Antioxidants and muscle growth in elderly

The effect of supplementation with vitamin C and E on muscle growth and maximal strength during 12 weeks of resistance exercise in elderly men

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ABSTRACT

INTRODUCTION: Supplementation with antioxidants could either facilitate or hamper adaptations to resistance exercise due to redox-sensitive signaling pathways that regulate protein synthesis. Thus, the *aim* of the present study was to investigate the effect supplementation with vitamin C and E on muscle growth and maximal strength during 12 weeks of resistance exercise in elderly men.

METHODS: Thirty-four elderly males (60 – 81 years) were randomized to either an antioxidant group (N=17; 1000 mg of vitamin C and 235 mg of vitamin E per day) or a placebo group (N=17). Muscle growth was assessed as changes in lean mass with dual-energy X-ray absorptiometry and local muscle thickness with ultrasound imaging. Maximal strength was measured as one-repetition maximum (1RM). All participants following a supervised undulating periodized program 3 times/week.

RESULTS: Total lean mass increased by 3.9% (95% confidence intervals 3.0-5.2) and 1.2% (0-3.6) in the placebo and antioxidant group, respectively; revealing larger gains in the placebo group (p=0.03). Similarly, results from the thickness of rectus femoris increased more in the placebo group (16.2% [12.8-24.1]) than in the antioxidant group (10.9% [9.8-13.5]; p=0.01). Changes in lean mass of trunk and arms, as well as muscle thickness of elbow flexors and vastus lateralis, did not differ significantly between groups. With no group differences, 1RM improved in the range of 15-21% in both groups (p<0.001).

CONCLUSION: Supplementation with vitamin C and E had no positive effects on the adaptation to resistance exercise in elderly men, but seemed on the contrary to hinder muscle growth.

KEYWORDS: Antioxidant supplementation, lean mass, muscle thickness, one-repetition maximum, undulating periodization.

Due to the word-limitation of the master thesis, results, discussion and conclusion of the present study are included in the attached article only.

SAMMENDRAG

INNLEDNING: Tilskudd av antioksidanter kan muligens øke eller redusere adaptasjon til styrketrening ved å påvirke redoks-sensitive signalveier som regulerer proteinsyntese. Målet med denne studien var derfor å undersøke effekten ved tilskudd av vitamin C og E på muskelvekst og maksimal styrke i løpet av en 12 ukers styrketreningsintervensjon hos eldre menn.

METODE: Trettifire eldre menn (60-81 år) ble randomisert til enten en antioksidant- (N = 17; vitamin C og vitamin E) eller placebogruppe (N = 17). Muskelvekst ble målt som endringer i lean mass ved bruk av dual-energy X-ray absorptiometry, og lokal muskeltykkelse ved bruk av ultralyd. Maksimal styrke ble målt som en-repetisjon maksimum (1RM). Alle deltakerne gjennomførte tre styrketreningsøkter i uken, etter en bølgeperiodiseringsprotokoll.

RESULTAT: Median endring i total lean mass var signifikant høyere i placebogruppen (3,9% [95% konfidensintervall 3,0 til 5,2]) sammenlignet med antioksidant-gruppen (1,2% [0 - 3,6], p =0,03). Lignende resultater ble vist ved ultralydbilder av rectus femoris, hvor muskeltykkelse i placebo gruppen økte med 16,2% (12,8 til 24,1) vs. 10,9% (9,8 til 13,5) i antioksidant-gruppen (p = 0,01). 1RM økte mellom 15 og 21% (p <0,001), uten signifikant forskjell mellom gruppene. Ingen signifikant forskjell ble oppdaget mellom gruppene i lean mass, lean mass av armer, samt muskeltykkelse av armbøyere og vastus lateralis.

KONKLUSJON: Tilskudd av vitamin C og E viste ingen positive effekter på adaptasjon til styrketrening hos eldre menn, men virket tvert imot å hemme muskelvekst. Begge gruppene hadde en robust økning i lean mass, muskel tykkelse og styrke etter styrketreningsintervensjonen.

NØKKELORD: Antioksidant-tilskudd, lean mass, muskeltykkelse, en-repetisjon maksimum, bølgeperiodisering.

På grunn av ordbegrensninger i masteroppgaven, vil resultater, diskusjon og konklusjon kun bli presentert i vedlagte artikkel.

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Kristiansand, Norway

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Thomas Bjørnsen

Contents

PART 1: THEORETICAL BACKGROUND AND METHODS

PART 2: PAPER

Part 3: APPENDIX

PART 1:

THEORETICAL BACKGROUND AND METHODS

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CONTENTS

Tables

Figures

1   INTRODUCTION

Demographics indicate that people aged 60 years and above, in the world's population, will more than triple within 50 years (24). This will probably present larger strains on the health care systems. Contributing to risks of diseases (83, 110) and a direct predictor of frailty and disability, is the age-related decline in skeletal muscle mass and strength, referred to as sarcopenia (22, 110). One of the main prevention strategies for the progression of sarcopenia is resistance exercise (11, 92, 93).

A combination of resistance exercise and nutritional interventions has been suggested as a promising candidate in combating sarcopenia (70). Nutrition is of obvious importance for health and for optimal training effect from intensive exercise (53). To boost effects of exercise a large assortment of nutritional supplements has been developed and a popular type of supplement available is antioxidants, e.g. vitamin C and E products. Vitamin supplementations with antioxidant properties are commonly thought to be beneficial for adaptation to exercise (117), but in recent years the widespread use of concentrated antioxidants (pills), have been questioned (47, 55). Scientists have reported both no effect (8, 123) and adverse effects (108) in young adults. In theory, antioxidant supplementation could either facilitate or hamper adaptations to resistance exercise by affecting the cellular redox balance (i.e. the balance between pro-oxidants and anti-oxidants) (84). Moderate and excessive shifts in redox status, resulting from a chronically higher oxidative stress, have been suggested to play an important role in the functional decline observed with aging (43, 60, 63). Subsequently, this can lead to inevitable deterioration of essential cellular functions, which can attenuate adaptations to resistance exercise, such as muscle growth (38, 59, 60, 112). However, the redox status also seems affect the redox sensitive signaling cascades regulating protein synthesis, as both reduced and increased levels of free radicals influence them (84, 130). Hence, it seems to exist a currently undefined optimal redox status. To the author's knowledge, only one study has specifically reported changes in fat-free mass or muscle mass in response to high-intensity resistance exercise combined with antioxidants in older adults. Bobeuf and co workers reported that antioxidants supplementation (1000 mg/day of vitamin C and 600 mg/day of vitamin E) resulted in higher increases of fat free-mass (14, 75) and beneficial effects on body composition (13), compared to resistance exercise only. Thus, we

anticipate that antioxidant supplementation can have protective effect in elderly subjects during resistance exercise.

1.1 Overall goals and study design

My working hypothesis was that an exercise-induced increase of oxidative stress overwhelms the redox balance and lead to suboptimal adaptations to resistance exercise. Supplementation with antioxidants could therefore facilitate adaptations to exercise by restoring a redox balance. Thus, the aim of this thesis is to investigate if supplementation with antioxidants vitamin C and E can accelerate adaptations to strength training in terms of muscle growth and increase in maximal strength in elderly men.

Hypotheses:

- *Supplementation with vitamin C and E in high daily dosages (1000 mg and 235 mg per day, respectively) will enhance adaptations to resistance training, i.e. increased muscle thickness, lean mass and one repetition maximum in the exercises: leg press, leg extension and scott curl.*

2   THEORETICAL BACKGROUND

Exposure to resistance exercise over time produces marked increase in muscular strength, which is attributed to a range of neurological and morphological adaptations (40). The primary morphological adaptations for both in young and older adults involve an increase in the volume of the muscle (94, 133). Importantly, the size of the muscle mass determines the force-generating capacity; i.e. maximal strength (27, 42).

2.1   Mechanisms of muscle growth

2.1.1   Resistance exercise induced muscle growth

Theoretically, muscle growth can be achieved either by an increase in the size of skeletal muscle fibers (hypertrophy), or by an increase in the number of fibers within the muscle (hyperplasia) (114). The methodological problems of measuring hyperplasia in whole human muscles *in vivo*, makes the investigation in humans extremely difficult. Evidence that hyperplasia occurs in humans is lacking, and if it does occur at all, the effect on muscle growth would appear to be minimal (31). Therefore, hypertrophy is generally regarded as the most important exercise-induced mechanism for muscle growth, and has been widely documented (97). Hypertrophy of individual fibers subsequent to traditional resistance training programs happens due to myofibrillar growth and proliferation, and can occur either by adding sarcomeres in series or in parallel (40).

2.1.2   Initiation of signaling pathways regulating protein-synthesis

Understanding the molecular basis of muscle hypertrophy is important to the development of targets for exercise interventions, which seeks to enhance performance or prevent muscle-wasting conditions such as sarcopenia (114). In order for hypertrophy to occur, additional contractile proteins must be manufactured and functionally integrated into the existing fibers and myofibrils (40). Muscle contraction activates numerous systems (i.e. mechanical tension and metabolic stress) that initiate signaling cascades important in regulation of protein synthesis through stimulating translation and transcription factors (21, 39).

Mechanical tension

Mechanical stimuli modulate cell function and directly affect tissue form and function (28). Technical limitations prevent accurate measurement of the effect of mechanical tension on mechanotransduction *in vivo* (28). However, mechanically induced tension produced both by

force generation and stretch of the muscle is considered essential to muscle growth, and the combination of these stimuli appears to have a pronounced effect (114). It is believed that tension disturbs the integrity of the skeletal muscle cells, which causes mechanochemically transduced molecular and cellular responses in myofibers and satellite cells (131). A cascade of upstream signaling events are thought to involve growth factors, cytokines, stretch-activated channels and focal adhesion complexes (114).

Metabolic stress

Numerous studies have emphasized the importance of metabolic stress mechanisms in the anabolic effect of resistance exercise (3, 4, 72, 85, 115). Some studies have even suggested that metabolic stress may be more important than high force and mechanical tension development in optimizing the hypertrophic response (85). The mechanism theorized to mediate the hypertrophic response from metabolic stress are numerous and include alterations in free-radical production (see section 2.3.2), hormonal milieu, cell swelling, muscular hypoxia, muscular ischemia, calcium concentrations ($[CA^2+]$), AMP/ADP-ratio, lactate concentrations, ion homeostasis and increased activity of growth-oriented transcriptional factors (115).

2.1.3   Regulation of protein synthesis

Skeletal muscle proteins are constantly synthesized and degraded, and the net protein balance is defined as the difference between protein synthesis and protein breakdown (7). Because of space limitations to this thesis, regulation of protein synthesis will be emphasized. Muscle fiber recruitment activates several systems that signal to a regulation of protein synthesis. Mechanical tension in muscle fibers and metabolic stress are two examples of such systems, subsequently activating several key kinases and phosphatases involved in signal transduction (96). In most cases the transduction network consists of a group of protein kinases that serve to signal the appropriate end point(s). However, signal transduction pathways are seldom linear and instead often have many branch points and multiple places at which signaling events can activate the kinase cascade (28). The protein kinase B (PKB; also known as AKT) - mechanistic target of rapamycin (mTOR) pathway (Akt-mTOR), and the mitogen-activated protein kinases (MAPK) pathways are two important anabolic signaling pathways that are activated by resistance exercise, and contribute to regulate protein synthesis in skeletal muscle (39).

Akt-mTOR and mitogen-activated protein kinases (MAPK) pathways

The Akt-mTOR pathway is shown to be crucial for adaptation to resistance exercise (109). Contractions of muscle fibers increase the Akt-mTOR pathway dramatically, and are important responses for increasing muscle protein synthesis, and subsequent growth (118). Activation of mTOR signaling subsequently phosphorylates downstream targets (i.e. p70 S6K and 4E-BP1), that, in turn, activates ribosomal proteins (i.e. S6) and translation initiation factors (i.e. eIF4E; figure 1). This ultimately enhances translation efficiency (i.e. messenger RNA (mRNA) translated per ribosome), increases protein synthesis, and promotes muscle growth.

