CHANGES IN MUSCLE CSA AND FORCE PRODUCTION OF LEG EXTENDERS DURING COMBINED STRENGTH AND ENDURANCE TRAINING IN YOUNG MEN AND WOMEN: IS THERE ANY ORDER EFFECT?

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ABSTRACT


In many sports strength training improves performance significantly more than bare sport specific training. Combined strength and endurance (SE) training can lead to similar cardiorespiratory and musculoskeletal adaptations compared to strength (S) or endurance (E) training alone. When both training types are performed in the same training session, strength training performed immediately after endurance training (E+S) may hinder strength development and improve endurance performance more than the opposite order (S+E). The purpose of this study was to investigate differences between S+E and E+S training during a 24-week combined training period in muscle CSA and force production.

A total of 32 men and 26 women participated to this study. Subjects were randomly assigned to four groups: men S+E, men E+S, women S+E, women E+S (mS+E, mE+S, wS+E, wE+S) and they performed two 12 weeks training periods with measurements at weeks 0(pre), 12(mid) and 24(post). Measurements included neuromuscular (1RM, isometric strength, MVC500, power), cardiorespiratory (VO$2_{\text{max}}$, time to exhaustion) and muscle size (ultrasound, DXA) measurements. Subjects trained twice a week during weeks 0–12 and five times in two weeks during weeks 13–24. In the first training period the strength training included loads of 40–60% (weeks 0–2) 60–80% (weeks 3–7) and 80–95% (weeks 8–12) of 1RM. The endurance training included two different training sessions. The first training session included continuous cycling around the aerobic threshold for 30–45min, whereas the second training session was interval training. The training program both for strength and endurance was similar during training period 2, but the loads were increased based on the development.

All training groups improved significantly 1RM (all groups p<0.001), isometric strength (mS+E and wS+E p<0.01, mE+S and wE+S p<0.001), MVC500 (mS+E, mE+S and wS+E p<0.01, wE+S p<0.001), power (mS+E p<0.001, mE+S, wS+E and wE+S p<0.01), time to exhaustion (all groups p<0.001), CSA (VL50% (mS+E p<0.001, mE+S, wS+E and wE+S p<0.01), and lean mass of legs (all groups p<0.001) during the 24-week training period. In VO$2_{\text{max}}$ all except mE+S improved significantly (mS+E and wS+E p<0.01, wE+S p<0.05). In the ultrasound measurement wE+S increased CSA in VL50% significantly more than wS+E (18.7±11.2% vs. 8.8±8.7%, p<0.05) and mE+S (18.7±11.2% vs. 10.4±8.4%, p<0.05). In time to exhaustion, wE+S improved significantly more than mE+S (21.7±10.9% vs. 13.1±8.6%, p<0.05).

According to this study, muscle CSA, muscle strength and endurance performance improved with combined single session strength and endurance training independently of the training order. In addition, wE+S showed larger muscle hypertrophy (CSA) than that of observed when training by the opposite order and larger than in men using the same E+S order.

Keywords: CSA, force production, combined single session training, order effect, men, women.
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Strength training can increase strength, power and muscle size. However, the qualities that increases the most, depends on the type of training, for example sets, repetitions, load, recovery time, intensity, total volume and performance technique. (Bomba 1999, 315–342, Campos et al. 2002.) According to Widrich et al. (2002) muscle size and absolute maximal strength have a linear relationship. However, Tan (1999) has shown in his study that it is possible to increase maximal force without hypertrophy. Moreover, in individuals without earlier experience of regular strength training, increases of strength may be caused by neural factors in the beginning of strength training period with minor hypertrophy (Moritani & deVries 1979, Häkkinen & Komi 1983, Häkkinen et al. 1998, Jones and Rutherford 1987). In turn, endurance training improves the capacity for respiratory control in skeletal muscle. Furthermore, it includes greater blood flow to muscles, greater fatty acid oxidation for energy, greater size and amount of mitochondria and decreased catecholamine release for the same absolute power output. (McArdle ym. 2007, 470–507.)

Concurrent strength and endurance (SE) training can lead to similar cardiovascular or musculoskeletal adaptations compared with strength (S) or endurance (E) training alone (Izquierdo et al. 2005 and Hendricson et al. 2010). However, some studies have reported attenuated cardiovascular and musculoskeletal results with combined SE training (Aagaard et al. 2011). Perhaps training adaptations after combined SE training, are just minor compared to strength or endurance training alone (Holviala et al 2010). According to some studies, also training intensity, frequency and duration have an effect on training adaptations, when combined SE training has been used (Karavirta et al. 2011).

Many researchers (Dudley & Djamil 1985, Glowacki et al. 2004, Hendrikson et al. 2010, Häkkinen et al. 2003) have found that concurrent SE training does not hinder strength development. Also Holviala et al. (2010) and Tan (1999) reported increased strength after combined training. However, they concluded that endurance training will
cap the upper limits of strength gains, due to minor changes of increase compared to the strength group (Holviala et al. 2010 and Tan 1999).

There are different results of peak power development after combined SE training. According to Häkkinen et al. (2003), combined SE training has an inhibiting effect on improvement of peak power production, whereas Hendricson et al. (2010) and Izquierdo et al. (2005) reported that combined SE training might not interfere with improvements in power. Furthermore, a same kind of dilemma concerns muscle hypertrophy. Some authors report that concurrent training does not effect in muscle hypertrophy (Häkkinen et al. 2003), while others report attenuated hypertrophy, especially, over a prolonged period of combined SE training (Aagaard et al. 2011, Karavirta et al. 2011, Losnegard et al. 2011, Putman et al. 2004).

Only a few studies have investigated differences between men and women in the development of strength and endurance after SE training. The same concerns adaptations to strength and endurance performances, when combined SE training is performed in two different orders (order effect): strength training performed before endurance training and strength training performed immediately after endurance training. According to Cadore et al. (2010) endurance training performed before strength training can hinder strength development and according to Chtara et al. (2005), the same order can improve endurance performance significantly more than opposite order or strength and endurance performed separately. Considering this, the purpose of this study was to find out, how results diverge when combined strength and endurance training is done in two different orders: S+E and E+S. Another interest was to investigate differences between young men and women during combined single session SE training.
2 NEUROMUSCULAR ADAPTATIONS TO STRENGTH TRAINING

The function of the nervous system is to determine the level and timing of muscle forces and send instructions to the muscles to coordinate body movement. In turn, the muscles produce the forces and move bones via the joints. The parts of the nervous and muscular system, which coordinate movements, compose the neuromuscular system. (Watkins 2010, 187–208.) The purpose of strength training is to increase the ability to create muscle strength which is possible through the development of the neuromuscular system (Bomba 1999, 315–342). The load, the amount of repetitions and sets, recovery time, performance technique and total load of training orders what kind of strength abilities are trained (Bomba 1999, 315–342, Campos ym. 2002, Cormie ym. 2009).

2.1 Muscle activation

The spinal cord is the major processor for muscular activity and distribution center for motor control (McArdle 2007, 402). The motor unit is the functional unit of skeletal muscle (Figure 1) (Watkins 2010, 219). It consists of alpha motor neuron and the specific muscle fibers it innervates. Alpha motor neuron include dendrites, cell body and axon, which together allow transmission of an electrochemical impulse from the spinal cord to the muscle. Dendrites bring the nerve impulses to the body cell, from which nerve impulses continue along axon to the muscle fibers. (McArdle 2007, 402–403.) Muscles associated with fine motor control have motor units with just a few muscle fibers to innervate and forceful movements need muscles with motor units, which innervate even 3000 muscle fibers (Watkins 2010, 219).
The increased number of motor units recruited and the increased frequency of motor unit discharged are the two mechanisms, which order the force of muscle action. The more motor units are recruited, the more muscle force is increased. Moreover, to produce the desired coordinated response, neural control selectively recruits and fires pattern of the fast-twitch and slow-twitch motor units. Slow-twitch motor units have the lowest threshold for activation and are recruited selectively during light and moderate effort. During rapid and powerful movements fast-twitch fatigue resistant (type 2a) units are progressively activated up through the fast twitch fatigable (type 2b) units at peak force. Motor unit firing is a major factor that separates skilled performance from unskilled and specific athletic groups. Weight lifters recruit many fast-twitch motor units simultaneously during lifting to generate force quickly for desired lift. In contrast, endurance athletes have just some motor units fired, while others recover, and they use predominantly slow-twitch motor units to continue performance with minimal fatigue. (McArdle 2007, 408–409.) Consequently, strength training will effect on muscle fiber type distribution, which changes the contractile properties of the muscle and finally affects the performance of the athlete. Therefore, strength training is nowadays used almost in all sports in which high intense work is conducted. (Andersen and Aagaard 2010.)
The changes in the nervous system function will cause rapid and large strength increase early in the training (Holtermann et al. 2007, McArdle 2007, 540–541). It includes greater efficiency in neural recruitment patterns, increased motor neuron excitability, increased central nervous system activation, improved motor unit synchronization and increased firing rates, lowering of neural inhibitory reflexes and inhibition of Golgi tendon organs. With these changes muscle contraction is faster and more effective, which regenerates muscle force output. (McArdle 2007, 540–541.) According to Holtermann et al. (2007), motor unit recruitment and discharge rate during maximal voluntary contraction (MVC) supports that with training achieved enhanced net excitatory synaptic input or excitability of motoneurons are more likely to increase rate of force development (RFD) than to contribute to gained strength.

