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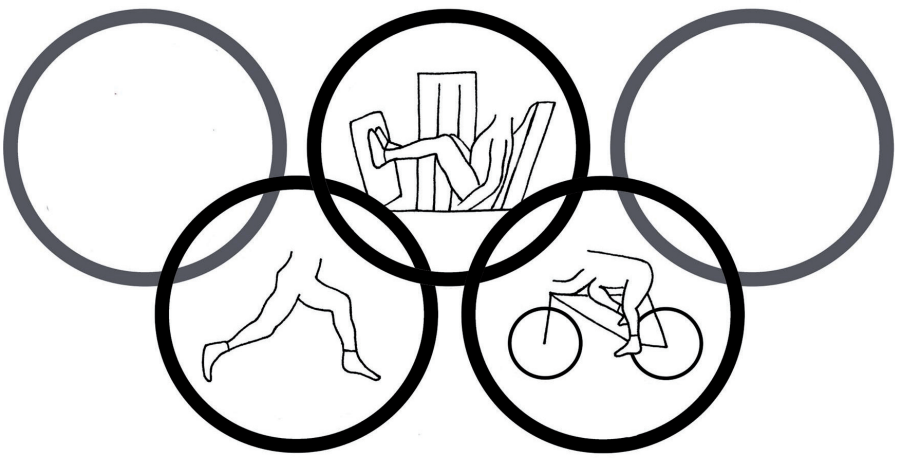
230

Moritz Schumann

Concurrent Endurance and Strength Training

Neuromuscular, Cardiorespiratory and Hormonal Effects of the Exercise Order in Previously Untrained and Recreationally Endurance Trained Men



JYVÄSKYLÄN YLIOPISTO

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UNIVERSITY OF JYVÄSKYLÄ

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"Inmitten der Schwierigkeiten liegt die Möglichkeit"
Albert Einstein

ABSTRACT

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The aim of the present thesis was two-fold. First, to investigate physiological adaptations to concurrent endurance and strength training performed in the same session with different exercise orders (i.e. commencing training with endurance or strength, respectively; E+S vs. S+E) in previously untrained men (n=42) (study 1). Second, to examine physiological adaptations when strength training was always performed immediately after endurance running in the same session (E+S) compared to endurance training alone (E) in recreational endurance runners (n=30) (study 2). In study 1, training consisted of 2 - 3 weekly combined endurance cycling and mixed hypertrophic and maximal strength training sessions for 24 weeks. In study 2, all subjects performed 4 - 6 weekly endurance training sessions for 24 weeks, while in E+S an additional strength training session was performed twice a week right after a strenuous endurance running session. In study 1, before the training intervention exercise order-specific differences were observed by statistically reduced testosterone concentrations during recovery following an experimental loading in E+S but not S+E. After 24 weeks of training, however, this initial reduction was no longer observed. Furthermore, increases in endurance and maximal strength performance, muscle cross-sectional-area and body composition as well as changes in basal hormone concentrations were similar in E+S and S+E, while rapid isometric force production was statistically increased in S+E only. In study 2, no statistical between-group difference was observed in endurance loading-induced acute force and hormone responses before or after the training intervention. In E+S, maximal strength was maintained and lean mass slightly increased, while also no statistical changes in electromyography and voluntary activation were observed. In E, maximal strength statistically decreased during training. Both groups improved endurance performance to a similar extent. The present thesis showed that in previously untrained men despite an initial between-group difference in testosterone concentrations during recovery, the exercise order did not affect training-induced adaptations in maximal strength and endurance performance or body composition. In recreational endurance runners, prolonged strength training performed always repeatedly after an intense endurance running session did not lead to enhanced endurance performance, possibly attributed to impaired neuromuscular adaptations.

Key words: order effect, body composition, endurance cycling, endurance running, combined training, coaching, testosterone, growth hormone, cortisol

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"There is nothing noble in being superior to your fellow men. True nobility lies in being superior to your former self."
Ernest Hemmingway

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Jyväskylä, November 2015
Moritz Schumann

LIST OF ORIGINAL PUBLICATIONS

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

I Schumann, M., Walker, S., Izquierdo, M., Newton, R.U., Kraemer, W.J. & Häkkinen, K. 2014. The order effect of combined endurance and strength loadings on force and hormone responses: Effects of prolonged training. *European Journal of Applied Physiology* 114 (4), 867-880.

II Schumann, M., Küüsmaa, M., Newton, R.U., Sirparanta, A.I., Syväoja, H., Häkkinen, A. & Häkkinen, K. 2014. Fitness and lean mass increases during combined training independent of loading order. *Medicine & Science in Sports & Exercise* 46 (9), 1758-1768.

III Schumann, M., Mykkänen O.P., Doma, K., Mazzolari, R., Nyman, K. & Häkkinen, K. 2015. Effects of endurance training only versus same-session combined endurance and strength training on physical performance and serum hormone concentrations in recreational endurance runners. *Applied Physiology, Nutrition, and Metabolism*, 40 (1), 28-36.

IV Schumann, M., Pelttari, P., Doma, K., Karavirta, L. & Häkkinen, K. 2015. Neuromuscular adaptations to same-session combined endurance and strength training in recreational endurance runners. *Submitted for publication.*

ABBREVIATIONS

ATP	Adenosine triphosphate
AUC	Area under the curve
CK	Creatine kinase
CSA	Cross-sectional area
DXA	Dual-energy x-ray absorptiometry
E	Endurance training only
EMG	Electromyography
EPOC	Excess post-exercise oxygen consumption
E+S	Endurance- immediately followed by strength training
F	Force
HDL-C	High-density lipoprotein
HR_{max}	Maximal heart rate
LDL-C	Low-density lipoprotein
OBLA	Onset of blood lactate accumulation
RM	Repetition maximum
SHBG	Sex hormone-binding globulin
S+E	Strength- immediately followed by endurance training
VA%	Voluntary activation percentage
VO_2	Oxygen uptake
VO_{2max}	Maximal oxygen uptake

CONTENTS

1 INTRODUCTION

Although physical exercise has been part of humanity since the antiquity, the science of progressive exercise training is a development of the 20th century. Inspired by physical principles, scientists slowly began to understand the importance of human movement in social and institutional settings but at this time did not use it to enhance performance capacity (Beamish & Ritchie 2005). In fact, it was not until the middle of the 20th century, when the first exercise training research was published. According to Todd et al. (2012), the first scientific papers of progressive overload resistance training were published in the end of World War II. In these studies, Thomas L. Delorme utilized progressive resistance training, defined as lifting multiple sets of the individual 10 repetition maximum (RM), for the rehabilitation of injured servicemen (Todd, Shurley & Todd 2012). Delorme's book "Progressive Resistance Exercise: Technic and Medical Application" as well as his academic publications are nowadays understood as the foundation for the science of strength training and were later applied to endurance training as well.

Interestingly, since the initial publications on resistance training, it was not until three decades later when the effects of combining endurance and strength training were scientifically explored. Robert C Hickson found in 1980 that strength but not endurance development may be compromised when a high frequency of intensive running and strength training sessions were performed concurrently (Hickson 1980). Ever since his pioneering study this phenomenon has been known as the "interference effect". Interestingly, Hickson's studies showed that concurrent training did not seem to blunt cardiorespiratory adaptations in a similar fashion to strength training adaptations. In fact, strength training appeared to enhance athlete's endurance performance (Hickson et al. 1988; Hickson 1980).

Following the initial studies on concurrent endurance and strength training, a number of scientists have further explored the "interference phenomenon" first in untrained and especially since the late 1990's in endurance trained athletes as well. However, in these studies endurance and strength training were typically performed on separate days, allowing for prolonged recovery between

subsequent training sessions. When performing endurance and strength exercises within the same training session, however, recovery may be compromised and residual fatigue from the first exercise (i.e. endurance or strength) may cancel out the positive physiological responses of the subsequent loading (i.e. strength or endurance). This phenomenon is known as the "acute hypothesis" and was brought forward by Craig et al. (1991). Importantly, while Craig et al. (1991) hypothesized that fatigue induced by the endurance exercise may compromise the ability to develop tension during the subsequent strength loading and, thus, impair chronic neuromuscular adaptations, their study did not exactly investigate the differences of the two exercise orders. However, as combining endurance and strength training within the same training session may provide a time effective strategy to help young adults adhering to regular physical activity, scientific investigation of the effect of the exercise order on physiological function is of utmost interest for sport and exercise scientists.

Two years later, Collins & Snow (1993) were the first to publish the effects of the exercise order on physiological adaptations when endurance running and heavy resistance training were performed within the same training session on 3 days a week. This study concluded that both neuromuscular and cardiorespiratory adaptations appeared to occur regardless of the exercise order following short-term (i.e. 7 weeks) concurrent endurance and strength training.

The first scientific study to investigate the effects of the concurrent exercise order on hormonal concentrations, however, was published rather recently by Cadore et al. (2012b). Interestingly, in this study endurance exercise performed prior to strength loading induced larger acute testosterone responses when compared to the opposite exercise order in young, recreationally strength-trained men. The authors concluded that training in this order may enhance anabolic stimuli for optimizing training adaptation but recovery measures were not performed. One year later, Taipale & Häkkinen (2013) reported significantly lower levels of testosterone concentrations at 24 h and 48 h of recovery when strength exercise preceded endurance running compared to the opposite exercise sequence in young endurance-trained men. However, as both studies only used randomized cross-over designs, the applications for prolonged exercise training remain unclear and require thorough investigation with longitudinal training interventions.

The present thesis, thus, aimed to investigate the neuromuscular, cardiorespiratory and hormonal responses and adaptations to concurrent endurance and strength training performed in the same session with different exercise orders (endurance followed by strength and vice versa) in previously untrained men. Second, it was aimed to investigate the physiological effects of same-session combined training, when strength training was always performed immediately after an endurance running session in recreational endurance runners.

2 REVIEW OF THE LITERATURE

2.1 The physiological foundation of exercise training

2.1.1 Neuromuscular bases of human movement

The coordination of human movement is dependent upon a complex interplay of neural and muscular actions. When engaging in systematic exercise training, much of the improvements in muscular strength are typically attributed to adaptations within the nervous system (Folland & Williams 2007; Aagaard 2003), indicating its importance for the initialization of muscular actions.

The human nervous system is divided into a central and a peripheral part. Centrally, the spinal cord provides the connection between the brain cells and the skin, joints and muscles. From the spinal cord, electric signals are transmitted via motor neurons to the muscle fibers. The anterior motor neuron and the specific muscle fiber it innervates are referred to as the motor unit, making up the functional element of movement (McArdle, Katch & Katch 2007, p. 384; Staudenmann et al. 2010). Peripherally, muscle fibers connect to the motor unit at the neuromuscular junction (motor endplate), which represents the interface between the nerve cell and the muscle (Enoka 2008, p. 229 - 232).

Signals are forwarded from the central nervous system to the motor unit by action potentials (waves of depolarization). Depending on the force requirement for a given movement, motor units usually appear to be activated in a set sequence. According to the size principle suggested by Henneman (1957), the motor units with the smallest motor neuron are recruited first, subsequently followed by larger motor units. This principle has been shown to exist among motor neurons that innervate the same muscle which are, thus, referred to as a motor neuron pool (Enoka 2008, p. 290). However, the force generated by a muscle is not only related to the number and size of motor neurons recruited but also largely dependent on the rate at which action potentials are discharged as well as the pattern of action potential activity (Folland & Williams 2007).

Muscle activation is often used as a term to describe factors related to muscular force generation. Typically this is evaluated through recording action potentials by electromyography (EMG) during maximal muscle contractions. Importantly, while EMG does reflect both the quality and quantity of electrical activity generated by muscle and, thus, is a measure of muscle activity it cannot be used to determine the cause of temporary or chronic changes in muscle activation (Rau, Schulte & Disselhorst-Klug 2004). Therefore, EMG recordings are typically performed concomitantly with electrical muscle or nerve stimulation in order to provide more precise information regarding the origin of neuromuscular responses or adaptations (e.g. voluntary activation).

2.1.2 Cardiorespiratory bases of physical exercise

The energy for physical exercise is derived from adenosine triphosphate (ATP), produced by macro-nutrient oxidation. Since only a very limited amount of ATP can be stored within the muscle cells, ATP must be constantly resynthesized at its rate of utilization (Åstrand et al. 1986). This can generally occur via anaerobic splitting of phosphate from phosphocreatine or the cellular oxidation of carbohydrate, lipid and protein macronutrients. During the aerobic production of ATP from carbohydrates, hydrogen ions are stripped from NAD^+ to form NADH which is then oxidized within the mitochondria to water and pyruvate (Silverthorn 2010, p. 108).

Aerobic glycolysis is the primary energy supply during sub-maximal exercise whereby hydrogen oxidation equals its production (Gastin 2001). However, when energy demands exceed the oxygen supply during strenuous exercise, the rate of hydrogen production surpasses its oxidation causing an excessive conversion of pyruvate to lactate (Silverthorn 2010, p. 109). Consequently, blood lactate concentrations begin to rise exponentially concomitantly with increases in exercise intensity, commonly referred to as the blood lactate threshold (Åstrand et al. 1986), or more recently, as the onset of blood lactate accumulation (OBLA) (McArdle, Katch & Katch 2007, p. 291).

In contrast to blood lactate accumulation, respiratory variables such as ventilation, oxygen uptake and carbon dioxide production typically increase linearly with concomitant increases in exercise intensity up until nearly maximal effort. Induced by increased blood lactate levels, a change in blood pH occurs, which causes carbon dioxide production to increase considerably, and thus, leads to a disproportional rise of minute ventilation in relation to VO_2. Consequently, elevation in pulmonary ventilation does no longer reflect the changes in oxygen uptake at the cellular level, typically known as the ventilatory threshold (Roecker et al. 2000).

At nearly maximal efforts, oxygen uptake can plateau or decrease slightly irrespective of increased exercise intensity, indicating maximal oxygen uptake (VO_{2max}) which is defined as the maximal amount of oxygen that can be utilized by the muscle (Hawkins et al. 2007). VO_{2max}, therefore, refers to the maximal cardiorespiratory capacity of an athlete and is commonly used as a predictor of endurance performance (Joyner & Coyle 2008). It is widely accepted that VO_{2max}

is dependent on the type of exercise performed and endurance runners often show higher VO_{2max} values compared to cyclists (Millet, Vleck & Bentley 2009), while exceptions may exist. However, VO_{2max} is only one aspect of cardiorespiratory function and its role as the most important predictor of endurance performance has previously been questioned, especially in homogenous groups of subjects with a similar maximal aerobic capacity (Atkinson et al. 2003). Thus, exercise economy defined as the oxygen uptake at a given sub-maximal power-output has been suggested as an additional determinant of cardiorespiratory function (Saunders et al. 2004).

2.1.3 Hormonal mechanisms related to physical exercise

Hormones are chemical substances synthesized by specific host glands belonging to the endocrine system, from where they are released into circulation and transported throughout the periphery in order to bind to specific target cells (McArdle, Katch & Katch 2007, p. 410). Thus, in addition to the nervous and cardiorespiratory systems, the endocrine system plays an integrative role in coordinating and initializing homeostasis.

Typically, hormones are grouped into anabolic and catabolic steroids as well as amino-acid based hormones and eicosanoids (Kraemer & Ratamess 2005). Anabolism and catabolism generally refer to the restructuring of bodily tissues with hormones acting as a major mediator in muscle growth (Bhasin, Woodhouse & Storer 2001). Subsequently, such hormones are of special interest to sport and exercise research.

Once bound to the target receptor, hormonal reactions may initiate widespread physiological effects to stabilize the body's internal environment by integrating and regulating bodily functions (Kraemer & Ratamess 2005). Hormones may, thus, alter cellular reactions of target cells by: i) modifying the rate of intracellular protein synthesis by stimulating DNA in the nucleus, ii) changing the rate of enzyme activity, iii) altering plasma membrane transport via a second messenger system and iiii) inducing secretory activity (McArdle, Katch & Katch 2007, p. 410). It should, however, be noted that circulating levels of hormones may not ultimately reflect changes in physiological function at the cellular levels. In fact, the effect of altered hormonal concentrations on subsequent physiological reactions cannot be observed until the cellular responses have been initialized by a receptor interaction. Thus, the final outcomes of hormonal reactions are dependent on availability and sensitivity of hormone receptors as well as the availability of substrates and materials necessary for adaptive responses (Tremblay & Chu 2000). Specific responses of target cells may therefore be observed after a period of seconds, minutes or even days (Keizer 1998, p. 146).

Previous studies have identified both endurance and strength training as powerful stimuli for acute and chronic endocrine reactions (Consitt, Copeland & Tremblay 2002; Hackney, Fahrner & Gulledge 1998; Kraemer & Ratamess 2005; Häkkinen et al. 1988a). Within the context of exercise training, biomarkers that have been identified to reflect physical exertion and initialization of physio-

logical processes include testosterone (Tremblay, Copeland & Van Helder 2005; Daly et al. 2005) and growth hormone (22-kda) (Näveri, Kuoppasalmi & Härkönen 1985; Kokalas et al. 2004) as anabolic hormones and cortisol as a catabolic hormone (Viru et al. 1996; Karkoulias et al. 2008). Thus, the present literature review will put emphasizes on these three hormones.

When interpreting hormonal data, it should be noted that transient and chronic changes in hormonal concentrations following prolonged exercise and training may not necessarily reflect alterations within the endocrine system as indicated by increased or decreased secretion rates but may be the result of: i) increased or reduced hepatic clearance, ii) alterations in plasma volume or fluid shifts or iii) increased or reduced degradation rates (Kraemer & Ratamess 2005). Furthermore, also factors related to biological variations (i.e. different hormonal concentrations due to differences in sex, race, age, body composition, circadian rhythms and mental health) and procedural differences (i.e. nutrition, stress and sleep, physical activity, participant posture, environment, geographical location, timing of specimen collection, and type of specimen collected) may affect obtained hormonal concentrations (Hackney & Viru 2008). Thus, despite hormonal concentrations being considered as an important tool to assess exercise-induced stress levels, caution should be given especially when comparing hormonal data between subjects as well as data coming from different study designs.

2.2 Endurance versus strength exercise and training

2.2.1 Acute neuromuscular responses and chronic adaptations

Endurance cycling and running typically involve prolonged alternated rhythmic flexion and extension of the limbs at low force levels (Häkkinen et al. 2003). Performance of strength protocols, on the other hand, requires a rhythmic bilateral or unilateral extension and flexion at high force levels and, thus, the expected neuromuscular responses and adaptations to endurance versus strength exercise and training are dissimilar. However, irrespective of the type of exercise (i.e. endurance or strength), neuromuscular fatigue may originate both centrally as indicated by lower recruitment rates and/or firing frequency (Gandevia 2001) and peripherally as characterized by changes in contractile processes (Bigland-Ritchie, Furbush & Woods 1986).

2.2.1.1 Endurance exercise and training

Acute exercise responses

The acute neuromuscular responses to endurance exercise generally occur on a smaller scale than those induced by resistance training. However, the reductions in strength loss following prolonged endurance exercise may still be significant, as acute decreases of 10 - 30% are typically observed (Millet, Vleck &

Bentley 2009). The magnitude of neuromuscular fatigue induced by a single session of endurance exercise seems to be related to the intensity and duration of the exercise as well as the type of endurance loading performed (e.g. endurance running vs. cycling). Endurance cycling is biomechanically similar to commonly performed lower body strength exercises such as leg press or squats (Fonda & Sarabon 2012) and may, dependent on the protocol (i.e. number of sets and repetitions), lead to a similar magnitude of fatigue as indicated, for example, by inhibited corticospinal output observed during a single isometric contraction (Seifert & Petersen 2010; Sidhu et al. 2013). From previous studies it appears that the acute strength loss following endurance running occurs in a dose-response manner to the exercise duration (Millet et al. 2003), while this relationship seems to be less consistent in endurance cycling (Millet & Lepers 2004).

Previous studies have indicated that the magnitude of contribution of the central nervous system to exercise-induced neuromuscular fatigue may differ between endurance running and cycling. Lepers et al. (2000) have shown similar reductions in EMG of vastii lateralis and medialis and the M-wave amplitude (~10%) in acute response to 2 h of continuous cycling in well trained cyclists, possibly indicating that central fatigue may have not been the cause for the overall 13% reductions in strength. In contrast, Millet et al. (2003) showed that indeed central fatigue was the major cause for strength loss after a prolonged 30 km run in trained male runners, as indicated by a decreased voluntary activation percentage and a lower ratio of vastus lateralis EMG divided by the M-wave amplitude.

Chronic training adaptations

The effects of prolonged endurance training on chronic neuromuscular adaptations have not been well studied. In contrast to strength training-induced neural adaptations, Cohen et al. (2010) showed that maximal strength and rate of force development seemed to remain unaffected in men who were systematically trained for swimming and triathlon. These findings are in line with findings of Vila-chã et al. (2010) following short-term training of endurance cycling (i.e. 6 weeks), while others have shown improvements in maximal strength following 16 weeks of moderate to vigorous intensity endurance cycling (Izquierdo et al. 2005). Similarly, data regarding changes in EMG amplitude are less conclusive as both no changes following short term training (Vila-Chã, Falla & Farina 2010) and increases during the first 30 ms of the isometric knee extension and flexion (Cohen et al. 2010) in endurance trained men have been observed. Moreover, Vila-chã et al. (2010) have shown enhanced resistance to fatigue after a training intervention, as indicated by a decreased motor unit discharge rate at the same relative sub-maximal force level during isometric knee extension. As a result of that, the authors concluded that more motor units must have been activated. This finding may in turn provide evidence that endurance training-induced neural adaptations are observed at sub-maximal rather than maximal force levels, as typically shown following prolonged strength training.

In addition to neural adaptations, previous studies have shown that prolonged training of endurance cycling may induce small but statistically significant increases in muscle cross-sectional area (CSA) (Mikkola et al. 2012) in physically active subjects with no experience in regular endurance or strength training. In contrast, while prolonged training of endurance running may theoretically improve muscle mass as has previously been shown in mice (Kemi et al. 2002), in humans statistically significant decreases in the size of type I and type IIc fibres have been observed following a prolonged endurance running intervention using high-intensity training (Kraemer et al. 1995). It should, however, be noted, that increases in muscle mass may not be desired by athletes of weight bearing endurance sports such as running due to the accompanied increases in bodyweight which may possibly be detrimental to endurance performance. Thus, it remains unclear whether endurance running-induced reductions in the size of type I and IIc fibres are indicative of a compromised training effect on a cellular level as was previously suggested (Kraemer et al. 1995).

2.2.1.2 Strength exercise and training

Acute exercise responses

The performance of heavy resistance loadings typically leads to drastic acute decreases in maximal neural activation, maximal force production and force-time characteristics of the muscles loaded (Häkkinen 1993; Häkkinen 1994). The magnitude of neuromuscular fatigue, however, seems to be dependent on the volume and intensity of the strength loading protocol (FIGURE 1) as well as on the exercise mode performed (Häkkinen 1994; Linnamo, Häkkinen & Komi 1997; McCaulley et al. 2009).

Following strenuous neural type of resistance loading protocols of 20 x 1RM in a horizontal leg press, acute reductions in maximal strength of up to 25% have previously been reported in strength trained men (Häkkinen 1993). Similar reductions have also been shown in both maximal and rapid force production following a maximal strength protocol consisting of 11 x 3 repetitions at loads of 90% of 1RM during parallel squats (McCaulley et al. 2009) (FIGURE 1). Hypertrophic-type loadings characterized by a larger number of repetitions at lower loads and shorter rest periods, on the other hand, may lead to much larger reductions in force production of up to 40% (Ahtiainen et al. 2003b) and when the load was further increased so that assistance was required in order to complete the set, acute reductions in maximal force greater than 50% were observed.

Following exercise cessation, maximal force production remained reduced for 2 days in the non-assisted protocol and was still reduced post 72 h following the assisted loading (Ahtiainen et al. 2003b). In contrast to these metabolic fatiguing protocols, the recovery of force production following explosive type of strength loadings characterized by lower loads and longer inter-set rests may already be completed after only few hours (Linnamo, Häkkinen & Komi 1997; McCaulley et al. 2009) (FIGURE 1).

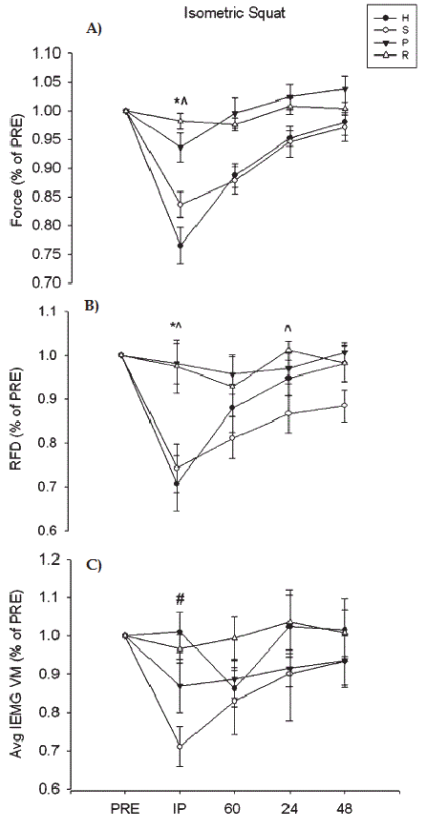

FIGURE 1 Acute decreases in maximal force (A), rate of force development (B) and EMG (C) during an isometric squat following hypertrophic (H), maximal strength (S), and power (P) loadings matched for total work. R (resting condition), IP (immediately post), 60 (60 minutes post), 24 (24 h post) and 48 (48 h post) (McCaulley et al. 2009). Copyright 2009 by Springer Publishing, reproduced with permission.

The observed reductions in force production may be the result of accompanied declines in muscle activation as indicated by reductions in maximal EMG. However, typically reductions in EMG amplitude are observed following maximal strength protocols (McCaulley et al. 2009) rather than hypertrophic loadings (McCaulley et al. 2009; Smilios, Häkkinen & Tokmakidis 2010) (FIGURE 1), possibly indicating the origin of fatigue to be centrally- rather than peripherally-oriented in such protocols.

Chronic training adaptations

Prolonged strength training typically leads to increases in maximal force production induced by significant neural and morphological changes. In early studies, it was shown that in young subjects with (Häkkinen & Komi 1983) and without (Moritani 1979) prior strength training experience the early improvements in muscle strength may be the primary result of neural adaptations,

while further improvements in maximal strength beyond the initial weeks of training may be accounted for by muscle growth. Interestingly, Kamen & Knight (2004) showed that maximal motor unit discharge rates of the vastus lateralis increased already after 7 days of hypertrophic strength training. Similarly, Vila-Chã et al. (2010) reported increases in motor unit discharge rates following three weeks of resistance training at loads of 60 - 75% of 1RM, indicating a very short time frame for initial neural adaptations to take place. Further neural adaptations following prolonged strength training generally include an increase in voluntary activation as a result of improved motor unit recruitment and/or firing frequency (Folland & Williams 2007). Morphological adaptations such as increases in whole muscle CSA, on the other hand, may be observed after 8 - 12 weeks of training (Folland & Williams 2007), while some evidence exists for muscle hypertrophy to be detectable already after 3 weeks of coupled concentric and eccentric resistance training (Seynnes, de Boer & Narici 2007).

The proportion of neural versus morphological adaptations, however, is dependent both on the training mode and -duration (Schoenfeld 2010, Folland & Williams 2007). Maximal and explosive strength training primarily leads to neural adaptations, while metabolic demanding hypertrophic strength training typically induces muscle growth (Kraemer & Ratamess 2004). In addition to chronic adaptations in neuromuscular function, previous studies have also shown that the acute responses to strength exercise may be altered by prolonged resistance training. Walker et al. (2010) found that 11 weeks of mixed maximal and explosive strength training (i.e. contrast training) led to significant acute decreases in squat jump performance induced by a contrasting strength protocol. These acute reductions in explosive strength performance were accompanied by a significantly larger decrease in maximal isometric force, rate of force development and EMG of vastus lateralis when post-training the same relative loads were used as before the training intervention. An increased magnitude of acute reductions in force production was also observed by Izquierdo et al. (2009a) when a strenuous hypertrophic strength protocol was performed after short-term heavy resistance training (i.e. 7 weeks) using both the pre-training loads as well as the new corresponding relative loads. These findings indicate that the rate of fatigue development was faster after training and, thus, the muscles were able to perform more work before task failure (i.e. improved resistance against fatigue). Interestingly, however, in the latter study the acute strength-loading induced changes in EMG amplitude and median frequency during isometric leg press were similar before and after the training intervention in both loading conditions. Thus, it was concluded that the mechanisms underlying the increased capacity to perform new relative loads were peripheral rather than central. However, whether this assumption can be applied to other loading protocols remains to be investigated.

2.2.2 Acute cardiorespiratory responses and chronic adaptations to endurance versus strength exercise and training

Theoretically, both endurance and strength exercise and training may potentially induce a variety of distinct responses and adaptations within the cardiorespiratory system (Hawley 2002). However, due to the vast energy demands during prolonged aerobic exercise, the magnitude of cardiorespiratory adaptations following prolonged endurance training is expected to be much larger than that induced by strength training.

Oxygen uptake kinetic during exercise

During the transition from rest to constant-load exercise of low or moderate intensity, oxygen uptake increases in a non-exponential manner until it plateaus after 2 - 3 minutes (Poole & Jones 2012). During strenuous exercise at intensities beyond the blood lactate threshold, on the other hand, the oxygen uptake may continue to rise despite a plateau in power output (Whipp 1994). The phenomenon of plateaued O_2 consumption is typically referred to as the VO_2 slow component. Carter et al. (2000) have shown that the oxygen uptake kinetics during cycling and running are generally similar. However the magnitude of the slow VO_2 component as typically observed during cycling exercise may be considerably lower in running at similar relative intensity, possibly related to different muscle contraction regimens between the two exercise modes (Carter et al. 2000).

In addition to possible differences in the oxygen uptake kinetics between endurance cycling and running, it has previously been shown that endurance running induces a higher oxygen uptake and possibly energy expenditure when compared to endurance cycling at the same relative exercise intensity (as controlled by the rate of perceived exertion and measured at values of 11 [fairly light], 13 [somewhat hard] and 5 [hard]) (Zeni, Hoffman & Clifford 1996). Furthermore, as the production of blood lactate is dependent on the oxidative demands of the exercise (i.e. exercise intensity), it is likely that the lactate kinetics are different between protocols performed on the treadmill or cycle ergometer as has previously been shown by differences of the exercise intensity at the blood lactate threshold (i.e. OBLA) (for review see Millet, Vleck & Bentley 2009). Similarly, Carter et al. (2000) showed that in subjects who are not specifically trained for cycling, the lactate threshold reported as a percentage of VO_{2max} is higher during running when compared to cycling.

Blood lactate kinetics during exercise

While the assessment of blood lactate concentrations has been proven useful, especially for guidance and evaluation of the endurance training progress, its significance as a physiological marker of metabolic stress during strength exercise requires caution. Significant increases in acute responses to strength exercise are typically expected following strength-endurance (Kang et al. 2005) and hypertrophic loading protocols (Raastad, Bjøro & Hallen 2000; Kang et al. 2005), both of which are characterized as metabolically demanding due to a larger

number of repetitions combined with shorter inter-set rest periods. On the other hand, explosive and maximal strength loadings typically stress the neural system more so than hypertrophic strength protocols. Thus, even though such protocols may result in a significant acute strength loss, they may induce only minor alterations in blood lactate concentrations (Linnamo et al. 2005; McCaulley et al. 2009).

Oxygen uptake and blood lactate kinetics during recovery

Depending on the exercise intensity, oxygen uptake and blood lactate concentrations may remain elevated above resting values following exercise cessation. Oxygen uptake may remain elevated above resting values for several hours (Drummond et al. 2005; McArdle, Katch & Katch 2007, p. 169), which is typically referred to as excess post exercise oxygen consumption (EPOC) and is described as the oxygen depth or recovery oxygen uptake (Gaesser & Brooks 1984). The increased energy demands (EPOC) have been shown both following endurance (Borsheim & Bahr 2003) and strength (Nagasawa 2008) exercise, presumably having an effect on prolonged cardiorespiratory adaptations if insufficient recovery is provided. The duration of EPOC following endurance running and cycling seems to relate linearly to the exercise intensity and duration (Borsheim & Bahr 2003). Interestingly, when comparing endurance and strength exercise matched for the overall energy cost (Gillette, Bullough & Melby 1994), oxygen uptake during 5 h of recovery was higher following the whole body strength protocol (5 x 8 - 12 repetitions at 70% of 1RM) compared to the moderate intensity (50% of VO_{2max}) endurance cycling. Similarly, when work was matched for oxygen uptake (Burleson et al. 1998), circuit resistance exercise seemed to produce a greater EPOC response during the first 30 minutes when compared to low-intensity treadmill running or walking but the reasons for these findings remain unclear.

In contrast to EPOC, the half-life of blood lactate concentrations has been suggested to be approximately 25 minutes (Hermansen et al. 1975). Thus, 95% of the accumulated blood lactate may be removed from the circulation after less than 90 minutes. During this early recovery, up to 60% of the accumulated blood lactate is aerobically metabolized while the remaining 40% is converted to glucose and protein and a small portion is excreted in the urine and sweat (Hermansen et al. 1975).

Chronic training adaptations

During prolonged endurance training, increases in cardiac output, along with an increased extraction of oxygen by the exercising muscles result in a greater VO_{2max} (Jones & Carter 2000). The magnitude of endurance training-induced increases in VO_{2max} seems to depend on a number of factors including the initial fitness status, the duration and the intensity of the training intervention as well as the duration and frequency of each individual training session (Wenger & Bell 1986).

The previous findings regarding exercise economy, on the other hand, are equivocal. Even though endurance-trained athletes are typically considered as more economical compared to less fit subjects (Morgan et al. 1995), training studies of shorter durations (i.e. 6 - 12 weeks) often fail to show significant changes in exercise economy. Jones & Carter (2000) speculated that the improved exercise economy may be related to the overall training volume as desirable economy values are typically found in older or more experienced subjects, indicated by a larger weekly training mileage.

In addition to direct markers of cardiorespiratory fitness, prolonged endurance training is likely to induce reductions in body fat mass, attributed to enhanced fat oxidation (Pratley et al. 2000). As previous studies have shown a strong association between changes in fat mass and blood lipids, prolonged endurance training is also likely to induce a positive blood lipid profile (Bobo & Bradley 1999; Sillanpää et al. 2009).

During prolonged strength training, on the other hand, typically no changes in VO_{2max} are expected (Hickson, Rosenkoetter & Brown 1980; Hurley et al. 1984). However, previous studies have shown that prolonged strength training may induce improvements in exercise economy especially in endurance trained subjects (Rønnestad & Mujika 2014) (for details see chapter 2.3). Furthermore, the prolonged strength training-induced cardiorespiratory adaptations may not be directly observable. Since lean body mass has been shown to be a major determinant of basal metabolic rate by representing 60 - 75% of an individual's daily energy expenditure (Sjödin et al. 1996), chronic increases in muscle and lean mass may have potential health benefits. Nevertheless, the direct effects of strength training on body fat and blood lipids have previously been shown to be minimal (Ghahramanloo, Midgley & Bentley 2009; Lemmer et al. 2001; Sillanpää et al. 2009) but small changes may be seen, especially in reductions of low-density lipoprotein (LDL-C) (Tambalis et al. 2009).

In addition to adaptations in oxygen uptake, body composition and blood lipids, prolonged endurance training may typically also lead to a decrease in blood lactate concentrations at sub-maximal power outputs and, thus, a delayed OBLA as shown by a right shift of the blood lactate curve (MacRae et al. 1992). As a consequence, endurance performance is enhanced as the blood pH can be maintained for a longer duration. However, while the mechanisms underlying the right-shift of OBLA are similar during prolonged endurance and strength training interventions, strength training has previously been shown to be less effective in delaying OBLA. Nevertheless, Warren et al. (1992) showed reductions in concentrations of blood lactate after already one week of high volume resistance training. In contrast to these findings, the magnitude of acute strength loading-induced increases in blood lactate concentrations may actually be larger following a period of prolonged strength training when compared to that observed pre-training (Walker et al. 2013). While this has been suggested to indicate a greater capacity to produce fatigue, such assumptions must be considered with caution as cellular buffering capacities may have increased as well as a result of the prolonged training inter-

vention. Thus, the meaning of acute exercise-increased lactate concentrations following prolonged training remains to be clarified.

2.2.3 Acute hormonal responses and chronic adaptations

The magnitude of acute endurance and strength-exercise-induced hormonal responses depends on the exercise intensity and volume, as well as the exercise mode performed (Kraemer et al. 1990; Häkkinen & Pakarinen 1995; Hackney et al. 2012; Stokes et al. 2013). Among other physiological functions, it is likely that the acute alterations in anabolic and catabolic hormone concentrations directly affect the rates of protein synthesis, red blood cell production and energy restoration (Shahani et al. 2009; Vingren et al. 2010), facilitating biological adaptations to prolonged training. However, although the hormonal responses to short term endurance and strength exercises are rather similar (Stokes et al. 2013), their physiological functions may differ due to the catabolic versus anabolic nature of both types of exercises.

2.2.3.1 Endurance exercise and training

Acute exercise responses

Short bouts of high intensity endurance exercise may induce acute elevations in both anabolic (e.g. testosterone, growth hormone) and catabolic (e.g. cortisol) hormone concentrations (Pritzlaff et al. 1999; Hackney et al. 2012; Stokes et al. 2013). Prolonged and physically demanding endurance performance (e.g. a marathon run), on the other hand, may in its final phases lead to decreases in testosterone and simultaneous increases in cortisol concentrations (Kuoppasalmi et al. 1980).

When systematically investigating the effect of endurance exercise duration on acute hormone responses, Tremblay et al. (2005) observed a significant relationship with both free and total testosterone in endurance runners. Testosterone levels showed an initial increase of about 20% during the first hour of an 80 and 120 minutes run at low intensity (i.e. at 55% of VO_{2max}), while 40 minutes of running at the same intensity affected serum testosterone concentrations to a much lower extent. Thus, testosterone concentrations seem to increase after 40 minutes of exercise (Tremblay, Copeland & Van Helder 2005), reach peak values at 60 - 80 minutes of running (Daly et al. 2005) (FIGURE 2A) and decline after marathon or ultra- marathon distances with durations beyond 4 h (Kuoppasalmi et al. 1980; Kraemer et al. 2008; Karkoulias et al. 2008) in endurance trained subjects.

During recovery, significant decreased testosterone levels for up to 6 h post-exercise following 45 - 90 minutes of distance running were observed, while after 24 h plasma testosterone had almost returned to baseline values (Kuoppasalmi et al. 1980). These findings are well in line with those of other studies showing reductions in testosterone concentrations for 3 h following 80 and 120 minutes of continuous running at low exercise intensities (Tremblay,

Copeland & Van Helder 2005) and up to 24 h following a treadmill run to volitional fatigue (Daly et al. 2005) (FIGURE 2A).

In contrast to endurance exercise-induced changes in testosterone concentrations, the relationship between the exercise intensity and duration and acute changes in cortisol concentrations seems to be less consistent. A review by Viru et al. (1996) showed that cortisol concentrations only increased in response to endurance loadings above an intensity threshold of 60 - 70% of VO_{2max}. Tremblay et al. (2005), on the other hand, observed increases in cortisol concentrations already at lower exercise running intensities (i.e. 55% of VO_{2max}) after a duration exceeding 80 minutes, while endurance rowing lasting less than 1 h at moderate intensity (i.e. at OBLA) did not seem to be sufficient to stimulate cortisol secretion (Kokalas et al. 2004). While these findings emphasize the importance of exercise intensity and duration, circulating cortisol concentrations may be altered by endurance exercise in a biphasic manner (Daly et al. 2005). According to their study, an initial increase in cortisol may occur during the first 10 - 20 minutes and a subsequent peak may be observed immediately following volitional fatigue (i.e. after 85 ± 4 minutes at the ventilatory threshold) (FIGURE 2B). This hypothesis was confirmed by another study in long-distance runners, which showed that increases in cortisol take place earlier if the intensity is higher (Vuorimaa et al. 2008), possibly due to a stimulation threshold of the pituitary-adrenal axis which controls the release of cortisol in response to stress (Karkoulias et al. 2008). During recovery of 24 h, cortisol concentrations may be significantly lowered compared to baseline values (Daly et al. 2005).

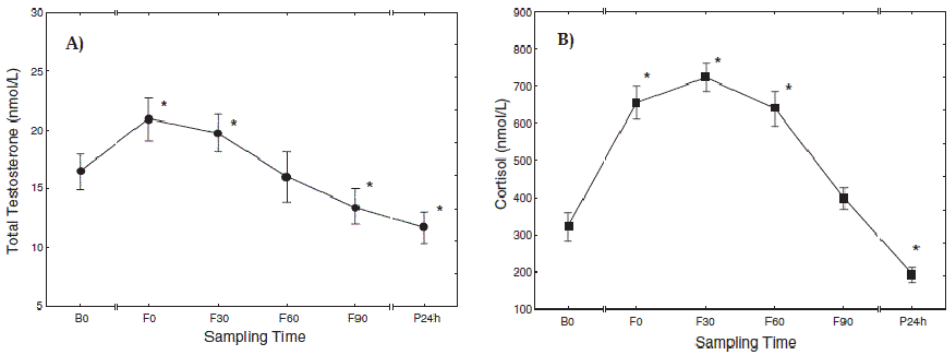

FIGURE 2 Testosterone (A) and cortisol (B) concentrations at baseline (B0), after a treadmill run to volitional fatigue (F0) as well as during recovery of 30 minutes (F30), 60 minutes (F60), 90 minutes (F90) and after 24 h (P24h) (Daly et al. 2005). Copyright 2005 by Springer Publishing, reproduced with permission.