MAPK signaling pathways are networks of several parallel phosphorylation cascades (figure 2) which have been identified as a candidate system that converts contraction-induced biochemical disturbances into appropriate intracellular responses (10). MAPK is considered a master regulator of redox status, gene expression and metabolism (79). Exercise activates several MAPK isoforms, including the extracellular signal-regulated kinase 1 and 2 (ERK 1/2) and the two stress-activated protein kinases, p38 MAPK and c-Jun NH_2-terminal kinase (JNK (79)). The ERK1/2 and p38 MAPK cascades have been reported to link cellular stress with an adaptive response in myocytes, modulating growth and differentiation (12). JNK has been shown to be the most responsive to mechanical tension and muscle damage of the MAPK pathways, and is particularly sensitive to eccentric exercise (12). MAPK activation can result not only in the production of transcription factors mediating gene expression, but can also stimulate the translation stage of protein synthesis (54).
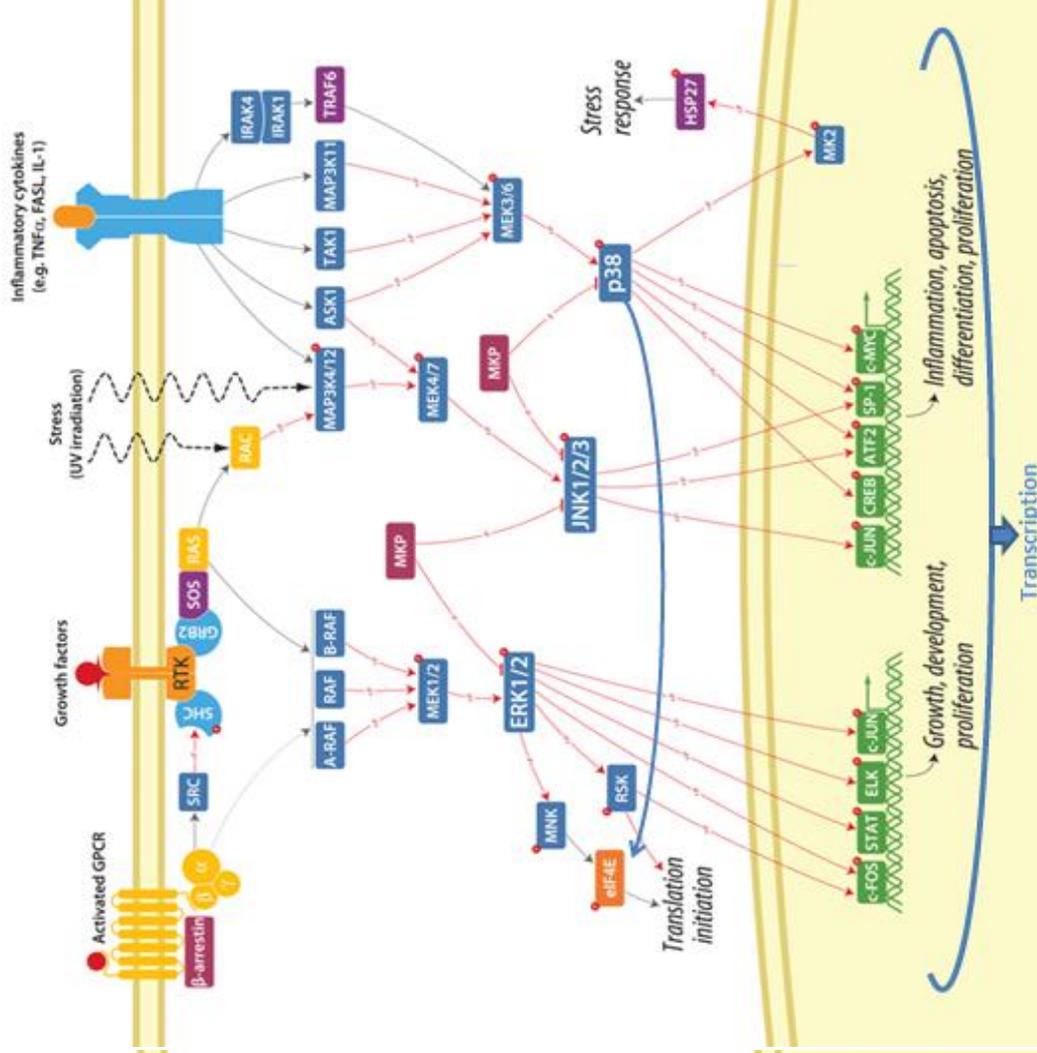
Figur 1 Brief overview of MAPK (ERK1/2, JNK and p38) pathways. Arrows signify a stimulatory response; blocked lines indicate an inhibitory response. Modified from Life Technologies (122).
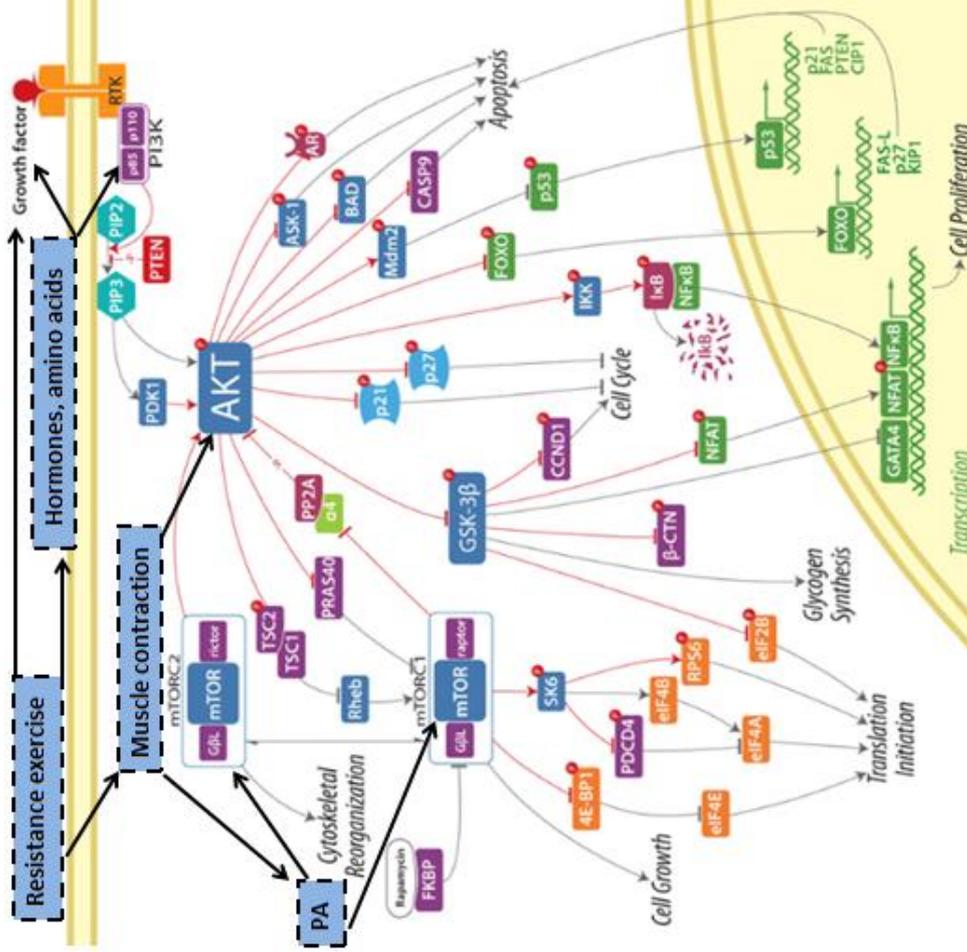
Figur 2 Brief overview of Akt–mTOR pathways. Arrows signify a stimulatory response; blocked lines indicate an inhibitory response. Modified from Life Technologies (121).

2.2 Elderly and sarcopenia

2.2.1 Characteristics and mechanisms of sarcopenia

The definition of sarcopenia has been thoroughly discussed by scientists to increase the clinical applicability of the concept. However, Cederholm et al. (22) defined sarcopenia as mainly, but not only, an age-related condition defined by the combined presence of reduced muscle mass and muscle function. Sarcopenia reflects a progressive withdrawal of anabolism and an increase in catabolism, which is characterized by a progressive loss of muscle mass and strength, increase of muscle fat and progressive decline of functional capacity with aging (110). This process leads to severe effects on quality of life in elderly people, since sarcopenia is the most frequent cause of frailty, dependency and increase in morbid-mortality (73). Combating physical frailty in old age, while maintaining an active role in life, is indeed extremely important, as we have entered the third millennium with a proportion of elderly citizens exceeding that of young people (24).

2.2.2 Protein synthesis in the elderly

It has been suggested that sarcopenia happens mainly due to a reduction in basal muscle protein synthesis, elevated basal-fasted rates of muscle protein breakdown, or a combination of these two (71). However, basal rates of muscle protein synthesis and breakdown seem to be unchanged with healthy aging (35, 49, 51, 73, 80, 120). It is important to acknowledge that current methods used to measure net muscle protein metabolism may not be sensitive enough to detect very small, but potential important changes. There may also be a failure to distinguish between healthy and frail elderly. However, the basal muscle protein turnover may be compromised in the frail elderly, potentially due to greater systemic inflammation and its associated co-morbidities (18). The understanding to the contribution of muscle protein turnover in sarcopenia has shifted from the perspective where basal muscle protein metabolism was thought to be compromised, to a new paradigm whereby protein synthesis in the muscles of the elderly seems blunted to anabolic stimuli, such as resistance exercise (18).

At the intracellular level, cytokines, in particular tumor necrosis factor-alpha (TNF-α), may impair muscle protein synthesis by blunting the phosphorylation of protein in the mTOR pathway (76). Kumar et al. (74) found a lower rate of muscle protein synthesis after acute resistance exercise, and showed that the phosphorylation of p70S6K and 4E-BP1 was blunted.

While Cuthbertson et al. (30) and Guillet et al. (50) showed that mTOR and downstream targets (i.e. p70S6K and 4E-BP1) are damped in elderly muscles in the presence of amino acids as compared with young. Thus, the sensitivity and/or response of intra-muscular signaling to resistance exercise and amino acids in the elderly may not be as efficient as younger individuals. It is not fully understood what causes this anabolic resistance in aging muscle, it may be that older people require a greater amino acid- and contraction volume to achieve a more robust response, but further research is required to elucidate these mechanisms.

2.2.3 Resistance exercise as a counter measure

Resistance exercise has demonstrated a great efficacy in the treatment of sarcopenia and is one of the main prevention strategies for the progression of sarcopenia (11, 92, 93). Although the anabolic response is generally blunted compared to younger adults, resistance exercise acutely increases muscle protein synthesis and, when practiced frequently over time, promotes hypertrophy in older adults (18, 73). To achieve these adaptations the research point out the importance of high intensity (80-90 % of one repetition maximum) and high volume (repetitions × force) in the resistance exercise protocol (57, 95, 119). A meta-analysis by Peterson et al. (95) looked at 49 intervention studies including 1328 participants that measured changes in lean body mass (LBM) in elderly (>50 years) after a period (mean duration = 20 wk.) of strength training. They found a mean increase in lean body mass of 1.1 kg (95% confidence interval = 0.9–1.2 kg), and that higher-volume interventions were associated with significantly greater increases in lean body mass. However, the clinical status of elderly people must be taken in to consideration and requires an individualization of the training program (20).

2.3 Oxidative stress, redox state, antioxidants and relevance to elderly

2.3.1 Oxidative stress and free radicals

Oxidative stress is a term that is used when the relationship between production of free radicals and the cellular antioxidant defense mechanisms become pro-oxidative (101). The production or formation of free radicals *in vivo* is primarily initiated by the consumption of oxygen molecules, which, due to its structure is in fact a radical specie itself (38). A free radical is an atom or a molecule that has one or more unpaired electrons in their orbital and present very pronounced chemical reactivity as a result (38). Although a multitude of free radicals exist (e.g. hydrogen atoms, transition metal ions, carbon centered radicals, and sulfur centered radicals), those derived from either oxygen (ROS) or nitrogen (RNS) represents the most important free radicals generated in living systems (101). These free radicals and non-radical species created

through an interaction with free radicals are collectively referred to as *reactive oxygen and nitrogen species* (RONS) (38). The primary free radicals generated in cells are superoxide ($O_2\cdot$) and nitric oxide (NO) (38). Because ROS is of most importance for skeletal muscle adaptation to exercise (64), this assignment will focus on the mechanisms of ROS.

2.3.2 Measurement of oxidative stress

A common approach to assess oxidative stress in mammalian tissues involves the measurement of increase or decrease in a redox-sensitive molecule that respond to oxidative stress (101). Reliable markers of oxidative stress also have to be chemically unique and detectable, possess relatively long half-lives, and cannot be impacted by other cellular processes (6). Many molecules that fit one or more of these criteria have been identified, and biomarkers of oxidative stress typically falls into one of four categories (figure 3).
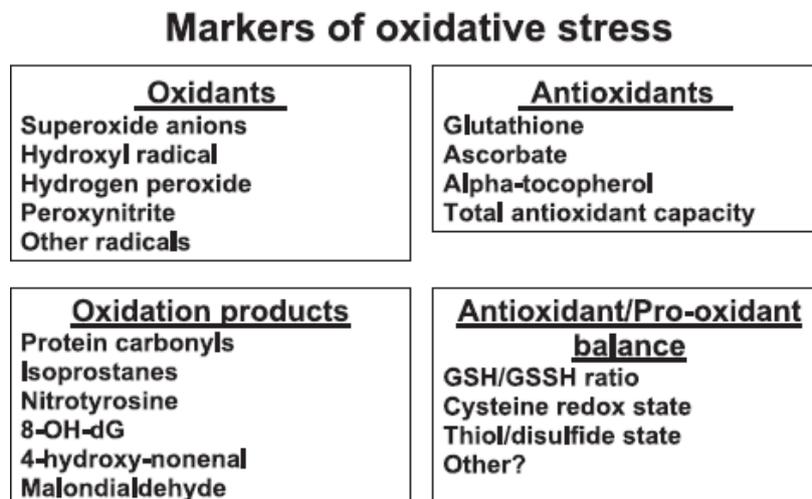
Figure 3   **Four broad classes of biomarkers used to assess cellular oxidative stress in tissues (101).**

Detection of oxidants is the first category of biomarkers. Unfortunately, direct measurement of ROS production in living cells is difficult because they are highly reactive and has a short half-life (38). Therefore, exogenous molecules (e.g. fluorescent) or spin traps are commonly used to measure oxidant production in cells (101). Nonetheless, because the use of spin traps and probes may disturb the biological system being investigated (6), and because an increase in ROS production does not necessarily define a pro-oxidant condition (128), measures of increased ROS production alone cannot be used as definitive markers of oxidative stress.

Measurement of antioxidants in tissues is a second group of biomarkers. In theory, a decrease in antioxidants will happen during oxidative insults. However, this approach is not without weaknesses. Other factors that can influence antioxidant levels in cells, such as changes in cellular metabolism and diet, and auto-oxidation during sample handling resulting in a reduction of antioxidant levels (44).

A third group of biomarkers involves the evaluation of oxidative modified molecules. ROS attack lipids, proteins and/or DNA, which generate uniquely oxidized biomolecules that can be used as markers to detect oxidative stress (128). This approach is suggested to be the most important biomarkers of oxidative stress (101). However, the measurement in biological systems is often difficult because oxidized molecules just exist in limited amounts after a period of oxidative stress, and because the oxidation products are subject to measurement artifacts if tissue samples are handled improperly (128).

The final and fourth category involves the measurement of cellular redox status. Redox status is a term that refers to the balance between pro-oxidants and antioxidants (38). Although this assessment is conceptually simple, experimental artifacts permitting auto-oxidation are common, and often occur during tissue removal and sample processing due to improper handling (128).