The electrical signal from motor neurons continues to muscle fibers as action potential. This electrical signal in muscle fibers can be recorded with an electromyogram (EMG). EMG can be used to estimate muscle force and identify adaptations in the neuromuscular system, when muscle force has changed. It can also be used to diagnose problems in the neuromuscular system or to determine the requirements of job-related tasks. EMG is measured with electrodes, which can be placed on the skin over the muscle (surface EMG) or in the muscle between muscle cells (subcutaneous EMG). Surface electrodes measures an action potential activity in the underlying muscle, whereas subcutaneous electrodes records single action potentials in a few adjacent muscle fibers. (Enoka 2002, 46–50.)

Neural adaptations occur during the initial phase of resistance training (Moritani & deVries 1979, Häkkinen & Komi 1983, Holtermann et al. 2007). In the study of Häkkinen et al. (1998), middle-aged and older people (men and women) had a progressive heavy-resistance training program combined with explosive types of exercises. Because in all groups the maximal voluntary activation of the agonist muscles increased to a much greater extent than muscle CSA and the coactivation of the antagonists reduced significantly in the elderly, they suggested that neural adaptations play a greater role than muscle hypertrophy when explaining large strength and power gains. (Häkkinen et al. 1998.) An intense 20-day period of eccentric training in previously untrained individuals caused also increased agonist activation and decreased antagonist coactivation in the study of Krentz and Farthing (2010). These activation
chances are early neural adaptations that reflect improved neural coordination of movement (Krentz and Farthing 2010).

### 2.2 Maximal strength and power

Strength is the ability of the neuromuscular system to produce force against an external resistance, whereas maximal strength is the force that a muscle or muscle group can generate. Changes in neural-, hormonal- and muscular system, causes the development in maximal force (Tan 1999). In addition, the maximal strength depends on the number of recruited motor units, the motor unit firing rate, the amount of motor unit synchronization, the use of the stretch-shortening cycle, the degree of neuromuscular inhibition, the muscle fiber type and the degree of muscle hypertrophy (McArdle 2007, 510–553). The use of the highest possible load and the highest possible movement velocity is the most effective way to develop maximal strength. The most notable increase in maximal force has been noticed, when used 4–8 movements in practice, 3–6 sets of every movement, 1–6 repetition in every set. Recovery time must last 1–5 minutes depending on loads and repetitions. Training loads are 85–100% of one repetition maximum (1RM), even 110%. While aiming to intense increase of maximal strength, trainings should take place 3–5 times a week and they should be divided to two separate training sessions a day. (Tan 1999.)

Maximal power in turn, is the product of speed and muscular strength (McArdle 2007, 510–553). To increase muscle power, intensity of training must be maximal and loads should be between 30–60% of 1RM. Moreover, 3–6 sets per exercise and 1–5 repetitions per set are advisable. Rest between sets should be 2–5 minutes to prepare for next maximal effort. (Kraemer & Häkkinen 2002, 20–36.) Maximal power can be expressed with force-velocity curve (Figure 2), where movement velocity decreases, when an external resistance increases. A resistance training program can focus to the high-force portion or high-velocity portion of the force-velocity curve. (McArdle 2007, 510–553.) Power improvements appear to be important especially to older adults, because it improves walking speed and short physical performance battery (Bean et al. 2010).
Progressive overload is needed to increase strength, which means that training variables should be manipulated as the muscle adapts to the training stimulus. (Bompa 2009, 259–284.) For example, in the study of Marshall et al. (2011), subjects were male and they had used 4-sets in their strength training. They were shared to 1, 4 and 8-sets groups. The purpose of the study was to compare improvements in one repetition maximum (1RM) squats, when three groups performed squats twice per week at 80% of 1RM. Strength improved significantly more in the 8-set group than in one-set group, therefore, they recommended multi-set resistance training programs for resistance trained individuals. (Marshall et al. 2011.)

High levels of muscular strength are significantly related to sport performance. Training programs should be constructed to transfer strength development into performance requirements. (Bompa 2009, 259–284.) In the study of Bogdanis et al. (2011), strength training with high loads and moderate load-program were compared in professional soccer players. This study showed that high loads is superior to a moderate-load program in soccer players, because with high loads strength increases without a change in muscle mass and running economy. Additionally, game-specific endurance and repeated sprint ability improved more than with moderate loads. (Bogdanis et al. 2011.)
2.3 Muscle hypertrophy

An increase in muscle hypertrophy is the most significant morphological change noted in response to increased workload in most resistance training studies (Bompa 2009, 266–267, Seynnes et al. 2007). To be more specific, muscle hypertrophy means significant increases in the cross-sectional area of the skeletal muscle fibers (Figure 3 & 4) and it can result in an increase in contractile materials and an increase in the pennation angle (Bompa 2009, 266–267). Genes, physical activity, diet, endocrine system, environment and neuromuscular activity have an impact on development and maintenance of muscle hypertrophy (McArdle et al. 2007). Typically in hypertrophic strength training 60–80% loads of 1RM has been used. Purpose is to achieve exhaustion in every set, which means 6–12 repetitions in one set. 4–6 sets of every movement have been used and recovery between sets has been short, 1–2 minutes. (Ahtiainen & Häkkinen 2009, Kraemer & Häkkinen 2002, 20–36.) In this type of training, the purpose is to achieve a high total load of training, which leads to extreme acute fatigue of the neuromuscular system, acute hormonal response and high level of lactate. Due to that, it takes quite a long time to recover from this type of training session. (Kraemer & Häkkinen 2002, 20–36.)

Muscle hypertrophy results from repeated training induced muscle fiber injury. More specifically, it means that mechanical stress on the components of the muscular system triggers signaling proteins to activate genes that activate translation of messenger RNA and stimulate protein synthesis in excess of protein breakdown (Chesley et al. 1992). In addition, the overcompensation of protein synthesis occurs especially, when effects of insulin are combined with adequate amino acid availability and it produces a net anabolic effect. The response achieves its peak approximately 24h following the training session and remains elevated for 36–48h. (Chesley et al. 1992, Komi 2003, 252–261, MacDougall et al. 1995, Phillips et al. 1997, Welle et al. 1999) Strength training also activates satellite cells, which have regenerative function on cellular growth and impact on muscle hypertrophy (Phelan & Gonyea 1997). As a result cell's myofibrils thicken and the amount of myofibrils and sarcomeres increases (Komi 2003). Likewise, anaerobic energy stores (intramuscular ATP, PCr and glycogen) increases considerably, which contribute to the rapid energy transfer required in resistance training. (McArdle 2007, 541–543.)
FIGURE 3. The structure of muscle (Modified from Komi 2003).

FIGURE 4. Muscle fiber hypertrophy: Strength training stimulates muscle hypertrophy. Hypertrophy can be found in single muscle fibers, but also in the whole muscle. (Modified from Komi 2003.)

In the initiation of resistance training, motor learning and coordination are primary adaptations. When learning effect decreases, hypertrophy and structural changes become more markable adaptations to increase muscle strength. (McArdle et al. 2007, 509–553.) According to Seynnes et al. (2007) changes in muscle size at macroscopic level were observed after only 3 weeks of resistance training, when bilateral leg extension were performed three times per week. Consequently, they suggested that neural factors contribute to the early strength gains as previously reported, but contribution of hypertrophy to strength gains during training occur earlier than previously reported. (Seynnes et al. 2007.)

High–tension eccentric (lengthening contraction) strength training will lead to the greatest increases in strength, when overload principle is strictly followed (Kraemer & Häkkinen 2002, 20–36). It causes delayed muscle soreness, which activates muscle regeneration. (Kraemer & Häkkinen 2002, 53; McArdle 2007, 541–543). However, in the study of Flann et al. (2011) eccentric exercise increased muscle size and strength independently of symptoms of muscle damage. Eccentric exercise has low energy
requirements and high force-production abilities, which is important for individuals who could benefit from increased muscle strength, but are exercise-limited (Gosker et al., 2000; Volpi et al., 2004). In turn, in previously untrained individuals intense eccentric training performed every second day led to prolonged impairment of muscle strength. Muscle thickness increased and strength decreased, what is a sign of muscle damage leading to swelling and impaired muscle function. (Krentz and Farthing 2010.) Nevertheless, according to Moore et al. (2012), contraction type has just little impact on the training-induced increase in muscle size and strength, when important training variables such as exercise intensity and volume of work are equivalent. However, with eccentric training, greater volume of work can be performed in fewer repetitions and possibly at a lower metabolic cost (Moore et al. 2012; Ryschon et al. 1997). Over a period of short-term training, eccentric training may provide a time and energy efficient strategy to enhance hypertrophic adaptations. (Moore et al. 2012.)

