Strength Training for Elderly: from a Functional, Structural and Molecular Perspective

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Dedicated to my Mother

Jung stirbt, wen die Götter lieben.

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Abstract

Sarcopenia is the age related loss of muscle mass and strength. Sarcopenia has been associated with an increased risk of falling and the development of metabolic diseases. Various training protocols as well as nutritional and hormonal interventions have been proposed to prevent sarcopenia.

This study explores the potential of continuous eccentric exercise to retard age-related loss of muscle mass and function and compared it to conventional strength training. Elderly men (n=26, 80.4±0.6y) and women (n=36, 80.9±0.6y) were randomly assigned to one of three training interventions, which demanded a training effort of two sessions (20 min of specific training each) per week for 12 weeks: 1) eccentric ergometer training, (EET; n=23) 2) conventional resistance training (RET; n=23) and 3) cognitive training (CT; n=16). EET was executed on a motor driven eccentric cycle ergometer where the subjects had to visually match to the eccentric target load being displayed on a screen. RET was carried out in the gym and consisted of four classical exercises for the lower extremity. EET has some advantages of particular benefit to the elderly: It demands a well aligned muscle coordination and it allows for high-load muscular training with moderate cardio-pulmonary stress due to the low metabolic costs of eccentric work. Before and after the study, subjects were tested for functional parameters and body composition. From a cohort of RET (6♀;7♂) and EET (7♀;7♂) subjects, biopsies were collected from the *vastus lateralis* muscle for the assessment of fiber composition, ultra-structure and gene expression. One year after completion of the training intervention, subjects were retested to monitor persistence of functional improvements and body composition. During this year, no guided training sessions were provided. Due to the advantageous features of EET (mechanical stimulus ↑, energy demand ↓, coordination ↑) we hypothesized EET being favourable for elderly to improve muscle coordination and leg strength.

As expected, maximal isometric leg extension strength (MEL: +8.4±1.7%) and eccentric muscle coordination (COORD: -43±4%) were significantly improved with EET but not with RET (MEL: +2.3±2.0%; COORD: -13±3%) and CT (MEL:-2.3±2.5%; COORD: -12±5%), respectively. We observed a significant loss of body fat (-5.0±1.1%) and thigh fat content (-6.9±1.5%) in EET-subjects only. Relative thigh muscle mass

increased with EET (+2.5±0.6%) and RET (+2.0±0.3%) but not with CT (+0.4±0.4%). With RET, changes in thigh muscle mass correlated with changes in fiber cross sectional area, suggesting fiber hypertrophy as the predominant mechanism for muscle growth. This correlation was not seen with EET, where muscle growth seems to occur by hyperplasia or addition of sarcomeres in series. Expression of transcripts encoding factors involved in muscle growth, repair and remodelling (e.g. IGF1, HGF, MYOG, MYH3) was increased by a greater magnitude with EET compared to RET. In contrast, with EET only, genes encoding mitochondrial and metabolic transcripts were consistently depressed. Expression of metabolic and mitochondrial gene transcripts correlated significantly with mitochondrial volume density. Micro RNAs are known to decrease protein abundance of target genes by translational repression and mRNA degradation. Muscle specific micro RNA-1 (miR-1) expression decreased independent of training modality and correlated negatively with changes in IGF-1 expression, which represents a potential target. IGF-1 is a potent promoter of muscle growth and its regulation by miR-1 seems to contribute to the gain of muscle mass observed in our subjects.

After one year without training, improvements in MEL were lost, as were loss in body fat and thigh fat content as well as gain in relative thigh muscle content. Surprisingly, EET subjects maintained the ability to precisely dose the eccentric target load on the eccentric bike, referred to as eccentric coordination. It remains to be investigated if and to which extent this ability can be transferred to other tasks and thereby be potentially useful in reducing the risk for falling.

We conclude that EET is more effective in improving body composition, muscle strength and coordination. Gain in muscle mass seems to occur by different mechanism with EET and RET. This is consistent with distinct gene expression profiles which point towards increased muscle remodelling and decreased oxidative capacity with EET. From a more general perspective, it can be concluded that two training sessions per week are the lower limit to induce measurable functional improvements in active elderly.

1. Introduction

Sarcopenia

Sarcopenia: Why and How

Summary

Human ageing is associated with cellular senescence and a decline in neuromuscular function and performance known as sarcopenia (Grimby and Saltin 1983; Doherty and Brown 1993; Doherty et al. 1993; Roos et al. 1997). Sarcopenia is characterised by an age-dependent progressive loss of muscle mass, impaired coordination of movements and declined strength. Loss of muscle mass occurs by atrophy and loss of individual muscle fibres and is accompanied by a loss of motor units followed by the infiltration of connective tissue and fat (Overend et al. 1993). It was suggested that the complex interaction of factors affecting neuro-muscular transmission, muscle architecture, fibre composition, excitation-contraction coupling and metabolism contribute to the onset and progression of sarcopenia. But also behavioural aspects such as an age-related decrease in physical activity and nutritional intake might contribute to a loss of strength and muscle mass (Vandervoort 2002; Doherty 2003).

Loss of Strength

Multiple studies compared knee extensor strength from subjects in their seventh and eighth decades and reported average decreases in strength between 20% and 40% compared to young subjects (Murray et al. 1980; Young et al. 1984; Murray et al. 1985; Young et al. 1985; Adamo and Farrar 2006). Study subjects older than 80 years showed considerably greater strength losses of 50% and more (Murray et al. 1980; Murray et al. 1985). Relative strength loss is similar in men and women, however, as men start at higher levels, their absolute strength loss is greater. The magnitude of the strength deficit with ageing depends on testing modalities (e.g. angular velocity, mode of contraction). Decreased strength is most pronounced using isokinetic testing devices at high angular velocities. This result is in accordance with the age-related slowing of muscle contractile properties (Campbell et al. 1973; Davies et al. 1986; Doherty et al. 1993; Doherty et al. 1993; Frontera et al. 2000; Klein et al. 2001; Raj et al. 2009). In marked contrast, under eccentric testing conditions, age-related loss of strength is less pronounced, manly at high angular velocities (Poulin et

al. 1992). Altered connective tissue content and composition, as well as shifts in muscle fiber type composition leading to altered muscle stiffness and contractile properties are possible explanations for this observation.

In a cross sectional study, Vandervoort et al. (Vandervoort and McComas 1986) examined young, middle aged and elderly subjects (20y to 100y) to evaluate the respective contribution of voluntary muscle activation and muscle mass to measured force generation. The authors concluded that loss of strength in the elderly was primarily caused by a decreased muscle mass and not by the inability to adequately activate muscle fibers. Loss of muscle quality as described by “force/cross sectional area” was investigated repeatedly; however, the results are inconclusive. Some scientists could detect differences among older and younger subjects while others could not (Young et al. 1984; Frontera et al. 1991; Reed et al. 1991; Brooks and Faulkner 1994). While measurements of CSA and muscle mass are assumed to be reasonably accurate (even though estimation of CSA in pennate muscles has some difficulties), estimation of maximal voluntary force is more difficult as voluntary aspects are hard to control (Frontera et al. 2000). Because there is a need for full central activation, a selective activation of defined muscles and negative effects of joint pain and other neuronal inhibitory factors can limit central drive. Muscle fiber type composition might also contribute to specific muscle strength as type II muscle fibers are able to generate greater forces than type I fibers (type IIX>type IIA>type I) (Slivka et al. 2008).

Causes of Sarcopenia

Loss of muscle mass during sarcopenia is caused by muscle fiber loss and by fiber atrophy. Average cross sectional area (CSA) of type I fibers is less affected than CSA of type II fibers (Doherty et al. 1993). Along with type II fiber atrophy, there is an increased occurrence of mixed muscle fibers (co-expression of myosin heavy chain isoforms in the same fiber) and fiber type grouping. The latter is consistent with a progressive de-nervation and re-innervation process (Essen-Gustavsson and Borges 1986; Oertel 1986; Andersen et al. 1999). The fact, that the loss of motor units and fibers follows the same time course and magnitude suggests similar underlying mechanisms (Faulkner et al. 2007). Decline of anabolic hormone levels (i.e. testosterone, dehydroepiandrosterone, growth hormone) accounts for a decrease in skeletal muscle mass (Harridge 2003). Menopause-associated decline in estrogen levels in women similarly contributes to a reduced anabolic action since estrogen is partly converted to testosterone (Roubenoff and Hughes 2000). Age-related reductions in

growth hormone (GH) and insulin-like growth factor (IGF) levels are consistently reported and largely contribute to the symptoms of sarcopenia (Lissett and Shalet 2003). The low levels of IGF are a consequence of the blunted GH stimulation, since local responsiveness is maintained with ageing (Lissett and Shalet 2003). Subclinical inflammation and oxidative stress promotes catabolic cytokines including interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor α (TNFα). It was suggested that transcription of transcripts encoding pro-inflammatory cytokines are driven by the redox-sensitive activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB). Cytokines directly mediate catabolic effects by promoting anorexia, insulin resistance, and reduced GH and IGF-1 levels (Jensen 2008). The balance of anabolic dehydroepiandrosterone (DHEA) and catabolic cortisol is disturbed with ageing resulting in an excess of cortisol favouring muscle wasting (Mayer and Rosen 1977; Brown 2008). The hormone myostatin (MSTN, GDF8) is a negative regulator of muscle mass. Animals and humans with defects or low levels of myostatin have a double muscled phenotype (i.e. texel sheep, belgian blue cattle, case report of a child with gross muscle hypertrohy) (McPherron and Lee 1997; Schuelke et al. 2004; Georges et al. 2006). Excess of myostatin levels could also contribute to age-related loss of muscle mass (Solomon and Bouloux 2006). “Anorexia of aging” describes a declined food intake being considered as an important factor in the development and progression of sarcopenia (Morley 2001; Morley et al. 2001). The complex mechanisms and interactions leading to decreased food intake with aging have recently been reviewed. They include early satiety due to decreased relaxation of the fundus, elevated release of cholecystokinin after fat intake, increased leptin levels (probably caused by an increase in body fat content with aging) and effects of neurotransmitters such as opioids and neuropeptides (Morley 2001; Morley et al. 2001). Comorbidity and deprivated appetite due to medical treatment might also account for hypocaloric food intake. It was suggested that the shortage of proteins and other dietary nutrients (i.e. creatine) could favour catabolic processes as occurring during sarcopenia (Morley et al. 2001). However, this hypothesis was recently questioned by Marzetti et al. (Marzetti et al. 2009) who claimed that moderate caloric restriction (not malnutrition!) ameliorates sarcopenia (manly in rodent models) by a variety of mechanisms including preservation of mitochondrial function and biogenesis, reduction of oxidative stress and modulation of apoptotic and autophagic signaling pathways (Marzetti et al. 2009). Calories in excess cause a condition known as “sarco-

penic obesity" were accumulation of fat mass accelerates loss of muscle mass and insulin resistance via secretion of pro-inflammatory cytokines such as TNFα (Frontera et al. 2000; Roubenoff 2004). In agreement with the caloric restriction theory, reactive oxygen species (ROS) were also suggested to play a key role in triggering sarcopenia. ROS are highly reactive ions or small molecules with unpaired valence shell electrons such as oxygen ions, free radicals, and peroxides. ROS are primarily generated in the mitochondrial respiratory chain, but also by enzymatic reactions (xanthine oxidase) and by immune cells (Morre et al. 2000; Sachdev and Davies 2008). ROS are important signaling molecules to initiate adaptation to exercise but at he same time being harmful resulting in oxidative stress that can damage cellular components such as DNA, proteins, lipids, etc. (McArdle et al. 2001; Fulle et al. 2004). As a consequence, membrane fluidity can be impaired leading to a disturbance in calcium homeostasis and excitation-contraction coupling. Damage of DNA is suggested to trigger sarcopenia by senescence and apoptosis. Mitochondrial DNA is especially sensitive to ROS due to its proximity to the source but also due to its limited repair mechanisms (Larsen et al. 2005; Druzhyna et al. 2008). ROS production is drastically increased during aging – likely by an impaired function of the electron transport chain and insufficient antioxidant defence mechanisms – suggesting a key role of ROS in the development of sarcopenia (Fulle et al. 2004).

Molecular Mechanisms of Muscle Atrophy

Muscle atrophy occurs when protein degradation exceeds protein synthesis. This condition is prevalent during sarcopenia, inactivity and disease (Jackman and Kandarian 2004; Reid 2005). Current understanding of skeletal muscle atrophy pathways involves at least three proteolytic systems: (1) calcium dependent calpains and caspases, (2) calcium independent cathepsins and (3) ubiquitin ligases. Proteases of the calpain and caspase families are responsible for myofibrillar disassembly and cleavage of contractile proteins (actin/myosin-complex) (Tischler et al. 1990; Du et al. 2004). Cathepsins are involved in lysosomal proteolysis and contribute to the degradation of membrane-spanning proteins including receptors, channels and transporters (Jackman and Kandarian 2004). Prior fragmentation of structural and contractile proteins by different proteases is required to enable destruction of the protein fragments by the universal ATP-dependent ubiquitin proteasome pathway (Kandarian and Jackman 2006). Proteins destined for degradation by the proteasome are specifically tagged by ubiquitin ligases (Kandarian and Jackman 2006).

Two muscle-specific E3 ubiquitin ligases, muscle atrophy F box (MAFBx, atrogin-1, FBXO32) and muscle ring finger protein 1 (MuRF1) are up-regulated during skeletal muscle atrophy (Bodine et al. 2001; Gomes et al. 2001; Franch and Price 2005; Glass 2005; de Palma et al. 2008). MAFBx and MuRF up-regulation was suggested to be driven by transcriptional control of forkhead (FoxO) transcription factors and TNFα-dependent activation of NFκB (Glass 2005). In general, FoxO and TNFα are negatively regulated by members of the IGF signaling pathway and inhibited during muscle hypertrophy (Stitt et al. 2004; Pelosi et al. 2007). Myostatin, a negative regulator of muscle mass, is suggested to play a role in muscle atrophy by suppressing satellite cell activation, proliferation and renewal (myostatin is further discussed on page 28) (Langley et al. 2002; McCroskery et al. 2003).

Epidemiologic Consequences

Due to improved medical care, life expectancy continues to increase and the number of senior citizens is expanding rapidly. In developed countries, fall-related fractures and injuries represent a significant health problem in the elderly. In an epidemiological cross sectional study with over 11’000 men and women aged 65 and older, accidents were systematically recorded using questionnaires with the aim of identifying socio-demographic aspects, health conditions, type of accidents and accident related circumstances (Beer et al. 2000). In Switzerland, the elderly have accidents mostly in the household situations and rarely while driving or as a consequence of sport related activities (<10%). Falls are the most common form of accident and a causal reason for death in 20% of seniors aged 65-69 years and in 80-90% of seniors aged 90 and older (Gass and Gutzwiller 1992). Fractures are the most frequent consequence of falls and compared to other age-related diseases and injuries, fractures led to 297’000 hospitalisation days (d) per year, the highest incidence (Switzerland, 2000), followed by chronic pulmonary disease (COPD, 126’000 d), strokes (88’000 d), heart failure (66’000 d) and diabetes (50’000 d). The cost of hospitalization due to fractures was estimated to total 400 Mio CHF per year (Switzerland, 2000), which highlights this economic burden (Lippuner et al. 1997). Concomitant with these enormous socioeconomic costs, the quality of life is dramatically reduced in affected people who often loose their independence as a consequence of falls. With regard to the importance of fall-related fractures in a population of elderly, prevention such as avoiding slippery surfaces, difficult stairs, bad lighting conditions, obstacles etc.

seems to be urgent (Beer et al. 2000). However, regular exercise also has positive impacts on the maintenance of bone density and improves the status of musculoskeletal fitness, both of which are important in preventing fractures (Beyer 2003). Exercise in particular is beneficial in preserving strength and muscle coordination, both of which are required for preventing falls (Runge and Hunter 2006). The maintenance of the musculoskeletal fitness is of particular importance since sarcopenia also affects healthy individuals with advancing age (Doherty 2003).

Physical and Pharmacological Interventions

Due to the various causes of sarcopenia, each individual patient should be analyzed and supported with a relevant therapy. In case of undernourished or anorectic people, nutritional support should be considered. In elderly hypogonadal men, testosterone replacement men produces increases in muscle mass and strength (Sih et al. 1997). However, these beneficial effects are modest, and not consistently observed (Borst 2004). Very high doses of sex steroids have not been administered fearing an increased risk of accelerating prostate cancer (Borst 2004; Wiren and Stattin 2008). A meta-analysis by Borst et al. (Borst 2004) screening studies for an effective sarcopenia treatment concluded that growth hormone (GH) supplementation in elderly subjects did not increase strength, neither did it augment strength gains resulting from resistance training (Thorner and Nass 2007). Alternative strategies to stimulate the endogenous GH/IGF pathway are promising, such as treatment with growth hormone releasing hormone (GHRH) or angiotensin converting enzyme inhibitors (ACE-inhibitors) (Giovannini et al. 2008). Nevertheless, Borst et al (Borst 2004) concluded that resistance training remains by far the most effective intervention for increasing muscle mass and strength in older people. Indeed, using an intensive training protocol with three heavy resistance training sessions (45 min each) per week for 12 weeks Kryger et al. (Kryger and Andersen 2007) reported increases of type IIA fiber cross sectional area and strength up to 40% in subjects aged 85 years and more.

Muscle Plasticity with Exercise and Training

Summary

Remodelling of skeletal muscle occurs in situations of altered use such as strength training, aging, denervation or immobilization with changes in mechanical, metabolic, neuronal, hormonal and immunological stimuli. Adjustments to mechanical and metabolic demands, elicit marked modifications of gene expression and protein synthesis that can lead to a gain (e.g. hypertrophy) or loss (atrophy) of muscle mass. Changes in muscle architecture, contractile and physiological properties are highly specific and depend on training intensity, duration, frequency, type of exercise (strength versus endurance) and contraction mode (eccentric versus concentric). The emphasis of mechanical, neural and metabolic stimuli is different between modes of contraction and type of exercise and is suggested to be a major determinant of the adaptive processes via fine tuned activation of distinct signaling pathways. The responses seen after single exercise bouts are thought to serve two purposes: (1) to restore homeostasis of the physiological systems (i.e. ion balance, pH, protein refolding) and (2) to adapt, and thus be “better” prepared to deal with similar future stress a.k.a. supercompensation (structural adaptation, contractile proprieties and energy/oxygen supply). The latter represents chronic adaptation to training which finally leads to changes in abundance, localisation and activity/function of proteins by transcriptional, post-transcriptional, translational and post-translational modifications. Chronic adaptation is supposed to reflect the accumulation of responses to many acute exercise bouts provoked by the formerly described homeostatic perturbations.

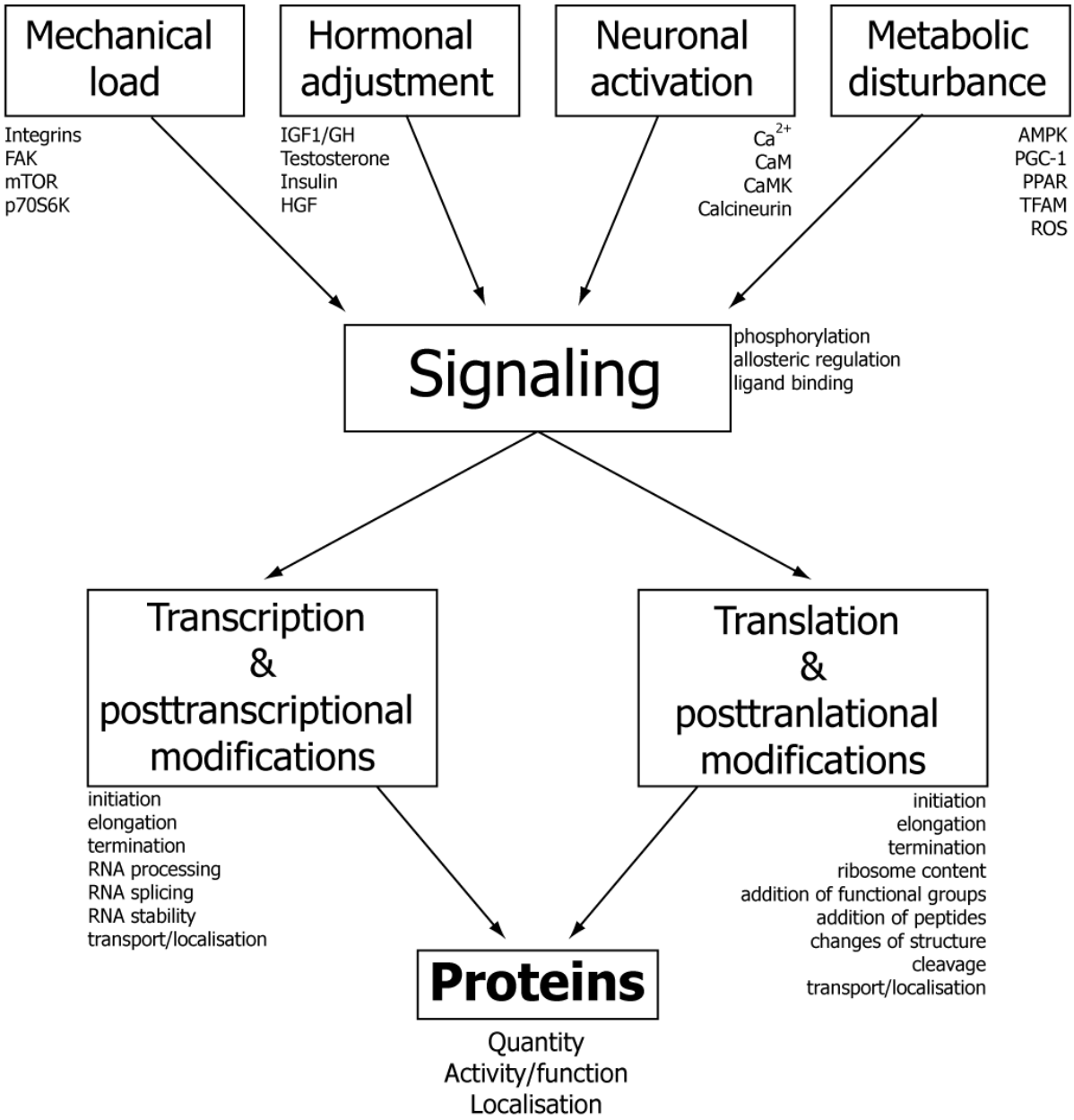

Figure 1: Schematic representation of the integration of distinct stimuli as executed by exercise. As a final read out, changes in specific protein quantity, activity and localisation contribute to the observed adaptations. See text for details.

From Genes to Structure and Function

Adaptive alterations in gene and protein expression by nuclear reprogramming of gene transcription are thought to be underlying mechanisms for the distinct phenotypic changes seen after prolonged periods of training (Pilegaard et al. 2000). The correlation between mRNA and protein levels with structural parameters such as mitochondrial content and functional read out (e.g. maximal oxygen consumption) has been described by Hoppeler et al. and underpins the dominant long term adaptation on RNA level in muscle tissue (Fluck and Hoppeler 2003; Hoppeler et al. 2003; Schmitt et al. 2003). In highly endurance trained athletes, mitochondrial and capillary density is doubled compared to sedentary individuals and represents a well orchestrated adaptation of the oxygen pathway from the lung to muscles (Hoppeler and Weibel 2000). Similarly, functional adaptation, as measured by maximal oxygen consumption during exercise, is increased by the same magnitude, as does muscle substrate storage in the form of intramyocellular lipids (IMCL) (Hoppeler 1986; Puntschart et al. 1995).

In an exemplary work, lasting more than 20 years, Weibel ER and Hoppeler H verified their hypothesis of symorphosis comparing sedentary and athletic species as well as humans in a trained and untrained state (Weibel et al. 1996). In contrast to the “bottleneck” hypothesis which assumes the existence of a limiting factor (=bottleneck) for exercise performance, the model of symorphosis is based on the assumption of a coordinated adaptation of function and structure. From an evolutionary perspective symorphosis is an energy-optimized adaptive concept which was favoured by selection when energy sources were limited. Applying the concept of symorphosis, it is reasonable that training causes changes in the periphery (muscle structure) accordingly to systemic adaptations. To match a higher energetic demand, the muscle increases fuel in the form of intracellular lipid droplets and the mitochondrial density. More mitochondria require an enhanced oxygen supply so the capillary density is also increased, while at a systemic level the cardiac output and hematocrit are also increased (Hoppeler and Weibel 1998).

The molecular mechanisms controlling the complex adaptive adjustments have not yet been resolved. However, it was suggested that the expression of key genes or clusters of genes that contribute to the altered phenotype after long-term training

programs are chronically altered in athletes compared with sedentary individuals (Schmutz et al. 2006). The orchestrated responses are commonly driven by so called master genes such as the peroxisome proliferator-activated receptor gamma, co-activator 1 alpha (PGC-1α), which regulates glucose and fatty acid metabolism, mitochondrial biogenesis, and muscle fiber type transformation from type II to type I fibers (Lin et al. 2002). These master genes usually encode transcription factors which induce clusters of genes responsible for specific pathways. Stepto et al. (Stepto et al. 2009) recently investigated the long term transcriptome adaptation of strength versus endurance training and observed an opposite regulation of transcripts related to mitochondrial function and oxidative capacity being decreased upon strength training and up-regulated with endurance training. Master regulators may also include micro RNAs since these small non-coding RNAs can affect a whole array of targets by binding 3' untranslated regions of mRNAs, thereby inhibiting translation and/or initiating degradation. McCarty et al. (McCarthy and Esser 2007) have recently observed decreased expression of micro RNA-1 (miR-1) and micro RNA-133a (miR-133a) during muscle hypertrophy. The proposed mechanisms include regulation of chromatin remodelling by targeting HDAC's expression and direct regulation of "hypertrophic factors" such as serum response factor (SRF), hepatocyte growth factor (HGF) and HGF-receptor (c-MET) as well as insulin like growth factor 1 (IGF-1) (see also pages 28 et seq.) (McCarthy and Esser 2007; Callis et al. 2008).