In summary, numerous approaches are used to assess oxidative stress in biological systems. Unfortunately, each category of biomarkers for oxidative stress has its limitations. Hence, although there are many measurements to quantify oxidative stress, it appears that no one biomarker best assesses oxidative stress and that in most cases, the measurement of multiple biomarkers is required to confirm the presence of oxidative stress in tissues (101).

2.3.3   ROS and oxidative stress induced by exercise

It is now well established that both resting and contracting muscle produce ROS (46, 58, 84, 102, 105). However, the sources of oxidative stress production from exercise are currently debated among scientists (102). There are many potential sources from which ROS may be produced, but surprisingly few studies have investigated the predominant source (102). This is likely due to both restricted access to most tissues in humans and the complex nature of exercise that involves several organic systems to meet the metabolic requirement of skeletal muscle (67).

Mitochondria and nicotinamide adenine dinucleotide phosphate (NADPH) oxidases

Inadequate coupling of electron transfer at complex I, II and III of the electron transport chain has been supposed to cause leakage of electrons to oxygen, which results in the formation of superoxide radicals (17). Since exercise increases the muscle metabolic rate up to 100 times higher than resting conditions, the elevated oxygen consumption and oxygen flux through mitochondria has been considered the major mechanism of exercise-induced formation of ROS (16). However, growing evidence argues against this conclusion, suggesting that mitochondria are not the primary source of RONS production in skeletal muscle during exercise (102).

ROS formation via NADPH oxidase isoforms exist in several cellular locations in muscle fibers (e.g. sarcoplasmic reticulum (SR), transverse tubules, sarcolemma and phagocytotic cells), where NADPH generates ROS by transferring electrons from NADPH to molecular oxygen (61). This ROS formation by NADPH oxidase seems to play an important role for modulation of redox sensitive signaling pathways (87, 103). However, the extent and importance of ROS formation by NADPH oxidase in skeletal muscle is not clear and needs to be elucidated (88, 102). Despite the initial suggestion that mitochondria are the predominant site for ROS generation during activity, a number of alternative potential sites have been identified (102). It is still unclear whether all of these multiple sites have a significant role in the increased ROS production observed during contractions or whether one site predominates. Clearly, additional research is required.
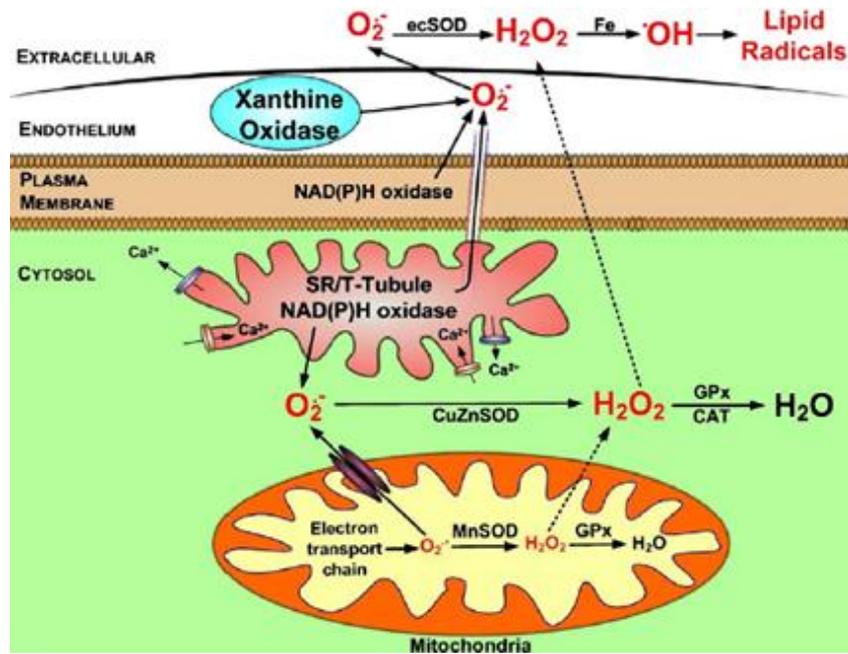
Figure 4   **Selected sites in skeletal muscle fibers that are capable of producing ROS (102).**

2.3.4   Hormesis, and ROS as signaling mechanisms

If the generation of ROS is to high or rapid, the antioxidant system may not react sufficiently (91). Indeed, several observations confirm that enhanced formation of ROS after prolonged or very intense exercise lead to oxidative modifications of lipids, proteins, DNA and other compounds (38). However, it has also become increasingly clear that ROS play an important role in adaptive responses to exercise, serving as a potential up-regulator to protein synthesis and antioxidant defense systems through redox sensitive pathways that stimulates cell transcription and translation (64, 65, 84, 100). The ROS activated signaling pathways that are of particular importance in skeletal muscle include the activation of phosphatase and tensin homolog (PTEN; subsequently stimulating the Akt-mTOR pathway), MAPKs (i.e. ERK 1/2, p38 and JNK) and nuclear factor kappa B (NF-κB) (103).
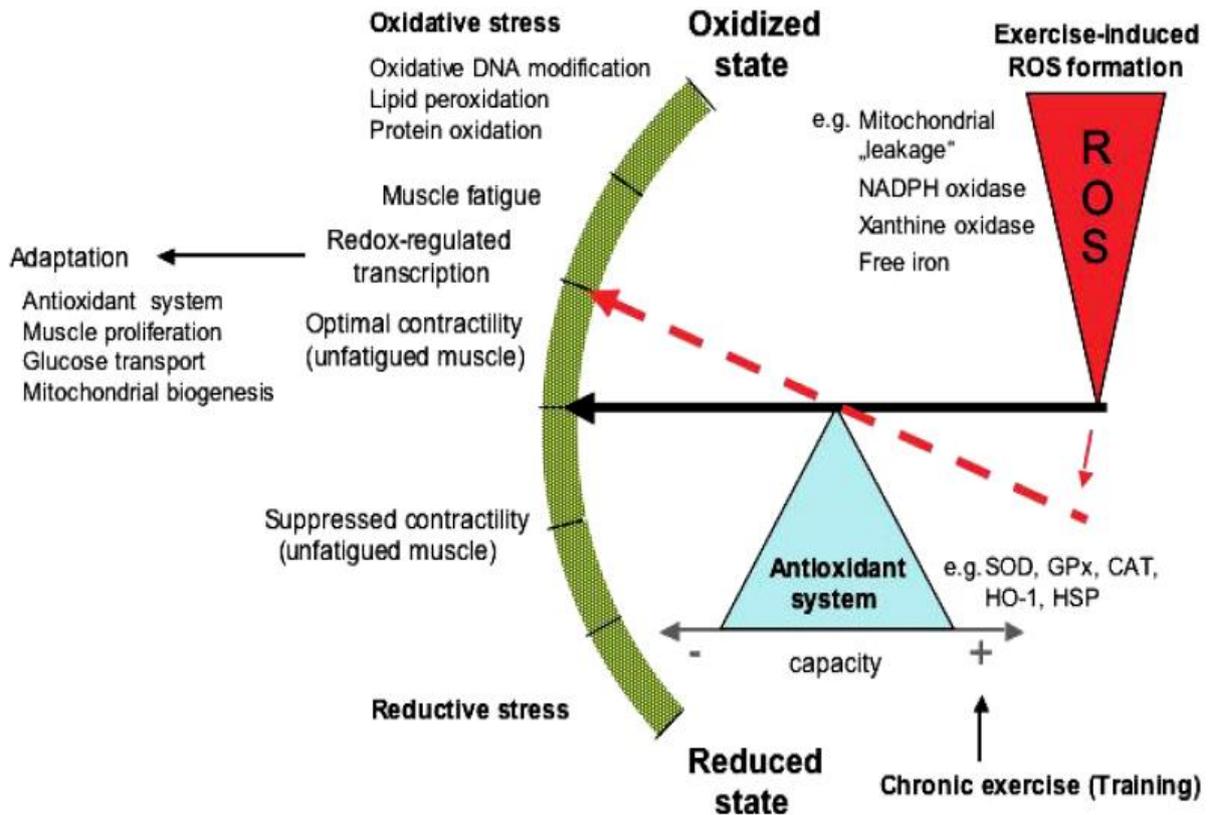
Figure 5 Potential determinants and consequence of exercise-related change in cellular redox-balance in skeletal muscle (84).
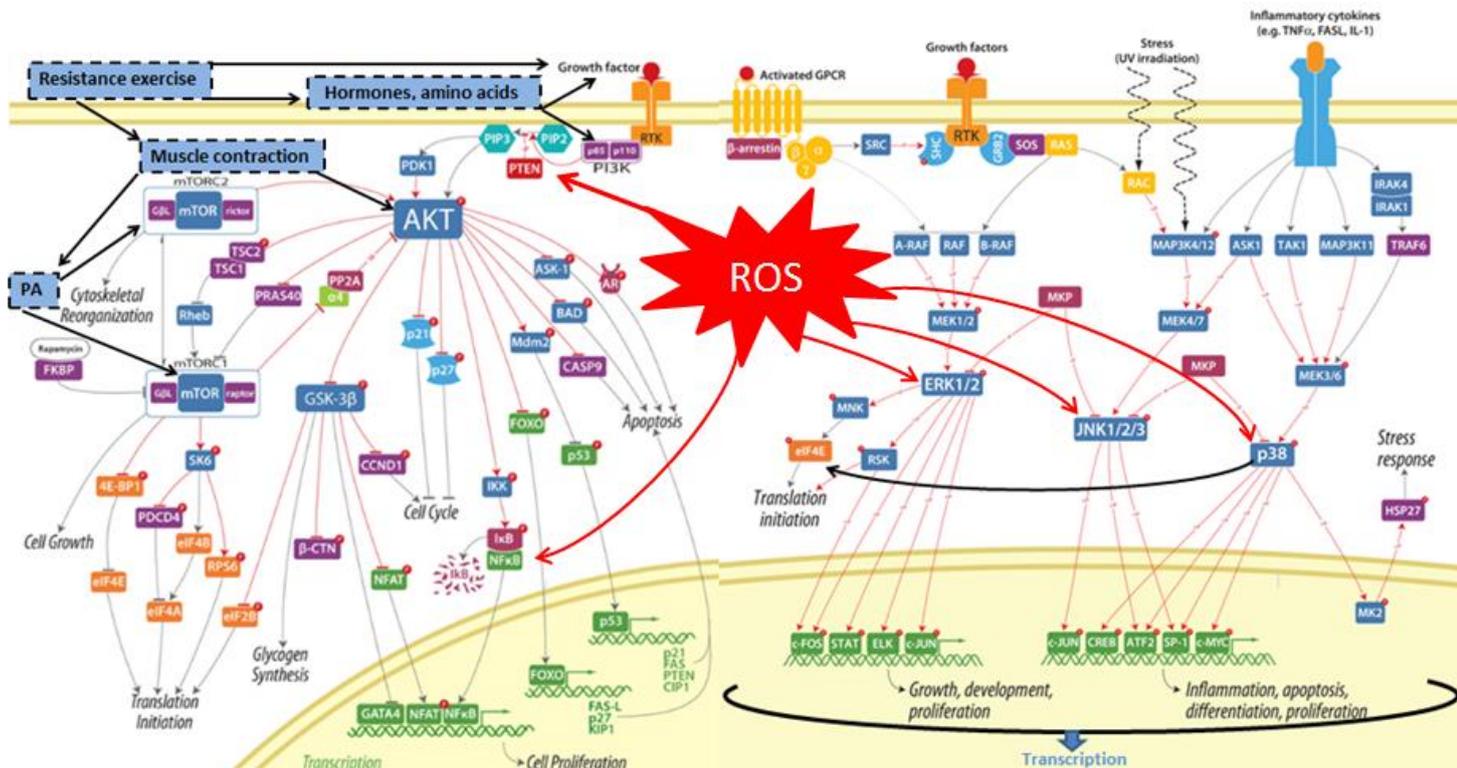
Figure 6 Brief overview of how ROS can affect the Akt–mTOR, MAPK and NF-κB pathways. Arrows signify a stimulatory response; blocked lines indicate an inhibitory response. Modified from Life Technologies (121, 122).

Taken together, exercise-induced oxidative stress may operate in a similar fashion to all other principles of exercise science. That is, in order for an adaptation to occur (e.g. increased muscle growth, maximal strength and antioxidant defense) the physiological stimulus applied (e.g. mechanical tension, metabolic stress, or in this case ROS production) must exceed a certain minimal threshold that overloads the system and subsequently expands or adapts the physiological capacity of the body (82). Although progress has been made in understanding the role of ROS as signaling molecules in muscle fibers, many unanswered questions remain. Future research is needed to clarify the specific role of redox-regulated pathways as an exercise-induced regulator of protein synthesis.

2.3.5   Antioxidants

The term *antioxidant* has been defined in many ways, a common definition of antioxidants is: *"any substance that delays or prevents the oxidation of a substrate (i.e. all molecules found in vivo)"* (101), and this will be used for further discussion in this assignment.