In contrast to testosterone and cortisol concentrations, growth hormone is secreted in a pulsatile manner and, thus, assessed concentrations are much more dependent on the timing of the sample collection. Most of the available literature on acute endurance exercise-induced growth hormone responses stems from high intensity sprint-type loadings. Pritzlaff et al. (1999) showed statistically significant increases in growth hormone concentrations only when an intensity equal or greater than the lactate threshold was performed in recreation-

ally active men during 30 minutes of treadmill running. Similar results have also been observed following 30 minutes of continuous running at 70% of VO_{2max} (Stokes et al. 2013) and following 60 minutes of rowing at OBLA in endurance-trained subjects (Kokalas et al. 2004), while 1 h endurance cycling at lower intensity (50% of VO_{2max}) (Wahl et al. 2010) and 90 minutes at moderate intensity running (4.3 $min \cdot km^{-1}$) (Näveri, Kuoppasalmi & Härkönen 1985) were not sufficient to induce significant changes in growth hormone concentrations. Growth hormone concentrations typically return to baseline shortly post-exercise (Näveri, Kuoppasalmi & Härkönen 1985; Kokalas et al. 2004).

Chronic training adaptations

During prolonged endurance training, equivocal findings regarding the changes in basal hormone concentrations have been observed. While it has been shown that the basal testicular testosterone production is lower in endurance-trained men compared to untrained subjects (Hackney, Szczepanowska & Viru 2003; Hackney 1996), Grandys et al. (2009) found increased basal concentrations of total testosterone by 17% and free testosterone by 26% after only 5 weeks of cycling training at 90% of the power output from the predicted lactate threshold. Thus, it is likely that changes in basal testosterone concentrations occur in a biphasic manner with initial increases being observed after the onset of training (Grandys et al. 2009) and reductions following prolonged training. In addition, it has previously been suggested that cortisol concentrations may be higher following prolonged endurance training (Kraemer et al. 1995) but decreases may also be observed, as has been shown after 24 weeks of rowing training (Purge, Jürimäe & Jürimäe 2006). Data regarding the changes in basal concentrations of growth hormone are rare but one year of endurance training above the lactate threshold has previously been shown to amplify the pulsatile release of growth hormone (Weltman et al. 1992).

Also the acute hormone responses to endurance exercise may be altered by prolonged endurance training. Kraemer et al. (1995) found significantly larger acute running-induced cortisol responses as indicated by an increased area under the curve following 12 weeks of high intensity running in physically trained men. On the other hand, endurance exercise-induced growth hormone responses have been shown to be statistically reduced following prolonged endurance training when the workload was similar to that performed before the training intervention (Weltman et al. 1997; Hartley et al. 1972). In addition, also a shift of the peak of growth hormone release post-exercise from 4 to 8 minutes has previously been observed (Craig et al. 1991).

2.2.3.2 Strength exercise and training

Acute exercise responses

Literature regarding the acute effects of strength exercise on hormonal concentrations is more consistent when compared to that investigating endurance exercise. Strength protocols of heavy loads and short inter-set rest periods (i.e. hypertrophic strength loadings) may result in acute increases in serum testos-

terone and growth hormone, as well as cortisol concentrations (Kraemer et al. 1990). On the other hand, maximal strength loadings with very heavy loads as well as explosive strength protocols requiring maximal movement velocity typically include prolonged inter-set rest and may not induce sufficient physiological stress to cause as large an increase in concentrations of anabolic or catabolic hormone concentrations (Kraemer et al. 1990; Häkkinen & Pakarinen 1993; Linnamo et al. 2005).

In a study by Häkkinen & Pakarinen (1993), significant increases in testosterone concentrations were observed following 10 x 10 repetitions of squats using 70% of 1RM loads but not when the same work was performed as 20 x 1RM. These early findings were more recently confirmed in a study by McCaulley et al. (2009). Importantly, while most studies have looked at total testosterone concentrations, it is the unbound fraction which is physiological important for inducing beneficial receptor interactions required to induce tissue remodeling (Kraemer & Ratamess 2005). Even though in many studies the free testosterone responses were similar to that of total testosterone (Ahtiainen et al. 2003b; Durand et al. 2003), in another study this has not been observed (Häkkinen et al. 1988a).

Besides the characteristics of the loading protocol, training status may also affect the magnitude of testosterone responses. Tremblay et al. (2004) showed that the acute elevation of free testosterone concentrations was greater in men who were resistance-trained compared to those trained for endurance. Furthermore, also the muscle mass involved in a specific exercise should be considered. Exercise involving a larger muscle mass, such as Olympic lifts or dead lifts, may cause greater acute elevations in testosterone compared to exercises involving much smaller muscle-mass (Kraemer & Ratamess 2005).

The acute exercise-induced increase in testosterone concentrations may be followed by a subsequent reduction in testosterone levels during a post-loading recovery period. Previous studies have reported lower values after 15 and 30 minutes (Ahtiainen et al. 2003a) as well as after 2 h (Häkkinen & Pakarinen 1995; Raastad 2000) and even up to 48 h following very strenuous protocols (Häkkinen & Pakarinen 1993) but the reasons for these observations remain to be investigated.

Similarly to strength exercise-induced changes in testosterone, cortisol concentrations may also be transiently increased during heavy strength loading (Kraemer & Ratamess 2005). Previous studies have shown significant correlations between blood lactate and serum cortisol concentrations, indicating metabolic stress as one cause for temporary elevations in cortisol levels (Ratamess et al. 2004; Kraemer et al. 1989). Thus, as with testosterone concentrations, highest acute elevations of cortisol are observed when heavy loads (i.e. hypertrophic loading protocols) with short inter-set rest periods and a large number of repetitions are performed (Smilios et al. 2003; Ratamess et al. 2004; McCaulley et al. 2009). In addition, endurance-trained athletes seem to show a smaller strength exercise-induced response in cortisol concentrations compared to strength-trained athletes (Tremblay, Copeland & Van Helder 2004). During recovery,

cortisol concentrations seem to return to baseline within 60 minutes (McCaulley et al. 2009) or 2 h post-loading (Häkkinen & Pakarinen 1995).

Most studies provided evidence that growth hormone reacts in a similar manner as testosterone and cortisol in response to resistance loadings. Thus, it appears that the overall load as well as the volume and the inter-set rest periods of the loading protocol seem to determine the magnitude of the growth hormone response (Häkkinen & Pakarinen 1993). Smilios et al. (2003) showed that the acute growth hormone response increased when performing 4 compared to 2 sets while a further increase to 6 sets did not intensify this response. Following loading, growth hormone concentrations may remain elevated for 30 - 60 minutes after which they return to baseline values (Häkkinen & Pakarinen 1993, Kraemer et al. 1990).

Chronic training adaptations

As with prolonged endurance training, previous findings regarding chronic changes in basal hormone concentrations following strength training are equivocal (Kraemer & Ratamess 2005). While in some studies no changes have been observed (Craig, Brown & Everhart 1989; McCall et al. 1999; Ahtiainen et al. 2003a; Häkkinen et al. 2000), others found increases (Häkkinen et al. 1985; Sallinen et al. 2007; Kraemer et al. 1999) or even decreases (Rankin et al. 2004; Fry et al. 1993) following prolonged training of more than 10 weeks. When investigating Olympic weight lifters over the course of one year, no changes in basal testosterone concentrations were observed (Häkkinen et al. 1987), while during the second year testosterone levels significantly increased (Häkkinen et al. 1988b). Ahtiainen et al. (2003a) showed increases in total and free testosterone concentrations in strength-trained athletes following 14 weeks of heavy resistance training but after a reduction of training volume and simultaneous increase in intensity during the subsequent 7 weeks of training testosterone concentrations were reduced. Thus, it is likely that basal hormone concentrations are altered parallel to changes in the training program, which provides an important methodological consideration for prolonged training studies.

The acute strength-loading-induced changes in serum hormone concentrations may also be altered by prolonged strength training. Previous studies have shown that testosterone (Kraemer et al. 1998) and growth hormone (Craig, Brown & Everhart 1989; Izquierdo et al. 2009b) concentrations may be acutely elevated following short-term (7 - 12 weeks) resistance training, while also no changes for both testosterone (Craig, Brown & Everhart 1989) and growth hormone (McCall et al. 1999) have been reported. In a study by Walker et al. (2015) in men, greater acute strength loading-induced growth hormone responses after training were associated with larger gains in leg lean mass. However, while this finding indicates an endocrine adaptation, the present data cannot rule out that observed training-induced increases in growth hormone responses are at least partly attributed to the increased muscle mass in these subjects.

In contrast to anabolic hormone responses, the acute strength exercise-induced cortisol response may actually be lower following a prolonged training

intervention (Hickson et al. 1994; Kraemer et al. 1999), even though some studies have shown maintained cortisol responses post-training (Kraemer et al. 1998; Izquierdo et al. 2009b; Walker et al. 2015). As cortisol is typically considered as a stress hormone, reduced cortisol responses after training may parallel training-induced fatigue resistance as has previously been shown in force responses after prolonged strength training (Izquierdo et al. 2011; Izquierdo et al. 2009a; Walker, Ahtiainen & Häkkinen 2010) and may, thus, indicate a positive training adaptation.

2.3 Concurrent endurance and strength exercise in previously untrained subjects and endurance athletes

2.3.1 Acute effects of endurance exercise on subsequent strength loadings

In line with the neuromuscular responses to endurance exercise (chapter 2.2.1.1), endurance performance may considerably affect the force production during subsequent strength loadings. Following an intense bout of 5 x 5 minutes cycling intervals at intensities of 40%, 60%, 80% and 100% of VO_{2peak}, significant reductions in the repetitions to failure were observed during the subsequent strength loading in physically active subjects (Leveritt & Abernethy 1999). In this study, the strength protocol consisted of isoinertial squat lifts at 80% of 1RM and the number of repetitions was reduced from 14 to 9 during set 1 and 10 to 9 during set 3. The authors associated these findings with a reduced blood pH induced by prior endurance exercise but the possible neural component was not investigated. Furthermore, it is possible that the strength loss observed in this study might be dependent on the contraction type and the angular velocity of the resistance loading protocol. While in the same study the torque at a 30° angle during isokinetic knee extension significantly decreased at both low ($60° \cdot s^{-1}$) and high ($300° \cdot s^{-1}$) contraction velocities (Leveritt & Abernethy 1999), another study has shown no strength loss at low velocities following a run to volitional exhaustion at the intensity of the lactate threshold in well-trained endurance runners (Denadai et al. 2007). The authors of the latter study justified their findings with the magnitude of running-induced muscle damage which would especially be reflected at higher angular velocities. However, such explanation is in contrast to the findings of Leveritt & Abernethy (1999), indicating the obvious need for further investigation.

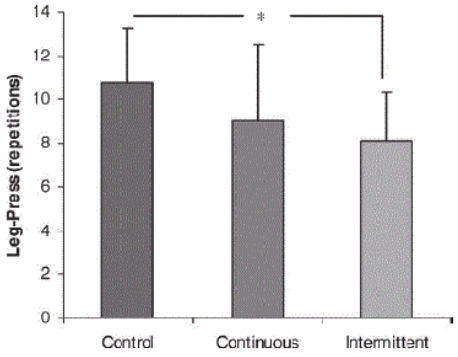

FIGURE 3 Number of repetitions during dynamic leg press performed at 80% of 1RM following rest (Control), a 5 km treadmill run at 90% of the anaerobic threshold (Continuous) and 5 km running performed intermittently 1:1 min at VO_{2max} (De Souza et al. 2007). Copyright Wolters Kluwer Health, Inc., reproduced with permission.

The strength loss following endurance exercise does seem to depend on the endurance exercise mode (De Souza et al. 2007) (FIGURE 3) as well as the recovery duration between the exercise bouts (Sporer & Wenger 2003). Interestingly, in the study by De Souza et al. (2007) (FIGURE 3) no detrimental effects of either endurance protocol on maximal strength (1RM) were observed. Furthermore, no changes in bench press performance were previously observed following endurance running in physically active (De Souza et al. 2007) and resistance-trained men (Reed, Schilling & Murlasits 2013) and after endurance exercise performed on an elliptical machine (Tan et al. 2014) in resistance trained men. Collectively, these findings seem to indicate that peripheral rather than central fatigue may account for the impaired performance during strength loadings conducted immediately after endurance exercise. The reductions in the quality of a strength loading may be apparent for up to 8 h post-endurance exercise but have no longer been observed after 24 h (Sporer & Wenger 2003).

2.3.2 Acute effects of strength exercise on subsequent endurance performance

Previous studies have indicated that the performance of strength loading may acutely alter the economy during subsequent endurance running (Palmer & Sleivert 2001; Doma et al. 2015; Marcora & Bosio 2007; Taipale et al. 2015; Burt et al. 2013) and cycling (Ratkevicius et al. 2006), while in other studies running economy remained unchanged (Doma & Deakin 2014; Paschalis et al. 2005; Conceição et al. 2014). The majority of studies investigating the acute effects of strength loading on subsequent cardiorespiratory function during endurance exercise were conducted in recreational endurance runners. Palmer & Sleivert (2001) showed that in well trained distance runners with at least 3 months of experience in resistance training, the sub-maximal oxygen uptake (economy) was

significantly increased at 1 and 8 h following a whole body hypertrophic resistance training session, while this was no longer observed at 24 h (FIGURE 4).

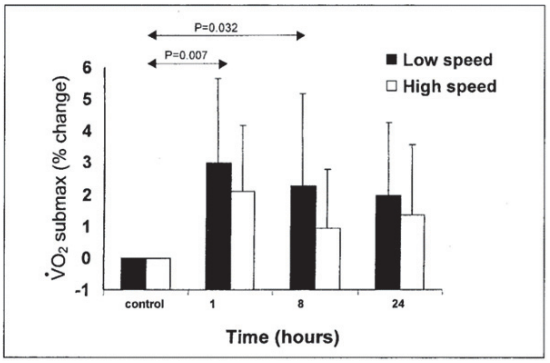

FIGURE 4 Relative changes in sub-maximal oxygen uptake at speeds of 0.56 $m \cdot s^{-1}$ (low) and 0.20 $m \cdot s^{-1}$ (high) below the blood lactate threshold at 1, 8 and 24 h following a whole body resistance workout of 3 x 8RM (Palmer & Sleivert 2001). Copyright 2001 Elsevier Inc., reproduced with permission.

Interestingly, the attenuated economy in this study was not accompanied by increases in heart rate, indicating that the resistance loading caused disturbances at a cellular level. Indeed, these findings are in line with reported results of Schuenke et al. (2002) and Nagasawa (2008) who showed that post-exercise oxygen consumption was significantly increased following resistance exercises, possibly attributed to exercise-induced muscle damage. Increased oxygen uptake above resting levels following a resistance loading session will in turn elevate the metabolic demands and thereby compromise the economy of subsequent endurance exercise. Furthermore, temporary perturbations in biomechanical variables, e.g. induced by neuromuscular fatigue may also subsequently increase aerobic demands, especially during running (Anderson 1996).

In contrast to heavy strength loadings, plyometric exercise protocols may not negatively affect the oxygen demands during subsequent running but may lead to reduced self-paced time trial performance (Marcora & Bosio 2007). From this study it was concluded that the reduced running performance was likely to be related to the sense of effort as running speed was lower and a significant correlation with the rate of perceived exertion (RPE) was found. Furthermore, these findings support a physiological difference between metabolic demanding hypertrophic resistance loading and plyometric (Marcora & Bosio 2007) as well as eccentric (Paschalis et al. 2005) protocols which are more prone to induce muscle damage. However, when hypertrophic and plyometric strength loadings were directly compared no differences in time to exhaustion during treadmill running were observed (Conceição et al. 2014).

The acute effects of strength exercise on the exercise economy during subsequent cycling have been investigated to a lesser extent. Crawford et al. (1991) found no changes in aerobic demands during cycling at 65% following

isokinetic leg exercise of 3 x 8RM in previously untrained men. When plyometric exercises were performed prior to the cycling test, however, significant increases in the oxygen demands were observed at intensities of ~40%, ~50% and ~65% of VO_{2max} (Ratkevicius et al. 2006). The authors in the latter study attributed their findings to an increased recruitment of type II fibers along with an impaired force transmission between the muscle fibers. This reduced neuromuscular function may have resulted from damaged structural proteins, which is especially pronounced following plyometric exercises characterized by intense stretch-shortening activities.

2.3.3 Neuromuscular, cardiorespiratory and hormonal responses to concurrent endurance and strength exercise

The literature regarding the acute responses to concurrent endurance and strength loadings (i.e. either performing endurance exercise followed by strength loading or vice versa) is very limited. In recreational endurance runners, it has been shown that the isometric force production and counter movement jump height acutely decreased to a similar extent when the combined loading was commenced with endurance or strength, respectively (Taipale et al. 2014b). Even though the decrease in vastus medialis EMG in this study was larger following the loading session starting with strength, no statistically significant between-group difference was observed. Maximal and explosive force production returned to baseline by 24 h post-loading.

Regarding the cardiorespiratory responses to concurrent loading sessions, EPOC has been the main variable investigated. The results of these studies, however, are equivocal. Di Blasio et al. (2012) found no significant difference between the two exercise orders in previously untrained women. Similar findings have also been observed in physically active men (Oliveira & Oliveira 2011; Vilacxa Alves et al. 2012), while another study using a similar protocol showed that in physically active men endurance exercise preceding the strength loading may lead to a greater EPOC response when compared to the opposite exercise order (Drummond et al. 2005). The authors of this study concluded that endurance running performed after strength exercise may actually act as an active recovery strategy by possibly enhancing lactate removal (Bond et al. 1991), which was then reflected in lower EPOC responses. Interestingly, the EPOC response following alternating endurance and resistance exercise (i.e. 3 x 10 minutes of treadmill running, each followed by one set of 8 exercises of circuit training) has been shown to be larger than that observed when endurance and strength exercise were performed subsequently (Di Blasio et al. 2012). The reasons for this phenomenon, however, remain to be investigated.

Few studies have investigated the acute hormonal responses to concurrent endurance and strength loadings. Depending on the exercise protocol, combining endurance and resistance exercise in the same session may become metabolic very demanding and lead to increased cortisol concentrations which potentially suppress elevations of testosterone post-loading (Brownlee, Moore & Hackney 2005). In fact, in recreational endurance athletes, a greater cortisol and

growth hormone response has been observed when the concurrent loading session consisting of a mixed maximal and explosive strength protocol and 60 minutes of track running was commenced by strength exercise (Taipale & Häkkinen 2013), while no significant increases in testosterone following either loading order were found. During recovery, total testosterone concentrations actually decreased for up to 48 h following the loading starting with strength exercise, possibly indicating prolonged recovery needs following this exercise sequence. The findings regarding growth hormone responses were in line with a previous study by Goto et al. (2005) who showed that endurance exercise performed before the strength loading may suppress the endocrine release of growth hormone, possibly due to the accumulation of fatty acids.

In addition to these findings, previous studies in recreationally strength-trained (Cadore et al. 2012b) and concurrently endurance and strength-trained athletes (Rosa et al. 2015) performing hypertrophic resistance loadings combined with endurance cycling (Cadore et al. 2012b) and running (Rosa et al. 2015) have shown increased testosterone concentrations only following the loading sequence which commenced with endurance exercise (cycling and running, respectively). Furthermore, Cadore et al. (2012b) showed that the cortisol concentrations were elevated after the first exercise modality (endurance and strength, respectively) in both loading sequences but returned to baseline during the second exercise (strength and endurance, respectively), while Rosa et al. (2015) observed increased cortisol and growth hormone concentrations following both loading conditions. While the authors of these studies concluded that the anabolic environment was optimized when endurance exercise preceded strength loading, the implications of these findings for chronic training adaptations remain to be investigated.

2.4 Neuromuscular, cardiorespiratory and hormonal adaptations to concurrent endurance and strength training

In the pioneering study by Hickson (1980), it has been demonstrated that the development of strength but not endurance performance may be compromised when a high volume of concurrent endurance and strength training (i.e. 5 weekly strength training sessions and 6 weekly endurance training sessions) was performed (FIGURE 5A). Importantly, however, it has later been shown that blunted adaptations in maximal strength development are not observed when the training frequency is reduced to 2 - 3 endurance and 2 - 3 strength training sessions per week (Wilson et al. 2012; Häkkinen et al. 2003) (FIGURE 5B).

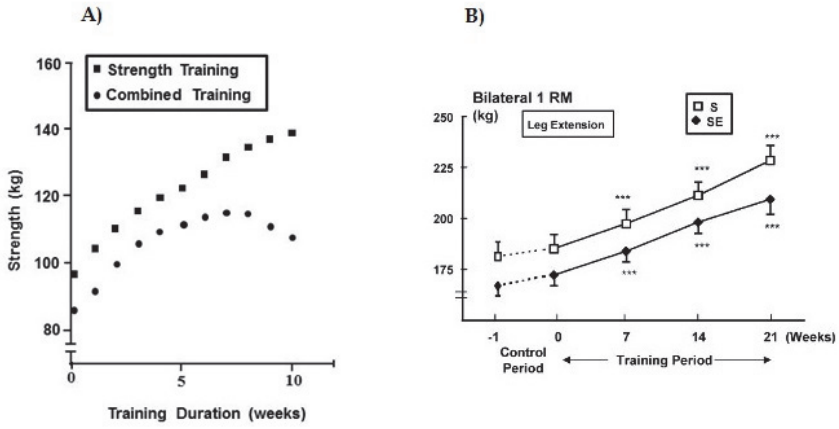

FIGURE 5 Leg strength development during concurrent endurance and strength training of a high (A) (Hickson 1980) and low (B) (Häkkinen et al. 2003) volume. Study A was performed with 5 strength and 6 endurance sessions per week, while study B included only 2 weekly endurance and strength sessions, respectively. Copyright 1980 and 2003 by Springer Publishing, reproduced with permission.

The observed impaired strength development during high volume concurrent endurance and strength training (i.e. "interference") has been explained by both a chronic and acute hypothesis. Hickson (1980) suggested that compromised strength development may occur due to the inability of muscles to adapt to both forms of exercise simultaneously. Craig et al. (1991), on the other hand, proposed that during training programs in which endurance and strength training are performed in close proximity, residual fatigue from the first exercise will detrimentally affect the quality of the subsequent loading, possibly compromising long-term adaptations (for review see chapter 2.3). Indeed, the recovery duration between endurance and strength exercise has previously been shown to affect long-term adaptations, indicating that even 6 h of recovery between subsequent training sessions may not be sufficient for optimal biological adaptations to occur (Robineau et al. 2014). Thus, when discussing chronic adaptations to concurrent training, it should be distinguished between programs in which endurance and strength training are performed on alternating days and those in which both types of training are combined within the same training session.

2.4.1 Adaptations to concurrent training performed on alternating days in previously untrained subjects

Chronic neuromuscular adaptations

Following the early study of Hickson (1980), numerous studies have further investigated the neuromuscular, cardiorespiratory and hormonal adaptations to

concurrent endurance and strength training in a variety of study designs and subject populations. From these studies it appears that the neuromuscular interference seems to be directly dependent on the overall training frequency and volume of endurance training performed (Wilson et al. 2012; Jones et al. 2013). In previously untrained subjects, strength gains were attenuated after 12 weeks when concurrent training was performed 6 days a week (Bell et al. 2000). These findings were in agreement with two previous studies (Kraemer et al. 1995; Dolezal & Potteiger 1998), while also no neuromuscular interference was observed when a similar volume of training was performed (Bell et al. 1991). When the training frequency is reduced to 4 - 6 sessions per week (i.e. 2 - 3 x endurance and strength, respectively), no compromised adaptations in maximal strength and muscle hypertrophy were observed when compared to strength training only (Häkkinen et al. 2003; McCarthy, Pozniak & Agre 2002). However, in the study by Häkkinen et al. (2003) explosive strength development and the average EMG of vastus lateralis during the first 500 ms of rapid isometric force production were significantly smaller in the concurrent training group compared to the strength training group. Similar findings were also observed in other studies (Hennessy & Watson 1994; Mikkola et al. 2012) and a meta-analysis (Wilson et al. 2012) has revealed that adaptations in explosive force production may be more susceptible to interference than strength or hypertrophy, even if the training frequency was low.

When discussing concurrent training studies with regard to adaptations in neuromuscular performance, it should be noted that the training volume in the concurrent training group is often two-fold of that of the endurance or strength training alone. Therefore, it is difficult to distinguish whether the negative effects on neuromuscular performance are potentially attributed to endurance training or are a result of an overall increase in training volume thereby causing overreaching (Hickson 1980). Also, the endurance training mode seems to have an influence on the magnitude of neuromuscular interference. Gergley (2009) directly compared the effects of time-matched endurance running versus cycling combined with resistance training in previously untrained men and found that the decrements in maximal strength were smaller when endurance training was performed by cycling compared to running. These findings are in line with a meta-analysis (Wilson et al. 2012) which also showed that the same was true for adaptations in muscle hypertrophy. It is likely that these findings are attributed to similar force-time characteristics during endurance cycling and lower body strength exercises (Fonda, B., Sarabon, N. 2012; Mann & Hagy 1980). Similarly, the differences in contraction types between cycling and running may also account for the distinct training adaptations as running consists of a larger eccentric component when compared to cycling, thereby causing a greater level of muscle damage (Koller et al. 1998).

Chronic cardiorespiratory adaptations

The influence of concurrent training on cardiorespiratory adaptations and endurance performance has not entirely been investigated (Wilson et al. 2012).

Only a few studies have indicated that VO_{2max} (Nelson et al. 1990; Kraemer et al. 1995) or maximal Watts (Dolezal & Potteiger 1998) may be compromised following prolonged concurrent endurance and strength training. The majority of prolonged training studies in previously untrained men, however, do not indicate compromised cardiorespiratory adaptations when concurrent training performed on alternating days is compared to endurance training alone (Hickson 1980; Bell et al. 2000; Kraemer et al. 1995; Izquierdo et al. 2005). Along with no impaired cardiorespiratory function, no perturbations in body fat mass and blood lipids have been observed from concurrent training. In physically active young men the overall improvements in blood lipids (Ghahramanloo, Midgley & Bentley 2009) and body fat (Ghahramanloo, Midgley & Bentley 2009; Dolezal & Potteiger 1998) following concurrent training have been shown to be larger than those observed after endurance or strength training only, while in older men only minor changes in blood lipids were found (Sillanpää et al. 2008). Yet, whether improved body composition and blood lipids are the result of the increased energy expenditure due to the greater training volume or an adaptation purely induced by the combination of endurance and strength training remains unclear.

Chronic hormonal adaptations

To date, data regarding the endocrine adaptations during prolonged combined training in young men is scarce. While some studies did not find statistical alterations in basal hormonal concentrations (Bell et al. 2000; Kraemer et al. 1995), others have shown significant increases in total and free testosterone to a similar magnitude as that observed after strength training alone (Shakeri et al. 2012). In addition, concurrent high intensity endurance and strength training has been shown to induce larger endurance-loading induced increases in testosterone and cortisol concentrations following 12 weeks of training when compared to the pre-training values (Kraemer et al. 1995). The findings in this study were explained by an "overtraining" response but the influence of the observed increases in anabolic and catabolic hormone concentrations on physiological function and performance remain unknown. More important than circulating hormonal levels are the androgen receptor concentrations. However, at least in old men during 21 weeks of concurrent training no changes in androgen receptor content in the trained muscles were previously observed (Ahtiainen et al. 2009).

2.4.2 Adaptations to concurrent training performed in the same session in previously untrained subjects

Following the indications provided by cross-sectional study designs, the systematic investigation of the physiological adaptations of endurance and strength training combined into the same training session provides some evidence of loading order-specific adaptations (TABLE 1).

In old men a previous study has shown that the force per unit of muscle mass of knee extensors increased to a larger extent when strength training was performed before endurance training (Cadore et al. 2012a). Similarly, lower

body strength gains and neuromuscular economy (normalized EMG at 50% of peak torque) were found when strength training preceded endurance training compared to the opposite exercise sequence, while no differences in muscle thickness were observed (Cadore et al. 2013). In young subjects, on the other hand, no statistical between-group differences in neuromuscular adaptations were observed (Gravelle & Blessing 2000; Collins & Snow 1993; Chtara et al. 2008; Makhlouf et al. 2015)

With regard to cardiorespiratory adaptations, studies have found limited increases in VO_{2max} following the order commencing with strength training in young women (Gravelle & Blessing 2000) and men (Chtara et al. 2005), while others have found no statistical between-group differences in young subjects (Chtara et al. 2005; Collins & Snow 1993; Makhlouf et al. 2015). Interestingly, while in old men no differences in VO_{2max} were observed, the load at the first ventilatory threshold was statistically increased only in the group commencing the training with strength (Cadore et al. 2012a). As in the same study also neuromuscular performance was optimized following the same exercise order, the authors concluded that improved muscular strength beneficially affected cycling economy.

TABLE 1 Training studies investigating the effects of the exercise order on markers of cardiorespiratory and neuromuscular function.

Study	Subjects	Training duration	Training mode	Cardio-respiratory function	Neuro-muscular function	Between-group difference
Collins & Snow 1993	untrained men and women E+S (n=15) S+E (n=15)	7 weeks	3 d·wk^{-1} E: 20 - 25 minutes running at 60 - 90% of heart rate reserve S: whole body, 2 x 3 - 12 repetitions at 50 - 90% of 1RM	E+S ↑ S+E ↑	E+S ↑ S+E ↑	No
Gravelle & Blessing 2000	active women E+S (n=6) S+E (n=7)	11 weeks	3 d·wk^{-1} E: 45 minutes at 70% of VO_{2max} S: lower body 2 – 4 x 6 - 10RM	E+S ↑ S+E →	E+S ↑ S+E ↑	C: Yes N: No
Chtara et al. 2005	male sport students E+S (n=10) S+E (n=10)	12 weeks	2 d·wk^{-1} E: Interval running on an indoor track S: whole body circuit including strength-endurance and explosive protocols	E+S ↑↑ S+E ↑	N/A	Yes
Chtara et al. 2008	male sport students E+S (n=10) S+E (n=10)	12 weeks	2 d·wk^{-1} E: Interval running on an indoor track S: whole body circuit including strength-endurance and explosive protocols	N/A	E+S ↑ S+E ↑	No

TABLE 1 Continued

Cadore et al. 2012a	untrained elderly men E+S (n=13) S+E (n=13)	12 weeks	3 d·wk^{-1} E: cycling, 20 - 30 minutes continuous at 80 - 90 of heart rate at second ventilatory threshold, interval cycling 6 x 4 minutes at second ventilatory threshold S: whole body, 2 – 3 x 6 - 20RM	E+S ↑↑ S+E ↑	E+S ↑ S+E ↑↑	Yes
Cadore et al. 2013	untrained elderly men E+S (n=13) S+E (n=13)		3 d·wk^{-1} E: cycling, 20 - 30 minutes continuous at 80 - 90 of heart rate at second ventilatory threshold, interval cycling 6 x 4 minutes at second ventilatory threshold S: whole body, 2 – 3 x 6 - 20RM	N/A	E+S ↑ S+E ↑↑	Yes
Makhlouf et al. 2015	male elite soccer players E+S (n=14) S+E (n=15)		2 d·wk^{-1} E: 2 x 12 – 16 x 15 seconds at 110 - 120% of max speed S:whole body 3 x 5 - 10RM	E+S ↑ S+E ↑	E+S ↑ S+E ↑	No

S: Strength training, E: Endurance training, C: Cardiorespiratory function, N: Neuromuscular function

2.4.3 Adaptations to concurrent training in endurance athletes

During recent years scientific evidence has emerged that inclusion of strength exercises into endurance training routines may beneficially affect performance of endurance athletes (Bazyler et al. 2015; Beattie et al. 2014; Rønnestad & Mujika 2014). Endurance capacity is known as the ability to maintain a constant power output over a given time (Paavolainen et al. 1999b; Stone et al. 2006). Thus, changes in neuromuscular function may substantially affect endurance performance (Paavolainen et al. 1999b), despite the fact that rather VO_{2max}, metabolic thresholds, and exercise economy are typically considered as determinants of cardiorespiratory fitness (Bassett & Howley 2000). Indeed, previous studies in which endurance and strength training was performed on alternating days have shown that strength training-induced improvements in endurance performance are attributed to adaptations within the neuromuscular system both in endurance runners (Millet et al. 2002; Storen et al. 2008; Taipale et al. 2010; Saunders et al. 2006; Turner, Owings & Schwane 2003; Guglielmo, Greco & Denadai 2009; Paavolainen et al. 1999a; Mikkola et al. 2007) and cyclists (Aagaard et al. 2011; Barrett-O'Keefe et al. 2012; Sunde et al. 2010; Rønnestad, Hansen & Raastad 2011).

Interestingly, the majority of studies has shown that the positive effects of strength training for endurance athletes may occur independently to changes in VO_{2max} (Paavolainen et al. 1999a; Storen et al. 2008). Instead, maximal and explosive strength training has been shown to improve running economy (Millet et al. 2002; Storen et al. 2008; Taipale et al. 2010; Paavolainen et al. 1999a),

velocity at the lactate threshold (Guglielmo, Greco & Denadai 2009; Mikkola et al. 2007; Paavolainen et al. 1999a), maximal running speed (Millet et al. 2002) and running time over a given distance (Spurrs, Murphy & Watsford 2003; Paavolainen et al. 1999a), while the positive effects of strength training on cycling economy are not as well documented (Rønnestad, Hansen & Raastad 2010b; Aagaard et al. 2011).

Typically, strength training-induced improvements in cardiorespiratory function and endurance performance occur with concomitant increases in maximal and/or explosive strength development, supporting the hypothesis that the beneficial effects on endurance performance may be induced by enhanced neuromuscular function (Mikkola et al. 2007; Taipale et al. 2010; Paavolainen et al. 1999a). However, it should be noted that due to the high volume of endurance training performed in these athletes, neuromuscular adaptations observed are of a smaller magnitude than those typically seen following strength training only (i.e. "interference effect").

According to Østerås et al. (2002) and Rønnestad & Mujika (2014) improvements in endurance performance induced by heavy resistance training may be attributed to increased muscle-tendon-unit stiffness, a postponed activation of less efficient type II fibers, improved neuromuscular efficiency as characterized by a greater capacity to store and release elastic energy which may lead to a right and upward shift of the force-velocity and force-power relationships and/or the conversion of fast twitch type IIX fibers into more fatigue resistant type IIA fibers. Similarly, improvements in the rate of force development have also been associated with improved movement economy (Paavolainen et al. 1999b; Saunders et al. 2006; Turner, Owings & Schwane 2003), indicating that explosive strength training may be similarly effective as maximal loads (Taipale et al. 2013). However, strength training has previously also been shown to stimulate mitochondrial biogenesis, possibly beneficially affecting endurance adaptations (Wilkinson et al. 2008).

Whether endocrine adaptations contribute to beneficial strength training-induced adaptations in endurance performance has to date not thoroughly been investigated. In one study, significant increases in serum testosterone concentrations were observed after 12 weeks of concurrent training in recreational endurance runners (Taipale et al. 2014a). This in turn may indicate that the strength training-induced changes in basal hormone concentrations may counteract an endurance training-induced catabolic state, possibly contributing to the beneficial effects of strength training for endurance athletes but further research is required in order to confirm this hypothesis.

3 PURPOSE OF THE THESIS

Concurrent endurance and strength training-induced changes in neuromuscular, cardiorespiratory and endocrine adaptations have previously been associated with positive effects on physical fitness and health. While the neuromuscular, cardiorespiratory and hormonal adaptations of concurrent training performed on separate days are relatively well studied both in previously untrained and endurance-trained subjects, information about the physiological adaptations of endurance and strength training combined into the same training session is still scarce. This training method, however, is very time effective and time constraints are among the major reasons for restraining from regular physical activity in young adults (Ruseski et al. 2011). In addition, in recreational endurance athletes, maintaining sufficient recovery between subsequent training sessions becomes challenging with increasing training demands. Performing concurrent endurance and strength training in the same session may, thus, help young adults to commit to regular physical activity, while it may be a necessity for endurance athletes. However, the few studies available on this topic have provided some indications of loading-order specific adaptations but were conducted in various subject populations with different training modes and volumes. Thus, it remains unclear which order should be performed in order to optimize neuromuscular, cardiorespiratory and hormonal adaptations in previously untrained subjects and recreational endurance athletes.

The present PhD thesis comprises of two study designs:

Study 1 investigated the exercise order-specific responses and adaptations to concurrent endurance and strength training performed in the same session (E+S vs. S+E) in previously untrained men. The specific aims of the two original papers were:

1) To investigate the acute force and hormone responses to concurrent endurance and strength loadings with different exercise sequences (E+S vs.

S+E) both before and after 24 weeks of order-specific training and its association with adaptations in endurance and strength performance. (I)

2) To assess exercise order-specific adaptations in training-induced changes in physical fitness, body composition, blood lipids and serum hormone concentrations following the prolonged training intervention. (I-II)

Hypothesis:
The leading hypothesis was that endurance cycling performed before strength training would reduce the quality of the subsequent strength protocol and, thus, would induce less favorable anabolic responses when compared to the opposite loading order. As a result, it was hypothesized to observe compromised neuromuscular adaptations in E+S compared to S+E, while no or only small between-group differences were expected in cardiorespiratory adaptations. Furthermore, only moderate reductions in total fat mass or blood lipids were expected in either training group.

Study 2 examined the adaptations to concurrent training where an endurance training session always immediately preceded the strength training versus endurance training only in recreational male endurance runners. The specific aims of the two original papers were:

3) To study the effect of acute force and hormone responses to endurance exercise both before and after 24 weeks of training and adaptations in basal hormonal concentrations on maximal endurance running performance. (III)

4) To examine the chronic neuromuscular adaptations and its effect on submaximal endurance performance following prolonged training. (III-IV)

Hypothesis:
Similarly to study 1, the primary hypothesis was that an endurance running session performed always immediately before the strength training session would compromise the anabolic effects of the added strength training. Thus, it was hypothesized to observe no between-group differences in endurance running-induced force and hormone responses after training. As the subjects assigned to the concurrent endurance and strength training group in this study were purposefully exposed to both chronic and acute interference, it was expected that the neuromuscular adaptations in this group would be compromised and, thus, only minimal beneficial effects on maximal and sub-maximal endurance performance would be observed.

4 METHODS

4.1 Subjects and ethical considerations

Forty-two previously untrained and 30 men recreationally trained in endurance running volunteered to participate in the present studies. In study 1, subjects were moderately active as characterized by irregular walking, cycling or occasional team sports at light to moderate intensity and not more than 3 times per week. Subjects did not engage in systematic or structured endurance or strength training prior to the study. In study 2, the subjects had performed endurance running for a minimum of 1 year with 2 - 6 sessions (at both moderate and high intensity) per week prior to inclusion. The study was conducted according to the Declaration of Helsinki and ethical approval was granted by the Ethics Committee of the University of Jyväskylä. Subjects were informed about possible risks of all study procedures before giving written informed consent. Subjects completed a health questionnaire which along with resting electrocardiogram was reviewed by a cardiologist prior to participating in the study. All subjects were reportedly free of acute and chronic illness, disease and injury and the use of medications that would contraindicate the performance of intense physical activity or interfere with neuromuscular or hormonal function. Due to drop-outs or a training adherence of less than 90% (in previously untrained subjects), not all subjects were included in the final analysis. The final subject numbers and anthropometrics of the original articles are presented in TABLE 2.

TABLE 2 Baseline anthropometric characteristics of the subjects (mean ± SD).

Paper	Group	N	Age (y)	Body mass (kg)	Body height (cm)	Body fat (%)
I	E+S	12	30 ± 5	79 ± 10	179 ± 6	22 ± 8
	S+E	17	30 ± 5	75 ± 9	179 ± 5	21 ± 5
II	E+S	16	30 ± 6	80 ± 12	178 ± 6	23 ± 8
	S+E	18	30 ± 4	75 ± 9	179 ± 5	21 ± 5
III+IV	E+S	13	32 ± 6	79 ± 6	179 ± 3	18 ± 5
	E	14	34 ± 7	78 ± 7	180 ± 7	19 ± 6

E+S: Endurance immediately followed by strength training, S+E: Strength training immediately followed by endurance training, E: Endurance training only

4.2 Research design

The research designs of studies 1 and 2 are presented in FIGURE 6. Following the pre-screening, in study 1 subjects were matched by baseline performance to an endurance immediately followed by strength training (E+S) or strength immediately followed by endurance training (S+E) group. In study 2, subjects were matched either to a concurrent endurance and strength training group (E+S), where strength training was always immediately preceded by an endurance running session or an endurance training only (E) group. As the purpose of this thesis was to investigate the differences between different training modes (E+S vs. S+E in study 1, E+S vs. E in study 2), a no-training control group was not included.

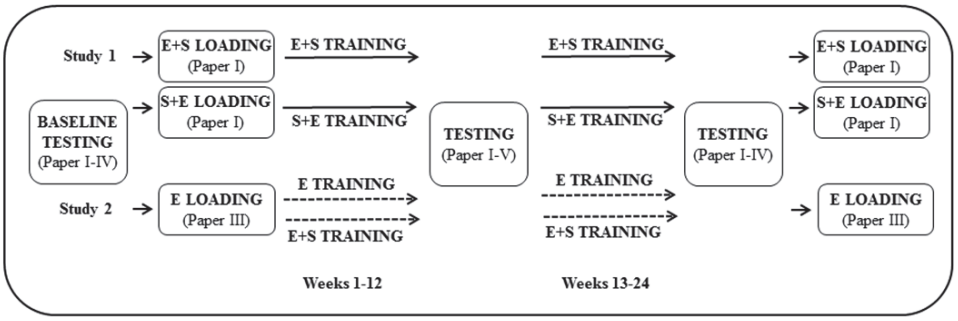

FIGURE 6 Experimental design of study 1 in previously untrained subjects and study 2 in recreational endurance runners.

For familiarization, in study 1 one combined training session in the order of the corresponding training group (E+S vs. S+E) was conducted prior to the baseline measurements and training. In study 2, this training familiarization was only performed in the E+S group. Thereafter, subjects of all training groups (both study 1 and 2) reported to the laboratory for a familiarization session during which the strength measurements were practiced and the equipment adjusted to the specifics of the subject. Testing of endurance and strength performance

and body composition were then performed on separate days prior to the start of the training (week 0). Baseline testing was repeated after 12 (II - IV) and 24 weeks and was performed at the same time of day within ± 1 h of the timing of baseline measurements. Baseline measures of both studies were performed during the fall, week 12 measurements during the winter and week 24 measurements during the spring.