In older individuals, the development of hypertrophy is more robust compared to young individuals, especially men (Kosek et al. 2006, Martel et al. 2006). Particularly the development of type I muscle fiber is more slow than in young individuals. In turn, young men appear to have greatest hypertrophic capacity compared to young women, older men and women. These differences in muscle hypertrophy may be due to varying expression of cellular components known to impact muscle fiber hypertrophy. All groups had hypertrophy also in type 2 fibers after heavy resistance training. (Martel et al. 2006.) However, the functional benefits of resistance training for older adults are remarkable. After 16 weeks of resistance training, older adults were capable to restore myofibers to the pretraining fiber sizes, and therefore, age-specific training programs should be evaluated. (Kosek et al. 2006.)

2.3.1 Muscle fiber size

An increase of the cross-sectional area of the skeletal muscle fibers is one reason for muscle hypertrophy (Bompa 2009, 266–267). The muscle fiber hypertrophy is typically observed with heavy resistance training (HRT) programs (Green et al. 1999; Wang et al. 1993). Type 2 muscle fibers have been noted to exhibit hypertrophy faster in response to resistance training and and also atrophy faster with detraining. Increase of type 2 fiber
size alters significantly the ratio of type 2 fibers to type 1, when considering muscle fiber cross-sectional area. This is beneficial to maximal strength and power generating capacity. (Bompa, 2009, 265–267.) Also in the study of Wang et al. (1993), type 2B fibers were hypertrophied to the greatest extent and transformed into type 2A fibers after 18 weeks of HRT, which altered the fiber type composition. The extent of these changes depends on the increase in the amount of myofibrils, mitochondria, lipid droplets and capillaries. (Wang et al. 1993.) In turn, after 12 weeks of the HRT period, Green et al. (1999) demonstrated that, depending of the different fiber types, either the capillarization or the oxidative potential is the way to cause decent fiber hypertrophy. However, the mechanisms, which links HRT with these adaptive changes is not clear, but apparently it is related to the muscle force development and duration of activity (Wang et al. 1993).

In the study of Hostler et al. (2001), elastic resistance training devices were used. For utilizing lower loads and higher amount of repetitions compared with free weight training. This method induced minor changes in fiber type composition, fiber size and capillarization. Despite a high number of repetitions in elastic resistance training, muscular adaptation appeared to be more similar to traditional resistance training than to aerobic forms of training. However, the extent of these changes was less than reported for short-term training programs using free weights. (Hostler et al. 2001.) An alteration in muscle fiber type is another positive morphological adaptation to resistance training. The change from type 2X to type 2A is the most consistent fiber type adaptation (Bompa, 2009, 266–267). McCall et al. (1996) studied twelve male subjects, who completed 12 week intensive resistance training for all major muscle groups. Subjects had recreational resistance training backgrounds. After the training period, there was hypertrophy in total muscle CSA and capillary changes were proportional to muscle fiber growth, but estimated fiber number did not changed. (McCall et al. 1996.)
2.3.2 Muscle cross-sectional area (CSA)

2.3.2.1 Measurement and growth of CSA

The greatest absolute force is generated by individuals with the largest muscle cross-sectional area (McArdle 2007, 515). Additionally, measurement of the CSA and volume of the human quadriceps femoris muscle is useful for monitoring the effects of immobilization as a consequence of sarcopenia as well as assessing the response of the muscle to physiotherapy and to training in sport (Hides et al. 1995, Reeves et al. 2004, Walton et al. 1997), which is important especially when the limb is painful and it is impossible to measure muscle strength. That being the case, evaluation of muscle strength must rely on measurements of muscle size (Walton et al. 1997). In addition to research studies which are focused on measuring changes in muscle size, direct measurements of muscle size are essential (Stokes 1985). Moreover, in the CSA measurement with ultrasound, the mid-thigh region CSA of the vastus lateralis muscle is reliable to measure, but in distal regions, it is difficult to get valid and repeatable images (Ahtiainen et al. 2010, Noorkoiv et al. 2010).

Strength training causes an increase in muscle anatomical cross-sectional area, in muscle fiber area and in fascicle angle after 10 weeks of resistance training. Moreover, these changes are also highly related to maximal strength. In turn, endurance training does not evoke these morphological changes, which supports that fiber hypertrophy and fascicle angle are interrelated (Figure 5) (Farub et al. 2012.) In the study of Lamas et al. (2012), eight weeks of training in the strength training and power training groups increased both maximal strength and CSA. However, there were no changes in muscle activation. Despite a non-significant interaction effect, there seemed to be a trend that the strength training group had greater muscle fiber hypertrophy (in 1, 2A and 2B fiber types) than power training group. In type 1 fibers, CSA decreased 5% after power training (Lamas et al. 2012.)

With increasing age, the decline in maximal strength could be related to the weakening in the muscle CSA (Häkkinen et al. 1996). According to Häkkinen et al. (1996), explosive strength may decrease even more than maximal strength with ageing, suggesting that atrophying effects of aging may be greater on fast-twitch muscle fibers than on slow-twitch fibers.
FIGURE 5. Fiber cross-sectional area of vastus lateralis muscle. The values are for mean fiber, type 1 and type 2 CSA after 10 weeks endurance (END) or resistance (RE) training. *Significant difference between pretraining and post training. (Farub et al. 2012.)

2.3.2.2 Muscle CSA in men and women

In the study of Jaworowski et al. (2002), men were significantly taller, heavier, had considerably larger muscle CSA and relatively larger type 1 and 2 fibers than women. Maximal activities of the glycolytic enzymes, lactate dehydrogenase (LDH) and phosphofructokinase (PFK), were also significantly higher in men than in women. This difference did not appear in activities of muscle enzymes involved in the oxidation of carbohydrates and fat. In conclusion, differences in enzyme activities were related to
height, weight, anatomical CSA and relative CSA of type 2 fibers (Jaworowski et al. 2002.)

Kanekisa et al. (1994), determined fat, muscle and bone tissues by ultrasound on the upper arm and thigh. Women had significantly larger fat CSA and smaller bone and muscle CSA than men. Regardless of the measurement site, the largest difference between men and women were found in fat CSA, and the differences in bone and muscle were larger in the upper arm compared to the thigh. When strength was expressed per unit of muscle CSA, there were no significant sex differences observed for the elbow flexors and extensors. Although the difference between sexes in muscle CSA was smaller in the thigh, men had significantly higher strength per muscle CSA compared to women for the knee flexors and extensors (Kanekisa et al. 1994.) Kanekisa et al. (1994) concluded that this might due to untrained women being less able to recruit motor units during dynamic muscle action and/or the differences between sexes in the musculoskeletal system.

According to Häkkinen et al. (1996), age does not change relationship between men and women in strength, because both men and women have an age related decline in the muscle CSA. However, the force per CSA in older women (W70) was significantly smaller than in other groups (W50, M50, M70) (Figure 6). This might due to a decrease in maximal voluntary neural input to the muscle and/or changes in the characteristics of the muscle tissue itself (Häkkinen et al. 1996.)
FIGURE 6. Left: Muscle CSA of the quadriceps femoris muscle for the left (A) and right (B) leg in the middle-aged (50yrs) and elderly (70yrs) men and women. (*p<0.05; **p<0.01; ***p<0.001) (Häkkinen et al. 1996). Right: Maximal force of the knee extensor muscles per cross-sectional area of the quadriceps femoris muscle averaged for the right and left leg in the middle-aged (50yrs) and elderly (70yrs) men and women (*p<0.05) (Modified from Häkkinen et al. 1996).
3 NEUROMUSCULAR ADAPTATIONS TO COMBINED STRENGTH AND ENDURANCE TRAINING

3.1 General neuromuscular effects

Usually maximum strength is combined with endurance training or power training, whereas single-mode training for maximum strength is rarely done. (Tan 1999.) In the studies concerning effect of combined strength and endurance training, subjects have usually been untrained-to-moderately trained individuals. Some of these studies have reported attenuated cardiovascular and musculoskeletal results with combined S and E training. In contrast, other studies have reported similar cardiovascular or musculoskeletal adaptations with concurrent S and E training compared with S or E alone. Only few studies have evaluated this effect in highly trained endurance athletes. (Aagaard et al. 2011.)

According to previous studies, combined strength and endurance training may interfere with optimal neuromuscular adaptation in young subjects, depending on the training intensity, frequency and duration (Karavirta et al. 2011). Despite that, McCarthy et al. (2002) found that concurrent strength and endurance (SE) training led to same results as strength-only training in all neuromuscular measures over the short term. Also Dudley & Djamil (1985), Glowacki et al. (2004), Hendrikson et al. (2010), Häkkinen et al. (2003) agree that concurrent SE training does not hinder strength development (Figure 7). Althought Holviala et al. (2010) also reported increased strength after combined training, increase was minor to changes in the strength group. Perhaps in combined training light endurance program does not bother maximal strength gains, but endurance training will cap the upper limits of strength gains (Tan 1999).
FIGURE 7. Percent changes in 1-RM squat, 1RM bench press, squat jump and bench press throw after 12 weeks of combined resistance and endurance training, resistance training, endurance training and no training (control) (Modified of Hendrikson et al. 2010).