Since exercise stimuli represent a mixture of metabolic, hormonal, neural and mechanical stimuli, single contributions are difficult to discriminate from each other (see also figure 1). Endurance exercise has a pronounced metabolic component whereas in eccentric exercise, mechanical stress predominates and the metabolic component is smaller. Given the fact, that sensors or integrators of metabolic adenosine monophosphate kinase (AMPK) and mechanical mammalian target of rapamycin (mTOR) stimuli are competitive, we expect a continuum of different adaptations depending on the magnitude of single contributing stimuli. In addition, the actual status of muscle cells potently contributes to the final outcome. For example, the energy status of muscle tissue (i.e. glycogen depleted) influences the activation of the main energy sensor AMPK which is an inhibitor of the mTOR/p70s6K stimulated protein synthesis. Strength exercise with either filled or depleted glycogen stores leads to a distinct immediate response pattern with regard to the molecular signaling events (Pilegaard et al. 2002).

Physical Training

Summary

Physical exercise represents a potent stimulus for physiological, structural and molecular adaptation, systemically and peripherally. In muscle tissue endurance training (ET) elicits drastic metabolic and morphological changes, including activation and/or repression of specific signaling pathways, mitochondrial biogenesis, fast-to-slow fiber-type transformation and pathways regulating exercise-induced gene expression and substrate metabolism (Holloszy et al. 1977; Hoppeler and Fluck 2003; Irrcher et al. 2003; Zierath and Hawley 2004). As opposed to strength training (ST), ET induces mitochondrial biogenesis and capillary proliferation without muscle hypertrophy (Henriksson 1992; Hoppeler and Fluck 2003). At the muscle cellular level, resistance training results in muscle fiber hypertrophy through an increase in contractile protein accumulation and net myofibrillar protein synthesis (MacDougall et al. 1979; Phillips et al. 1997; Tang et al. 2008; Kumar et al. 2009). Although, ET and ST share common signaling pathways their activation differs drastically in kinetics and magnitude due to the competitive and inhibitory nature of major signal integrators (i.e. AMPK, mTOR).

Mode of Contraction: Eccentric versus Concentric Contractions

Every day activities such as walking and running consist mostly of a blend of concentric, isometric and eccentric muscle work depending on muscle group and activity (i.e. walking uphill or downhill). From the force-velocity relationship (figure 2) it is evident that during eccentric contractions at similar angular velocities, muscles are able to generate greater forces than during concentric contractions. This allows for overloading muscles with loads up to 150% of the concentric one repetition maximum. The force-velocity relationship is also valid for single muscle fibers. As a consequence, less muscle fibers have to be activated during eccentric contractions compared to concentric contractions for the same load (Asmussen 1953) as reflected in the much lower electromyograpic (EMG) activity (Seger and Thorstensson 2005). Furthermore this is a reason why eccentric work has a lower energy demand than concentric work for the same workload. Eccentric training is thus especially convenient for elderly to achieve a high mechanical stimulus despite a limited cardiopulmonary performance capacity (Meyer et al. 2003; Mueller et al. 2009). Ageing does not only decrease muscular strength, but also contraction velocity leading to

changes in force-velocity and power-velocity relationships (Raj et al. 2009). Due to high preservation of eccentric force, eccentric exercise does allow an overload of elderly subjects to a relatively high degree despite a strong reduction in isometric and concentric strength (fig. 2) (Poulin et al. 1992). A recent meta analysis by Roig et al. (Roig et al. 2008) comparing eccentric with concentric strength training designs, reported greater effects with regard to muscle hypertrophy and strength gain with eccentric training.

Delayed onset muscle soreness (DOMS) is the pain or discomfort often felt 24 to 72 hours after unaccustomed eccentric exercise and lasting up to 7 days. DOMS likely represents inflammatory processes associated with remodelling initiated with muscle stretch. Yu et al. postulated a causal relation of DOMS was the addition of extra sarcomeres in series leading to elongation of muscle fibers (Yu et al. 2004). Elongation of fibers leads to an increase in physiological cross sectional area (CSA) in pennate muscles if the pennation angle is preserved.

Recruitment pattern of muscle fiber types during concentric contractions is based on the size principle of motoneurons according to Henneman et al. (Henneman et al. 1965; Henneman et al. 1965). The size principle of motoneurons predicts that the smaller motor units, containing type I fibers, are recruited at low levels of force. With increasing tension, larger motor units containing type IIA and IIX fibers, are recruited (Henneman et al. 1965; Henneman et al. 1965). Fiber recruitment order during eccentric work is less profoundly investigated and results are conflicting. While some studies concluded a reversal of the muscle fiber activation with a preferential activation of type II fibers during eccentric muscle work, others reported no difference with regard to muscle fiber activation between concentric and eccentric contractions (Nardone et al. 1989; Enoka 1996; Linnamo et al. 2003; Beltman et al. 2004).

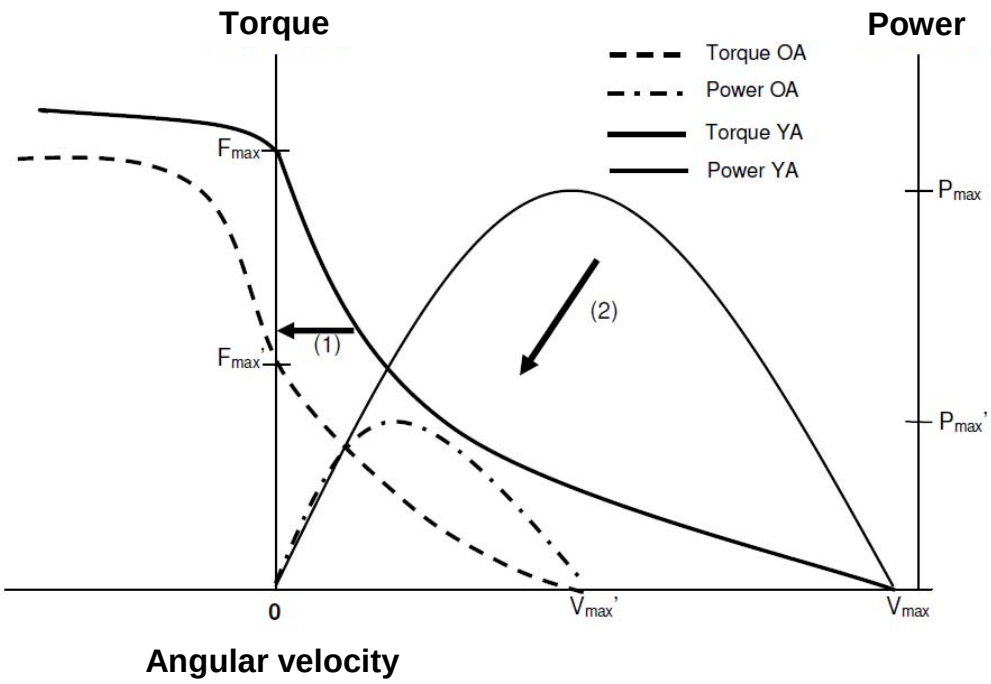

Figure 2: Adapted from Raj et al., Exp. Gerontol. 2009 (Raj et al. 2009): Changes in the force-velocity (1) and power-velocity (2) relationship with ageing. OA = old adults; YA = young adults; Pmax = maximal power; Vmax = maximum contraction velocity; Fmax = peak isometric torque; abbreviations with apostrophes belong to old adults.

Endurance Training

Endurance training (ET) consists of exercise sessions with low torque, repetitive contractions performed over prolonged periods from minutes to hours. ET is the best studied training regime due to its ease of application and its well documented benefits in preventing cardiovascular disease and obesity. ET causes both, central and peripheral adaptations influencing cardiac output, blood composition as well increased peripheral oxidative capacity (Hoppeler and Fluck 2003). Along with increased oxidative capacity (mitochondrial density and capillarity), ET results in elevated muscle glycogen and fat stores. Substrate selection is shifted towards fat oxidation with glycogen sparing during submaximal performance where energy to regenerate ATP derives manly from oxidation of fatty acids. Further physiological adjustments include improved pH buffering capacity accompanied by a higher lactate clearance partly due to an increased expression of monocarboxylate transporters (Sahlin and Henriksson 1984; Dubouchaud et al. 2000). Muscle fiber type composition is shifted towards a greater type I fiber proportion (myosin heavy chain 1(MHC-1) expression) concomitantly with an increased capillarity and mitochondrial density leading to a higher muscular oxidative capacity (Howald et al. 1985).

Oxidative Capacity: Angiogenesis and Mitochondrial Biogenesis

The skeletal muscle response to a higher energy demand is to increase the mitochondria volume density. This in turn requires increased oxygen supply via a higher capillarity. Angiogenesis occurs upon triggers such as local hypoxia, shear stress, stretch (as applied during exercise) by sprouting and/or intusuception and relies on angiogenic factors such as vascular endothelial growth factor (VEGF), reactive oxygen species (ROS) and nitric oxide (NO) among others (Hudlicka and Brown 2009). Mitochondrial biogenesis is a highly regulated and complex process which requires the coordinated expression of many genes encoded by the nuclear and mitochondrial genome (Hood 2009). Endurance exercise induces signaling pathways leading to activation of transcriptional co-activators and transcription factors for mitochondrial biogenesis. Some of these control transcription of "nuclear" genes encoding mitochondrial proteins that have to be imported into mitochondria before processing and assembling. Expression of mitochondrial DNA (mtDNA) must be precisely coordinated since some multi-subunit complexes are composed of both mitochondrial and nuclear gene products (i.e. cytochorme C oxidase) (Hood 2009). PGC-1α is the most prominent transcriptional co-activator of mitochondrial biogenesis. PGC-1α is involved in co-activation of multiple mitochondrial transcription factors including PPARγ. PPARγ primarily regulates lipid homeostasis by induction of genes involved in mitochondrial fatty acid oxidation (Oberkofler et al. 2002; Finck and Kelly 2006; Hood et al. 2006). PGC-1α has a variety of additional functions, among them regulation of antioxidant defences and the response to oxidative stress (St-Pierre et al. 2006). PGC-1α also activates the production of NRF-1 and NRF-2, two nuclear respiratory factors responsible for mitochondrial biogenesis (see figure 3, page 23). Indeed, NRF-1 is a potent transcriptional promoter of TFAM, being responsible for the duplication of mitochondrial DNA (Puigserver et al. 1998). NRF-2 promotes transcription of a variety of nuclear encoded mitochondrial proteins (Hood 2009). Along with mitochondrial biogenesis, PGC-1α also regulates muscle fiber type composition (Handschin et al. 2007). Mechanisms leading to increased levels of PGC-1α and mitochondrial proteins are not fully understood. Daitoku et al. (Daitoku et al. 2003) reported that forkhead transcription factor O1 (FoxO1) promotes transcription of PGC-1α. The calcineurin/Ca^{2+}/calmodulin-dependent protein kinase (CamK) pathway was suggested to be induced by elevated calcium levels as occurring during sustained muscle contraction. With gene promoter studies using cultured myoblasts, Wu et al.

(Wu et al. 2002) reported that, indeed, CamK induced PGC-1α expression. Nitric oxide (NO) produced by NO synthase (NOS) was also suggested to control PGC-1α expression via activation of guanylat cyclase and generation of cGMP (Bossy-Wetzel and Lipton 2003). Reactive oxygen species as produced during exercise are potent activators of MAP kinases and NFκB which promote up-regulation of mitochondrial superoxide dismutase 2 (SOD2) and NOS isoforms (Vina et al. 2009). Besides mitochondrial de novo synthesis, their size and number is controlled by mitofusins, proteins promoting mitochondrial fusion (Santel and Fuller 2001; Santel et al. 2003). Mitofusin-1 a prominent member of this family is up-regulated following endurance exercise probably in via PGC-1α (Soriano et al. 2006). Along with the increased density of mitochondria, expression of genes coding for metabolic proteins (i.e. transporters and enzymes) increases following ET and contributes to the development of an endurance like muscle phenotype (Pilegaard et al. 2000; Mahoney and Tarnopolsky 2005).

The immediate response upon a single bout of endurance exercise shows an initial drop in muscle specific transcript abundance followed by a rapid recovery and supercompensation within 4 to 8 hours. Transcription and expression of metabolic genes (carbohydrate and fat metabolism) consistently peak in the initial hours of recovery and return to resting levels within 24 hours (Pilegaard et al. 2000; Schmutz et al. 2006). Based on this observation it has been postulated that the cumulative effect of a transient up-regulation with many bouts of exercise may be an underlying mechanism for exercise-induced adaptation with endurance training (Pilegaard et al. 2000; Fluck and Hoppeler 2003).

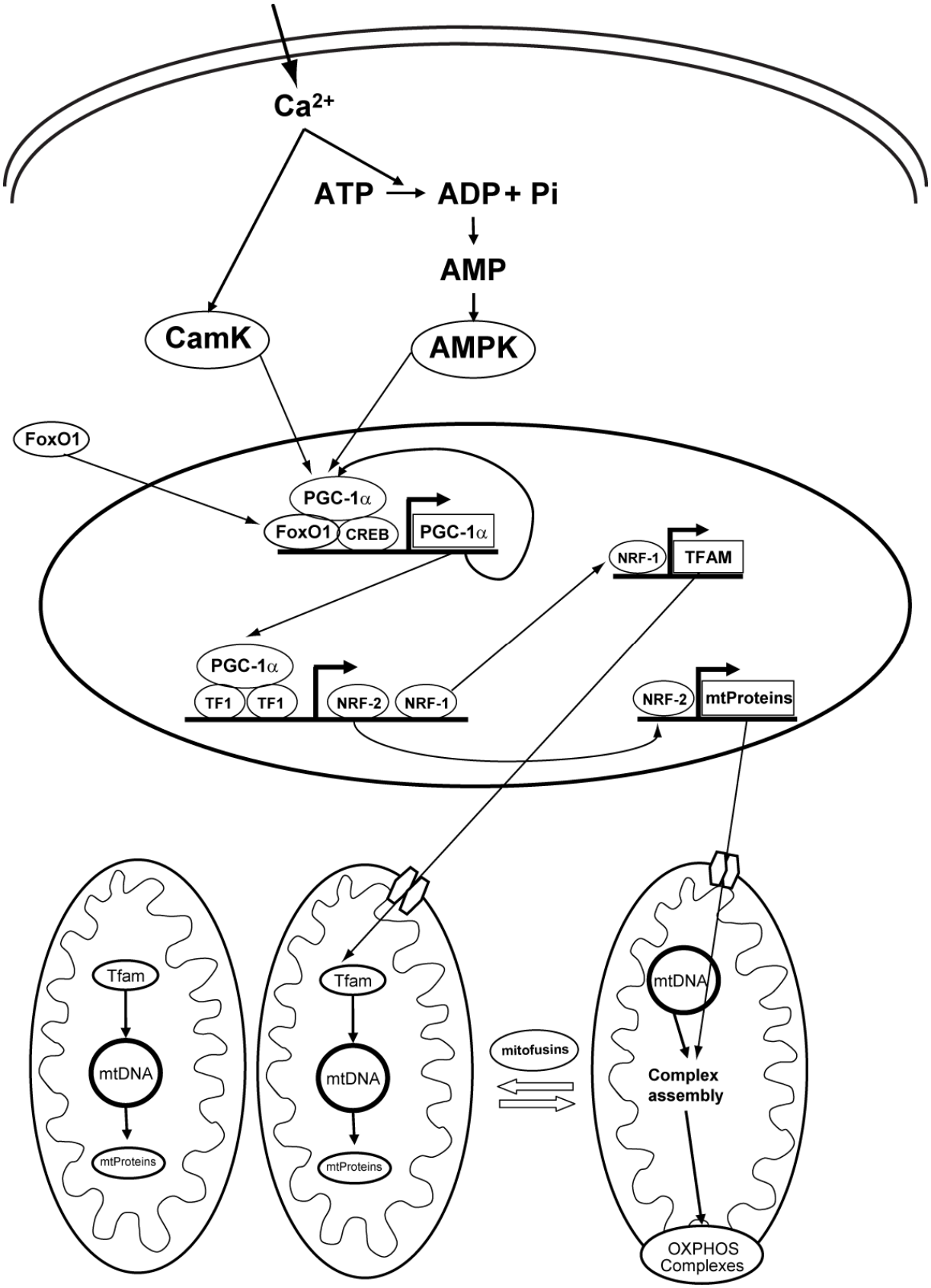

Figure 3: A simplified depiction of the pathways and key factors regulating mitochondrial biogenesis. Modified from (Hood et al. 2006). For details see text pages 21 et seq.

Strength Training

Strength training consists of high force, low volume repetitive contractions with the aim of mechanically overloading the muscles. Muscle strength is dependent on the physiological cross sectional area (CSA) of muscles and neuronal activation mechanisms. Strength training (ST) improves neuronal activation strategies and CSA if applied with appropriate intensity. Many ST regimes are successfully applied: concentric, isometric and eccentric exercises with different number of repetitions, breaks and series but all with the common theme, emphasizing mechanical stress on muscles. Eccentric training seems to be favourable with regard to muscle growth and strength gains. This is because muscles can be overloaded by up to 150-200% of the concentric one repetition maximum, and because of its' low energy demand (Roig et al. 2008) (see also pages 18 et seq.).

Response upon Mechanical Stress

Mechanical stress must be translated into biochemical signals to initiate intracellular responses. This process is called mechano-transduction. Mechano-transduction acts via the engagement of different mechanical sensors. Among them, focal adhesion kinase (FAK, sensitive to shear stress) has been shown to specifically phosphorylate and activate mTOR and downstream effectors upon mechanical stimulation, resulting in acute changes of the rate and specificity of protein synthesis and degradation (Klossner et al. 2009). The mTOR/ p70Ss6-Kinase pathway is one of the key regulators of muscle mass upon mechanical stress (Wilkinson et al. 2008). mTOR integrates signals from growth factors, nutrients, energy status and mechanical stress to activate downstream targets/effectors such as p70s6-Kinase (see also figure 4, page 25 and figure 5, page 68). The mTOR pathway is activated in a PI3-Kinase (PI3K)/Akt (aka protein kinase B, PKB) dependent manner via growth factors (IGF1; MGF, Insulin), where the engagement of membrane bound receptors activate PI3K to generate phosphatidylinositol 3-phosphate (PIP3) which binds to and recruits Akt to the plasma membrane. Akt is activated via phosporylation by different kinase-complexes and promotes protein synthesis by mTOR activation and inhibition of glycogen synthase kinase 3β (GSK3 β). mTOR activates p70s6K, resulting in an increased translational capacity by the phosphorylation of mRNA's encoding elongation factors and ribosomal proteins. p70s6K also initiates translation by inhibition of 4E-BP1, a translational repressor (Ruvinsky and Meyuhas 2006). Most notably,

strength exercise specifically increases myofibrillar protein synthesis and to a lesser extent sarcoplasmic protein synthesis rate (Cuthbertson et al. 2006). However, the control of this specificity is not yet understood.

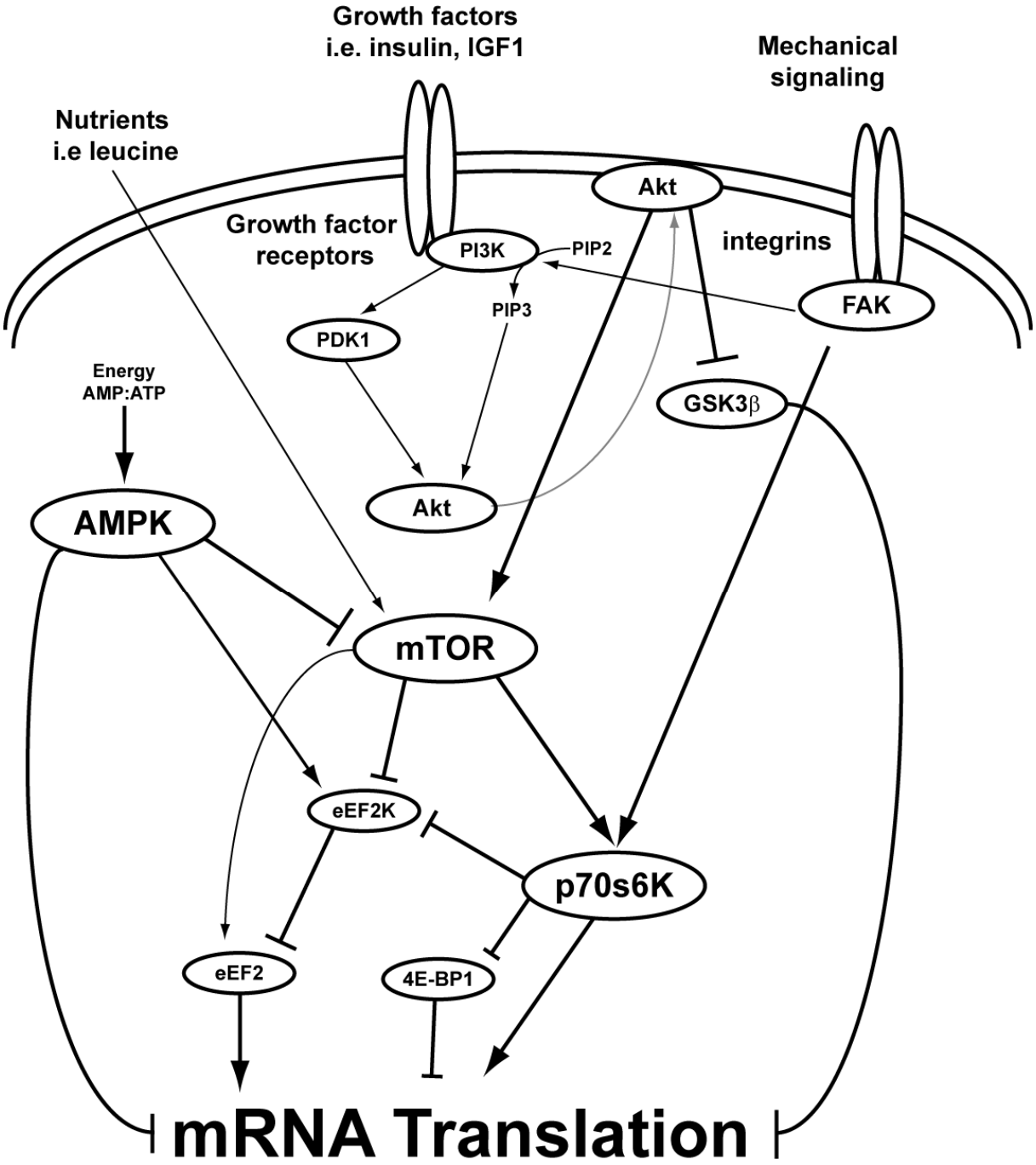

Figure 4: Simplified diagram of the signaling pathways leading towards increased protein synthesis. For explanations see text.

It was also suggested that mechanical stress directly regulates gene expression in the nucleus (Lange et al. 2005). Titin a giant protein spanning half of the sarcomere is involved in recruitment and phosphorylation of MuRF1 its kinase domain located at the M-band signalosome. The kinase domain of titin is active only in conditions of applied stretch and muscle contraction. Under normal conditions the signalosome is dissociated and muscle ring finger protein 2 (MuRF2) translocates to the nucleus where it inhibits serum response factor (SRF), a transcription factor that usually promotes growth and differentiation. During exercise though, MURF2 resides at the sarcomere and SRF can act as a transcription factor. Muscle ankyrin repeat proteins (MARP) are also hypothesized to link the sarcomere to the nucleus. MARP's are located at the I-band in complexes with calpain3 (p94), titin and connection (Hayashi et al. 2008). In response to stretch (as occurring during eccentric contractions), akyrin repeat domain protein 2 (Ankrd2, a MARP family member) translocates to the nucleus where it induces transcription of target genes (Pallavicini et al. 2001; Kojic et al. 2004). The involvement of p94 in releasing Ankrd2 is hypothesized and seems reasonable since p94 is very sensitive to calcium concentrations and activated by elevated calcium levels as occurring during sustained muscle contractions (Murphy 2009).

Phenotypical Adaptation: Muscle Hypertrophy

A main characteristic of a strength exercise response is an immediate elevation of protein synthesis manly driven by increased mRNA translation. However, long term rate of protein synthesis can be controlled by changing the abundance of mRNA transcripts, by rate of transcription and degradation (stability). Downstream of mRNA levels, protein synthesis is controlled by modulation of translation initiation, elongation and termination. Additionally, cellular ribosome content controls translation efficiency (proteins per mRNA) (Farrell et al. 2000; Richter and Sonenberg 2005). As formerly described, translation initiation is suggested to be controlled in a short term by mechanical stress activated kinases as well as in the longer term by growth hormones such as insulin like growth factor 1 (IGF-1) (Richter and Sonenberg 2005). Most of the circulating IGF-1 is produced by the liver as a result of growth hormone (GH) stimulation via GH-receptors. Other organs including muscle tissue are also able to produce IGF-1 in a GH dependent manner. As a consequence of mechanical stimulation muscle specific IGF-1Ea (similar to a systemic isoform) is alternatively spliced and translated into a protein with a slightly altered C-terminus and called a

mechano growth factor (MGF)(Goldspink 2005). Exclusively MGF, but not IGF-1Ea, is up-regulated with eccentric exercise. MGF is also hypothesized to be more potent in activating satellite cells than IGF-1Ea (Hameed et al. 2008). Hameed et al. reported a blunted MGF transcript up-regulation with resistance training in elderly suggesting an age-related desensitization to mechanical loading. Another mechanism for reduced responsiveness to IGF-1 in elderly could include a lower expression of IGF-1 receptors or IGF-1 binding proteins, however, there is evidence that the local responsiveness is maintained with ageing (Lissett and Shalet 2003). Lower levels of circulating GH might also contribute to reduced levels of hepatic and extra-hepatic synthesized IGF-1. Exogenous supplementation of GH increases IGF-1 but not MGF mRNA levels. However, combined with resistance training splicing of IGF-1 towards MGF is favoured (Hameed et al. 2003). Due to inconsistent reports in the literature with regard to IGF-1 and MGF, these will be collectively mentioned IGF-1 henceforth. IGF-1 promotes initiation of translation via activation of the PI3K/Akt/ mTOR-pathway and is thought to be the major factor controlling general protein synthesis with strength training (Shen et al. 2005; Vary 2006). Besides the direct action of IGF-1 towards translationally enhanced protein synthesis it causes also satellite cell activation, recruitment, proliferation and increased life span (Chakravarthy et al. 2000; Chakravarthy et al. 2000). Satellite cell activation is accompanied by the expression of myogenic regulatory factor family of transcription factors (MRF) and cell cycle kinases being responsible for the activation of quiescent satellites and promoting proliferation and differentiation (Zammit et al. 2006). Messenger RNA expression of myogenic differentiation factor (MyoD) and myogenin (MyoG), the two most prominent members of the MRF family is increased in response to mechanical overload in skeletal muscle and significantly correlate with the observed muscle growth (Ishido et al. 2004; Petrella et al. 2006). Skeletal muscle MyoD and MyoG mRNAs are already up-regulated after a single bout of resistance exercise and remain elevated with long-term resistance training (Adams et al. 1999; Haddad and Adams 2002; Bickel et al. 2005; Kosek et al. 2006). These factors might contribute to the observed continuous muscle growth with resistance training. Hepatocyte growth factor (HGF) is another growth factor which activates quiescent satellite cells. Active HGF is stored in the extracellular matrix (ECM) and then released upon exercise with muscle contraction (Tatsumi and Allen 2004). Androgens such as testosterone are well known to induce muscle hypertrophy and are consequently abused in competition sports. Androgens

act via the engagement of androgen receptors expressed by satellite cells and increase expression of myogenic factors. Androgens also have glucocorticoid antagonistic effects (Zhao et al. 2004; Solomon and Bouloux 2006). Using animal models, androgens were suggested to act by increasing muscle specific expression of IGF-1 (Lewis et al. 2002).