In order to determine acute force and hormone responses and recovery in previously untrained subjects (I), all subjects performed one experimental session of combined endurance and strength loadings in the order of the corresponding group (E+S or S+E) and returned to the laboratory for recovery measurements at 24 h and 48 h. This experimental loading was then repeated after the 24-week training intervention. In recreational endurance runners, acute force and hormone responses were determined following an endurance loading before and after 24 weeks of training (III). The experimental loading in study 1 and 2 at weeks 0 and 24, respectively was performed at the same time of day (± 1 h).

4.3 Measurement procedures

4.3.1 Body composition and muscle mass

4.3.1.1 Total and partial body composition

Whole body tissue composition and body mass were assessed by Dual-energy X-ray Absorptiometry (DXA) (Lunar Prodigy Advance, GE Medical Systems, Madison, USA) (II and IV). To control the experimental conditions, each scan was conducted in the morning after 12 h of fasting. Legs were secured by non-elastic straps at knees and ankles and arms were aligned along the trunk with palms facing the thighs. All metal objects were removed from the subject prior to the scan. Automatic analyses (Encore, version 14.10.022) provided total and upper body lean (including muscle) and fat mass. Automatic generated regions of the legs were manually adjusted by the same investigator to include hamstrings and gluteal muscles. Thus, legs were separated from the trunk by a horizontal line right above the iliac crest providing lean and fat mass for legs and upper body separately.

Abdominal fat mass (II) was calculated by manually defining a range of interest (ROI) confined cranially by the upper end plate of the first lumbar vertebra, laterally by the ribs and caudally by the iliac crest (Tallroth, Kettunen & Kujala 2013). This customized ROI was then copied to the DXA scans obtained at weeks 12 and 24, respectively in order to assure analyses were conducted from the same areas at all measurement times.

4.3.1.2 Muscle cross-sectional area

Anatomical muscle cross-sectional area (CSA) of vastus lateralis was measured by the extended field of view mode (Ahtiainen et al. 2010), using a B-mode axial-plane ultrasound (model SSD-a10, Aloka Co Ltd, Yokohama City, Japan) with a 10 MHz linear array probe (II and IV). A customized convex-shaped probe support was used to assure a perpendicular measurement and to constantly distribute pressure on the tissue. The transducer was moved manually from lateral to medial along a marked line on the skin. Three panoramic CSA images were taken at 30%, 50% and 70% of the femur length (lateral aspect of the distal diaphysis to the greater trochanter), respectively and CSA was analyzed manually using Image-J software (version 1.44p, National Institute of Health, Bethesda, MD, USA). The mean of the two closest values (at 30%, 50% and 70%, respectively) was used for statistical analyses. To assess total CSA of vastus lateralis, values of the three measurement points were averaged.

4.3.2 Neuromuscular function

4.3.2.1 Isometric strength performance

Maximal isometric bilateral leg press force was assessed by a horizontal leg press dynamometer (Häkkinen et al. 1998) (Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland) in order to determine both acute (I and III) and chronic (I - IV) changes in neuromuscular performance. In addition, maximal isometric unilateral knee extension and flexion forces of the right limb were measured by a customized dynamometer (Häkkinen et al. 1998) (David 200, David Health Solutions, Helsinki, Finland) (IV). During all isometric actions, subjects were seated at a hip and knee angle of 110 and 107 degrees, respectively. On verbal command, subjects were instructed to produce maximal force as rapidly as possible and maintain maximal tension for 3 - 4 seconds. During the execution of each maximum trial, subjects were required to grasp handles located by the seat of the dynamometer, as well as to keep constant contact with the seat and the backrest and verbal encouragement was given to promote maximal effort. During isometric unilateral knee extension and flexion, subjects were secured by a non-elastic strap at the hip and a pad across the knee to prevent extraneous movement. In addition to maximal force production, rapid isometric force production was calculated from the force curve obtained during isometric leg press (II and IV). Rapid force production was defined as the average force produced during the first 500 ms of the maximal contraction (Häkkinen et al. 2003). The force signal of all isometric measurements was low-pass filtered (20 Hz) and analyzed using customized, automated scripts (Signal, version 4.04, Cambridge Electronic Design Ltd., Cambridge, UK).

For the determination of training-induced adaptations (II and IV), at least 3 trials separated by a rest period of 1 minute were conducted and up to 2 additional trials were performed if the maximum force during the last trial exceeded

the previous attempt by 5%. The trial with the highest maximal force was used for statistical analysis.

During the assessment of acute force responses (I and III) and recovery (I), the baseline force level was determined by performing 3 trials separated by a resting period of 1 minute prior to the start of the experimental loading session. Similarly, during recovery at 24 h and 48 h, at least 3 trials separated by 1 minute were conducted. If the maximum force during the last trial exceeded the previous attempt by 5%, an additional trial was performed. To assess acute changes in maximal and rapid force production during (i.e. after E or S, respectively; in paper I) and after the loading session (i.e. after E+S or S+E in paper I and after E in paper III) only two maximal trials separated by 10 – 15 seconds were performed. The best performance trial in terms of maximal force measured at each time point was used for statistical analysis.

To assess the changes in force per cm^2 of cross-sectional area (IV) the force/CSA-ratio (F/CSA-ratio) was calculated by dividing maximal isometric knee extensor force by total CSA of vastus lateralis as was previously done in a concurrent endurance and strength training study (Cadore et al. 2012a).

4.3.2.2 Dynamic strength performance

One repetition maximum (1RM) of leg extensors was determined using a dynamic horizontal leg press device (David 210, David Health Solutions, Helsinki, Finland) (Häkkinen & Komi 1983). Following a warm up (1 set of 5 repetitions at 70% of estimated 1RM, 1 set of 2 repetitions at 80 - 85% of estimated 1RM, 1 set of 1 repetition at 90 - 95% of estimated 1RM) a maximum of 5 trials was allowed to obtain a true 1RM. The device was set up so that the knee angle in the initial flexed position was approximately 60 degrees and a successful trial was accepted when the knees were fully extended (~180 degrees). The greatest load that the subject could lift to full knee extension at an accuracy of 1.25 kg was accepted as 1RM.

Maximal power (III) was determined by a counter movement jump (CMJ) on a force plate (Komi & Bosco 1978) (Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland). Subjects were asked to keep their hands on the waist throughout the movement and were instructed to jump as high as possible on verbal command. Force data was collected and manually analyzed (Signal, version 4.04, Cambridge Electronic Design Ltd., Cambridge, UK). Jumping height was calculated from the take-off velocity using the formula:

$$h=v^2/2g$$

where h refers to jumping height and v refers to take-off velocity (Komi & Bosco 1978). The best trial in terms of jumping height measured in cm was used for statistical analysis.

4.3.2.3 Surface Electromyography

Muscle activity of the vastii lateralis and medialis as well as biceps femoris muscles of the right leg was monitored through surface electromyography (IV). Placement of two adhesive electrodes (bipolar configuration Al/AgCl electrodes with an inter-electrode resistance < 5 kΩ, Blue Sensor N ECG Electrodes, Ambu A/S, Ballerup, Denmark) was defined according to the SENIAM guidelines (Hermens et al. 1999). The correct position was marked by subcutaneous ink in order to replicate measurements after 12 and 24 weeks (Häkkinen & Komi 1983). The electrodes were placed aligned with the estimated pennation angle of the muscle, as close as possible to each side of the mark. The activity of the 3 muscles was recorded during the isometric muscle actions (isometric bilateral leg press, isometric unilateral knee extension and flexion) and electrical muscle stimulation (isometric unilateral knee extension). The raw EMG signals of isometric leg press, knee extension and flexion were amplified by a factor of 1000 and sampled at 3000 Hz. The signals were passed from a portable transmitter via a receiver box (Telemyo 2400R, Noraxon, Scottsdale, AZ, USA) to an analog-to-digital converter (Micro 1401, Cambridge Electronic Design, Cambridge, UK). Analysis of the isometric EMG and conversion into integrated EMG (iEMG, mV·s) was performed using a customized, automated script (Signal, version 4.04, Cambridge Electronic Design Ltd., Cambridge, UK). The raw EMG signals were band-pass filtered (20 – 350 Hz) before applying further analyses. For statistical analyses, average maximum iEMG (mV·s) was determined during the 500 - 1500 ms time period after the onset of the contraction, representing the peak force phase. Agonist-Antagonist co-activation was calculated by expressing the maximal activation of biceps femoris during isometric unilateral knee extension relatively to the EMG of biceps femoris obtained during isometric unilateral knee flexion (Häkkinen et al. 1988b). As an indicator of rapid maximal activation, average maximum iEMG (mV·s) of vastii lateralis and medialis was also analyzed for 0 – 500 ms during the maximal contraction of isometric bilateral leg press (Häkkinen et al. 2003).

4.3.2.4 Voluntary activation

Voluntary activation percentage (VA%) of the right limb (IV) was assessed during isometric unilateral knee extension on a customized dynamometer (Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland) (Walker et al. 2009). Subjects were seated upright with a knee joint angle of the right limb at 107°. To prevent extraneous movement, subjects were secured by a non-elastic belt at the hip and a pad strapped over the right knee. The ankle was strapped to the device 2 cm above the right lateral malleolus with a Velcro strap which was connected to a strain gauge. Subjects were instructed to increase force gradually, reaching maximum voluntary force in approximately 3 seconds and maintaining the maximal force level for approximately 4 seconds. Three maximal trials separated by 1 minute of rest were performed. To assess VA%, electrical muscle stimulation was delivered to the

quadriceps femoris during the maximal unilateral knee extension using the interpolated twitch technique. Four galvanically paired self-adhesive electrodes (6.98 cm Vtrodes, Mettler Electronics Corp, Anaheim, CA, USA) were placed on the proximal and intermediate regions of the quadriceps muscle belly of the right limb. To determine stimulation intensity, rectangular single pulses of 1 ms were delivered at rest. The intensity was increased in 5 mA increments with a constant-current stimulator (400V, Model DS7AH, Digitimer Ltd, Welwyn Garden City, UK) until a plateau in the stimulation-induced force was observed. To the maximal stimulation current determined at rest an additional increase of 25% was added in order to assure maximal effect during the performance trials. During each measurement trial, the supra-maximal single-pulse electrical stimulation was delivered to the muscle at three separate times (Merton 1954): 3 seconds before the onset of voluntary force production (i.e. at rest), during the plateau of maximal force (i.e. super-imposed twitch), and 5 seconds after contraction cessation. The trial with the highest voluntarily achieved force prior to the electrical muscle stimulation was used for statistical analyses. VA% was calculated by the following formula (Bellemare & Bigland-Ritchie 1984):

$$VA\% = (1 - (P_{ts} / P_t)) \times 100$$

where P_{ts} is the amplitude of twitch elicited by the electrical stimulation on top of the voluntary exerted force and P_t is the electrical stimulus delivered to the resting muscle 5 seconds after the maximal voluntary contraction. Force signals were recorded at 2000 Hz and processed with a low-pass filter of 20 Hz. VA% was analyzed manually (Signal, version 4.04, Cambridge Electronic Design, Cambridge, UK).

4.3.3 Cardiorespiratory capacity

4.3.3.1 Endurance performance

In study 1, endurance performance was assessed by a graded protocol on a cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany). The protocol begun at 50 W and increased by 25 W every 2 minutes. Subjects were asked to maintain a pedaling frequency of 70 rpm throughout the test. The test was stopped when the subjects failed to maintain the required cadence for more than 15 seconds. Heart rate was monitored throughout the test (Polar S410, Polar Electro Oy, Kempele, Finland) and recorded as the average of the last 5 seconds at each stage. Blood lactate concentrations were determined during the final seconds of every power output. Maximal power output (Watts) was calculated using the equation (Kuipers et al. 1985):

$$\text{Maximal power output} = W_{com} + (t/120)*25$$

where W_{com} is the load of the last completed stage and t is the time of the last incomplete stage (in seconds). Time to exhaustion was defined as the total duration of the test.

In study 2, endurance performance was measured both by an incremental treadmill (weeks 0 and 24) and field (weeks 0, 12 and 24) test. The treadmill (Telineyhtymä, Kotka, Finland) test begun with a velocity of 9 $km \cdot h^{-1}$ and increased by 1 $km \cdot h^{-1}$ every 3 minutes, while the incline was kept constant at 0.5°. The treadmill was stopped every 3 minutes for 20 seconds in order to collect capillary blood samples from the fingertip for the determination of blood lactate concentrations. Time to exhaustion was defined as the total duration of the test. Running performance was also determined by an incremental field test of 6 x 1000 m (1 minute inter-set rest) performed on a 200 m indoor running track. The initial speed for all subjects was 6 $min \cdot km^{-1}$ and the speed was increased by 30 seconds every 1000 m. The test was performed in small groups and velocity was controlled every 100 m. The final 1000 m were performed at individual maximal running speed and the time of this trial was used for statistical analysis. Blood lactate concentrations were determined at the end of each stage.

4.3.3.2 Maximal and sub-maximal oxygen uptake and cycling efficiency

In study 1, oxygen uptake was determined continuously breath-by-breath throughout the incremental cycle ergometer test using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). On each testing day, air flow calibration was performed using a manual flow calibrator. Before each test, automatic air flow calibration was performed and the gas analyzer was calibrated using a certified gas mixture of 16% O_2 and 4% CO_2. VO_{2max} was accepted when the oxygen uptake plateaued despite a further increase in power output and when the respiratory exchange ratio exceeded 1.05. VO_{2max} used for statistical analysis was calculated as the highest VO_2 value averaged over 60 seconds.

Sub-maximal oxygen uptake was calculated as the average VO_2 during the second minute at power outputs completed by all subjects (i.e. 50, 75, 100, 125, 150 and 175 W, representing ~20%, ~30%, ~40%, ~50% and ~60% of maximal power output at week 0 and ~15%, ~25%, ~35%, ~45%, ~50% and ~60% of maximal power output at week 24). In addition, gross efficiency was calculated from the same power outputs from the ratio of O_2 uptake and CO_2 production, averaged over the second minute at each power output as previously done by Moseley and Jeukendrup (2001):

Mechanical efficiency = (Power output [W]) / Energy expended ($J \cdot s$-1) x 100%

4.3.3.3 Metabolic thresholds

In order to determine intensities for the endurance training in study 1, subjects' individual aerobic and anaerobic thresholds were determined using deflection points obtained by plotting the curves of blood lactate concentrations, ventilation, oxygen uptake and carbon dioxide production (Aunola & Rusko 1986). For

group wise comparisons, power output (study 1) and velocity (study 2) at a blood lactate concentration of 4 mmol·l^{-1} (Heck et al. 1985) were calculated from the cycle ergometer test (weeks 0, 12 and 24) or treadmill test (week 0 and 24), respectively by linear interpolation.

4.3.4 Blood sampling and analysis

4.3.4.1 Blood lactate concentrations

Blood lactate concentrations were determined during the incremental cycling test and the experimental combined loading (study 1) as well as during the incremental treadmill and field tests (study 2). Twenty µl of blood were collected by small capillaries, inserted into reaction capsules containing a hemolyzing and anticoagulant agent and lactate concentrations were analyzed using a Biosen analyzer (C_line Clinic, EKF, Magdeburg, Germany).

4.3.4.2 Serum hormone concentrations and creatine kinase

Acute hormone responses and chronic changes in basal hormone concentrations as well as acute alterations in creatine kinase (CK) were determined from venous blood (I and III). Prior to the assessment of basal hormone concentrations, subjects were fasting for 12 h and asked to rest for at least 8 h during the preceding night. Moreover, subjects were required to restrain from strenuous physical activity for at least 48 h. Blood samples were drawn from the antecubital vein into serum tubes (Venosafe, Terumo Medical Co., Leuven, Belgium) using standard laboratory procedures. Whole blood was centrifuged at 3.500 rpm (Megafuge 1.0 R, Heraeus, Hanau, Germany) for 10 minutes after which serum was removed and stored at -80°C until analysis (approximately 4 - 8 weeks). Analysis of total serum testosterone, growth hormone (22-kDa), cortisol and sex hormone-binding globulin (SHBG) were performed using chemical luminescence techniques (Immulite 1000, Siemens, New York City, NY, USA) and hormone specific immunoassay kits (Siemens, New York City, NY, USA). The sensitivities for serum hormones were: testosterone 0.5 nmol·l^{-1}, growth hormone 0.03 mlU·l^{-1}, cortisol 5.5 nmol·l^{-1} and SHBG 0.2 nmol·l^{-1}. The intra-assay coefficients of variation for testosterone, growth hormone, cortisol and SHBG were 8.7 ± 2.7%, 6.0 ± 0.5%, 7.1 ± 1.1% and 6.4 ± 1.7%, respectively. The inter-assay coefficients of variation for testosterone, growth hormone, cortisol and SHBG were 10.6 ± 3.2%, 15.8 ± 0.3%, 7.9 ± 1.2% and 7.6 ± 1.4%, respectively. Basal testosterone/cortisol and testosterone/SHBG ratios were also calculated. Furthermore, during the measurement of acute loading responses plasma volume changes were estimated from changes in hematocrit and hemoglobin (Dill & Costill 1974).

4.3.4.3 Blood lipids

Concentrations of total cholesterol, low density lipoprotein (LDL-C), high density lipoprotein (HLD-C) and triglycerides were determined from fasting blood samples (i.e. serum) by spectrophotometry (Konelab 20XTi, Thermo Fisher Scientific, Vantaa, Finland). LDL-C was estimated using the following equation (Friedewald, Levy & Fredrickson 1972):

LDL-C=total cholesterol - HDL-C - (triglycerides/2.2)

4.3.5 Nutrition

To control nutritional intake, all subjects (studies 1 and 2) received both verbal and written dietary recommendations and were asked to maintain nutritional intake constant throughout the 24 weeks of training. In addition, in preparation for all baseline and experimental loading measurements, subjects were required to consume a light meal 2 – 3 h prior to the start of each test-session or experimental loading and asked to keep nutritional intake similar prior to the measurements at each time point (weeks 0, 12 and 24, respectively). Caffeine intake was prohibited for 12 h and alcohol intake for 24 h prior to each test. During the combined training sessions, a standardized low dose of glucose (according to bodyweight 2 - 4 tablets, each containing 2.1 g of glucose) was provided after half of the training session was completed (i.e. after E or S, respectively), while water was allowed ad libitum.

In study 1 (II), food diaries were collected for three consecutive days including one weekend day at weeks 0, 12 and 24, respectively. Subjects received instructions on how to report nutritional intake in the diaries. The food diaries were analyzed by nutrient analysis software (Nutriflow, Flow-team Oy, Oulu, Finland).

4.4 Experimental loading sessions

4.4.1 Concurrent endurance and strength loading

The study design for the experimental loading (I) is presented in FIGURE 7. Within the experimental loading sessions (at weeks 0 and 24, respectively), maximal isometric leg press force and concentrations of hormones (testosterone, growth hormone and cortisol), CK and blood lactate were determined. Force data and blood samples were collected at the following time points: Prior to the start of the experimental loading (PRE), immediately following the first loading (MID, after E or S, respectively) as well as immediately after the completed combined session (POST). At MID and POST, the venous blood sample was drawn always immediately after the assessment of force responses. Recovery of

force as well as hormone (only testosterone and cortisol) and CK concentrations was assessed after 24 h and 48 h at ± 1 h from the end of each completed session.

Hydration status was controlled by instructing the subjects to commence the experimental loading in a hydrated state. Subjects were instructed to ingest 2 dl of water after completion of the first exercise modality (i.e. after E or S, respectively), immediately after the venous blood sample was taken.

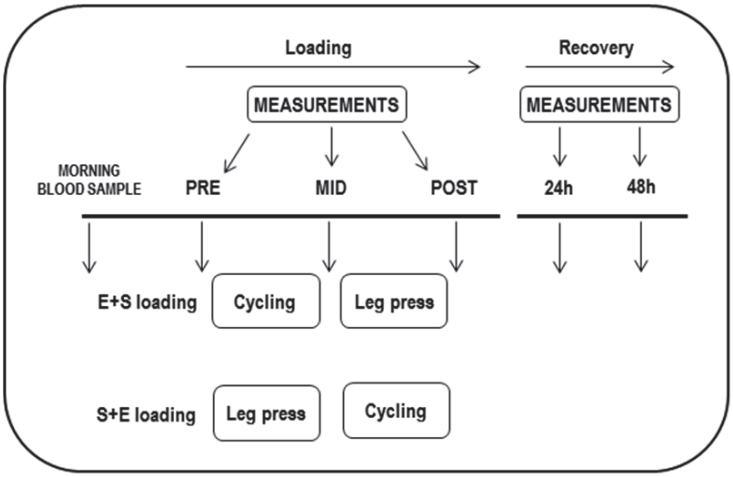

FIGURE 7 Experimental design for the determination of acute exercise responses and recovery in study 1 (I).

The endurance protocol was performed on a bike ergometer (Ergomedic 839E, Monark Exercise AB, Vansbro, Sweden). Subjects cycled for 30 minutes at 65% of subject's individual maximal power output (achieved during the baseline measurement at weeks 0 and 24, respectively). Subjects were required to keep the revolutions constant at 70 rpm. In case the subjects were unable to maintain this frequency, intensity was reduced by 15 W until the subjects were able to complete the loading.

The strength protocol focused on leg extensors and was performed on a dynamic leg press device (David 210, David Health Solutions Ltd., Helsinki, Finland). The starting knee angle for all exercises was similar to that used for the determination of 1RM strength during the baseline measurements (≤60 degrees). The strength loading was composed of 3 parts including explosive, maximal and hypertrophic strength protocols. The loading was initiated by 3 x 10 repetitions at 40% of 1RM. During these sets, subjects were instructed to fully extend their legs while producing force through intended maximal velocity. Thereafter, subjects performed 1 x 3 repetitions at 75% of 1RM, followed by 3 x 3 repetitions at 90% of 1RM. The strength protocol was concluded by performing 4 x 10 repetitions at 75% and 80 - 85% of 1RM (first and last set 75%, second and third set 80 - 85%). The inter-set rest periods during all explosive and maximal sets were 3 minutes, while rest periods during the hypertrophic sets were only 2 minutes. The loads were calculated from the individual 1RM

strength at weeks 0 and 24, respectively. However, in order to standardize the loading conditions between all subjects, additional load was added to achieve at least one set of a true repetition maximum (i.e. 3RM and 10RM, respectively) during the maximal and hypertrophic sets. Thus, during these sets, failure was allowed and in case the subjects were unable to complete the required amount of repetitions, assistance was provided so that all subjects performed similar loadings.

4.4.2 Endurance loading

Exercise-induced force and hormone responses in recreational endurance runners (III) were determined before and after the incremental treadmill test to voluntary exhaustion (at weeks 0 and 24, respectively). The treadmill test was followed by a cool-down of 5 minutes at the initial speed of 9 $km \cdot h^{-1}$. Isometric bilateral leg press force and serum hormone concentrations (testosterone, cortisol and growth hormone) were determined both before the exercise and immediately after the cool-down, as described previously.

4.5 Endurance and strength training programs

All subjects (Studies 1 and 2) were asked to maintain their habitual physical activity (light walking, cycling and occasional team sports) throughout the study period. In study 1, the training was designed to reflect a program typically recommended for physically active populations (Thompson, Gordon N.F. & Pescatello 2010). The main objective was to improve both endurance and strength performance through a periodized program including moderate and vigorous intensity aerobic exercise (Helgerud et al. 2007; Daussin et al. 2007) combined with hypertrophic and maximal strength protocols (Kraemer & Ratamess 2004). To assure the correct execution of the training prescribed, all training sessions were supervised by qualified instructors.

Prior to the start of the intervention (study 1), previously untrained subjects performed one preparatory training session in the order of the corresponding group (E+S or S+E). Thereafter, subjects performed 2 combined training sessions (2 x [1E + 1S] or 2 x [1S + 1E]) per week during weeks 1 - 12 and 2 - 3 combined training sessions (2 - 3 x [1E + 1S] or 2 - 3 x [1S + 1E]) per week during weeks 13 - 24. Subjects were required to proceed from the first loading (i.e. E or S, respectively) to the subsequent loading (i.e. S or E, respectively) within 5 - 10 minutes. To reflect tapering before testing, both weeks 12 and 24 were conducted with maintained training frequency but reduced training volume (Bosquet et al. 2007). This was achieved by reducing the number of sets during the strength training and simultaneously reducing the total duration of the endurance training.

In study 2, recreational endurance runners performed identical endurance training 4 - 6 times per week irrespective of the training group (i.e. E+S and E).

In the E+S group, subjects performed additional strength training twice a week (once per week in weeks 12 and 24), always immediately after a standardized endurance training session. Similarly to study 1, subjects of the E+S group were required to transition from endurance running to the strength protocol within a maximum of 5 - 10 minutes.

4.5.1 Endurance training

In study 1, endurance training was performed on a cycle ergometer. The intensity was controlled by heart rate zones determined from subjects' individual aerobic and anaerobic threshold obtained during the baseline measurement at weeks 0 and 12, respectively. Subjects were asked to maintain a constant pedaling frequency at about 70 - 80 rpm during each training session, while the magnetic resistance of the ergometer was used to achieve the prescribed exercise intensity. The endurance program consisted of both steady-state and interval cycling and the intensity was progressively increased from low (below the aerobic threshold) to high (above the anaerobic threshold) throughout both 12-week periods.

During weeks 1 - 7 steady-state cycling of low to moderate intensity (below and above the aerobic threshold) was performed and during the remaining weeks, additional high-intensity interval sessions (below and above the anaerobic threshold) were incorporated into the training program. The duration of endurance cycling progressively increased throughout the 12 weeks of training from 30 to 50 minutes. During the second 12-week period, the major endurance program structure was maintained, while both training volume and intensity were further increased. The aerobic threshold represented an intensity (percentage of HR_{max}) of 65 ± 5% and 67 ± 6% in E+S and 68 ± 8% and 67 ± 6% in S+E at weeks 0 and 12, respectively. The anaerobic threshold represented an intensity of 85 ± 5% and 86 ± 5% in E+S and 82 ± 8% and 86 ± 5% in S+E at weeks 0 and 12, respectively.

In study 2, the endurance training program was created based on the polarized training approach (Muñoz et al. 2014). The endurance exercises were mainly performed by running but alternative endurance types such as cycling and cross country skiing were occasionally permitted for specific low-intensity sessions in order to minimize the risk of injuries (TABLE 3).

While two training sessions per week were supervised, the remaining endurance training sessions were performed individually. In case of sickness, subjects were either required to catch up missing training sessions or the overall training period was extended in order to standardize training volume between subjects. The training intensity was based on heart rate zones calculated from maximal heart rate determined during the incremental treadmill protocol (except for short intervals for which intensities were calculated based on the individual maximal 1000 m time, TABLE 3). Training intensity, duration and distance were consistently controlled and recorded by heart rate monitors (RS800cx, Polar Electro Oy, Kempele, Finland), using manually pre-

programmed exercise files. The endurance training intensity and volume increased throughout the two 12-week periods.

TABLE 3 Endurance training sessions performed in study 2.

	Weeks 1 - 12	**Weeks 13 - 24**
Incremental run	2 x/week, running only 35 - 45 minutes/65 - 85%	2 x/week, running only 40 - 45 minutes/65 - 85%
Long workout	1 x/week, free 70 - 120 minutes/60 - 65%	1 x/week, free 85 - 125 minutes/60 - 65%
Long intervals	1 x/week, running only 4 – 5 x 5 minutes/80 - 85%	1 x/week, running only 4 – 6 x 5 minutes/80 - 85%
Short intervals		1 x/week, running only (track) 3 - 6 x 400 m + 3 - 6 x 800 m/85%
Light run	1 x/week, running only 35 - 40 minutes/60 - 65%	1 x/week, running only 40 minutes/60 - 65%
Optional workout	Optional 1 x/week, free 35 - 40 minutes/70 - 75%	

Free refers to a choice of running, cycling or cross-country skiing; intensities are % of HR_{max} and for short intervals % of 1000 m running time

4.5.2 Strength training

In study 1, the strength training program included exercises for all major muscle groups, while special attention was given to the lower extremities. The loads used during the strength training were determined by the number of repetitions and execution velocity and progressively increased throughout the two 12-week periods. Exercises for the lower body were bilateral dynamic leg press as well as bilateral and unilateral dynamic knee extension and flexion. Additional exercises for the upper body included dynamic seated vertical press and lateral-pull down as well as exercises commonly used to improve trunk stability (crunches, torso rotation and lower back extension). During the first two weeks training was performed as a circuit using 2 – 4 x 15 - 20 repetitions at 40 - 60% of 1RM. Thereafter, protocols aiming for muscle hypertrophy (2 – 5 x 8 - 10 repetitions at 80 - 85% of 1RM, 1.5 - 2 minutes inter-set rest) and maximal strength (2 – 5 x 3 - 5 repetitions at 85 - 95% of 1RM, 3 - 4 minutes inter-set rest) as well as during the last 2 weeks protocols targeting explosive strength (2 x 8 - 10 repetitions at 40% of 1RM with maximal velocity, 3 - 4 minutes inter-set rest) were performed. During the second 12-week period the major strength program structure was maintained, while both training volume and frequency were slightly increased in order to maximize fitness and health outcomes and to avoid a training plateau. The overall duration of the strength protocol within each combined training session was 30 - 50 minutes, resulting in a total duration of ~60 - 100 minutes for each combined training session (i.e. E+S and S+E, respectively).

In study 2, the strength training in E+S was performed always after the incremental endurance run (35 - 45 minutes by progressively increasing intensity from 65 - 85%, TABLE 3), with at least 48 h in between two subsequent com-

bined training sessions. Subjects were instructed to rest or perform the light run (35 - 40 minutes, 60 - 65%) on the day before the combined E+S training session. The strength training consisted of mixed maximal (~80% of total strength training volume) and explosive (~20% of total strength training volume) strength training sessions and was focused on the lower limbs, while additional exercises for the upper body were included. The loads of each exercise were determined by the number of repetitions and execution velocity which was progressively increased throughout the two 12-week periods. Exercises for the lower body included bilateral leg press, bilateral and unilateral knee flexion and calf raises. In addition, jumping and hopping exercises commonly used to improve explosive force production were performed (loaded and unloaded squat jumps, drop jumps, leaps, step-ups) and introduced in a progressive manner. During the final weeks of the second training period (i.e. weeks 21 - 24) hurdle jumps and resisted knee lifts were also incorporated into the strength training program. Exercises for the upper body included dynamic seated vertical press, biceps curls as well as exercises commonly used to improve trunk stability (crunches, torso rotation, and lower back extension). As the subjects were not accustomed to strength training, low loads (2 – 3 x 15 - 20 repetitions at 40 - 50% of 1RM, 1 - 2 minutes inter-set rest) were utilized during weeks 1 - 4. Thereafter, the strength training intensity progressed to heavier loads and a lower number of sets (2 – 5 x 5 - 12 repetitions at 60 - 85% of 1RM, 1 - 3 minutes inter-set rest). During the second 12-week period the major strength program structure was maintained, while both training volume and loads were increased to maximize maximal and explosive strength improvements.

4.6 Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (version 20.0 - 22.0, IBM Inc., Chicago, IL, USA). Data are presented as mean ± SD and shown as relative changes from the pre-loading values (I and III) or baseline values at week 0 (I - IV), unless indicated. Normality of distribution was assessed by the Shapiro-Wilk test. Data that did not match the criteria for normality were log-transformed before parametric tests were applied. In paper I, non-parametric tests were performed for selected within- (Wilcoxon signed-rank test) and between-group (Mann-Whitney U-test) comparisons and the results adjusted according to Bonferroni by multiplying all pair-wise p-values with the number of comparisons. Acute loading responses (I and III) of normally distributed variables at weeks 0 and 24 were analysed by a mixed ANCOVA design with repeated measures, using the corresponding pre-loading values as covariates. Training- or loading-induced within-group differences in these papers were analysed by a paired t-test using relative changes (weeks 24 vs. week 0). Within and between-group differences of basal measures were assessed by a mixed ANOVA design (I - IV).

In order to assess relationships between dependent variables within experimental groups, bivariate correlations were computed using the Pearson product-moment correlation coefficient (I - IV). Partial correlations with adjustment for groups were performed to determine relationships between dependent variables across all experimental subjects (II). In order to compare changes induced in sub-maximal oxygen uptake, cycling efficiency and blood lactate concentrations determined during the incremental cycle ergometer test in previously untrained subjects (study 1), area under the curve (AUC) analyses was conducted for all power outputs achieved by all subjects (i.e. 50, 75, 100, 125, 150 and 175 W), after which values of each time point (i.e. 0, 12 and 24) were analysed by two-way ANOVA. In study 2, the same method was also used to analyse changes in lactate and heart rate curves obtained from the 5 pre-determined increments (i.e. 6.0, 5.5, 5.0, 4.5 and 4.0 $min \cdot km^{-1}$) of the incremental field test in recreational endurance runners. The alpha level for all presented data was set at $p=0.05$.

5 RESULTS

5.1 Training adherence, nutrition and body mass

In previously untrained men (study 1), the training adherence was 99% in both the E+S and S+E groups with 2 - 3 weekly combined endurance and strength training sessions. In recreational endurance runners (study 2), the weekly average endurance training time was 4.7 ± 0.5 h and 4.9 ± 0.2 h in E+S and E, leading to a total training time of 111.8 ± 10.8 h and 116.5 ± 4.5 h, respectively. The weekly average running distance was 33.5 ± 7.9 km and 36.6 ± 5.6 km in E+S and E, leading to a total mileage of 804 ± 189.3 km and 879.5 ± 133.3 km, respectively. The number of weekly endurance training sessions in both the E+S and E group was 5 ± 1. No statistically significant between-group differences were observed. In E+S (study 2), the training adherence for strength training was 100%.

The nutritional data were analysed from previously untrained men (II). Total energy intake at weeks 0, 12 and 24 was 9.3 ± 1.8 MJ, 10.2 ± 2.6 MJ and 9.5 ± 2.6 MJ in E+S and 9.4 ± 2.0 MJ, 9.3 ± 1.7 MJ and 7.9 ± 1.7 MJ in S+E. The average nutritional intake as percentage of total energy for carbohydrates, fat and protein was 42 - 45%, 31 - 36% and 17 - 19% in E+S and 42 - 44%, 33 - 36% and 18% in S+E throughout the 24 weeks of training. No statistically significant within or between-group differences were observed.

In previously untrained men, a significant increase in body mass was observed in S+E only (1.7 ± 2.4% and 1.7 ± 2.6% at weeks 12 and 24 respectively, $p=0.026$ and 0.043, respectively). In recreational endurance runners, a statistically significant reduction in body mass was observed at week 24 in the E group only (-3 ± 2%, $p=0.006$).

5.2 Concurrent endurance and strength exercise-induced acute responses before and after training

5.2.1 Isometric force

Before the training intervention, statistically significant acute reductions in maximal isometric leg press force were observed in E+S at MID (p=0.005), while force production further decreased at POST (p=0.001) compared to PRE (FIGURE 8A). In S+E, maximal force production statistically decreased at MID (p<0.001) and remained reduced at POST (p<0.001) compared to PRE. The relative change at MID was statistically larger in S+E compared to E+S (-20 ± 13% vs. 11 ± 7%, p=0.037) but no statistically significant between-group difference was observed at POST. Both E+S and S+E recovered from POST to 24 h, so that the observed values obtained at 24 h and 48 h of recovery were not statistically different from PRE.

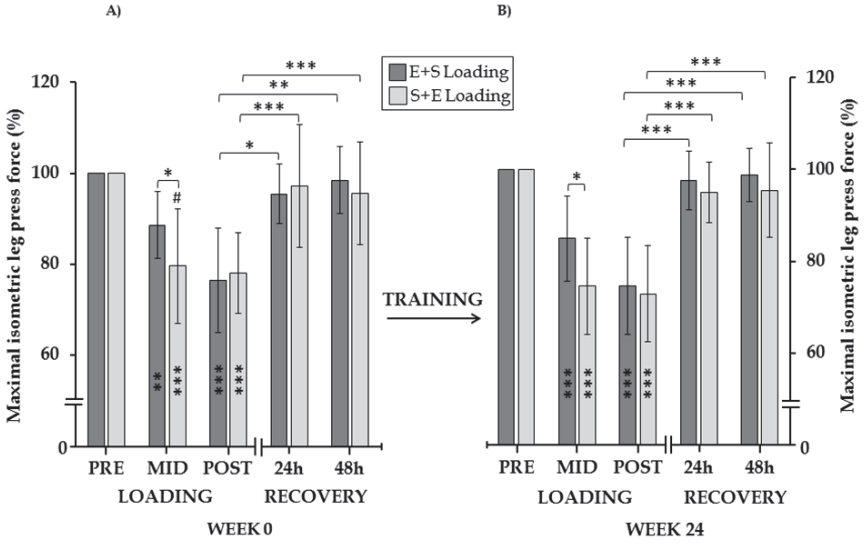

FIGURE 8 Acute relative changes in maximal isometric bilateral leg press force during and after the two combined loadings before (A) and after (B) the training intervention in previously untrained men. *, **, *** p<0.05, 0.01, and 0.001, respectively. When marked within the bar the comparison is made to PRE. # p<0.05 from corresponding time point of week 24.

After the training intervention (FIGURE 8B), E+S and S+E statistically decreased maximal isometric leg press force at MID (both p<0.001) and POST (both p<0.001) to a similar magnitude as the reductions observed at week 0. Similarly to week 0, the decrease at MID was significantly larger in S+E compared to E+S (-25 ± 11% vs. -15 ± 9%, p=0.011), while at POST no statistical between-group difference was observed. The reduction in maximal force production from PRE to MID in S+E was statistically larger at week 24 compared to

week 0 (-25 ± 11% vs. -20 ± 13%, p=0.039). Both E+S and S+E recovered from POST to 24 h so that the observed values at 24 h and 48 h did not statistically differ from PRE.

5.2.2 Serum hormone concentrations

Total serum testosterone

Before the training intervention, statistically significant increases in total serum testosterone concentrations (FIGURE 9A) were observed in E+S at MID only (p=0.018), leading to a statistical between-group difference at this time point (18%, p=0.017). In S+E, the increase from MID to POST was statistically significant (p=0.050), even though the concentrations at POST did not statistically differ from PRE. During recovery, testosterone concentrations statistically decreased in E+S both at 24 h (p=0.007) and 48 h (p=0.001) compared to PRE but remained statistically unaltered in S+E. Thus, a statistically significant between-group difference was observed at both 24 h (-23 ± 14% vs. -1 ± 32%, p=0.037) and 48 h (-21 ± 11% vs. -4 ± 21%, p=0.017).

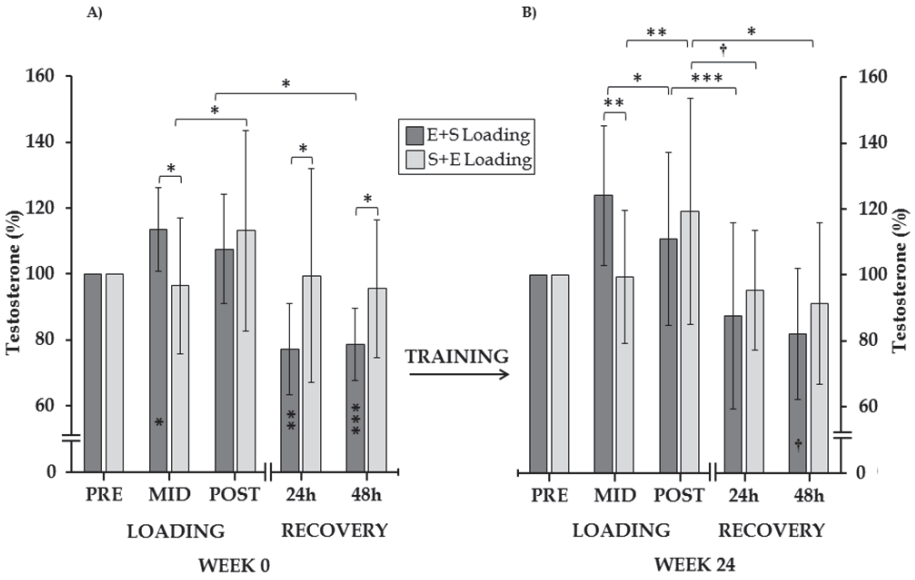

FIGURE 9 Acute relative changes in total serum testosterone concentrations during and after the two combined loadings before (A) and after (B) the training intervention in previously untrained men. *, **, *** p<0.05, 0.01, and 0.001, respectively. † p<0.06. When marked within the bar the comparison is made to PRE.

After the training intervention, testosterone concentrations (FIGURE 9B) remained statistically unaltered during the two loadings at MID and POST. Similarly to week 0, however, the difference between E+S and S+E at MID was statistically significant (+24 ± 21% vs. -1 ± 20%, p=0.004). In E+S, testosterone sta-

tistically decreased from MID to POST (p=0.031) but increased in S+E (p=0.007), while no statistical between-group difference at POST was observed. During recovery, testosterone concentrations were statistically unaltered in E+S and S+E at 24 h and S+E at 48 h compared to PRE, while the reduction in E+S at 48 h approached statistical significance (p=0.052). The acute responses and recovery kinetics of total testosterone concentrations after the training intervention did not statistically differ from those observed before the training in either of the two groups.

Serum growth hormone

Before the training intervention, a statistically significant increase in growth hormone concentrations both at MID and POST in E+S (+250 fold, p=0.006 and 57 fold p=0.012, respectively) and S+E (+49 fold, p=0.003 and +300 fold, p=0.001, respectively) compared to PRE was observed. The difference between the two groups was statistically significant both at MID (p=0.014) and POST (p=0.001).

Similarly, after the training intervention, growth hormone concentrations statistically increased both at MID and POST in E+S (+330 fold, p=0.006 and +80 fold, p=0.006, respectively). In S+E, increases in growth hormone concentrations approached statistical significance at MID (+53 fold, p=0.069) and were statistically altered at POST (+340 fold, p=0.001) compared to PRE. The difference between the two groups was statistically significant both at MID (p=0.001) and POST (p=0.018). However, the growth hormone responses after the training intervention did not statistically differ from those observed before training in either of the two groups.

