACTH, etc) cannot be excluded, since the present study does not allow to differentiate between the suggested mechanisms. Further research is also needed to establish the minimum frequency of repetition of bouts of exercise needed to maintain the present time-of-day-specific adaptation for cortisol.

In total serum testosterone, a significant diurnal rhythm was detected in all three groups before and after the time-of-day-specific training, in line with numerous previous reports (for example, Veldhuis et al. 1987, Deschenes et al. 1998, Keenan and Veldhuis 1998). This could mean that neither the preparatory nor the time-of-day-specific training period chronically altered the diurnal rhythm of testosterone in the training groups. Limited results from the acute hormonal response studies may imply phase-shifting properties of strength exercise. Strength exercise performed in the afternoon (McMurray et al. 1995, Nindl et al. 2001b), but not in the morning (Kraemer et al. 2001), has been reported to temporarily alter overnight testosterone release. Nevertheless, the present study does not allow us to evaluate possible training-induced phase-shifts in the diurnal rhythms of total testosterone and cortisol, mainly due to the low sampling frequency. In addition, we hypothesized that the 10-week preparatory training period and the subsequent time-of-day-specific training period would not be sufficient to induce a significant phase shift on testosterone and cortisol rhythms, mainly due to the rather low training frequency. Subsequent sessions were always separated by two or three days, to allow sufficient recovery and avoid overreaching, overtraining, and/or injury. Rest days may have provided sufficient time to reset any possible phase-shifting effects of the morning or afternoon exercise, since the subjects were exposed to other strong environmental and social synchronizers (sun light-dark cycle, work schedule, social contacts etc) of circadian rhythms. Seasonal variation and age of the subjects could be neglected as possible confounding factors in the present study (for further details see manuscript II).

As expected, 10 weeks of strength training, performed either in the morning or in the afternoon, resulted in significant increases in quadriceps femoris muscle size. The absolute quadriceps femoris volume after the preparatory training period agreed with values previously reported for young healthy men by Ivey et al. (2000) and Morse et al. (2007). The relative increase of 2.7% (the morning group) and 3.5% (the afternoon group) in average QF CSA during the time-of-day-specific training was at the lower end of range typically reported in the literature for strength training similar to that in the present study (for example, 1.1 – 17.3% over a 10-week period in previously untrained subjects, Wernbom et al. 2007). Slightly lower gains in QF size in the present study could be mainly due to the effect of the preparatory training period. Although the preparatory training period did not include typical protocols for inducing muscle hypertrophy, the high load and high speed protocol most likely did this to some extent (Wernbom et al. 2007) and so might narrow the adaptation window during the actual time-of-day-specific training (Deschenes and Kraemer 2002). An interesting finding was the difference in the relative QF volume increase, although statistically non-significant and minor (0.8%) in magnitude, between the training groups in favour of the afternoon group. This increase in QF volume was more pronounced (1% – 1.5%) when considering the mid-section cross-sectional areas only. The present study does not allow differentiation between true time-of-day effects (e.g., via time-of-day-dependent differences in the steroid hormones circulatory levels - Veldhuis et al. 1987) and/or in hormonal response to a heavy bout of exercise - Häkkinen et al. 1988a, Nindl et al. 2001a, Bird and Tarpenning 2004) or the influence of possible confounding factors. For instance, the 07:00 h testosterone concentrations were within the normal physiological range in all subjects. However, subjects with testosterone levels closer to the upper limits could have an advantage over those closer to the lower limit, as there is evidence that an increase in muscle strength and size may be positively related to resting total serum testosterone concentrations (Bhasin et al. 2001, Kvorning et al. 2006). Furthermore, differences in the timing and amount of protein (amino acids) ingested shortly before and after the morning and afternoon training sessions (Volek 2004, Hulmi et al. 2005), the inter-individual difference in responsiveness to training due to distribution of fibre types (Thorstensson et al. 1976, Campos et al. 2002) and/or greater pre-training muscle satellite cell presence (Petrella et al. 2008) might be among potential confounding factors in the present study.

In summary, traditional low-frequency strength training performed in the morning, but not in the afternoon hours, was found to attenuate the typical diurnal variation in maximum strength. However, the absolute increase in maximum strength and muscle size did not differ statistically with the time of day of training. In addition, the level of adaptation to time-of-day-specific training varied greatly between individuals. Adaptations also probably occurred at the psychological level, by attenuating the anticipatory stress level prior to the morning testing when repeated morning training was performed. Future research is needed firstly, to address the effects of resistance training programs over a longer training period and their impact on long-term muscle

mass and strength gains. Secondly, resistance training protocols of shorter duration and volume but of high training frequency (daily), ideally under strictly controlled forced desynchronisation or ultra-short sleep-wake cycle protocols, could be used. This approach would allow a more precise examination of the patterns of time-of-day-specific adaptation and possible phase shifting properties of resistance exercise/training on the circadian timing system.