Myostatin (MSTN, also known as growth differentiation factor 8, GDF8) belongs to the transforming growth factor β (TGFβ) family of cytokines that inhibit muscle differentiation and growth. Myostatin is primarily produced by skeletal muscle and secreted into the bloodstream. Circulating myostatin acts on skeletal muscle tissue by binding membrane-bound activin type II receptors and influencing subsequent smad signaling. Myostatin was suggested to function mainly by inhibiting satellite cell activation via activation of smad2, smad3 and smad4 (Zhu et al. 2004). The latter smad transcription factors and FoxO1 directly promote transcription of the myostatin gene by binding to its promoter region (Allen and Unterman 2007). Follistatin, an endogenous antagonist of myostatin, contributes to satellite cell activation and inhibition of myostatin action (Gilson et al. 2009). Defects in the myostatin gene or defects which suppress myostatin expression or effects may lead to double muscled phenotype as seen in Belgian blue cattle, texel sheep, and this has also described in humans (McPherron and Lee 1997; Schuelke et al. 2004; Georges et al. 2006). Fiber type specific adaptations with regard to “strength type” mechanical signaling have not yet been elucidated but due to the predominant responsiveness of type II fibers to strength training it is obvious that hypertrophic signal integration occurs mostly in these fibers.

Micro RNA’s

Micro RNA’s are small non-coding RNA’s characterized by the association with Argonaute (AGO) proteins and by their function to guide these RNA/protein complexes to specific nucleic acid sequences (Ambros 2004; Meister and Tuschl 2004; Sontheimer and Carthew 2005). MiRNAs base pair with complementary sequences of untranslated regions from target mRNA’s leading to the formation of the so called RNA induced silencing complex (RISC). The RNA-mediated silencing machinery functions by silencing RNA in various regulatory networks, thus regulating gene expression by the degradation of RNA, inhibition of translational initiation and elongation, co-translational protein degradation, and premature termination of translation

(ribosome drop-off). Vital functions of the RNA silencing comprise also the control of transposable elements and viruses, epigenetic alterations in DNA and chromatin structure as well as accurate chromosome segregation during cell division (Meister and Tuschl 2004; Wassenegger 2005; Valencia-Sanchez et al. 2006; Eulalio et al. 2008). More than 500 human miRNAs are currently known, of which most are evolutionarily conserved among species. Computer-based calculations estimate that miRNA's make up to 2%–3% of all genes in the genome but functions and targets of most miRNAs are still unknown (Griffiths-Jones 2004). It was hypothesized that up to one third of all human genes are targets for endogenous small RNAs (Lewis et al. 2005).

Micro RNAs are processed similar to messenger RNAs. They are transcribed by RNA polymerase II as long primary transcripts that are capped, polyadenylated and spliced (Rodriguez et al. 2004; Kim 2005). They are further processed in two steps by the nuclear (Drosha) and cytosolic RNase III-type endonuclease (Dicer) leading to 19–22 nucleotide long duplex mature miRNA. In the RISC, one of the strands of mature miRNA is eliminated and the remaining base pairs with its seed bind to specific sequences in their target mRNAs.

MiRNAs that are localized within genes (intronic or exonic) are co-ordinately expressed with their host-genes. Those localized in non-coding regions function as independent transcriptional units and posses their own promoter regions (Rodriguez et al. 2004). While some miRNAs are ubiquitously expressed, others show a cell or tissue specific expression pattern (Lagos-Quintana et al. 2002). Nowadays, miRNAs are seen as key regulators in controlling development, tissue differentiation and maintenance of tissue specificity during embryogenesis and adult life (Ambros 2004; Filipowicz 2005).

Most of basic muscle specific miRNA research was done using C2C12 myoblasts as a model system in which over-expression and knockdown experiments have been performed to study miRNA functions (Chen et al. 2006; Kim et al. 2006). Muscle specific miRNAs miR-1, miR-133, miR-206, and miR-208, are largely regulated by evolutionarily conserved muscle transcriptional networks involving serum response factor (SRF), myogenic differentiation factor (MyoD), myocyte enhancer factor 2C (MEF2C),

and myocardin (Zhao et al. 2005; Chen et al. 2006; Rao et al. 2006; Rosenberg et al. 2006; van Rooij et al. 2007). MiR-133 expression which is restricted to muscle tissue is under the control of muscle specific transcription factors. Promoter analyses revealed that the miR-133 locus has upstream enhancers with SRF binding sites, and that its expression is controlled by MyoD (Zhao et al. 2005; Chen et al. 2006). Similarly, miR-206 expression is initiated by MyoD a transcription factor which is involved in the terminal differentiation of skeletal muscle (Rosenberg et al. 2006). Cardiac muscle specific expression of miR-208 is due to its intronic localization in the alpha myosin heavy chain gene (αMHC) constitutively expressed in the heart (van Rooij et al. 2007). In skeletal muscle tissue, miR-1/miR-206 and miR-133 have opposite effects with regard to proliferation and differentiation. Over-expression of miR-133 repressed myoblast differentiation but promoted proliferation and vice versa with over-expression of miR-1 and/or miR-206. Interestingly, miR-1 and miR-133 derive from the same miRNA polycistron and are transcribed simultaneously but have obviously opposing effects which could lead to a fine tuned balance between cell proliferation and differentiation. In a functional mouse model of skeletal muscle hypertrophy, expression levels of miR-1 and miR-133 were decreased after muscle overload (McCarthy and Esser 2007). Expression levels of exportin 5 and drosha were elevated in this overload model, excluding the possibility of a general down-regulation of miRNA processing (McCarthy and Esser 2007). Histone deacetylase 4 (HDAC4) is a verified target of miR-1 and was reported to inhibit skeletal muscle differentiation and gene expression partly by repressing MEF2C (Lu et al. 2000; Lu et al. 2000). In marked contrast, miR-133 enhances myoblast proliferation by reducing protein levels of SRF (Miano 2003). Down-regulation of miR-1 and miR-133 may result in increased protein abundance of important muscle growth/differentiation regulators such as c-Met, HGF, IGF-1 and SRF (all potential targets)(Callis et al. 2008). IGF-1 is a very potent initiator of muscle hypertrophy and has potential binding site for miR-1 in its 3'-untranslated region (3'-UTR) (Callis et al. 2008). However, it remains to be elucidated if IGF-1 is really a bona fide target. The fact that, exclusively IGF-1 protein levels, but not IGF-1 mRNA levels are significantly elevated 12h after overload in mice, support the hypothesis of predominantly miRNA-1 based translational regulation (Callis et al. 2008).

MiR-206 was reported to contribute to the double-muscled phenotype of Texel sheep. Quantitative trait loci (QTL) mapping revealed Texel sheep having a single nucleotide polymorphism (SNP) creating a functional miR-206 target site the 3'-UTR of the myostatin mRNA (Georges et al. 2006). Consequently, translation of the myostatin mRNA is repressed by miR-206, resulting in a loss of approximately 70% of myostatin in the serum of Texel sheep. This latter case identifies the de novo creation of a target site (by a single nucleotide polymorphism (SNP)) which usually doesn't exist. Myostatin does therefore not represent a bona fide target of miR-206.

Expression of miRNA's with strength exercise

MiRNAs are undoubtedly important regulators of developmental programs in different tissues including muscle tissue. Research has focused on proliferation, differentiation and regeneration but to our knowledge there exist to date no data regarding short term response of miRNA expression upon a single bout of strength exercise. Bioinformatic modelling as well as observations using cell culture experiments and animal models suggest mechanisms how micro RNA's could efficiently orchestrate the coordinated expression of target genes responsible for muscle hypertrophy, i.e. IGF-1 (Callis et al. 2008). Nevertheless, the existence and relevance of such mechanisms still needs to be explored with molecular tools in human studies.

2. Methods and Results

Within the National Research Programme 53 "musculoskeletal health, chronic pain", we performed a study in which we compared functional, structural and molecular outcomes of conventional resistance (RET) and eccentric ergometer training (EET). A control group performed a non-physical cognitive training on computers to account for the social interactions which could positively affect subject's performance and health (Buschkuehl et al. 2008). EET was carried out on a motor driven eccentric cycling ergometer where the appropriate matching of the eccentric target load had to be self-monitored on a screen by the subjects. RET consisted of four classical exercises for the lower extremity while control subjects (CT) did a non-physical computer based cognitive training task (see (Mueller et al. 2009)). Subjects were tested before and after the training intervention for functional, structural and molecular parameters. For the estimation of muscle structure and muscle specific gene expression, biopsies were collected from a knee extensor muscle (*musculus vastus lateralis*). Subjects were additionally tested one year after the training intervention to monitor persistence of the training related benefits. During this year, no guided training session were provided.

In the first paper we reported improvements with EET in eccentric coordination (-43% RMS), maximal isometric extension strength of the legs (+8.4±1.7%) and body composition (-5.0±1.1% body fat, -6.9±1.5% thigh fat). RET and EET increased the relative thigh lean mass by 2% and 3% respectively. Type IIX/type II muscle fiber content significantly decreased with EET (-22±14%) and correlated with body composition parameters. Results are discussed in the following paper which has been published in the European journal of applied physiology.

Paper I: "*Different response to eccentric and concentric training in older men and women*"

Mueller, M., F. A. Breil, M. Vogt, R. Steiner, K. Lippuner, A. Popp, S. Klossner, H. Hoppeler and C. Däpp (2009). Eur J Appl Physiol. (referred to as (Mueller et al. 2009)). Pages 33 to 41.

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ORIGINAL ARTICLE

Different response to eccentric and concentric training in older men and women

Matthias Mueller · Fabio Andreas Breil · Michael Vogt · Roger Steiner · Kurt Lippuner · Albrecht Popp · Stephan Klossner · Hans Hoppeler · Christoph Däpp

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Abstract Sarcopenia is the age-related loss of muscle mass and strength and has been associated with an increased risk of falling and the development of metabolic diseases. Various training protocols, nutritional and hormonal interventions have been proposed to prevent sarcopenia. This study explores the potential of continuous eccentric exercise to retard age-related loss of muscle mass and function. Elderly men and women (80.6 ± 3.5 years) were randomized to one of three training interventions demanding a training effort of two sessions weekly for 12 weeks: cognitive training (CT; $n = 16$), conventional resistance training (RET; $n = 23$) and eccentric ergometer training (EET; $n = 23$). Subjects were tested for functional parameters and body composition. Biopsies were collected from *M. vastus lateralis* before and after the intervention for the assessment of fiber size and composition. Maximal isometric leg extension strength (MEL: +8.4 ± 1.7%) and eccentric muscle coordination (COORD: −43 ± 4%) were significantly improved with EET but not with RET (MEL: +2.3 ± 2.0%; COORD: −13 ± 3%) and CT (MEL: −2.3 ± 2.5%; COORD: −12 ± 5%), respectively. We observed a loss of body fat (−5.0 ± 1.1%) and thigh fat (−6.9 ± 1.5%) in EET subjects only. Relative thigh lean mass increased with EET (+2.5 ± 0.6%) and RET (+2.0 ± 0.3%) and correlated negatively with type IIX/type II muscle fiber ratios. It was concluded that both RET and EET are beneficial for the elderly with regard to muscle functional and structural improvements but differ in their spectrum of effects. A training frequency of only two sessions per week seems to be the lower limit for a training stimulus to reveal measurable benefits.

Keywords Eccentric · Elderly · Strength · Fat · Coordination · Fibertyping

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Introduction

Sarcopenia is a condition of muscle tissue characterized by the loss of muscle fibers and fiber atrophy (Doherty 2003; Lexell 1995) accompanied by increased infiltration of non-contractile components such as connective tissue and fat (Overend et al. 1993). These structural changes along with impaired neuronal functions result in loss of muscle strength (Skelton et al. 1994). The mechanisms underlying muscle atrophy and loss of innervation are not fully understood. Reduced physical activity, decline in anabolic hormone levels (dehydroepiandrosterone, testosterone, growth hormone) concomitant with a chronic low-grade inflammation (increased tumor necrosis factor α and cortisol serum levels) contribute to the loss of muscle mass (Doherty 2003; Vandervoort 2002). Improvement of leg strength by means of strength training is a broadly applied strategy to reduce the risk of falling, since these two parameters seem causally associated (Perry et al. 2007; Shigematsu et al. 2006). Heavy resistance training can successfully be applied in the elderly (Hruda et al. 2003; Wieser and Haber 2007). However, it can result in significant cardiovascular as well as substantial mechanical stress on single joints

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(Hungerford and Barry 1979; Kaufman et al. 1991). On the other hand, endurance ergometer training, carried out in a closed muscle chain at high-angular velocities, has a broad application, improving body composition and insulin sensitivity (Hersey et al. 1994), but with minor benefits for strength and muscle mass, as mechanical stress on muscles remains low in endurance cycling.

It is well documented that strength training in the elderly results in substantial strength gain and muscle hypertrophy (Kryger and Andersen 2007). A recent meta-analysis by Roig et al. (2008) evaluated 20 studies comparing eccentric and concentric strength training and concluded that subjects profited more from eccentric than concentric training with regard to strength gain and muscle hypertrophy. These studies compared maximal eccentric training protocols applied to young adults and trained athletes and draw attention to the prospects of eccentric exercise modalities. In view of potential benefits of eccentric exercise we explored a training protocol of continuous eccentric exercise and compared it to established conventional strength training protocols. The eccentric exercise training (EET) consisted of resisting the pedal movement of a motor driven recumbent ergometer. As the energy cost of eccentric work is approximately four times less than that of concentric work of a comparable external load (Lastayo et al. 1999) large torques can be exerted at a manageable metabolic cost. The ensuing low stress on the cardiovascular system is particularly important for elderly, since they are characterized by a reduced aerobic capacity (Lotscher et al. 2007). The objective of using EET thus was to achieve a high mechanical load on muscle tissue with a restricted aerobic demand. Similar to concentric ergometer training, EET is executed in a closed muscle chain at relatively high-angular velocities, minimizing peak forces on single joints (Ericson and Nisell 1986, 1987). Due to these favourable features, eccentric exercise has been applied to people with a limited tolerance for conventional strength training (LaStayo et al. 2003), chronic obstructive pulmonary disease (Rooyackers et al. 2003) and coronary disease (Steiner et al. 2004). As a drawback, eccentric exercise is potentially associated with delayed onset muscle soreness (DOMS) due to muscle tissue damage (Friden et al. 1983). In the present study, muscle damage was avoided by carefully increasing muscle load over repeated exercise sessions beginning with very low eccentric loads.

It has been suggested that strength gain in the elderly is more related to neural mechanisms and less to muscle hypertrophy (Moritani and deVries 1980). Using an intensive exercise protocol, Kryger and Andersen (2007) still reported a significant increase of the type IIA fiber area and an impressive 37% strength gain in subjects, aged 85 and older, after a 12 week training period with three heavy resistance training sessions (45 min each) per week, indicating the maintenance of muscular plasticity on a structural as well as on a functional level even in very old people.

The aim of this study was to investigate EET as an alternative to conventional resistance training for the elderly to increase leg strength and leg muscle mass. This was done in a setting in which subjects were asked to perform only two sessions/week to maximize adherence. Based on the study of LaStayo et al. (Lastayo et al. 2002), we hypothesized that EET would be more effective in increasing muscle strength and mass than conventional resistance training. We further expected larger improvements of muscle strength in females than in males due to their initial lower fitness level (Lotscher et al. 2007). We did not expect any changes in muscle fiber type composition as the imposed training regime (2×20 min effective training time per week for 12 weeks) seemed to be insufficient to produce fiber type changes.

Materials and methods

Subjects and study design

A total of 62 subjects (71–89, average 80.6 years) with stable medication and health conditions were included in the study. Subjects with severe neuromuscular disease, instable coronary disease or severe hip or knee arthritis were excluded (Lotscher et al. 2007). The study was part of the National Foundation Program 53 ''Musculoskeletal health and chronic pain'' and carried out in accordance with the guidelines and the approval #190/04 of the "Kantonale Ethische Kommission".

The training period lasted for 12 weeks and comprised two guided training sessions (45 min each) per week. In order to familiarize the subjects with the functional tests they were sham tested 2 weeks before the real testing procedure. Subjects from whom biopsies were collected were randomized to one of the physical intervention groups and all others were randomly distributed as follows:

1. Cognitive training (CT) consisted of computer-guided cognitive training. The subjects (10 women, 6 men) did not perform any physical training and served as a control to account for the influence of social aspects of the training sessions.
2. Conventional resistance training (RET) was performed by 23 subjects (13 women, 10 men). RET was carried out in a gym and comprised four exercises for the lower extremity (leg press, knee extension, leg curl, hip extension). The sessions consisted of a 10-min warm-up with cardiovascular activation and gymnastics, 20 min training and 10 min cool-down with stretching. For the first six sessions the individual loads were set very low to familiarize subjects with the exercises.

Exercises included three sets with ten repetitions and loads were gradually increased during this time. The subsequent sessions consisted of one warm up set and two sets with eight to ten repetitions. If subjects were able to do ten repetitions or more, the load was increased in the next session. The load was not increased if people suffered from DOMS as indicated by scores ≥3 on a visual analog scale (VAS) (Langley and Sheppeard 1985) or when rating of the perceived exertion (RPE) of the whole training session was >13 according to BORG (Borg et al. 1987). VAS ranges from 0 to 10, where 0 is no soreness and 10 is the highest perceived muscle soreness. DOMS ratings of subjects were in the interval between 0 and 4.

3. Eccentric ergometer training (EET) was carried out by 23 subjects (13 women, 10 men) on a custom-built motor-driven ergometer (Meyer et al. 2003). The trainings started with a 10-min warm-up on a conventional ergometer with minimal loads (females 10 W, males 20 W) and closed with 10 min cool-down with stretching, while the actual EET lasted 20 min. The initial load on the eccentric bike was set very low (females 30 W, males 50 W). Initially, subjects exercised for only 5 min to prevent severe DOMS. During the first sessions the training duration was gradually increased in 5-min steps until it reached 20 min, before the imposed load was ramped. Load was ramped in consecutive sessions by 20% of the individual maximal power output achieved in the initial ergometer ramp test to exhaustion (Lotscher et al. 2007). Contraindications to increase the workload were the same as those for RET (DOMS; RPE).

Some of the subjects had to be partly or entirely excluded from physical tests due to illness such as *herpes zooster* (1 woman EET), *appendicitis* (1 woman CT), *osteoporosis* (1 woman CT), progressive *morbus Alzheimer* (1 woman CT, 1 woman RET) or injuries and persisting joint pain (1 man CT, 1 woman RET, 1 man RET, 1 man EET). Other subjects were excluded because they were unable to complete the required test (MEL: 1 women CT, 1 man RET, 1 woman EET) or because they were unable to follow the training protocol (1 woman EET was not able to dose the eccentric ergometer). Compliance in the sessions was secured by coaches (at least one coach per two subjects). Subjects attended on average 89 ± 2% of the training sessions.

Subjects were specifically instructed to continue their usual diet. However, no written reports on the dietary regime were obtained.

Specific training loads

Changes of training loads were assessed by the comparison of the loads after the 3 weeks of habituation to those at the very end of the intervention for RET and EET. For RET the average loads of the four exercises were compared.

Timed up & go and Berg balance scale

The Berg balance scale (BBS) (Berg et al. 1992) and the timed up & go (TUG) (Shumway-Cook et al. 2000) are functional tests designed for elderly people in order to assess their risk of falling (Lotscher et al. 2007). These tests were carried out at the beginning and at the end of the training period.

Body composition, muscle biopsies

Whole body composition (lean and fat tissue mass) was determined by dual energy X-ray absorptiometry (DEXA) (QDR-4500A, Hologic Inc., Bedford, USA). Thigh was defined as the part from *tuber ischiadicum* to the distal end of the femur, whereas the leg included the distal section of the limb below the *tuber ischiadicum*. Fat and lean values of thighs and legs include right and left extremities.

Biopsies were taken from 27 subjects (RET: 6 women, 7 men; EET: 7 women, 7 men) using the Bergström technique (Bergstrom 1975) from the mid thigh position of the M. *vastus lateralis* before and after the 12-week training period in a resting state, 48–72 h after the last exercise bout. Pre- and post-biopsies were collected from the same leg with the incision approximately 2 cm apart. For ethical reasons muscle biopsies were taken from subjects of the physical intervention groups only. Muscle samples were immediately frozen in liquid nitrogen cooled isopentane and stored in liquid nitrogen until required for further analysis.

Histochemistry

Selective myofibrillar ATPase inactivation and subsequent staining was processed at 12 µm cryostat cross-sections with preincubation at pH 4.5 and 10.5 as described by Billeter et al. (1980). Fibers were classified as type I, type IIA and type IIX. The type II fiber population consists of the sum of the type IIA type IIX fibers. On average, 351 fibers were counted per biopsy. Reliability of the technique and the technician was assessed by the test–retest method with 10 randomly chosen biopsies. Pearson product–moment correlation coefficient was 0.95. For the estimation of fiber type specific cross sectional areas a 30 × 30 µm grid was overlaid and points on fibers were counted. This procedure was applied in areas that appeared reasonably cross-sectioned (80 fibers per biopsy on average).

Maximal isometric extension of the legs (MEL)

Strength testing was performed as described by Lötscher et al. (2007). Subjects were fixed in a sitting position

(90° angle; ankle-knee-hip) on a force platform (Quattro Jump, Kistler Instrumente AG, Winterthur, Switzerland). They were verbally encouraged to push maximally against the platform for about 4 s. The force was permanently recorded with a resolution of 500 Hz. The best trial out of three was evaluated by determining the highest mean force over a one-second period. Normalization to the subject's body mass resulted in relative MEL.

Eccentric coordination

Estimation of eccentric coordination was carried out on the eccentric ergometer (Meyer et al. 2003). This parameter estimates a subject's ability to adjust the power of braking the pedals to the eccentric target load [W]. The appropriate load is self-monitored on a screen where the actual and the target load are graphically displayed in "real time" (Fig. 1). The deviation of actual from target load is estimated by the Root Mean Square (RMS; indicated by the hatched area in Fig. 1). The target load in the testing was set to 30 W for females and 50 W for males; subjects were tested over a 5-min period. In the EET group the post-test for eccentric coordination was carried out at the individual training load of each subjects' last training session (Table 1, 2).