The body's antioxidant system consists of both endogenous (e.g. bilirubin, uric acid, superoxide dismutase (SOD), catalase, glutathione) and exogenous (e.g. carotenoids, tocopherols, ascorbate, bioflavonoids) compounds, and contains both enzymatic and non-enzymatic antioxidants that work together to regulate ROS and resist redox disturbances within the cell (91). Moreover, these antioxidants are strategically located in both in extracellular and vascular space, as well as cytoplasm and various organelles within the skeletal muscle fiber (91). Numerous antioxidant strategies exist and are used to protect against ROS toxicity, for example some agents (e.g. catalase) convert ROS into less active molecules and prevent the transformation to a more deleterious form (25). Another antioxidant strategy is to minimize the availability of pro-oxidants such as free iron and copper ions through metal binding proteins (25). Furthermore, numerous low-molecular-weight agents are capable of scavenging ROS through a donation of electrons (25).
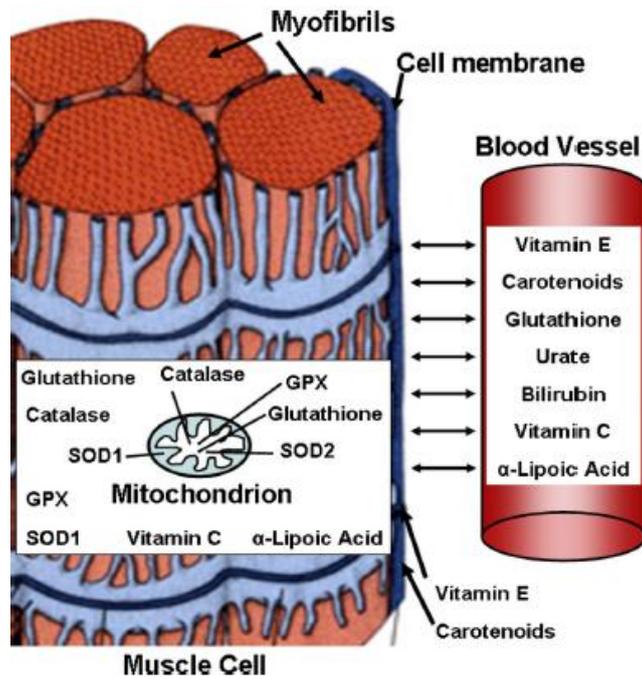
Figure 7   **Locations of primary cellular enzymatic and non-enzymatic antioxidants (101).**

2.3.6 Supplementation of vitamin E and C

Antioxidants supplementation is often assumed to provide benefits including better health, anti-aging effects and improved exercise performance (117), as evidently demonstrated by the nutritional supplement industry. However, as discussed in section 2.3.2, growing evidence shows that increased ROS production are important for a wide range of exercise-related physiological processes and can promote adaptive responses. Thus, a reduction of ROS through antioxidant supplementation may blunt training responses (29, 69, 108).

Among a plethora of dietary antioxidants, vitamin C and E are considered as two of the most important (36). The generic term vitamin E refers to at least eight structural isomers of tocopherols and tocotrienols, and is the primary chain-breaking antioxidant in cell membranes (36). Of these isomers, α-tocopherol possesses the highest antioxidant activity (36). In contrast to vitamin E, vitamin C (ascorbic acid) is hydrophilic and functions better in an aqueous environment (36). Ascorbate (vitamin C) is allegedly widely distributed in human tissues (101), and its role as an antioxidant is twofold. Vitamin C can directly scavenge free radicals as superoxide, hydroxyl and lipid hydroperoxide, but also plays an important role in the recycling of vitamin E (62). As vitamin C serves as an electron donor to vitamin E (62), and due to their different subcellular locations, a combination of Vitamin E and C has been shown to have a better antioxidant effect than either of the two vitamins alone (36). The majority of studies

15

regarding supplementation of vitamin C and E have used measures of oxidative stress as their main outcome, and most of them have demonstrated an attenuated exercise-induced increase in oxidative stress (36, 113). However, experimental evidence to support beneficial effects on physical performance and adaptations to resistance exercise are lacking (55). Given that reactive species play an important role in the adaptation to exercise, their elimination with high doses of antioxidants may result in negative effects on muscle function and adaptation to exercise (46, 47). Thus, it looks like endogenous human antioxidant defense systems are adequate to prevent excessive oxidative stress in healthy adults under "normal" conditions (47). The antioxidant defense systems appear to control levels of free radicals, rather than eliminating them completely, which seems logical as free radicals are thought to have numerous physiological functions (66, 105). Nevertheless, exercise at very high intensity or prolonged duration may lead to a free radical production that overwhelms the antioxidant defenses (105, 130). Moreover, aging, as well as numerous diseases, has an etiology that involves a free radical production that overwhelms the antioxidant defense (15, 78, 111).

2.3.7 Oxidative stress, antioxidant supplementation and muscle growth in elderly

One of the major characteristics of aging is the progressive accumulation of molecular damage in nuclei acids, proteins, lipids, and other macromolecules due to increased oxidative stress in the elderly (15, 43, 78). Subsequently, this leads to inevitable deterioration of essential cellular functions, which can attenuate adaptations to resistance exercise in elderly, such as muscle growth (78). The cause of this is uncertain, it has been suggested that there is a failure in ROS activation of redox-sensitive signaling pathways such as akt-mTOR, MAPK and $NF_{K}B$ (60), which subsequently leads to a lack of complete activation of appropriate translation and transcription factors regulating the endogenous antioxidant system and protein synthesis (60).

Although resistance exercise is promoted to increase fat-free mass in older adults (section 2.2.3), some clinical trials report no meaningful effects (127). One potential explanation is that the chronically increased oxidative stress attenuates adaptations to resistance exercise (60). Thus, antioxidant supplementation has been suggested to provide additional protection in elderly individuals due to the increased risk of oxidative damage (70). Labontè et al. (75) and Bobeuf et al. (13, 14) used a double-blinded randomized controlled study to investigate the effect of antioxidant supplementation during resistance exercise in healthy elderly individuals (60-75 years). Labonte et al. (75) reported that 6 months of high intensity resistance exercise (3

* 8 repetitions at 80% of one-repetition maximum, 3 times a week) had no significant effects on fat-free mass (0.5 ± 1.4kg) while resistance exercise combined with antioxidant supplementation (600 mg vitamin E and 1000 mg vitamin C per day) provided a significant increase in fat-free mass (1.3 ± 1.4kg; $p<0.01$). However, significant treatment was observed for both groups when assessing appendicular FFM and estimating muscle mass by urinary excretion of creatinine ($p<0.01$), with no difference between the groups (13). Furthermore, both groups showed a similar antioxidant/pro-oxidant profile before and after the intervention and a similar significant increase in maximal strength (all $p<0.001$). These preliminary findings indicate that antioxidants may provide a significantly greater effect on FFM when combined with resistance exercise, supporting the hypothesis that vitamin C and E can reduce damage induced by contractions and/or increase protein synthesis. To the author's knowledge, no other studies have specifically examined changes in fat-free mass in response to high-intensity resistance exercise combined with antioxidants in older people. ROS formation and metabolism are clearly disrupted with increased age, and there is evidence that these changes in ROS contribute to the loss of muscle mass and function that occur with aging (60). Whether dysregulation of ROS is the prime cause of aging, or a consequence of it, remains an open question.

3   MATERIALS AND METHODS

3.1   Design

The present study is an extension of "Smartfish, Antioxidants, Recovery and Adaptation" (SARA; Paulsen et al. Manuscript in preparation), which was a research project conducted to investigate how high dosage with antioxidants and a Smartfish drink affects adaptation to resistance exercise and endurance exercise in young to middle aged subjects (18-45 years) and elderly men (60-81). The design of the SARA-elderly study will briefly be presented below (figure 8). However, the present master thesis is limited to investigate how supplementation with high dosage of vitamin C and E affects muscle growth and maximum strength after 12 weeks of resistance exercise in elderly men.

The present study design was conducted as a double-blinded randomized placebo-controlled experiment. Volunteers were invited to an information meeting, where questionnaires (appendix 4) regarding the inclusion criteria's (section 3.2) was distributed and subsequently screened by the researchers. Following, included subjects (see section 3.2) started with a 2 weeks familiarization period without supplementation, which focused on proper exercise techniques and "wash out" previous training regimes, as well as familiarization to the 1RM tests. The exercise volume increased progressively from 1-2 warm-up/technique (40-60% of 1RM) sets and one exercise set (10RM) to 1-2 warm-up and 2-3 exercise sets per exercise (10RM). To minimize the learning effect all subjects conducted familiarization to the one-repetition maximum (1RM) tests at the end of the 2 weeks period. Subjects were then randomized to three groups: *antioxidant group* (vitamin C and vitamin E), *Smartfish group* (Smartfish drink) or *placebo group* (cellulose and dicalsium phosphate), stratified upon one-repetition maximum (1RM) familiarization tests.

After the familiarization period the subjects were handed out a schedule over appointments for the different tests within the next two weeks. All the measurements and tests were performed by the same test leader, and in approximately the same order each time. Furthermore, oral and written instructions were given in conjunction to preparations prior for testing. Pre and post-tests were taken over three separate days; one day at Southern Norway Hospital Trust, Agder, measuring lean mass with dual-energy X-ray absorptiometry (DXA), and two days at the University of Agder testing 1RM, taking blood samples, and measuring muscle thickness with ultrasound imaging. Upon arrival of the last test day, instructions were provided for a four-day

diary registration (5) that was carried out to assess their energy intake, protein intake and intake of vitamin C and E. At the same time, habitual physical activity was recorded in the four consecutive days with the activity monitor SenseWear Pro3 Armband. Subjects were instructed to maintain usual food and physical activity habits. All the following described measurements were performed before and after the intervention. Ultrasound imaging and 1RM tests were also conducted two times during the 12 weeks resistance exercise intervention (week 4 and 8; figure 8), at two separate days each time.

The study complied with the standards set by the Declaration of Helsinki and was approved by the regional committee for medical and health research ethics, south-east, before initiation (appendix 2). The nature and goals of the study were thoroughly explained, and all subjects provided a written informed consent (appendix 3).
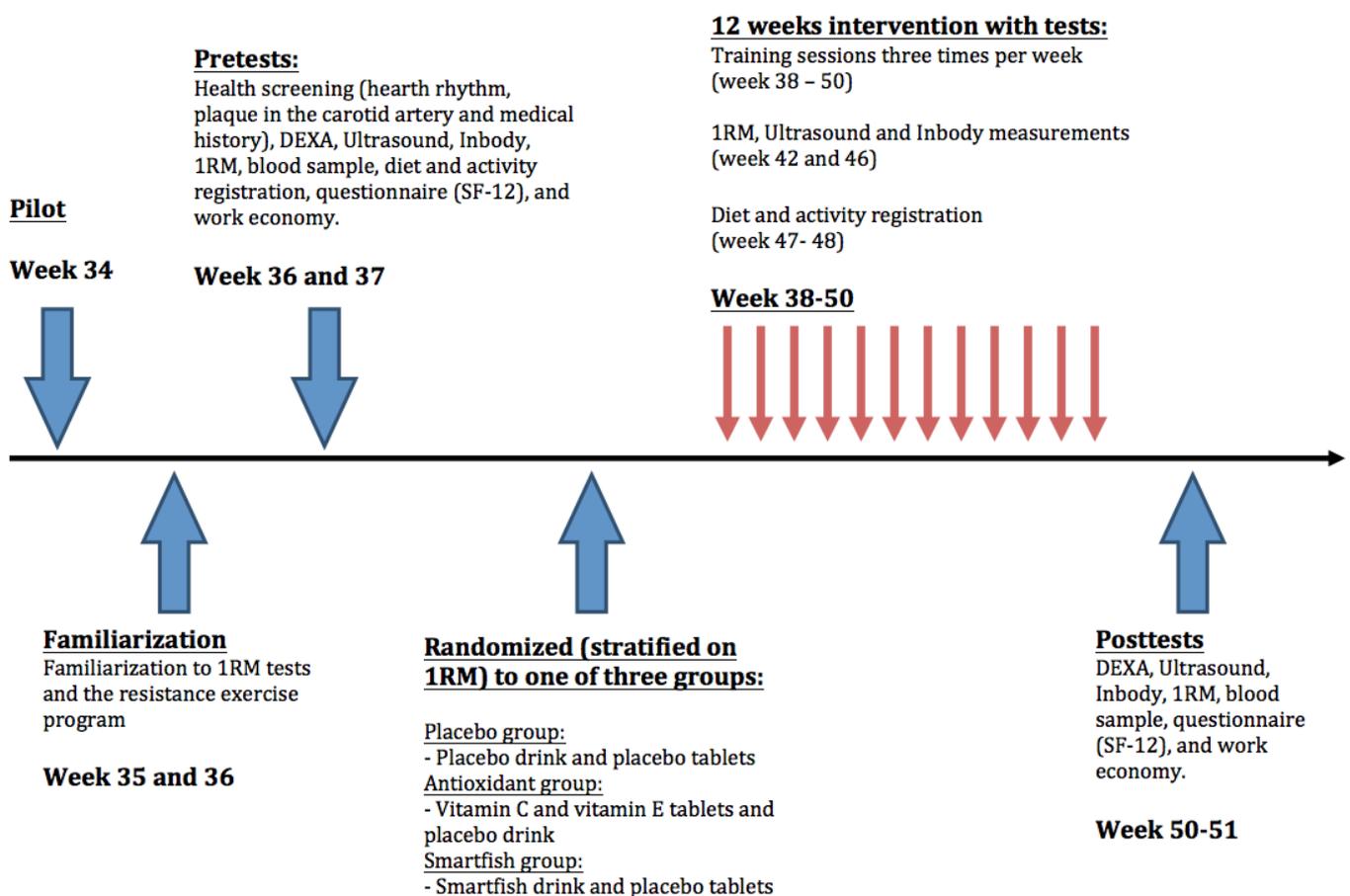
Figure 8   **Timeline over the SARA-elderly study.**

3.2 Subjects

Since elderly women have shown to respond poorly on resistance exercise (57), and a have a higher resting antioxidant level (45); we considered males as the appropriate choice to test our hypothesis. Thus, elderly males (60 – 81 years) were recruited through advertising, from the local community in Kristiansand; Norway. Both newspaper and flyers were used (appendix 1). To ensure that the subjects were healthy and able to participate in heavy resistance exercise, a cardiologist conducted a medical screening at Southern Norway Hospital Trust before entering the study. Inclusion criteria were any overt disease (COPD etc.), disabilities to perform resistance exercise, or use of medication or supplements that could interfere with the measurements. None of the subjects conducted systematic resistance exercise (>1 time per week) during the last 6 months before entering the study. The sample size was calculated to ensure statistical power when comparing groups. Based on an expected standard deviation (SD) of 15, we had 80 % power to detect a true mean group difference of 11% in muscle mass and maximal strength with a minimum of 15 participants in each group (alpha: 0.05; two-tailed). We considered such difference to be well within the physiological meaningful range, as the expected mean group changes are >25% (41).