Dudley & Djamil (1985) and Häkkinen et al. (2003) suggested that concurrent SE training (even lowfrequency) will hinder explosive strength development, which might due to the limitations of rapid voluntary neural activation of the trained muscles (Häkkinen et al. 2003). In contrast, Izquierdo et al. (2005) suggested that prolonged low frequency combined SE training in untrained middle-aged men hinder leg strength development, but it might not affect leg muscle power compared to strength training alone. Also Hendrikson et al. (2010) reported that an 8 week concurrent training period does not interfere with improvements in power. Bell et al. (2000) suggested that long term concurrent SE training may lead to an elevated catabolic state, decreased skeletal muscle hypertrophy and impaired strength gains. On the contrary, short term (less than 7±10 weeks) concurrent training will promote increases in many aspects of strength and endurance. However, also Häkkinen et al. (2003) reported that high overall frequency and/or volume of concurrent SE training will lead to large strength gains during initial weeks of training, but only with limited strength development during later months of training.
Ronnestad et al. (2012) studied well-trained cyclists and recreationally active individuals. The cyclists composed combined S+E group (heavy strength training was added to their normal endurance training) and recreationally active individuals composed S group (the same strength training as S+E, but without endurance training). During the 12 week training period both groups increased thigh muscle CSA, squat jump performance and one repetition maximum (1RM), but the relative improvements in S were greater than in S+E. S increased also peak rate of force development (RFD), but S+E did not. (Ronnestad et al. 2012.) Ronnestad et al. (2012) concluded that strength training response on 1RM strength, thigh muscle CSA and squat jump performance is attenuated in well-trained endurance athletes during a period of concurrent S+E training. Also Häkkinen et al. (2003) studied differences between S+E and S groups after 21-week training period. Although the S group improved CSA a bit more than the S+E group (S7%, S+E9%), there were no significant differences between groups (Häkkinen et al. 2003).

In the study of Sunde et al. (2010), competitive road cyclists performed maximal strength training for 8 weeks supplement to their normal training. Cyclists improved cycling economy and efficiency and increased time to exhaustion at maximal aerobic power, without change in maximal oxygen uptake, cadence, or body weight (Sunde et al. 2010). Also according to Cadore et al. (2011) and Storen et al. (2008), strength training concurrently with endurance training may improve the neuromuscular economy more than endurance training alone. This can be seen in decreased electrical activity in the quadriceps muscle during aerobic activity, which means that fewer motor units were recruited for same load (Cadore et al, 2011).

According to Putman et al. (2004), 12 weeks of strength training (S) and combined strength and endurance training (SE) induced similar increases in CSA of type 2A fibers. In contrast, S group increased CSA of type 1 fibers more than SE group (Putman et al. 2004). Putman et al. (2004) concluded that concurrent SE training resulted in greater fast-to-slow fibre type transition and attenuated hypertrophy of the type 1 fibers. Also Karavirta et al. (2011) indicate diminished muscle hypertrophy in previously untrained 40–67-year-old men, when combined SE training are combined over a prolonged period. When maximal muscle strength is elevated without muscle fiber hypertrophy after concurrent SE training, strength appears to be a result of
neuromuscular adaptation. This is interesting to top-level endurance athletes, where muscle forces are generated against gravity (for example, running and uphill cycling) and where gains in body mass are undesirable. (Aagaard et al. 2011.)

Losnegard et al. (2011) investigated the effects of high volume endurance training and heavy strength training in elite cross country skiers. During a 12 week period, strength improved in leg and upper body muscles, but CSA in the thigh muscles changed only a little. Also VO\(_{2}\text{max}\) improved. (Losnegard et al. 2011.)

### 3.2 Order effect of neuromuscular adaptation

In the study of Cadore et al. (2010), 65±4 years old men were studied and they were assigned into three groups: concurrent, strength and endurance group. All groups trained three times per week during 12 weeks. Strength enhancements in this study show that endurance training performed before strength training can hinder strength development in elderly men (Figure 8), when the same muscle group is activated in both types of training. At least in part, neural adaptations seem to explain interference effect in concurrent E+S training, because a catabolic state in hormonal concentrations was not observed. (Cadore et al. 2010.)

![FIGURE 8. Lower-body 1 RM values before and after 12 weeks of combined strength and endurance training (CG), strength training (SG) and endurance training (EG). *Significant difference from pre training values (Cadore et al. 2010.)*](image)
Also Cadore et al. (2012a) and Cadore et al. (2012b) studied 65±4 year old men, who trained three times per week during 12 weeks. They were placed into two combined training groups: strength prior to (SE) or after (ES) endurance training. In both of the studies (Cadore et al. 2012a and Cadore et al. 2012b), both training groups increased muscle thickness with no difference between groups. In the group that performed strength training prior to endurance training, a significantly greater improvement in the lower-body 1RM and in the force per unit of muscle mass was observed. (Cadore et al. 2012a.) Also, in the study of Cadore et al. (2012b) both groups increased upper- and lower-body 1RM and increase in lower-body 1RM was higher in SE than ES. In addition, the maximal EMG and neuromuscular economy of vastus lateralis increased in both SE and ES, but the neuromuscular economy of rectus femoris improved only in SE group. (Cadore et al. 2012b.) According to Cadore et al. (2012b), performing strength prior to endurance exercise during concurrent training resulted in greater lower-body strength gains as well as greater changes in the neuromuscular economy in elderly.

Chtara et al. (2008) examined the influence of the sequence order of high-intensity endurance training and circuit training on changes in muscular strength and anaerobic power. The subjects were students of physical education who were divided into four groups, (strength, endurance, S+E and E+S groups) in which they trained for 12 weeks. In this study no markable differences were noticed, whether endurance training were performed before circuit training (E+S) or conversely (S+E). Group, which trained only strength, improved muscular strength and explosive strength and power significantly more than combined training groups, regardless of the intrasession sequencing. (Chtara et al. 2008.)
4 CARDIORESPIRATORY ADAPTATIONS TO COMBINED STRENGTH AND ENDURANCE TRAINING

4.1 General cardiorespiratory effects

Studies of combined strength and endurance training have different results of cardiorespiratory adaptations when compared to endurance training alone. In the study of Bell et al. (2000), Dudley & Djamil (1985) and Hendrikson et al. (2010), gains in VO\(_{2\text{max}}\) were similar with concurrent training, but according to Glowacki et al. (2004) concurrent training may hinder improvements in aerobic capacity. In addition, Izquierdo et al. (2005) suggested that prolonged low frequency combined training in untrained middle-aged men might not affect cardiovascular fitness. In ageing men, oxygen uptake increased in combined SE training group, however, increase remained minor compared to endurance training group. However, combined SE training might be superior compared to S or E training, when it is about performance of load carrying walking exercise test, where both strength and endurance are beneficial. (Holviala et al. 2010.)

Bastiaans et al. (2001) recommended replacing a portion of endurance training by explosive strength training, because it prevents a decrease in short-term performance without decrease in endurance performance of trained cyclists. In another study of cyclists combined ES training improved maximal isometric strength and prevented the decrease in the cadence, which was mostly observed due to exercise duration (Hauswirth et al. 2010). Garcia-Pallares et al. (2009) found that 12-week periodized combined heavy strength and endurance training is effective for improving both cardiovascular and neuromuscular markers of highly trained top-level athletes. This can be seen also in the study of Mikkola et al. (2007), where young distance runners performed concurrent explosive strength and endurance training. They improved anaerobic and selective neuromuscular performance characteristics without decrease in aerobic capacity, which could be primarily explained by neural adaptations. (Mikkola et al. 2007.) Also in the study of Paavolainen et al. (1999), explosive strength training was included to sprint and endurance training, which did not change VO\(_{2\text{max}}\) in well-trained
endurance athletes. Instead, muscle power, running economy improved, which approved
5km running performance significantly (Paavolainen et al. 1999).

Taipale et al. (2010), Aagaard and Andersen (2010) and Aagaard et al. (2011) reported
same kind of results. According the study of Taipale et al. (2010) maximal and
explosive strength training together performed concurrently with endurance training are
more effective in improving strength, power and muscle activation in recreational
endurance runners than concurrent circuit and endurance training. Strength, power and
muscle activation improvements seemed to change also endurance performance by
improving velocity at VO_{2max} (vVO_{2max}) and running economy (RE). (Taipale et al.
2010.) In turn, Aagaard and Andersen (2010) studied that concurrent heavy-resistance
training and endurance training can lead to enhanced long-term (30 min) and short-term
(15 min) endurance capacity both in well-trained individuals and highly trained top-
level endurance athletes. Aagaard et al. (2011) noticed the same in 45-min time-trial
performance in top-level (National Team) endurance athletes. In both studies these
changes were accompanied to increased type 2A muscle fiber proportions and gains in
maximal voluntary contraction (MVC) and rate of force development (RFD). Aagaard et
al. (2011) suggested that these increases are responsible for the adaptive changes in 45-
min performance, because the group with endurance training only did not have these
changes. In this study muscle fiber area and capillarization remained unchanged.
(Aagaard et al. 2011.) In contrast, Bell et al. (2000) reported that concurrent ES training
may increase capillarization more than endurance training alone. When maximal
strength training is focused on neural adaptation, strength, particularly rate of force
development has improved. As a consequence work economy has enhanced, which will
lead to improved aerobic endurance performance (Hoff et al. 2002).