Data analysis

Data are presented as mean ± SE. Interaction of training modality (CT, RET, EET) on functional parameters such as TUG, BBS, MEL and eccentric coordination was verified with an analysis of variance (ANOVA) for repeated measures and Tukey's Honest Significant Difference (HSD) post hoc test. For the ANOVA, the level of significance was set to $P < 0.05$ and marked with * and ** for $P < 0.01$, respectively. *P*-values in the figures are from Tukey's HSD and indicate the probability that differences between pre and post-intervention occur randomly. Analysis of sex specific improvements in MEL was analyzed in the eccentric group only using ANOVA with repeated measures. For the comparison of pre-post differences between groups we applied a Kruskal–Wallis ANOVA and verified significant results ($P < 0.05$) with Mann–Whitney *U* post hoc testing

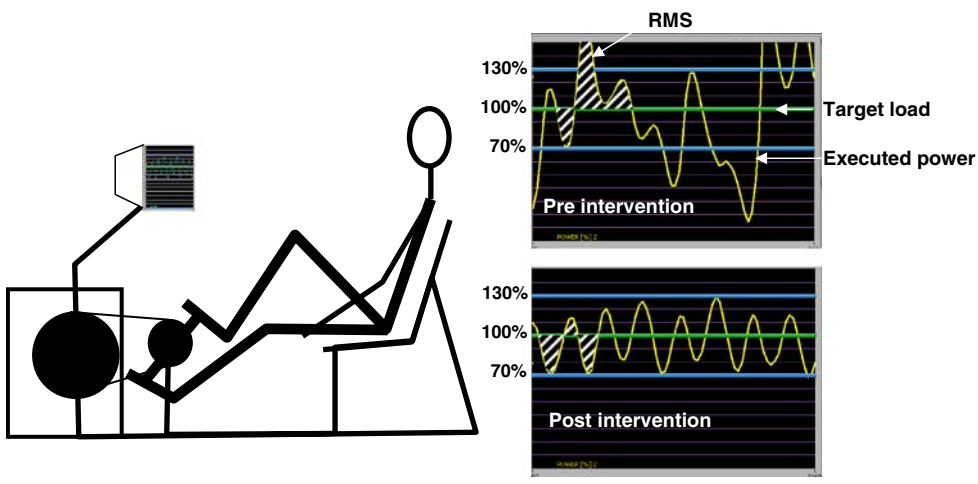

Fig. 1 Schematic set-up of the eccentric ergometer and evaluation of eccentric coordination. Root mean square (*RMS*) represents the hatched area comprised by the target load and the executed power. The smaller the RMS, the better the eccentric coordination. Representative illustrations of pre- and post-test graphs from EET subjects. Note the difference of the match of the executed power and the target load in the post-test

Table 1 Anthropometric characteristics of the study subjects

	Women (36)	Men (26)	CT (16)	RET (23)	EET (23)
Age (years)	80.4 ± 0.6	80.9 ± 0.6	81.8 ± 0.8	80.1 ± 0.8	80.3 ± 0.7
Height (cm)	161 ± 1	176 ± 1	166 ± 3	167 ± 2	168 ± 2
VO_{2max} (ml/min)	1210 ± 40	1940 ± 90	1380 ± 150	1640 ± 110	1500 ± 80
Body mass (kg)	65.5 ± 1.9	71.1 ± 1.7	70.5 ± 2.8	67.7 ± 2.6	66.1 ± 1.8
BMI (kg/m^2)	25.3 ± 0.7	23.1 ± 0.6	25.6 ± 0.9	24.3 ± 1.0	23.5 ± 0.6
Lean (kg)	42.7 ± 1	53.6 ± 1.3	48.4 ± 2.5	47.3 ± 1.7	46.5 ± 1.6
Fat (kg)	21.0 ± 1.3	15.4 ± 1.1	18.9 ± 1.3	19.1 ± 2.0	17.6 ± 1.3
Fat rel (%)	31.7 ± 1.1	21.2 ± 1.3	29.3 ± 2.1	26.7 ± 1.9	26.5 ± 1.6

Parameters are displayed as mean values ± SE. VO_{2max} = maximal oxygen uptake, BMI = body mass index, Lean = whole body lean content, Fat = whole body fat content, Fat rel = relative whole body fat content. Mean values did not differ among groups (ANOVA) but among sex (all except age) according to a two tailed student's *t* test ($P < 0.05$)

Table 2 Estimation of subjects' fiber type composition: parameters are displayed as mean values ± SE

	EET		RET	
	Pre	Post	Pre	Post
Number (%)				
Type I	55.2 ± 3.3	57.5 ± 2.2	52.3 ± 3.7	54.3 ± 3.9
Type IIA	34.4 ± 2.0	35.6 ± 1.9	32.7 ± 1.6	31.4 ± 2.4
Type IIX	10.3 ± 2.0	6.9 ± 1.6	15.1 ± 4.2	14.2 ± 4.9
Area (%)				
Type I	62.9 ± 2.7	63.1 ± 2.5	56.6 ± 3.3	56.5 ± 3.4
Type IIA	31.6 ± 2.2	32.6 ± 2.3	34 ± 1.7	35.2 ± 2.0
Type IIX	5.5 ± 1.2	4.2 ± 1.1	9.3 ± 3.3	8.4 ± 3.7
Area (μm^2)				
Type I	4,250 ± 210	4,392 ± 200	4,050 ± 240	4,030 ± 270
Type IIA	3,890 ± 340	3,570 ± 290	3,990 ± 490	4,430 ± 490
Type IIX	2,320 ± 230	2,220 ± 210	2,070 ± 200	1,950 ± 160

Top panel displays individual fiber numbers in percentage, middle panel displays the cross sectional areas of the same fiber population in percentage of all fibers and the last panel shows the estimated average cross sectional area in square micrometers

(M–W *U* test). The sex difference in eccentric coordination was verified with a two tailed student's *t* test. The level of significance was set to $P < 0.05$. Coefficients of correlation were calculated using Pearson Product Moment Correlation for pooled pre- and post-training datasets. All statistical analyses were carried out with the Statistica software package 6.1 (StatSoft (Europe) GmbH, Hamburg, Germany).

Results

Training specific improvements

Statistically significant improvements of the training loads could be recorded for RET and for EET subjects. RET subjects improved leg extension on average by 84.8% from 30.2 ± 2.2 to 55.8 ± 3.7 kg, while EET subjects increased average training load from 69.6 ± 4.3 to 314.8 ± 27.0 W (+352%). The increased training loads in EET overestimate the training progress since the subjects were ramped carefully to avoid muscle injury.

Timed up & go (TUG), Berg balance scale (BBS)

Subjects improved significantly in TUG from 7.37 ± 0.16 to 6.88 ± 0.16 s (−6.7 ± 0.2%) independent of training modality (EET −7.5 ± 0.2%; RET −7.3 ± 0.2%; CT −4.9 ± 0.5%). No significant improvements could be recorded for BBS (EET +1.7 ± 0.3%; RET +0.7 ± 0.3%; CT +0.7 ± 0.4%) since the study-subjects had on average already achieved 53.8 of maximal 56 points in the pre-test.

Body composition

The EET group experienced a reduction in whole body fat (−5.0 ± 1.1%) and thigh fat content (−6.9 ± 1.5%) not observed in RET (body: −0.6 ± 1.0%, thigh: −2.7 ± 0.9%) and CT (body: +1.4 ± 1.2%, thigh: +0.6 ± 1.9%). Subjects' relative thigh muscle mass increased significantly with EET (+2.5 ± 0.6%) and RET (+2.0 ± 0.3%) but not with CT (+0.4 ± 0.4%) (Fig. 2). Pre-Post differences between RET and EET were verified for body ($P = 0.002$) and thigh fat ($P = 0.03$) by a Mann–Whitney *U* test (M–W *U* test).

Muscle fiber types

A significant reduction of the type IIX/type II ratio was recorded for EET subjects (−22 ± 14%) but not for RET subjects (−8 ± 14%) (Fig. 3a). Type IIX/type II fiber ratio correlated significantly ($P < 0.01$) with body composition parameters: positively with body fat ($R = 0.75$) and thigh fat ($R = 0.70$) and negatively with relative thigh lean mass ($R = 0.69$). Biopsies from women displayed a significantly higher type IIX/type II fiber ratio than biopsy samples from men (women: 29 ± 5%; men: 19 ± 3%; two-tailed students *t* test, $P < 0.05$). Pre-post differences between RET and EET were not significant ($P = 0.2$; M–W *U* test).

Maximal isometric extension strength of the legs (MEL)

The EET group significantly improved MEL (+7.5 ± 1.7%), whereas no significant strength changes were noticed for RET (+2.3 ± 2.0%) and CT (−2.3 ± 2.5%). Improvements of EET subjects were even more pronounced when MEL was normalized to body mass (+8.4 ± 1.7%) (Fig. 4). However, pre-post differences in MEL were not significant between EET and RET ($P = 0.1$; M–W *U* test). Focusing on EET subject's relative MEL, exclusively women improved significantly (from 13.7 ± 2.8 to 14.4 ± 2.8 N/kg; +13.8 ± 2.5%), whereas in men the observed increase was not significant (i.e. from 16.9 ± 3.5 to 17.2 ± 3.6 N/kg; +4.8 ± 1.4%). The sex specific analysis was verified with a two tailed student's *t* test with a level of significance offset at 5%.

Eccentric coordination

The ability to match instantaneous muscle torque to eccentric target load was improved significantly by EET subjects (−43 ± 6% RMS) but not by RET (−13 ± 3%) and CT subjects (−12 ± 5%) (Fig. 5). The initial inferior coordination performance of women compared to men (Pre: women: 79.6 ± 7.3 RMS; men: 55.4 ± 6.7 RMS) was lost following EET (Post: women: 24.9 ± 1.0 RMS; men: 22.9 ± 2.3 RMS). Pre-Post differences between RET and EET were significant ($P = 0.02$) according to M–W *U* test.

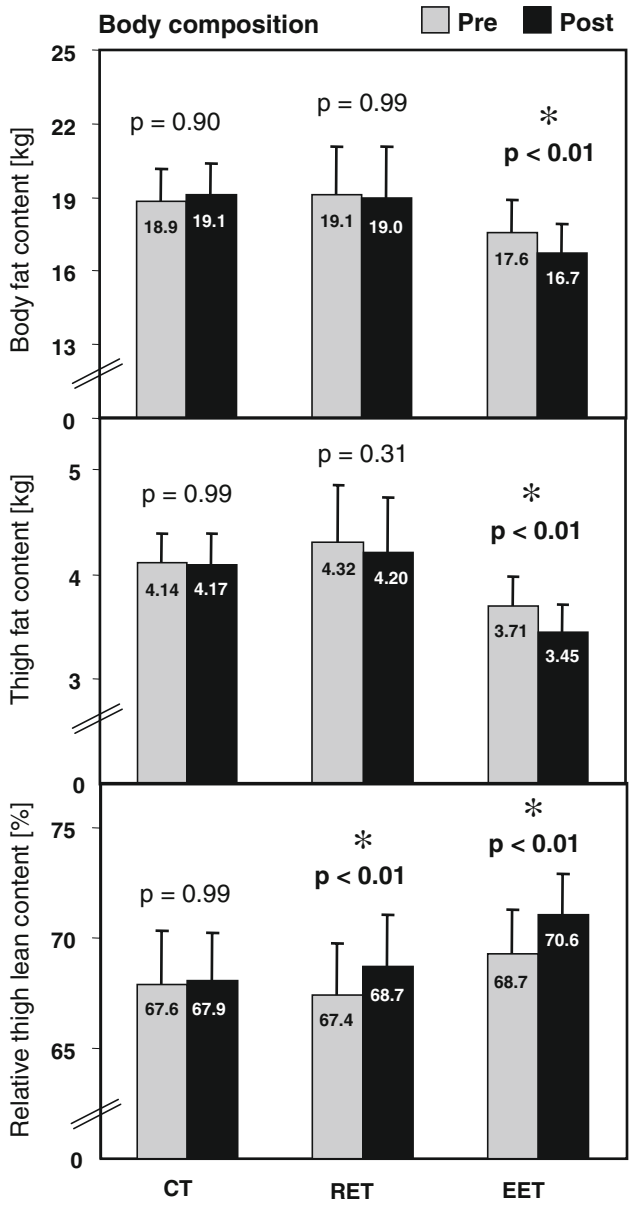

Fig. 2 Whole body fat, thigh fat and relative thigh lean content as assessed by dual energy X-ray absorptiometry. *Bars* (*grey* pre; *black* post) represent mean contents ± SE in kg (fat) and % (lean) of CT ($n = 14$), RET ($n = 21$) and EET subjects ($n = 19$). (ANOVA with repeated measures; $*P < 0.05$; indicated P-values of Tukey's HSD Post hoc test)

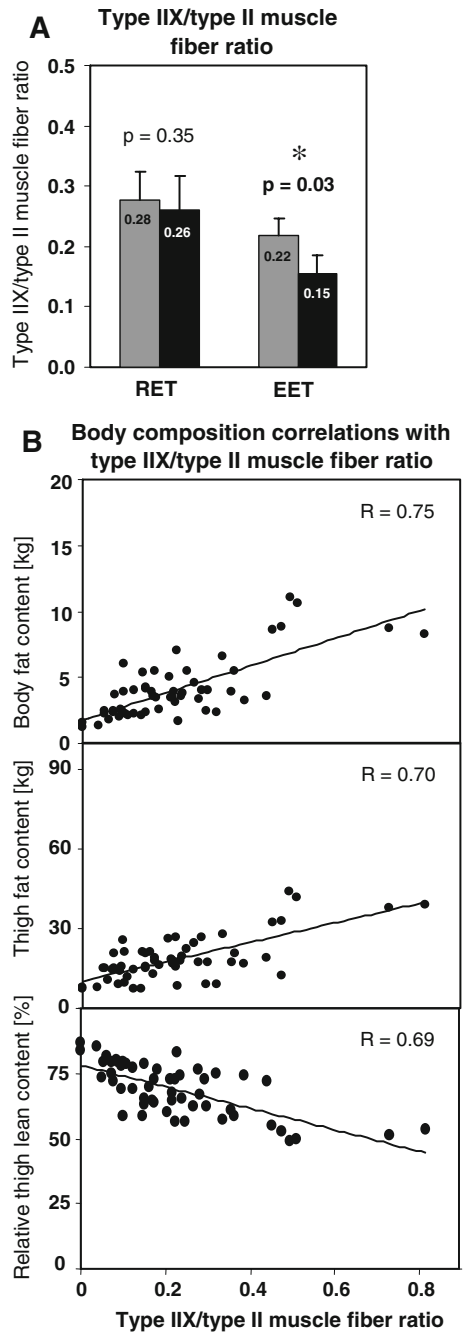

Fig. 3 **a** Ratio of type IIX/type II muscle fiber content of EET ($n = 14$) and RET ($n = 13$). *Bars* represent means of pre- (*grey*) and post-values (*black*) ± SE. (Indicated P-values of one tailed paired student's t test; $*P < 0.05$). **b** Highly significant ($P < 0.01$) correlation of fibertype composition with body fat, thigh fat and relative thigh lean content. Data points consist of individual pre- or post-values of body composition parameters with the corresponding type IIX/type II muscle fiber ratio evaluated from muscle biopsies ($n = 56$)

Discussion

This study shows eccentric ergometer exercise and resistance training to be well tolerated by elderly. Despite a low training frequency of just two sessions per week we observed a moderately positive outcome. EET improved leg muscle strength, body composition and eccentric muscle coordination in elderly. The positive effects of RET were similar in magnitude but mostly statistically not significant. The fact that strength gain in our study was measured on a training independent device underlines the task specific improvements in most other studies which don't necessarily reflect the "useful" benefits.

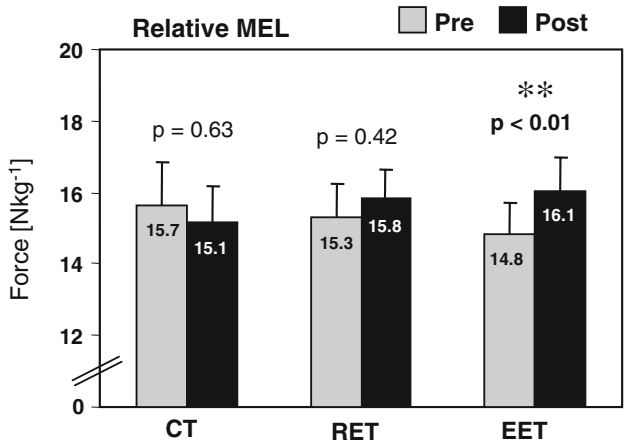

Fig. 4 Maximal isometric extension strength of the legs (*MEL*) normalized to body weight. *Bars* (*grey* pre; *black* post) represent mean leg force in N kg^{-1} of CT ($n = 13$), RET ($n = 18$) and EET subjects ($n = 19$) ± SE. (ANOVA with repeated measures; $^{**}P < 0.01$; indicated *P*-values of Tukey's HSD Post hoc test)

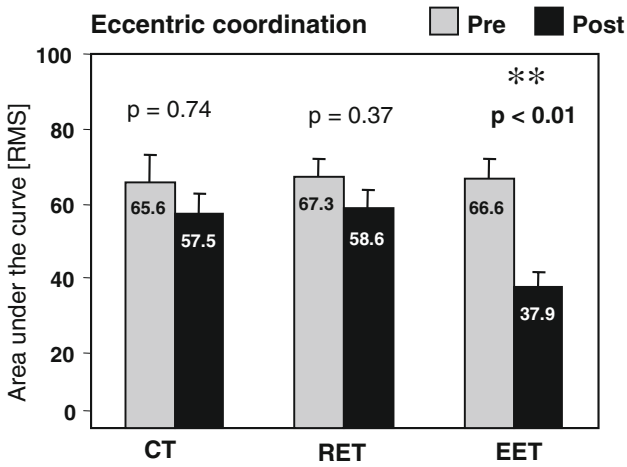

Fig. 5 Eccentric muscle work load as assessed on the eccentric ergometer. *Bars* represent root mean square (*RMS*) of CT ($n = 14$), RET ($n = 19$) and EET ($n = 19$) ± SE. (ANOVA with repeated measures; $^{**}P < 0.01$; indicated *P*-values of Tukey's HSD Post hoc test)

A major limitation of our study was the low training frequency of our subjects which was chosen to maximize adherence for independently living volunteers, characterized by an active lifestyle and concomitant duties and obligations. Together with the exceptionally good physical condition of our subjects, functional tests such as TUG and BBS were not sensitive enough to detect differences. The modest increase in strength (as measured by MEL) seen in our study when compared to strength increases reported in other studies with elderly subjects (Hauer et al. 2001; Kryger and Andersen 2007) has several reasons: (1) our subjects were already in exceptional physical condition. (2) We used a low training frequency with just two sessions of 20 min specific training per week. We believe this to be a realistic sustainable training program for this population. (3) Our force measurement setting was devised to mimic an every day situation and thus independent from the training procedure for both training modalities. The randomisation procedure had to be adapted because not all of our subjects agreed to get biopsied. Due to medical reasons (i.e. use of anticoagulants) we could not biopsy some of the subjects. Subjects who fulfilled biopsy criteria were then randomized to one of the physical intervention groups (RET, EET). We did not collect biopsies from CT subjects as this would have represented an unwarranted risk. Since there were no significant initial functional differences between the groups we think it unlikely that the de facto stratified randomisation influences our results.

A major concern in this study was to avoid the negative consequences of eccentric exercise, consisting of muscle damage i.e. DOMS (Friden et al. 1983). To this end, we chose very low initial training loads (30 and 50 W for women and men, respectively) and short training times (5 min). Reported discomfort after eccentric exercise was thus, between 0 and 1 and never exceeded a value of 4 on a VAS scale of 0–10. The prerequisite for safe eccentric exercise is to tailor and monitor eccentric load individually. This was achieved with a computer-based visual feedback system in which subjects were matched the instantaneous training load to a target load displayed on a computer screen (Fig. 1). Matching of the training load to a target load turned out to be a demanding coordination task which required coaching and some practice and turned out to be feasible for all except one subject. The software for our eccentric ergometer allows for quantitative assessing the deviation of the eccentric performance from the required load (eccentric coordination; see Fig. 1). Not surprisingly, only EET-trained subjects were able to improve eccentric coordination (by 43%) significantly over the entire training period. The initial difference in coordination between better performing males than females was lost after training, as women improved their eccentric coordination more than men. It is currently difficult to assess the relevance of the massive improvement of eccentric coordination as defined in our setting. Matching eccentric performance to a target load is a complex task involving the integration of visual feedback with motor control. In a previous study using the same eccentric ergometer on world cup level alpine skiers, a positive correlation was found between the eccentric coordination and success in ski slalom races (Vogt et al. 2003). Whether improvements in eccentric coordination results in a lowered risk of falling needs further evaluation.

In the current study, the risk of falling was assessed by standard tests such as TUG and the BBS. All our subjects ranked in the lowest risk category at the outset of the study. For TUG, subjects performing the task in less than 14 s belong to the category of people with no increased risk of

falling. The subjects in our study performed this task in the pre-test in 7.4 s. For TUG we still found a significant 7% improvement, independent of training modality. Other studies (Hauer et al. 2001; Kryger and Andersen 2007; Lastayo et al. 2002) in which frail or reconvalescent elderly subjects were trained showed improvements of TUG and leg strength in the order of 50%. We see the failure of the standard risk assessment tools TUG and BBS, to demonstrate large improvements as a consequence of the good physical condition of our subjects at the outset of the study (Lotscher et al. 2007).

Leg strength was assessed in this study by measuring Leg strength developed in a restrained sitting position (MEL). Relative MEL increased significantly by 8.4% in subjects only after eccentric training. We believe that the small but significant improvement in MEL in EET subjects is biologically relevant. Small gains in maximal performance may cause larger improvements in submaximal performance as a consequence of the non-linear relationship of power versus time or maximal versus repeated activity (Dufour et al. 2006; Wilkie 1985). Maximal performance testing has the advantage of yielding reliable results (Schroeder et al. 2007), while submaximal performance is difficult to assess reliably, but it is more relevant in order to characterize practical benefits for the subjects.

The assessment of body composition showed an unexpected but significant 5% decrease in body fat content along with a significant 6.9% decrease in thigh fat content in EET subjects only. Both RET and EET subjects showed a significant increase in relative thigh muscle mass after training. This relative increase was due to an increase in thigh muscle mass (more pronounced with RET), combined with a decrease in thigh fat mass (significant with EET). From these estimates we assume that the observed increase in leg extension strength in EET (reported above) cannot entirely be attributed to a structural change of muscle tissue, but seems to be a consequence of functional (i.e. neural) improvements. It is difficult to assess the potential benefit of a 5% decrease in body fat content in an elderly population with EET; however, a simultaneous gain of muscle mass with a decrease of body fat counteracts the sarcopenia that usually develops with age.

The muscle fiber type analysis showed two major results: a significant correlation between the type IIX/type II ratio and body composition and a decrease of the type IIX/type II ratio exclusively with EET. The type IIX/type II ratio describes the fraction of the type IIX fibers from the type II fiber pool. It can be assumed that the lower typeIIX/type II ratio of leaner subjects is related to physical activity and lifestyle. It has previously been described that physical activity in elderly leads to a decrease of type IIX fibers (representing the most anaerobic muscle fiber type) in favor of more aerobic type IIA fibers (Herbison et al. 1982). Since types I and IIA muscle fibers possess a larger oxidative capacity than type IIX fibers (Herbison et al. 1982) they are better able to couple ATP regeneration with fatty acid and carbohydrate catabolism. In fact, Kriketos et al. (1996) showed a significant positive correlation of relative body fat content with type IIX fibers as well as negative correlations with oxidative enzyme activity (citrate synthase, hexokinase) and insulin sensitivity. This result is in accordance with the limited capacity of type IIX fibers to utilize fatty acids as substrates for ATP regeneration due to their relatively small mitochondrial density (Gueguen et al. 2005). The observed changes of the type IIX/type II ratio with individual changes in body composition parameters indicates the maintenance of plasticity of muscle fibers and body composition as influenced by physical training into old age even at low exercise frequencies.

The higher proportion of type IIX fibers in women may reflect their initial lower fitness possibly due to their less active lifestyle. Due to their greater potential for improvements (strength, coordination) we find women to profit more from training (MEL, eccentric coordination) than men. It has been suggested that muscle fiber composition is implicated in the correlation of body fat content and risk for non-insulin-dependent diabetes mellitus (NIDDM) (Jensen et al. 2007). This view is compatible with our findings, as we find a positive influence of physical training (EET) on fiber composition and potentially reducing the risk of developing age-dependent metabolic diseases such as NIDDM.

In conclusion, at the low training frequency of our study EET was similarly successful as RET in improving muscle functional and structural parameters analyzed in this study. EET significantly improved MEL, body composition and eccentric coordination. EET further showed the persistence of muscular plasticity in elderly as evidenced by a decrease of the type IIX/type II muscle fiber ratio. The latter was found to correlate with body composition in all the subjects studied. These findings suggest that eccentric exercise modalities, given their low metabolic costs, merit further evaluation with regard to their potential to improve muscle motor and metabolic functionality in elderly.

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The second paper was submitted to Gerontology and reports additional ultra-structural and molecular data. As expected, increase of muscle fiber cross sectional area (hypertrophy) paralleled gain in leg muscle mass with resistance training (RET) but surprisingly not with eccentric ergometer training (EET). Changes of mitochondrial volume density correlate with the expression of genes encoding mitochondrial and metabolic transcripts which were significantly depressed with EET but remained stable with RET.

Paper II: *"Different molecular and structural adaptations with eccentric and conventional strength training in elderly men and women"*

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Different molecular and structural adaptations with eccentric and conventional strength training in elderly men and women

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Abstract

Reprogramming of gene expression contributes to structural and thus functional adaptation of muscle tissue in response to altered use. The aim of this study was to investigate mechanisms for observed improvements in leg extension strength, gain in relative thigh muscle mass and loss of body and thigh fat content in response to eccentric and conventional strength training in elderly men (n=14) and women (n=13; average age men and women: 80.1±3.7y) by means of structural and molecular analy-ses. Biopsies were collected from M. vastus lateralis in the resting state before and after 12 weeks of training with two weekly resistance exercise sessions (RET) or eccentric ergometer sessions (EET). Gene expression was analyzed using custom designed low density PCR arrays. Muscle ultrastructure was evaluated using EM morphometry.

Gain in thigh muscle mass was paralleled by an increase of muscle fiber cross sectional area (hypertrophy) with RET but not with EET, where muscle growth is likely occurring by the addition of sarcomeres in series or by hyperplasia. The expression of transcripts encoding factors involved in muscle growth, repair and remodelling (e.g. IGF1, HGF, MYOG, MYH3) was increased to a larger extent after EET than RET. Micro RNA-1 (miR-1) expression was decreased independent of the training modality and was paralleled by an increased expression of IGF-1 which represents a potential target. IGF-1 is a potent promoter of muscle growth and its regulation by miR-1 may have contributed to the gain of muscle mass observed in our subjects. EET depressed genes encoding mitochondrial and metabolic transcripts. The changes of several metabolic and mitochondrial transcripts correlated significantly with changes in mitochondrial volume density. Intramyocellular lipid content (IMCL) was decreased after EET concomitantly with total body fat. Changes in IMCL content correlated with changes in body fat content with both, RET and EET.

In elderly, RET and EET lead to distinct molecular and structural adaptations which might contribute to the observed small quantitative differences in functional tests and body composition parameters. EET seems to be particularly convenient for elderly with regard to improvements in body composition and strength but at the expense of reducing muscular oxidative capacity.

Keywords: adaptation, eccentric, elderly, gene expression, physical performance, exercise training, mitochondria, sarcopenia.