A total of 200 were interested and attended an information meeting at University in Agder. Of the 200 interested, 71 were invited to participate in the study. Sixteen of the subjects did not meet the requirements set by a cardiologist after the health screening at the Southern Norway Hospital Trust, and were excluded from the study. In addition, two subjects decided to drop out of the study because of personal circumstances. The remaining 53 subjects were randomized in three groups (antioxidant group, smartfish group or placebo group), stratified upon the one-repetition maximum familiarization tests (Figure 9). The two groups investigated in the present thesis consisted of an *antioxidant group* (n=17; vitamin C and vitamin E tablets), and a *placebo group* (n=17; sugar water/cellulose tablets; table 1). During the intervention, one participant dropped out of the study because of a broken ankle. Furthermore, one of the subjects was excluded from the 1RM test in leg extension because of a painful knee, but is included in all other analyses.
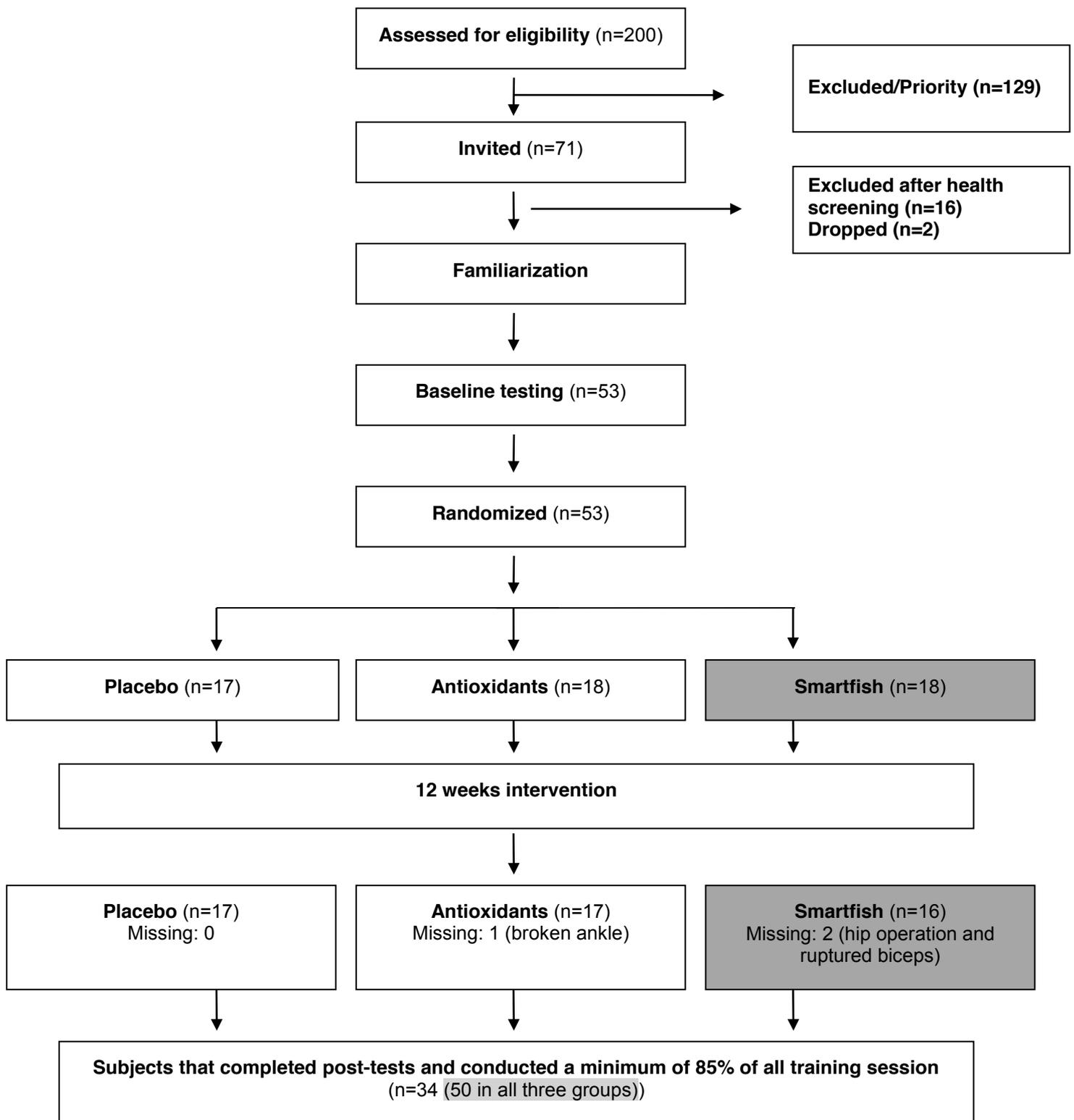
Figure 9 **Flowchart showing inclusion and exclusion of participants in the SARA-elderly study**

3.2.1 Supplement

The C and E vitamin and placebo pills were produced under Good Manufacturing Practice (GMP) requirements at Petefa AB (Västra Frölunda, Sweden). Each vitamin pill contained 250 mg of ascorbic acid and 58.5 mg DL-alpha-tocopherol acetate (table 1). The placebo pills had identical shape and appearance as the vitamin pills. The participants ingested 2 pills 1-3 hours before every training session and 2 pills in the hour after training. On non-training days the participants ingested 2 pills in the morning and 2 pills in the evening. The intake of pills was confirmed in a training diary to ensure compliance regarding the supplementation. These daily doses have been shown to alter the oxidative profile of physical active adults (45). Subjects were provided with approximately one-week-rations of supplements, and the amount of supplements they had left was registered. A person which was not part of the physical testing, nor any type of analyses were responsible for the randomization and for concealing the group identity of each subject through the intervention.

Table 1   Content of the supplements

Vitamin C and E	Placebo capsules
Capsules: Vitamin C: 250 mg Vitamin E: 88 IU	Capsules containing cellulose and dicalsium phosphate; same appearance as the C+E vitamin capsules
2 pills twice pr. day; before and after training or morning and evening	2 pills twice pr. day; before and after training or morning and evening
Total dosage pr. day: Vitamin C: 1000mg Vitamin E: 350 IU (235mg)	

Note, although the dosage of vitamin C and E used are well within the range of intake believed to be safe for healthy adults (32, 41, 48, 52), we provided subjects with schemes (training diary) on which they noted any side effects which they believed were due to the supplementation.

3.3   Resistance exercise

The 12 weeks resistance exercise program had an undulating periodized profile (68, 81). The protocol included 3 full-body sessions per week, emphasizing free weight exercises where all the major muscle groups were included (table 2). Two of the sessions each week were "moderate" (8-10 rep, with 1 min break between sets), and one was varied between "heavy" (3-5 rep, with 2 min break between sets) and "light" (13-15 rep, with 45 seconds break between sets)" every second week. The resistance exercise program was designed to give large metabolic stress (i.e., oxidative stress) and the intention was to stimulate as much muscle growth as possible. The number of sets per exercise was increased progressively from 1 to 4 sets during the first 10 weeks, and then reduced with 1 set each of the last 2 weeks of the intervention (tapering). The subjects conducted one additional "warm-up" set at 50 % of their target weight in each exercise before the main sets started. Two experienced instructors supervised all resistance exercise sessions and the loads were weekly adjusted. Breaks intervals between sets in exercises were timed and synchronized for the all participants. The movement velocity was also instructed to a minimum of 1 second in the concentric phase, and 2 seconds in the eccentric phase. All subjects received an individual exercise diary before each session, with a target weight for every exercise. The last set of each exercise in one of the "moderate" sessions each week, and in every light and heavy session, was performed as a maximum repetition test at the given weight that was written down in the prefilled diary. If the maximum repetition result fell outside the given repetition interval (table 2), the resistance was adjusted before the next session.

Table 2   Resistance exercise program

	Session 1	Session 2	Session 3
1 wk	8-10 rep. **1 (warm-up) + 1** series 1 min rest between set **and exercises**	13-15 rep. 1 + 1 series 45 sec rest between set **and exercises**	3-5 rep. 2 min rest between set **and exercises**
2 wk	**1 (warm-up) + 2** series		1 + 1 series
3 wk	**1 (warm-up) + 2** series	1 + 2 series	
4 wk	**1 (warm-up) + 2** series		1 + 2 series
5 wk	**1 (warm-up) + 3** series	1 + 2 series	
6 wk	**1 (warm-up) + 3** series		1 + 3 series
7-8 wk	**1 (warm-up) + 3** series	1 + 3 series	1 + 3 series
9 wk	**1 (warm-up) + 4** series	1 + 3 series	
10 wk	**1 (warm-up) + 4** series		1 + 4 series
11 wk	**1 (warm-up) + 3** series	1 + 3 series	
12 wk	**1 (warm-up) + 2(3)** series		1 + 2(3) series

	Exercises:		
	Bulgarian squat	"Sumo" deadlift w/ kettlebells	Leg-extension
	Squat	Lunges	Leg press
	Bench-press	Step up	Chest-press
	Pull-down narrow grip	Flyes	Pull-down wide grip
	Upright row	Seated row machine	Arnold-press
	Calf raise	Lateral raises	Bench-press narrow grip
	French press	Triceps pushdown	Scott curl
	Standing biceps curl w/dumbbells	Scott curl	Side-plank
	Quadruped exercise	Plank	

3.4 Measurements

3.4.1   Dual-energy X-ray absorptiometry (DXA)

Body composition measured by one experienced observer was assessed by dual-energy X-ray absorptiometry (DXA; GE-Lunar Prodigy, Madison, WI, USA). Both weight and height was measured before the test, and bodily ornaments (watches, jewelry etc.) were removed. The participants were told to lay down at the DXA machine after the test leaders instruction, with hands along the side, slightly away from the body and with the legs straight and internally rotated. Participants were scanned from head to toe in supine position, providing values for non-bone lean tissue in total body, as well as in arms, legs and trunk separately. Test-retest analyses from 62 scans in 31 participants demonstrated intra class correlation from 0,95 to 0.99 (all with $p<0,001$) for total lean mass, lean mass in arms, lean mass in legs, and lean mass in

the trunk. Limits of agreements (mean difference ± 1,96 standard deviations (SD) of the difference) were -0,23 ± 1,76 kg for total lean mass, 0,1 ± 0,6 kg for lean mass in legs, 0,1 ± 0,6 kg for lean mass in arms and 0,6 ± 1,1 mm for lean mass in the trunk, with 94-97 % of the values within two SD. The coefficient of variation (CV) was 1,5 % in total lean mass, 3,08 % in lean mass of the legs, 3,23% in lean mass of the arms and 2,99 % in lean mass in the trunk.

3.4.2 Ultrasound measurements (US)

A single, previously trained examiner performed all of the ultrasound measurements. Muscle thickness was measured in vastus lateralis, rectus femoris and in the elbow flexors (biceps brachii and brachialis) using a brightness mode (B-mode) ultrasonographic apparatus (LogicScan 128 CEXT-1Z kit, Telemed, LT), with linear probe of 40mm width and an excitation frequency of 9 MHz. Ultrasound settings such as focal depth, image depth, power and gain were optimized to best identify the collagenous tissue that defines the outer border of the muscle. All subjects were instructed to lay down on a bench with their legs relaxed in a supine position. Muscles of the dominant arm or foot were analyzed. Excessive use of gel and great care used to ensure that minimal pressure was applied to the muscle tissue when an image was scanned. The vertical diameter of vastus lateralis and rectus femoris were measured at a distance equal to 40% of the femur length, distally. Scan locations for vastus lateralis and rectus femoris were located between the lateral epicondylus and the great trochanter major of the femur. Ultrasound images of the elbow flexors were taken in two places; 50 %, and 30% of the humerus length, distally. Scan locations were found between the epicondylus lateralis and the proximal part of the humerus. Locations were identified using ultrasound when possible. Probe position in each measurement were recorded on acetate paper to ensure identical placement, and sets of three consecutive US images were scanned for later analyses in each position.

Analyses of US images

The images where analyzed with the program ImageJ (version 1.46r, National Institutes of health, USA) widely used for this purpose (2, 106). All the images where analyzed blinded and at random order, by the same investigator. The vertical diameter of each muscle was measured on the inner edge of the muscle in three locations. The average of the measurements in three pictures of each position was used as the muscles thickness in rectus femoris and vastus lateralis, and further analyzed. The same was done in elbow flexors, but additionally the average of the two locations was used. Test-retest analyses from 20 scans in 10 subjects

demonstrated intra class correlation from 0,98 to 0.99 (all with p<0,001) for muscle thickness of elbow flexors, rectus femoris and vastus lateralis. Limits of agreements (mean difference ± 1,96 standard deviations (SD) of the difference) were 0,2 ± 1,0 mm for the elbow flexors (biceps brachii and brachialis), 0,1 ± 0,6 mm for rectus femoris, and 0,6 ± 1,1 mm for vastus lateralis, with 90-100 % of the values within two SD. The coefficient of variation (CV) was 1,55 % in elbow flexors, 1,58 % in rectus femoris, and 2,45 % in vastus lateralis.

3.4.3   One-repetition maximum (1RM)

1RM was measured in leg extension, leg press and scott curl (TechnoGym, Italy; as previously described in Paulsen et al. (89)). The warm-up consisted of four sets with gradual increasing load and descending repetitions (12 [~50%], 5-7 [~70%], 2-4 [~80%], 1 [~90%]). Following, the participants had four test attempts, where the first attempt was customized to around 5 % under expected 1RM after the familiarization. After the first attempt the load was increased until maximum was found and the lift was accepted. All subjects performed the 1RM tests in the same order; first at leg extension, moving on to scott curl and then finally leg press. Three participants conducted the 1RM tests simultaneously. The rest interval between each 1RM test attempts was 2 minutes. Each test was conducted with one limb at the time, with approximately 30 seconds between right and left leg/arm. The minimum increase of load at leg extension was 2,5 kg, 5 kg in leg press, and 1 kg in scott curl. All tests had the same test-leader each time, and individual adjustments were noted for each subject at the first test. The 1RM test was conducted pre and post, and in addition two times (figure x) under the intervention period. The summation of the results for the right and left limb was used for further analysis in each 1RM test.