4.2 Order effect of cardiorespiratory adaptation

Chtara et al. (2005) studied adaptive responses of the intra-session order of strength and
endurance training. Male sport students trained 12 weeks in four training groups
(running endurance training, strength circuit training, S+E and E+S, who combined the
two programmes in a different order during the same training session). The E+S group,
which performed circuit training immediately after endurance training, improved
endurance performance and aerobic capacity significantly more than opposite order or each of the training programmes performed separately. (Chtara et al. 2005.)

In contrast, Cadore et al. (2012a) studied older (65±4 years old) men, who trained three times per week during 12 weeks. They were placed into two combined training groups: strength prior to (SE) or after (ES) endurance training. However, the intra-session exercise sequence had little influence on endurance markers (Figure 9). They did not find significant differences between SE and ES, when the maximal aerobic power, maximal workload, and workload at the anaerobic threshold were observed. (Cadore et al. 2012.)

FIGURE 9. Mean values of the peak oxygen uptake (VO$_{2\text{peak}}$) before and after 12 weeks of concurrent training. SE, strength prior to endurance training; ES, endurance prior to strength training. * Significant difference from pre training values (Cadore et al. 2012.)
5 STRENGTH TRAINING INDUCED ADAPTATIONS IN MEN AND WOMEN

5.1 Muscle strength and power development

The greatest absolute force is clearly generated by individuals with the largest muscle cross sections. With reference measurements such as body mass, fat free mass (FFM), muscle cross section, limb volume or girth, it is possible to evaluate strength performance differences between individuals. Women produce about 50% lower upper body strength and about 30% lower leg strength than men. (McArdle 2007, 515–517.) The clearer difference in the upper limb muscles might be due to the fact that women tend to have a lower proportion of their lean tissue distributed in the upper body (Miller et al. 1993).

When muscle strength of men and women are divided by body mass, there is only minor gender differences left. Therefore, the gender differences are rather caused by muscle volume than muscle fiber architectural characteristics or metabolic functions. When maximal strength is compared to proportions of fat free mass (FFM), men and women do not differ significantly in either upper- or lower-body strength. (McArdle 2007, 515–517.) However, in the study of Miller et al. (1993) men tended to have stronger muscles than women in upper and lower limb muscles, although units were relative to lean body mass. Also Lovell et al. (2011) reported that men were significantly stronger and more powerful in the upper body than women, although results were relative to body mass.
However, there were no significant gender differences in the number of muscle fibers, the number of motor units, the ability to activate motor units and strength per CSA in the study of Miller et al. (1993). According to that, the greater strength of the men is primarily due to the larger fibers (Figure 10) and this study suggests that it is largely caused by innate gender differences. (Miller et al. 1993.) In turn, Lovell et al. (2011) supposed that there might be some gender differences in force per cross-sectional area or in the manner in which the genders produce ATP during high intensity activity, which also affects differences in maximal strength and power.

In the study of Delmonico et al (2005), men and women trained unilateral knee extension strength during 10-weeks. As a result, both men and women increased significantly their absolute and relative peak power. When the absolute results were normalized by muscle volume, only women increased peak power. Both men and women increased their absolute peak velocity and decreased their relative velocity, when results were not normalized for muscle volume. However, in men reductions were significantly greater than in women. This study suggests that strength training induced increases in peak power depending on muscular hypertrophy in men, but not in women. (Delmonico et al. 2005.)

5.2 Muscle hypertrophy

According to Ivey et al. (2000), men are able to gain approximately twice as great muscle mass as women, when expressed in absolute terms. Because men have larger initial muscle mass, men experience a greater absolute change in muscle size. Nonetheless, muscular enlargement on a presentage basis remains similar between genders (McArdle 2007, 547–548). Ivey et al. (2000) noted that men also experience larger losses in response to detraining than women. However, Clark et al. (2009) found that maximal strength recovery is slower in women than men after three weeks of limb immobilization. In the study of Ivey et al. (2000), where groups of young men, young women, old men and old women were compared to each other, young men were the only group that maintained muscle volume adaptation after 31 weeks of detraining (Ivey et al. 2000).
5.3 Adaptations to combined strength and endurance training

In combined training studies concerning men, combined group gained the same results as strength group in strength (Glowacki et al. 2004; Häkkinen et al. 2003; Karavirta et al. 2010; McCarthy et al. 2002) and muscle hypertrophy (Häkkinen et al. 2003; McCarthy et al. 2002). However, in the study concerning only men (Karavirta et al. 2011) and in the studies concerning both men and women (Losnegard et al. 2011), combined training had interference effect on muscle hypertrophy compared to strength training only. Results in muscle power among men are conflicting. For example, according to Häkkinen et al. (2003), combined training hinders explosive strength development, but according to Izquierdo et al. (2005), it does not. Similarly in the study where both sexes were in the same group, combined training reduced the magnitude of increase in muscle power (Dudley & Djamil 1985). In most combined training studies subjects are men or both sexes are in the same groups. In the study of Hendrickson et al. (2010), where subjects were women, combined strength and endurance training did not have an effect on improvements in strength or power compared to strength training alone.

Ferketich et al. (1998) and Hendrickson et al. (2010) have shown that resistance training together with endurance training does not hinder cardiovascular adaptations compared to endurance training only, when subjects were only women. In men, only Cadore et al. (2011) found same increases in VO\textsubscript{2max} with combined training than with endurance training only. In the study of Paavolainen et al. (1999), the endurance group increased VO\textsubscript{2max}, but any increases were observed in the combined group. In turn, Glowacki et al. (2004) found out that combined training may even hinder development of VO\textsubscript{2max}. Mikkola et al. (2007) and Storen et al. (2008) studied distance runners, which included both men and women. In these studies no changes were observed in VO\textsubscript{2max} (Mikkola et al. 2007 and Storen et al. 2008).
6 RESEARCH PROBLEMS AND HYPOTHESIS

The purpose of the study was to find out how the results diverge when a single session combined strength and endurance training is done in two different orders: S+E and E+S. The interest is focused on maximal leg strength, size of lower limb muscles and maximal oxygen uptake. Another interest concerns differences between young men and women according to combined SE training and order effect.

The present research problems and hypotheses are:

1. Is there any difference between S+E training and E+S training after 24 weeks of training on muscle hypertrophy?

   H1a: Strength training before endurance training (S+E) develops more muscle hypertrophy than strength training after endurance training (E+S). The increases in muscle anatomical cross sectional area, in muscle fiber area and in fascicle angle are highly related to maximal strength (Farub et al. 2012). Endurance training before strength training can hinder strength development, when the same muscle group is activated in both types of training in the single session (Cadore et al. 2010).

   H1b: There is no earlier information of differences between men and women in single session combined training. According to earlier information, it can be expected that men will demonstrate relatively more muscle hypertrophy than women. Combined training does not hinder development of muscle hypertrophy in men (Häkkinen et al. 2003; McCarthy et al. 2002), but in women combined training has interference effect on muscle hypertrophy (Losnegard et al. 2011). Young men appear to have the greatest hypertrophic capacity compared to young women, older men and women. This may be due to varying expression of cellular components known to impact muscle fiber hypertrophy. (Martel et al. 2006.) For example, maximal activities of the glycolytic enzymes such as lactate dehydrogenase (LDH) and phosphofructokinase (PFK) were significantly higher in men than in women,
which were related to height, weight, anatomical CSA and relative CSA of type 2 fibers. (Jaworowski et al. 2002.)

2. Is there any difference between S+E training and E+S training after 24 weeks of training in the leg muscle strength?

H2a: Strength training before endurance training (S+E) develops more muscle strength than strength training after endurance training (E+S). Endurance training performed before strength training can hinder strength development (Cadore et al. 2010). A significantly greater improvement in the lower-body 1RM, in the force per unit of muscle mass and in the neuromuscular economy is observed, when strength training is performed prior to endurance training (Cadore et al. 2012a and Cadore et al. 2012b).

H2b: There is no earlier information of single session combined training between men and women. According to earlier information it can be expected that in men muscle strength improves relatively more than in women. Combined training has no negative effects on strength development neither in women (Hendrickson et al. 2010), nor in men (Glowacki et al. 2004; Häkkinen et al. 2003; Karavirta et al. 2010; McCarthy et al. 2002). Men have significantly higher strength per muscle CSA compared to women for the knee flexors and extensors. This might be due to the fact that untrained women are less able to recruit motor units during dynamic muscle action and/or the differences between sexes in the musculoskeletal system (Kanehisa et al. 1994.)

3. Is there any difference between S+E training and E+S training after 24 weeks of training on maximal oxygen uptake?

H3a: Maximal oxygen uptake will improve more when endurance training is performed prior to strength training. In the study of Chtara et al. (2005), E+S single
session training group improved endurance performance and aerobic capacity significantly more than S+E after 12 weeks of training.