Introduction

Exercise always invokes a mixture of metabolic, hormonal, neural and mechanical stimuli, the challenge is that their individual contributions are difficult to discriminate. Endurance exercise has a pronounced metabolic component while in pure eccentric exercise mechanical stress is predominant and the metabolic component is thought to be small. Given that sensors/integrators of metabolic (AMPK) and mechanical stress (mTOR) are thought to signal in an antagonistic manner, a continuum of adaptations can be expected, depending on the relative contributions of the stimuli which are dependent on training intensity, duration, frequency, type of exercise (strength vs. endurance) and contraction mode (eccentric vs. concentric). Moreover, the actual status of muscle cells potently contributes to the final response such as the energy status of muscle tissue (i.e. glycogen depleted) which influences activation of 5' adenosine monophosphate-activated protein kinase (AMPK) an inhibitor of the mammalian target of rapamycin (mTOR)/p70s6-Kinase (p70s6k) stimulated protein synthesis. Endurance exercise in a muscle glycogen depleted state leads to an enhanced transcriptional activation of some metabolic genes (PDK4, UCP3) possibly by an increased activation of AMPK [1] .

Exercise training, by “accumulation” of the many stimuli, leads to specific adjustments of gene and protein expression, partly by nuclear reprogramming of gene transcription, which eventually results in the phenotypic adaptation seen after prolonged periods of strength or endurance training [2]. Athletes with a several year training history were found to differ in the expression of key regulators compared to sedentary individuals which is thought to be a key part in the adaptation to the trained muscle phenotype [3]. At least some of these orchestrated adaptations are thought to be driven by so called “master” genes which mostly encode transcription factors or transcriptional co-activators that promote transcription of a whole cluster of genes responsible for specific functions and pathways, respectively. The peroxisome proliferator-activated receptor gamma, co-activator 1 alpha (PGC-1α) is induced with endurance training. PGC-1α regulates genes involved in glucose and fatty acid metabolism, mitochondrial biogenesis and muscle fiber type shifts [4]. Stepto et al. [5] investigated the transcriptome adaptation in response to long term strength or endurance training and observed contrasting regulation of transcripts related to oxidative metabolism, which were increased after endurance and decreased after strength training.

Good correlations between mRNA and protein levels as well as structural and functional parameters, e.g. mitochondrial density and maximal oxygen consumption have been described in Hoppeler et al. [6].This points towards a dominant long term adaptation at the mRNA level for these systems [7-9]. In the muscles of highly endurance-trained athletes mitochondrial and capillary density are double compared to sedentary individuals, illustrating the well orchestrated adaptations of the oxygen pathway from lung to muscles [10-11]. In agreement with the concept of symorphosis, maximal oxygen consumption was found to be increased to the same extent as muscle substrate storage in the form of intramyocellular lipids (IMCL) [12-14].

Micro RNA’s are thought to be another group of “master modulators” since these small non-coding RNAs can affect a whole array of targets by binding 3’-untranslated regions of specific mRNAs, thereby inhibiting translation and/or initiating degradation. McCarty et al. [15] have observed decreased expression of micro RNA 1 (miR-1) and micro RNA 133a (miR-133a) during muscle hypertrophy. Proposed downstream effects include regulation of chromatin remodelling by targeting expression of histone deacetylases (HDAC) as well as a direct influence on the generation of transcription factors, growth factors and growth factor receptors such as serum response factor (SRF), hepatocyte growth factor (HGF), HGF-receptor (c-MET) as well as insulin-like growth factor 1 (IGF-1) [15-16].

Eccentric strength training has been proposed to be most effective to increase strength, due to the higher mechanical forces that can be exerted during

eccentric contractions compared to concentric ones [17]. A recent meta-analysis confirmed the greater extent of strength gain and muscle hypertrophy upon eccentric training [18] (for further discussion see also [19]). Most of these studies have used high loads. Using more "endurance like" chronic eccentric exercise, Zoll et al. [20] compared eccentric and concentric cycling ergometry in a population of coronary disease patients. The low energy demand of this training regime allowed for a high mechanical stimulus with low oxygen demand thus avoiding a high cardiopulmonary load. Comparing eccentric with concentric cycling using the same relative metabolic load, Meyer et al. [21] have shown that the eccentrically exercising subjects trained with approximately four times higher workloads than those training concentrically. On the level of gene expression these authors reported a differential adaptation of gene transcripts involved in mitochondrial biogenesis. The mRNAs of transcription factor A (TFAM) and cytochrome C oxidase (COX-4) were both down-regulated after eccentric but not concentric training supporting the hypothesis of a dominant mechanical stimulus with eccentric training [22].

In this study, we compared chronic molecular and structural adaptations in a steady state condition of EET with conventional RET in subjects of advanced age. These two training modes were chosen because RET is recommended to prevent age related muscle atrophy and the functional read out of eccentric contractions is closer to strength training than other modalities [19].

Materials and Methods

Subjects and Study Design

The subjects described in this study were participating in a larger trial whose training programs and subject characteristics have been described in detail previously [19]. In brief, 62 elderly subjects (80.2 years on average) with stable medication and health conditions were randomly assigned to one of three training groups: Cognitive Training (CT), Conventional Resistance Training (RET) and Eccentric Ergometer Training (EET). Cognitive training (CT) consisted of non–physical computer-guided cognitive training (10 women, 6 men). RET was carried out by 23 subjects (13 women, 10 men) in a gym and comprised four exercises for the lower extremity (leg press, knee extension, leg curl, hip extension). The sessions consisted of a 10-minute warm-up, 20 minutes specific training and 10 minutes cool-down with stretching. Exercises consisted of three sets with 8-10 repetitions. EET was carried out by 23 subjects (13 women, 10 men) on a custom-built motor-driven ergometer [21]. The trainings started with a 10-minute warm-up and closed with a 10-minute cool down, while the actual EET lasted 20 minutes. To avoid severe DOMS, the initial load on the eccentric bike was set low (females 30 Watts, males 50 Watts) for 5 min and duration was gradually increased in 5-minute steps until it reached 20 minutes. Afterwards the load was ramped in consecutive sessions by 20% of the individual maximal power output achieved in the initial incremental exercise test to exhaustion on a cycling ergometer [19,23]. All groups trained for 12 weeks. Muscle biopsies were collected from M. *vastus lateralis* from RET (n=13) and EET-subjects (n=14) prior to the start of the training intervention and 48 to 72 hours after the last training bout. This is the subgroup of subject this manuscript describes. Their anthropometric and morphological data are reported in table 1. The biopsy portions subsequently used for histochemistry and RNA isolation were immediately frozen in liquid nitrogen cooled isopentane and stored in liquid nitrogen until required for further analysis. The portions processed for morphometric analysis were fixed in 6.25% glutaraldehyde as described previously [24].

RNA Isolation and Reverse Transcription

Total RNA was isolated with the RNeasy All Prep Kit from Qiagen (Hombrechtikon, Switzerland) with the protocol adapted for the simultaneous extraction of small RNA's (such as micro RNA's) and mRNA. See QIAGEN supplementary protocol (http://www1.qiagen.com/literature/protocols/pdf/ry26.pdf). In order to relate the total RNA content to the amount of input tissue, the amino acid as well as the DNA content of the whole proteinase K digested lysate was esti-

mated. Amino acid analysis was performed by the Department of Chemistry and Biochemistry (University of Bern, Switzerland) using total acid hydrolysis followed by high-performance liquid chromatography on a silica column. DNA was collected on a silica membrane column. The concentration of its eluate was measured as absorbance at 260 and 280 nm on a Nanodrop photospectrometer (Wilmington, USA). RNA was collected according to the manufacturer's protocol for total RNA isolation from fibrous tissue with a proteinase K digestion after muscle tissue. Total RNA yield was between 4.3 and 20.6 μg. For reverse transcription of mRNAs, the High Capacity RNA reverse transcription kit from Applied Biosystems (Foster City, USA) was used, with 700 ng RNA input per sample (download protocol at http://www3.appliedbiosystems.com/cms/groups/mcb_support/documents/generaldocuments/cms_042557.pdf). Reverse transcription for micro RNA analysis was carried out with the miScript reverse transcription kit from Qiagen according to the manufacturer's protocol, as were the other procedures (http://www1.qiagen.com/literature/render.aspx?id=104793). cDNA was aliquoted and stored at -20°Celsius and only thawed immediately prior to use. Repeated freeze thaw cycles were avoided.

Gene Expression and Micro RNA Analysis

For PCR quantitation, TaqMan arrays (microfluidic cards) Applied Biosystems (Foster City, USA) were used. Two different 384-well microfluidics cards in format 96a were custom designed, allowing the relative amount of 187 muscle specific mRNA transcripts to be determined for each biopsy, using cDNA from 400 ng reverse transcribed RNA as input. A complete list of transcripts can be seen in the supplementary table 4. Cycling conditions and procedure were followed as recommended by the manufacturer. TaqMan arrays measure the content of each cDNA relative to the amount of this cDNA in an calibrator sample that is run identically on each array. For this study, the calibrator cDNA was generated as a mixture containing cDNA from each biopsy in order to ensure coverage of all expressed transcripts. Relative expression was estimated by the ΔΔCt method using 18S cDNA normalisation. We have chosen 18S as a standard because we can exclude significant fluctuations in the yield of total RNA, based on our measurements of amino acid as well as DNA content, which were determined as described above. Ribosomal RNA content makes 80% or more of total RNA and can assumed to be stable if total RNA content remains unchanged. Transcripts that were expressed with a cycle threshold (CT) ≤35 were included in the analysis. The miScript primer assay detection system from Qiagen (Hombrechtikon, Switzerland) was used to analyze micro RNA expression. Micro RNA-1 expression was normalized to U6 small nuclear RNA [25].

Biopsy Sampling, Morphometry, Histochemistry and estimation of Body Composition

Biopsy sampling of M. *vastus laterlis* and subsequent histochemical analysis were performed as described previously [19]. Ultrastructural parameters were determined by morphometric analysis as described by Steiner et al. [24]. Body composition parameters were assessed by dual energy X-ray absorptiometry (DEXA) as described previously [19].

Statistical Analysis

Differences in muscle ultra-structure and muscle fiber type composition were verified using the two-tailed student's t test. Interactions of training modality (RET, EET) with transcript levels was checked with analysis of variance (ANOVA) for repeated measures with Tukey's honest significant differences post-hoc test. Coefficients of correlation were calculated using Pearsons product moment correlation. The probability level for statistical significance was set at $p<0.05$. All statistical analyses were carried out with the Statistica software package 6.1 [StatSoft (Europe) GmbH, 20253 Hamburg, Germany]. The analysis of functional data has been described previously by Mueller et al. [19].

Results

Anthropometry and Muscle structure

The anthropometric data of the study subjects are reported in table 1, the data on their muscle morphology in table 2. Thigh muscle mass was increased by 2.8% with RET and 2.1% with EET along with a reduction of thigh fat content (RET: -2.8%; EET: -5.4%). Body fat content decreased by -0.2% (RET) and -3.1% (EET), respectively. When pooled (n = 27), all changes were statistically significant. Most muscle ultra-structural parameters did not change significantly over time, only intramuscular lipid (IMCL) content per muscle fiber was decreased in eccentrically trained subjects after the training period (table 2, fig. 1A). The changes in (post/pre ratio) IMCL content correlated with the changes in body fat content (fig. 1B). Fiber cross sectional area did not significantly change with training, nor did volume density of mitochondria or capillary density. In agreement with the classic concept of muscle hypertrophy, the individual changes in fiber cross sectional area correlated well with the changes in thigh muscle content with RET. However, this was not the case with EET, were changes in thigh muscle mass were not related to changes in fiber cross sectional area (fig. 2).

Gene- and micro RNA expression analysis

Our custom designed low density arrays contained primer and probe pairs for detection of 187 different gene transcripts including the 18S ribosomal RNA used for normalisation (transcripts are listed in supplementary table 4). 178 transcripts yielded an average cycle threshold (Ct) of ≤ 35 over all subjects and were therefore included in the analysis. The mean Ct of all gene transcripts was 27. As already indicated, our normalisation to 18S expression was based on the observation that we obtained similar yields of total RNA from all biopsy portions when related to their protein content measured by amino acid analysis. The amount of total RNA did not significantly change with training (EET: +9%; RET: -6%).

Among the 178 analysed transcripts, 60 transcripts were significantly changed with training. Four transcripts were exclusively changed with RET, 29 with EET and 27 reached the level of significance only when subjects were pooled. Those transcripts that significantly changed in the same direction in response to strength training (both groups taken together) are indicated as “ALL” in table 3. The transcripts are grouped according to their function. Those coding for metabolic enzymes and mitochondrial proteins were consistently down-regulated with EET, in contrast to transcripts coding for factors involved in repair and remodelling, which were up-regulated after EET. The individual values for the expression of metabolic and mitochondrial genes correlated highly significantly with each other (see supplementary figure 6). In addition, the levels of transcripts involved in mitochondrial function also correlated strongly ($p<0.01$) with mitochondrial density (figure 4 and supplementary figure 5).

The expression of miR-1 was decreased after training, with no significant differences between the changes in the two groups (Fig. 3A). An analysis of potential mRNA targets (miRanda, www.microrna.org) of miR-1 revealed 3343 potential targets, among them IGF-1, a potent modulator of muscle hypertrophy [26-27]. We observed a significant correlation between changes in miR-1 and IGF-1 expression (Fig 3B). From other potential targets we could detect significant correlations with fibronectin 1 (FN1; R=-0.6), chemokine (C-C motif) ligand 2 (CCL2; R=-0.7) and platelet-derived growth factor alpha (PDGFA; R=-0.6).

Discussion

This study reports the quantitative structural and molecular analyses of data obtained from muscle biopsies of aged men (n=14) and women (n=13; 80.1 ± 3.8 years) before and after eccentric (EET) and conventional resistance training (RET) for 12 weeks. The functional and body composition data from our larger group of training subjects have been reported previously [19]. In this larger group, we found significant but small increases in relative thigh muscle mass after both modes of training as well as significant losses in body and thigh fat content in EET subjects only. The strength gains in an isomet-

ric test were relatively small in EET (+8.9%) and RET (+2.3%; but significant only for EET). Compared to conventional strength training studies, these changes are relatively small, but nevertheless important, given that the testing procedures were exercise independent and our subjects performed only 2 training sessions per week and were of very good physical condition for their age [19]. The subset of subjects with biopsies did not reach significant group specific functional and body composition improvements, although the data point in that direction. The pooled data from the subjects in both training groups showed significantly increased thigh muscle mass and strength (table 1). Thigh fat mass was reduced with both training modalities (significant for pooled data) but this was more pronounced with EET. We therefore assume the subgroup of subjects discussed in this analysis to have adapted in the same manner as the larger cohort, but with individually large variations that prevent a statistically significant demonstration of functional improvements on the basis of our conventional functional tests.

Normalisation procedures of gene expression data: Although the actual calculations were performed by normalising the PCR data to 18S cDNA, we interpret our gene expression data as relative measures of tissue content for a given mRNA. By estimating the RNA, DNA and amino acid contents from the same muscle biopsy specimens we were able to control eventual fluctuations of total RNA (rRNA, mRNA , tRNA, micro RNA) as well as DNA content (nuclear domains, infiltration of non-muscle cells) by standardising to protein content. Changes in RNA and DNA levels were not significant. Our normalisation procedure is therefore independent of housekeeping genes [9]. In addition, we used the ultrastructure morphometry of mitochondria as a measure of specific protein content to reflect bulk structural consequence of transcriptomic changes observed. The significant correlations of mitochondrially (MT-ATP5, MT-CO1, MT-ND1) as well as nuclear coded transcripts (ATP5B, COX5B, PGC-1α) with mitochondrial volume (Fig. 4 and suppl. Fig. 5) further validate our normalisation strategy and illustrate the value of gene expression markers.

The gains in relative thigh muscle mass (as determined by DEXA) were similar after RET (2.8%) and EET (2.1%). The increases were small and reached the level of significance only for RET and EET combined. A more in depth analysis of the individual data suggests that the muscle mass increased via different mechanisms in the two groups. In RET but not EET, we found a significant correlation between the change in thigh muscle mass and the increase in fiber cross-sectional area (Fig 2). This is compatible with the hypothesis that RET leads to fiber hypertrophy [28]. With eccentric training muscle growth seems to occur independent of changes in fiber CSA, possibly driven by hyperplasia [29-30] or by addition of sarcomeres in series, i.e. by lengthening of muscle fibers [31-32]. Reeves et al. [33] have shown that eccentric exercise increases fascicle length in pennate muscles, leading to increased physiological CSA. They used a protocol of 3 training sessions per week for 14 weeks in subjects aged an average of 67 years. Using a training mode independent isometric testing procedure, they also found strength gains of comparable size to our study with eccentric and concentric training regimes. Given the strikingly different correlations between gains in fibre area and thigh muscle mass between RET and EET, we suggest that in our study, EET may have led to muscle gain via increased fascicle, i.e. fibre length.

Neither EET nor RET significantly changed the mitochondrial content of the skeletal muscle fibers analysed. Moreover, the proportion of subsarcolemmal and intermyofibrillar mitochondria remained unchanged after either training intervention. Using a more aggressive but otherwise similar training protocol in younger subjects a significantly decreased skeletal muscle mitochondria in response eccentric exercise training was found [24]. In that study, the decrease in oxidative capacity was paralleled by a decrease in IMCL content (-24%, not significant) and increased fiber/muscle area (+2.76%, p=0.01) (unpublished results). The data in

our current study point in the same direction. Intramuscular lipid content was found to be significantly reduced in EET subjects (Fig 1A). Among individuals, the decrease in intramyocellular lipid was significantly correlated with the decrease in body fat content (Fig 1B). Using concentric exercise training protocols, it has been consistently found that intracellular lipids are increased concurrent with the increases of mitochondrial volumes [34]. Eccentric exercise has been associated with lowered blood triacylglycerol after a high fat meal in the post exercise period [35]. When older subjects (average age 66 years) were subjected to a hyperglycemic clamp 48 h after a bout of eccentric exercise, their lipid oxidation increased at the expense of carbohydrate oxidation compared to the rested control state. In contrast, a cohort of younger subjects (average 23 years) showed the expected increased carbohydrate oxidation at the expense of lipid oxidation [36]. Eccentric exercise is known to induce transient insulin resistance, possibly due to the muscle's inflammatory state during the repair phase [37]. It is possible that in elderly, the reduced insulin sensitivity could be compensated sufficiently via increased insulin secretion to the glucose challenge. The subjects in our study are distinctly older (average 80 years), thus it is conceivable that even a balanced meal could have been sufficient to elicit enhanced lipid oxidation. Molecular mechanisms of fat loss are discussed at page 14.

An increase in steady state gene expression is thought to be the main mechanism of muscle plasticity in endurance type training situations [9]. As such, we expected significant time (pre vs. post) and group (RET vs. EET) differences. Most strikingly, the biopsies of the EET subjects showed a significantly decreased expression of transcripts coding for mitochondrial proteins and metabolic enzymes (see table 3) which was not observed in RET subjects. This finding is in accordance with a previous eccentric training study in which Zoll et al. [38] observed a decrease in the steady state expression of mitochondrial transcripts with EET (TFAM, COX4). Monitoring the immediate transcriptome response after a single bout of eccentric ergometry, Klossner et al. [39] observed a significant depression of a large number of metabolic gene transcripts over a 24 hour period. In contrast, using a single bout of concentric, endurance type exercise, Schmutz et al [3] observed a significant up-regulation of a range of metabolic and mitochondrial gene transcripts after an initial drop in mRNA abundance in the first hour post-exercise. The short term response of metabolic transcript expression therefore corresponds to the long term (steady state) adaptation seen after both prolonged concentric and eccentric exercise training [40]. In the present study, although not statistically significant, the trends observed as changes of the mitochondrial volume density are compatible with steady state changes of the respective mRNAs (Table 3). Further support for the idea that eccentric training tends to decrease mitochondria comes from our observation that the overall abundance of most of mitochondrial transcripts correlated significantly with mitochondrial volume density if all biopsies are considered (Fig 4 and suppl. Fig 5). This apparent mitochondrial decrease was subtle and not expected in light of the potentially increased lipid metabolism and the consistent reports of perceived increased fitness and energy levels after the training period by the participants in this study. It has to be pointed out, however, that the established, exhaustive endurance tests such as VO_2 max were not performed with these subjects for ethical reasons.

In accordance with the higher mechanical stress experienced by the muscles of the EET subjects, up-regulation of transcripts coding for ECM components was more pronounced with EET than with RET. While expression of COL4A1 and COL6A1 mRNAs increased independent of training regime, COL1A1 and COL3A1 mRNAs were elevated almost 4 fold significantly with EET. Assuming an increase in interstitial tissue components with EET, these findings are in line with the observation that EET generates a stiffer muscle phenotype [41-42]. The expression of embryonic myosin heavy chain (MYH3) and alpha cardiac myosin heavy chain (MYH6), both markers of repair, as well as transcripts encoding proteins involved in cell cycle and

DNA repair (CCND1, CDKN2D, GADD45) were all elevated independent of training mode. GADD45 (Growth arrest and DNA-damage-inducible) encodes a DNA repair enzyme which is elevated after general stress. The stronger induction of GADD45 after EET (+140%) supports the hypothesis that EET has a more distinct molecular signature due to larger mechanical stress. Remodelling of muscle tissue both as neoformation or hypertrophy of muscle fibers requires activation of quiescent satellite cells. Activated satellite cells express a variety of unique markers such as CDH15 (M-cadherin), hepatocyte growth factor (HGF) and Pax7. As expected, mRNA expression of all these markers was increased after training with both training regimes, suggesting an increased remodelling of muscle tissue in strength trained subjects. Expression of MYH1, almost exclusively expressed in type IIX muscle fibers, was decreased with training. This is in line with the observed loss of type IIX fiber content more pronounced with eccentric training [19].

Marker transcripts involved in muscle growth such as myoD, myogenin, IGF-1 and HGF are significantly up-regulated at the mRNA level with training in pooled RET and EET subjects (see table 3). Myogenin, IGF-1 and HGF were induced by more than 70% in EET, but by only 20-40% in RET-subjects (statistically not significant). Of the micro RNAs, we found miR-1 to be down-regulated, which correlated significantly inversely with the upregulation of IGF-1 mRNA, a potential inhibitory target of miR-1 (see figure 3A and 3B). The down-regulation of miR-1 is an expected result [15], however, the mechanism by which miR-1 could influence protein abundance of IGF-1 is not clear and could include translation repression as well as mRNA degradation. Since the potential priming site for miR-1 is present in the 3'-UTR of IGF-1, both a translational inhibition and effects on mRNA abundance seem possible mechanisms. Our finding of increased IGF-1 mRNA is compatible with miR-1 influencing also its abundance. The expression of other potential targets such as SRF and HGF did not correlate well with miR-1 expression in our analysis. This suggests that the translation of these mRNAs could be inhibited or may not represent bona fide targets of miR-1 [15]. Work from Rao et al. [43] has shown that myoD and myogenin bind to the promoter region of miR-1 and can promote its transcription. In our study we find both myogenin and myoD mRNA expression increased while miR-1 abundance was decreased in response to training. This apparent discrepancy might be explained by a different availability of co-factors at the two time points in this study.

Our data show a trend in the reduction of fatty acid synthase mRNA by approximately 60% in the EET subjects with a concomitant up-regulation of FAT by 33% (not significant). We did not find increased expression of uncoupling proteins which have in other studies been linked to enhanced mitochondrial fat oxidation [44-45]. In light of the decreased mitochondrial protein transcripts and the tendency to decreased mitochondrial volumes, we would not have expected increased fatty acid synthase or UCP mRNAs. On the basis of our mRNA data, we would suggest increased basal but not maximal fatty acid breakdown in response to the eccentric training. Further studies will be necessary to confirm this.

Conclusions

Both EET and RET training regimes showed similar functional improvements, but distinct patterns of marker transcript accumulations. Pronounced mechanical stress combined with the smaller metabolic load of EET resulted in depressed expression of mitochondrial transcripts along with increased transcripts involved in remodelling and repair. RET led to a lesser disturbance of the muscle gene expression profile with smaller increases in expression of remodelling and repair genes and unchanged expression of mitochondrial and metabolic transcripts. While EET seems to be at least as effective as RET with regard to the functional outcome of the strength training intervention, the depression of metabolic genes is at first sight undesirable. It also contradicts the decrease in IMCL content in response to EET (which remarkably correlated with body fat), which may well be a consequence of reduced insulin sensitivity in older humans. Given the unanimously

positive response we had in terms of improved life quality in response to the training from the participants, it looks as if for a cohort of this age, fitness is more limited by muscle mass than endurance capacity.

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Table 1

	Women (13)	Men (14)	ALL (27)	RET (6♀;7♂)	EET (7♀;7♂)
Age [yr]	80.1 ± 1.2	80.2 ± 0.8	80.1 ± 0.7	79.9 ± 1	80.3 ± 1
Height [cm]	162.1 ± 1.5*	175.6 ± 1.6*	168.9 ± 1.7	169.8 ± 2.4	168 ± 2.4
Body mass [kg]	69.3 ± 3.7	73.6 ± 2.4	71.5 ± 2.2	74.9 ± 3.4	68.5 ± 2.7
Body fat pre [kg]	21.9 ± 2.4*	15.8 ± 1.7*	19 ± 1.6#	20.2 ± 2.9	17.8 ± 1.3
Body fat post [kg]	22.1 ± 2.6*	15.3 ± 1.6*	18.7 ± 1.6#	20.1 ± 3.1	17.3 ± 1.2
Thigh fat pre [kg]	5.4 ± 0.7*	3.1 ± 0.3*	4.2 ± 0.1#	4.6 ± 0.8	3.9 ± 0.3
Thigh fat post [kg]	5.2 ± 0.7*	3 ± 0.3*	4.1 ± 0.1#	4.5 ± 0.8	3.7 ± 0.3
Thigh muscle pre [kg]	9.1 ± 0.4	9.6 ± 0.3	9.3 ± 0.3#	9.4 ± 0.4	9.2 ± 0.3
Thigh muscle post [kg]	9.3 ± 0.4	9.8 ± 0.3	9.6 ± 0.3#	9.7 ± 0.4	9.4 ± 0.3
relative MEL pre [N/kg]	14.1 ± 1.1	14.7 ± 1.4	14.4 ± 0.9#	14.9 ± 1.4	14 ± 1.1
relative MEL post [N/kg]	15.3 ± 1.1	15.9 ± 1.1	15.6 ± 0.7#	15.9 ± 1	15.3 ± 1.1

Parameters of RET (n=13) and EET (n=14) subjects are displayed as mean values±SE. Relative MEL=Maximal isometric leg extension strength relative to body weight. The changes from pre to post training were significant for pooled subjects (EET+RET, n=27) for body fat, thigh fat, thigh lean and relative MEL (ANOVA with repeated measures, $p<0.05$, Tukey's post-hoc analysis, indicated as #). Height, body fat and thigh fat content were significantly different between women and men (ANOVA, $p<0.05$, indicated as *).