In *leg extension*, the leg pad was adjusted to a position just above the shoe, with the other leg outside the pad. The back support was adjusted in a position where the knee fitted at the edge of the seat and hands were placed around handles at the apparatus. A piece of tape marked the height of an approved attempt.

In *scott curl*, the height of the seat was adjusted to ensure that the chest, axillary, upper arm and elbow all were in contact with the pillow in seated position. The arm was placed in a supine position, between two markers to ensure a lift without rotation. The attempt was approved if the arm went from an extended position, to vertical position without any rotation of the arm or movement in the upper-body.

In *leg press*, the seat was adjusted so that the angle of the knee was measured to be 80 degrees in the start position before the lift. The back support was always in the same upright position, with shoulder pads individually adjusted. The foot was placed on a marked spot at the panel, with the other foot placed outside the panel. An approved lift was satisfied when the leg was fully stretched.

3.5 Pilot study

A pilot study was conducted prior to the intervention in an attempt to ensure that the exercise regime and test protocol fitted to elderly males. Six of the 200 interested elderly males (figure 9) that were excluded were asked to participate in the pilot study. All six subjects conducted each of the three resistance exercise sessions (table 2) two times, and were tested in 1RM, and ultrasound imaging.

3.6 Statistics

Baseline variables showed overall normal distribution (Gaussian distribution), whereas some variables measuring changes in muscle thickness, lean mass and 1RM had a skewed distribution at certain time points, especially 1RM measures in the knee extension exercise. Hence, descriptive data are presented as mean and standard deviation (SD), whereas results are presented as median with 95% confidence intervals, if it not otherwise stated in the text. Due to the non-normality non-parametric tests were used when analyzing muscle thickness, lean mass, and muscle strength. Mann-Whitney u-test was used to detect statistical differences between the antioxidant group and the placebo group. Wilcoxon signed rank test was applied to evaluate differences between baseline and post-intervention measurements in each group. Spearman's correlations coefficient was used to evaluate relationships between the strength and measurements of muscle growth.

Statistics were performed with IBM SPSS Statistics 19.0 (Statistical Product and Service Solutions, Chicago, IL, 2010). The sample size was calculated using G*Power (version 3.1.2 software). Variables were considered significant when $p \leq 0.05$.

4   METHOD DISCUSSION

4.1   Design

Experimental research attempts to establish cause-and-effect relationships, where an independent variable is manipulated to judge its effect on a dependent variable (125). Thus, experimental design seems to be the most appropriate method to investigate the main *aim* of the present study (section 1.1). Cause-and-effect relationships can only be established by a well-designed experiment, and it is crucial that no other reasonable explanation exists for the changes in the dependent variable except the manipulation of the independent variable. However, because there exists a large number of possible variables that can influence adaptations to resistance exercise, and due to limitations in resources and economics in the present study, it is impossible to fully control all possible variables. A double-blinded randomized placebo-controlled design was used as a method for controlling such variables. The participants were randomized to allow the assumption that the groups did not differ at the beginning of the experiment. Furthermore, we used stratified randomization to ensure that the groups were equal in regards to maximal strength at baseline. In an attempt to control the psychological effect, placebo supplements were given to the control group and both researchers and participants were blinded. Lastly, we registered several other variables that possibly could affect our dependent variables (i.e. muscle growth and maximal strength), such as energy consumption, protein consumption, physical activity level and plasma vitamin status.

4.2   Study sample

With a standard deviation (SD) of 15 in the main study we had 80% power to detect a true mean group difference of 11%, with a minimum of 15 participants in each group (alpha: 0.05; two-tailed), which is considered to be well within the physiological meaningful range, as the expected mean group changes were likely to be >25% (41). The study sample in the present study included 34 participants that completed the intervention; 17 in the placebo group and 17 in the antioxidant group. Thus, we considered to have enough power to detect significant changes in the outcome measures. When considering if we can generalize our results, is essential to determine whether the actual elderly population in southern Norway would differ significantly from the participating sample in the present study. The participants in the present study were generally in good health before entering the study. Furthermore, the inclusion criteria ruled out smokers, elderly with physical injuries, or use of medication and supplements that could interfere with the measurements. Finally, one could assume that elderly interested in

participating in such a training intervention are more healthy and active compared to the general elderly population. Therefore, the present study sample probably had a better physical health than the actual elderly population in southern Norway. In the present sample, both seniors and elderly still working were included.

4.3 Resistance exercise and supplementation

Several considerations should be made when evaluating the resistance exercise protocol, as many different variables (i.e. resistance exercises, volume [set * rep * number of exercises], intensity, rest period between sets, supervised exercise and baseline levels) may contribute to affect the adaptations following resistance exercise (133). The present study used an undulating periodized resistance exercise protocol and included mainly free-weight exercises, with the intension to give large metabolic stress (i.e. oxidative stress) and stimulate as much muscle growth as possible. A meta-analysis by Rhea and Brandon (107) investigated the effect of periodization on strength and power gains during resistance exercise, and showed that periodization is superior compared to nonperiodized resistance exercise for men and women, individuals of varying training backgrounds, and for all age groups. Furthermore, Jiménez (68) compared resistance exercise periodization models, and concluded that researchers and exercise professionals should include undulating periodization models during resistance exercise to induce optimal gains in strength, power, motor performance, and/or muscle hypertrophy. Finally, the undulating periodized resistance exercise protocol in the present study emphasizes a relatively high resistance exercise volume (set * rep * number of exercises), which is supported in a meta-analysis by Peterson (95), showing that higher-volume interventions were associated with significantly greater increases in lean body mass of elderly individuals (>50 years). Hence, we consider the resistance exercise protocol in the present study efficient to induce optimal gains in lean mass and maximal strength in the elderly.

It is possible that the dosages of vitamin C and E used in the present study (235 mg vitamin E and 1000 mg vitamin C) were not optimal to favorably affect the redox status and subsequently accelerate the adaptation to resistance exercise for the studied population. However, investigations have reported that very high dosage of vitamin C and E administered as a dietary supplement to adult humans exhibits a pro-oxidant, as well as an antioxidant, effect in vivo (90, 99). Furthermore, these daily dosages have been shown to be effective in altering the oxidative profile of physically active adults (45). The present study maintained close control of adherence regarding the supplementation.

4.4 Measurements

In general, testing in sports science is necessary to identify the effects of an exercise intervention. Several factors are fundamental in testing: 1) that the test is valid and reliable, 2) control of the work conditions, 3) accurate measures from equipment, and 4) same standardized protocol before, during and after the test (125). To increase the validity and reliability of the measurements, the tests were conducted at approximately the same time of day both pre and post intervention for the participants. Moreover, to maintain a high degree of reproducibility the same test leader supervised the same tests each time.

4.4.1 Dual-energy X-ray absorptiometry (DXA)

When evaluating the validity of body composition measurements by DXA, one would refer to the difference between fat-mass and lean-mass estimation by DXA and the true fat mass and lean mass, which can be measured in cadavers. However, to the author's knowledge, such cadaver studies have only been done in animals (26). The studies that have assessed the validity and reliability of body composition measurements by DXA in humans are evaluated against the 4-compartment model. The 4-compartment model is currently regarded the "gold standard" of body composition measurements (126). The different compartments are measured using hydrodensitometry or air displacement plethysmography to determine fat mass and fat free mass, isotope dilution to determine total body water, and DXA to measure bone mineral (56, 134). However, this model is very time consuming, and the equipment was not available for use in the present study. Although the primary use of DXA is the measurement of bone mineral density to diagnose osteoporosis and other bone diseases (126), several studies have proven that DXA can provide valid and reliable assessments of body composition i.e., the measurement of fat and bone-free lean mass (19, 23, 104).

4.4.2 Ultrasound imaging

Brightness-mode ultrasound imaging has gained importance as a simple, inexpensive and reliable morphological measurement of human muscle *in vivo* (34). In fact, several studies have evaluated the validity and reliability of ultrasound imaging, and concluded that it is a good alternative to Magnetic resonance imaging (MRI) (34, 106, 116, 124). MRI is considered to be the "gold standard" in measurement of muscle thickness, volume and length *in vivo* (34). However, this technique is expensive, and was not available in the present study. Errors in

ultrasound measurements such as tissue compression from the transducer, and poor manual processing of images, are common (106). This can be avoided if the investigator is properly trained (106). In the present study one previously trained examiner performed all measurements, and excessive use of gel, as well as great care was used to ensure that minimal pressure was applied to the muscle tissue when an image was scanned. Pathology, or measures of deep muscles, can pose a problem for ultrasound imaging. If muscles become partly fused to other muscles by pathology, or the deep auponerousis of the muscle is of poor solution, it can be hard to identify. In the present study, the focus was in superficial muscles in the quadriceps, and the total thickness of the elbow flexors (biceps and brachialis), which have clearly identifiable borders. Furthermore, the researcher who conducted the measurement was previously trained in both ultrasound image acquisition and image processing. Unfortunately, to the author's knowledge there is currently no automatic technique to process muscle images by ultrasound that is able to minimize errors of manual processing (9). Other sources of error which are more difficult to control could be lack of uniformity in muscle growth along the muscle (33, 86), acute swelling of the muscle by physical activity prior to the measurement (106), or different hydration levels of the subjects, as reported in cadavers by Ward & Lieber (132). However, in an attempt to control such errors subjects were instructed not to conduct physical activity before testing, and were tested at approximately the same time a day each time. Lastly, test-retest of 10 subjects in the present study showed overall good reliability.

4.4.3   One-repetition maximum (1RM)

All subjects performed the 1RM test with the standardized protocol described in section 3.4.3. The participants were instructed eat normally, without conducting any strenuous exercise the day before 1RM testing, to ensure recovery of the capacity of force production in the elderly participants would be similar (37). The participants were not informed about their previous results when performing a 1RM test, due to psychological interference. The relatively high interval in increase of load of 1RM tests (Scott curl: 1kg, leg press: 5 kg and leg extension: 2.5 kg) could attenuate the sensitivity of the measurement. Even with a minimum of 2 minutes rest between each 1RM attempt, one may notice some degree of fatigue in the working muscle, and thus confounding results. The use of maximal voluntary contraction (MVC) is an alternative method for assessing maximal strength. This method has shown a better test-retest reliability (129), due to a high level of assessments control (1). Although MVC is considered the ''gold standard'' for the assessment of muscle strength *in vivo* (129), one-repetition maximum testing is proven to be a valid and reliable method to measure maximal strength in adults (77) and

older individuals (98) when using a sufficient stringent protocol (77). Due to equipment and practical implications 1RM was conducted in the present study.

4.5   Main strengths and limitations

The *main strengths* of the present study were the strong research design, high attendance in resistance exercise sessions and close control of adherence for both resistance exercise and antioxidant supplementation. Nevertheless, the present study had a relative short duration of the resistance exercise intervention and a small sample size, which in turn could lead to type II errors. However, we had sufficient statistical power according to the calculations of sample size. Lastly, we did not measure underlying cellular mechanisms (i.e. translation and transcription factors) or markers of oxidative stress and redox state. The participating study sample's good health was important for the safety throughout the high intensity resistance exercise intervention using free weight exercises and a large training volume. Further research is needed to investigate the effect of antioxidants combined with resistance exercise in frail elderly.

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PART 2:

PAPER

Antioxidants and muscle growth after resistance exercise in elderly

The following paper is, with minor exceptions, written after the standards of the journal:

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Effect of antioxidants on muscle growth and strength induced by resistance exercise in elderly men

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ABSTRACT

PURPOSE: To investigate the effect of vitamin C and E on muscle growth and strength during 12 weeks of resistance exercise in elderly men.

METHODS: Thirty-four elderly males (60 – 81 years) were randomized to either an antioxidant group (N=17; 1000 mg of vitamin C and 235 mg of vitamin E per day) or a placebo group (N=17). Muscle growth was assessed as changes in lean mass with Dual-energy X-ray absorptiometry and muscle thickness of rectus femoris, vastus lateralis and elbow flexors (biceps brachii and brachialis) with ultrasound imaging. Strength was measured as one-repetition maximum (1RM) in leg press, leg extension and scott curl. All participants following a supervised undulating periodized program 3 times/week.

RESULTS: Total lean mass increased by 3.9% (95% confidence intervals 3.0-5.2) and 1.2% (0-3.6) in the placebo and antioxidant group, respectively; revealing larger gains in the placebo group (p=0.03). Similarly, the thickness of rectus femoris increased more in the placebo group (16.2% [12.8-24.1]) than in the antioxidant group (10.9% [9.8-13.5]; p=0.01). Changes in lean mass of trunk and arms, as well as muscle thickness of elbow flexors and vastus lateralis, did not differ significantly between groups. With no group differences, 1RM improved in the range of 15-21% (p<0.001).

CONCLUSION: The resistance exercise training induced a robust increase in lean mass, local muscle thickness and strength. Supplementation with vitamin C and E had no positive effects on the adaptation to resistance exercise in elderly men, but seemed on the contrary to hinder muscle growth.

KEYWORDS: Antioxidant supplementation, lean mass, muscle thickness, one-repetition maximum, undulating periodization.

INTRODUCTION

Paragraph number 1 Demographics indicate that people aged 60 years and above in the world's population will more than triple within 50 years (7). This will probably present large strains on the health care systems. Contributing to risks of diseases, and a direct predictor of frailty and disability, are the age-related decline in skeletal muscle mass and strength, referred to as sarcopenia (23). One of the main prevention strategies for the progression of sarcopenia is resistance exercise (4, 29, 30).