H3b: There is no earlier information about single session combined training between men and women. According to earlier information, it can be expected that women will improve VO2max relatively more than men. In men combined strength and endurance training may hinder the development of VO2max (Paavolainen et al. 1999 and Glowacki et al. 2004), but in women combined training does not negatively influence on the magnitude of the increase in VO2max (Mikkola et al. 2007 and Storen et al. 2008).
7 METHODS

7.1 Subjects

A group of young men and women, a total of 58 volunteers were recruited for the current study from the Jyväskylä region. Subjects were physically active, but previously systematically untrained. Initially, they had to be free of acute illness and injuries and secondly could not be overweight (BMI<31kg/m²). The exclusion criteria included impaired glucose tolerance or metabolic syndrome as well as any form of cardiovascular or obstructive pulmonary diseases including use of medication for both. Type 2 diabetes or musculoskeletal diseases which may restrict the participation in prolonged physical activity were also defined as exclusion criteria. The subjects gave a written informed consent to participate in the study and they had the right to withdraw from the study at any time. The study was approved by the Ethics Committee of the University of Jyväskylä.

<table>
<thead>
<tr>
<th>Table 1. Background information of the subjects.</th>
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<tr>
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<tr>
<td>Exp. S+E men (n=18)</td>
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<tr>
<td>Exp. E+S men (n=14)</td>
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<tr>
<td>Exp. S+E women (n=12)</td>
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<tr>
<td>Exp. E+S women (n=14)</td>
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<tr>
<td>Control group (n=8)</td>
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7.2 Study protocol

The present study included two 12 weeks training periods and 3 measurement phases (Figure 11). The first measurement phase (pre measurements) was performed before the training periods, second measurements phase (mid measurements) was performed after the first training period and the third measurement phase (post measurements) was performed after the second training period. Prior to the pre measurements, subjects had a familiarization session in the lab to prepare them for the upcoming measurements and to adjust the measurement devices. Subjects were randomly assigned into four groups: 1. Experimental S+E male (n=18), 2. Experimental S+E female (n=12), 3. Experimental E+S male (n=14), 4. Experimental E+S female (n=14). Ten subjects were randomly selected from the training groups to the control group, which performed the measurements also three weeks before the first measurement phase.

FIGURE 11. Overview of the study design.
7.3 Training programs

Subjects performed single session combined strength and endurance training over a period of 24 weeks. Half of the men and women performed strength training every time before endurance training and the other half performed strength training invariably immediately after endurance training. During weeks 0–12, subjects had two training session per week and during weeks 13–24 subjects had five training sessions every two weeks.

7.3.1 Strength training program

Strength training focused on knee flexors and extensors, although all major muscle groups were trained. The loads for strength training were determined by testing subjects’ individual one repetition maximum (1RM). The first combined training period began with low loads of 40–60% of 1RM during the first 2 weeks and progressing to 60–80% 1RM loads during weeks 3–7 and 80–95% during weeks 8–12. Explosive power exercises were included in weeks 8–12 with the loads between 30 and 40% 1RM. In the second combined training period the loads of the combined training period 1 were repeated, but percentages were calculated from 1RM results of the second measurement phase.

7.3.2 Endurance training program

Endurance training was performed with bicycle ergometer and intensity was controlled by heart rate zones that were determined after the initial maximal endurance test on a cycle ergometer. During the combined training period 1 there were two different endurance trainings sessions. The first training session included continuous cycling around the aerobic threshold for 30–45min and the second training session was interval training, where the heart rate started below aerobic threshold and raised over the anaerobic threshold. During the combined training period 2, the same training program was repeated with new thresholds.
7.4 Measurements

All strength tests were performed in the same test session, and there were no more than five days between the last training session and strength tests. The endurance test was performed on the other test day, which was two days before or two days after the strength tests. The anthropometric measurements were performed in a rested state after one day of total rest. However, DXA was performed after 12 hours fast and before 10.30 am.

7.4.1 Neuromuscular measurements

*Isometric strength and MVC500.* Maximal isometric voluntary strength and force at 500 ms timepoint were measured with the dynamometer and the analyses were done with the Signal 2.15, Cambridge Electronic Design Ltd. 1997–2004. Subjects were instructed to press with their whole foot, to push as fast and as hard as possible in the beginning and to hold the maximal force for approximately 3 seconds. Subjects were also instructed to keep their back and pelvis in contact with the bench throughout the movement and grasp the handle throughout the measurement. Subjects performed 2–3 warm up trials, where they were instructed to push with sub-maximal effort. After that 3 maximal trials with one minute rest were performed.

*1RM leg press.* One repetition maximum was assessed using the leg press (David 210, David Fitness and Medical LTD., Helsinki, Finland). The correct technique was explained to subjects and it was controlled during the measurements (similarly as in isometric strength). To determine 1RM, warm up was started with approximately 70% of perceived capacity (5 repetitions), after that 2 repetitions with 80–85%, one repetition with 90–95% and finally 1RM. 50% from isometric maximal force was determined as the reference value for dynamic 1RM. After that the increase of the weight was either 5 or 2.5 kg and subjects had as many trials as 1RM was found. Between the trials, subjects were allowed to rest for one minute.
**Leg press power.** Leg press power was assessed using the leg press (David 210, David Fitness and Medical LTD., Helsinki, Finland), and the analyses were done with the Signal 2.15, Cambridge Electronic Design Ltd. 1997–2004. The technique was the same as in 1RM leg press, but now it was highlighted to push as fast as possible. The load was 50% of the assessed 1RM, and it was calculated from the new 1RM in every measurement point. The subjects had three trials, and between the trials they had one minute rest.

7.4.2 Cardiorespiratory measurements

**VO\textsubscript{2max} and time to exhaustion.** Maximal oxygen uptake (VO\textsubscript{2max}) and time to exhaustion for untrained subjects were measured using an incremental cycling protocol. Moreover, the subjects were briefed on the cycle ergometer protocol including starting speed, progression of the test and procedures for taking blood lactate. Subjects were also told to be free to stop the test for any reason if they would feel that it is necessary. Cycling was started at a load of 50 watts for both men and women and was increased by 25 watts every 2 minutes. The test was performed until voluntary exhaustion. Blood lactate was measured and subjects personal RPE recorded after every stage. After exhaustion subjects had a 5 min cool down at a self selected intensity. Aerobic (AerT) and anaerobic (AnT) thresholds were determined using blood lactate, ventilation, VO\textsubscript{2} and VCO\textsubscript{2} (production of carbon dioxide) according to Aunola and Rusko (1986).

7.4.3 Muscle CSA and legs lean mass

**Ultrasound.** Cross sectional area of the knee extensor muscles (vastus lateralis and rectus femoris) were measured using ultrasound (model SSD-2000, Aloka, Tokyo). Cross sectional images were conducted at 30%, 50% and 70% of the femur length. The three clear images from every measurement point were saved for analysis. A tattoo point were marked to subjects (place at the 50% of the femur length) to ensure that the measurement places are always the same. In every measurement, straight lines perpendicular to the measurement table were drawn along the thigh with a marker pen to ensure that the probe is moved along the straight line. Images were analysed with ImageJ (National Institute of Health, USA, version 1,42) program, and CSA were
measured by marking manually the lines of the muscles to the image. The two closest values of vastus lateralis and rectus femoris muscles were taken to account and the averages were calculated.

**Dual-energy X-ray Absorptiometry (DXA).** Lean mass for lower body extremities was analysed from the results of Lunar Prodigy Advance (GE Medical Systems – Lunar, Madison WI USA) machine. During the measurement subject was lying on the measurement table for a few minutes, and at the same time generator was moving over the whole body from head to toes. The DXA measurement is based on two energy x-rays, which goes through the body and can recognize bone, fat and lean mass because of their different densities. The measurement program makes its own analysis of the image, but it is not strict and that is why it should be done also manually. In the analysis, the body is divided to different segaments with lines. The segments include head (not analysed), backbone, hands, legs, torso, lower and upper part of the body (line at the sacroiliac bones). The program calculates, for example, bone density and lean mass (includes everything else than fat and bone) to every segment.

### 7.5 Statistical analysis

Microsoft Office 2007 Excel-program was used to calculate averages and standard deviations. The normality of the data was verified with Shapiro-Wilk normality test. Changes in the variables before and after training were compared with Paired-Samples T Test, if the data was normally distributed. If the data was not normally distributed, Wilcoxon Test of the Related Samples Nonparametric Tests was used. To compare differences between training groups Independent-Samples T Test were used, when the data was normally distributed. However, if the data was not normally distributed, Kruskal-Wallis Test of the Related Samples Nonparametric Tests was used. To calculate correlations between variables Pearson Correlation test were used. The statistical significance level was 0.05. The star symbols were used to illustrate statistical significance in the figures and tables (** =p<0.01, * =p<0.05). In the text, results are presented as mean ± SD.
8 RESULTS

8.1 Effects of combined training on maximal strength and power

Before the 24-week training period, the S+E and E+S training groups did not show significant differences in strength, either in men or women. After the 24 week training period, one repetition maximum, isometric leg press, maximal voluntary contraction and average leg press power improved significantly in all training groups (Figure 12, Table 2). The relative changes were compared between the groups and no significant differences existed between the groups in any variables. Maximal strength and power correlated considerably both in men and in women. In the control group, 1RM was significantly larger at week 3 than at week 0 (162.9±26.6kg vs. 153.8±28.8, p<0.05), while no significant differences were observed in power.

TABLE 2. The relative changes from week 0 to week 24 in strength variables.