Table 2

	Eccentric (EET)		Resistance (RET)	
	Pre	Post	Pre	Post
Vv(mc,f)	4.6 ± 0.9%	4.32 ± 0.65%	4.86 ± 0.24%	5.17 ± 0.22%
Vv(ms,f)	0.6 ± 0.99%	0.54 ± 0.06%	0.72 ± 0.4%	0.65 ± 0%
Vv(mt,f)	5.19 ± 1.88%	4.85 ± 0.6%	5.59 ± 0.64%	5.82 ± 0.23%
Vv(li,f)	0.54 ± 0.32%	0.3 ± 0.12% *	0.44 ± 0.03%	0.49 ± 0.17%
Vv(fi,f)	82.94 ± 2.72%	84.29 ± 2.04%	82.6 ± 2.96%	82.3 ± 1.93%
Vv(re,f)	11.33 ± 0.52%	10.55 ± 2.52%	11.38 ± 3.57%	11.39 ± 1.98%
NN(c,f)	1.05 ± 0.08	1.14 ± 0.08	1.26 ± 0.15	1.24 ± 0.12
a(f)	3125.48 ± 182.99 µm2	3266.06 ± 210.89µm2	3489.13 ± 388.71µm2	3714.1 ± 329.81µm2

Morphological parameters as estimated from M. vastus lateralis biopsies by electron microscopy of RET (n=13) and EET (n=14) subjects. They are displayed as mean values±SE. Vv(mc,f)= Central mitochondrial volume per fiber volume, Vv(ms,f)= Subsarcolemmal mitochondrial volume per fiber volume, Vv(mt,f)= Total mitochondrial volume per fiber volume, Vv(li,f)=Volume of intramyocellular lipid per fiber volume, Vv(fi,f)=Volume of myofibers per fiber volume, Vv(re,f)= "Residual" volume per fiber volume, Nn(c,f)=Number of capillaries per fiber, a(f)=mean cross sectional area per fiber. A significant decrease in Vv(li,f) could be recorded with EET (two tailed paired student's t test; $p=0.02$).

GENE	FUNCTION	RET	EET	ALL
COL1A1	Extracellular matrix	0.81	3.78	1.76
COL3A1	Extracellular matrix	1.15	3.50	2.03
COL4A1	Extracellular matrix	1.48	1.36	1.42
COL6A1	Extracellular matrix	1.20	1.52	1.33
LAMA2	Extracellular matrix	1.23	1.29	1.25
CDH15	Cell adhesion	1.20	1.13	1.16
ITGB1	Cell adhesion	1.22	1.23	1.23
VCL	Cell adhesion	1.34	1.27	1.31
ACTA1	Cytoskeleton	1.18	0.86	1.01
ACTN1	Cytoskeleton	1.83	1.43	1.63
DAG1	Cytoskeleton	1.36	1.16	1.26
DES	Cytoskeleton	1.06	0.75	0.89
DMD	Cytoskeleton	1.07	0.74	0.91
MYH1	Cytoskeleton	0.85	0.46	0.73
MYH3	Cytoskeleton	1.31	2.55	2.06
MYH6	Cytoskeleton	1.43	1.92	1.68
TNNC2	Cytoskeleton	0.96	0.62	0.79
ANGPT1	Angiogenesis	1.37	1.22	1.29
ANGPT2	Angiogenesis	1.37	1.25	1.32
VEGFB	Angiogenesis	1.16	0.94	1.05
CDKN2D	Cell cycle	1.37	1.10	1.23
CCND1	Cell cycle	1.29	1.22	1.26
GADD45	DNA repair	1.74	2.38	2.09
FGFR4	Cell Growth	0.98	1.82	1.38
HGF	Cell Growth	1.36	1.79	1.56
IGF1	Cell Growth	1.22	1.71	1.43
IGFBP5	Cell Growth	0.84	0.75	0.80
RPSA	Translation	0.97	0.81	0.89
MEF2C	Transcription factor	1.23	1.10	1.16
MYOD1	Transcription factor	1.49	1.18	1.32
MYOG	Transcription factor	1.40	1.73	1.58
Pax7	Transcription factor	1.21	1.30	1.26
SRF	Transcription factor	1.18	0.90	1.05
CAMK2B	Kinase	1.02	0.76	0.88
DMPK	Kinase	1.09	1.57	1.34
CKM	Metabolism	1.05	0.66	0.85
CS	Metabolism	1.12	0.68	0.89
GAPDH	Metabolism	1.10	0.73	0.91
DCI	Metabolism	0.95	0.76	0.86
MDH2	Metabolism	0.96	0.76	0.87
ALDOA	Metabolism	1.04	0.65	0.86
HADH	Metabolism	0.97	0.72	0.85
ACADVL	Metabolism	1.05	0.80	0.92
HADHB	Metabolism	1.06	0.78	0.92
ACAT1	Metabolism	1.06	0.73	0.90
ACADL	Metabolism	0.94	0.79	0.87
COX5B	Mitochondria	1.03	0.81	0.92
MT-ND1	Mitochondria	1.06	0.71	0.88
MT-CO1	Mitochondria	1.09	0.75	0.91
ATP6	Mitochondria	1.08	0.82	0.95
ATP5B	Mitochondria	0.89	0.76	0.83
CYC1	Mitochondria	0.98	0.83	0.90
PPARGC1A	Transcription factor mt	1.26	0.68	0.95
TFAM	Transcription factor mt	1.17	0.90	1.04
SLC27A1	fatty acid transporter	0.88	0.82	0.85
CD36	FAT	1.13	1.33	1.23
CAPN3	Protease	1.09	0.70	0.90
SOD2	Redox/pH	1.22	0.91	1.06
NOS1	Signaling	1.09	0.62	0.86
MB	Oxygen carrier	0.86	0.77	0.82

Table 3

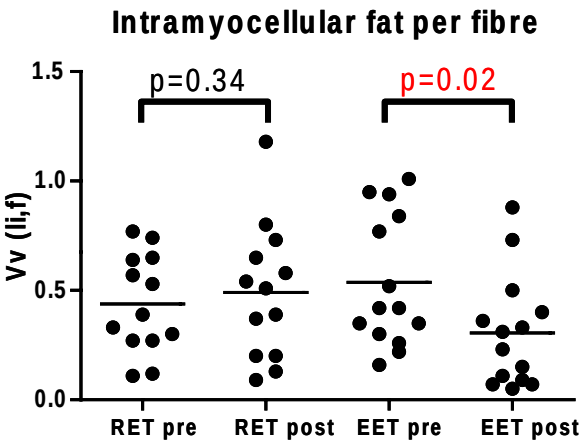
Table 3 shows significantly changed expression of gene transcripts with training. Numbers represent post/pre ratio of RET (n=13), EET (n=14) and all trained subjects (ALL) (n=27), respectively. Black indicates significant up-regulation, grey significant down-regulation. Pre-post differences were verified with an ANOVA with repeated measures and Tukey's HSD post hoc analysis with a 5% level of significance. Transcript names are according to U.S. National Library of Medicine (NCBI).

Supplementary table 4

Lists all 187 gene transcripts which were analyzed with the microfluidic cards.

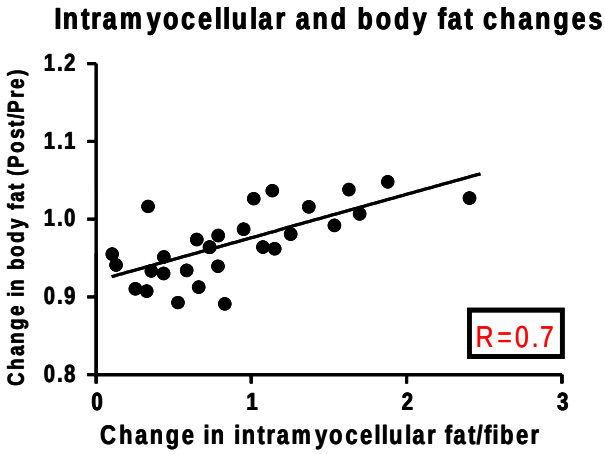
Gene	Gene Name	Gene	Gene Name
18S	ribosomal RNA	IL6	interleukin 6
ACADL	acyl-Coenzyme A dehydrogenase	IL6R	interleukin 6 receptor
ACADM	acyl-Coenzyme A dehydrogenase	IL8	interleukin 8
ACADVL	acyl-Coenzyme A dehydrogenase	IL8RA	interleukin 8 receptor
ACAT1	acetyl-Coenzyme A acetyltransferase 1	INSR	insulin receptor
ACE	angiotensin I converting enzyme	ITGA2	integrin, alpha 2
ACTA1	alpha sarcomeric actin	ITGA7	integrin, alpha 7
ACTB	beta-actin	ITGB1	integrin, beta 8
ACTN1	actinin, alpha 1	JUN	jun oncogene
ACTN2	actinin, alpha 2	KDR	VEGF-Receptor 2
ADMR	adrenomedullin receptor	LAMA2	laminin alpha 2
ADORA1	adenosine A1 receptor	LAMA4	laminin alpha 4
AGRIN	agrin	LDHA	lactate dehydrogenase A
AKT1	protein Kinase B, PKB	LDHB	lactate dehydrogenase B
ALDOA	aldolase A	LGALS1	lectin, galactose binding, soluble 1
ALDOC	aldolase C	LIPE	lipase, hormone sensitive
ANGPT1	angiopoietin 1	LPL	lipoprotein lipase
ANGPT2	angiopoietin 2	MAPK8	mitogen-activated protein kinase 8
ANKRD2	ankyrin repeat domain 2 (stretch responsive muscle)	MB	myoglobin
AR	androgen receptor (dihydrotestosterone receptor)	MDH2	malate dehydrogenase 2
ATP2A1	ATPase, Ca++ transporting, fast twitch 1	MEF2B	myocyte enhancer factor 2B
ATP2A2	ATPase, Ca++ transporting, slow twitch 2	MEF2C	myocyte enhancer factor 2C
ATP5B	F1FO ATPase, beta subunit	MET	hepatocyte growth factor receptor
ATP6	ATP synthase 6, mt encoded	MMP11	matrix metallopeptidase 11 (stromelysin 3)
BCKDHA	branched chain keto acid dehydrogenase E1	MMP2	matrix metallopeptidase 2 (gelatinase A)
BDNF	brain-derived neurotrophic factor	MMP8	matrix metallopeptidase 8 (neutrophil collagenase)
CA3	carbonic anhydrase III	MMP9	matrix metallopeptidase 9 (gelatinase B)
CAMK2B	CaM kinase II beta	MT-CO1	mitochondrially encoded cytochrome c oxidase I
CAPN1	calpain 1	MT-ND1	NADH gene 1, mt encoded
CAPN2	calpain 2	MUSK	muscle, skeletal, receptor tyrosine kinase
CAPN3	calpain 3, p94	MYF6	myogenic factor 6 (herculin)
CAST	calpastatin (calpain inhibitor)	MYH1	myosin heavy chain, 2X
CCL2	chemokine (C-C motif) ligand 2	MYH2	myosin heavy chain 2A
CCNA1	cyclin A1	MYH3	myosin heavy chain, embrionic
CCND1	cyclin D1	MYH4	myosin heavy chain, 2B
CD163	CD163 molecule, macrophage marker	MYH6	myosin heavy chain 6, cardiac muscle, alpha
CD34	CD34 molecule, satellite cell marker	MYH7	myosin heavy chain 6, cardiac muscle, beta
CD36	muscular fatty acid transporter, FAT	MYH8	myosin heavy chain 8, perinatal
CD68	CD68 molecule, macrophage marker	MYOD1	myogenic differentiation 1
CDH15	cadherin 15	MYOG	myogenin (myogenic factor 4)
CDKN1A	cyclin-dependent kinase inhibitor 1A, p21	MYOT	myotilin
CDKN2D	cyclin-dependent kinase inhibitor 2D (p19, inhibits CDK4)	NCAM1	neural cell adhesion molecule 1
CKM	creatine kinase	NEB	nebulin
CNTF	ciliary neurotrophic factor	NGFB	nerve growth factor, beta polypeptide
COL1A1	collagen, type I, alpha 1	NID1	nidogen 1
COL3A1	collagen, type III, alpha 1	NOS1	nitric oxide synthase 1 (neuronal)
COL4A1	collagen, type IV, alpha 1	NR4A3	Nor1, Transcription factor
COL6A1	collagen, type VI, alpha 1	NRG1	neuregulin 1
COX4I1	cytochrome c oxidase subunit IV isoform 1	NTF3	neurotrophin 3
COX5B	cytochrome c oxidase subunit Vb	NTF5	neurotrophin 5 (neurotrophin 4/5)
CPT1B	carnitine palmitoyltransferase I	Pax7	paired box 7, satellite cell marker
CPT2	carnitine palmitoyltransferase II	PDGFA	platelet-derived growth factor alpha polypeptide
CS	citrate synthase	PDHA2	pyruvate dehydrogenase (lipoamide) alpha 2
CYC1	cytochrome c-1	PDHB	pyruvate dehydrogenase (lipoamide) beta
DAG1	dystroglycan	PECAM1	platelet/endothelial cell adhesion molecule (CD31)
DCI	dodecenoyl-Coenzyme A delta isomerase	PFKM	phosphofructokinase
DES	desmin	PGK1	phosphoglycerate kinase 1
DMD	dystrophin (muscular dystrophy)	PMP22	peripheral myelin protein 22
DMPK	dystrophia myotonica-protein kinase	PPARA	peroxisome proliferator-activated receptor alpha
ECH1	enoyl Coenzyme A hydratase 1	PPARG	peroxisome proliferator-activated receptor gamma
ECHS1	enoyl Coenzyme A hydratase	PPARGC1A	peroxisome proliferator-activated receptor gamma
EEF2	eukaryotic translation elongation factor 2	PTK2	PTK2 protein tyrosine kinase 2
EIF4E	eukaryotic translation initiation factor 4E	RELN	reelin
EPAS1	endothelial PAS domain protein 1	RPL11	ribosomal protein L11
ESR1	estrogen receptor 1	RPS9	ribosomal protein S9
FABP3	fatty acid binding protein 3	RPSA	ribosomal protein SA
FASN	fatty acid synthase	SCP2	sterol carrier protein 2
FBXO32	F-box protein 32, Atrogin-1	SIRT1	sirtuin
FGF2	basic fibroblast growth factor	SLC16A1	monocarboxylic acid transporter 1, MCT-1
FGFR1	fibroblast growth factor receptor 1	SLC16A4	monocarboxylic acid transporter 4, MCT-4
FGFR4	fibroblast growth factor receptor 4	SLC27A1	solute carrier family 27 (fatty acid transporter)
FN1	fibronectin 1	SLC2A1	Glucose transporter 1, GLUT1
FST	follistatin	SLC2A4	Glucose transporter 4, GLUT4
GADD45	growth arrest and DNA-damage-inducible 45	SOD1	superoxide dismutase 1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	SOD2	superoxide dismutase 2
GDF8	growth differentiation factor 8, myostatin	SOD3	superoxide dismutase 3
GHRL	ghrelin/obestatin preprohormone	SRF	serum response factor
GSPT2	G1 to S phase transition 2	TCP1	t-complex 1, molecular chaperone
GYS1	glycogen synthase 1 (muscle)	TFAM	transcription factor A, mitochondrial
HADH	hydroxyacyl-Coenzyme A dehydrogenase	TGFB1	transforming growth factor beta 1
HADHB	dehydrogenase/thiolase/hydratase (trifunctional)	TNC	tenascin C
HGF	hepatocyte growth factor	TNF	tumor necrosis factor
HIF1A	hypoxia-inducible factor 1	TNFRSF1A	tumor necrosis factor receptor superfamily
HK2	hexokinase 2	TNNC1	troponin C type 1 (slow)
HNF4A	hepatic nuclear factor 4, alpha	TNNC2	troponin C type 2 (fast)
HSPA4	heat shock 70kDa protein 4	TTN	titin
IGF1	insulin-like growth factor 1 (somatomedin C)	TUBA1	tubulin
IGF1R	insulin-like growth factor 1 receptor	UBC	ubiquitin c
IGF2	insulin-like growth factor 2 (somatomedin A)	UBTF	upstream binding transcription factor
IGF2R	insulin-like growth factor 2 receptor	UCP3	uncoupling protein 3, mitochondrial
IGFBP4	insulin-like growth factor binding protein 4	VCL	vinculin
IGFBP5	insulin-like growth factor binding protein 5	VEGF	vascular endothelial growth factor
IL1B	interleukin 1 beta	VEGFB	vascular endothelial growth factor beta
IL1R1	interleukin 1 receptor		

Figure 1A



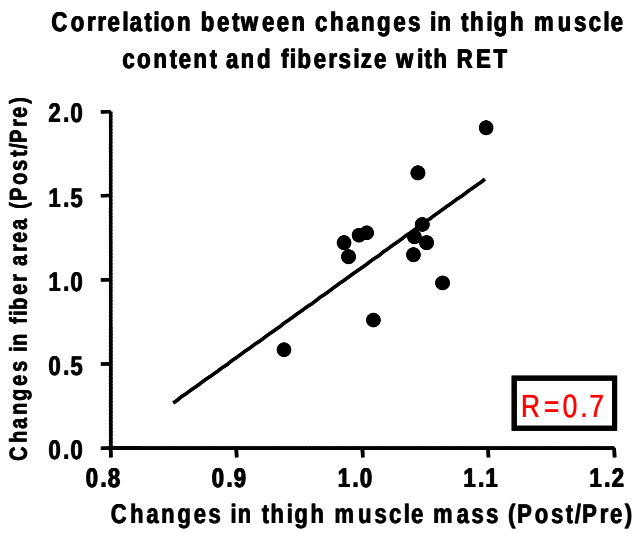
Intramusclular lipid (IMCL) volume per fiber volume in RET (n=13) and EET (n=14) subjects, pre and post-training, respectively. A statistically significant change was found in the biopsies of EET subjects who lost 43% of IMCL with training. Indicated p-values were from a two-tailed paired student's t test.

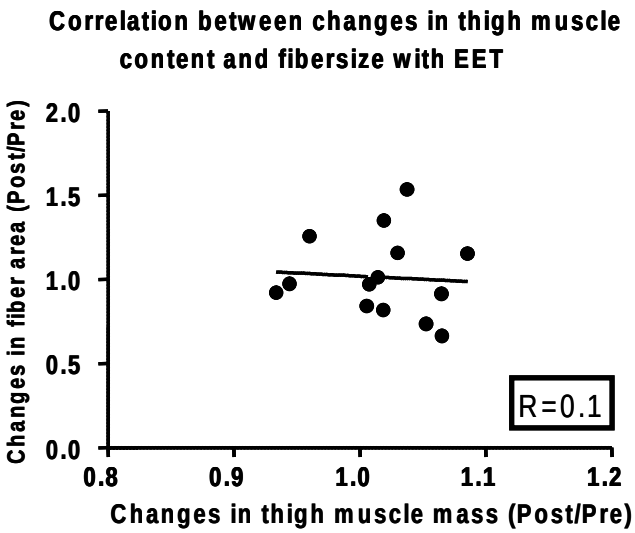
Figure 1B



This figure illustrates the highly significant ($P < 0.01$) positive correlation of change (post/pre) in body fat content with change in IMCL content over all subjects (n=27). The Pearson coefficient for the correlation is 0.7.

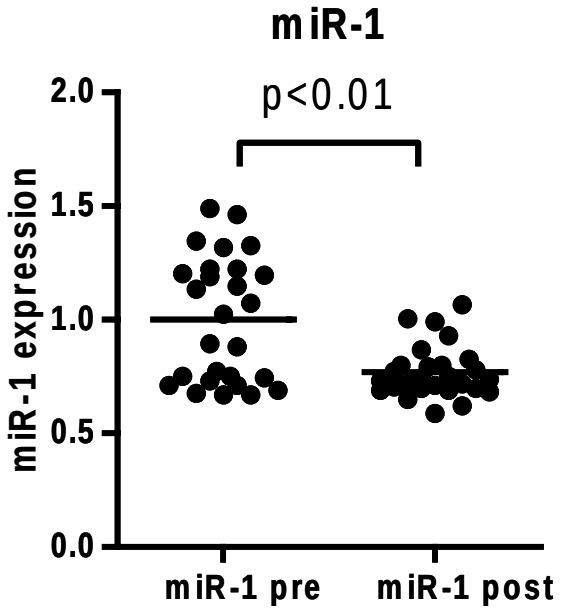
Figure 2





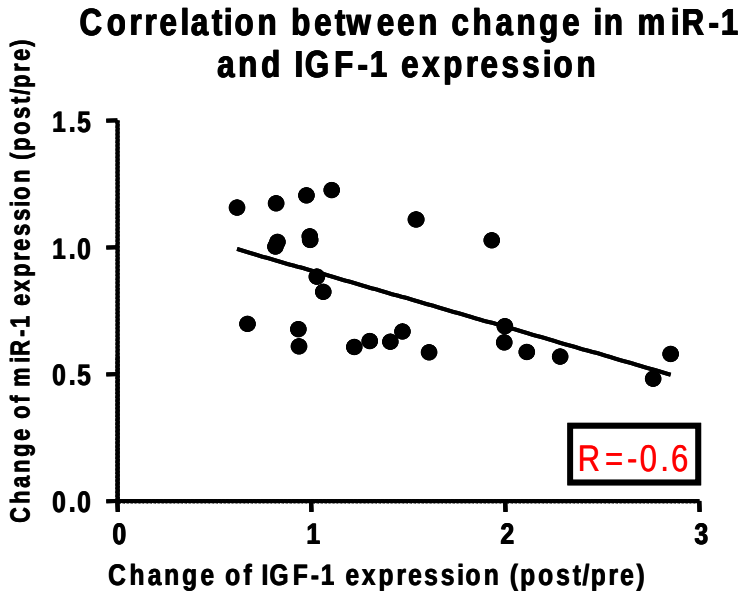
A highly significant correlation between changes in thigh lean mass content and mean fiber cross sectional are (post/pre) could be observed exclusively in the RET subjects (n=13) but not in EET subjects (n=14). R indicates the Pearson coefficient.

Figure 3A



Expression of micro RNA 1(miR-1) before and after training in the biopsies of all subjects (RET and EET group, n=27). A significant drop in response to training is indicated (paired two-tailed student's t test).

Figure 3B



Illustrates the significant negative correlation (p<0.01) between the changes (post/pre) in miR-1 and insulin like growth factor 1 (IGF-1) mRNA of pooled RET and EET subjects (n=27).The Pearson coefficient was -0.6.

Figure 4

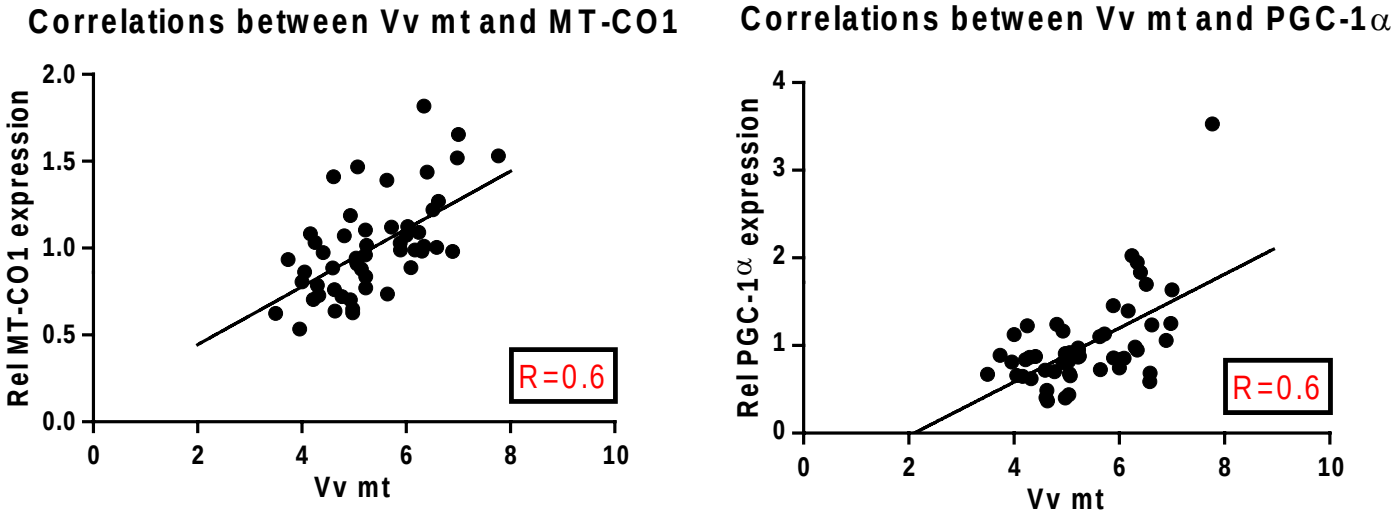


Figure 4 Shows the correlations (p < 0.01) between mitochondrial volume density (Vv mt) and the mitochonrially encoded cytochrome c oxidase 1 mRNA (MT-CO1) in the left panel and between mitochondrial volume density (Vv mt) and the nuclear encoded transcriptional coactivator promoting mitochondrial biogenesis (PGC 1α) in the right panel. Both Pearson coefficients were 0.6 RET and EET subjects were pooled (n=27, 54 datapoints).