Paragraph number 2 A combination of resistance exercise and nutritional interventions has been suggested as a promising candidate in combating sarcopenia (20). Nutrition is of obvious importance for health and for optimal training effect of exercise (13). To boost effects of exercise a large assortment of nutritional supplements has been developed and a popular type of supplement available is antioxidants, e.g. vitamin C and E products. Vitamin supplementations with antioxidant properties are commonly thought to be beneficial for adaptation to exercise (38), but in recent years the widespread use of concentrated antioxidants (pills), have been questioned (12, 14). Scientists have reported both no effect (3, 39) and adverse effects (36) in young adults. In theory, antioxidant supplementation could paradoxically both facilitate and hamper muscular adaptations to resistance exercise by affecting the cellular redox balance (i.e. the balance between pro-oxidants and anti-oxidants) (25). A right shift in redox status, resulting from a chronically higher oxidative stress, has been suggested to play an important role in the functional decline aging muscles (18). Subsequently, this can lead to inevitable deterioration of essential cellular functions, which can attenuate adaptations to resistance exercise, such as muscle growth (8, 16, 18). However, the redox status also seems to affect the redox sensitive signaling cascades regulating protein synthesis, as both reduced and increased levels of free radicals influence them (25, 40). Thus, it seems to exist a currently undefined optimal redox

status. To the authors' knowledge, only one study has specifically reported changes in fat-free mass or muscle mass in response to high-intensity resistance exercise combined with antioxidants in older adults. Bobeuf and co-workers (5, 6, 21) reported that vitamin C (1000 mg/day) and vitamin E (600 mg/day) supplementation resulted in a larger increase in fat-free mass (6, 21) and beneficial effects on body composition (5), compared to resistance exercise only. Considering the health benefits of gained muscle mass in elderly individuals (30), such alleged effects of a vitamin C and E supplement could indeed prove very valuable, both on the individual level and for society.

Paragraph number 3 The *aim* the present study was to investigate if supplementation with antioxidants vitamin C and E could accelerate adaptations to 12 weeks of resistance exercise in terms of muscle growth and increase in maximal strength in elderly men. We hypothesized that the vitamin supplementation would favorably affect the redox status in our elderly participants and accelerate muscle growth during 12 weeks with resistance exercise 3 times per week.

MATERIALS AND METHODS

Subjects

Paragraph number 4 Thirty-five elderly males aged between 60 and 81 (68 ± 6years; mean ± standard deviation (SD)) were recruited through advertising, from the local community in Kristiansand; Norway. To ensure that the subjects were healthy and able to participate in heavy strength training, a cardiologist conducted a medical screening before entering the study. Exclusion criteria were any overt disease (COPD, cancer, known heart disease etc.), disabilities to perform resistance exercise, or use of medication or supplements that could interfere with the measurements. None of the subjects conducted systematic resistance exercise during the last 6 months before entering the study. The sample size was calculated to ensure statistical power

when comparing groups. Based on an expected standard deviation (SD) of 15, we had 80 %

power to detect a true mean group difference of 11% in muscle mass and maximal strength

with a minimum of 15 participants in each group (alpha: 0.05; two-tailed). We considered such

difference to be well within the physiological meaningful range, as the expected mean group

changes are >25% (9). One of the thirty-five participants did not complete the study, and one

was excluded from 1RM test of leg extension because of a painful knee, but was included in all

other analyses.

Study procedure

Paragraph number 5 The present study design was conducted as a double-blinded randomized

placebo-controlled experiment. Volunteers were invited to an information meeting, where

questionnaires regarding the inclusion criteria were distributed and subsequently screened by

the researchers. Following, the included subjects started with a 2 weeks familiarization period

to the resistance exercise program and the 1RM tests, without supplementation. Subjects were

then randomized in two groups: *antioxidant group* [vitamin C and vitamin E]; n=17 or *placebo*

group [cellulose and dicalsium phosphate]; n=17), stratified upon one-repetition maximum

(1RM) familiarization tests. After the familiarization period measurements of lean mass with

dual-energy X-ray absorptiometry (DXA), muscle thickness with ultrasound imaging, one-

repetition maximum (1RM) tests and blood samples were taken over three separate days. All

measurements were performed by the same test leader and in the same order each time.

Furthermore, oral and written instructions were given in conjunction to preparations prior for

testing. Upon arrival of the last test day, instructions were provided for a four-day diary

registration (2) that was carried out to assess their energy intake, protein intake and intake of

vitamin C and E. At the same time, habitual physical activity was recorded in the four

consecutive days with the activity monitor SenseWear Pro3 Armband. Subjects were instructed

to maintain their usual food and physical activity habits. All the following described measurements were performed before and after the intervention. Ultrasound imaging and 1RM tests were also conducted two times during the 12 weeks resistance exercise intervention (week 4 and 8). The study complied with the standards set by the Declaration of Helsinki and was approved by the regional committee for medical and health research ethics; south-east, before initiation. The nature and goals of the study were thoroughly explained, and all subjects provided a written informed consent.

Antioxidant supplementation

Paragraph number 6 The C and E vitamin and placebo pills were produced under Good Manufacturing Practice (GMP) requirements at Petefa AB (Västra Frölunda, Sweden). Each vitamin pill contained 250 mg of ascorbic acid and 58.5 mg DL-alpha-tocopherol acetate. The placebo pills had identical shape and appearance as the vitamins pills. The participants ingested 2 pills (500 mg of vitamin C and 117.5 mg vitamin E) 1-3 hours before every training session and 2 pills in the hour after training. On non-training days the participants ingested 2 pills in the morning and 2 pills in the evening. The intake of pills was confirmed in a training diary. Thus, the daily dosage was 1000 mg of vitamin C and 235 mg vitamin E. These daily doses have been shown to alter the oxidative profile of physical active adults (11). Subjects were provided with approximately one-week-rations of supplements, and the amount of supplements they had left was registered to ensure compliance.

Resistance exercise

Paragraph number 7 The 12 weeks resistance exercise program had an undulating periodized profile (19). The protocol included 3 full-body sessions per week, emphasizing free weight exercises where all the major muscle groups were included. Two of the sessions each week

were "moderate" (8-10 rep, with 1 min rest between sets), and one varied between "heavy" (3-5 rep, with 2 min rest between sets) and "light" (13-15 rep, with 45 seconds rest between sets)" every second week. The strength-training program was designed to give large metabolic stress (i.e., oxidative stress) and the intention was to stimulate as much muscle growth as possible. The number of sets per exercise was increased progressively from 1 to 4 sets during the first 10 weeks, and then reduced with 1 set each of the last 2 weeks of the intervention (tapering). The subjects conducted one additional "warm-up" set at 50 % of their target weight in each exercise before the main sets started. The last set of each exercise was performed with maximal number of repetition, and if the number of repetitions exceeded the sessions target repetitions the load was adjusted for the next weeks sessions. Two experienced instructors supervised all resistance exercise sessions and the loads were weekly adjusted.

Lean mass measurements

Paragraph number 8 Lean mass measured by one experienced observer was assessed by dual-energy X-ray absorptiometry (DXA; GE-Lunar Prodigy, Madison, WI, USA). Participants were scanned from head to toe in supine position, providing values for non-bone lean tissue in total body, as well as in arms, legs and trunk separately. Test-retest analyses from 62 scans (31 participants) demonstrated intra class correlation from 0.95 to 0.99 (all with $p<0.001$) for total lean mass, lean mass in arms, lean mass in legs, and lean mass in the trunk. Limits of agreements (mean difference $\pm$ 1.96 standard deviations (SD) of the difference) were -0.23 $\pm$ 1.76 kg for total lean mass, 0.1 $\pm$ 0.6 kg for lean mass in legs, 0.1 $\pm$ 0.6 kg for lean mass in arms and 0.6 $\pm$ 1.1 kg for lean mass in the trunk.

Ultrasound measurements (US)

Paragraph number 9 One examiner performed all ultrasound measurements. Muscle thickness was measured in vastus lateralis, rectus femoris and in the arm flexors (biceps brachii and brachialis) using a brightness mode (B-mode) ultrasonographic apparatus (LogicScan 128 CEXT-1Z kit, Telemed, LT), with linear probe of 40 mm width and an excitation frequency of 9 MHz. Ultrasound settings such as focal depth, image depth, power and gain were optimized to best identify the collagenous tissue that defines the outer border of the muscle. All subjects were instructed to lay down on a bench with the legs relaxed in a supine position. Muscles of the dominant arm or foot were analyzed. The vertical diameter of vastus lateralis and rectus femoris was measured at a distance equal to 40% of the femur length, distally. Ultrasound images of the elbow flexors were taken in two places: 30% and 50% of the humerus length, distally. Later, the average of the two locations was used for further analysis. Probe position in each measurement were recorded on acetate paper to ensure identical placement. The images were analyzed with the program ImageJ (version 1.46r, National Institutes of health, USA), widely used for this purpose (1, 34). The average of the vertical diameter measured in three locations on the inner edge of each muscle was used as the muscles thickness, and further analyzed. Test-retest analyses from 20 scans in 10 subjects demonstrated intra class correlation from 0.98 to 0.99 (all with $p<0.001$) for muscle thickness of elbow flexors, rectus femoris and vastus lateralis. Limits of agreements were 0.2 ± 1.0 mm for the elbow flexors (biceps brachii and brachialis), 0.1 ± 0.6 mm for rectus femoris, and 0.6 ± 1.1 mm for vastus lateralis. The coefficient of variation (CV) was 1.55 % in elbow flexors, 1.58 % in rectus femoris, and 2.45 % in vastus lateralis.

One-repetition maximum (1RM)

Paragraph number 10 1RM was measured in leg extension, leg press and scott curl (TechnoGym, Italy; as previously described in Paulsen et al. (27)). The warm-up consisted of four sets with gradual increasing load and descending repetitions (12 [~50%], 5-7 [~70%], 2-4 [~80%], 1 [~90%]). After the warm-up, the participants had four test attempts. The rest interval between each 1RM test attempt was 2 minutes. Each test was conducted with one limb at the time, with approximately 30 seconds between right and left leg/arm. The minimum increase of load was 2.5 kg in leg extension, 5 kg in leg press, and 1 kg in scott curl. All tests were administered by the same test-leaders, and individual adjustments were noted for each subject at the first test. The summation of the results for the right and left limb was used for further analysis in each 1RM test.

Statistical analysis

Paragraph number 11 Baseline variables showed overall normal distribution (Gaussian distribution), whereas some variables measuring changes in muscle thickness, lean mass and 1RM had a skewed distribution at certain time points, especially 1RM measures in the knee extension exercise. Hence, descriptive data are presented as mean and SD, whereas results are presented as median with 95% confidence intervals, if not otherwise stated in the text. Mann-Whitney u-test was used to detect statistical differences between the antioxidant group and the placebo group. Wilcoxon signed rank test was applied to evaluate differences between baseline and post-intervention measurements in each group. Statistics were performed with IBM SPSS Statistics 19.0 (Statistical Product and Service Solutions, Chicago, IL, 2010). Variables were considered significant when $p \leq 0.05$.

RESULTS

Paragraph number 12 The mean attendance in resistance exercise sessions was 94 % and all of the subjects reached the adherence minimum of 85 % completed training sessions during the intervention. Baseline characteristics of the subjects are presented in table 1. There were no significant differences between groups in any variables at baseline (table 1).

Lean mass

Paragraph number 13 Both groups had a significant increase in total lean mass, and lean mass of the arms after 12 weeks of resistance exercise (p<0.05). However, only the placebo group showed a significant increase in lean mass of the legs and trunk from baseline to post exercise (figure 1). Median changes in total lean mass and in lean mass of legs were significantly increased in the placebo group (2191 gram [g] [95% confidence intervals 1517 - 3296]; and 727 g [575 - 1221]; respectively) compared to antioxidant group (818 g [0-1820]; p<0.03 and 330 g [223-804]; p<0.02, respectively). A similar tendency was shown in lean mass of the trunk, with an increase of 1091 g (265 - 1790) for the placebo vs. 41 g (379 -950) for the antioxidant group, although the group differences did not reach statistical significance (p=0.08). No differences were found in lean mass of arms between the placebo group (561 g [152 - 689]) and the antioxidant group (453 g [231 - 670]).

Muscle thickness

Paragraph number 14 The time course of % changes in muscle thickness is presented in figure 2. Both groups had a significant increase of muscle thickness in all three measures of muscle thickness from baseline to post exercise (p<0.001). Significant differences between groups were found in ultrasound images of rectus femoris, as the muscle thickness increased with 3.4 mm (2.4 – 4.2) for the placebo group vs. 1.9 mm (1.3 – 3.2) for the antioxidant group (p=0.01).

However, no significant differences were found between the placebo group vs. the antioxidant group for elbow flexors (1.9mm [1.7 – 2.8] vs. 2.1mm [1.3 – 2.6], respectively) and vastus lateralis thickness (1.5mm [1.1 – 2.6] vs. 1.9mm [1.3 – 2.3], respectively).

One repetition maximum (1RM)

Paragraph number 15 The time course of % changes in 1RM is presented in figure 3. Both the placebo group and the antioxidant group showed a significant (p=0.001) increase after 12 weeks of resistance exercise in 1RM measures of Leg extension (12.5 kg [10 – 17.5] vs. 12.5 kg [10 – 20], respectively), Leg press (50 kg [40 – 70] vs. 60 kg [40 – 80], respectively) and Scott curl (4 kg [2 – 6] vs. 4 kg [2 – 5], respectively); however, no group differences were identified.