Serum cortisol

Before the training intervention, cortisol concentrations remained statistically unaltered during the two loadings at MID and POST (FIGURE 10A). In S+E, the increase from MID to POST was statistically significant (p<0.001). During recovery at 24 h and 48 h, cortisol concentrations decreased in both E+S (p=0.023 and p=0.001, respectively) and S+E (p=0.009 and p=0.001, respectively) compared to PRE. No statistically significant between-group differences in the acute responses or during recovery were observed.

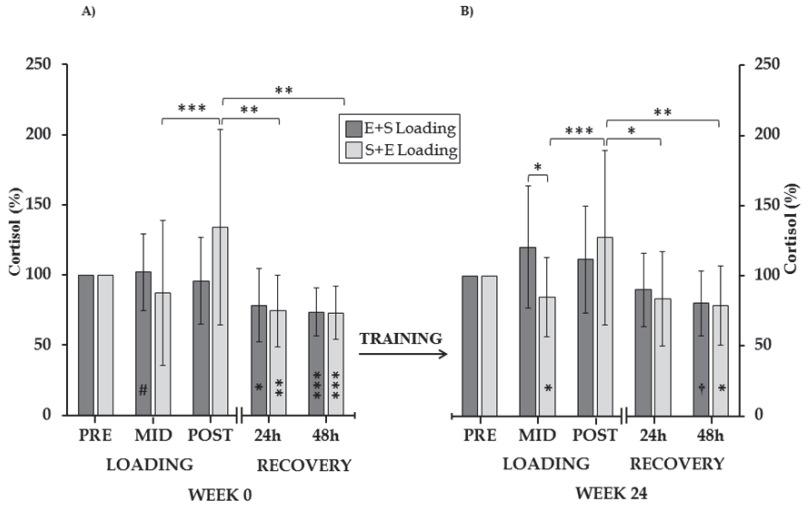

FIGURE 10 Acute relative changes in serum cortisol concentrations during and after the two combined loadings before (A) and after (B) the training intervention in previously untrained men. *, **, *** $p<0.05$, 0.01, and 0.001, respectively. † $p<0.06$. When marked within the bar the comparison is made to PRE. # $p<0.05$ from corresponding time point of week 24.

After the training intervention, cortisol concentrations (FIGURE 10B) remained statistically unaltered in E+S at MID and POST but were significantly decreased in S+E at MID ($p=0.012$) and increased from MID to POST ($p=0.001$) in the same group. Thus, the difference between E+S and S+E at MID was statistically significant (+20 ± 44% vs. -15 ± 28%, $p=0.015$). Furthermore, the relative change in cortisol concentrations in E+S at MID (Fig. 4b) was statistically larger compared to that observed before the training intervention (+20 ± 44% vs. +2 ± 27%, $p=0.043$). During recovery, cortisol concentrations remained statistically unaltered in the two groups at 24 h but at 48 h the reduction in E+S approached statistical significance ($p=0.057$), while it was statistically significant in S+E ($p=0.016$) compared to PRE. However, no statistical between-group difference was observed.

5.2.3 Blood lactate and creatine kinase

Before the training intervention, blood lactate concentrations (TABLE 4) increased in E+S and S+E at MID ($p=0.002$ and $p<0.001$, respectively) and POST (both $p<0.001$) to a similar magnitude. In addition, the increases in CK concentrations (TABLE 4) were similar in both E+S and S+E at MID ($p=0.006$ and $p=0.001$, respectively) and POST ($p=0.006$ and $p=0.001$, respectively). The largest relative increase in CK was observed during recovery at 24 h and 48 h, statistically significant at 48 h in S+E ($p=0.029$), while large standard deviations in the two groups were observed.

TABLE 4 Absolute values of blood lactate and creatine kinase concentrations in response to the two combined loadings before and after the training intervention in previously untrained men.

		Week 0		Week 24	
		Lactate ($mmol \cdot l^{-1}$)	CK ($mlU \cdot l^{-1}$)	Lactate ($mmol \cdot l^{-1}$)	CK ($mlU \cdot l^{-1}$)
E+S Loading	PRE	1.1 ± 0.4	169 ± 98	1.0 ± 0.2	135 ± 79
	MID	5.8 ± 2.8**	197 ± 105*	6.2 ± 2.0***	160 ± 93*
	POST	8.3 ± 3.2***	209 ± 98*	9.2 ± 3.9***	171 ± 94*
	24 h		405 ± 229		314 ± 200*
	48 h		276 ± 128		243 ± 198
S+E Loading	PRE	1.4 ± 0.4	160 ± 119	1.5 ± 0.8	107 ± 52
	MID	8.0 ± 2.3***	186 ± 140***	9.0 ± 2.3***	138 ± 83**
	POST	7.2 ± 2.0***	214 ± 155***	7.9 ± 2.1***	175 ± 99***
	24 h		290 ± 170**		173 ± 124***##
	48 h		221 ± 129		123 ± 61##

*, **, *** $p<0.05$, 0.01 and 0.001, respectively from corresponding PRE values; ## $p<0.01$ from measurements of week 0

After the training intervention, blood lactate concentrations (TABLE 4) were statistically increased in both loadings at MID (both $p<0.001$) and POST (both $p<0.001$). In addition, CK concentrations (TABLE 4) were statistically increased in both E+S and S+E at MID ($p=0.006$ and $p=0.003$, respectively) and POST ($p=0.006$ and $p=0.001$, respectively). The relative increase at POST was statistically larger in S+E compared to E+S (+70 ± 92% vs. +29 ± 15%, $p=0.037$). The largest relative increases in CK concentrations were observed during recovery in both E+S and S+E (+155 ± 60%, $p=0.009$ and +57 ± 56%, $p=0.001$), while the increase in E+S was statistically larger than that of S+E ($p=0.046$).

While no training-induced differences in acute blood lactate responses were observed, the absolute values of CK in S+E during recovery at 24 h and 48 h were statistically lower after the training intervention compared to the corresponding pre-training values (24 h 173 ± 124 $mlU \cdot l^{-1}$ vs. 290 ± 170 $mlU \cdot l^{-1}$, $p=0.002$; 48 h 123 ± 61 $mlU \cdot l^{-1}$ vs. 221 ± 129 $mlU \cdot l^{-1}$, $p=0.002$). Furthermore, after the training intervention, the relative increase in S+E from PRE to 24 h and 48 h was statistically smaller than that observed from pre-training (24 h 157 ± 56% vs. 200 ± 81%, $p=0.039$; 48 h 137 ± 39% vs. 153 ± 57%, $p=0.015$).

5.2.4 Plasma volume

No statistical between-group differences in plasma volume shifts were observed before or after the training intervention. Plasma volume shifts in the two groups ranged from -10% to -5% during loading and +1% to +7% during recovery, both compared to PRE.

5.3 Endurance exercise-induced responses before and after training

5.3.1 Force responses

The acute endurance exercise-induced changes in maximal isometric leg press force were similar in the two groups both before (FIGURE 11A) and after (FIGURE 11B) 24 weeks of training. Statistically significant acute decreases occurred during the endurance loading in E+S and E both before ($p=0.005$ and $p=0.001$, respectively) and after ($p=0.032$ and $p=0.030$, respectively) the training intervention. No statistically significant training-induced changes in the acute force responses were observed.

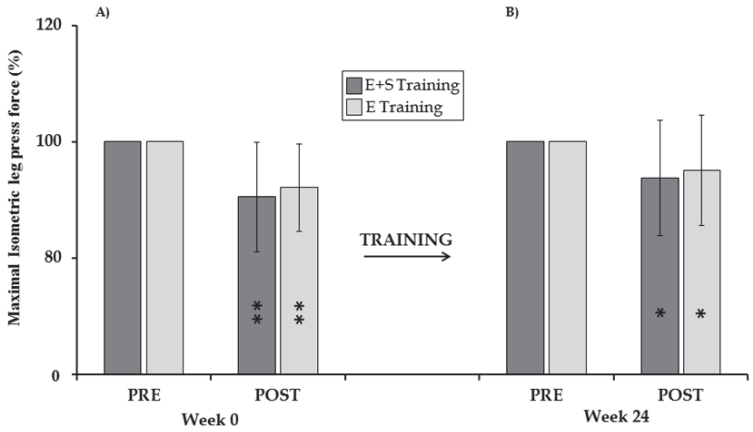

FIGURE 11 Acute relative changes in maximal isometric bilateral leg press force following the incremental treadmill run to voluntary exhaustion before (A) and after (B) the training intervention in recreational endurance runners. *, ** $p<0.05$ and 0.01, respectively compared to PRE.

5.3.2 Hormone responses

Total serum testosterone

Total serum testosterone concentrations (FIGURE 12A) statistically increased during the endurance loading in E+S and E both before ($p=0.012$ and $p=0.010$, respectively) and after ($p=0.013$ and $p=0.006$, respectively) the training intervention. No statistically significant training-induced changes in acute testosterone responses were observed in either of the two groups.

Serum growth hormone

Serum growth hormone concentrations statistically increased during the endurance loading in E+S and E both before (208 fold, $p<0.001$ and 227 fold, $p<0.001$,

respectively) and after (210 fold, p<0.001 and 341 fold, p<0.001, respectively) the training intervention. No statistically significant training-induced changes in the acute growth hormone responses were observed in either of the two groups.

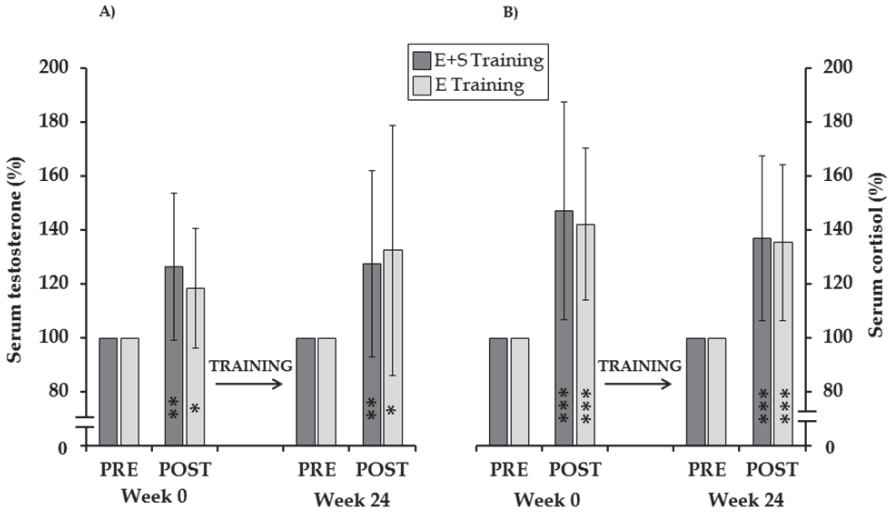

FIGURE 12 Acute relative changes in serum testosterone (A) and cortisol (B) concentrations following the incremental treadmill run to voluntary exhaustion before and after the training intervention in recreational endurance runners. *, **, *** p<0.05, 0.01 and 0.001 respectively compared to PRE.

Serum cortisol

Serum cortisol concentrations (FIGURE 12B) statistically increased during the endurance loading in E+S and E both before (both p<0.001) and after (both p<0.001) training but no statistically significant training-induced changes in acute cortisol responses were observed in either of the two groups.

5.3.3 Plasma volume

No statistical between-group differences in acute endurance loading-induced plasma volume shifts were observed at either measurement time. Loading-induced plasma volume shifts before and after the training intervention ranged from -6% to -7% in the two groups.

5.4 Adaptations in basal neuromuscular, cardiorespiratory and hormonal function

5.4.1 Neuromuscular adaptations

5.4.1.1 Dynamic and isometric strength performance

Previously untrained men

Baseline values of dynamic and isometric strength performance in previously untrained men (study 1) are presented in TABLE 5.

TABLE 5 Baseline dynamic strength and isometric force in previously untrained men.

	E+S	S+E
Maximal dynamic leg press strength (kg)	157 ± 30	143 ± 23
Maximal bilateral isometric leg press force (N)	2648 ± 689	2338 ± 540
Rapid bilateral isometric leg press force (N)	1828 ± 485	1616 ± 315

Both E+S and S+E statistically increased 1RM strength (FIGURE 13A) at week 12 (both $p<0.001$) and 24 ($p=0.001$ and $p<0.001$, respectively). The increase from weeks 12 to 24 was statistically significant in both groups (both $p<0.05$). Maximal bilateral isometric leg press force (FIGURE 13B) statistically increased in E+S and S+E at weeks 12 ($p=0.010$ and $p=0.019$, respectively) and 24 ($p=0.025$ and $p=0.024$, respectively) while rapid isometric force (FIGURE 13C) increased significantly in S+E only, both at weeks 12 ($p=0.002$) and 24 ($p=0.005$).

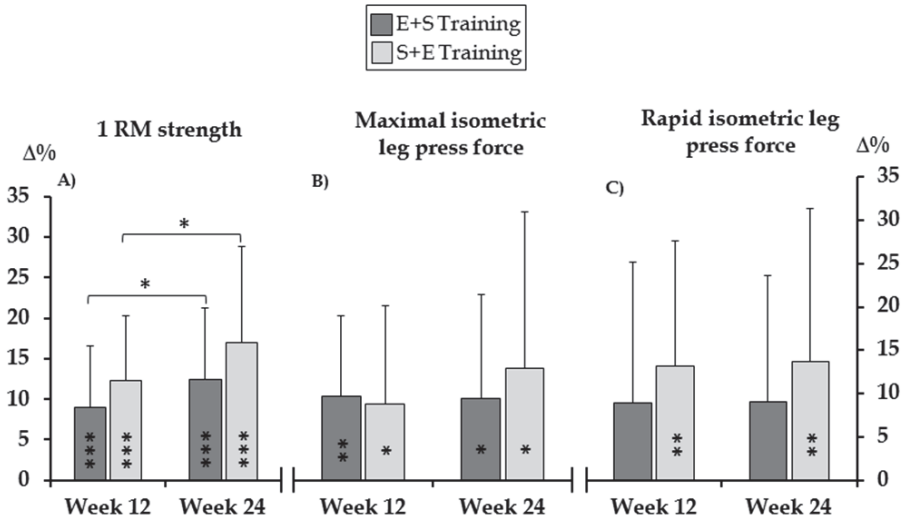

FIGURE 13 Chronic relative changes in maximal dynamic strength (A), maximal isometric bilateral leg press force (B) and rapid isometric bilateral leg press force (C) following 12 and 24 weeks of E+S and S+E training in previously untrained men. *, **, *** $p<0.05$, 0.01, and 0.001, respectively. When marked within the bar the comparison is made to week 0.

Recreational endurance runners

Baseline values of dynamic and isometric strength performance in recreational endurance runners (study 2) are presented in TABLE 6.

TABLE 6 Baseline dynamic strength and isometric force in recreational endurance runners.

	E+S	E
Maximal dynamic leg press strength (kg)	164 ± 21	148 ± 25
Maximal bilateral isometric leg press force (N)	2824 ± 552	2571 ± 456
Rapid bilateral isometric leg press force (N)	1911 ± 329	1732 ± 308
Maximal unilateral isometric knee extension force (N)	888 ± 186	774 ± 65*
Maximal unilateral isometric knee flexion force (N)	445 ± 45	337 ± 49**
Countermovement jump height (cm)	28.4 ± 4.0	28.0 ± 2.4

*, ** p<0.05 and 0.01, respectively between groups

Dynamic 1RM strength (FIGURE 14A) remained statistically unaltered in E+S at weeks 12 and 24 but statistically decreased in E at week 24 (p=0.014). Thus, the difference between the two groups was statistically significant both at weeks 12 (+5 ± 7 vs. -2 ± 6%, p=0.014) and 24 (+1 ± 6 vs. -5 ± 5%, p=0.011). In addition, the individual values of baseline 1RM strength performance statistically correlated with the corresponding change in 1RM strength during the first 12-week period (r=-0.622, p=0.023) in E+S but not E. Maximal and rapid isometric leg press force remained statistically unaltered in both E+S and E at week 12 and 24. Similarly, maximal unilateral isometric knee extension force did not statistically change in the two groups throughout the training but isometric unilateral knee flexion force statistically decreased in E+S at week 24 (p=0.031), while it remained statistically unaltered in E.

Counter-movement jump height (FIGURE 14B) remained statistically unaltered in both E+S and E at weeks 12 and 24, but the change in jump height at week 12 was statistically greater in E+S compared to E (+3 ± 8% vs. -4 ± 7%, p=0.025).

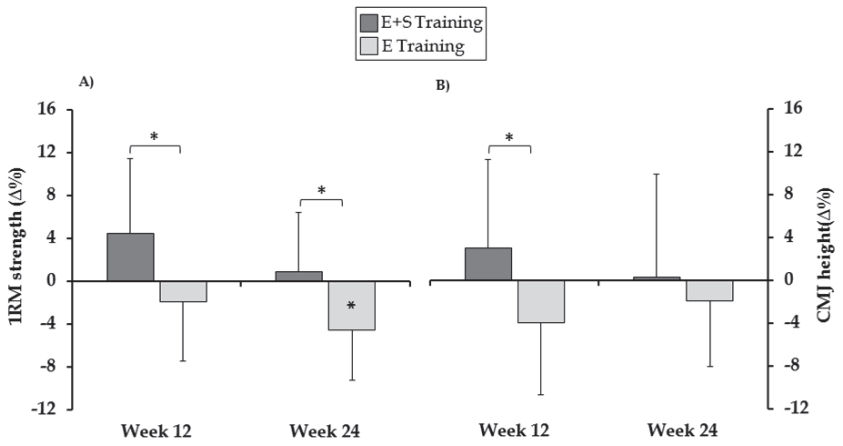

FIGURE 14 Chronic relative changes in maximal dynamic strength (A) and countermovement jump height (B) following 12 and 24 weeks of E+S and E training in recreational endurance runners. * p<0.05.

5.4.1.2 Muscle cross-sectional area and lean body mass

Previously untrained men

Baseline values of average muscle cross-sectional area and lean body mass in previously untrained men (study 1) are presented in TABLE 7.

TABLE 7 Baseline muscle cross-sectional area and lean body mass in previously untrained men.

	E+S	S+E
CSA (cm^2)	21.4 ± 3.2	20.6 ± 2.7
Total lean mass (g)	57963 ± 5116	56406 ± 4942
Leg lean mass (g)	29085 ± 2768	27953 ± 2491
Upper body lean mass (g)	28878 ± 2798	28453 ± 2705

CSA: Average muscle cross-sectional area of vastus lateralis

Average muscle cross-sectional-area of vastus lateralis (FIGURE 15) statistically increased in E+S and S+E at week 12 ($p=0.002$ and $p<0.001$, respectively) and further at week 24 (from week 0 $p=0.001$ and $p<0.001$, respectively; from week 12 both $p<0.001$). No statistical between-group differences were observed. Based on the pooled data of both groups, the individual changes in muscle cross-sectional area were statistically correlated with the corresponding changes in 1RM strength at week 24 ($r=0.629$, $p=0.007$).

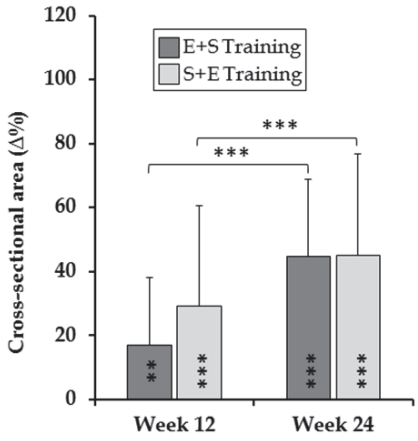

FIGURE 15 Chronic relative changes in muscle cross-sectional area of vastus lateralis following 12 and 24 weeks of E+S and S+E training in previously untrained men. **, *** $p<0.01$ and 0.001, respectively. When marked within the bar the comparison is made to week 0.

Statistically significant increases were also observed in total lean mass (FIGURE 16A) both at weeks 12 and 24 (E+S $p=0.042$ and $p=0.001$, respectively; S+E $p<0.001$ and $p=0.001$, respectively), leg lean mass (FIGURE 16B) in both groups at weeks 12 and 24 (E+S $p=0.024$ and $p<0.001$, respectively; S+E $p<0.001$ and

p=0.001, respectively) and upper body lean mass (FIGURE 16C) in E+S only at week 24 (p=0.005) but S+E at both weeks 12 and 24 (p=0.022 and p=0.025, respectively). The increase in leg lean mass from weeks 12 to 24 was statistically significant in E+S only (p=0.002). No statistical between-group differences were observed. When data of both groups were pooled, the individual changes in leg lean mass were statistically correlated with the corresponding changes in 1RM strength at week 24 (r=0.476, p=0.037).

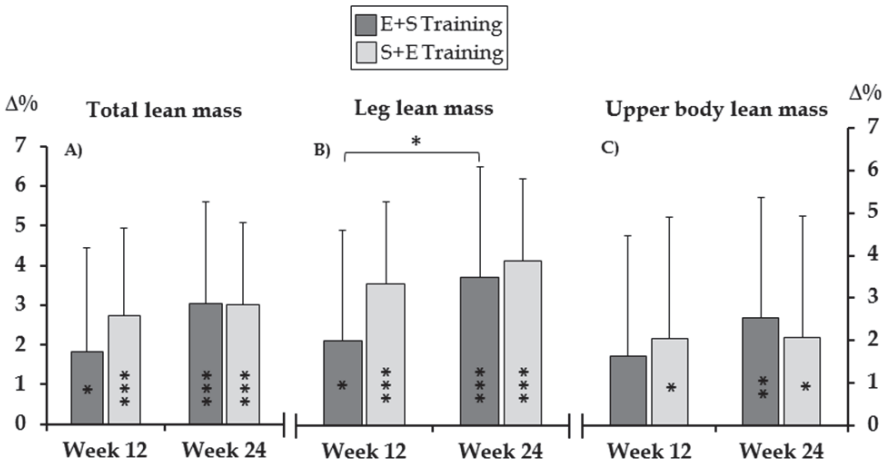

FIGURE 16 Chronic relative changes in total lean mass (A), leg lean mass (B) and upper body lean mass (C) following 12 and 24 weeks of E+S and S+E training in previously untrained men. *, **, *** p<0.05, 0.01, and 0.001, respectively. When marked within the bar the comparison is made to week 0.

Recreational endurance runners

Baseline values of average muscle cross-sectional area and lean body mass in recreational endurance runners (study 2) are presented in TABLE 8.

TABLE 8 Baseline muscle cross-sectional area and lean body mass in recreational endurance runners.

	E+S	E
CSA (cm²)	22.2 ± 3.9	19.5 ± 2.9
Total lean mass (g)	57378 ± 4553	54646 ± 4240
Leg lean mass (g)	30444 ± 2468	29229 ± 2298
Upper body lean mass (g)	26934 ± 2328	25418 ± 2210

CSA: Average muscle cross-sectional area of vastus lateralis

Average muscle cross-sectional-area of vastus lateralis (FIGURE 17) remained statistically unaltered throughout the training in both E+S and E but a statistical between-group difference was observed at weeks 12 (+6 ± 8% vs. -5 ± 6%, p<0.001) and 24 (+7 ± 7% vs. -6 ± 5%, p<0.001).

The F/CSA-ratio remained statistically unaltered in both E+S and E throughout the training. However, a statistical difference between E+S and E was observed both at weeks 12 (-5 ± 10% vs. +3 ± 8%, p=0.049) and 24 (-8 ± 9% vs. +3 ± 10%, p=0.006).

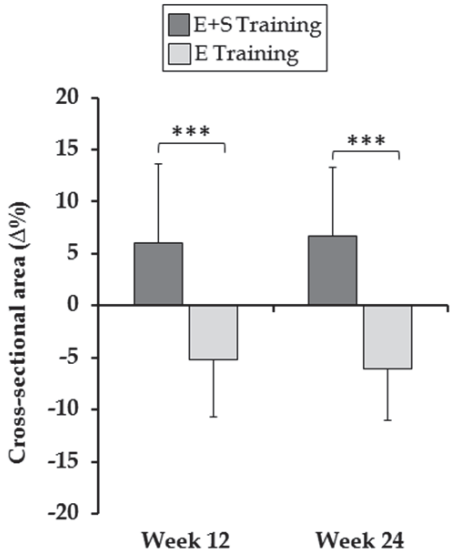

FIGURE 17 Chronic relative changes in muscle cross-sectional area following 12 and 24 weeks of E+S and E training in recreational endurance runners. *** p<0.001.

Total (FIGURE 18A) and leg lean mass (FIGURE 18B) statistically increased in both groups at week 12 (E+S p=0.002 and p=0.001, respectively; E p=0.015 and p=0.043, respectively) but only in E+S at week 24 (p=0.021 and p=0.016, respectively). The increases in both total and leg lean mass at week 12 were statistically larger in E+S compared to E (+3.0 ± 2.3% vs. +1.2 ± 1.3%, p=0.02 and 3.0 ± 2.2% vs. 1.1 ± 1.5%, p=0.017). Upper body lean mass (FIGURE 18C) statistically increased in E+S at week 12 only (p=0.024).

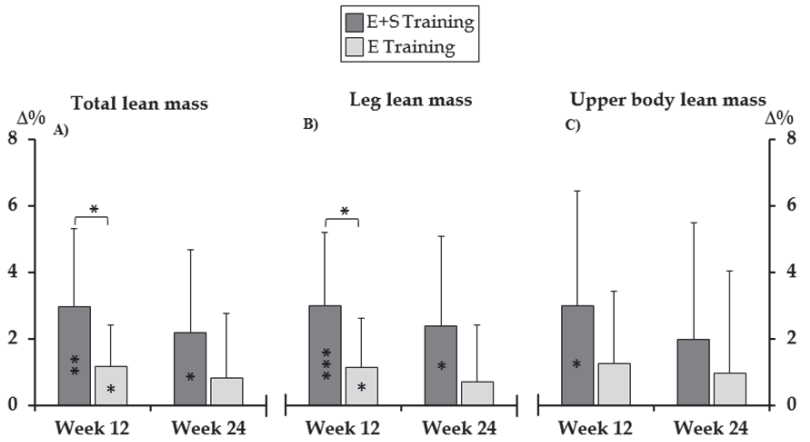

FIGURE 18 Chronic relative changes in total lean mass (A), leg lean mass (B) and upper body lean mass (C) following 12 and 24 weeks of E+S and E training in recreational endurance runners. *, **, *** $p<0.05$, 0.01, and 0.001, respectively. When marked within the bar the comparison is made to week 0.

5.4.1.3 Voluntary activation and surface EMG

In recreational endurance runners, voluntary activation percentage (FIGURE 19A) remained statistically unaltered throughout the training in E+S (n=10, 93 ± 5% at week 0) and E (88 ± 7% at week 0). However, the training-induced changes in the E+S group were distinct on an individual level (FIGURE 19B) and the correlation between the individual basal levels of voluntary activation and the corresponding relative changes from weeks 0 to 12 approached statistical significance (r=-0.568, p=0.087).

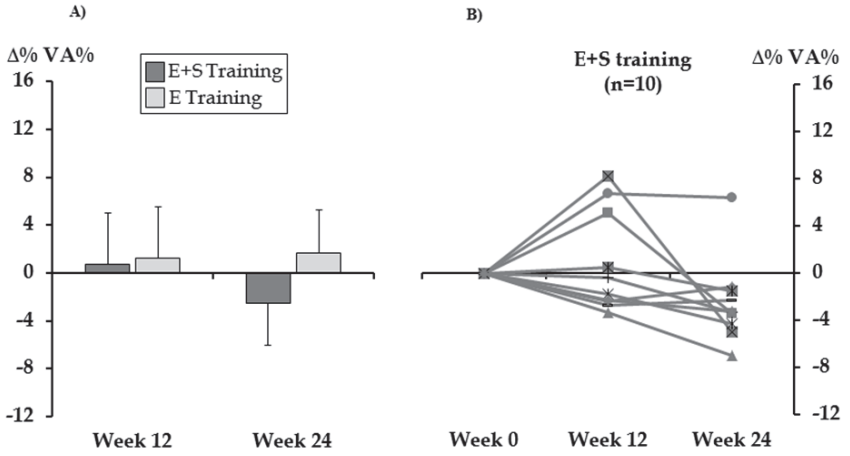

FIGURE 19 Chronic relative changes in voluntary activation in E+S and E (A) and individual changes in the E+S group (B) following 12 and 24 weeks of training in recreational endurance runners.

Average EMG of vastii lateralis and medialis during maximal and rapid isometric leg press and maximal isometric knee extension as well as average EMG of biceps femoris during isometric knee flexion remained statistically unaltered in both E+S and E. No statistically significant changes in agonist-antagonist co-activation of the leg extensors and flexors were observed in either of the two groups.

5.4.2 Cardiorespiratory adaptations

5.4.2.1 Endurance performance and maximal oxygen uptake

Previously untrained men
Baseline endurance performance and maximal oxygen uptake in previously untrained men are presented in TABLE 9.

TABLE 9 Baseline endurance performance and oxygen uptake in previously untrained men.

	E+S	S+E
Time to exhaustion (minutes)	19.3 ± 3.2	17.8 ± 2.8
Maximal power output (W)	268 ± 40	245 ± 35
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	42.2 ± 7.2	43.8 ± 7.4

Both the E+S and S+E groups statistically increased time to exhaustion (FIGURE 20A) at weeks 12 ($p=0.003$ and $p<0.001$, respectively) and 24 (both $p<0.001$) as well as maximal power output (FIGURE 20B) at weeks 12 ($p=0.011$ and $p<0.001$, respectively) and 24 (both $p<0.001$). The increases in maximal power output in both groups from weeks 12 to 24 were statistically significant ($p<0.01$ - 0.001). Maximal oxygen uptake statistically increased in both groups at weeks 12 (E+S 5 ± 7%, $p=0.051$; S+E 7 ± 8%, $p=0.003$) and 24 (E+S 6 ± 8%, $p=0.041$; E 6 ± 12%, $p=0.006$).

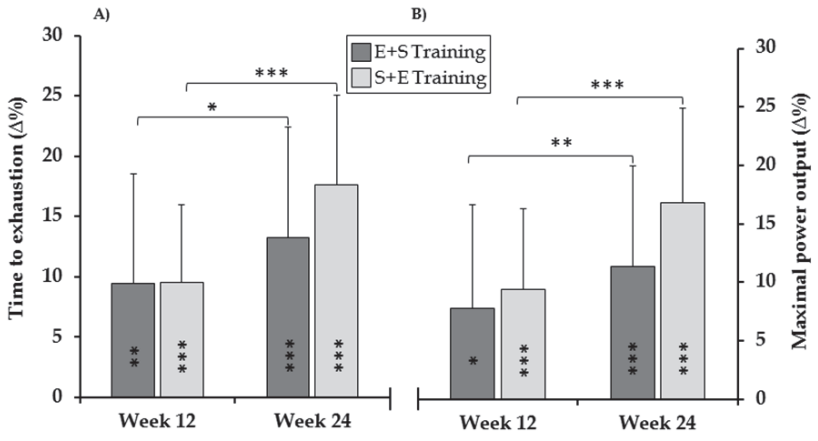

FIGURE 20 Chronic relative changes in time to exhaustion (A) and maximal power output (B) following 12 and 24 weeks of E+S and S+E training in previously untrained men. *, **, *** $p<0.05$, 0.01, and 0.001, respectively. When marked within the bar the comparison is made to week 0.

Recreational endurance runners

Baseline endurance performance in recreational endurance runners is presented in TABLE 10.

TABLE 10 Baseline endurance performance in recreational endurance runners.

	E+S	E
Time to exhaustion (minutes)	27.3 ± 2.8	24.9 ± 3.4
1000 m running time (minutes)	3.6 ± 0.4	3.8 ± 0.3

Maximal 1000 m running time (FIGURE 21) statistically decreased from weeks 0 to 12 ($p<0.001$) in E+S but plateaued thereafter. The running time at week 24 was statistically shorter compared to that at week 0 ($p=0.001$). In E, the running time statistically decreased from weeks 0 to 12 as well ($p=0.001$) but further decreased from weeks 12 to 24 ($p=0.012$). The running time at week 24 was statistically shorter compared to that at week 0 (-13 ± 5%, $p<0.001$). No statistical between-group differences were observed.

Time to exhaustion determined during the incremental treadmill test statistically increased at week 24 in both E+S (7 ± 7%, $p=0.011$) and E (10 ± 7%, $p<0.001$), while no statistical between-group differences were observed.

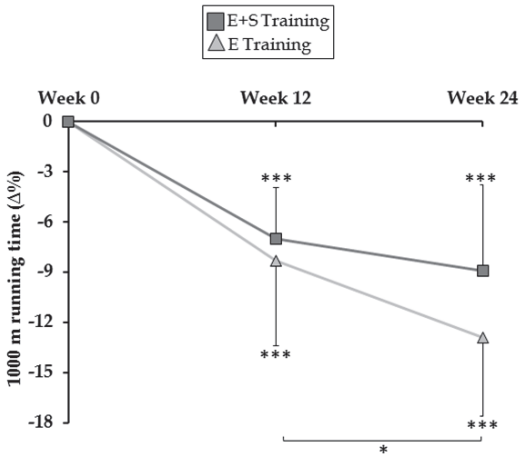

FIGURE 21 Chronic relative changes in maximal 1000 m running time during E+S and E training in recreational endurance runners. *** $p<0.001$ compared to week 0, * $p<0.05$ compared to week 12.

5.4.2.2 Metabolic thresholds and blood lactate, oxygen and heart rate curves

Previously untrained men

In previously untrained men (study 1), statistical increases in the load at 4 mmol·l^{-1} of blood lactate were observed in S+E only, both at weeks 12 (16 ± 20%, p=0.018) and 24 (25 ± 21%, p<0.001). Furthermore, AUC of blood lactate concentrations (FIGURE 22) statistically decreased at week 24 in E+S (p=0.025) and S+E (p=0.004). AUC of sub-maximal oxygen uptake remained statistically unaltered in both E+S and S+E. A statistical increase in AUC of gross efficiency was observed only in E+S at week 24 (+3 ± 5%, p=0.046).

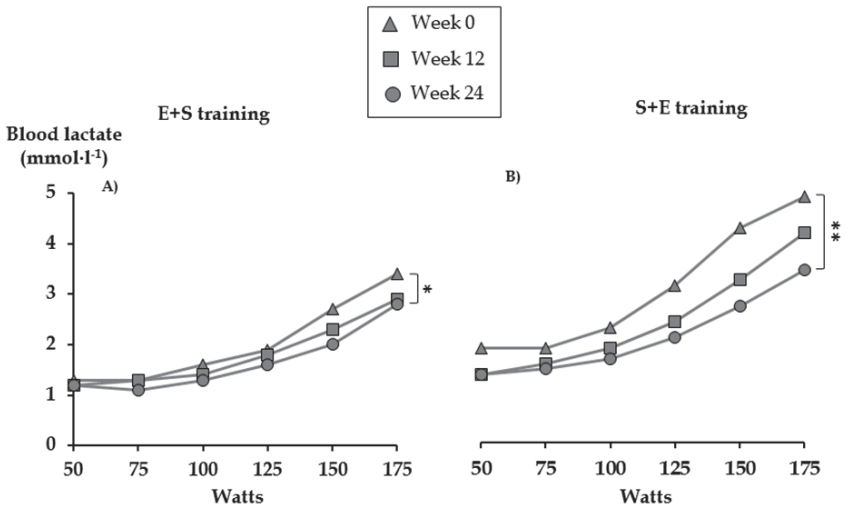

FIGURE 22 Chronic absolute changes in blood lactate concentrations during the first 6 stages of the incremental cycle ergometer test in previously untrained men. Area under the curve *, ** p<0.05 and p<0.01, respectively.

Recreational endurance runners

In recreational endurance runners (study 2), treadmill test determined velocity at 4 mmol·l^{-1} of blood lactate statistically increased at week 24 in E+S (6 ± 6%, p=0.003) and E (8 ± 9%, p=0.013), while no statistical between-group differences were observed. AUC of heart rate (FIGURE 23) statistically decreased from weeks 0 to 12 in both E+S (p=0.005) and E (p=0.002) and plateaued thereafter in both groups. The reduction of heart rate AUC at week 24 was statistically significant both in E+S (-3 ± 4%, p=0.014) and E (-6 ± 4%, p=0.003). AUC of blood lactate concentrations remained statistically unaltered in E+S but significantly decreased in E both at weeks 12 (-11 ± 5%, p=0.024) and 24 (-21 ± 29%, p=0.011). However, no statistically significant between-group difference was observed in AUC of heart rate and blood lactate concentrations.

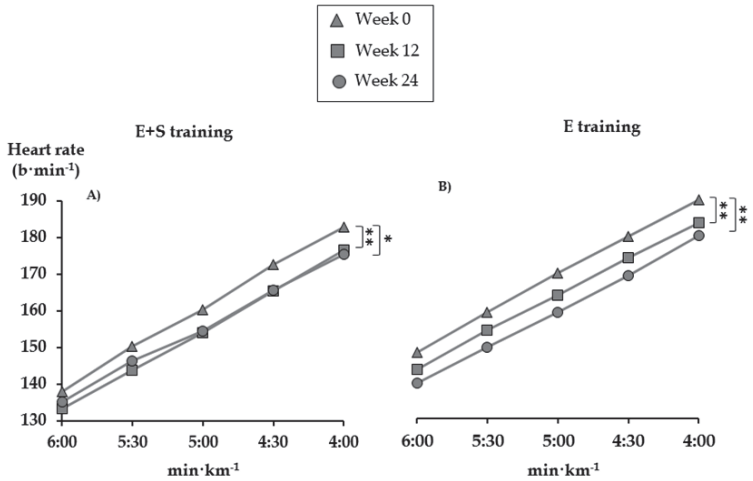

FIGURE 23 Chronic absolute changes in heart rate during the first 5 stages of the incremental field test in the E+S (A) and E (B) groups of recreational endurance runners. Area under the curve *, *** $p<0.05$ and 0.001, respectively.

5.4.2.3 Body fat mass and blood lipids

Previously untrained men

In previously untrained men (study 1), no statistical changes in body fat percentage, total fat mass or abdominal fat mass were observed in E+S or S+E at either weeks 12 or 24. However, when data of the two groups were pooled, the individual values of body fat percentage and total fat mass at baseline statistically correlated with the corresponding relative changes at week 24 ($r=-0.450$, $p=0.006$ and $r=-0.364$, $p=0.037$, respectively). In addition, the individual values of both the total fat and abdominal fat mass at baseline statistically correlated with the relative change in body fat percentage at week 24 ($r=-0.458$, $p=0.006$ and $r=-0.431$, $p=0.006$, respectively).

In addition, only minor changes in total cholesterol, HDL-C, LDL-C and triglycerides were observed after 24 weeks of training (TABLE 11). A statistically significant difference between E+S and S+E was observed for LDL-C at week 12 (-8 ± 14 vs. +3 ± 15%, $p=0.043$) but was diminished after 24 weeks of training.

TABLE 11 Chronic changes in absolute values of blood lipids in previously untrained men.

Group	Week 0 (mmol·l^{-1})	Week 12 (mmol·l^{-1})	Week 24 (mmol·l^{-1})	Week 0 - 12 (Δ mmol·l^{-1})	Week 0 - 24 (Δ mmol·l^{-1})
Total Cholesterol					
E+S	4.83 ± 0.93	4.55 ± 0.82	4.62 ± 0.98	-0.28 ± 0.46	-0.21 ± 0.45
S+E	4.58 ± 0.77	4.63 ± 0.64	4.62 ± 0.64	0.04 ± 0.56	0.04 ± 0.30
LDL-C					
E+S	2.80 ± 0.85	2.53 ± 0.73	2.69 ± 0.86	-0.27 ± 0.44	-0.11 ± 0.34
S+E	2.75 ± 0.57	2.84 ± 0.73$^{\gamma}$	2.74 ± 0.53	0.09 ± 0.40	-0.01 ± 0.41
HDL-C					
E+S	1.59 ± 0.38	1.58 ± 0.50	1.54 ± 0.44	-0.02 ± 0.19	-0.05 ± 0.17
S+E	1.40 ± 0.30	1.30 ± 0.30*	1.37 ± 0.34	-0.1 ± 0.1	-0.03 ± 0.12
Triglycerides					
E+S	0.96 ± 0.34	0.98 ± 0.28	0.82 ± 0.30	0.0 ± 0.2	-0.14 ± 0.31
S+E	1.36 ± 1.03	1.19 ± 0.79	1.13 ± 0.53	-0.2 ± 0.6	-0.24 ± 0.63

LDL-C: Low density lipoprotein, HDL-C: High density lipoprotein; * $p<0.05$ compared to corresponding value at week 0; $^{\gamma}$ $p<0.05$ between the two groups at corresponding time point.

Recreational endurance runners

In recreational endurance runners (study 2), body fat percentage statistically decreased in E+S and E at weeks 12 (-2.1 ± 2.3%, p=0.003 and -1.5 ± 1.3%, p=0.019, respectively) and 24 (-2.3 ± 2.0%, p<0.001 and -2.6 ± 1.7%, p=0.003, respectively). In addition, statistically significant reductions were also observed in total fat mass in the two groups at weeks 12 (E+S -11.8 ± 16.2%, p=0.007; E -10.5 ± 10.1, p=0.042) and 24 (E+S -15.0 ± 16.9%, p<0.001; E -17.6 ± 11.4%, p=0.004). The training-induced changes in body fat percentage and total fat mass did not statistically differ between the two groups.

In addition, no statistical alterations in total cholesterol, HDL-C and LDL-C (TABLE 12) were observed after 24 weeks of training. In E+S, a statistically significant reduction in concentrations of triglycerides was observed at week 24 (-11 ± 24%, p=0.035) and, thus, the change at week 24 differed statistically between E+S and E (-11 ± 24 vs. +11 ± 33%, p=0.002).

TABLE 12 Chronic changes in absolute values of blood lipids in recreational endurance runners.