<table>
<thead>
<tr>
<th></th>
<th>Change %</th>
<th>P-value</th>
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<tbody>
<tr>
<td><strong>1RM</strong></td>
<td></td>
<td></td>
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<tr>
<td>Women S+E</td>
<td>17.0±11.2</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Women E+S</td>
<td>15.1±11.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Men S+E</td>
<td>17±11.8</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Men E+S</td>
<td>12.6±8.3</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Isometric leg press</strong></td>
<td></td>
<td></td>
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<tr>
<td>Women S+E</td>
<td>12.7±13.8</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Women E+S</td>
<td>17.7±13.7</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Men S+E</td>
<td>13.2±18.4</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Men E+S</td>
<td>11.5±9.8</td>
<td>p&lt;0.001</td>
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<tr>
<td><strong>MVC500</strong></td>
<td></td>
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<tr>
<td>Women S+E</td>
<td>17.5±17.3</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Women E+S</td>
<td>29.6±22.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Men S+E</td>
<td>19.3±23.2</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Men E+S</td>
<td>20.6±24.1</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>Average leg press power</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women S+E</td>
<td>13.1±10.1</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Women E+S</td>
<td>12.7±10.2</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Men S+E</td>
<td>11.0±9.6</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Men E+S</td>
<td>12.8±13.3</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>
FIGURE 12. 1RM, isometric leg press, MVC500 and average leg press power at week 0, week 12 and week 24 in every group separately.

8.2 Effects of combined training on VO\textsubscript{2max} and endurance performance

Before the 24-week training period, no significant differences were noted in endurance variables between the training groups either in men or in women. Maximal oxygen consumption improved significantly from week 0 to week 24 in the women S+E group from 35.7±4.3ml/kg/min by 9.2±7.7% (p<0.01), in the women E+S group from 32.5±4.3ml/kg/min by 7.5±9.8% (p < 0.05) and in the men S+E group from 43.8±7.3ml/kg/min by 6.1±7.8% (p<0.01). Both women groups improved VO\textsubscript{2max} significantly from week 0 to week 12 (women S+E from 35.7±4.3ml/kg/min by 6.8±9.2%, p<0.05, women E+S group from 32.5±4.3ml/kg/min by 7.3±10.4%, p<0.05), but not from week 12 to week 24. In men, no significant improvements occurred from week 0 to 12 or from 12 to 24 in VO\textsubscript{2max}. When comparing the relative changes (Figure 13) between groups, no significant differences were observed. Time to exhaustion improved significantly in all groups from week 0 to week 12 (men S+E and women E+S p<0.001, men E+S p<0.01, women S+E p<0.05), from week 12 to week 24 (men E+S p<0.01, other groups p<0.001) and from week 0 to week 24 (all groups p<0.000). The
women E+S group improved time to exhaustion significantly more than the men E+S group (21.7±10,9% vs. 13.1±8.6%, p<0.05) (Figure 13). No significant differences were observed between the other groups. The values of strength variables did not correlate with the respective ones of endurance variables.

FIGURE 13. Relative changes (mean+SD) in VO\textsubscript{2max} and time to exhaustion after 24 weeks of training in all training groups.

### 8.3 Effects of combined training on muscle cross-sectional area (CSA)

The cross-sectional area of the training groups did not differ significantly before the 24 week training period in men. In women, the S+E group had a significantly larger CSA in the vastus lateralis muscle at the 50% measurement point than the E+S group before the training period (21.7±3.7cm\textsuperscript{2} vs. 18.0±3.4cm\textsuperscript{2}, p<0.05). In the vastus lateralis muscle, there was no significant group difference at the 70% measurement point in women. In the control group, there were no significant difference in the control (-3 week) and pre (0 week) measurements in VL50% and VL70%. Both groups in men and women increased significantly CSA of the vastus lateralis muscle at the 50% and 70% measurement points during 24 weeks of training (Table 3). At the 50% measurement
point, men E+S and women E+S increased CSA significantly during both training periods, but men S+E increased only during the first training period (0–12 week) and women S+E increased only during the second training period (13–24 week). At the 70% measurement point men S+E, men E+S and women S+E increased CSA significantly during both training periods, but women E+S increased only during the second training period (13–24 week).

**TABLE 3.** Muscle cross-sectional area (cm$^2$) of the vastus lateralis muscle (50% and 70% measurement point of femur length) before, after 12 weeks and after 24 weeks of combined training in every training group.

<table>
<thead>
<tr>
<th>Vastus lateralis 50%</th>
<th>PRE (cm$^2$)</th>
<th>MID (cm$^2$)</th>
<th>POST (cm$^2$)</th>
<th>p-value PRE-POST</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women S+E</strong></td>
<td>21.7±3.7</td>
<td>22.6±3.8</td>
<td>23.5±3.6</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>Women E+S</strong></td>
<td>18.0±3.4</td>
<td>19.4±2.9</td>
<td>21.2±3.3</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>Men S+E</strong></td>
<td>26.2±4.0</td>
<td>28.8±3.4</td>
<td>29.9±4.1</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Men E+S</strong></td>
<td>27.9±3.6</td>
<td>29.4±4.7</td>
<td>30.8±4.2</td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vastus lateralis 70%</th>
<th>PRE (cm$^2$)</th>
<th>MID (cm$^2$)</th>
<th>POST (cm$^2$)</th>
<th>p-value PRE-POST</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Women S+E</strong></td>
<td>16.8±2.5</td>
<td>18.5±2.5</td>
<td>19.5±2.4</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Women E+S</strong></td>
<td>15.6±2.6</td>
<td>16.7±1.9</td>
<td>18.7±1.9</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td><strong>Men S+E</strong></td>
<td>22.4±3.9</td>
<td>24.9±4.2</td>
<td>26.6±3.7</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td><strong>Men E+S</strong></td>
<td>24.3±4.5</td>
<td>26.7±4.5</td>
<td>28.5±4.8</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

The relative changes in muscle CSA were compared between the S+E and E+S groups separately in men and women. Moreover, the comparison between men and women were done separately in the S+E group and in E+S group (Figure 14). In women, the E+S group increased muscle CSA of vastus lateralis 50% significantly more than the S+E group, (18.7±11.2% vs. 8.8±8.7%, p<0.05). In the E+S group, women increased vastus lateralis 50% CSA more than men (18.7±11.2% vs. 10.4±8.4%, p<0.05). No other significant differences were observed in muscle CSA between the training groups. Men showed a significant correlation between strength and muscle CSA, whereas women did not.
8.4 Effects of combined training on lean mass of legs

Before the training period no significant group differences were observed in men or in women in lean mass of legs. Lean mass of legs increased in all training groups significantly from week 0 to week 24 (women S+E 16.3±1.6kg vs. 17.1±1.6kg (p<0.001), women E+S 16.9±2.1kg vs. 17.5±2.0kg (p<0.001), men S+E 23.9±2.5kg vs. 25.3±2.6kg (p<0.001), men E+S group 23.5±2.2kg vs. 24.6±2.4kg (p<0.001). Lean mass increased also from week 0 to week 12 in all training groups (men S+E p<0.05, men E+S and women E+S p<0.01, women S+E p<0.001) and from week 12 to week 24 in all but the men S+E group (men E+S p<0.01, women S+E and women E+S p<0.05). All in all, when comparing the relative improvements, no significant differences between the groups were observed (Figure 15).
FIGURE 15. Relative changes (mean±SD) of lean mass of legs from week 0 to week 24 in every training group.
9 DISCUSSION

9.1 Main findings

The purpose of this study was to find out, how the results diverge after 24 weeks of training when combined strength and endurance training is performed in two different orders: S+E and E+S. According to the present results, thigh muscle CSA, maximal strength and endurance performance increased significantly in all groups. However, there were some differences between the groups so that wE+S showed larger muscle hypertrophy (CSA) than that of observed when training by the opposite order and larger than in men using the same E+S order.

9.2 Differences between training groups in muscle hypertrophy

All the training groups increased significantly muscle CSA in the 50% and 70% measurement points. The women E+S group increased VL50% significantly more than the women S+E and the men E+S groups. Thus, hypothesis one and two did not come true. Moreover, in women the hypothesis one went conversely. One reason might be due to the fact that our subjects did not have a systematic strength training background and, therefore, a large part of the strength development might be contributed by the nervous system (Moritani & deVries 1979, Häkkinen & Komi 1983, Holtermann et al. 2007). Althought every group increased muscle CSA significantly, only women had a significant group difference in VL50%. We have to take into account that the present resistance training program included maximal, explosive and hypertrophic strength training. Thus, our training program was not optimal for muscle hypertrophy, because the load, the amount of repetitions and sets, recovery time, performance technique and total load of training did not focus only on hypertrophy (Bomba 1999, 315–342, Campos ym. 2002, Cormie ym. 2009). In addition, the endurance training consisted of cycling, which as such might be intensive enough for women to promote some muscle hypertrophy. The cycling load for 30 – 45 minutes training sessions was controlled with heart rate zones. Women E+S did endurance trainings every time in a rested state, while
wS+E did it after strength exercise, which might influence to heart rate during the endurance exercise. However, that might affect to some extent to the cycling loads. That is why we could assume that the total training load for legs was harder for wE+S than for wS+E, which might in part explain the group differences. In the case of men, our results support the studies of Cadore et al. (2012a) and Cadore et al. (2012b), where both training groups (SE and ES) increased muscle thickness with no difference between the groups.