7 PRIMARY FINDINGS AND CONCLUSIONS

The main findings and conclusions of the present study can be summarized as follows:

1) Diurnal variation in selected neuromuscular and physiological variables
Under normal environmental and dietary conditions, a typical diurnal variation, with lower morning levels of maximum voluntary isometric and dynamic strength compared to the rest of the day, was observed.

a) However, the diurnal patterns were affected by test order-related confounding factors. In isometric maximal voluntary contractions, anticipatory stress and higher arousal prior to and during the first session seemed to be the major confounding factor, probably causing added benefits in strength production compared to the following test sessions. This effect occurred regardless of timing of the first session - whether it was exclusively in the morning hours during the test design across two consecutive days or in a test design with the first test session counterbalanced to begin at any time throughout the day. For dynamic, multi-joint explosive tests, learning and/or improved inter-/intra-muscular coordination due to frequent and repeated testing seemed to be the major confounding effect regardless of test design. Also, training background could play a role as these confounding factors were more pronounced in untrained subjects. However, repeated testing did not induce any significant day-to-day carryover influence of cumulative fatigue when testing occurred throughout two consecutive days. However, two separate familiarization sessions were not sufficient in preventing the effects of confounding factors; therefore, the familiarization procedure should perhaps mimic the actual test session and so be perceived by the subjects as an actual testing session.

b) Regarding the possible mechanisms involved, neither EMG activity nor muscle tone could fully explain the diurnal variation in maximum strength. It is plausible that processes within the muscle tissue and affecting its contractile properties are partly responsible for the diurnal variation. Based on the EMG

data from the muscle tone study, however, diurnal changes in neural drive, motor unit and/or muscle membrane properties cannot be fully disregarded as possible additional sources of variation.

c) Diurnal variations in serum total cortisol and oral temperature were typical for the studied subject population. However, anticipatory stress prior to the first test session most likely induced a masking effect, resulting in transient elevation of both cortisol concentration and temperature during the first morning test session compared to that the following morning. Total serum testosterone was not significantly affected by anticipatory stress and its diurnal variation was in line with previous findings.

2) Training adaptations to the time-of-day-specific resistance training
The present results confirm previous findings that time-of-day-specific adaptation to resistance training may exist.

a) Traditional, low-frequency resistance training performed in the morning, but not in the afternoon, attenuated the typical diurnal variation in maximum strength. However, the absolute increase in maximum voluntary strength was similar regardless of the time of day that training took place. The level of adaptation to time-of-day-specific training varied considerably between individuals. Some individuals, by training repeatedly in the morning, might be able to improve typically poor morning strength and power to the same or even a higher level than their normal daily peak, typically observed in the late afternoon. In contrast, other individuals would still perform poorly in the morning in spite of the training at this time. It is suggested that the chronotype of a subject may be partly responsible for the inter-individual variation in responsiveness to time-of-day-specific training.

b) Time-of-day-specific training adaptations also occurred at the psychological level, most likely by attenuating the anticipatory stress response (measured as cortisol concentration) prior to the first morning test session, but only in the group of subjects who repeatedly trained in the morning hours. Subjects in the afternoon and control groups showed similarly elevated cortisol levels prior to the first morning test session as were observed before the time-of-day-specific training. It can be concluded that, in order to maintain the acute attenuation of the anticipatory stress response over a period of weeks, the exercise stimuli must be applied repeatedly and be time-of-day-specific (i.e., applied in the morning). No statistically significant adaptation was observed in diurnal variation of testosterone following time-of-day-specific training.

c) Both the morning and afternoon training periods induced significant muscle hypertrophy. However, the magnitude of muscle gain (especially in the mid-section of quadriceps femoris) was somewhat larger in the subjects training in the afternoon hours, though this difference did not reach statistical significance. It remains unclear whether time-of-day-specific training performed over longer

periods of time (>3 months) would cause systematic differences in the gain of muscle mass.

d) It is suggested that time-of-day-specific strength training may be beneficial when maximum strength performance needs to be achieved at a particular time of day, especially in the morning hours. Strength and power athletes required to compete at a certain time of day (e.g., morning qualifications) may be advised to train repeatedly at that particular time of day for several weeks prior to the competition.

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