Group	Week 0 (mmol·l^{-1})	Week 12 (mmol·l^{-1})	Week 24 (mmol·l^{-1})	Week 0 - 12 (Δ mmol·l^{-1})	Week 0 - 24 (Δ mmol·l^{-1})
Total Cholesterol					
E+S	4.90 ± 0.90	4.72 ± 0.83	4.56 ± 0.87	-0.18 ± 0.43	-0.34 ± 0.54
E	4.88 ± 0.54	4.73 ± 0.72	4.72 ± 0.63	-0.15 ± 0.48	-0.01 ± 0.42
LDL-C					
E+S	2.89 ± 0.71	2.80 ± 0.63	2.73 ± 0.67	-0.09 ± 0.46	-0.16 ± 0.57
E	2.90 ± 0.11	2.77 ± 1.16	2.76 ± 1.21	-0.13 ± 0.42	-0.14 ± 0.40
HDL-C					
E+S	1.59 ± 0.28	1.73 ± 0.27	1.50 ± 0.24	0.14 ± 0.22	-0.09 ± 0.14
E	1.61 ± 0.38	1.62 ± 0.38	1.59 ± 0.37	0.01 ± 0.16	-0.02 ± 0.19
Triglycerides					
E+S	0.98 ± 0.34	0.93 ± 0.45	0.75 ± 0.18*	-0.05 ± 0.55	-0.23 ± 0.33^{YY}
E	1.12 ± 0.46	1.10 ± 0.40	1.20 ± 0.48	-0.02 ± 0.22	0.08 ± 0.23

LDL-C: Low density lipoprotein, HDL-C: High density lipoprotein; * $p<0.05$ compared to corresponding value at week 0; ^{YY} $p<0.01$ between the two groups at corresponding time point.

5.4.3 Adaptations in basal hormone concentrations

Previously untrained men

Baseline concentrations of serum hormones in previously untrained men are presented in TABLE 13.

TABLE 13 Baseline serum hormone concentrations in previously untrained men.

	E+S	S+E
Testosterone (nmol·l^{-1})	12.7 ± 3.0	13.8 ± 3.7
Growth hormone (mlU·l^{-1})	1.0 ± 1.7	2.1 ± 6.3
Cortisol (nmol·l^{-1})	522.0 ± 107.6	525.1 ± 114.1
SHBG (nmol·l^{-1})	27.5 ± 7.0	31.4 ± 8.7
Testosterone/Cortisol-ratio	0.025 ± 0.004	0.027 ± 0.10
Testosterone/SHBG-ratio	0.489 ± 0.161	0.444 ± 0.076

SHBG: Sex-hormone-binding-globulin

Statistically significant increases were observed for basal testosterone concentrations (FIGURE 24A) in E+S and S+E both at weeks 12 ($p=0.027$ and $p=0.004$, respectively) and 24 (both $p<0.001$). The increase from weeks 12 to 24 was statistically significant in E+S only ($p=0.024$). Concentrations of growth hormone, cortisol and SHBG in the two groups remained statistically unaltered. The change in testosterone/cortisol-ratio (FIGURE 24B) approached statistical significance in E+S at week 12 ($p=0.058$) and was significantly altered in S+E ($p=0.005$), while at week 24 a statistically significant increase was observed both in E+S and S+E ($p=0.001$ and $p=0.012$, respectively). Similarly, statistically significant increases were also observed in the changes in testosterone/SHBG-ratio

in E+S at week 24 (47 ± 39%, p<0.001) and S+E both at weeks 12 (37 ± 39%, p=0.003) and 24 (34 ± 38%, p=0.001).

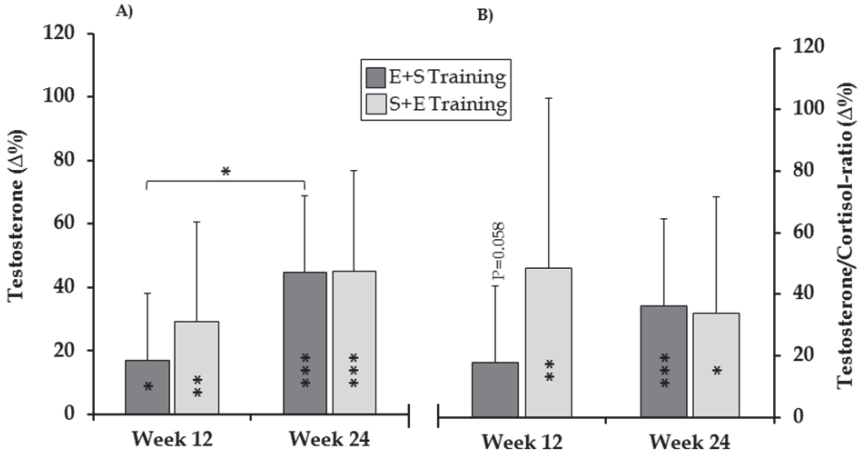

FIGURE 24 Chronic relative changes in total serum testosterone concentrations (A) and the testosterone/cortisol-ratio (B) following 12 and 24 weeks of E+S and S+E training in previously untrained men. *, **, *** p<0.05, 0.01, and 0.001, respectively. When marked within the bar the comparison is made to week 0.

Recreational endurance runners

Baseline concentrations of serum hormones in recreational endurance runners (III) are presented in TABLE 14.

TABLE 14 Baseline serum hormone concentrations in recreational endurance runners.

	E+S	E
Testosterone (nmol·l^{-1})	19.7 ± 7.9	16.9 ± 6.4
Growth hormone (mIU·l^{-1})	1.0 ± 1.7	0.5 ± 0.6
Cortisol (nmol·l^{-1})	504.4 ± 130.9	499.9 ± 85.2
SHBG (nmol·l^{-1})	33.6 ± 9.8	29.6 ± 11.3
Testosterone/Cortisol-ratio	0.040 ± 0.013	0.034 ± 0.012
Testosterone/SHBG-ratio	0.614 ± 0.248	0.601 ± 0.181

SHBG: Sex-hormone-binding-globulin

Serum concentrations of testosterone, growth hormone, cortisol, SHBG and the testosterone/cortisol-ratio remained statistically unaltered in both groups. The testosterone/SHBG-ratio statistically decreased in E+S at week 12 (-19 ± 26%, p=0.006) but remained statistically unaltered in E. Even though the testosterone/SHBG-ratio was no longer statistically altered in E+S at week 24, the change in testosterone/SHBG-ratio from weeks 12 to 24 was statistically larger in E+S compared to E (+42 ± 47% vs. -5 ± 33%, p=0.006).

6 DISCUSSION

The purpose of this thesis was two-fold. First, to investigate neuromuscular, cardiorespiratory and hormonal responses and adaptations to concurrent endurance and strength training performed in the same session with different exercises orders (endurance followed by strength training and vice versa) in previously untrained young men. Second, to assess physiological effects of same-session combined training when strength training was always performed immediately after an endurance running session in recreational endurance runners.

6.1 Previously untrained men

6.1.1 Acute force and hormone responses before and after training

Acute force responses

The overall magnitude of acute reductions in isometric force production in response to the experimental loadings was similar in E+S and S+E both before and after 24 weeks of training. Similarly, maximal force returned to pre-loading levels already within 24 h in both groups before and after the training intervention. Interestingly, at week 0, endurance cycling performed before strength loading (i.e. in E+S) led to a reduction in maximal force of 11% while in the opposite order endurance cycling performed after the strength loading did not further reduce force production. Hence, while the present strength loading produced neuromuscular fatigue when performed both before and after endurance exercise, endurance cycling did not add to the overall magnitude of fatigue when performed in a pre-fatigued condition.

This observed plateau in fatigue was previously also shown in studies investigating the acute neuromuscular responses to prolonged performance of strength loadings only (Häkkinen & Pakarinen 1993; Ahtiainen et al. 2003b). The reasons for this phenomenon observed in the present thesis may be mani-

fold. During endurance cycling of moderate intensity both type I and type IIa fibres of the quadriceps muscle are typically active (Vøllestad, Vaage & Hermansen 1984). Although muscle activation was not measured in the present study, it is likely that the strength loading activated high threshold motor units characterized by high fatigability (Henneman, Somjen & Carpenter 1965), while the subsequent cycling only led to additional recruitment of fatigue-resistant slow twitch fibers, apparently not increasing the magnitude of overall fatigue. The underlying mechanisms for the present finding, however, may also be metabolic in nature and were not examined in detail.

The reduction of maximal force in S+E at MID (i.e. after S) was significantly larger post-training compared to the corresponding change observed before the intervention. Although not reflected in blood lactate concentrations, these results indicate an enhanced ability to produce fatigue as previously shown in acute responses to strength loadings after periods of heavy resistance training (Izquierdo et al. 2009b; Izquierdo et al. 2011; Walker, Ahtiainen & Häkkinen 2010). Vice versa the findings of these previous studies as well as that of the present S+E group indicated that the subjects were able to sustain a larger magnitude of both mechanical and metabolic stress. The present findings, thus, suggest strength loadings performed immediately before endurance cycling to be more favorable over the reverse loading order in previously untrained men.

Acute hormonal responses

Acute exercise loading-induced reductions in force production are typically accompanied by temporary alterations in hormonal concentrations. Statistically significant acute changes in hormone responses to the present two combined loading sessions (i.e. E+S vs. S+E), however, only occurred in serum growth hormone both before and after the training intervention and in total serum testosterone in E+S at MID only at week 0. Since the highest concentrations of growth hormone both before and after the training intervention were found in E+S at MID (i.e. after E) and S+E at POST, it appears that the present steady-state cycling at moderate- to high-intensity rather than the strength protocol induced large increases in serum 22-kDa growth hormone concentrations. Previous findings by Goto et al. (2005) showed that endurance exercise may attenuate the growth hormone response during subsequent strength loading as observed in the present E+S group. However, it should be emphasized that in the present study it was not investigated whether the concurrent loading induced significant changes in other growth hormone aggregates or variants (Kraemer et al. 1990).

When interpreting the findings of this thesis, one must bear in mind that significant strength loading-induced hormonal responses are typically observed following metabolic demanding protocols, such as hypertrophic loading sessions characterized by larger number of repetitions with short inter-set rest periods (Häkkinen & Pakarinen 1993). The intensity and volume of the present experimental loadings, however, was purposefully chosen to i) account for the capabilities of previously untrained subjects and ii) to represent the overall pe-

riodized training program by combining moderate to high intensity continuous cycling of a relatively short duration with a mixture of explosive, maximal and hypertrophic leg press protocols. Thus, only 2 out of the total 11 sets of the strength protocol were performed as a hypertrophic-style loading. In agreement with previous studies as well as that indicated by the relatively low concentrations of blood lactate in this thesis, the present combined loading likely did not produce sufficient physiological stress to stimulate increases in serum testosterone and cortisol concentrations (Kraemer et al. 1990; Häkkinen & Pakarinen 1993; Linnamo et al. 2005; McCaulley et al. 2009).

However, despite comparable acute testosterone and cortisol responses were observed in the present group of previously untrained men, serum cortisol concentrations were statistically reduced during recovery at 24 h and 48 h before the training intervention in both groups and testosterone following the E+S loading only. Previous studies have shown that prolonged endurance performance may lead to reduced cortisol concentrations for at least 24 h in endurance trained subjects (Daly et al. 2005). Similarly, testosterone concentrations have previously been shown to be reduced following heavy resistance loadings for up to 48 h in strength-trained athletes (Häkkinen & Pakarinen 1993), following an intermittent endurance loading for 12 h in endurance athletes (Hackney et al. 2012) and following a combined loading session for up to 48 h in recreational endurance runners (Taipale & Häkkinen 2013). The present findings may, thus, indicate the E+S loading protocol conducted before the training intervention to be physiologically more demanding for previously untrained men, possibly prolonging the recovery needs.

However, the detailed mechanisms for the present decreased hormonal concentrations during recovery are not yet conclusively understood. While the biological functions of transiently reduced concentrations of cortisol vs. testosterone may differ due to the catabolic vs. anabolic nature of these hormones, reduced hormonal concentrations during recovery have generally been linked with both an up-regulation of androgen receptors accompanied by increased target tissue uptake or an inhibited production of these hormones in the releasing gland or at the hypothalamus level (Vingren et al. 2010). However, since the kinetics of androgen receptor regulation and its association with circulating testosterone concentrations following strenuous exercise sessions has not yet been fully elucidated, the biological meaning of reduced concentrations of serum testosterone and cortisol during recovery has to be further examined.

Interestingly, the present initial decreases in serum cortisol and testosterone during recovery at week 0 diminished after the 24 weeks of training. The magnitude of loading-induced hormone responses, however, was similar before and after the training intervention in both groups. The latter finding is in agreement with previous studies investigating chronic adaptations in strength loading-induced hormone concentrations following prolonged strength training (Kraemer et al. 1990; Häkkinen et al. 2000; Ahtiainen et al. 2003a). The training-induced diminished reductions of both catabolic and anabolic hormone concentrations during recovery in the present thesis, however, suggest adaptations on

the endocrine level which apparently were especially pronounced in the E+S group. It is, however, worth noting that after the training intervention the reductions in testosterone concentrations at 48 h of recovery in E+S approached statistical significance. In addition, also in cortisol concentrations a statistic trend for reductions was found in E+S at 48 h, while in S+E cortisol concentrations were statistically reduced at the same time point. Therefore, it cannot be ruled out that the present training intervention affected the time course for hormonal changes and systematic hormonal reductions would have been observed beyond the 48 h recovery period.

6.1.2 Basal neuromuscular, cardiorespiratory and hormonal adaptations

Accumulated concentrations of anabolic hormones dramatically increase the likelihood for androgen receptor interactions (Kraemer & Ratamess 2005). Repeated loading-induced acute increases in these hormones during prolonged training have been shown to be associated with positive adaptations in muscle hypertrophy and strength development during pure strength training (Häkkinen et al. 2000; Rønnestad, Nygaard & Raastad 2011). While few authors have questioned the relationship of exercise loading-induced testosterone concentrations with chronic training adaptations (West et al. 2010), several studies have shown statistically significant correlations between both basal and loading-induced concentrations of circulating testosterone and chronic development of muscle mass and strength during strength training in men (McCall et al. 1999; Ahtiainen et al. 2003a; Kvorning et al. 2006; Rønnestad, Nygaard & Raastad 2011). Thus, it may be expected that reduced concentrations of testosterone and cortisol during recovery, especially in the present E+S group, likely affected the chronic neuromuscular, cardiorespiratory and hormonal adaptations to prolonged training in these previously untrained men. However, in the present thesis, no correlations were found between loading-induced testosterone, growth hormone or cortisol concentrations and chronic physiological adaptations and both exercise orders led to similar neuromuscular, cardiorespiratory and hormonal adaptations after 24 weeks of training. It has to be acknowledged that the training frequency in the present study was rather low, allowing for at least 2 full days of rest between consecutive training sessions. The reduced testosterone concentrations in E+S, however, were monitored for 48 h only and may have, thus, not affected chronic training adaptations. The present results indicate that the previously untrained men in this study adapted to both the endurance and strength training stimuli simultaneously with similar magnitude.

Neuromuscular adaptations

The adaptations in dynamic 1RM strength and maximal isometric leg press force were similar in the two groups, while a statistic increase in rapid force production was observed in the S+E group only. It should be emphasized that endurance training was performed on a cycle ergometer. Previous studies have shown that endurance cycling is biomechanically similar to many of the strength exercises performed in the present study (Fonda, B., Sarabon, N. 2012;

Escamilla et al. 2001) and may essentially lead to a similar magnitude of fatigue as indicated, for example, by inhibited neuromuscular performance (Sidhu et al. 2013; Seifert & Petersen 2010). Together with other findings which showed that prolonged training of endurance cycling may also lead to small but statistically significant increases in muscle CSA (Mikkola et al. 2012) and strength (Izquierdo et al. 2005) in previously untrained subjects, it is possible that the present endurance training combined with the hypertrophic and maximal strength training protocols led to synergistic rather than adverse effects on strength performance.

However, in another data set from our research group which was analyzed from the same group of previously untrained subjects, it was shown that the neural adaptations, as indicated by measures of voluntary activation and EMG, differed between the E+S and S+E group (Eklund et al. 2015). Along with the leading hypothesis of the present thesis that residual fatigue from endurance training possibly compromises the quality of the subsequently performed strength loading, our previous study found that voluntary activation was statistically increased in the S+E group only. Furthermore, the individual changes in voluntary activation during the latter half of the training in E+S were statistically correlated with the individual changes in maximal isometric knee extension force. Therefore, it was concluded that neural inhibition indeed seemed to have occurred in the E+S group especially during the second training period but this was at least after 24 weeks not reflected in maximal strength gains but possibly in rapid force production which remained statistically unaltered in the E+S group of the present thesis.

One possible explanation for similar maximal strength gains despite dissimilar neural adaptations in the E+S and S+E groups may lie in the morphological adaptations. The present individual increases in 1RM strength were significantly correlated with individual increases in anatomical muscle cross-sectional area and leg lean mass in all subjects across the two training groups. Both training groups statistically increased muscle CSA as well as leg, upper body and total lean mass after the 24 weeks of training independent of the loading order which may have compensated for possible neural deficits. Although animal studies have shown that endurance and strength training might induce distinct genetic and molecular pathways critical for muscle hypertrophy (Atherton et al. 2005; Hawley 2009), it has recently been hypothesized that anabolic responses to combined loadings may be unimpeded (Fyfe, Bishop & Stepto 2014). This has been attributed to studies showing that the time course of metabolic signaling responses to endurance exercise that possibly inhibit protein synthesis are of much shorter duration when compared to anabolic responses to strength training (Drummond et al. 2011; Lee-Young et al. 2008). However, other studies have indicated the cumulative effect of both loadings to possibly compromise beneficial morphological adaptations (Hawley 2009; Coffey et al. 2009). Coffey et al. (2009) found in a randomized cross-over design that neither of the two loading orders (E+S vs. S+E) showed superior signaling responses over the other but concluded that endurance and strength training performed

in close proximity did not induce optimal activation of pathways to promote significant anabolic processes. While the magnitude of interference when compared to strength training alone was beyond the scope of the present thesis, these previous findings possibly explain why no between-group differences in muscle growth were present in previously untrained subjects.

Cardiorespiratory adaptations

In the present study, no statistically significant between-group differences in the changes of time to exhaustion, maximal power output and maximal oxygen uptake were observed. Furthermore, AUC of sub-maximal oxygen uptake remained statistically unaltered in both groups throughout the training, while gross efficiency significantly increased in E+S only. The overall magnitude of efficiency changes in E+S, however, was nearly similar to that observed in S+E. Previous cross-sectional studies have shown that movement economy may be reduced for several hours following typical (Doma & Deakin 2014; Burt et al. 2013) and plyometric strength loadings (Ratkevicius et al. 2006). Possibly as a result, Chtara et al. (2005) showed that in young men endurance performance and cardiorespiratory fitness increased to a larger extent following E+S compared to S+E training. As stated earlier, however, the low weekly training frequency of 2 - 3 sessions in the present study may have provided sufficient recovery between each training session, optimizing adaptations irrespective of the exercise order.

Typically, the metabolic cost of cycling at sub-maximal loads decreases when endurance performance is increased by prolonged training. In addition, strength training has previously been shown to induce positive adaptations in cycling economy in previously untrained men (Loveless et al. 2005). These strength training-induced adaptations have been attributed to metabolic adaptations such as increased phosphocreatine, lower lactate and higher glycogen content during exercise (Goreham et al. 1999), increased peak muscle tension (Marcinik et al. 1991), increased tendon cross-sectional area (Rønnestad, Hansen & Raastad 2012) or delayed muscle fatigue (Barclay 1996). Furthermore, Cadore et al. (2011) have shown that strength training may improve neuromuscular economy as reflected by decreased EMG at different power outputs during cycle ergometry in elderly subjects. While such measurement was beyond the scope of the present study, all training groups significantly improved 1RM strength performance after 24 weeks of training but this was not reflected in the sub-maximal oxygen uptake in either of the two training groups. However, the AUC of blood lactate concentrations significantly decreased in both E+S and S+E, indicating that positive training adaptation at a cellular level occurred.

Despite the non-statistical differences between the two groups, the magnitude of improvement of the load at 4 $mmol \cdot l^{-1}$ in E+S was only half as large (10 ± 15%) as that observed in S+E (25 ± 21%). Previous studies have shown that the oxygen demands following combined training sessions are increased when endurance exercise preceded strength loadings (Drummond et al. 2005; Di Blasio et al. 2012). However, this has typically been considered as a positive ex-

ercise response by supporting adenosine triphosphate and creatine phosphate resynthesis, the replenishment of glycogen and oxygen stores and lactate removal (Borsheim & Bahr 2003). In contrast, Drummond et al. (2005) suggested that endurance running performed immediately after a strength training session may actually act as an active recovery strategy for the strength loading by enhancing lactate removal (Bond et al. 1991). Our findings of compromised development of the load at the metabolic threshold following E+S training in previously untrained subjects, thus, emphasizes the need for further investigation as to what may be the possible causes.

Aerobic exercise has been considered as being effective in inducing increases in fat oxidation during and in the hours following an exercise loading (Tambalis et al. 2009). However, in the present study no significant reductions in body fat percentage and total or abdominal fat mass in either training group were observed. Furthermore, no significant changes in total cholesterol as well as high- (HDL-C) and low (LDL-C) density lipoproteins or triglycerides were found. One possible explanation for these findings may be related to the endurance training mode chosen for the present study. Achten et al. (2003) showed that running induces higher rates of fat oxidation when compared to cycling and according to a meta-analysis (Wilson et al. 2012) a combination of running, as opposed to cycling, may be more beneficial in reducing body fat. Furthermore, the duration of each training session in the present study was limited to a maximum of 100 minutes leading to a total of maximal 200 minutes during weeks 0 - 12 and 200 - 300 minutes during weeks 13 - 24, half of which was performed aerobically. Thus, the overall duration and intensity of aerobic training may have not been sufficient (as also observed by the relatively small increases in VO_{2max}) to result in significant reductions of body fat and changes in blood lipids. Furthermore, when interpreting the present results, one must bear in mind that the subjects of the present study were normal weight, moderately active and healthy males with normal blood lipid levels which in turn provided a relatively small window for adaptations. Moreover, the nutritional intake was controlled but not restricted and the analysis of food diaries revealed that the subjects in both training groups maintained their nutritional intake constant throughout the 24 weeks of training and the reported caloric intake at baseline was considered normal. However, the observed correlations of the pooled data between individual baseline values of fat mass and body fat percentage and the corresponding relative reductions in these variables observed after 24 weeks of training, indicate that the present training program was especially effective for subjects with an initially higher percentage of body fat.

Hormonal adaptations

The adaptations in physical fitness and lean body mass were accompanied by significant increases in basal total testosterone concentrations as well as a in the testosterone/cortisol and testosterone/SHBG-ratio. It has previously been proposed that concurrent training may compromise the anabolic effects typically observed following pure strength training, due to the catabolic nature of endur-

ance training (Bell et al. 2000; Leveritt et al. 1999). Even though there was no pure strength training group in the present study, both concurrent training regimens in previously untrained men led to significant increases in testosterone concentrations already after the first 12 weeks. Interestingly, while the increase in E+S was linear throughout 24 weeks, no further statistical increase was observed during the second 12-week period in S+E.

Importantly, in the E+S group, significant reductions in testosterone concentrations during recovery of 24 h and 48 h were observed during the experimental loading before the training intervention. Even though these loading-specific differences diminished after 24 weeks of training, the exact time course for the adaptations in E+S cannot be elucidated by the present study design. Even though the difference in basal testosterone concentrations between E+S and S+E at week 12 was not significant, the lower concentrations observed in E+S may be the result of initially less favorable anabolic adaptations when endurance cycling preceded strength training. This finding may also be supported by the observed changes in the testosterone/SHBG ratio which was significant in E+S at week 24 but not after 12 weeks.

6.2 Recreational endurance runners

6.2.1 Acute force and hormone responses in recreational endurance runners before and after training

Acute force responses

Strength training-induced improvements in maximal endurance running performance in endurance athletes may in part be attributed to increased fatigue resistance allowing sustained repetitive cycles of stretch-shortening contractions over a prolonged period of time (Paavolainen et al. 1999b; Damasceno et al. 2015; Bertuzzi et al. 2014). This greater level of resistance against neuromuscular fatigue in response to endurance loading would be expected after a prolonged period of combined endurance and strength training compared to endurance training only. However, the endurance loading-induced acute reductions in maximal bilateral leg press force were similar in the present E+S and E groups both before and after the training intervention (~10%). The overall magnitude of reduction in the two groups is well in line with a previous review by Millet & Lepers (2004) reporting the strength loss induced by prolonged running to be directly related to both the duration and intensity of the preceding endurance run.

It should, however, be emphasized that the endurance loading was performed with relative maximal loads (i.e. time to exhaustion based on the current training status at weeks 0 and 24, respectively) and both training groups significantly increased time to exhaustion after 24 weeks of training. In light of the resulting increases in loading volume, the observed acute reductions in maximal force at week 24 may actually indicate a training adaptation. However,

as the magnitude of reductions was similar in E+S and E, the findings indicate these positive adaptations to be induced by the prolonged endurance training rather than the added strength training which was performed always immediately after an endurance running session.

Acute hormonal responses
Similar to the observed acute force responses, no training-induced changes in endurance loading-induced acute responses of total testosterone, growth hormone and cortisol were observed. Both groups significantly increased anabolic and catabolic hormone concentrations before and after the training intervention to a similar extent. While these findings are in contrast to a study by Kraemer et al. (1995) who found significantly larger testosterone responses to endurance loading after combined training performed on alternating days in physically active subjects, the present findings are in line with results of Craig et al. (1991). In their study, acute endurance running-induced growth hormone responses were examined in an endurance training only group and a concurrent endurance and strength training group, where endurance running always preceded strength training in the same session. As in the present study, they found no differences in the magnitude of growth hormone responses before and after training in either of the two groups. However, as with the acute force responses observed in the present study both before and after the training intervention, it should be kept in mind that the present improvements in treadmill running time to exhaustion may have masked any potential training-induced changes in acute endurance loading-induced serum hormone concentrations.

6.2.2 Basal neuromuscular, cardiorespiratory and hormonal adaptations

Neuromuscular adaptations
The present strength training performed always immediately after an endurance running session (35 - 45 minutes by progressively increasing intensity from 65 - 85% of HR_{max}) led to small but statistically significant increases in lean mass but not to statistical improvements in neuromuscular performance. This was reflected by maintained dynamic and isometric strength performance, electromyography during maximal and rapid isometric force production or maximal voluntary activation during isometric knee extension. These findings differed from those recently observed by Psilander et al. (2015) who used a similar research design but investigated moderately trained cyclists. In their study, increases in strength performance were observed in the concurrent training group even though strength training was consistently performed immediately following 60 minutes of continuous endurance cycling at a workload corresponding to 90 - 95% of the mean power output determined during a 40 minute time trial.

Despite a high volume of endurance training, additional strength training performed in endurance athletes has typically been shown to induce positive adaptations in neuromuscular function (Bazyler et al. 2015; Beattie et al. 2014; Taipale et al. 2013). These strength-training induced increases in neuromuscular performance are thought to aid beneficial changes in maximal and sub-maximal

endurance performance via improved exercise efficiency (Beattie et al. 2014). However, in contrast to most studies, strength training in the present study was performed always immediately after a strenuous endurance running session. Thus, it is likely that the preceding endurance training sessions may have compromised the anabolic effects of subsequent strength trainings sessions e.g. due to residual fatigue (Craig et al. 1991; Eklund et al. 2015). This assumption was also supported by the observed acute reductions in maximal force production of ~10% following the incremental treadmill run in the present study both before and after the training intervention.

Previous studies in untrained young (Chtara et al. 2008; Eklund et al. 2015) or old (Cadore et al. 2013) subjects have indicated that endurance exercise performed right before strength training may compromise chronic strength gains possibly due to inhibition of neural adaptations. Indeed, prolonged endurance running has previously been shown to induce a large central activation deficit, indicating severely impaired cortical motoneurons which are no longer able to fire at optimal frequency (Temesi et al. 2014). These neural mechanisms may have also contributed to the acute reductions in force production observed in the present experimental loadings before and after training as well as to findings by other cross-sectional studies of acutely decreased strength performance after endurance cycling (Leveritt & Abernethy 1999) and incremental running (Denadai et al. 2007), the latter of which was very similar to the protocol performed in the present study. Although no acute neural responses were assessed in the present study, the lack of chronic improvements in muscle strength, electromyography and voluntary activation in the present study may be attributed to a combination of both chronic (Hickson 1980) and acute (Craig et al. 1991) interference.

The endurance intensity of the incremental run performed immediately before the strength training sessions, however, was high (i.e. at or above the anaerobic threshold). Thus, it remains to be investigated whether similar detrimental effects on strength adaptations would be observed when the preceding endurance running is performed at a much lower intensity. In addition, it should be noted that the adaptations in voluntary activation measured during isometric knee extension in the present E+S group in recreational endurance runners appeared to be distinct on an individual level. Moreover, the negative correlation between the individual absolute activation levels and the corresponding relative changes in E+S approached statistical significance ($p=0.087$) during the first 12 weeks. These findings, thus, show that the present strength training was indeed beneficial for the endurance athletes with initially low activation levels. As this correlation was no longer observed during the latter half of the training, our findings further indicate that the possible neural inhibition seemed to be pronounced during the second training period, possibly attributed to the increased volume and intensity of both endurance and strength training. This was also supported by the significant correlation between the individual basal levels of dynamic 1RM strength and the corresponding changes in maximal strength performance during the first 12 but not 24 weeks. Although

not statistically significant, after 12 weeks of training the E+S group had improved maximal strength performance by 5 ± 7% and countermovement jump height by 4 ± 10%, which was diminished at week 24, despite a progressive increase in training load.

In line with the lack of improvements in muscle strength and neural function, no significant changes in muscle cross-sectional-area of vastus lateralis were observed in either of the two groups. Even though the direction of changes differed in E+S and E both at weeks 12 and 24, as indicated by a statistically significant between-group difference at these time points, the E+S group did not significantly improve CSA. It should be pointed out that the magnitude of increases in vastus lateralis CSA, determined by ultrasound, was smaller than that typically observed following 6 months of pure strength training as reviewed by Wernborn et al. (2007) as well as following concurrent endurance and strength training as observed in the present study 1 (II) in previously untrained subjects. Furthermore, it should be kept in mind that the measurement error of CSA assessed by ultrasound may be ± 5% (Ahtiainen et al. 2010). Thus, the observed non-significant increase of 6 and 7% in the present E+S group at weeks 12 and 24, respectively may not be considered as physiologically meaningful, even though the increase of total leg lean mass assessed by DXA was statistically significant (2 - 3%). This hypothesis was supported by the observed statistical between-group difference in the ratio of force to cross-sectional area, indicating no strength advantage of the gained muscle mass shown by a slight decrease in the E+S group. Furthermore, total body mass remained statistically unaltered in this group and, thus, the small increases in muscle mass were not likely to have negatively affected maximal and sub-maximal endurance performance as shown by similar improvements in 1000 m running time and heart rate curves compared to E.

In the present recreational endurance runners, a statistically significant between-group difference in training-induced adaptations in dynamic strength but not isometric force was observed. It should be noted that the present training program consisted of both dynamic maximal (~80% of total strength training volume) and explosive (~20% of total strength training volume) strength training. Thus, due to the training specificity (Folland et al. 2005), no large changes in isometric strength were expected but the inclusion of such measurements was necessary in order to asses neuromuscular function by means of electromyography and muscle stimulation. Nevertheless, a statistical reduction was observed in isometric unilateral knee flexion force in E+S but not E after 24 weeks of training. As running at higher speeds places high demands on the hamstring muscles (Schache et al. 2014) and the present strength training program included exercises for both leg extensors and flexors, the observed decline in isometric knee flexion force may provide some indications for a possible overload, due to the high training volume (i.e. 4 - 6 x endurance and 2 x strength training per week). However, as the biceps femoris activation assessed by electromyography remained statistically unaltered, such assumption as well as its impact on possible performance outcomes remains speculative.

Cardiorespiratory adaptations

Typically, endurance training supplemented by maximal and explosive strength training has previously been shown to beneficially affect endurance performance in endurance athletes. Although added strength training may further induce endurance development due to greater training volume compared to endurance training alone, improvements in running performance have also been shown with reduced endurance training volume compensated by added strength training (Paavolainen et al. 1999a). In general, however, beneficial adaptations in cardiorespiratory function and endurance performance following concurrent training have typically occurred with concomitant increases in maximal and/or explosive strength development, indicating that strength training-induced endurance development may occur as a result of enhanced neuromuscular performance (Mikkola et al. 2007; Taipale et al. 2010; Rønnestad & Mujika 2014; Paavolainen et al. 1999a).

Treadmill running time to exhaustion and maximal 1000 m running time increased to the same extent in both the E+S and E groups possibly as a result of only minor neuromuscular adaptations. Similarly, the AUC analysis of the incremental field tests indicated similar improvements in heart rate curves in both groups and the lactate curve in the E group only. Accordingly, the present same-session combined endurance and strength training protocol did not appear to provide additional benefits for endurance development in recreational endurance runners. While this may be attributed to the present design in which strength training was always immediately preceded by an endurance running session, possibly impairing optimal neuromuscular adaptations, also other factors such as mitochondrial biogenesis may account for the present findings. In a study by Psilander et al. (2015) where endurance training was performed by continuous cycling, improvements in 1RM strength and peak power during a Wingate test were observed although no superior improvements in endurance performance occurred. Furthermore, as Psilander et al. (2015) found no changes in markers of muscle oxidative capacity, acute interference on endurance development may possibly also occur at a cellular level. Whilst such mechanisms were beyond the scope of the present thesis, the data obtained by Psilander et al. (2015) may aid in explaining the present findings.

It should, however, also be noted that the improvement in running time in the present E group was linear throughout both the first and second 12-week period, while in the E+S group, endurance performance plateaued after 12 weeks. Although speculative, overreaching induced by high training volume in the E+S group may have caused the observed plateau in endurance development as also indicated by the reduction in isometric knee flexion force. Thus, a reduction in the overall training volume may have been necessary in order to avoid plateau in endurance performance beyond 12 weeks of training (Taipale et al. 2010; Paavolainen et al. 1999a). This may be supported by findings of other studies which showed that only one weekly session of strength training with low volume and high intensity may be sufficient to maintain previously achieved strength training adaptations (Rønnestad, Nymark & Raastad 2011;

Rønnestad, Hansen & Raastad 2010a). However, one should also bear in mind that the initial endurance performance level was slightly higher in E+S compared to E (i.e. longer time to exhaustion and faster 1000 m running time). Even though this difference was not statistically significant, it may have contributed to the observed plateau in maximal 1000 m running time after 12 weeks in E+S but not E.

In addition to similar improvements in endurance performance, both groups significantly decreased body fat percentage and body fat mass after 12 and 24 weeks to a similar degree. These findings are in line with findings of Ghahramanloo et al. (2009) who found that reductions in body fat mass were similar between an endurance training only and a concurrent training group in previously untrained subjects, indicating that added strength training may not provide additional benefits for reductions in fat mass.

The findings of reduced fat mass in the two groups are in contrast to those observed in previously untrained men (study 1). However, it should be emphasized that the endurance training volume in the present recreational endurance runners was much larger than that of the study in untrained subjects. Moreover, the endurance training in study 2 was conducted by running which has previously been shown to induce larger rates of fat oxidation when compared to endurance cycling (Achten, Venables & Jeukendrup 2003) as performed in study 1.

Interestingly, significant reductions in triglycerides in the present study were observed in the E+S group only. Typically, concurrent training is more prone to induce changes in LDL-C and HDL-C rather than reductions in triglycerides (Tambalis et al. 2009). However, most of these studies were conducted in elderly subjects. It should be noted that the present E+S group performed up to 8 training sessions per week (i.e. 4 - 6 x endurance, 2 x strength), while the training volume in the E group was smaller (4 - 6 x endurance only). As with reductions in fat mass, changes in lipoproteins have previously been shown to be dependent on the overall training volume (Kraus et al. 2002), which somewhat support the present findings.

Hormonal adaptations

Strength training-induced changes in basal hormone concentrations may counteract an endurance training-induced catabolic state. This possibly contributes to the beneficial effects of strength training for endurance athletes, typically observed in previous studies. In the present study, however, only small fluctuations in basal hormone concentrations were observed, supporting the lack of effects of strength training on endurance performance, when performed immediately after high-intensity endurance running sessions.

Interestingly, a significant reduction in the testosterone/SHBG-ratio was observed after 12 weeks of training in the present E+S group and this initial decrease was followed by a large increase thereafter. Thus, a statistically significant between-group difference in the magnitude of changes in the testosterone/SHBG-ratio from week 12 to 24 was observed. As this ratio correlates with free available testosterone concentrations (Selby 1990) and may therefore

reflect an anabolic state, the observed reductions in the E+S group during the first 12 weeks may indicate increased uptake of testosterone by the target cells (Vingren et al. 2010). Although receptor content was not assessed in this study, the observed reduction in the testosterone/SHBG-ratio during the first 12 weeks of training may, thus, reflect a positive response to a new training stimulus since the subjects were recreationally endurance trained but not accustomed to strength training.

6.3 Methodological strengths and limitations

The present studies incorporated both the measurement of acute exercise responses as well as chronic training adaptations. The measurements were conducted by trained staff members and all training sessions were supervised. Within subjects, the measurements were performed at the same time of day and both verbal and written instructions about the measurement preparations were provided which allowed for standardized measurement conditions. As the presented studies were part of a larger study design, the timing for measurements and training was randomized. While this may have slightly biased results of training improvements (Sedliak et al. 2007), it is unlikely that between-group comparisons were influenced due to randomization.

Both training interventions spanned over 24 weeks, which provided a realistic time frame for the investigation of prolonged training adaptations. However, with the exceptional duration of the studies also methodological difficulties need to be considered. Baseline measurements were conducted in the fall and post-training measurements carried out in the spring and, thus, possible seasonal variations in serum hormone concentrations should be considered when interpreting the present basal hormonal data (Svartberg et al. 2003). Furthermore, due to organizational constraints, the acute exercise responses were assessed before and after 6 months of training only and, thus, it was not possible to elucidate the exact timing of possible endocrine adaptations. Related to that, recovery in untrained subjects was followed up for 48 h only and it cannot be ruled out whether the observed loading-specific differences in hormonal concentrations diminished after 48 h. Last, due to the large number of subjects, each group (E+S and S+E) performed the experimental loading in one exercise order only. Implementing a cross-over design would have aided in further confirming the present findings.

In contrast to the study in previously untrained subjects, no recovery measurements for the loading-induced force and hormone responses were conducted in recreational endurance runners. Considering that the main findings in the present study 1 were observed at 24 h and 48 h of recovery, the missing recovery measurements in study 2 need to be emphasized as a limitation of the present thesis. In addition, the inclusion of a group training with the S+E order or performing endurance and strength training on alternating days would have strengthened the present findings. While the strength training program utilized

in study 2 was similar to that previously used in studies of our Department (Taipale et al. 2010; Mikkola et al. 2007) and recommended by a recent review (Rønnestad & Mujika 2014), it cannot be conclusively stated that the present program would have beneficially affected endurance performance when performed on alternating days. Furthermore, it should be acknowledged that no tapering period was included in the present study 2. As indicated by previous concurrent training studies (Taipale et al. 2010; Paavolainen et al. 1999a), a reduction in total training volume prior to the measurements may possibly have led to larger endurance and/or strength performance improvements. Last, even though it has previously been shown that strength training-induced adaptations in endurance performance may occur independent of changes in VO_{2max} (Spurrs, Murphy & Watsford 2003; Storen et al. 2008; Taipale et al. 2010; Paavolainen et al. 1999a), the inclusion of gas analysis would have allowed for the assessment of running economy which, in turn, would have provided further explanations as to why no beneficial effects on endurance performance were observed in the present E+S group.

7 MAIN FINDINGS AND CONCLUSIONS

The purpose of the present thesis was 2-fold. First, to investigate the neuromuscular, cardiorespiratory and hormonal responses and adaptations to concurrent endurance and strength training performed in the same session with different exercises orders (E+S vs. S+E) in previously untrained men. Second, to assess physiological effects of same-session combined training when strength training was always performed immediately after an endurance running session in recreational endurance runners. The specific findings of the present studies can be summarized as follows:

Study 1

1) In previously untrained men, the acute force and hormone responses to the present E+S and S+E loadings were similar before and after 24 weeks of training. However, while the recovery of force was mainly completed at 24 h in both groups before and after the training intervention, at baseline statistically reduced serum testosterone concentrations at 24 h and 48 h of recovery were found in E+S but not S+E. After the training intervention, however, this initial loading-specific difference during recovery was no longer observed.

2) Both E+S and S+E led to similar statistical increases in maximal strength and cardiorespiratory fitness, muscle cross-sectional area, lean body mass and serum testosterone concentrations after 24 weeks of training. However, statistical increases in rapid isometric force production were only observed in the S+E group. Furthermore, no reductions in total body or abdominal fat mass, body fat percentage or blood lipids were found in either of the two groups.

In study 1, it was shown that despite an initial between-group difference in loading-induced changes in testosterone concentrations during recovery, the order of combined endurance and strength training did not affect long-term adaptations of maximal strength and endurance performance, body composition and basal hormone concentrations in previously untrained men. However,

this study also showed that performing E+S loadings may, especially in the early phase of the training, lead to prolonged recovery needs which may have a negative impact on training outcomes especially when the training frequency is high. In addition, it was shown that rapid force production increased in the S+E group only which may extend the current findings of acute hormone responses with indications of additional neural inhibition in the E+S group. However, it should be noted that the present findings are limited to a rather low training frequency similar to that of the present study (i.e. 2 - 3 combined training sessions per week) and caution must be taken when performing much greater training volumes and/or frequencies and/or longer durations of combined training interventions.

Study 2

1) In recreational endurance runners, the acute force and hormone responses to endurance loading were similar in E+S and E both before and after the 24 weeks of training. Similarly, basal hormonal concentrations were maintained throughout the training intervention. Both groups improved maximal endurance performance to a similar extent.

2) In E+S, dynamic maximal strength and muscle-cross-sectional area were maintained throughout the 24 weeks of training, while maximal strength was reduced in E. Muscle activity and maximal voluntary activation remained statistically unaltered in both groups. No statistical between-group differences were observed in the training-induced changes in sub-maximal endurance performance.