Supplementary figure 5

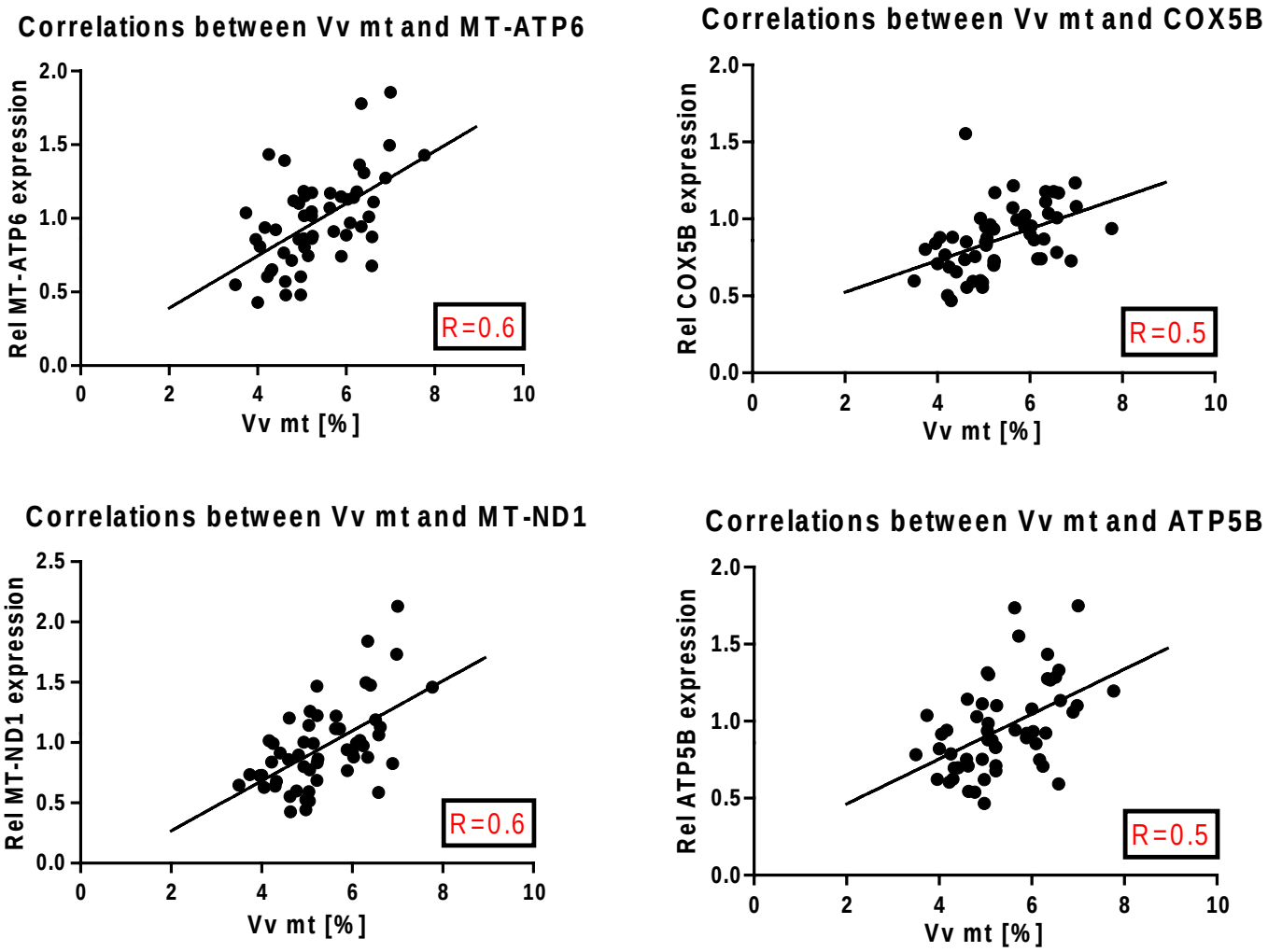
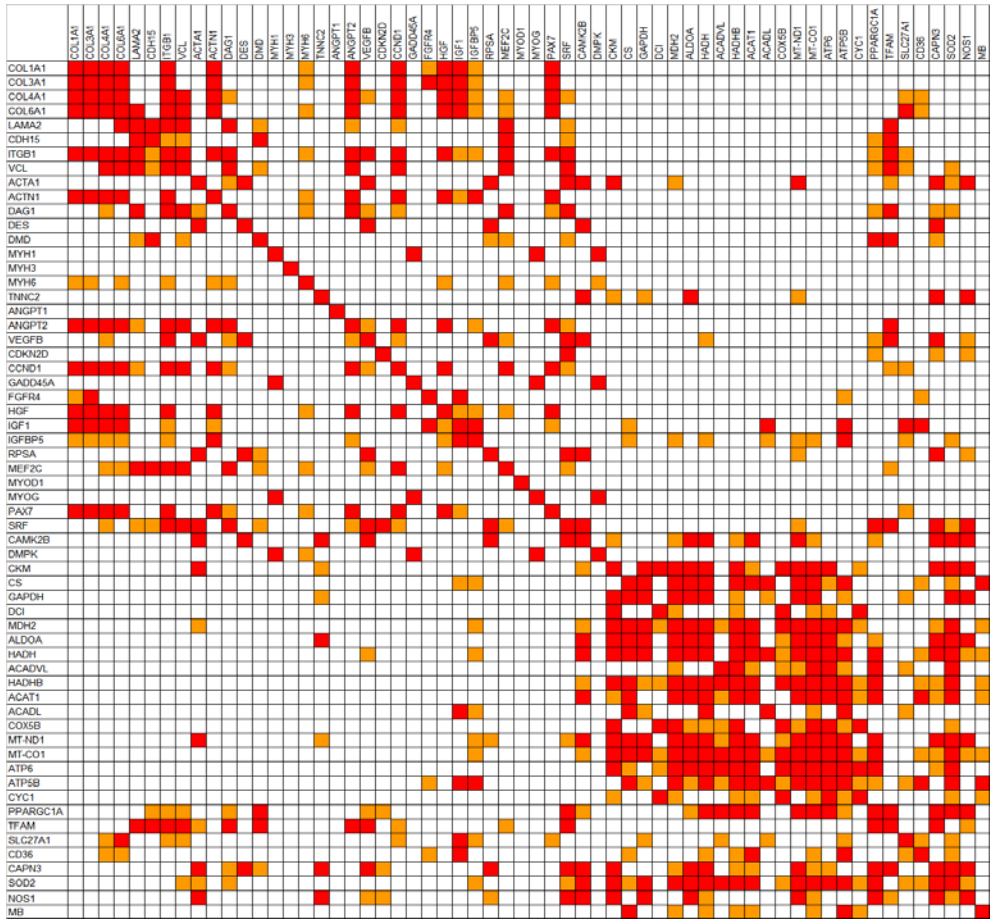
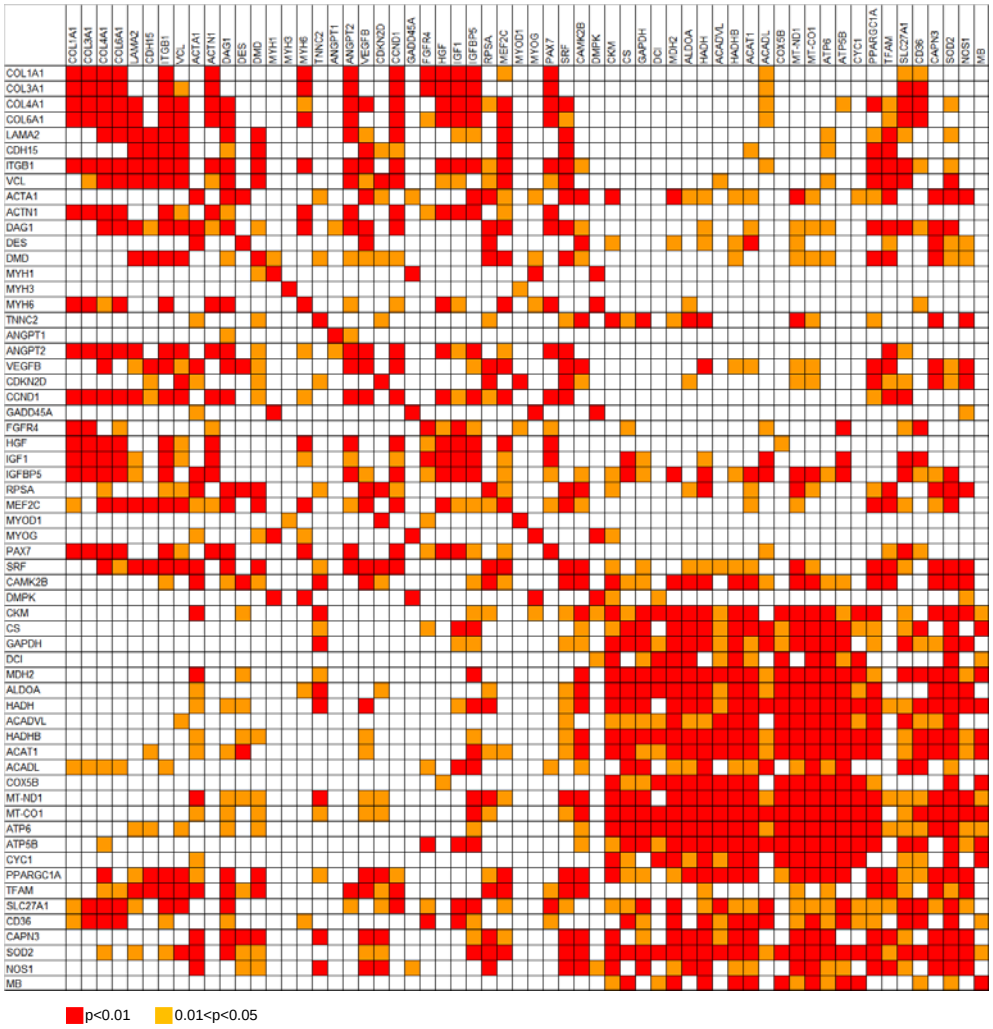


Figure 5 displays a highly significant (P<0.01) positive correlation between mitochon-drial volume density and expression of mitochondrially encoded ATP-6, ND1 and nuclear encoded COX5B and ATP5B. RET and EET subjects were pooled (n=27, 54 datapoints). Respective Pearsons coefficients of correlation are indicated in the figures.



Supplementary figure 6

Top panel: correlation matrix with colour coded coefficients of correlation. The color value of each little square is an indication of the strength of the correlation between the two transcripts whose lines cross at that point. Only those genes that were significantly changed with training are displayed with the same grouping as in table 3.



Bottom panel: p-values of the the respective correlations (see top panel). Coloured spots represent significant (p<0.05) correlations between the two transcripts whose lines cross at that point.

One Year Follow Up

Subjects were tested one year after finishing the training intervention for functional parameters and body composition. During this year no guided training session were provided. Activity was qualitatively assessed using questionnaires. Physical activity level was maintained during this year, but most subjects stopped the regular strength training.

	Cognitive (CT)			Resisitance (RET)			Eccentric (EET)		
Time point	Pre	Post	FU	Pre	Post	FU	Pre	Post	FU
MEL [N/kg]	16.2 ± 1.09	15.8 ± 0.98	15.4 ± 1.09	15.5 ± 1.01	16 ± 0.88	15.6 ± 0.93	15.5 ± 0.9	**16.7 ± 0.9***	15.7 ± 0.81
Coordination [RMS]	66.3 ± 12.7	56.4 ± 8.3	56.5 ± 9	68.4 ± 5	58.9 ± 5	59.4 ± 4.7	66.6 ± 6.1	**37.8 ± 4.1***	**31.8 ± 3.2***
Body fat [%]	27 ± 2.7	27.2 ± 2.4	27.8 ± 2.5	27.7 ± 2.1	27.2 ± 2.1	27.7 ± 2	25.1 ± 2	**24.2 ± 1.9***	24.9 ± 1.9
Thigh musle [%]	68.1 ± 3.16	68.2 ± 2.8	65.7 ± 5.2	67.9 ± 2.7	**69 ± 2.7***	68.4 ± 4.7	69.4 ± 2.3	**71.5 ± 2***	70.2 ± 2.6

Table 1: Parameters are displayed as mean values±SE. CT (n=7), RET (n=17), EET (n=17), Pre=pre training, Post=post training, FU=one year follow up, MEL=maximal isometric extension strength of the legs, Coordination=eccentric coordination on the eccentric bike, Body fat=relative whole body fat content, Thigh muscle= relative thigh muscle content. Bold* marked values significantly differ from Pre-values (ANOVA with repeated measures; Tukey's HSD post-hoc test; $p<0.05$)

As expected, improvements in leg strength and body composition of EET subjects were lost upon cessation of training. Surprisingly, eccentrically training subjects maintained the ability to match the eccentric target load on the eccentric bike. Subjects did not practice this kind of exercise for one year and were still able to execute this highly demanding task with the same accuracy as directly after the intervention. We don't know whether or to which extent an improved eccentric coordination may be beneficial with regard to fall prevention. A prospective study with a large sample size would be necessary to answer these questions.

3. Discussion and Outlook

Summary

The basic aim of our study was to compare different training regimes in a population of older men and women with the object of maximizing leg extension strength. Functional, structural and molecular adaptations were assessed in order to elucidate the underlying mechanisms for observed improvements. The persistence of the functional and body composition improvements was assessed with a one year follow-up test. The screened population consisted of independently living active elderly with an average age of 80 years (ranging from 71 to 92 years). Training duration and frequency were low to moderate (2x45min/week) such that the extent of the training load could realistically be fitted into the busy schedule of our elderly subjects. Despite this limitation, we observed beneficial functional outcomes with EET and RET. On the one hand, EET was more successful than RET in improving leg (1) strength, (2) body composition and (3) eccentric coordination. On the other hand, EET resulted in a significant depression of transcripts coding for proteins involved in metabolism and mitochondrial function. The decrease of oxidative capacity on the mitochondrial level was not significant, but individually correlated with the expression of mitochondrial transcripts (Mueller et al. 2009; Mueller et al. 2010).

Study Limitations

Our study design required subjects who lived independently and were able to train twice weekly in the gym or at our institute. This simple requirement resulted in a selection bias, such that subjects displayed better physical and psychological condition on average than their peers. Inclusion of subjects was restricted to individuals with stable medication. Compliance with the training programmes was good with an average attendance of 89%. Every day activity before, during and after the training intervention was not quantitatively assessed. We assume that activity was very heterogeneous among subjects and might also have interfered with our training advice. Institutionalized subjects allow for better survey, but usually subjects reside initially in a poorer health condition and therefore do not represent our population of interest. We chose our study subjects with the knowledge the following drawbacks: (1) due to their good initial fitness the response to the training regime would be less pronounced, (2) it would be very difficult to control their daily activity and diet (and was not attempted

as a consequence in our study), (3) the population was less homogeneous and required paired analyses and individual correlations (responders vs. non-responders).

Stable medication was one of the inclusion criteria. Medication included beta blockers, statins, diuretics, Ca^{2+} channel blockers, ACE inhibitors, angiotensin II antagonists, coumarins, anti-depressant drugs and acetyl-salicylic acid. There were no apparent influences of medical treatments on the functional or molecular results, probably also due to the fact that groups of subjects with similar medication were very small (Lotscher et al. 2007). Subjects included were in a stable health condition and did not suffer from symptomatic non-insulin dependent diabetes mellitus (NIDDM). However, we could not exclude subjects suffering from mild forms of age related insulin resistance. Over all, health condition of our subjects was good and it was either improved or maintained during the intervention.

A major concern during the follow up was the health condition of subjects, which can rapidly change in the elderly. Not only the personal health condition but also disease or death of partners, friends or relatives is much more frequent in the social environment of elderly and strongly affects their own welfare and health but also their behaviour with regard to nutrition and physical activity. Therefore follow up results have to be interpreted with caution since they do not only reflect the discontinuation of training but also depend largely on behaviour and lifestyle. Assessment of physical activity and quality of life were only approximations used for the estimation of factors contributing to the follow up results.

Technical Limitations

Analysis of muscle biopsies by means of stereological tools has extensively been applied and validated in our institute and it still reflects the gold standard in morphological ultra-structural examination. The experience with biopsy sampling was indispensable as this particular point strongly affects outcome and interpretation of data. A major concern in biopsy sampling and analysis is the so called “reference trap”. The term "reference trap" describes the problem of estimating relative data without taking absolute values into account. Based on the assumption of homogeneous muscle adaptations (fiber types, ultrastructure, gene expression), it is possible to extrapolate sampled data and to obtain absolute values using additional measures such as muscle mass and CSA. However, the assumption of homogenous muscle architecture is not entirely vaild: in some muscles gradients in fiber type distribution can be found (i.e. surface versus bone proximity). Muscle biopsies in our study were collected from the midportion of M. *vastus lateralis*, which is known to be relatively homogenous with regard to fiber type distribution. Nevertheless, with this sampling method we risked missing adaptations that occurred locally at the proximal or distal portions of M. *vastus lateralis* as reported by Seyennes et al. (Seynnes et al. 2007). The same might be true for the estimation of muscle specific gene expression which was suggested to parallel local muscle growth (Fluck and Hoppeler 2003).

Beside the dependence of localisation, the time point of biopsy sampling after the intervention might strongly affect gene expression, but not the structural changes. Since we were interested in steady state adaptations we collected biopsies between 48 and 72 hours after the last training bout. To rule out the possible interference of the last training bout (especially in the eccentric mode), we chose to sample biopsies at least 48 hours after the last training session. This was based on previous observations in our lab monitoring the eccentric cycling ergometer single bout response over 24 hours. Klossner et al. (Klossner et al. 2007) reported an incomplete recovery of initially decreased muscle specific mRNA transcript expression. To guarantee steady state measurements and not simply a response to the last bout of training, it would be best to collect multiple biopsies 1, 2, 3, 7 and 14 days after the intervention. Due to ethical reasons this was not possible and we were forced to take only one single post time-point. The control with morphological correlates confirmed our gene expression data and supported the decision to sample between 48 and 72 hours after the last bout. The mechanisms of the repeated bout effect are still largely unknown

and therefore it would be of interest to monitor a single exercise bout response in a trained and untrained state using our eccentric cycling ergometer.

Normalisation procedure for gene expression data deals with even more complex problems analogous to the “reference trap” for morphometrical analyses. Standardization of gene expression data to relative values is already problematic, because reverse transcription is difficult to standardize and needs to be controlled endogenously. Usually, PCR based gene expression data are normalized to single endogenous housekeeping genes such as Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), β-2-microglobulin (B2M), β -actin (ACTB), cyclophylin (Cyp), whose expression is assumed to be stable (Garma et al. 2007). However, with interventions causing perturbations that affect expression levels of most genes we could not assume stable expression of these common housekeeping genes. We applied different standardisation procedures including a globalisation approach in which we normalized individual transcripts to the mean expression of all transcripts, thereby assuming a stable over all expression of the measured transcripts from pre to post training. In another approach we standardized using combinations of different housekeeping genes including GAPDH, citrate synthase (CS), ACTB and glucose transporter 1 (GLUT1). Finally we have chosen 18S as a standard to account for different reverse transcription efficiencies for two reasons: (1) total RNA content remained unchanged from pre to post intervention (see (Mueller et al. 2010)). (2) Total RNA amount is best reflected by ribosomal RNA which makes up to 80% of total RNA content (Hamurcu et al. 2006). 3) Results obtained with 18S standard fitted best with structural correlates such as mitochondrial volume density. Extrapolation of relative gene expression to absolute values did not qualitatively change our results. For the group comparison (RET versus EET), absolute values were not of interest since changes in muscle mass were of the same magnitude and direction. Most importantly, verification with morphometrical correlates corroborated our normalisation procedure as these are estimates of protein quantity (i.e. mitochondrial proteins).

Gene expression analysis with muscle homogenates is always confounded by the fact that muscle tissue is not made up exclusively of muscle cells, but also with non-muscle cells such as fibroblasts, endothelial cells and blood derived cells including immune cells. Approximately 50% of all nuclei occurring in muscle tissue belong to

non-muscle cells. This has the potential to drastically influence gene expression analysis. Mitochondrial and metabolic genes are mostly transcribed by muscle cells since these contain a high mitochondrial content and metabolic activity. However, extracellular matrix components (which are considerably up-regulated with EET) derive from fibroblasts residing within the ECM of muscle tissue. Transcripts coding for myogenic factors are mostly up-regulated by satellite cells located between sarcolemmal and basement membrane. With laser capture microscopy, also known as laser capture micro-dissection (LCM) it is possible to isolate and collect small numbers of cells (i.e. satellite cells) or tissue samples from frozen or formalin-fixed tissue sections. LCM techniques rely on slides covered with thermo labile membranes where tissue sections are sampled. Focused laser energy is used to melt the membrane and to cut boarders of samples, which then can be captured in a vial. This technique allows for the selection of cells or tissue sections with a minimal resolution of several micrometers. DNA, RNA, protein, and lipid samples may be isolated and analyzed from micro-dissected samples. An obvious use LCM would be to analyze muscle samples in order to screen for single fiber types or to track one individual fiber longitudinally. RNA or protein expression of single cells or gradients longitudinally along single fibers can be assessed without contaminants, which may heavily influence experimental results. We established laser capture micro-dissection to analyze different muscle fiber types. We chose an approach using serial sections to identify fiber types using ATPase staining. Specific fiber-sections were collected with LCM from the subsequent 10 μm sections, which were processed with an adapted hematoxylin and eosin staining kit to prevent RNA degradation prior to dehydration (Arcturus, Bucher Biosciences, Basel, Switzerland). Preservation of RNA is the most difficult part of the application of LCM to analyze gene expression. Isolation of RNA from LCM samples requires a very sensitive protocol due to the small amount of tissue. A visual check of muscle tissue prior to isolation of RNA was not only advantageous with regard to quality but also helped to normalize transcript expression results to a known sample volume. Knowing thickness and the area of the collected section, sample volume could easily be calculated and served to standardize RNA content. Due to a shortage of muscle biopsy samples we were not able do gene expression analysis in the current study. However, we were able to show that the application of LCM is feasible and might be applied in future studies. We are aware of the fact that a larger number of samples are required to estimate the reliability of LCM in order to

guarantee a high local resolution of gene or protein expression. We did not assess RNA integrity in our pilot study but we have observed more consistent results analyzing small RNA's such as mirco RNA's. This could be due to the fact that these were less affected by degradation probably by protective binding of argonaute proteins. Our straight-forward RNA isolation strategy allowed for maximizing transcript yield but implicated reduced control for RNA abundance and quality.

Molecular Training Specificity

Both, endurance and strength training yield specific benefits such as fatigue resistance and strength and may also help to control body weight and composition. However, these two training regimes interfere with each other. Endurance exercise negatively affects strength training induced mechanical signaling of muscle tissue and thereby inhibits any gain in strength and muscle mass. Even though the molecular key players are identical, the magnitude of activation strongly depends on the kind of training regime. Protein synthesis, as boosted by strength training is limited by eukaryotic elongation factor 2 (eEF2), mediating translocation of ribosomes along mRNAs. eEF2 action is inhibited by phosphorylation via eEF2-Kinase (eEF2K) in response to increased energy demand or energy depletion (see figure 5, page 68) (Browne and Proud 2002). Additionally, activation of eEF2K is regulated via calmodulin and AMPK-mediated signaling, both of which are activated by endurance training (Ryazanov 1987; Horman et al. 2002). eEF2 seems to be a major signal integrator of metabolic and mechanical stimulation and can promote or inhibit protein synthesis, respectively. Activation of the mTOR/p70S6K-pathway following resistance training inactivates eEF2K and decreases eEF2 phosporylation thereby promoting protein synthesis and muscle hypertrophy (Wang et al. 2001; Browne and Proud 2002). eEF2 activity is regulated by endurance and strength exercise and represents a key determinant of the control of protein synthesis. PGC-1α is a master regulator of mitochondrial biogenesis, which is promoted by endurance training. The induction of PGC-1α is not well understood and can be promoted by different transcription factors (see introduction) among them FoxO1 (Daitoku et al. 2003). Nuclear abundance and activity of FoxO1 is negatively regulated by Akt which is activated following resistance exercise (Bodine et al. 2001; Sandri 2008). The subsequent inhibition of FoxO1 (translocation to the cytosol) leads not only to a down-regulation of PGC-1α but also ubiquitin ligase gene expression promoting muscle hypertrophy and

inhibiting mitochondrial biogenesis (Southgate et al. 2005). With regard to endurance and strength exercise, FoxO1 activity and localisation seems to be a point of divergence for mitochondrial biogenesis and muscle hypertrophy. Atherton et al. (Atherton et al. 2005) claimed the AMPK-Akt 'master-switch' hypothesis explains training specific phenotypical adaptation. Using a rat model, muscles were electrically stimulated to either mimic endurance (prolonged low frequency) or strength exercise (short high frequency). After low frequency stimulation (endurance) an increase in AMPK activity, PGC-1α gene expression, and an inhibition of mTOR-mediated translational initiation could be observed. In contrast, after high frequency stimulation (strength), hypertrophy was promoted by Akt activation concomitant with a decrease in AMPK activity. It seems that AMPK and Akt represent master regulators that direct skeletal muscle adaptation towards oxidative and hypertrophic phenotype, respectively. In conclusion, endurance and strength type of exercise interfere with each other due to their common signaling pathways. Alternating strength and endurance exercise likely reduces the individual stimulation of muscle hypertrophy or mitochondrial biogenesis compared with single mode training. Since EET executes a strong mechanical stimulus with a small metabolic stress it belongs certainly to strength training modalities even if it is carried out continuously with medium torque contractions.