Our hypothesis was that men would increase relatively more muscle CSA than women, because the earlier studies have shown that men have significantly higher pre-exercise and exercise-induced serum testosterone levels, which will promote muscle growth (Häkkinen & Pakarinen 1995, Kraemer et al. 1991, Vingren et al. 2010). In addition, men recover faster than women after three weeks of limb immobilization (Clark et al. 2009) and they experience larger losses than women in response to detraining (Ivey et al. 2000). However, in the E+S group women increased CSA relatively more than men, but in the S+E group there were no significant differences between the groups. Regarding this, maybe the present strength training program was not hard enough for men or maybe endurance training inhibited muscle hypertrophy in men. On the other hand, as mentioned earlier, cycling might be hard enough for previously untrained women to promote some muscle hypertrophy in thigh muscles. According to that, we can expect that women in the E+S group received more intensive training for the leg muscles than men, which might explain difference between men and women. For example, in the study of Losnegard et al. (2011) combined endurance and strength training led to improved strength with minor changes in muscle CSA. However, this study lasted 24 weeks, which is according to Bell et al. (2000) a too long a period of combined strength and endurance training. Bell et al. (2000) have suggested that long term concurrent SE training may lead to an elevated catabolic state, decreased skeletal muscle hypertrophy and impaired strength gains, while short term SE training will promote increases in strength and endurance (Bell et al. 2000). Despite the contrary, in the study of Häkkinen et al. (2003), there was no interference effect either in strength or in hypertrophy, although they had a 21 week training period. Hickson (1980) has shown that also short term training might impaire strength gains if the amount of training is too high.
In this study muscle CSA was measured with ultrasound, which has been studied to be valid and repeatable for assessing the changes in skeletal muscle CSA (Ahtiainen et al. 2010). However, we have to remember that this measurement has some defects. The main mistakes of the ultrasound measurement are caused by the movement of the probe which depends on an examiner. These errors are caused by pressure and angle of the probe. Pressure might cause compression and too small angle might cause overestimation (Esformes et al. 2002, Reeves et al. 2004). Besides, if the probe diverges of the line, this would result in scanning a different region of the muscle resulting as an invalid image (Reeves et al. 2004). The field of view is relatively limited in ultrasound (Ahtiainen et al. 2010) and identifying the fascia between the two muscle groups is difficult in some conditions (Martinson and Stokes 1991). In the present study results of VL30% and RF50% are not reported, because according to the control measurements and literature (Noorkoiv et al. 2010), the whole rectus femoris muscle and the distal parts of vastus lateralis muscle are too small to obtain reliable results in the ultrasound measurement. However, results of vastus lateralis 50% and 70% measurement points should be reliable according to the control measurements and literature (Ahtiainen et al. 2010, Noorkoiv et al. 2010).

In the DXA measurement lower limb muscle mass increased significantly in all training groups with no differences between the groups. The DXA results did not correlate with strength or CSA, although all variables improved significantly in all our training groups. In our strength training program leg extensors were the main focus, but training included all major muscle groups. DXA analyzes the entire lean body mass of the legs including all muscles of the thigh (Sillanpää et al. 2008). Thus, besides the knee extensors, DXA analyzes also flexors, adductors and abductors. In turn, ultrasound measures CSA in specific sites of individual muscles (Sillanpää et al. 2008). In this study the specific site was in the vastus lateralis muscle, which is one of the leg extensor muscles. We can estimate muscle mass with ultrasound and DXA, however, the procedure is so different that these two methods are hardly comparable (Sillanpää et al. 2008).
9.3 Differences between training groups in maximal strength and power

Both maximal strength and power variables improved significantly in all training groups, but there were no significant differences between the groups in any variables. In addition, maximal strength and power showed significant correlations in both men and in women. Because the subjects did not have any systematic training background, neural factors could have had a big role in strength development (Moritani & deVries 1979, Häkkinen & Komi 1983, Holtermann et al. 2007). Furthermore, in untrained subjects, neural adaptations contribute to both strength and power development (Häkkinen et al. 1997), which might explain the significant correlation between strength and power. That might also be explained by our strength training program, which included not only maximal, but also power and hypertrophic training.

Our study supports the studies of Dudley & Djamil (1985), Glowacki et al. (2004), Hendrikson et al. (2010), Häkkinen et al. (2003) and Holviala et al. (2010), because strength and power improved significantly during the training period. Thus, this amount of combined strength and endurance training did not inhibit the strength and power development. However, we did not have the pure strength group, when it is difficult to evaluate, if the endurance training hindered the strength and power development (Hickson 1980). In the studies of Cadore et al. (2012a), Cadore et al. (2012b) and Cadore et al. (2010), S+E training led significantly greater improvements in the lower-body strength than E+S training. In these studies, the training period lasted 12 weeks and in our study the training period lasted 24 weeks. Consequently, this might have an influence on the results, because long term concurrent SE and short term concurrent SE training with a high volume of training may lead to impaired strength gains (Bell et al. 2000, Hickson 1980).

On the other hand, our study supports the study of Chtara et al. (2008), where no markable differences were noticed between E+S and S+E groups, although the training period lasted only 12 weeks. Men showed the significant correlation between maximal strength and muscle CSA, whereas women did not. With the variables of this study, it is difficult to say, did the men have a better association between development of muscle activation and development of muscle hypertrophy than women? In addition, there were no systematic correlation between maximal strength and muscle CSA in men or in women, when every group was regarded separately.
9.4 Differences between training groups in VO$_{2\text{max}}$ and endurance performance

Time to exhaustion improved significantly in all training groups. Also VO$_{2\text{max}}$ increased significantly in the women S+E, women E+S and men S+E groups, but in men the E+S group VO$_{2\text{max}}$ did not improve significantly. However, there were no significant group differences in time to exhaustion or in VO$_{2\text{max}}$, but in the E+S group women improved time to exhaustion significantly more than men. In many studies (Aagaard et al. 2011, Aagaard and Andersen 2010, Holviala et al. 2010, Hauswirth et al. 2010, Mikkola et al. 2007, Hoff et al. 2002, Paavolainen et al. 1999) endurance performance has improved with combined endurance and strength training even with only minor changes in VO$_{2\text{max}}$, because the economy of performance has improved. In this study, women in the E+S group increased time to exhaustion and also CSA significantly more than men. That might suggest that women improved also cycling economy more than men in the E+S group. However, either women or men showed significant correlations between time to exhaustion and CSA. Moreover, no significant differences between the groups were observed in strength development, since both wE+S and mE+S improved strength significantly. Chtara et al. (2005) showed that the E+S group improved endurance performance and aerobic capacity significantly more than opposite order or each of the separately performed training programmes. The study of Cadore et al. (2012a) showed that the order of strength and endurance in combined training had just a little influence on endurance variables.

It seems that time to exhaustion improved relatively more than VO$_{2\text{max}}$, which supports earlier studies (Aagaard and Andersen 2010, Aagaard et al. 2011, Mikkola et al. 2007, Paavolainen et al. 1999 and Taipale et al. 2010), where endurance performance, velocity at VO$_{2\text{max}}$ and economy have improved without remarkable changes in VO$_{2\text{max}}$. Despite that, time to exhaustion and VO$_{2\text{max}}$ correlated significantly in the other groups, but not in women E+S. In this study we did not find any significant correlation between endurance and hypertrophy or between endurance and strength. The study of Holviala et al. (2010) supports this finding, because it suggests that combined training does not inhibit the development of strength or endurance, however, the development is minor compared to strength or endurance training alone.
9.5 Strengths and limitations

Overall, the strength of this study was the possibility to study long term changes and group differences in combined single session strength and endurance training. Earlier studies of the order effect have lasted 12 weeks, whereas in this study the training period was twice as long. Besides, this study included training groups for men and women which gives the possibility to compare differences between sexes. The subjects in this study were recreationally active people, without any systematic training background, which may have been the limit for the accuracy of this study. Therefore, the training background should have been limited more accurately, because of the diverse training backgrounds of the subjects. On the other hand, this study gave us general aspects of recreationally active individuals. In this study, the strength training only and the endurance training only groups were missing. Although the purpose was only to compare the training order, these separate groups would have possibly further helped to explain the results of this type of training program.

9.6 Future directions

In the future it would be interesting to study order effect in different sports with sports specific training programs, where strength or endurance play a larger role. The sports specific study would give the opportunity to study subjects with a same type of training backgrounds, whereas athletes would get more specific information about training order for their own sport. It would be also interesting to clarify, whether men have a stronger association between the development of maximal strength and muscle CSA than women. In this study, men had the significant correlation between strength and muscle CSA during combined training, but women did not. However, it is difficult to argue that with the variables of this study. Furthermore, future studies should investigate, whether women can tolerate combined SE training better than men, because in this study training seemed to be most effective for women E+S group.
9.7 Conclusions

According to the present study, subjects with no systematic training background increased muscle CSA and improved muscle strength and endurance performance with combined single session strength and endurance training with both training orders of S+E and E+S. Additionally, in this study combined training seemed to be most effective for the wE+S group in terms of CSA and cycling performance, because that group improved CSA more than by the opposite order as well as more than men with the same order.
REFERENCES