In study 2, it was shown that same-session combined training (i.e. where strength training was repeatedly preceded by a high-intensity endurance running session) did not lead to superior maximal and sub-maximal endurance performance benefits when compared to endurance running only in recreational endurance runners. It is likely that this was attributed to factors such as the impaired neuromuscular development, maintained basal hormone concentrations and similar acute force and hormone responses compared to E. Although in the present thesis no group utilizing other combined endurance and strength training modes were included, these results suggest that recreational endurance athletes should separate their endurance and strength training sessions or possibly reduce the intensity of endurance running sessions performed immediately prior to strength training in order to maximize benefits of the added strength training. Whether combined endurance and strength training sessions performed in the opposite exercise order (i.e. commencing with strength) may lead to beneficial neuromuscular and cardiorespiratory adaptations in endurance runners remains to be investigated.

YHTEENVEETO (FINNISH SUMMARY)

Yhdistetty kestävyys- ja voimaharjoittelu: Vaikutukset hermo-lihasjärjestelmään, verenkierto- ja hengityselimistöön ja hormonipitoisuuksiin sekä harjoitusjärjestyksen vaikutus harjoittelemattomilla ja kestävyysharjoitelleilla miehillä

Tämän väitöskirjatutkimuksen tavoite oli kaksiosainen: 1) tutkittiin samassa harjoituskerrassa toteutettujen kestävyys- ja voimaharjoitusten keskinäisen järjestyksen (ts. harjoituksen aloittaminen kestävyys- tai voimaharjoittelulla; K+V vs. V+K) vaikutusta akuutteihin vasteisiin ja pitkäaikaisadaptaatioihin hermolihasjärjestelmässä, verenkierto- ja hengityselimistössä sekä hormonaalisiin muuttujiin aiemmin harjoittelemattomilla mieskoehenkilöillä (n=42), 2) Tutkittiin eroja fysiologisissa adaptaatioissa kestävyysharjoitelleilla miehillä (n=30), kun harjoitettiin pelkkää kestävyyttä (K) tai kun kestävyysharjoittelu aina edelsi voimaharjoittelua samassa harjoituskerrassa (K+V). Ensimmäisessä tutkimuksessa harjoittelu koostui 2-3 viikottaisesta yhdistelmäharjoituskerrasta (ts. 2 - 3 x [1K + 1V] tai 2 – 3 x [1V + 1K]) 24 viikon ajan. Kestävyysharjoittelu suoritettiin polkupyöräergometrilla ja koostui sekä matalan intensiteetin harjoittelusta että korkean intensiteetin intervalliharjoittelusta. Voimaharjoittelu keskittyi alaraajoihin intensiteetin edetessä hypertrofisesta harjoittelusta maksimivoimaharjoitteluun. Kaikki kestävyystaustaiset koehenkilöt suorittivat 4-6 viikoittaista kestävyysharjoitusta vaihtelevilla intensiteeteillä 24 viikon ajan. Lisäksi voimaharjoittelua tehtiin kahdesti viikossa välittömästi korkean intensiteetin kestävyysharjoituksen jälkeen K+V-ryhmällä. Voimaharjoittelu koostui sekä maksimivoima- (n. 80% kokonaisharjoitteluvolyymista) että nopeusvoimaharjoittelusta (n. 20% kokonaisharjoitteluvolyymista) ja keskittyi alaraajoihin.

Akuutteja, järjestysspesifisiä eroja havaittiin ennen harjoitusjaksoa kuormituksen jälkeisen palautumisen aikana aiemmin harjoittelemattomilla koehenkilöillä: K+V -ryhmällä testosteronikonsentraatio oli tilastollisesti merkitsevästi alentunut kahden vuorokauden ajan mutta V+K -ryhmällä tätä ei ilmennyt. Harjoitusjakson jälkeen tätä ei enää havaittu, vaan ryhmien välinen ero poistui ja testosteronipitoisuudet pysyivät kummallakin ryhmällä muuttumattomina kahden vuorokauden palautumisen aikana. Alkutilanteen järjestyseroavaisuuksista huolimatta ryhmien välisiä eroja ei havaittu harjoitusjakson jälkeen voima- tai kestävyyssuorituskyvyn kehittymisessä, lihaksen poikkipintaalassa, kehonkoostumuksessa eikä hormonipitoisuuksissa K+V- ja V+K ryhmien välillä. Nopeusvoimaominaisuudet paranivat kuitenkin vain V+K ryhmällä. Kestävyysharjoitelleilla koehenkilöillä tilastollisesti merkitseviä ryhmien välisiä eroja ei havaittu kestävyyssuorituksen aiheuttamissa akuuteissa voima- ja hormonivasteissa ennen tai jälkeen harjoitusjakson. K+V -ryhmässä maksimivoimatasot pysyivät ennallaan ja kehon lihasmassa oli lisääntynyt. Tilastollisesti merkitseviä muutoksia ei havaittu maksimaalisen voimantuoton aikaisessa elektromyografiassa tai lihaksen maksimaalisessa tahdonalaisessa aktivaatiossa. K-ryhmässä maksimivoima laski harjoitusjakson aikana.

Tämä väitöskirjatutkimus osoitti, että alkutilanteen ryhmienvälisestä erosta huolimatta, yhdistetyn voima- ja kestävyysharjoittelun harjoituksen sisäisellä suoritusjärjestyksellä ei näyttäisi olevan merkitsevää vaikutusta voima- tai kestävyyssuorituksen pitkäaikaisadaptaatioihin aiemmin harjoittelemattomilla henkilöillä. Tämä tutkimus kuitenkin osoitti, että K+V -järjestys saattaa etenkin harjoittelun alkuvaiheessa johtaa palautumisen pitkittymiseen, mikä saattaa vaikuttaa negatiivisesti harjoitusadaptaatioihin etenkin, kun harjoitustiheys on korkea. Lisäksi tutkimus osoitti, että nopea voimantuotto parani vain V+K -ryhmällä, mikä tukee akuuttien hormonivasteiden löydöksiä ja saattaa viitata K+V -ryhmän lisääntyneeseen hermostolliseen inhibitioon. Tämän tutkimuksen tuloksien johtopäätöksissä on otettava huomioon tutkimusasetelman kohtuullisen matala harjoitustiheys (ts. 2 - 3 yhdistettyä voima- ja kestävyysharjoitusta viikossa) ja korkeampien harjoitustiheyksien osalta tarvitaan lisätutkimuksia.

Lisäksi tämä väitöskirjatutkimus myös osoitti, että kestävyysharjoittelutaustaisten henkilöiden submaksimaalinen tai maksimaalinen kestävyyssuorituskyky ei parane yhdistetyllä voima- ja kestävyysharjoittelulla enempää kuin pelkällä kestävyysjuoksuharjoittelulla, kun voimaharjoitus suoritetaan aina kestävyysharjoituksen jälkeen. Tämä on todennäköisesti yhteydessä hermolihasjärjestelmän kehittymisen heikentymiseen, hormonitasojen muuttumattomuuteen sekä samankaltaisiin akuutteihin hormonivasteisiin, joita nähtiin pelkän K-harjoittelun yhteydessä. Vaikka tässä väitöskirjatutkimuksessa ei ollut V+K -järjestyksen omaavaa ryhmää kestävyysharjoitelleille koehenkilöille, tulosten perusteella voidaan olettaa, että kestävyysharjoitelleiden yksilöiden kannattaa eriyttää voima- ja kestävyysharjoittelu toisistaan, jotta voimaharjoittelun tuomat edut kestävyyssuorituskyvylle voidaan optimoida. Tulevaisuudessa tulisi vielä tutkia sitä, johtaisiko kestävyys- ja voimaharjoitusten suorittaminen päinvastaisessa järjestyksessä (ts. V+K) suotuisempiin adaptaatioihin hermolihasjärjestelmässä tai hengitys- ja verenkiertoelimistössä ja näin ollen kestävyyssuorituskyvyssä.

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ORIGINAL PAPERS

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THE ORDER EFFECT OF COMBINED ENDURANCE AND STRENGTH LOADINGS ON FORCE AND HORMONE RESPONSES: EFFECTS OF PROLONGED TRAINING

by

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The order effect of combined endurance and strength loadings on force and hormone responses: Effects of prolonged training

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Running title: Training-induced responses to combined loadings

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ABSTRACT

Purpose: To examine acute responses and recovery of force and serum hormones to combined endurance and strength loadings utilizing different orders of exercises before and after training.

Methods: Physically active men were matched to an order sequence of endurance+strength (E+S, n=12) or strength+endurance (S+E, n=17). The subjects performed one experimental loading consisting of steady-state cycling and a leg press protocol before and after 24 weeks of order-specific combined training.

Results: No between-group difference in acute reductions of force was observed at week 0 (E+S -23%, $p<0.001$; S+E -22%, $p<0.01$) and 24 (E+S -25%, $p<0.001$; S+E -27%, $p<0.001$) and recovery in force was completed after 24h in both groups. Concentrations of growth hormone (22-kDa) increased post-acute loading at week 0 (E+S, +57 fold, $p<0.05$; S+E, +300 fold, $p<0.001$; between-groups $p<0.001$) and 24 (E+S, +80 fold, $p<0.01$; S+E, +340 fold, $p<0.05$; between-groups $p<0.05$). No significant acute responses in concentrations of testosterone were observed at week 0 or 24. However, at week 0 testosterone was reduced during recovery following the E+S loading only (24h -23%, $p<0.01$; 48h -21%, $p<0.001$; between-groups at 24h and 48h, $p<0.05$), but was no longer observed after training. 1RM strength improved similarly in E+S (13%, $p<0.001$) and S+E (17%, $p<0.001$).

Conclusions: This study showed an order effect (E+S vs. S+E) in concentrations of testosterone during 2 days of recovery at week 0, which was diminished after training at week 24. This initial difference in testosterone concentrations during recovery did not seem to be associated with strength development.

KEY WORDS: fatigue; testosterone; recovery; endurance cycling; concurrent training; combined training; training adaptations

LIST OF ABBREVIATIONS

C	-	cortisol
CK	-	creatine kinase
E	-	endurance
ECG	-	electrocardiogram
ES	-	effect size
E+S	-	endurance+strength
GH	-	growth hormone (22-kDa)
MVC_{max}	-	maximal isometric bilateral leg press force
S	-	strength
SD	-	standard deviation
S+E	-	strength+endurance
T	-	testosterone
TSH	-	thyroid stimulating hormone
$\dot{V}O_{2max}$	-	maximal oxygen consumption
1RM	-	one repetition maximum

INTRODUCTION

Acute responses to exercise loading create the biological foundation for the development of chronic adaptations (Kraemer and Ratamess 2005). While the magnitude of loading-induced stress can be quantified by temporary declines in performance and biological function, the anabolic and catabolic processes of tissue remodeling following exercise loadings are typically reflected by acute and chronic changes in hormonal concentrations (Hackney and Viru 2008). Due to the important biological functions for tissue growth and degradation, concentrations of testosterone (T), growth hormone (GH), thyroid stimulating hormone (TSH) and cortisol (C) are often utilized as indicators of loading induced tissue remodeling (Kraemer et al. 1990, Häkkinen and Pakarinen 1993, Hackney et al. 2012).

The magnitude of both endurance (E) and strength (S) loading-induced hormonal responses in men depend on the intensity and volume, as well as the exercise mode performed. Short bouts of high intensity endurance loadings may induce acute elevations in both anabolic (e.g. T, TSH, GH) and catabolic (e.g. cortisol) hormone concentrations (Pritzlaff et al. 1999, Hackney et al. 2012), while prolonged and physically demanding endurance performance (e.g. a marathon run) may in its final phases lead to decreases in testosterone and simultaneous increases in cortisol concentrations (Kuoppasalmi et al. 1980).

On the other hand, strength loading protocols utilizing heavy resistance, combined with short inter-set rest periods (i.e. hypertrophic strength loadings), result in acute increases in serum testosterone and GH, as well as cortisol concentrations (Kraemer et al. 1990). However, maximal strength loadings with high loads and low number of sets as well as explosive strength protocols utilizing maximal movement velocity typically require a prolonged inter-set recovery and may not be sufficiently physiologically demanding to induce as large increases in concentrations of anabolic or catabolic hormone concentrations (Kraemer et al. 1990; Häkkinen and Pakarinen 1993; Linnamo et al. 2005).

When combining endurance and strength loadings into one training session the question of exercise order arises (i.e. endurance+strength [E+S] vs. strength+endurance [S+E]). Previous studies have emphasized the sensitivity of strength performance to preceding endurance loadings (Leveritt and Abernethy 1999; Lepers et al. 2008), leading to reduced force production and possibly compromised long-term adaptations when compared to the

reverse loading order (Chtaha et al. 2008). Furthermore, it has been shown that force and hormone responses to combined loadings depend on the training status of the subjects and the specificity of the combined protocol performed (Cadore et al. 2012; Schumann et al. 2013; Taipale and Häkkinen 2013). In physically active men, a recent cross-sectional study showed reduced serum testosterone concentrations during a recovery period of (at least) 2 days when the strength loading was immediately preceded by endurance cycling (Schumann et al. 2013). However, the possible influence of prolonged training on acute force and hormone responses as well as the biological effects of acute loading-induced endocrine changes on long term strength development remains to be investigated.

Therefore, the purpose of the present study was to investigate acute responses and recovery of force and serum hormone concentrations (i.e. T, TSH, GH and C) to a combined endurance and strength loading protocol with different loading orders (E+S vs. S+E) performed before and after 24 weeks of combined training. A secondary purpose of this study was to examine whether loading order-induced differences in these acute responses are related to strength development.

In agreement with the above mentioned previous findings, it was postulated that a combined endurance and strength loading protocol typically utilized by physically active subjects (i.e. endurance cycling of moderate intensity and rather short duration and a mixed maximal, explosive and hypertrophic leg press protocol) may only lead to modest acute increases in anabolic and catabolic hormone concentrations (e.g. Häkkinen and Pakarinen 1993; Linnamo et al. 2005) but would still indicate loading order specific differences in hormonal responses (Schumann et al. 2013). Based on this assumption it was hypothesized that performing endurance cycling immediately before a strength loading protocol (E+S) would lead to less favorable hormonal responses when compared to the reverse loading order (S+E) and that this loading specific difference would be maintained after long-term training. Thus, it was further hypothesized that 1RM strength may be developed to a lesser extent in the E+S group compared to S+E training group.

METHODS

Subjects

Forty-two physically active men volunteered to participate in this study. The subjects were free of acute and chronic illness, disease and injury and reported not using medication that would contraindicate the performance of intense physical activity or affect endocrine metabolism and neuromuscular function. A standardized phone interview was conducted to initially assess subjects' health and activity status. The subjects reported to perform light physical activity such as walking, cycling or occasionally team sports for not more than 3 times per week but did not train systematically for endurance or strength training prior to inclusion into the study. Verbal and written instructions about the study procedures and possible risks were provided to the subjects before giving informed consent. In addition, a completed health questionnaire and resting ECG measurement were reviewed by a cardiologist prior to the first exercise testing and training. Following the pre-screening process subjects were matched according to age and physical performance at baseline to either of two training groups: Endurance+Strength (E+S n=21) or Strength+Endurance (S+E n=21). To be included in the data analysis, subjects were required to complete at least 90% of the supervised training sessions prescribed during a 24-week training period. Thus, out of the 42 originally recruited subjects, 13 subjects did not complete the study, mostly due to personal reasons (i.e. occupational changes) possibly attributed to the exceptional length of the study period. The demographic characteristics of the remaining 29 subjects (E+S n=12; S+E n=17) included in the data analysis were as follows (mean±SD): E+S age 30±5 years, height 179±6 cm, body mass 79±10 kg; S+E age 30±5 years, height 179±5 cm, body mass 75±9 kg. The study was conducted according to the Declaration of Helsinki and ethical approval was granted by the ethics committee at the University of Jyväskylä.

Experimental design

To investigate the training adaptations in acute responses and recovery to combined endurance and strength loadings with different loading orders (i.e. endurance + strength [E+S] vs. strength + endurance [S+E]), a longitudinal research design was used and loading-specific responses and recovery patterns of force production and hormonal concentrations determined before and after the combined training of 24 weeks (Fig 1). As this study directly compared the order effect, no control group was included. Before the experimental loading, subjects were familiarized with the measurement procedures (day 1) and tested for baseline endurance (day 2) and strength (day 3) performance. Thereafter, all subjects performed one experimental session of combined endurance and strength loadings in the order of the corresponding group (E+S or S+E) and returned to the

laboratory for recovery measurements at 24h and 48h (Fig 1). To allow for sufficient recovery, all testing sessions (except for recovery measurements) were separated by at least 48h. Both the baseline and the experimental loading and recovery measurements were repeated after 24 weeks of combined training in the loading order specific to the corresponding group. Due to financial and time constraints, a cross over design was not possible and each group performed only one experimental loading both before and after the training (i.e. only E+S or S+E).

+++ *Figure 1 somewhere near here* +++

Strength and endurance loading

The strength and endurance loading protocols have been described in detail elsewhere (Schumann et al. 2013). Briefly, the strength loading (30min) was performed on a dynamic leg press device (David 210, David Health Solutions Ltd., Helsinki, Finland) and included sets aimed for explosive strength (3x10 repetitions at 40% of 1RM with 3 min rest between sets), maximal strength (1x3 repetitions at 75% of 1RM and 3x3 repetitions at 90% of 1RM with 3 min rest between sets) and muscle hypertrophy (1x10 repetitions at 75% of 1RM and 3x10 repetitions at 80-85% of 1RM with 2 min rest between sets). The loads were derived from subject's individually determined 1RM (at week 0 and 24, respectively) but additional load was added or assistance provided to achieve at least one set of a true repetition maximum during the maximal and hypertrophic sets (i.e. 3RM and 10RM, respectively). Based on both previous literature (Cadore et al. 2012) and a pilot study, the endurance loading was conducted on a cycle ergometer (Ergomedic 839E, Monark Exercise AB, Varberg, Sweden) over 30 minutes of steady-state cycling at 65% of subjects' individual maximal aerobic power (Watts), determined during an incremental ergometer test at week 0 and 24, respectively. Subjects were required to keep pedaling frequency constant at 70 rpm but for instances when the subjects failed to keep up the required frequency, intensity was reduced by 15 Watts every minute until the subject could complete the loading.

Baseline and loading measurements

To control the experimental conditions, subjects received both verbal and written instructions about the measurement preparation in order to minimize physical and mental stress and to allow for at least 7-8h of sleep on the day before as well as throughout the baseline and loading measurements. In addition, to assure the

resting state of the subjects basal morning concentrations of serum hormones and creatine kinase (CK) were determined by drawing venous blood samples on the days of the experimental sessions (at week 0 and 24, respectively) after 12h of fasting, between 7:00 a.m. and 9:00 a.m.

Within the experimental loading sessions (at week 0 and 24, respectively), maximal isometric strength (horizontal bilateral isometric leg press) and concentrations of serum hormones (T, TSH, GH and C), creatine kinase (venous blood samples), and blood lactate (capillary blood) were determined. In order to obtain acute changes in these variables, force measurements and blood samplings were conducted at the following time points (Fig 1); prior to the start of the experimental loading (PRE), immediately following the first loading (MID, after the endurance or strength loading, respectively) as well as immediately after the completed combined session (POST). In addition, recovery of force as well as hormone (T, TSH and C) and CK concentrations were measured after 24h and 48h at ±1h from the end of each completed session. To control for circadian variations in force production and hormone concentrations, experimental loading and recovery measurements of each subject were performed at the same time of day with an accuracy of ±1h at week 0 and 24, respectively. The testing times of the experimental loadings at week 0 were (mean±SD): E+S 9:27 a.m. ± 1:38h; S+E 9:12 a.m. ± 2:25h. The corresponding recovery measurements in both groups were (mean±SD): at 24h in E+S 11:48 a.m. ± 1:45h; in S+E 11:29 a.m. ± 2:23h; at 48h in E+S, 11:48 a.m. ± 1:45h; in S+E, 11:25 a.m. ± 2:22h).

Isometric leg press: Maximal isometric bilateral leg press force (MVC_{max}) was measured on a horizontal leg press dynamometer (Department of Biology of Physical Activity, University of Jyväskylä, Finland) in a seated position at a hip and knee angle of 110 and 107 degrees, respectively (Häkkinen et al. 1998). On verbal command, subjects were instructed to produce maximal force as rapidly as possible with the entire foot against the force plate and maintain maximal tension for 3-4 seconds (as observed from the force trace by the researcher). During the execution of each maximum trial, subjects were required to grasp handles located by the seat of the dynamometer, as well as to keep constant contact with the seat and the backrest and verbal encouragement was given to promote maximal effort. Prior to the start of the experimental loading session, as well as at both recovery measurements (at 24h and 48h), three trials separated by a resting period of 1 minute were conducted. If the maximum force during the last trial was greater than 5% compared to the previous trial,

an additional attempt was performed. To assess acute force responses, at MID (after E or S in each experimental loading, respectively) and POST, **only two** maximal isometric trials were performed and **separated** by only 10-15 seconds. The best performance trial in terms of maximal force measured in Newtons, at PRE, MID, POST, 24h and 48h was used for statistical analysis. The force signal was low-pass filtered (20Hz) and analyzed (Signal software, version 4.04, Cambridge Electronic Design Ltd., Cambridge, UK).

One repetition maximum: Subjects' one repetition maximum (1RM) of leg extensors was determined using a seated dynamic horizontal leg press (David 210, David Health Solutions, Helsinki, Finland). Prior to attempting 1RM, subjects completed a warm up consisting of 5 repetitions **at** 70% of the estimated maximal load, 2 repetitions at 80-85% and 1 repetition at 90-95% with 1 minute rest between the sets (i.e. 3 warm up sets). Following this warm up, no more than 5 trials were allowed to achieve 1RM. The starting knee angle for all subjects was (mean±SD) 58 ± 2 degrees. Subjects were instructed to grasp the handles located by the seat of the dynamometer and to keep constant contact with the seat and backrest during complete extension to 180 degrees knee angle. To promote maximal effort, verbal encouragement was given. The greatest weight that the subject could successfully lift (knees fully extended) at an accuracy of 1.25 kg was accepted as 1RM.

Maximal power output: Aerobic power and maximal oxygen consumption were determined during a graded cycle ergometer test (Ergometrics 800, Ergoline, Bitz, Germany). The initial load for all subjects was 50 Watts and was increased by 25 Watts every 2 minutes. Heart rate was monitored throughout the test (Polar S410, Polar Electro Oy, Kempele, Finland) and recorded as the average of the last 5 seconds at each stage. Oxygen uptake was determined continuously breath-by-breath using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). On each testing day, air flow calibration was performed using a manual flow calibrator and the gas analyzer was calibrated using a certified gas mixture of 16% O_2 and 4% CO_2. The $\dot{V}O_{2max}$ was taken as the highest 60-s $\dot{V}O_2$ value. To assure that $\dot{V}O_{2max}$ was achieved, other criteria such as heart rate, blood lactate and respiratory exchange ratio (RER) were monitored throughout the test. Aerobic power (Watts) used for the determination of the endurance intensity during the experimental loadings was calculated using the equation: $W_{max}= W_{com}+(t/120)*25$, where W_{com} is the load of the last completed stage and t is the time of the last incomplete stage. Subjects' individual aerobic and anaerobic thresholds used to determine intensities for the

endurance training were determined using deflection points obtained by plotting the curves of blood lactate, ventilation, oxygen consumption and production of carbon dioxide (Aunola and Rusko 1986).

Venous blood samples and blood lactate: Venous blood samples (10 ml) for the determination of serum hormone concentrations and CK were collected by a qualified lab technician, using sterile needles into serum tubes (Venosafe, Terum Medical Co., Leuven, Belgium). Whole blood was centrifuged at 3.500 rpm (Megafuge 1.0 R, Heraeus, Germany) for 10 minutes after which serum was removed and stored at -80°C until analysis (approximately 4-8 weeks). Analysis of total serum testosterone, TSH, GH (22-kDa) and cortisol were performed using chemical luminescence techniques (Immunlite 1000, Simens, New York, USA) and hormone specific immunoassay kits (Siemens, New York, USA). The sensitivity for serum hormones were: T 0.5 $nmol \cdot l^{-1}$, TSH 0.004 $mlU \cdot l^{-1}$, GH 0.03 $mlU \cdot l^{-1}$ and C 5.5 $nmol \cdot l^{-1}$. The intra-assay coefficients of variation for T, TSH, GH and C were 8.7±2.7%, 7.1±4.6 %, 6.0±0.5% and 7.1±1.1%, respectively. The inter-assay coefficients of variation for T, TSH, GH and C were 10.6±3.2%, 11.1±4.3%, 5.8±0.3% and 7.9±1.2%, respectively. While being aware that loading induced changes in plasma volume shift may influence hormonal concentrations (Kargotich et al. 1998), we believe that the concentrations of hormones the receptors are exposed to are most critical for the initiation of tissue remodeling (Kraemer and Ratamess 2005). Therefore, plasma volume changes were estimated from changes in hematocrit and hemoglobin (Dill and Costill 1974) but were not used to correct obtained serum hormone concentrations.

Capillary blood samples for the determination of blood lactate concentrations were taken from the fingertip at the described time points. The amount of 20 µl of blood was inserted into pre-filled reaction capsules containing a hemolyzing agent and blood lactate concentrations were analyzed using a Biosen lactate analyzer (C_line Lab+, EKF, Magdeburg, Germany).

Training

Subjects were asked to maintain their habitual physical activity (light walking, cycling and occasional team sports) throughout the study period. In addition to training diaries completed during all prescribed training sessions, subjects were asked to record recreational physical activity in a standardized activity log.

The training was designed to reflect a program typically recommended for physically active populations

(Thompson et al. 2010). The main objective was to improve both endurance and strength performance through a periodized program including both moderate and vigorous intensity aerobic loadings (Helgerud et al. 2007, Daussin et al. 2007) combined with hypertrophic and maximal strength loading protocols (Kraemer and Ratamess 2004). To assure the correct execution of the training prescribed, all training sessions were supervised by qualified instructors.

In order to familiarize the subjects with the equipment and exercises to be used during the consecutive 24 weeks of training, a 1-week preparatory period was conducted prior to the start of the experimental loading sessions and training. During the first 12 weeks of training, the subjects performed according to their corresponding training group either 2x [1E+1S] or 2x [1S+1E]) per week. During the second 12 weeks, the frequency was increased so that 2 combined training sessions were performed in every 1^{st} and 4^{th} week and 3 combined training sessions in every 2^{nd} and 3^{rd} week (i.e. 2x [1E+1S] or 2x [1S+1E] or 3x[1E+1S] or 3x [1S+1E], respectively).

The strength training program included exercises for all major muscle groups with special consideration to the lower extremities. Exercises for the lower body consisted of bilateral dynamic leg press, as well as both bilateral (weeks 1-7 and 13-18) and unilateral (weeks 8-12 and 19-24) dynamic knee extension and flexion. Additional exercises for the upper body included shoulder press and lat-pull down, as well as exercises commonly used to improve core stability. The overall duration of the strength loading within each combined training session was 30-60 min. During weeks 1-2, all exercises were conducted as a circuit using 2-4 sets of 15-20 repetitions at an intensity of 40-60% of 1RM. During the following 10 weeks of training, protocols aiming for muscle hypertrophy (2-5 x 8-10 repetitions at 80-85% of 1RM, 1.5-2 min rest between the sets) and maximal strength (2-5 x 3-5 repetitions at 85-95% of 1RM, 3-4min rest between the sets), as well as during the last 2 weeks protocols targeting explosive power (2 x 8-10 repetitions at 40% of 1RM, 3-4 min rest between the sets) were incorporated into the training program. During the second 12-week period, the strength training program was further intensified by increasing both training volume and frequency while the major program structure was maintained. The strength training loads were controlled by the number of repetitions and execution velocity and increased progressively throughout the two 12-week periods.

Endurance training was performed on a cycle ergometer. The intensity was controlled by heart rate zones

determined from subjects' individual aerobic and anaerobic threshold obtained during the baseline measurement at week 0 and 24. Subjects were asked to maintain a constant pedalling frequency at about 70-80 rpm during each training session, while the magnetic resistance of the ergometer was used to achieve the prescribed cycling intensity. The endurance program consisted of both steady-state and interval exercise sessions while the intensity was progressively increased from low (below the aerobic threshold) to high (above the anaerobic threshold) throughout both 12-week periods. The duration of cycling was between 30 and 60 min per combined session, leading to a total duration of 60-120 min for each combined training session (i.e. E+S and S+E, respectively).

Nutrition

To control nutritional intake, subjects received both verbal and written nutritional recommendations and were asked to maintain dietary intake constant throughout the 24 weeks of training. In preparation for all baseline and loading measurements, subjects were required to consume a light meal 2-3 h prior to the start of each test-session or experimental loading and asked to keep nutritional intake prior to the measurements similar at week 0 and 24. Furthermore, to control for hydration status during each experimental loading, subjects were instructed to begin each combined loading in a hydrated state and were allowed to ingest 2 dl of water during the combined loading at MID, immediately after the venous blood sample was taken.

Statistical analyses

Within- and between-group analyses were conducted in order to investigate 1) acute loading responses and recovery before the training intervention, 2) acute loading responses and recovery after 24 weeks of training and 3) training- or loading-induced changes in acute loading responses and recovery. Data are presented as mean±SD and shown as relative changes from the pre-loading values unless indicated. All baseline and pre-loading data obtained before the training intervention were checked for normality. Concentrations of serum CK and GH were not normally distributed even after log transformation. Therefore, data of CK and GH were analyzed using non-parametric tests for all within- (Wilcoxon signed-rank test) and between-group (Mann-Whitney U-test) comparisons using Bonferroni adjustments by multiplying all pair-wise p-values with the number of comparisons. Within-group differences for all remaining variables before (week 0) and after (week

24) the training were analyzed with absolute values using repeated measurement analysis of co-variance (ANCOVA) with 5 levels (PRE, MID, POST, 24h and 48h). Training- or loading-induced within-group differences were analyzed by a paired t-test using relative changes (week 24 vs. week 0). Between-group differences were analyzed by an independent t-test using relative changes. The statistical significance for all tests was set at 0.05, where *=p<0.05, **=p<0.01 and ***=p<0.001 and effect size (ES) for both within and between-group comparisons is reported as Cohen's d (cliff's delta for CK and GH). Statistical analysis was conducted using IBM SPSS 20.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

The training adherence was 99% in both the E+S and S+E groups. All subjects completed at least 90% of the prescribed training sessions. Baseline endurance and strength performance as well as basal concentrations of serum hormones and CK at week 0 and 24 are presented in Table 1.

+++ Table 1 somewhere near here +++

Both the E+S and S+E group significantly increased 1RM strength after 24 weeks of training (E+S +13±8%, p<0.05, ES=0.683; S+E +17±12%, p<.0.05, ES=0.998). No significant between-group difference in 1RM strength development was found.

Acute loading responses at week 0

Maximal force production

In E+S, MVC_{max} was significantly decreased at MID (-11±7%, p<0.01, ES=-0.773) and further decreased at POST (-23±12%, p<0.001, ES=-1.453) compared to PRE (Fig. 2a). In S+E, MVC_{max} significantly decreased at MID (-20±13%, p<0.001, ES=-0.848) and remained reduced at POST (-22±9%, p<0.005, ES=-0.878) compared to PRE. The relative change at MID was significantly larger in S+E compared to E+S (-20±13% vs. 11±7%, p<0.05, ES=0.867). No significant between-group difference was found at POST. Both E+S and S+E significantly recovered from POST to 24h (E+S ES=1.161; S+E ES=0.753) and 48h (E+S ES=1.342; S+E ES=0.698), respectively, so that the MVC_{max} values obtained at 24h and 48h of recovery were not statistically different from PRE (p>0.05).

+++ *Fig 2a and 2b somewhere near here* +++

Serum hormone concentrations

Concentrations of serum T (Fig 3a) at MID were significantly increased in E+S only (+13±6%, $p<0.05$, ES=0.438) and did not statistically differ from PRE in either of the two groups at POST. The increase of serum T in S+E from MID to POST was significant (+17±18%, $p<0.05$, ES=0.517). A significant between-group difference was observed at MID (18%, $p<0.05$, ES=1.003) but not at POST. During recovery, concentrations of serum T decreased in E+S at 24h and 48h compared to PRE (at 24h -23±14%, $p<0.01$, ES=-0.834; at 48h -21±11%, $p<0.001$, ES=-0.884) but were not significantly different from PRE in S+E. The difference between E+S and S+E observed at 24h and 48h was significant (at 24h -23±14% vs. -1±32%, $p<0.05$, ES=0.891; at 48h -21±11% vs. -4±21%, $p<0.05$, ES=1.011).

+++ *Fig 3a and 3b somewhere near here* +++

Concentrations of serum TSH remained statistically unaltered during the two loadings at MID and POST. During recovery at 24h and 48h, serum TSH significantly decreased at 24h in E+S (-33±13%, $p<0.001$, ES=-1.317) and at 48h in S+E (-24±27%, $p<0.01$, ES=-0.582) compared to PRE. No significant between-group difference in acute responses or recovery was observed.

Concentrations of serum GH (Table 2) significantly increased in the two loadings at MID (E+S +250 fold $p<0.01$, ES=0.972; S+E +49 fold, $p<0.01$, ES=0.734) and POST (E+S +57 fold $p<0.05$, ES=0.888; S+E +300 fold, $p<0.001$, ES=0.953) compared to PRE. A significant between-group difference was observed at MID ($p<0.05$, ES=0.552) and POST ($p<0.001$, ES=0.719).

+++ *Table 2 somewhere near here* +++

Concentrations of serum C remained statistically unaltered during the two loadings at MID and POST (Fig. 4a). The increase from MID to POST in S+E was significant (+47±36%, $p<0.001$, ES=1.385). During recovery of 24h and 48h, concentrations of serum C significantly decreased in both E+S and S+E compared to PRE (E+S at 24h -22±26%, $p<0.05$, ES=-0.940; E+S at 48h -27±17%, $p<0.001$, ES=-1.093; S+E at 24h -26±26%, $p<0.01$,

ES=-0.966; S+E at 48h -27±19%, p<0.001, ES=-0.926). No significant between-group difference in acute responses or recovery was observed.

+++ Fig 4a and 4b somewhere near here +++

Blood lactate and serum CK concentrations

Blood lactate concentrations (Table 2) significantly increased at MID (E+S +560±297%, p<0.01, ES=2.369; S+E +610±258%, p<0.001, ES=3.198) and POST (E+S +753±485%, p<0.001, ES=3.104; S+E +557±256%, p<0.001, ES=4.041) compared to PRE. Concentrations of serum CK (Table 2) significantly increased during both loadings at MID (E+S ES=0.236; S+E ES=0.215) and POST (E+S ES=0.320; S+E ES=0.368) compared to PRE. The largest relative increase of CK concentrations was observed during recovery at 24h and 48h (significant only at 48h in S+E +53±57%, p<0.05, ES=0.418) compared to PRE, while large standard deviations were observed.

Acute loading responses at week 24

Maximal force production

In E+S, MVC_{max} was significantly decreased at MID (-15±9%, p<0.01, ES=-0.604) and further decreased at POST (-25±11%, p<0.001, ES=-1.123) compared to PRE (Fig. 2b). In S+E, MVC_{max} significantly decreased at MID (-25±11%, p<0.001, ES=-1.259) and remained reduced at POST (-27±10%, p<0.001, ES=-1.160) compared to PRE. The decrease at MID was significantly larger in S+E compared to E+S (-25±11% vs. -15±9%, p<0.05, ES=1.045) while at POST no between-group difference was observed. Both E+S and S+E significantly recovered from POST to 24h (E+S ES=1.174; S+E ES=0.944) and 48h (E+S ES=1.240; S+E ES=0.910), so that the observed values at 24h and 48h did not statistically differ from PRE (p>0.05).

Serum hormone concentrations

Concentrations of serum T (Fig 3b) remained statistically unaltered during the two loadings at MID and POST. However, since the concentrations of serum T at MID somewhat increased in E+S (ES=0.634) but remained unaltered in S+E (ES=-0.072), the difference between the two loadings at MID was significant (between-group difference 25%, p<0.01, ES=1.196). Serum T significantly decreased from MID to POST in E+S (-13±11%,

p<0.05, ES=-0.303) and significantly increased in S+E (+18±23%, p<0.01, ES=0.527). During recovery, concentrations of serum T were only slightly reduced at 24h and 48h compared to PRE in both E+S and S+E while the reduction in E+S at 48h was nearly significant (-18±20%, p=0.052, ES=-0.636) but no significant between-group difference was observed.

Concentrations of serum TSH remained statistically unaltered during the two loadings at MID and POST. During recovery, serum TSH concentrations significantly decreased at 24h in both loadings (E+S -22%, p<0.05, ES=-0.612; S+E -17%, p<0.05, ES=-0.597) and 48h in E+S only (-21%, p<0.05, ES=-0.692) compared to PRE. No significant between-group difference in acute responses or recovery was observed.

Concentrations of serum GH (Table 2) significantly increased at MID (E+S +330 fold, p<0.01, ES=0.972; S+E +53 fold, p>0.05, ES=0.637) and POST (E+S +80 fold, p<0.01, ES=0.847; S+E +340 fold, p<0.001, ES=0.990) compared to PRE. A significant between-group difference at MID (p<0.001, ES=0.740) and POST (p<0.05, ES=0.531) was observed.

Concentrations of serum C (Fig. 4b) remained significantly unaltered in E+S at MID and POST but were significantly increased in S+E from MID to POST (+42±50%, p<0.01, ES=1.382). The difference between E+S and S+E at MID was significant (+20±44% vs. -15±28%, <0.05, ES=0.960). During recovery at 24h and 48h, concentrations of serum C were slightly decreased in both E+S and S+E (at 48h E+S -20±23%, p=0.057, ES=-0.729; S+E -21±28%, p<0.05, ES=-0.932) compared to PRE but did not significantly differ between the two groups.

Blood lactate and serum CK concentrations

Concentrations of blood lactate (Table 2) significantly increased at in both loadings at MID (E+S +688±314%, p<0.001, ES=3.622; S+E +717±305%, p<0.001, ES=4.480) and POST (E+S +978±735%, p<0.001, ES=2.980; S+E +6161±224%, p<0.001, ES=3.998) compared to PRE. Concentrations of serum CK (Table 2) significantly increased in both loadings at MID (E+S +19±8%, p<0.05, ES=0.236; S+E +31±23%, p<0.01, ES=0.242) and POST (E+S +29±15%, p<0.05, ES=0.285; S+E +70±92%, p<0.001, ES=0.500) compared to PRE. The increase at POST was significantly larger in S+E compared to E+S (+70±92% vs. +29±15%, p<0.05, ES=0.469).

Highest concentrations of serum CK were observed during recovery at 24h in both groups (E+S +155±60, $p<0.05$, ES=0.597; S+E +57±56, $p<0.001$, ES=0.422).

Differences in acute responses and recovery between the measurements at week 0 and 24

In S+E the reduction in MVC_{max} from PRE to MID (Fig. 2a) was significantly larger at week 24 compared to week 0 (-25±11% vs. -20±13%, $p<0.05$, ES=0.435).

No significant training or loading-induced changes were found for changes in serum T, TSH and GH concentrations in either of the two groups. In E+S, the relative change in serum C at MID (Fig. 4b) was significantly larger after the training intervention (+20±44 vs. +2±27%, $p<0.05$, ES=0.504).

Absolute values of CK in S+E (Table 2) during recovery at 24h and 48h were significantly lower at week 24 compared to week 0 (24h 173±124 $mlU \cdot l^{-1}$ vs. 290±170 $mlU \cdot l^{-1}$, $p<0.01$ ES=-0.570; 48h 123±61 $mlU \cdot l^{-1}$ vs. 221±129 $mlU \cdot l^{-1}$, $p<0.01$, ES=-0.566). In addition, the relative increase from PRE to 24h and 48h in S+E was significantly smaller at week 24 compared to week 0 (24h 157±56% vs. 200±81%, $p<0.05$, ES=-0.352; 48h 137±39% vs. 153±57%, $p<0.05$, ES=-0.398).

Plasma volume

No between-group differences in plasma volume shifts were observed at either week 0 or 24. Plasma volume shifts in the two groups ranged from -10% to -5% during loading and +1% to +7% during recovery, both compared to PRE.

DISCUSSION

The main findings of this study were: 1) Both loading protocols led to similar acute reductions in maximal force production at POST both before and after the prolonged combined training period. 2) The magnitude of reductions in maximal force production in the two groups at POST was similar before and after the training and recovery of force production was already completed at 24h after the two loading protocols at week 0 and 24. 3) Significant acute loading-induced hormone responses were found only in serum GH in both loadings before and after the training and serum T in E+S at MID before the training intervention only. 4) Concentrations of serum cortisol and TSH were reduced compared to pre-loading concentrations during recovery of (at least) 48h after

both loading protocols and serum testosterone after the E+S loading only. Thus, a significant between-group difference (order effect) was found in concentrations of serum T during recovery at 24h and 48h. After training for 24 weeks, reductions of serum hormonal concentrations during recovery were no longer observed in either of the two groups. 5) Both training groups significantly improved 1RM strength after 24 weeks of training independent of the loading order.

Acute reductions in strength performance following strenuous exercise loading may result from both central and peripheral fatigue initiated by repetitive cycles of muscle contractions. In the present study, no significant between-group differences in the magnitude of acute reductions in maximal force production before or after the 24-week training period were observed. After the initial acute decrease in force production, strength performance returned to pre-loading levels already within 24h in both loading protocols at week 0 and 24. Since both repeated bouts of strength loadings and prolonged endurance cycling have been shown to result in decreased force production (Leveritt and Abernethy 1999; Moore et al. 2005; Schumann et al. 2013), the present findings are not surprising. Due to the nature of the present cycling and leg press protocol, the magnitude of loading-induced reductions in maximal force production, however, was relatively low (22-27%) and different results may possibly be observed by modifying the experimental loading performed.