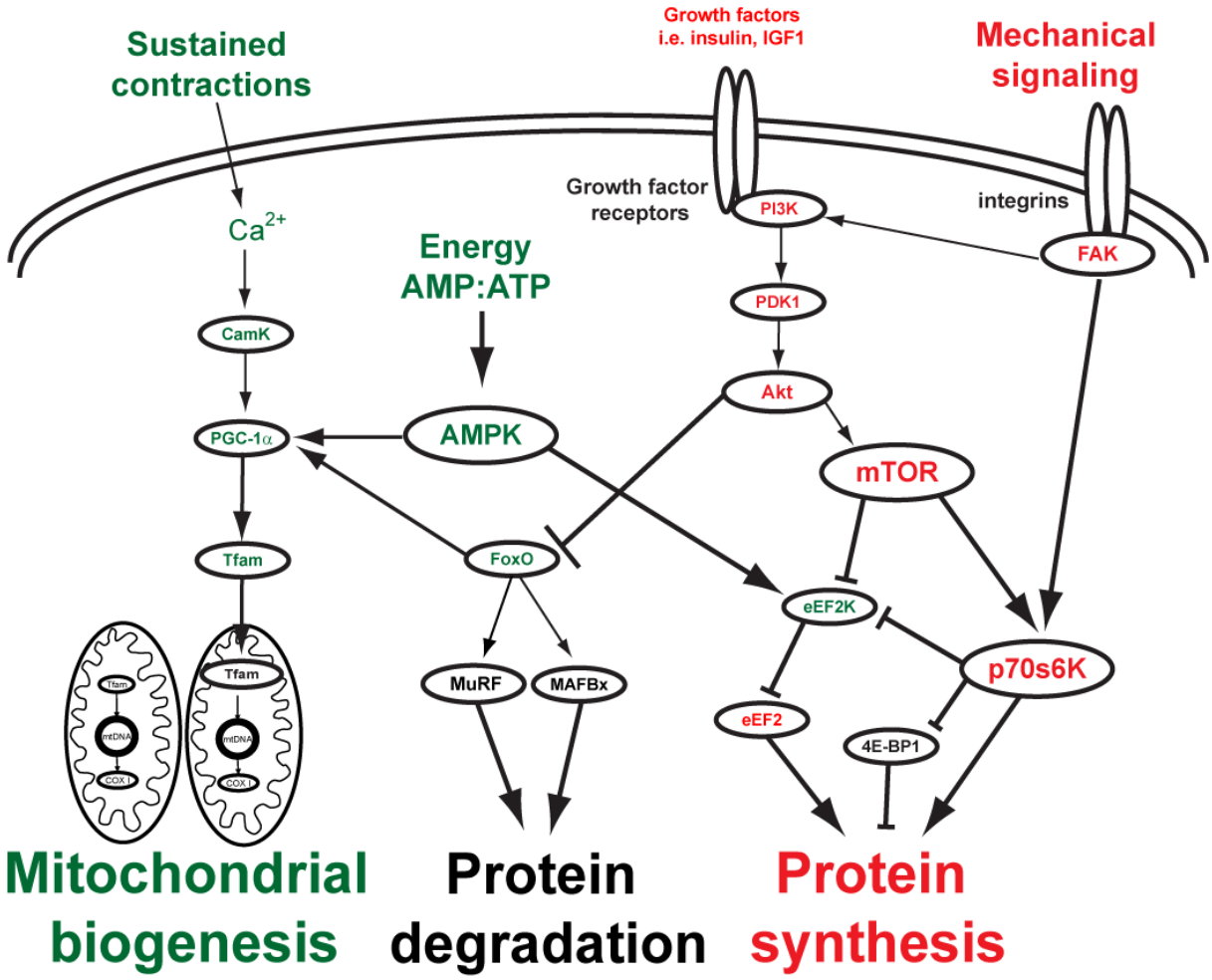


Figure 5: Schematic representation of the predominant molecular signaling events with endurance (green) and strength (red) training. For a more detailed explanation see pages 66 et seq.

Muscle Mass and Strength

Relative thigh muscle mass was increased by a similar magnitude with RET and EET but obviously by different mechanisms. Muscle growth by fiber hypertrophy was suggested to occur with RET where changes in fiber CSA correlate with changes in muscle mass (Mueller et al. 2010). With EET this relation could not be observed. Muscle growth was suggested to be driven by neoplasia, addition of serial sarcomeres or splitting of fibers (Antonio and Gonyea 1993; Kelley 1996; Shoepe et al. 2003; Yu et al. 2003; Yu et al. 2004; Mueller et al. 2010). An increase of fascicle length of pennate muscles with eccentric training leads to an enlarged physiological CSA. This mechanism could account for the increase in strength observed in our

eccentrically study subjects (Reeves et al. 2009). The observed difference between RET and EET subjects with regard to strength gain cannot be explained by structural data. It could be caused by improved muscle coordination which is practiced with EET. The resulting improvement of eccentric coordination could be an indicator of improved neuronal muscle activation. However, we currently don't know if, or to what extent this ability may be transferred to other tasks such as isometric or concentric leg extension. The increase in isometric leg extension strength with EET was of the magnitude as reported by Reeves et al. (Reeves et al. 2009). In their study they also used training independent testing procedures in a population of elderly (average age 67y). For further discussion see (Mueller et al. 2009; Mueller et al. 2010).

Reduction of Oxidative Capacity with EET

One of the most striking results was the depression of mitochondrial and metabolic transcripts with EET. However, oxidative capacity, as estimated by mitochondrial volume density, was not statistically significant. This could be due to several reasons: (1) Estimation of real time PCR based mRNA expression is technically more robust than morphometric analysis because it includes a greater muscle volume; (2) mRNA changes are more pronounced than structural changes; (3) Structural adaptations show a delayed time course. Our current data do not support the hypothesis of a higher technical robustness of real time PCR data since the morphometric estimates had less measurement variation than gene expression data. To which extent a delayed time course or a stronger amplitude of mRNA levels contribute to the observed quantitative discrepancy remains to be investigated. Nevertheless, a reduction of oxidative capacity by EET has been observed consistently and is obviously a potential drawback of EET (Steiner et al. 2004; Zoll et al. 2006). Reduction of oxidative capacity at the expense of increased myofibrillar content was also supported by the morphometric analysis, although this was not statistically significant (myofibrillar content per fiber Vv(fi,f); EET: +1.62%, p=0.1; RET: -0.35%, p=0.69; see also (Mueller et al. 2010)). The reason for a decreased oxidative capacity is not known, but based on the strong mechanical and relatively small metabolic component of eccentric work, stimulation of mitochondrial biogenesis (i.e. activation of AMPK) seems to be insufficient to maintain mitochondrial content during muscle growth. Even in absolute terms, mitochondrial content was reduced with EET (not significant). In addition to oxidative phosphorylation, mitochondria function as calcium sinks in conditions of disturbed cellular calcium levels as occurred during eccentric contractions. Sequestering of

calcium is an important mitochondrial function that guarantees proper cell functioning. It is not yet clear how calcium is bound in mitochondria since protein levels of calreticulin or calsequestrin are low in mitochondria. It was suggested that calcium is most likely bound to membrane phospholipids and/or that calcium precipitates with phosphate ions (Ganitkevich 2003). To what extent high calcium levels can harm mitochondria is not yet clear. Additionally, calcium was shown to regulate mitochondrial ATP synthesis in an organ specific manner. Muscle contraction during exercise produces an increase in cytosolic calcium levels. Ca^{2+} is sequestered by mitochondrial Ca^{2+} transporters leading to an increase in mitochondrial Ca^{2+} level. ATP production is subsequently boosted to meet the increased energy demand (Griffiths and Rutter 2009). For concentric work the energy requirement is high as compared to the increase in cytosolic Ca^{2+} concentration. In contrast, during eccentric work, cytosolic Ca^{2+} increases while energy consumption is remains relatively low (Vissing et al. 2008). This could result in an excess of Ca^{2+} being sequestered by mitochondria. Severe Ca^{2+} overload may extinguish the mitochondrial membrane potential, especially if paralleled by high oxidative stress (Duchen 2000). To what extent Ca^{2+} could be involved in triggering mitochondrial induced apoptosis is not clear. Susceptibility to calcium overload was reported to depend on the level of PGC-1α, which decreases mitochondrial Ca^{2+} uptake (Bianchi et al. 2006; Hajnoczky et al. 2006). To obtain an insight into the mechanisms by which eccentric exercise decreases mitochondrial content it is necessary to analyze immediate mitochondrial responses such as respiration, ATP generation, Ca^{2+} concentrations, membrane potential etc.

Loss of Body Fat and Intramuscular Lipid Content

The observed loss of body fat with eccentric training was completely unexpected since the metabolic training load was low with just two 20-min sessions per week. We did not assess energy consumption neither with EET nor with RET. Estimating an average workload of 150 Watts per session, subjects would have expended approximately 300 Kilojoules (kJ) per session assuming 95% cycling efficiency and 65% energy efficiency (conversion of ATP hydrolysis to yield kinetic energy). With an average of 22 attended sessions, a total of ca. 6'600 kJ, equivalent to 175 g of fat. Using approximate calculations for concentric cycling we estimate energy expenditure during the training intervention to be 20'000 kJ, equivalent to the energy of 530 grams of fat. From these estimates (which are based on concentric cycling modality) it is obvious that the loss of body fat by far exceeds the estimated exercise-related

energy expenditure., Even if exercise and a plus of physical activity led to an increase in energy expenditure of 33'000 kJ (= 870g fat) during the 12 weeks, we would not expect changes in body composition assuming the participants followed an isocaloric diet (Strasser et al. 2007). Loss of body fat with a concomitant increase in muscle mass is suggestive of systemic and peripheral mechanisms such as hormonal alterations in favour of anabolism. This could include an increase in testosterone, growth hormone and IGF-1, and also a lowering of cortisol and TNFα levels. An improved local responsiveness (i.e. insulin sensitivity) might also contribute to an altered body composition. The decrease of fat tissue reduces levels of adipokines, some of which being responsible for muscle wasting (Ronti et al. 2006). In previous training studies with younger subjects performing EET, a loss of body fat and intramuscular lipid content was not observed. This result suggests that a disturbed hormonal balance, a chronic low grade inflammation or decreased insulin sensitivity are necessary preconditions for significant improvements. Changes in anabolic hormone levels may indeed favour changes in body composition, presumably more pronounced than increased energy expenditure or energy restriction (Waters et al. 2008). It is generally known that ageing is accompanied by the development of insulin resistance with its consequences, also known as the metabolic syndrome (Denys et al. 2009). If peripheral glucose uptake into fat and muscle tissue is impaired due to partial insulin resistance, an expanding fat mass creates an increased sink for glucose disposal (Virtanen et al. 2005). Other authors assume a disturbed body composition or insulin receptor imbalance as an initial trigger for intrinsic insulin resistance due to an insufficient clearing of blood glucose and occurrence of hyperglycemia and hyperinsulinemia (Eaton et al. 2009). Although adipose tissue is less efficient in glucose uptake than muscle tissue, it accounts for a significant proportion of up to 20% of muscular glucose uptake (Virtanen et al. 2005). Adversely, insulin-resistant adipose tissue has a reduced capacity to re-esterify free fatty acids (FFAs) which then circulate at higher levels in the bloodstream. Under high-insulin conditions, FFAs can be taken up by muscle tissue and accumulate as intramuscular lipid droplets (Schrauwen-Hinderling et al. 2006). Therefore, IMCL content is a predictor of insulin resistance in untrained individuals (Krssak et al. 1999; Machann et al. 2004). Insulin resistant adipose tissue also releases increased amounts of cytokines with insulin-desensitizing activity (e.g. TNFα) and reduced amounts of adiponectin, an insulin-sensitizing cytokine (Weyer et al. 2001). This cycle is responsible for the develop-

ment of the metabolic syndrome. In insulin resistant subjects, an exercise induced-increase in insulin sensitivity could lead to a decrease of adipose tissue due to improved clearing of blood glucose by muscle tissue (Dela and Kjaer 2006). Interestingly, endurance and resistance training improve insulin sensitivity by the same magnitude, however, eccentric exercise is known to cause transient insulin resistance (Kirwan et al. 1992; Asp et al. 1996; Del Aguila et al. 2000; Ryan 2000). In elderly who are less sensitive to insulin, eccentric exercise bouts may augment insulin resistance to a degree that inhibits the anti-lipolytic effects of insulin thus favouring lipid oxidation (Krishnan et al. 2003). The loss of oxidative capacity observed in our subjects does not fortify the latter hypothesis but favours the explanation of increased insulin sensitivity on a longer term, leading to a reduced body fat content. Improvements of insulin sensitivity on a longer term were reported by Drexel et al. (Drexel et al. 2008) studying continuous eccentric training (downhill walking) a training comparable in intensity and duration to EET. A transient insulin resistance as occurring with heavy eccentric exercise could potentially result in a more sensitive insulin response on a longer term by positive feedback signaling. The observed loss of fat content with EET requires further investigations with a broader analysis of parameters including systemic blood hormone and cytokine levels but also estimation of insulin sensitivity. On a molecular level, CD36 and GLUT4 translocation with exercise and training should be monitored. A comprehensive study set-up should include pre and post steady state testing, and also monitor exercise responses in the trained and untrained state, respectively. In depth knowledge of EET action with regard to insulin sensitivity and oxidative capacity is indispensible to guarantee safety concerning the development of the metabolic syndrome.

Even though oxidative capacity (=maximal substrate oxidation) is reduced with EET, the loss of body fat clearly demonstrates that fat is oxidized rather than stored during the training period. A recent study reported a higher resting metabolic rate (RMR) as a result of sustained strength training, suggesting a mechanism for loss of body fat content (Kirk et al. 2009). RMR is strongly dependent on lean mass but might also be influenced by diet and exercise (Donahoo et al. 2004). RMR was not measured in our study subjects but their small gain in lean mass (which was similar in both groups) could not fully account for an increase in RMR leading to the observed loss in fat mass. On a molecular level expression of the muscular fatty acid transporter CD36

was increased (+33%, p=0.1) while expression of fatty acid synthase was decreased (FAS; -60%, p=0.3) with EET but not with RET, thus supporting the idea of a preferential uptake of FFAs for fueling skeletal muscle during recovery from EET (Harasim et al. 2008; Holloway et al. 2008). An obvious explanation for a decrease in body fat and IMCL content suggests a higher degree of uncoupling mitochondrial respiration with ATP production, most likely by an elevated expression of uncoupling proteins such as uncoupling protein 3 (UCP3). However, UCP3 mRNA expression remained unchanged with EET and RET. Transient mitochondrial uncoupling is also caused by fatty acids and depends on the lipid composition of the inner mitochondrial membrane (Kadenbach 2003). Mitochondrial membrane composition could be altered in response to eccentric training: Mahoney et al. (Mahoney et al. 2008) reported an increased expression of proteins and enzymes responsible for membrane repair and remodelling during recovery from eccentric exercise. Intrinsic uncoupling of electron transport and proton pumping was described for complex IV (cytochrome c oxidase) (Kadenbach 2003). The physiological relevance of this mechanism is not yet clear.

Body fat content correlates with IMCL content in sedentary individuals but not in athletes where intramuscular lipids serve as energy stores and do not represent disposal of excess plasma fatty acids (Sinha et al. 2002; van Loon and Goodpaster 2006; Dube et al. 2008). With EET, loss of body fat correlated with the decrease in IMCL content. Oxidative capacity was similarly reduced with EET suggesting that IMCL were not used during exercise. Decrease of mitochondrial content is usually associated with negative consequences regarding the risk to develop NIDDM (type II diabetes). As the reduction of mitochondrial content went along with a decrease in intramuscular lipid content, the ratio of IMCL to mitochondrial content remained unchanged. A recent study has shown that the degree of physical association between mitochondria and lipid droplets in muscle tissue is a valuable predictor of the risk of developing NIDDM (Schrauwen 2007). This is in accordance with the functional point of view since intramuscular lipid droplets must topologically be related to mitochondria in order to serve as substrate during submaximal work. An excess of IMCL without mitochondrial association reflects disposal of excess FFAs.

Recommendations for Practice

The feasibility and beneficial functional effects of EET have been reported repeatedly and consistently. The possibility to accurately adjust the demanded load allows for exercising virtually all possible subjects (elderly, coronary disease patients, athletes) and to ramp the load appropriately avoiding muscle damage but concomitantly to guarantee a sufficiently high mechanical stimulation to induce muscle adaptation. Potential negative effects such as a depression of mitochondrial and metabolic transcripts along with a reduced mitochondrial content was reported previously and has also been observed in our study (Zoll et al. 2006; Mueller et al. 2010). Attenuation of mitochondrial density is not a desired result particularly with regard to insulin sensitivity and NIDDM (for further discussion see previous paragraph). To counteract the loss of muscular oxidative capacity, concomitant endurance training to stimulate mitochondrial biogenesis could help to maintain the mitochondrial content. A loss of mitochondria in old age is certainly not desired but its' maintenance should not be at the expense of muscle mass and strength, since a minimum of strength is required to maintain basic every day activities and allow the elderly to live independently.

Conclusions

Eccentric and concentric training regimes are both convenient for elderly to reduce symptoms of sarcopenia and eventually lower the risk of falls. EET, as applied in our study, was more efficient in improving strength, body composition and eccentric coordination. Gain in muscle mass occurs by different mechanisms with RET inducing hypertrophic growth and EET inducing neoplastic growth. A distinct gene expression profile was observed with RET and EET, highlighting the strong mechanical and relatively low metabolic load.

4. Acknowledgements

I am especially grateful to Hans Hoppeler for giving me the opportunity to do my PhD thesis under his supervision. I particularly appreciated his general interest in research and extensive knowledge ranging from molecular biology to human and animal physiology. I was inspired by his ambition not only to describe conditions but also to unravel the mechanisms by which organisms adapt and evolve in response to the environment and training. Being an enthusiastic sportsman himself with great skills in skiing and cycling, he has understood and supported my passion for climbing and allowed me to work flexibly and independently.

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6. Abbreviations

ACE	Angiotensin converting enzyme
ACTB	Beta-actin
ADP	Adenosine diphosphate
AGO	Argonaute proteins
Akt	Protein kinase B (PKB)
αMHC	Alpha myosin heavy chain
AMPK	Adenosine-5'-monophosphate-activated protein kinase
Ankrd2	Akyrin repeat domain protein 2
ATP	Adenosine-5'-triphosphate
B2M	Beta-2-microglobulin
CamK	Ca^{2+}/calmodulin-dependent protein kinase
Ca^{2+}	Calcium
CAPN3	Calpain 3, p94
CD36	Cluster of Differentiation 36, muscular fatty acid transporter, FAT
cGMP	Cyclic guanosine monophosphate
c-MET	Hepatocyte growth factor (HGF) receptor
COPD	Chronic pulmonary disease
CREB	cAMP response element binding protein
CS	Citrate synthase
CSA	Cross sectional area
CT	Cognitive training
Cyp	Cyclophylin
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
DOMS	Delayed onset muscle soreness
ECM	Extracellular matrix
eEF2	Eukaryotic elongation factor 2
eEF2K	Eukaryotic elongation factor 2-kinase
EET	Eccentric ergometer training
EMG	Electromyography
ET	Endurance training
FAAs	Free fatty acids
FAS	Fatty acid synthase
FAK	Focal adhesion kinase
FoxO	Family of forkhead box transcription factors
FOXO1	Forkhead box O1
FBXO32	F-box only protein 32, Atrogin-1, muscle atrophy F box (MAFBx)
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDF8	Myostatin, MSTN

GH	Growth hormone
GHGR	Growth hormone releasing hormone
GLUT1	Glucose transporter 1, SLC2A1
GLUT4	Glucose transporter 4, SLC2A4
GSK3β	Glycogen synthase kinase 3 beta
HDACs	Histone deacetylases
HDAC4	Histone deacetylase 4
HGF	Hepatocyte growth factor
IGF-1	Insulin-like growth factor 1
IL-1	Interleukin 1
IL-6	Interleukin 6
IMCL	Intramyocellular lipid content
kJ	Kilojoules
LCM	Laser capture microdissection
MAFBx	Muscle atrophy F box, F-box only protein 32 (FBXO32), Atrogin-1
MAPK	Mitogen-activated protein kinase
MARP	Muscle ankyrin repeat proteins
MEF2C	Myocyte enhancer factor 2C
MEL	Maximal isometric extension strength of the legs
MGF	Mechano growth factor
MHC-1	Myosin heavy chain 1
miR	Micro RNA
miRNA	Micro RNA
mRNA	Messenger Ribonucleic acid
MSTN	Myostatin, GDF8
mtDNA	mitochondrial desoxyribonucleic acid
MRF	Myogenic regulatory factor family of transcription factors
mTOR	Mammalian target of rapamycin
MuRF1	Muscle ring finger protein 1
MuRF2	Muscle ring finger protein 2
MyoD	Myogenic differentiation factor
MyoG	Myogenin
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NIDDM	Non-insulin dependent diabetes mellitus (Type II)
NO	Nitric oxide
NOS	Nitric oxide synthase
NRF-1	Nuclear respiratory factor 1
NRF-2	Nuclear respiratory factor 2
OXPHOS	Oxidative phosphorylation
PGC-1α	Peroxisome proliferator-activated receptor gamma, co-activator 1 alpha
PIP3	Phosphatidylinositol 3-phosphate

PI3K	Phosphoinositide 3-kinase
PKB	Protein kinase B, Akt
PPARγ	Peroxisome proliferator-activated receptor gamma
p70s6K	p70s6-Kinase
p94	Calpain 3, CAPN3
QTL	Quantitative trait loci
RET	Resistance exercise training
RISC	RNA induced silencing complex
RMR	Resting metabolic rate
ROS	Reactive oxygen species
RNA	Ribonucleic acid
SNP	Single nucleotide polymorphism
SOD2	Mitochondrial superoxide dismutase 2
SRF	Serum response factor
ST	Strength training
Tfam	Transcription factor A, mitochondrial
TGFβ	Transforming growth factor beta
TNFα	Tumor necrosis factor alpha
UCP3	Uncoupling protein 3
VEGF	Vascular endothelial growth factor
3'-UTR	3’-untranslated region

7. Appendix

Curriculum Vitae

Personal Data

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Civil status: single

Born in Frutigen on the 13th of July 1979

Occupational History

1986-1995	Obligatory school in Reichenbach i.K
1995-1999	Gymnasium in Interlaken with major focus in science
1999-2001	Semi-professional climber, working on construction
2001-2004	Study of biology at the University of Bern, major in medical microbiology and immunology
2004-2005	Master thesis: Regulation of Glucocorticoid Synthesis in the Intestinal Epithelium, Professor Dr. Thomas Brunner, Immunopathology, Institute of Pathology, University of Bern
2006	Semi-professional climber, working on steel constructions as an acrobatic worker
2007-2010	PhD student in the group of Professor Dr. Hans Hoppeler, Functional Anatomy, Institute of Anatomy, University of Bern

Publications

Mueller M, Cima I, Noti M, Fuhrer A, Jakob S, Dubuquoy L, Schoonjans K, Brunner T (2006) *The nuclear receptor LRH-1 critically regulates extra-adrenal glucocorticoid synthesis in the intestine*. J Exp Med 203: 2057-2062

Mueller M, Atanasov A, Cima I, Corazza N, Schoonjans K, Brunner T (2007) *Differential regulation of glucocorticoid synthesis in murine intestinal epithelial versus adrenocortical cell lines.* Endocrinology 148: 1445-1453

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Mueller M, Breil FA, Lurman G, Klossner S, Flück M, Billeter R, Däpp C, Hoppeler H (2010) *Different molecular and structural adaptations with eccentric and conventional strength training in elderly men and women*, Gerontology, submitted

Buschkuehl M, Jaeggi SM, Hutchison S, Perrig-Chiello P, Dapp C, **Mueller M**, Breil F, Hoppeler H, Perrig WJ (2008) *Impact of working memory training on memory performance in old-old adults.* Psychol Aging 23: 743-753

Schmutz S, Däpp C, Wittwer M, Durieux AC, Weinstein F, **Mueller M**, Vogt M, Hoppeler H, Flück M (2009) *A Hypoxia Complement Differentiates The Muscle Response To Endurance Exercise.* Experimental Physiology, in revision

Draeger A, Sanchez-Freire V, Monastyrskaya K, Hoppeler H, **Mueller M**, Breil FA, Mohaupt MG, Babiychuk EB (2009) *Statin therapy affects the expression of genes that regulate calcium homeostasis and membrane repair in skeletal muscle,* Journal of Pathology, submitted

Hoppeler H, Lurman G, **Mueller M**, Klossner S, Baum O (2010) *Molecular Mechanisms of Muscle Plasticity with acute and chronic exercise,* Handbook of Physiology, in preparation

Hoppeler H, **Mueller M**, Vogt M (2009) *Skeletal muscle tissue changes with hypoxia*, Handbook article, submitted

Scientific Congresses

Annual Congress of the Schweizerische Gesellschaft für Allergologie und Immunologie (SGAI) (2005), Bern, Switzerland

1st International Workshop on Animal Models in Apoptosis Research (2006), Obergurgl, Austria

Swiss Apoptosis Meeting (2006), Bern, Switzerland

Omics: Assembling System(s) Biology (2007), Ascona, Switzerland

Research Day of the Departement der klinischen Forschung (DKF) (2008), Bern, Switzerland

Annual Congress of the European College of Sport Science (ECSS) (2008), Estoril, Portugal

Annual Congress of the Schweizerische Gesellschaft für Sportmedizin (SGSM) (2008), Fribourg, Switzerland

Swiss RNA Workshop (2009), Bern, Switzerland

Scientific Prizes

Poster Prize at the Annual Congress of the Schweizerische Gesellschaft für Sportmedizin (SGSM) (2008), CHF 2500

Declaration of Originality

Last name, first name: **Müller Matthias**

Matriculation number: **01-127-786**

I hereby declare that this thesis represents my original work and that I have used no other sources except as noted by citations.
All data, tables, figures and text citations which have been reproduced from any other source, including the internet, have been explicitly acknowledged as such.
I am aware that in case of non-compliance, the Senate is entitled to divest me of the doctorate degree awarded to me on the basis of the present thesis, in accordance with the "Statut der Universität Bern (Universitätsstatut; UniSt)", Art. 20, of 17 December 1997.

Place, date	Signature
Bern, 15.12.2009	
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