Interestingly, at week 0 the endurance cycling in the E+S loading led to a reduction in MVC_{max} of 11% while in the S+E protocol endurance cycling performed after strength loading did not further reduce maximal force, demonstrating a plateau in fatigue as observed previously during prolonged performance of strength loadings only (Häkkinen and Pakarinen 1993; Ahtiainen et al. 2003a). Hence, while strength loading produces neuromuscular fatigue when performed both before and after an endurance loading, cycling may only induce fatigue when performed in an unfatigued state. Even though steady-state cycling and both maximal and hypertrophic strength protocols mainly recruit different fiber types (Kraemer et al. 1995) and the number and size of motor units recruited depends on the intensity and activity performed (Henneman et al. 1965), some overlapping may occur between both types of loadings. Although muscle activation was not measured in the present study, it is likely that the strength loading activated high threshold motor units characterized by a high fatigability (Henneman et al. 1965), while the subsequent cycling only led to additional recruitment of fatigue-

resistant slow twitch fibers, apparently not increasing the magnitude of overall fatigue. The underlying mechanisms for the present finding, however, may also be metabolic in nature and were not examined in detail.

The magnitude of acute reductions in maximal force at POST in both loading groups after 24 weeks of training was similar to that observed at week 0. In addition, no within group differences in the recovery of force production were observed before or after the training period. However, the reduction of maximal force in S+E at MID (i.e. after S) was significantly larger post-training compared to the corresponding change observed before the training intervention. Although not reflected in blood lactate concentrations, these results indicate an improved fatigue-resistance as previously shown in acute responses to strength loadings after periods of heavy resistance training only (Izquierdo et al. 2009, 2011; Walker et al. 2010). As increased fatigue-resistance allows subjects to sustain a larger magnitude of both mechanical and metabolic stress, the present findings would suggest strength loadings performed immediately before endurance cycling to be more favourable over the reverse loading order. However, these positive adaptations were not reflected in 1RM strength development after 24 weeks of training in this study. Therefore, the role of exercise order with regard to chronic neuromuscular adaptations needs further investigation, for example by modifying the frequency, volume and type of training and loading protocols.

Acute reductions in force production in response to endurance or strength loadings are typically accompanied by loading-induced changes in hormonal concentrations. Hypertrophic type strength loadings characterized by short rest periods as well as endurance exercise of short duration and high intensity may lead to acute increases in serum testosterone, growth hormone and cortisol concentrations (Kraemer et al. 1990; Häkkinen and Pakarinen 1993; Stokes et al. 2013). Similarly, serum TSH as a precursor of thyroid hormones T_3 and T_4 may also significantly increase following both endurance and strength loadings (Hackney et al. 2012). In agreement with our hypothesis, significant acute hormone responses to the present two combined loading sessions, however, were only found in GH both before and after training and in T in E+S at MID only before the training. Since the highest concentrations of GH at both week 0 and week 24 were found in E+S at MID (i.e. after E) and S+E at POST, it appears that the present steady-state cycling at moderate- to high-intensity induced large increases in serum 22-kDa GH concentrations. The strength loading consisting of mixed explosive, maximal and hypertrophic leg press protocols, on the other hand, may not have been sufficiently metabolically

demanding to stimulate GH responses (Häkkinen and Pakarinen 1993). Whether the present endurance and strength loading induced significant changes in other GH aggregates or variants (Kraemer et al. 1990) has not been examined.

When interpreting these results one must bear in mind that the intensity and volume of the present combined loading was purposefully chosen to 1) account for the capabilities of relatively untrained subjects and 2) to represent the overall periodized training program by combining moderate to high intensity steady-state cycling of a relatively short duration with a mixture of explosive, maximal and hypertrophic leg press protocols. In fact, only 2 out of the total 11 sets of the strength loading design were conducted using a purely hypertrophic protocol. In agreement with previous studies, and indicated by the low concentrations of blood lactate in this study, the present combined loading did not produce sufficient physiological stress to stimulate increases in serum testosterone, TSH and cortisol concentrations (Kraemer et al. 1990: Häkkinen and Pakarinen 1993; Linnamo et al. 2005; McCaulley et al. 2009).

However, even though no significant changes in serum concentrations of testosterone, TSH and cortisol in immediate response to the two loading protocols were observed, serum cortisol concentrations were significantly reduced during recovery at 24h and 48h at week 0, independent of the loading protocol. Furthermore, a significant reduction in serum TSH concentrations was observed at 24h in E+S and 48h in S+E. As shown previously, prolonged endurance performance may lead to reduced concentrations of serum cortisol for at least 24h in endurance trained subjects (Daly et al. 2005) and may induce a temporal non-pathological hypothyroidism lasting for 12h to 72h (Moore et al. 2005; Hackney et al. 2012). Although in-line with previous investigations, the decreased concentrations of cortisol and TSH in the present study appeared not to be loading specific. These findings may, therefore, indicate that the concentrations of these hormones are not sensitive enough to reflect differences in the order of combined endurance and strength loadings.

Interestingly, a significant decrease in concentrations of serum testosterone during recovery at 24h and 48h at week 0 was observed in the present E+S group only. Therefore, in line with our hypothesis, the present study showed a significant between-group difference (order effect) before the training. Previous studies have demonstrated reduced concentrations of testosterone during recovery of (at least) 48h in strength athletes following intensive and voluminous strength loadings only (Häkkinen and Pakarinen 1993), in endurance

athletes following an intermittent endurance loading during recovery of 12h (Hackney et al. 2012) and in recreational endurance athletes during recovery of 48h following a combined loading session (Taipale and Häkkinen 2013). The present findings may, thus, indicate the E+S loading protocol conducted before the training period to be physiologically more demanding for physically active men, leading to a requirement for prolonged recovery.

The detailed mechanisms for the present decreased basal hormonal concentrations during recovery are not yet conclusively understood. Loading or training induced changes in serum hormone concentrations may be associated with adaptations within the endocrine system but temporary fluctuations in circulating blood hormone levels can also result from 1) increased or decreased secretion, 2) increased or reduced hepatic clearance, 3) alterations in plasma volume or fluid shift or 4) increased or reduced degradation rates (Kraemer and Ratamess 2005). While the biological functions of transiently reduced concentrations of cortisol vs. TSH and testosterone may differ due to the catabolic vs. anabolic nature of these hormones, reduced concentrations of hormones during recovery have generally been linked with both an up-regulation of androgen receptors accompanied by increased target tissue uptake or an inhibited production of these hormones in the releasing gland or at the hypothalamus level (Vingren et al. 2010). However, since the kinetics of androgen receptor regulation and its association with circulating testosterone concentrations following strenuous exercise sessions has not yet been fully elucidated, the biological meaning of reduced concentrations of serum testosterone during recovery has to be further examined.

Interestingly, the present initial decreases in serum cortisol, TSH and testosterone at 24h and 48h of recovery at week 0 diminished after the 24 weeks of training. The magnitude of immediate acute responses in both catabolic and anabolic hormone concentrations within each loading group, however, was similar at week 24 compared to week 0. The latter finding is in agreement with previous studies investigating chronic adaptations in loading- induced hormone concentrations following strength loadings and training only (Kraemer et al. 1990; Häkkinen et al. 2000; Ahtiainen et al. 2003b). The diminished reductions of both catabolic and anabolic hormone concentrations during recovery, however, suggest adaptations within the endocrine system which were especially pronounced in the E+S training group. Notably, after the 24-week training period a significant trend for decreases in serum testosterone at 48h of recovery in the E+S loading protocol was found and serum

cortisol was significantly reduced in S+E and nearly significantly reduced in E+S at the same time point. Therefore, the present results may also indicate that the time course of hormonal concentrations to return to baseline levels after the training period was prolonged. It would have, thus, been interesting to measure the concentrations of these hormones after recovery of 72h.

Accumulated concentrations of anabolic hormones dramatically increase the likelihood for androgen receptor interactions and repeated loading-induced acute increases in these hormones during training have been shown to be associated with positive adaptations in muscle hypertrophy and strength development during pure strength training (Häkkinen et al. 2000). It is, therefore, reasonable to assume that reduced concentrations of anabolic and catabolic hormones during recovery may also impact on long-term strength development. However, although in the present study order-specific differences in hormonal concentrations between the E+S and S+E loading protocol at week 0 were found, both training programs led to similar increases in 1RM strength after 24 weeks of order-specific combined endurance and strength training. While few authors have questioned the relationship of loading-induced testosterone concentrations with chronic training adaptations (West et al. 2010), several studies have shown significant correlations between both basal and loading-induced concentrations of circulating testosterone and chronic development of muscle mass and strength during strength training only both in men (McCall et al. 1999; Ahtiainen et al. 2003b; Kvorning et al. 2006) and women (Häkkinen et al. 1992). In the present study, however, no correlations were found between basal or loading-induced concentrations of the hormones examined and improvements in 1RM strength during the combined endurance and strength training period.

In contrast to studies investigating endurance or strength training only, one must consider the role of possible acute and chronic interference (Wilson et al. 2012) when interpreting the present findings. Since the endurance part of the combined loading possibly reduced the anabolic effects of the strength loading, a combination of both endurance and strength may in fact dilute possible correlations between loading-induced hormonal concentrations and chronic strength development. Furthermore, it has to be acknowledged that the training frequency in the present study was rather low, allowing for at least 2 full days of rest between consecutive training sessions. Since differences in hormonal concentrations during recovery before training were monitored for 48h only, this would be in-line with the finding that both groups developed 1RM strength to a similar

extent. Finally, the present design including experimental loadings before and after a comparably long training period of 6 months was not able to elucidate the exact timing of endocrine adaptations. It is possible that initial differences in serum testosterone concentrations during recovery were diminished already in an early phase of the training program (for example after a few weeks) and, thus, the possible impact on strength development after 24 weeks was not observed.

CONCLUSIONS

This study has demonstrated that the acute force and hormone responses to combined endurance+strength vs. strength+endurance loadings were similar when compared before and after combined training. While the recovery of force was mainly completed after 24h at pre and post-training in the two loading groups, the order effect was reflected by significantly reduced serum testosterone concentrations at 24h and 48h of recovery in the E+S but not S+E group before the training period. This initial loading-specific difference during recovery was diminished after 24 weeks of combined endurance and strength training and both groups developed 1RM strength to a similar extent. Therefore, the present findings indicate that despite an initial order effect, the order of combined training does not seem to influence long-term adaptations of strength development in physically active young men. However, this study also showed that performing E+S loadings may, especially in the early phase of the training, lead to prolonged recovery needs which may have a negative impact on training outcomes especially when the training frequency is high. Therefore, the present findings are limited to the training volume and frequency performed and should be applied to physically active young men only.

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CONFLICTS OF INTEREST

The authors of this manuscript do not have any conflicts of interest.

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TABLES

Table 1 Baseline values of endurance and strength performance and blood markers. Physical performance data were obtained on separate days before the loading measurements at week 0 and week 24, respectively. Serum hormone and CK concentrations were obtained in the morning of each loading after fasting for 12 hours

Group / **Variable**	E+S week 0	E+S week 24	S+E week 0	S+E week 24
1RM (Kg)	158±30	177±27***	143±24	165±21***
Aerobic power (W)	274±36$^{\#}$	302±34***	247±36	285±38***
MVC_{max} (N)	2628±692	2943±801*	2357±549	2599±580*
Basal Testosterone (nmol·l^{-1})	13±3.1	18.9±4.8***	14.3±3.5	19.9±4.2**
Basal Cortisol (nmol·l^{-1})	529.9±114.5	574±98*	534.5±113.2	597.4±139.5
Basal TSH (nmol·l^{-1})	2.6±0.8$^{\#}$	2.2±1.3	2.0±0.6	1.5±0.7
Basal GH (mlU·l^{-1})	1.2±1.8	2.2±4.6	2.4±6.7	0.9±1.5
Basal CK (mlU·l^{-1})	166.7±98	132.3±78.8	158.4±116.5	103.6±51.6*

$^{\#}$significant different from S+E at corresponding time point, p<0.05; *,**,***, significant different from measurements at week 0 (p<0.05,0.01 and 0.001, respectively)

Table 2 Serum growth hormone, blood lactate and serum creatine kinase concentrations before, during and after the two combined loadings obtained before and after loading order-specific combined training

		Week 0			**Week 24**		
		GH (mlU·l^{-1})	**Lactate** (mmol·l^{-1})	**CK** (mlU·l^{-1})	**GH** (mlU·l^{-1})	**Lactate** (mmol·l^{-1})	**CK** (mlU·l^{-1})
E+S Loading	**PRE**	1.2±1.8	1.1±0.4	168.5±98.1	2.2±4.6	1±0.2	134.8±79.2
	MID	56.3±29**†	5.8±2.8**	197.3±104.8*	68.6±43.5**†††	6.2±2.0***	159.8±92.8*
	POST	13.7±8*†††	8.3±3.2***	209±97.8*	19.1±18.6**†	9.2±3.9***	170.7±93.5*
	24h			404.8±229.3			313.8±199.6*
	48h			276±127.6			242.9±198
S+E Loading	**PRE**	2.4±6.7	1.4±0.4	160.4±118.5	0.8±1.5	1.5±0.8	106.6±52.1
	MID	15±27.5**	8±2.3***	185.7±139.7***	7.7±12.3	9±2.3***	137.8±82.5**
	POST	54.4±32.3***	7.2±2.0***	214.3±155***	56.7±37.5***	7.9±2.1***	174.8±99.4***
	24h			290.4±170**			172.6±123.6***$^{\#}$
	48h			221.3±128.8			122.8±61.2$^{\#}$

†,†††significant different from S+E at corresponding time point, (p<0.05 and p<0.001, respectively); *,**,***, significant different from corresponding PRE values(p<0.05,0.01 and 0.001, respectively), $^{\#}$significant different from measurements of week 0

FIGURE CAPTIONS

Fig 1 Experimental design for the examination of acute force and hormone responses and recovery to combined endurance and strength loadings conducted before and after the loading order-specific combined training. Baseline measurements consisted of tests for endurance and strength performance as well as the determination of serum hormone and CK concentrations

Fig 2 Acute responses and recovery of maximal isometric leg press force (MVC_{max}) before (a) and after (b) the combined training. *p<0.05, **p<0.01, ***p<0.001 within the bar compared to PRE; outside the bar as indicated; # significant different from corresponding time point at week 24 (p<0.05)

Fig 3 Serum testosterone concentrations during loading and recovery before (a) and after (b) the combined training. *p<0.05, **p<0.01, ***p<0.001 within the bar compared to PRE; outside the bar as indicated; † refers to a significant trend p<0.06; within the bar compared to PRE, outside the bar as indicated

Fig 4 Serum cortsiol concentrations during loading and recovery before (a) and after (b) the combined training. *p<0.05, **p<0.01, ***p<0.001 within the bar compared to PRE; outside the bar as indicated; # significant different from corresponding time point at week 24 (p<0.05); † refers to a significant trend p<0.06 compared to PRE

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FITNESS AND LEAN MASS INCREASES DURING COMBINED TRAINING INDEPENDENT OF LOADING ORDER

by

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Fitness and lean mass increases during combined training independent of loading order

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ABSTRACT

Purpose: Although the benefits of combined endurance (E) and strength (S) training for the development of physical fitness and health are well known, scientific examination of the effect of loading order when E and S are combined into the same training session (E+S vs. S+E) is rare. This study investigated the effects of moderate frequency E+S versus S+E training on physical fitness, body composition and blood lipids. **Methods:** Physically active and healthy young men performed E+S (n=16) or S+E (n=18) training**,** 2-3 $x \cdot wk^{-1}$ for 24 weeks. Endurance (by incremental bike test) and strength (by dynamic leg press) performance as well as body composition (by DXA), muscle cross-sectional area of vastus lateralis (by ultrasound) and blood lipids were determined before and after the intervention. **Results:** Time to exhaustion, aerobic power (W) and 1RM strength significantly increased in the two groups at week 24 (E+S 12-15%, p=0.003-0.001; S+E 16-17%, $p<0.001$) but no between-group difference was observed. Similarly, the two groups significantly increased total lean mass (E+S 3%, S+E 3%, both p=0.001) and muscle cross-sectional area (E+S 14%, p=0.001; S+E 16%, $p<0.001$) at week 24 to a similar extent. No significant changes in body fat or blood lipids were observed in either of the two groups at week 24. **Conclusion:** These results showed that moderate frequency (2-3 $x \cdot wk^{-1}$) combined E+S or S+E training led to significant improvements in physical fitness and lean body mass but did not induce significant changes in body fat or blood lipids. Furthermore, as no between-group differences were observed, these results indicate that loading order does not seem to affect training adaptations of healthy moderately active young men.

Key words: order effect, aerobic training, resistance training, concurrent endurance and strength training, muscle cross-sectional area, body composition, hypertrophy, health

INTRODUCTION

The benefits of combined endurance (E) and strength (S) training for the maintenance and development of physical fitness and body composition have been extensively investigated and their importance especially for sedentary and moderately active populations is well known (15, 19, 21, 23, 34). Over recent years, a growing body of scientific knowledge strongly suggests that regular performance of aerobic and resistance training is a major factor in the prevention and treatment of cardiovascular disease including risk factors such as obesity, diabetes and blood lipid levels (23).

The long-term physiological adaptations of endurance and strength training are dissimilar in nature. Prolonged endurance training may enhance oxidative energy metabolism and simultaneously increase whole-body rates of fat oxidation (2), which may lead to decreases in total and abdominal body fat (1) and total cholesterol, as well as a more positive distribution of low (LDL-C) and high (HDL-C) density lipoproteins (38). While endurance training may directly decrease total body fat and weight, strength training can positively affect body composition via increases in muscle cross-sectional area and lean body mass (1, 36).

The suggested amount of weekly physical activity commonly ranges from 150 to 300 $min \cdot wk^{-1}$ (1, 18) of combined aerobic and resistance training. However, research findings have repeatedly emphasized impaired biological adaptations (interference) when a high volume of endurance and strength training are combined over a longer period of time (40). It appears that this interference may be more pronounced in strength or power development (21) but to some extent also in muscle growth (24, 40), reducing the positive effects of physical training on fitness, body composition and health. Combined training utilizing a low volume and frequency of training sessions (i.e. 2-3 $x \cdot wk^{-1}$), on the other hand, may not have inhibitory effects on neuromuscular and morphological adaptations (24, 28). A recent study by Fisher et al. (2013) (15) has shown that a combined training frequency of 1 x wk^{-1} aerobic and 1 x wk^{-1} resistance training led to similar improvements in overall fitness as a twice or three times higher training frequency in older women. The exact amount of exercise needed to achieve favorable training adaptations seems to depend on the size and type of the expected outcome variables (i.e. modest vs. large reductions in body fat or body weight, reductions in blood lipids vs. increases in

muscle size etc.), intensity and volume of training performed, as well as on dietary prescriptions (i.e. diet restriction vs. freely chosen diet) and the population studied (1, 23, 38).

The biological adaptations of concurrent training performed on separate days were previously examined in numerous studies. However, scientific examination of the physiological adaptations of endurance and strength training combined into the same training session are still rare but provide some evidence of loading order-specific adaptations due to the missing recovery when loadings are performed consecutively (9-11, 13, 20). As this training method can be considered as extremely time effective and time constraints are among the major reasons for restraining from regular physical activity in young adults (31), the aforementioned training regimen may help young adults to commit to regular physical activity while allowing sufficient time for other responsibilities.

The purpose of this study was two-fold to determine; 1) whether a moderate volume of endurance and strength training combined into the same training session is sufficient to yield significant changes in physical fitness, body composition and blood lipids and 2) whether loading order-specific adaptations (order effect) in these variables can be observed. Consequently, while the magnitude of possible interference was beyond the scope of this study, the focus of this study was to investigate the order effect of endurance followed by strength (E+S) and strength followed by endurance (S+E) training performed over 24 weeks. We hypothesized that our training volume will induce significant improvements in physical fitness, body composition and blood lipids but the magnitude of these adaptations will be related to the loading order performed.

METHODS

Subjects

Forty-two healthy men were recruited to participate in this study. Subjects' initial health and activity status was assessed by a standardized phone interview. The subjects were moderately physically active as characterized by irregular performance of walking, cycling or occasionally team sports at light to moderate intensity and duration for not more than 3 times per week and did not engage systematically in any endurance or strength training prior to inclusion into the study. Subjects were informed about possible risks of all study procedures before giving written informed consent. A completed health questionnaire and resting ECG were reviewed by a

cardiologist prior to the first exercise testing and training. All subjects were free of acute and chronic illness, disease and injury and did not report use of any medications that would contraindicate the performance of intense physical activity. Out of the 42 originally recruited subjects, 8 did not complete the study or were not included in the data analysis due to a training adherence of less than 90%. Demographic characteristics of all included subjects were as follows (mean±SD): age 30±5 years, height 179±6 cm, weight 78±11 kg, BMI 24±3. An additional group of subjects undergoing the same pre-screening process was recruited for reproducibility tests of the measurement procedures (n=21, age 30±6 years, height 180±7 cm, weight 82±9 kg, BMI 25±2). The study was conducted according to the Declaration of Helsinki and ethical approval was granted by the ethics committee at the University of Jyväskylä

Study design

Following the pre-screening, all experimental subjects were assigned to an Endurance+Strength (E+S, n=16) or Strength+Endurance (S+E, n=18) training group. The subjects performed either endurance immediately followed by strength training (E+S) or strength immediately followed by endurance training (S+E) for 24 weeks. As this study was aimed to investigate the order effect of combined training, a no-training control group was not used. For familiarization, one combined training session in the order of the corresponding training group (E+S vs. S+E) was conducted prior to the baseline measurements and training. Thereafter, subjects reported to the laboratory for a second familiarization session during which the strength measurements were practiced and the equipment adjusted to the specifics of the subject. Testing of physical fitness, body composition and blood lipids were then performed on three separate days prior to the start of the training (wk 0). To allow for sufficient recovery, endurance, strength as well as body composition and blood tests were separated by at least 48 h of rest. All measurements were repeated after 12 and 24 weeks and were performed at the same time of day within ±1h of the timing of baseline measurements. The additional subjects recruited for measurement reproducibility testing (n=21) were familiarized with the measurement procedures in the same manner as the intervention groups and were tested both before and after a 12-week period without undergoing prescribed training but maintaining their habitual activities of daily living.

Testing procedures

Strength performance

Subjects' one repetition maximum (1RM) of leg extensors was determined using a dynamic horizontal leg press device (David 210, David Health Solutions, Helsinki, Finland). Following a warm up (1 set of 5 repetitions at 70% of estimated 1RM, 1 set of 2 repetitions at 80-85% of estimated 1RM, 1 set of 1 repetition at 90-95% of estimated 1RM) a maximum of 5 trials was allowed to obtain a true 1RM. The device was set up so that the knee angle in the initial flexed position was approximately 60 degrees (mean±SD, 58 ± 2 degrees) and a successful trial was accepted when the knees were fully extended (~180 degrees). The greatest load that the subject could lift to full knee extension at an accuracy of 1.25 kg was accepted as 1RM. In addition, a horizontal leg press dynamometer (Department of Biology of Physical Activity, University of Jyväskylä, Finland) was used to determine maximal isometric bilateral leg press force (MVC_{max}). Subjects were seated with a hip and knee angle of 110 and 107 degrees, respectively and were instructed to produce maximal force as rapidly as possible on verbal command and to maintain the force plateaued for 3-4 seconds. At least three trials separated by a rest period of 1 minute were conducted and up to two additional trials were performed if the maximum force during the last trial was greater by 5% compared to the previous attempt. The trial with the highest maximal force measured in Newtons was used for statistical analysis. The force signal was low-pass filtered (20Hz) and analyzed (Signal software, version 4.04, Cambridge Electronic Design Ltd., Cambridge, UK). Rapid force production (MVC_{500}) was calculated from the force curve and defined as the average force produced during the first 500ms of the maximal contraction.

Endurance performance

A graded protocol on a cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany) was used to determine $\dot{V}O_{2max}$. The initial load for all subjects was 50 Watts and increased by 25 Watts every 2 minutes. Subjects were asked to maintain a pedaling frequency of 70 rpm throughout the test. The test was stopped when the subjects failed to keep up the required rpm for more than 15 s. Heart rate was monitored throughout the test (Polar S410, Polar Electro Oy, Kempele, Finland) and recorded as the average of the last 5 seconds at each stage. Oxygen uptake was determined continuously breath-by-breath using a gas analyzer (Oxycon Pro, Jaeger, Hoechberg, Germany). On each testing day, air flow calibration was performed using a manual flow calibrator. Before each test, automatic air flow calibration was performed and the gas analyzer was calibrated using a certified gas

mixture of 16% O_2 and 4% CO_2. $\dot{V}O_{2max}$ was accepted when $\dot{V}O_2$ plateaued despite a further increase in power and when the respiratory exchange ratio exceeded 1.05. $\dot{V}O_{2max}$ used for statistical analysis was calculated as the highest $\dot{V}O_2$ value averaged over 60 s. In addition, maximal aerobic power (W) was calculated using the equation (25): Aerobic power= W_{com}+(t/120)*25, where W_{com} is the load of the last completed stage and t is the time of the last incomplete stage (in s) and time to exhaustion was defined as the total duration of the test. Blood lactate concentrations were determined by capillary blood samples taken from the fingertip during the final seconds of each load. Twenty µl of blood were collected by small capillaries, inserted into reaction capsules containing a hemolyzing and anticoagulant agent and lactate concentrations were analyzed using a Biosen analyzer (C_line Clinic, EKF, Magdeburg, Germany). Subjects' individual aerobic and anaerobic thresholds were determined using deflection points obtained by plotting the curves of blood lactate concentrations, ventilation, oxygen consumption and carbon dioxide production (6).

Body composition and venous blood sampling

Anatomical muscle cross-sectional area (CSA) of vastus lateralis (VL) was measured by the extended field of view mode (3), using a B-mode axial-plane ultrasound (model SSD-a10, Aloka Co Ltd, Japan) with a 10-MHz linear-array probe. A customized convex-shaped probe support was used to assure a perpendicular measurement and to constantly distribute pressure on the tissue. The transducer was moved manually from lateral to medial along a marked line on the skin. Three panoramic CSA images were taken at 30%, 50% and 70% of the femur length (lateral aspect of the distal diaphysis to the greater trochanter), respectively and CSA was analyzed manually using Image-J software (version 1.44p, National Institute of Health, USA). The mean of the two closest values (at 30%, 50% and 70%, respectively) was used for statistical analyses. To assess total CSA of VL, values of the three measurement points were averaged.

Whole body tissue composition was assessed by DXA (Lunar Prodigy Advance, GE Medical Systems, Madison, USA). To control the experimental conditions, each scan was conducted in the morning after 12 hours of fasting. Legs were secured by non-elastic straps at knee and ankles and arms were aligned along the trunk with palms facing the thighs. All metal objects were removed from the subject prior to the scan. Automatic analyses (Encore, version 14.10.022) provided total and upper body lean (including muscle) and fat mass.

Automatic generated regions of the legs were manually adjusted by the same investigator to include hamstrings and gluteal muscles. Thus, legs were separated from the trunk by a horizontal line right above the iliac crest providing lean and fat mass for legs and upper body separately. Abdominal fat mass was calculated by manually defining a range of interest (ROI) confined cranially by the upper end plate of the first lumbar vertebra, laterally by the ribs and caudally by the iliac crest (37). This customized ROI was then copied to the DXA scans obtained at week 12 and 24, respectively in order to assure analyses were conducted from the same areas at all measurement times.

Venous blood samples were drawn after 12h of fasting in order to obtain concentrations of total cholesterol, low density lipoprotein (LDL-C), high density lipoprotein (HLD-C) and triglycerides. Subjects were asked to rest for at least 8 h during the preceding night and were required to restrain from strenuous physical activity for at least 48 h. Blood samples were taken from the antecubital vein into serum tubes (Venosafe, Terumo Medical Co., Leuven, Hanau, Belgium) using standard laboratory procedures. Serum samples were stored for 10 min after which they were centrifuged at 3 500 rpm (Megafure 1.0 R, Heraeus, Germany) and immediately analyzed by spectrophotometry (Konelab 20XTi, Thermo Fisher Scientific, Vantaa, Finland). LDL-C was estimated using the Friedwald (1972) (17) equation: LDL-C=total cholesterol - HDL-C - (triglycerides/2.2).

Combined endurance and strength training

Subjects were asked to maintain individual habitual physical activity (e.g. light walking, cycling and occasional team sports) throughout the study period. All prescribed training in the study was consistently supervised by qualified instructors. The training was designed to reflect a program aimed for physically active populations according to recommendations outlined by the American College of Sports Medicine (39) but modified to reduce overall training volume and frequency. The main objective was to improve physical fitness and health through a periodized program including both moderate and vigorous intensity aerobic exercises combined with hypertrophic and maximal strength exercise protocols. The endurance training was conducted on a cycle ergometer and the strength training program included exercises for all major muscle groups with a major focus on the lower extremities. Subjects were asked to proceed from one loading (i.e. E or S, respectively) to the subsequent loading (i.e. S or E, respectively) after a maximum of 10 minutes of rest.

During the first 12 weeks, the subjects performed according to their corresponding training group 2x (1E+1S) or 2x (1S+1E) per week. The frequency was then increased during the second 12 weeks so that 2 combined training sessions were performed in every 1st and 4th week and 3 combined training sessions in every 2nd and 3rd week (i.e. 2x [1E+1S] or 2x [1S+1E] or 3x[1E+1S] or 3x [1S+1E], respectively). To reflect tapering before testing, both week 12 and week 24 were conducted by maintaining the training frequency but reducing training volume and intensity by reducing the number of sets and lowering the loads during the strength loading as well as a reducing both the total duration and time spent at high intensity (i.e. above the anaerobic threshold) during endurance cycling.

The intensity of the endurance training was controlled by heart rate (HR) (Polar S410, Polar Electro Oy, Kempele, Finland) associated with subject's individual aerobic and anaerobic threshold determined during measurements at week 0 and 12, respectively. Subjects were instructed to maintain a constant pedalling frequency at approximately 70 rpm during each training session, while the magnetic resistance of the ergometer was adjusted to achieve the required exercise intensity. During weeks 1-7 steady-state cycling of low to moderate intensity (below and above the aerobic threshold) was performed and during the remaining weeks, additional high-intensity interval sessions (below and above the anaerobic threshold) were incorporated into the training program. The duration of endurance cycling progressively increased throughout the 12 weeks of training from 30 to 50 minutes. During the second 12-week period, the major endurance program structure was maintained, while both training volume and intensity were further increased. The aerobic threshold represented an intensity (% of HR_{max}) of 65±5 % and 67±6 % in E+S and 68±8 and 67±6 % in S+E at week 0 and 12, respectively. The anaerobic threshold represented an intensity of 85±5 % and 86±5 % in E+S and 82±8 % and 86±5 % in S+E at week 0 and 12, respectively.

The loads used during the strength training were determined by the number of repetitions and execution velocity and progressively increased throughout the two 12-week periods. Exercises for the lower body were bilateral dynamic leg press as well as bilateral (weeks 1-7 and 13-18) and unilateral (weeks 8-12 and 19-24) dynamic knee extension and flexion. Additional exercises for the upper body included dynamic seated vertical press and lat-pull down as well as exercises commonly used to improve trunk stability (crunches, torso rotation and lower back extension). During the first two weeks training was performed as a circuit using 2-4 sets of 15-

20 repetitions at an intensity of 40-60% of 1RM. Thereafter, protocols aiming for muscle hypertrophy (2-5 sets of 8-10 repetitions at 80-85% of 1RM, 1.5-2 min inter-set rest) and maximal strength (2-5 sets of 3-5 repetitions at 85-95% of 1RM, 3-4min inter-set rest) as well as during the last 2 weeks protocols targeting explosive strength (2 sets of 8-10 repetitions at 40% of 1RM with maximal velocity, 3-4 min inter-set rest) were performed. During the second 12-week period the major strength program structure was maintained, while both training volume and frequency were slightly increased in order to maximize fitness and health outcomes and to avoid a training plateau. The overall duration of the strength protocol within each combined training session was 30-50 min, resulting in a total duration of ~60-100 min for each combined training session (i.e. E+S and S+E, respectively).

Dietary intake

To control nutritional intake, food diaries were collected for three days including one weekend day at week 0, 12 and 24. Subjects received both verbal and written nutritional recommendations and were instructed on how to report nutritional intake in the diaries. The food diaries were analyzed by nutrient analysis software (Nutriflow, Flow-team Oy, Finland). Subjects were asked to maintain constant dietary intake throughout the study period. In preparation for all testing, subjects were instructed to consume a light meal 2-3 h prior to the start of each test and were asked to maintain similar nutritional intake prior to the measurements at week 0, 12 and 24. During each training session, a standardized low dose of glucose (according to bodyweight 2-4 tablets, each containing 2.1g of glucose) was provided at the mid-point of each combined session (after E or S, respectively), while water was allowed ad libitum.

Statistical analyses

Data were analyzed using the Statistical Package for the Social Sciences (version 20.0, IBM Inc., Chicago, IL, USA). All results are presented as absolute values (Tables 1 and 2) and relative changes from week 0 as means with standard deviations (±SD). Normality of distribution was determined by the Shapiro-Wilk test at a significance level of $p<0.05$. To achieve normality, all body composition and blood variables were log transformed. Within and between-group differences were assessed by a mixed ANOVA design with repeated measures. Effect sizes (ES) are given as Cohen's d. Partial correlations with adjustment for groups were performed to determine relationships between dependent variables across all experimental subjects. Measures

of reliability are presented as intra-class correlations (ICC) of absolute agreement for single measures. Significance for all tests was defined as p=0.05, while values <0.06 were accepted as a significant trend.

RESULTS

The training adherence was 99±2% in both the E+S and S+E training group. All subjects completed at least 90% of the overall training volume.

Measurement reproducibility

The analysis of reliability revealed an ICC > 0.7 for all test measures, indicating high reproducibility. The intra-class correlations of endurance and strength performance, body composition and blood lipid measures were 0.737 - 0.955, 0.786 - 0.975 and 0.763 - 0.866, respectively.

Nutrition

Total energy intake at week 0, 12 and 24 was 9.3±1.8 MJ, 10.2±2.6 MJ, 9.5±2.6 MJ in E+S and 9.4±2.0 MJ, 9.3±1.7 MJ, 7.9±1.7 MJ in S+E. The average nutritional intake as percentage of total energy for carbohydrates, fat and protein was 42-45%, 31-36% and 17-19% in E+S and 42-44%, 33-36% and 18% in S+E throughout the 24 weeks of training. No significant within or between-group differences were observed.

Physical Fitness

Absolute values of physical fitness at week 0 and 24 are presented in Table 1. *S*ignificant main effects for time were observed in 1RM (F=73, p<0.001), MVC_{max} (F=14, p<0.001) and MVC_{500} (F=15, p<0.001). Both groups significantly improved 1RM strength (Fig. 1) at week 12 (E+S 9±8 %, p<0.001, ES=0.456; S+E 12±8%, p<0.001, ES=0.772) and 24 (E+S 12±9%, p=0.001, ES=0.620; S+E 17±12%, p<0.001, ES=1.032). The increase from week 12 to 24 was significant in both groups (p<0.05). Similarly, MVC_{max} significantly increased in both groups at week 12 (E+S 10±10%, p=0.010, ES=0.345; S+E 9±12%, p=0.019, ES=0.337) and 24 (E+S 10±12%, p=0.025, ES=0.302; S+E 13±18%, p=0.024, ES=0.482) while MVC_{500} increased significantly in S+E only at week 12 (13±15%, p=0.002, ES=0.623) and 24 (14±18%, p=0.005; ES=0.620).

+++ Fig 1 somewhere near here +++

Significant main effects for time were observed in time to exhaustion (F=83, p<0.001), maximal aerobic power (F=71, p<0.001) and $\dot{V}O_{2max}$ (F=12, p<0.001). Both groups significantly improved time to exhaustion (Fig. 2a) at week 12 (E+S 9±9%, p=0.003, ES=0.387; S+E 10±6%, p<0.001, ES=0.551) and 24 (E+S 15±9%, p<0.001, ES=0.859; S+E 17±7%, p<0.001, ES=1.027) as well as maximal aerobic power (Fig. 2b) at week 12 (E+S 8±9%, p=0.011, ES=0.820; S+E 9±7%, p<0.001, ES=0.630) and 24 (E+S 13±9%, p<0.001, ES=0.830; S+E 16±7%, p<0.001, ES=1.074). The increases in aerobic power in both groups from week 12 to 24 were significant (p<0.01-0.001). The observed increases in $\dot{V}O_{2max}$ were significant at both week 12 (E+S 4.8±7%, p=0.051, ES=0.266; S+E 7.3±8%, p=0.003, ES=0.339) and 24 (E+S 6.1±8%, p=0.041, ES=0.366; S+E 6.4±12%, p=0.006, ES=0.396). No significant between-group differences were obtained for the measures of physical fitness.

+++ Fig 2a and 2b somewhere near here +++

Body composition

Absolute values of body composition measures at week 0 and 24 are presented in Table 1. A significant increase in bodyweight and BMI was observed in S+E only (1.7±2.4% and 1.7±2.6% at week 12 and 24 respectively, p<0.05). No significant changes in body fat %, total fat mass or abdominal fat mass were observed in the two groups at either week 12 or week 24. A significant main effect for time was observed for muscle CSA at 30% (F=18, p<0.001), 50% (F=50, p=0.001) and 70% (F=60, p<0.001) of vastus lateralis (Fig. 3). Both groups significantly improved average CSA of VL at week 12 (E+S 8±7%, p=0.002, ES=0.490; S+E 9±7%, p<0.001, ES=0.643) and 24 (E+S 14±7%, p=0.001, ES=0.822; S+E 16±8%, p<0.001, ES=1.178) whereby the increase from week 12 to 24 was significant (both groups p<0.001).

+++ Table 1 somewhere near here +++

+++ Fig 3 somewhere near here +++

A significant main effect for time was observed for total lean mass (F=8, p=0.001), upper body lean mass (F=13, p<0.001) and leg lean mass (F=49, p=0.001). Both groups significantly increased total lean mass (Fig. 4a) at week 12 (E+S 2±3%, p=0.042, ES=0.203; S+E 3±2%, p<0.001, ES=0.310) and week 24 (E+S 3±3%, p=0.001, ES=0.329; S+E 3±2%, p=0.001, ES=0.342). Similarly both groups increased upper body lean mass

(Fig. 4b) at week 12 (significant in S+E only, 2±3%, p=0.022, ES=0.212) and 24 (E+S 3±3% p=0.005, ES=0.253; S+E 2±3%, p=0.025, ES=0.218) and leg lean mass (Fig 4c) both at week 12 (E+S 2±3%, p=0.024, ES=0.210; S+E 3±2%, p<0.001, ES=0.373) and 24 (E+S 4±3%, p<0.001, ES=0.361; S+E 4±2% p<0.001, ES=0.427). The increase in leg lean mass from week 12 to 24 was significant in E+S only (p<0.05). No significant between-group differences for the measures of body composition were obtained.

+++ Fig 4a – 4c somewhere near here +++

Blood lipids

Only minor changes in total cholesterol, HDL-C, LDL-C and triglycerides were observed after 24 weeks of training (Table 2). A significant between-group difference was observed for LDL-C at week 12 (p<0.05) but was diminished after 24 weeks of training.

+++ Table 2 somewhere near here +++

Correlations of physical fitness and body composition across all experimental subjects

All absolute values of physical fitness at baseline (1RM, MVC_{max}, MVC_{500}, aerobic power, time to exhaustion and $\dot{V}O_{2max}$) were significantly correlated with the corresponding relative changes obtained at week 12 and 24 (r=-0.376 to -0.725, p=0.031 to <0.001). Similarly, significant correlations at week 24 were also found for body fat % at baseline and the relative change in body fat % (r=-0.450, p=0.006) as well as for the absolute values of total fat at baseline and the corresponding relative change (r=-0.364, p=0.037) and total fat and abdominal fat mass at baseline and the relative change in body fat % (r=-0.458, p=0.006 r=-0.431, p=0.006, respectively). In addition, absolute values of 1RM strength at baseline were significantly correlated with relative changes in body fat % as well as relative changes of total and abdominal fat mass obtained at week 12 and 24 (r=-0.365 to -0.456, p=0.025-0.006). Similarly, changes in 1RM strength performance and changes in leg lean mass and VL CSA were significantly correlated (r=0.476-0.629, p=0.037-0.007) at week 24.

DISCUSSION

Physical fitness, body composition and blood lipids are strongly associated with health and mortality even in relatively young and healthy subjects (23, 30). The purpose of the present study was to assess the effects of

exercise order of moderate frequency (2-3 x·wk^{-1}) endurance and strength training combined into the same training session (E+S vs. S+E) on physical fitness, body composition and blood lipids in moderately active and healthy young men. This study showed that both training orders (E+S and S+E) led to significant increases in muscular and cardiorespiratory fitness, muscle cross-sectional area and lean body mass after 12 and 24 weeks of training, but no reductions in total body or abdominal fat mass, body fat % or blood lipids were observed in either of the two training groups. In addition, the magnitude of training-induced adaptations did not differ between the two groups.

Compared to concurrent training performed on separate days, endurance and strength training combined into the same training session does not allow any recovery between the two modes, leading to the loading performed second to be adversely affected by fatigue induced by the first loading. In recent studies, these adverse effects were reflected by increased work economy when endurance loading was performed immediately after a strength loading (14) and reduced neuromuscular performance measured immediately following intensive running or cycling (27), possibly influencing physiological training adaptations. As previous studies of combined endurance and strength training have shown possible compromised adaptations in strength and power but not endurance performance (21), it is likely that the acute effects of endurance loading on strength performance are more critical for the long-term development of physical fitness than the acute effects of strength loading on work economy during endurance performance.

Interestingly, the present E+S and S+E training groups significantly improved physical fitness as reflected in 1RM strength (12-17%), MVC_{max} (10-13%), time to exhaustion (15-17%), aerobic power (13-16%) and $\dot{V}O_{2max}$ (7%) to a similar extent and no between-group differences were observed. Our findings are in line with results of Collins and Snow (1993) (13) and Chtara et al. (2008) (10) who also reported that either loading order was similarly effective in improving endurance and strength performance following prolonged combined E+S or S+E training. However, other studies have found limited increases in VO_{2max} following the E+S order in women (20) or S+E order in men (11) as well as impaired strength adaptations following E+S training in older men (9) when compared to the reverse loading order. Despite these findings of studies combining endurance and strength training into the same training session and those which report diverse biological adaptations

induced by endurance and strength training alone (22), the present results indicate that our subjects adapted to both training stimuli simultaneously and to the similar magnitude.

When combining endurance and strength into the same training session, it seems that the type of endurance training performed needs to be carefully considered. Endurance cycling is biomechanically similar to many of the strength exercises performed in the present study (16) and may essentially lead to a similar magnitude of fatigue as indicated, for example, by inhibited neuromuscular performance observed during a single isometric contraction (32, 33), suggesting similar acute neural responses to both types of loadings. Furthermore, previous studies have shown that endurance cycling training may also lead to small but significant increases in muscle CSA (28) and strength (24) in physically active subjects with no experience in regular endurance or strength training. Therefore, it is possible that the present endurance cycling combined with the hypertrophic and maximal strength training protocols led to synergistic rather than adverse effects on strength and endurance performance. This hypothesis may also be supported by the review by Wilson et al. (2012) (40) who revealed that endurance running may be more detrimental to strength adaptations when compared to endurance cycling, possibly related to a larger magnitude of muscle damage induced by the eccentric components of prolonged running (29).

The present increases in 1RM strength were significantly correlated with increases in anatomical muscle-cross-sectional area and leg lean mass in all subjects across the two training groups. Both training groups significantly increased muscle CSA after the 24 weeks of training independent of the loading order. Although animal studies have shown that endurance and strength training might induce distinct genetic and molecular pathways critical for muscle hypertrophy (5, 22), other studies of human subjects have indicated the cumulative effect of both loadings to possibly compromise beneficial morphological adaptations (12, 22). Coffey et al. (2009) (12) found in an acute study that neither of the two loading orders (E+S vs. S+E) showed superior signaling responses over the other but concluded that endurance and strength training performed in close proximity did not induce optimal activation of pathways to promote significant anabolic processes. While the magnitude of interference when compared to strength training alone was beyond the scope of this study, these previous findings possibly explain why no between-group differences in muscle growth were observed.

Similar to anatomical muscle CSA, leg, upper body and total lean mass were increased in the present two groups during the 24 weeks of training independent of loading order. Muscle strength and possibly muscle mass have been associated with reduced mortality even in young subjects (30). Since lean body mass has been shown to be a major determinant of basal metabolic rate by representing 60-75% of an individual's daily energy expenditure (35), increases in muscle and lean mass may have potential health benefits by inducing enhanced fat oxidation (1, 36). Our present findings are thus, of great importance as they show that a moderate volume of combined endurance and strength training may be beneficial in significantly increasing muscle strength and lean mass whereby the loading order does not seem to influence the magnitude of these adaptations.

However, the positive adaptations in physical fitness and lean body mass were not accompanied by significant reductions in body fat % and total or abdominal fat mass in either training group. Furthermore, no significant changes in total cholesterol as well as high- (HDL-C) and low (LDL-C) density lipoproteins or triglycerides were observed. Previous studies have shown a strong association between body fat and blood lipids (7), indicating that a reduction in fat mass positively correlates to changes in blood lipids. Typically aerobic exercise has been considered as being most effective to induce reductions in fat oxidation during and in the hours following an exercise loading (1, 38), while the direct effects of strength training on reductions in body fat and blood lipids are minimal (19, 26). Studies combining endurance and strength training on separate days often show reductions in both variables with varying training frequency and volume in young (19) and old men (34) as well as old women (15). Therefore, our present results may indicate endurance and strength training combined into the same training session to be less favorable for reductions in body fat and blood lipids than combined training performed on separate days. In contrast to our study, however, it needs to be noted that most of the previous studies were performed with endurance running. Achten et al. (2003) (2) showed that running induces higher rates of fat oxidation when compared to cycling and a meta-analysis by Wilson et al. (2012) (40) found combined training programs in which the aerobic training is carried out by running to be possibly more beneficial in reducing body fat when compared to endurance cycling which may provide additional explanations for our findings compared to previous investigations.

In addition, the important difference of the present study design compared to combined studies in which endurance and strength training was performed on separate days is that by performing both types of loadings

subsequently in the same training session, the total training frequency is essentially reduced (2-3x 1E+S or 2-3x 1S+E per week =2-3 total sessions instead of 2-3x 1S +2-3x per week = 4-6 total sessions). While energy expenditure (as measured by post-exercise oxygen consumption) during exercise increases in proportion to the work performed, it does not return to baseline immediately post-exercise but may remain elevated for a prolonged time (8). Previous studies have shown a dose-response relationship between the duration and magnitude of post-exercise oxygen consumption and the duration and intensity of both endurance and strength loadings performed (8) but very few studies have directly compared the effects of splitting exercise sessions compared to the similar workload performed during only one session. From these studies it, however, appears that performing prolonged endurance cycling (4) may lead to a smaller overall increase in post-exercise oxygen consumption when compared to the same workload performed in two separate exercise sessions. As we decreased the overall training frequency in the present study by combining endurance and strength training into the same training session, it is possible that the overall weekly energy expenditure was lower than that observed during conventional concurrent training programs (i.e. separate day combined training). However, as post-exercise oxygen consumption or energy expenditure were not measured in this study, these speculations remain to be investigated.

Further possible explanations for our findings of no significant reductions in body fat and blood lipids may be related to the present endurance training program. In line with our purpose to provide a moderate volume training program, we limited the duration of each training session to a maximum of 100 min leading to a total of maximal 200 min during weeks 0-12 and 200-300 min during weeks 13-24. As only half of the total training time was performed as endurance cycling, the overall duration and intensity of aerobic training may have not been sufficient (as also observed by the relatively small increases in $\dot{V}O_{2max}$) to result in significant reductions of body fat and changes in blood lipids.

Last, when interpreting the present results one must bear in mind that the subjects of the present study were normal weight, moderately active and healthy males with normal blood lipid levels which in turn provided a relatively small window for adaptations (1). Moreover, the nutritional intake was controlled but not restricted and the analysis of food diaries revealed that the subjects in both training groups maintained their caloric intake constant throughout the 24 weeks of training which may support that no significant changes in fat mass and

blood lipids were observed. However, the observed correlations between the present absolute values of fat mass and body fat % and the relative reductions in these variables observed after 24 weeks of training in all subjects independent of the training group indicate that our training program was especially effective for subjects with an initially high percentage of body fat, suggesting that the present training program may be desirable for overweight or obese populations.

In conclusion, this study demonstrated that both endurance training immediately followed by strength training and the reversed loading order are beneficial in enhancing physical fitness and body composition in healthy moderately active subjects even when the training frequency and volume is moderate. Although no significant reductions in body fat and blood lipids were observed, the significant increases in lean body mass may provide prolonged health benefits with the present training design. However, further studies should compare endurance and strength training combined into the same training session to that performed on separate days by possibly modifying the type and volume of endurance training performed, providing dietary restrictions or including additional populations such as overweight, obese or elderly subjects.

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CONFLICT OF INTERESTS

The authors do not have conflicts of interests and state that the results of the present study do not constitute endorsement by ACSM.

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FIGURE AND TABLE LEGENDS

Table 1. Absolute values of physical fitness and body composition in the two groups at week 0 and 24. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to corresponding value at week 0.

Table 2. Absolute values of blood lipids in the two groups at week 0, 12 and 24. *$p<0.05$ compared to corresponding value at week 0; ^{y} $p<0.05$ between the two groups at corresponding time point.

Fig 1. Relative changes in 1RM strength after 12 and 24 weeks of combined E+S or S+E training. * $p<0.05$, *** $p<0.001$; within the bar compared to pre-training values, outside the bar as indicated.

Fig 2. Relative changes in time to exhaustion and maximal aerobic power (W) after 12 and 24 weeks of combined E+S or S+E training. * $p<0.05$, ** $p<0.01$, *** $p<0.001$; within the bar compared to pre-training values, outside the bar as indicated.

Fig 3. Absolute values of muscle cross-sectional area at 30%, 50% and 70% of VL (bars) and relative changes of average VL (line) after 12 and 24 weeks of combined E+S or S+E training. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ within the bar (next to the SD bars in the line diagram) compared to pre-training values, † $p<0.05$, †† $p<0.01$, ††† $p<0.001$ compared to week 12.

Fig 4. Relative changes in total, leg and upper body lean mass after 12 and 24 weeks of combined E+S or S+E training. * $p<0.05$, ** $p<0.01$, *** $p<0.001$; within the bar compared to pre-training values, outside the bar as indicated.

III

EFFECTS OF ENDURANCE TRAINING ONLY VERSUS SAME-SESSION COMBINED ENDURANCE AND STRENGTH TRAINING ON PHYSICAL PERFORMANCE AND SERUM HORMONE CONCENTRATIONS IN RECREATIONAL ENDURANCE RUNNERS

by

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Effects of endurance training only versus same-session combined endurance and strength training on physical performance and serum hormone concentrations in recreational endurance runners

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ABSTRACT

This study investigated the effects of endurance training only (E, n=14) and same-session combined training, when strength training is repeatedly preceded by endurance loading (E+S, n=13) on endurance (1000 m running time during incremental field test) and strength performance (1RM in dynamic leg press), basal serum hormone concentrations, and endurance loading-induced force and hormone responses in recreationally endurance trained men. E was identical in the two groups and consisted of steady-state and interval running, 4-6 x wk^{-1} for 24 weeks. E+S performed additional mixed maximal and explosive strength training (2 x $week^{-1}$) immediately following an incremental running session (35-45min, 65-85% HR_{max}). E and E+S decreased running time at week 12 (-8±5%, p=0.001 and -7±3%, p<0.001) and 24 (-13±5%, p<0.001 and -9±5%, p=0.001). Strength performance decreased in E at week 24 (-5±5%, p=0.014) but was maintained in E+S (btw-groups at week 12 and 24, p=0.014 and 0.011). Basal serum testosterone and cortisol concentrations remained unaltered in E and E+S but testosterone/SHBG-ratio decreased in E+S at week 12 (-19±26%, p=0.006). At week 0 and 24, endurance loading-induced acute force (-5 to -9 %, p=0.032 - 0.001) and testosterone and cortisol responses (18-47%, p=0.013 - p<0.001) were similar between E and E+S. This study showed no endurance performance benefits when strength training was performed repeatedly after endurance training compared to endurance training only. This was supported by similar acute responses in force and hormonal measures immediately post endurance loading after the training with sustained 1RM strength in E+S.

Key words: concurrent training, acute interference, testosterone, cortisol, endurance running, endocrine adaptations,

54 INTRODUCTION

55 High frequency and volume combined endurance and strength training has previously been
56 shown to impair maximal strength development in untrained subjects (Hickson 1980). While
57 detrimental effects on endurance performance are typically not observed in these subjects,
58 heavy and explosive strength training added to endurance training of moderately and highly
59 trained endurance runners may lead to beneficial adaptations in running economy, running
60 speed at VO_{2max} and time to exhaustion, when adequate recovery between each training mode
61 is provided (Beattie et al. 2014).

62 The superior effects of combined training on maximal and sub-maximal endurance running
63 performance in endurance athletes are often attributed to improved neuromuscular efficiency,
64 increased force generating capacity, and delayed recruitment of type II fibres as well as a
65 conversion of fast-twitch type IIx fibres into more fatigue resistant type IIa fibers (Ronnestad
66 and Mujika 2014). Although neuromuscular adaptations induced by combined training
67 typically occur in conjunction with changes in endocrine function, only little is known
68 regarding the contribution of the endocrine system when combining strength and endurance
69 training (Kraemer et al. 1995, Taipale et al. 2010).

70 Previous studies have indicated that both endurance and strength exercises can transiently
71 increase hormone concentrations (Kraemer et al. 1990; Häkkinen and Pakarinen 1993;
72 Hackney et al. 2012), such as testosterone (T), growth hormone (GH) and cortisol (C).
73 Among other physiological functions, it is likely that these acute alterations in anabolic and
74 catabolic hormone concentrations directly affect the rates of protein synthesis, red blood cell
75 production and energy restoration (Shahani et al. 2009; Vingren et al. 2010), facilitating
76 biological adaptations to exercise training. As part of the adaptation process during prolonged
77 endurance and/or strength training, exercise induced acute hormone responses (Keizer et al.

1987; Kraemer et al. 1995; Häkkinen et al. 2000) and basal hormone concentrations (Kraemer et al. 1995; Häkkinen et al. 2000; Ahtiainen et al. 2003; Hackney et al. 2003; Taipale et al. 2010) may be observed.

Although the hormonal responses to short term endurance and strength exercises are rather similar (Stokes et al. 2013), their physiological functions may differ due to the catabolic vs. anabolic nature of both types of exercises, possibly contributing to the beneficial effects of strength training on endurance performance. In previous studies, endurance and strength training have typically been performed on separate days allowing for prolonged recovery between subsequent training sessions (Ronnestad and Mujika 2014; Beattie et al. 2014). However, several studies have shown that heavy strength training sessions caused acute detrimental effects on subsequent running performance for 6-24 hours (Doma and Deakin 2013; Palmer & Sleivert 2003) owing to the possibility that strength training may in fact compromise endurance development for running performance. On the other hand, previous studies have also shown acute reductions in strength performance following endurance running (de Souza et al. 2007), possibly reducing the beneficial long-term effects of strength training on endurance performance. As decrements in endurance running performance may still be observed 24h following a strenuous strength loading (Doma and Deakin 2013) while recovery following endurance loadings of moderate duration and intensity is much shorter (Bentley et al. 2000, Millet & Lepers 2004), performing endurance training immediately prior to strength training may minimize acute interference and optimize strength training-induced endurance development.

The purpose of the present study was to investigate 1) the adaptations in endurance and strength performance as well as basal serum hormone concentrations and 2) the adaptations in acute force and hormone responses to endurance loading, following prolonged endurance training only versus same-session combined training when strength training is repeatedly

103 preceded by endurance loading. A secondary purpose of this study was to investigate whether
104 acute endurance loading-induced changes in force production and serum hormone
105 concentrations are associated with endurance and strength performance development.

106 MATERIALS AND METHODS

107 *Subjects*

108 Twenty-seven recreationally endurance-trained males participated in this study. The subjects
109 had performed endurance running for a minimum of 1 year with 2-6 sessions (at both
110 moderate and high intensity) per week prior to the start of the study. Before giving informed
111 consent, all subjects received information about possible risks of all study procedures. A
112 completed health questionnaire and resting ECG were reviewed by a cardiologist prior to the
113 first exercise testing. All subjects were free of acute and chronic illness, disease and injury
114 and did not report use of medications that would contraindicate the performance of intense
115 physical activity or would interfere with endocrine function. Demographic characteristics of
116 all subjects were as follows (mean±SD): age 33±7years, body height 179±6 cm and body
117 weight 78±7 kg. The study was conducted according to the Declaration of Helsinki and
118 ethical approval was granted by the Ethics Committee at the local University.

119 *Study design*

120 Following health-screening, subjects were assigned to an endurance only (E, n=14) or same-
121 session combined endurance and strength training (E+S, n=13) group. All subjects performed
122 identical endurance training for 24 weeks but additional strength training was added to the
123 E+S training program and was performed always immediately after a standardized endurance
124 running protocol.

Prior to the commencement of E and E+S training, baseline testing of endurance (incremental field test) and strength performance (dynamic leg press and counter movement jump [CMJ]) was conducted and concentrations of serum hormones (T, GH, and C) and sex hormone binding globulin (SHBG) were assessed. Acute force and hormone responses were determined by measuring maximal force (bilateral isometric leg press) and serum hormone concentrations before and after an incremental treadmill protocol (Fig 1). To ensure sufficient recovery, all tests were separated by at least 48 h of rest. The measurements of loading responses were repeated after 24 weeks, while the baseline measurements of endurance and strength performances as well as serum hormone concentrations were also conducted after 12 weeks. All post-training measurements (at week 12 or 24, respectively) were performed at the same time of day within ±1h of the testing time at week 0. To control the experimental conditions, subjects received both verbal and written instructions about the measurement preparation in order to minimize physical and mental stress and to allow for at least 7-8 h of sleep on the day before each testing. Basal concentrations of serum hormones were assessed in the morning (between 7:00 a.m. and 9 a.m.) after 12 h of fasting.

+++Figure 1 somewhere near here+++

Testing procedures

Strength and power performance

One repetition maximum (1RM) using the dynamic horizontal leg press device (David 210, David Health Solutions, Helsinki, Finland) was determined at week 0, 12 and 24, respectively. Following a warm up (1 set of 5 repetitions at 70% of estimated 1RM, 1 set of 2 repetitions at 80-85% of estimated 1RM, 1 set of 1 repetition at 90-95% of estimated 1RM), a maximum of 5 trials were allowed to obtain a true 1RM. The device was set up so that the knee angle in the initial flexed position was approximately 60 degrees (as measured by a

manual goniometer) and a successful trial was accepted when the knees were fully extended (~180 degrees). The greatest load that the subject could lift to full knee extension was accepted as 1RM.

Maximal power was determined by a counter movement jump (CMJ) on a force plate (Department of Biology of Physical Activity, Jyväskylä, Finland) at week 0, 12 and 24, respectively. Subjects were asked to keep their hands in contact with their hips throughout the movement and were instructed to jump as high as possible on command. Force data was collected and analyzed by Signal software (Signal 4.04, Cambridge Electronic Design Ltd., Cambridge, UK). Jumping height was calculated from the take-off velocity using the formula $h=v^2/2g$, in which h refers to jumping height and v refers to take-off velocity (Komi and Bosco 1978). The best trial in terms of jumping height measured in cm was used for statistical analysis.

To assess acute endurance loading-induced force responses at week 0 and 24, maximal isometric bilateral leg press force (MVC_{max}) was assessed by a horizontal leg press dynamometer (Department of Biology of Physical Activity, University of Jyväskylä, Finland). Subjects were seated with a hip and knee angle of 110 and 107 degrees, respectively and were instructed to produce maximal force as rapidly as possible on verbal command and to maintain the force plateaued for 3-4 seconds. Before the treadmill protocol three trials separated by 1 minute of rest were conducted, while after the exercise only two trials separated by 15 seconds were performed. The trial with the highest maximal force measured before or after the loading, respectively was used for statistical analysis. The force signal was low-pass filtered (20Hz) and analyzed (Signal software, version 4.04, Cambridge Electronic Design Ltd., Cambridge, UK).

Endurance performance measures and endurance loading

Running performance was determined by an incremental field test of 6x1000m (1 minute inter-set rest) performed on a 200 m indoor running track at week 0, 12 and 24, respectively. The initial speed for all subjects was 6 $min \cdot km^{-1}$ and the speed was increased by 30 seconds every 1000 m. The test was performed in small groups and velocity was controlled every 100 m. The final 1000 m were performed at individual maximal running speed and the time of this trial was used for statistical analysis.

At week 0 and 24, a graded protocol on a motorized treadmill was used to measure endurance loading-induced acute force and hormone responses. The initial velocity for all subjects was 9 $Km \cdot h^{-1}$ and increased by 1 $Km \cdot h^{-1}$ every 3 minutes, while the incline was kept constant at 0.5º. The treadmill was stopped every 3 minutes for 20 seconds in order to collect capillary blood samples from the fingertip for the determination of blood lactate concentrations. Twenty µl of blood were collected by small capillaries, inserted into reaction capsules containing a hemolyzing and anticoagulant agent and lactate concentrations were analyzed using a Biosen analyzer (C_line Clinic, EKF, Magdeburg, Germany). Time to exhaustion was used for statistical analysis and was defined as the maximal testing time until voluntary exhaustion. Furthermore, the velocity at a blood lactate concentration of 4 $mmol \cdot l^{-1}$ (V_4) was used as an indicator of running economy (Heck et al. 1985). Following voluntary exhaustion, a 5 minute cool down at the initial speed (9 $Km \cdot h^{-1}$) was performed. In order to determine acute force and hormone responses, MVC_{max} and serum hormone concentrations were assessed before the start of the treadmill protocol and after the cool down (Fig 1).

Venous blood sampling

Venous blood samples (10 ml) for the determination of serum hormone concentrations (basal concentrations at week 0, 12 and 24; endurance loading-induced acute responses at week 0 and 24) were collected by a qualified lab technician. Whole blood was centrifuged at 3,500

rpm (Megafuge 1.0 R, Heraeus, Germany) for 10 minutes after which serum was removed and stored at -80°C until analysis (approximately 4-8 weeks). Analysis of total serum testosterone (T), GH (22-kDa), sex hormone binding globuline (SHBG) and cortisol (C) were performed using chemical luminescence techniques (Immunlite 1000, Simens, New York, USA) and hormone specific immunoassay kits (Siemens, New York, USA). The sensitivity for serum hormones were: T 0.5 $nmol \cdot l^{-1}$, GH 0.03 $mlU \cdot l^{-1}$, SHBG 0.2 $nmol \cdot l^{-1}$ and C 5.5 $nmol \cdot l^{-1}$. The intra-assay coefficients of variation for T, GH, SHBG, and C were 8.7±2.7%, 7.1±4.6 %, 6.4±1.7%, 6.0±0.5% and 7.1±1.1%, respectively. The inter-assay coefficients of variation for T, GH, SHBG and C were 10.6±3.2%, 11.1±4.3%, 5.8±0.3%, 7.6±1.4 and 7.9±1.2%, respectively. Basal T/C- and T/SHBG-ratios were also calculated. Plasma volume changes were estimated from changes in hematocrit and hemoglobin (Dill and Costill 1974) but were not used to correct obtained serum hormone concentrations (Kraemer and Ratamess 2005).

Endurance training

The subjects were required to maintain habitual physical activity throughout the study period. The prescribed endurance training program was identical in the two groups and consisted of both continuous and interval training sessions 4-6x per week (Table 1), based on the polarized training approach (Muñoz et al. 2014). The endurance exercises focused on running but alternative endurance types such as cycling and cross country skiing were occasionally permitted for specific exercises in order to minimize the risk of injuries (Table 1). While two training sessions per week were supervised, the remaining endurance training sessions were performed individually. In case of sickness, subjects were required to catch up missing training sessions to standardize training volume between subjects. The training intensity was based on heart rate zones calculated from maximal heart rate determined during the incremental treadmill protocol (except for short intervals for which intensities were calculated

based on the determined best 1000 m time, Table 1). Training intensity, duration and distance were consistently controlled and recorded by heart rate monitors (RS800cx, Polar Electro Oy, Kempele, Finland), using manually pre-programmed exercise files. The endurance training intensity and volume increased throughout the two 12-week periods (Table 1).

+++Table 1 somewhere near here+++

Strength training

In the E+S group, additional strength training was performed twice a week (once a week at week 12 and 24, respectively) and was conducted always after the incremental endurance run (35-45 min by progressively increasing intensity from 65-85%, Table 1), with at least 48 h in between two combined training sessions. Subjects were instructed to rest or perform a light run (35-40 min, 60-65%, Table 1) on the day before the combined E+S session. A maximum of 10 minutes was allowed between transitions from the running session to the strength training session.

The strength training consisted of mixed maximal and explosive (~20% of strength training volume) strength training sessions and was focused on the lower limbs, while additional exercises for the upper body were included. The loads of each exercise were determined by the number of repetitions and execution velocity which was progressively increased throughout the two 12-week periods. Exercises for the lower body included bilateral leg press, bilateral and unilateral knee flexion and calf raises. During weeks 1-12 and 13-20 jumping exercises commonly used to improve explosive force production were performed (loaded and unloaded squat jumps, drop jumps, leaps, step-ups). During weeks 21-24 hurdle jumps and resisted knee lifts were also incorporated into the strength training program. Exercises for the upper body included dynamic seated vertical press, biceps curls as well as exercises commonly used to improve core stability (crunches, torso rotation, and lower back extension).

As the subjects were not accustomed to strength training, low loads (15-20x 40-50% of 1RM, 1-2min inter-set rest) were utilized during weeks 1-4. Thereafter, strength training intensity progressed to heavier loads and a lower number of sets (5-12x 60-85% of 1RM, 1-3min inter-set rest). During the second 12-week period the major strength program structure was maintained, while both training volume and loads used were increased to maximize maximal and explosive strength improvements.

Statistical analyses

Data are presented as mean±SD and shown as relative changes from the pre-loading values unless otherwise indicated. The normality of distribution was assessed using the Shapiro-Wilk test and log transformation was performed when necessary. Within and between-group differences of basal measures were assessed by a mixed ANOVA design with repeated measures. Within and between-group differences of acute loading responses were assessed by a mixed ANCOVA design, using the pre-loading values as covariates. Bivariate correlations were computed using the Pearson product-moment correlation coefficient. The statistical significance for all tests was set at 0.05. Statistical analysis was conducted using IBM SPSS 20.0 (SPSS, Inc., Chicago, IL, USA).

RESULTS

The weekly average training time was 4.9±0.2 h and 4.7±0.5 h in E and E+S, leading to a total training time of 116.5±4.5 h and 111.8±10.8 h, respectively. The weekly average running distance was 36.6±5.6 km and 33.5±7.9 km in E and E+S leading to a total mileage of 879.5±133.3 km and 804±189.3 km, respectively.

Endurance and strength performance adaptations

1000 m running time (Fig 2) significantly decreased in E at week 12 (from 3.8±0.3 $min \cdot km^{-1}$ to 3.5±0.3 $min \cdot km^{-1}$, $p=0.001$) and week 24 (to 3.3±0.2 $min \cdot km^{-1}$, $p<0.001$). Similarly, significant reductions in 1000 m time were also observed in E+S at week 12 (from 3.6±0.4 $min \cdot km^{-1}$ to 3.4±0.4 $min \cdot km^{-1}$, $p<0.001$) and week 24 (to 3.3±0.2 $min \cdot km^{-1}$, $p=0.001$) and no significant between-group differences in the changes of 1000 m time were found.

Time to exhaustion determined during the incremental treadmill test significantly increased at week 24 in E (from 24.9±3±4 min to 27.2±3.1min, 10±7%, $p<0.001$) and E+S (from 27.3±2.8 min to 29.1±2.5 min, 7±7%, $p=0.011$), while no significant between-group differences in the changes of time to exhaustion were observed.

Similarly, V_4 significantly increased in E at week 24 (from 3.7±0.5 $m \cdot s^{-1}$ to 3.9±0.3 $m \cdot s^{-1}$, 8±9%, $p=0.013$) and E+S (from 3.9±0.4 $m \cdot s^{-1}$ to 4.1±0.4 $m \cdot s^{-1}$, 6±6%, $p=0.003$), while no significant between-group differences in the changes of V_4 were observed.

+++Figure 2 somewhere near here+++

Dynamic leg press 1RM strength (Fig 3a) remained statistically unaltered in E at week 12 but significantly decreased at week 24 (from 148±25 kg to 141±23 kg, $p=0.014$). In E+S 1RM strength remained significantly unaltered at both week 12 and 24 and the between-group difference in the changes of 1RM strength was significant at week 12 ($p=0.014$) and 24 ($p=0.011$). Baseline 1RM strength performance significantly correlated with the corresponding change in 1RM strength during the first 12-week period ($r=-0.622$, $p=0.023$) in E+S but not E.

CMJ height (Fig 3b) remained statistically unaltered in both E and E+S at week 12 and 24, while the change in CMJ height at week 12 was significantly greater in E+S than E (3±8 % vs. -4±7 %, $P=0.025$).

+++Figure 3a and 3b somewhere near here+++

Basal hormone concentrations

No significant changes in basal serum concentrations (Table 2) of T, GH, cortisol, SHBG and the T/C-ratio were observed in either group at week 12 or 24. The T/SHBG-ratio (Fig 4) significantly decreased in E+S at week 12 (-19±26%, p=0.006) but was no longer significantly altered at week 24. The change in T/SHBG-ratio from week 12 to 24 was significantly larger in E+S compared to E (42±47% vs. -5±33%, p=0.006).

+++Table 2 somewhere near here+++

Acute force responses

In MVC_{max} (Fig 5) significant acute decreases occurred during the endurance loading in E and E+S at week 0 (-8±8%, p=0.001 and -9±9%, p=0.005, respectively) and 24 (-5±9%, p=0.03 and -6±10%, p=0.032, respectively). No significant training-induced changes in acute force responses to endurance loading at week 24 were observed.

+++Figure 4 somewhere near here+++

Acute hormone responses

Serum T (Fig 6a) significantly increased during the endurance loading in E and E+S at week 0 (18±22%, p=0.01 and 26±27%, p=0.012, respectively) and week 24 (32±46%, p=0.006 and 27±35%, p=0.013, respectively). No significant training-induced changes in acute serum T responses at week 24 were observed.

Serum C (Fig 6b) significantly increased during the endurance loading in E and E+S at week 0 (47±40%, p<0.001 and 37±28%, p<0.001, respectively) and week 24 (42±31%, p<0.001

and 35±29%, p<0.001, respectively) but no significant training-induced changes in acute serum C responses at week 24 were observed.

+++Figure 5a and 5b somewhere near here+++

Serum GH significantly increased during the endurance loading in E and E+S at week 0 (227 fold, p<0.001 and 208 fold, p<0.001, respectively) and week 24 (341 fold, p<0.001 and 210 fold, p<0.001, respectively). No significant training-induced changes in acute serum GH responses at week 24 were observed.

Plasma volume

No between-group differences in basal plasma volume changes at week 12 and 24 were observed. Basal plasma volume shifts in the two groups ranged from -1 to +4%. Similarly, no between-group differences in acute endurance loading-induced plasma volume shifts were observed at either measurement time. Loading-induced plasma volume shifts at week 0 and 24 ranged from -6 to -7% in the two groups.

DISCUSSION

The main findings of this study were: 1) both groups improved maximal and sub-maximal endurance performance to a similar extent; 2) 1RM strength was significantly decreased in E after the training period but was maintained in E+S, leading to the between-group difference at week 12 and 24; 3) the T/SHBG-ratio significantly decreased in E+S at week 12 and the change from week 12 to 24 was significantly larger in E+S than in E; 4) the endurance loading-induced acute force and hormone responses were similar in the two groups before and after the training period and no training-induced changes in acute loading responses were observed.

Previous studies have shown that maximal and explosive strength training added to endurance training improved running economy (Millet et al. 2002; Paavolainen et al. 2003; Storen et al. 2008), velocity at the lactate threshold (Mikkola et al. 2007; Guglielmo et al. 2009), maximal running speed (Millet et al. 2002) and running time over a given distance (Paavolainen et al. 2003; Spurrs et al. 2003), while only small or no effects on maximal oxygen consumption (VO_{2max}) were reported (Paavolainen et al. 2003; Spurrs et al. 2003; Storen et al. 2008; Taipale et al. 2010). Although added strength training may further induce endurance development due to greater training volume compared to endurance training alone, improvements in running performance have also been shown with reduced endurance training volume compensated by added strength training (Paavolainen et al. 2003). In general, however, beneficial adaptations in cardiorespiratory function and endurance performance following concurrent training have typically occurred with concomitant increases in maximal and/or explosive strength development, indicating that strength training-induced endurance development may occur as a result of enhanced neuromuscular performance (Paavolainen et al. 2003; Mikkola et al. 2007; Taipale et al. 2010).

In contrast to these studies, the present investigation found similar improvements in maximal and sub-maximal endurance performance for both training groups with no additional effects of the supplemented strength training in the E+S group. However, while the present E group significantly decreased 1RM strength after 24 weeks, maximal leg strength was maintained but not increased in the E+S group and a similar tendency was observed for CMJ height.

The present research design purposefully differed from previous studies since the strength loading was always performed immediately after an exhausting endurance running session, and therefore, every strength training session may have been affected by residual fatigue. While several cross-sectional studies have shown acute detrimental effects on strength performance (Leveritt and Abernethy 1999; de Souza et al. 2007) and anabolic hormone

responses (Goto et al. 2005) when strength loading was immediately preceded by endurance cycling or running, this has, to the best of our knowledge, only been supported by few longitudinal training studies. Craig et al. (1991) failed to observe lower body strength gains when strength training was repeatedly preceded by endurance running and, in line with our results, endurance performance increased to a similar magnitude in their combined and E training group. However, compared to the study of Craig et al. (1991), our E group significantly reduced maximal strength performance after 24 weeks of training, while our E+S group was able to maintain their basal strength performance which may in turn provide benefits over long-term.

Strength training-induced increases in maximal endurance running performance may in part be attributed to increased fatigue resistance allowing sustained repetitive cycles of stretch-shortening contractions over a prolonged period of time (Paavolainen et al. 1999). This greater level of resistance against neuromuscular fatigue in response to endurance loading would be expected after a prolonged period of combined endurance and strength training compared to endurance training only. However, the endurance loading-induced acute reductions in MVC_{max} were similar between E and E+S groups in the present study when comparing before and after the 24-week training period, supporting the finding that both groups experienced similar improvements in endurance adaptations. However, caution should be taken since the endurance loading was performed with relative maximal loads (i.e. time to exhaustion based on current training status at weeks 0 and 24) and both training groups significantly increased time to exhaustion after 24 weeks of training. In light of the resulting increases in loading volume, the observed acute reductions in maximal force at week 24 may actually indicate a training adaptation but as the magnitude of reductions was similar in E and E+S, our findings indicate these positive adaptations to be induced by the prolonged endurance training rather than the added strength training.

Interestingly, in the E+S group a significant correlation was observed between the basal levels of 1RM strength and the corresponding changes in maximal strength performance during the first 12 but not 24 weeks. Although not statistically significant, after 12 weeks of training the E+S group had improved strength performance by 5±7% which was diminished at week 24, despite a progressive increase in training load. While the observed correlation indicates the importance of strength training especially for the weaker endurance runners, our results suggest a biphasic response to the performed strength training which may indicate the strength loading stimulus to be less effective during the second 12 weeks of training. As both the strength and endurance training volume and intensity progressively increased during the latter half of the training, it may be possible that the unfavorable effects of preceding endurance loading on the quality of the subsequent strength training session were intensified during the second 12-week period and, thus, resulting in further impairment of strength development.

However, this finding was not accompanied by significant changes in basal hormone concentrations. Typically, the training induced endocrine adaptations differ between the types of training performed (Kraemer et al. 1995). Prolonged strength training may lead to increases in basal levels of anabolic hormone concentrations at least in previously untrained subjects (Häkkinen et al. 2000; Ahtiainen et al. 2003), while the basal concentrations of these hormones may actually be decreased following endurance training only (Hackney et al. 2003). Combined endurance and strength training studies in which endurance and strength were performed on separate days, on the other hand, have shown small increases in basal serum testosterone concentrations in untrained (Kraemer et al. 1995) and significant increases in endurance trained subjects (Taipale et al. 2010). These previous findings indicate that the strength training-induced changes in basal hormone concentrations may counteract an endurance training-induced catabolic state, possibly contributing to the beneficial effects of

409 strength training for endurance athletes. In the present study, however, only small fluctuations
410 in basal hormone concentrations were observed, supporting the lack of effects of strength
411 training on endurance performance, when performed immediately after endurance running
412 sessions.

413 Interestingly, in our E+S group a significant reduction in the T/SHBG-ratio was observed
414 after 12 weeks of training and this initial decrease was followed by a large increase thereafter,
415 leading to a significant between-group difference in the magnitude of changes in the
416 T/SHBG-ratio from week 12 to 24. As the T/SHBG-ratio correlates with free available
417 testosterone concentrations (Selby 1990) and may therefore reflect an anabolic state, the
418 observed reductions in the E+S group during the first 12 weeks may indicate increased uptake
419 of testosterone by the target cells (Vingren et al. 2010). Although receptor content was not
420 assessed in this study, the observed reduction in the T/SHBG-ratio during the first 12 weeks
421 of training may reflect a positive response to a new training stimulus since the subjects were
422 recreationally endurance trained but not accustomed to strength training. Similar
423 improvements in endurance performance between the E+S and E groups in conjunction with
424 small increases in maximal strength for the E+S group at the 12-week time point suggests that
425 the present strength training method may be effective in inducing strength development for at
426 least 12 weeks. In a previous study the beneficial effects of strength training on running
427 economy were apparent not during the actual combined endurance and strength training
428 intervention but after a reduction in strength training volume (Taipale et al. 2010). It may also
429 be possible that a reduction in strength training volume after the present 12 weeks of training
430 would have been necessary in order to induce improvements in maximal and sub-maximal
431 endurance performance.

432 Similar to the maintained serum hormone concentrations during the 24 weeks of training, no
433 training-induced changes in endurance loading responses of testosterone, growth hormone

434 and cortisol were observed. Both groups significantly increased endurance loading-induced
435 acute anabolic and catabolic hormone concentrations before and after the training to a similar
436 extent. While these findings are in contrast to a study by Kraemer et al. (1995) who found
437 significantly larger testosterone responses to endurance loading after combined training in
438 physically active subjects, our findings are in line with results of Craig et al. (1991). In their
439 study, endurance running-induced growth hormone responses were examined between an
440 endurance training only group and a combined strength and endurance training group, where
441 endurance training always preceded strength training in the same session. In line with our
442 results, they found no differences in growth hormone responses. However, similar to our
443 observed acute force responses, the improvements in treadmill running time to exhaustion
444 found in the present study may have blunted any potential changes in acute endurance
445 loading-induced serum hormone concentrations. Furthermore, due to the length of the study
446 where pre-training measurements were conducted in the Fall and post-training measurements
447 carried out in the Spring, possible seasonal variations in serum hormone concentrations
448 should be considered when interpreting the present findings (Svartberg et al. 2003).

449 Although training- or loading-induced alterations in serum hormone concentrations may be
450 associated with chronic increases in strength performance, the possible role of neural
451 interference in respect to the present findings should not be neglected. While a thorough
452 investigation of neuromuscular mechanisms was beyond the scope of this study, it is possible
453 that residual fatigue from the preceding endurance session affected neural activation of the
454 exercised muscles during the subsequent strength training sessions, as shown in our previous
455 study in untrained men (Eklund et al. 2014). Due to the high volume of endurance training
456 performed in the present study, it is possible that such a neural inhibition may have
457 contributed to the lack of expected increases in strength performance in the present E+S
458 group.

In conclusion, the present study showed that same-session combined training where strength training was repeatedly preceded by endurance loading did not lead to superior endurance performance benefits in recreational endurance runners, when compared to endurance running only. It is likely that this was attributed to the impaired strength development, despite consistent progressive strength training in the E+S group. In support of this assumption, no between-group differences in training-induced changes in acute force and hormone responses to endurance loading were observed and basal hormone concentrations were maintained in the two groups. Although in the present design no group utilizing other combined endurance and strength training modes were included, these results suggest that endurance athletes should separate their endurance and strength training sessions in order to maximize benefits of the added strength training.

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571 TABLES

572 **Table 1.** Prescribed endurance training program for the two groups.

	Weeks 1-12	Weeks 13-24
Incremental run	2x/week, running only 35-45min/65-85%	2x/week, running only 40-45min/65-85%
Long run	1x/week, running, cycling or skiing 70-120min/60-65%	1x/week, running, cycling or skiing 85-125min/60-65%
Long intervals	1x/week, running only 4-5x5min/80-85%, rest 3min <65%	1x/week, running only 4-6x5min/85%, rest 3min <65%
Short intervals		1x/week, running only (on the track) 3-6x400m + 3-6x 800m/85%, rest 2min <65%
Light run	1x/week, running only 35-40min/60-65%	1x/week, running, cycling or skiing 40min/60-65%
Optional run	Optional 1x/week, running, cycling or skiing 35-40min/ 70-75%	

573 Intensity zones are % of HRmax except for short intervals (% of 1000m time).

574 In the E+S group, strength training was performed twice a week after the incremental

575 endurance run.

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581 **Table 2.** Basal concentrations of serum hormones throughout the 24 weeks of training.

	E			E+S		
	Week 0	**Week 12**	**Week 24**	**Week 0**	**Week 12**	**Week 24**
Testosterone ($nmol \cdot l^{-1}$)	16.9±6.4	15.3±4.1	15.6±5.2	19.7±7.9	15.9±5.3	19.1±5.9
GH (22-kDa) ($mlU \cdot l^{-1}$)	0.5±0.6	0.6±1.3	0.6±0.7	1.0±1.7	2.6±7.5	1.2±2.1
SHBG ($nmol \cdot l^{-1}$)	29.6±11.3	29.1±10.5	32.2±11.8	33.6±9.8	36.4±13.0	33.0±9.9
Cortisol ($nmol \cdot l^{-1}$)	499.9±85.2	469.2±92.8	514.0±44.2	504.4±130.9	466.4±104.7	498.2±93.2
T/SHBG-ratio ($nmol \cdot l^{-1}$)	0.6±0.2	0.6±0.2	0.5±0.1	0.6±0.3	0.5±0.2**	0.6±0.2
T/C-ratio ($nmol \cdot l^{-1}$)	0.034±0.012	0.033±0.010	0.030±0.010	0.040±0.013	0.034±0.008	0.040±0.016

582 GH=growth hormone; SHBG=sex hormone binding globulin; T/SHBG-ratio=testosterone/SHBG-

583 ratio; T/C-ratio=testosterone/cortisol-ratio; **p<0.01 compared to week 0.

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FIGURE CAPTIONS

Figure 1. Study design. Baseline tests included the determination of endurance (incremental field test) and strength performance (1RM during dynamic leg press and CMJ height) as well as the determination of basal hormone concentrations.

Figure 2. Changes in maximal 1000 m running time determined during an incremental field test of 6x1000m. ***p<0.001 compared to values obtained at week 0.

Figure 3. Changes in 1RM strength (A) and CMJ height (B). *p<0.05, within bar compared to values obtained at week 0; outside the bar as indicated.

Figure 4. Changes in T/SHBG ratio. **<0.01, inside the bar compared to values obtained at week 0; outside the bar as indicated.

Figure 5. Endurance loading-induced acute reductions in isometric maximal force (MVC_{max}) at week 0 and week 24. *p<0.05, **p<0.01 compared to obtained pre-loading values at week 0 and 24, respectively.

Figure 6. Endurance loading-induced acute changes in serum testosterone (A) and cortisol (B) concentrations. *p<0.05, **p<0.01, ***p<0.001 compared to obtained pre-loading values at week 0 and 24, respectively.

IV

NEUROMUSCULAR ADAPTATIONS TO SAME-SESSION COMBINED ENDURANCE AND STRENGTH TRAINING IN RECREATIONAL ENDURANCE RUNNERS

by

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