DISCUSSION

Paragraph number 16 The purpose of the present study was to investigate the effects of antioxidants supplementation on the adaptations to a period of resistance exercise in elderly men. Supplementation with high dosages of vitamin C and E did not accelerate adaptations to resistance exercise. On the contrary, the placebo group had a significantly larger increase in total lean mass, lean mass of the legs and muscle thickness in rectus femoris, compared to the antioxidant group. Furthermore, only the placebo group showed a significant increase in lean mass of the legs and trunk, although the group differences did not reach statistical significance in lean mass of the trunk (p=0.08). Changes in lean mass of the arms, muscle thickness in elbow flexors and vastus lateralis, as well as all 1RM measures, did not differ between groups.

Paragraph number 17 To the authors knowledge, only one study has reported the effect of antioxidants combined with high-intensity resistance exercise on changes of fat-free mass

(FFM) in older individuals. With a study design similar to the present, Labontè et al. (21) and

Bobeuf et al. (5, 6) reported that 6 months of high intensity resistance exercise (3 * 8

repetitions at 80% of one-repetition maximum, 3 times a week) in healthy elderly individuals

(60-75 years) had no significant effect on fat-free mass (0.5 ± 1.4kg) while high intensity

resistance exercise combined with antioxidant supplementation (600 mg vitamin E and 1000

mg vitamin C per day) provided a significant increase in fat-free mass (1.3 ± 1.4kg; p<0.01).

However, significant treatment was observed for both groups when assessing appendicular

FFM and estimating muscle mass by urinary excretion of creatinine (p<0.01), with no

difference between the groups (5). Moreover, both groups showed a similar antioxidant/pro-

oxidant profile in the circulation before and after the intervention and a similar significant

increase in maximal strength (all p<0.001). Several considerations should be mentioned when

comparing the results in the present study with the study conducted by Bobeuf and co-workers

(5, 6, 21). The present study included a small sample size, but had sufficient statistical power

according to the calculations of sample size. Whereas, Bobeuf and co-workers (5) aimed to

complete the study with a minimum of 16 participants per group based on their sample size

calculations. However, the number of participants completing the study per group did not meet

these calculations, as three of the four groups had lower number participants. Furthermore, the

present study used an undulating periodized resistance exercise protocol, included mainly free-

weight exercises and had a relatively high training volume (set * rep * number of exercises),

with the intention to give large metabolic stress (i.e., oxidative stress) and stimulate as much

muscle growth as possible. Whereas Bobeuf and co-workers used nonperiodized resistance

exercise including eight exercises targeting large and small muscle groups. This may explain

the larger increase in lean mass compared to the study by Bobeuf and co-workers (2.2 kg and

0.7 kg [present study] vs. 0.5 kg and 1.3 kg [Bobeuf and co-workers], in the placebo group and

antioxidant group), especially when adjusting for a shorter duration (12 weeks in the present

study vs. 6 months in Bobeuf and co-workers study). These results are in line with previous research investigating periodization of resistance exercise (19, 35) and resistance exercise volume in elderly (31).

Paragraph number 18 Since moderate and excessive shifts in redox status resulting from a chronically higher oxidative stress seems to occur with aging (16), antioxidant supplementation has been suggested to facilitate adaptations to resistance exercise in elderly individuals (6). Thus, we hypothesized that the vitamin supplementation would favorably affect the redox status in our elderly participants and accelerate muscle growth during 12 weeks of resistance exercise. However, the results of the present study did not show accelerated adaptations to resistance exercise, but seemed on the contrary to attenuate resistance exercise induced increases in muscle growth. It is possible that the dosage of vitamin C and E used in the present study (235 mg vitamin E and 1000 mg vitamin C) was not optimal to favorably affect the redox status and subsequently accelerate the adaptation to resistance exercise for the studied population. In fact**,** investigations have reported that high dosage of vitamin C and E administered as a dietary supplement to adult humans exhibits a pro-oxidant, as well as an antioxidant, effect in vivo (28, 32). Furthermore, the redox status in our physically healthy elderly could have been favorable for adaptations to resistance exercise when entering the study, or a sufficient compensatory increase in antioxidant defense occurred in response to the exercise-induced oxidative stress. In support of the latter, Parise et al. (26) showed that antioxidant enzyme activity was up-regulated after resistance exercise in older adults (71 ± 7 years).

Paragraph number 19 Unfortunately, we did not measure markers of muscular oxidative stress, redox status or underlying cellular mechanism. However, previous research indicates

that oxidative stress induces a shift in the redox state, which is important for initiation of redox sensitive signaling pathways, subsequently regulating protein synthesis and the enzymatic antioxidant defense (33). Ito et al. (15) demonstrated that the highly reactive oxidant, peroxynitrite, which is formed by superoxide with nitric oxide, regulates skeletal muscle hypertrophy induced by overload. Furtheremore, in a review of "Strategies for reducing oxidative damage in ageing skeletal muscle" Jackson et al (17) suggested that use of high dose antioxidant supplementation to modulate ageing processes would suppress physiologically important ROS-mediated actions in addition to their putative effects on the ROS generation that causes age-related oxidative damage. In support of this, Makanae et al. (22) demonstrated that oral administration of vitamin C combined with chronic mechanical overload of the plantaris muscle attenuated hypertrophy in rats, which appeared to be related to the modulation of intracellular signaling pathways of muscle protein metabolism.

Paragraph number 20 Assuming that the attenuation of total lean mass and muscle thickness of rectus femoris is due to the antioxidant effect of vitamin C and E, the lack of significant differences between groups in lean mass of the trunk and arms, as well as muscle thickness of vastus lateralis and elbow flexors, may suggest a minor role for reactive oxygen species (ROS) in skeletal muscle growth. Furthermore, a relatively short duration of the intervention and lack of sensitivity in measurements to detect the small differences between groups could lead to a type II error. Indeed, these specific measures of lean mass and muscle thickness in which no group differences were detected showed the lowest absolute change from pre to post intervention. Moreover, this region specific change of muscle thickness the quadriceps muscle, where the greater significant change was in the rectus femoris muscle, is in line with previous training studies (24, 37).

Paragraph number 21 It is noteworthy that no group-differences were detected in 1RM measures. Indeed, because of the direct proportionality between muscle size and force (10), one would expect that the differences between groups that were detected in muscle growth also would be reflected in the 1RM measurements. However, the summation of the increase in the 1RM measures (18 %) was considerably higher compared with the increase in muscle thickness (9 %) or in total lean mass (2.5 %) during the 12 weeks with resistance exercise. This suggests that neural factors accounted for a major portion of the observed strength gain, which is in line with previous research (24, 37). Moreover, we found no association between the variation of increases in strength and lean mass or muscle thickness (data not shown). Hence, it could be speculated that the failure to detect differences between groups in 1RM was caused by a relatively large adaptation in neural factors.

Paragraph number 22 The *main strengths* of the present study were the strong research design, high attendance in resistance exercise sessions and close control of adherence for both resistance exercise and antioxidant supplementation. Nevertheless, the present study had a relative short duration of the resistance exercise intervention and a small sample size, which in turn could lead to type II errors. However, we had sufficient statistical power according to the calculations of sample size. Lastly, we did not measure underlying cellular mechanisms (i.e. translation and transcription factors) or markers of oxidative stress and redox state.

Paragraph number 23 *In conclusion*, our results demonstrated that supplementation with vitamin C and E did not have any positive effects on the adaptation to resistance exercise, but seemed rather to attenuate muscle growth. However, a 12 weeks resistance exercise intervention using an undulating periodized model was an efficient method for increasing lean mass, muscle thickness and strength in elderly men. Thus, our study supports the importance of

resistance exercise as a prevention strategy for the progression of sarcopenia. There is still no conclusive evidence on how exercise-induced oxidative stress and antioxidant supplementation affect adaptations to resistance exercise in the elderly. Future research is suggested to use interventions with longer duration and include sufficient measurements of cellular mechanisms to examine the long-term effects of the combination between resistance exercise and antioxidants.

ACKNOWLEDGEMENTS

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TABLE AND FIGURE LEGENDS

Table 1. Descriptive characteristics of the participating subjects.

The values are presented as mean ± standard deviation (SD).

Figure 1

Percent changes in lean mass after 12 weeks of resistance exercise in the placebo-controlled group (□) and the antioxidant group (▲), measured as total lean mass (A) and in three locations; lean mass in both arms (B), lean mass in both legs (C) and lean mass in trunk (D). Data are median; error bars are inter-quartile range. *: Significant difference between groups ($p < 0.05$). #: significantly different from baseline ($p < 0.05$).

Figure 2

Percent changes in muscle thickness after four weeks, eight weeks and post exercise in the placebo-controlled group (□) and the antioxidant group (▲), measured in three locations; Rectus femoris (A), Vastus lateralis (B) and Armflexors (C). Data are median; error bars are 95 % confidence intervals. *: Significant difference between groups ($p < 0.05$). #: Significantly different from baseline ($p < 0.01$).

Figure 3

Percent changes in 1RM after four weeks, eight weeks and post exercise in the placebo-controlled group (□) and the antioxidant group (▲), measured in three resistance exercises; Scott curl (A), Leg extension (B) and Leg press (C). Data are median; error bars are 95 % confidence intervals. #: Significantly different from baseline ($p < 0.001$).

Table 1

| Variables	Control group	Antioxidant group
Age (years)	67 (5)	69 (7)
Height (cm)	176 (8)	177 (5)
Weight (kg)	84 (15)	82 (13)
Fat mass %	27.5	26.5
Total lean mass (kg)	5.8 (7.9)	57.3 (6.6)
Lean mass in legs (kg)	18.9 (2.6)	18.8 (2.6)
Lean mass in trunk (kg)	27.7 (4.2)	27.6 (3.3)
Lean mass in arms (kg)	7.0 (1.1)	6.8 (1.0)
Muscle thickness rectus femoris (mm)	17 (3)	17 (4)
Muscle thickness vastus lateralis (mm)	25 (3)	22 (4)
Muscle thickness armflexors (mm)	34 (4)	33 (4)
1RM in leg extension (kg)	85 (14)	83 (19)
1RM in scott curl (kg)	21 (4)	20 (4)
1RM in leg press (kg)	316 (64)	315 (74)
Protein consumption (g/day)	86 (20)	102 (30)
Energy intake (KJ/day)	8.6 (1.9)	10.1 (3.0)
Moderate-vigorous physical activity (h/day)	5.2 (1.7)	5.3 (1.7)

Figure 1
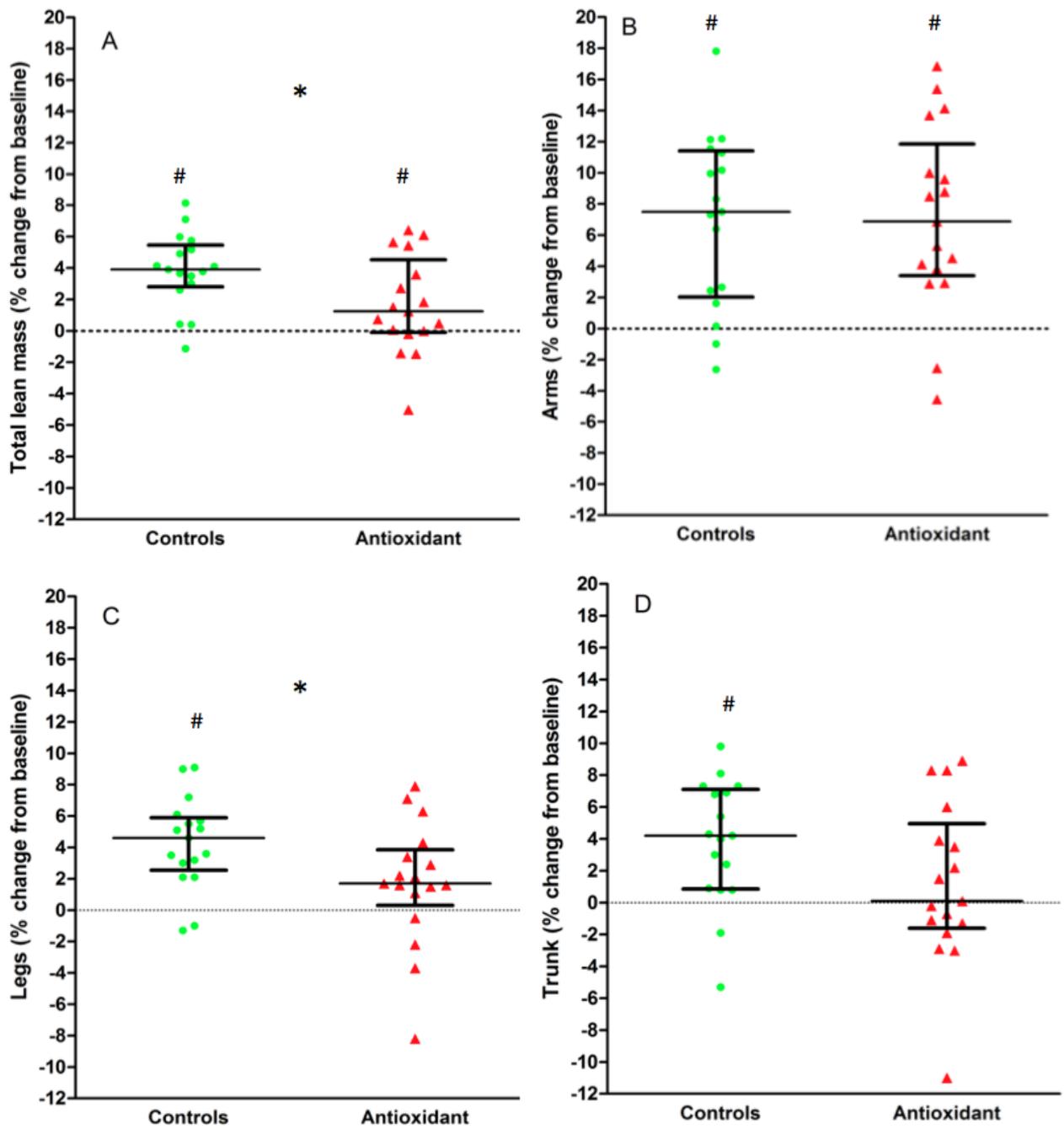
Figure 2
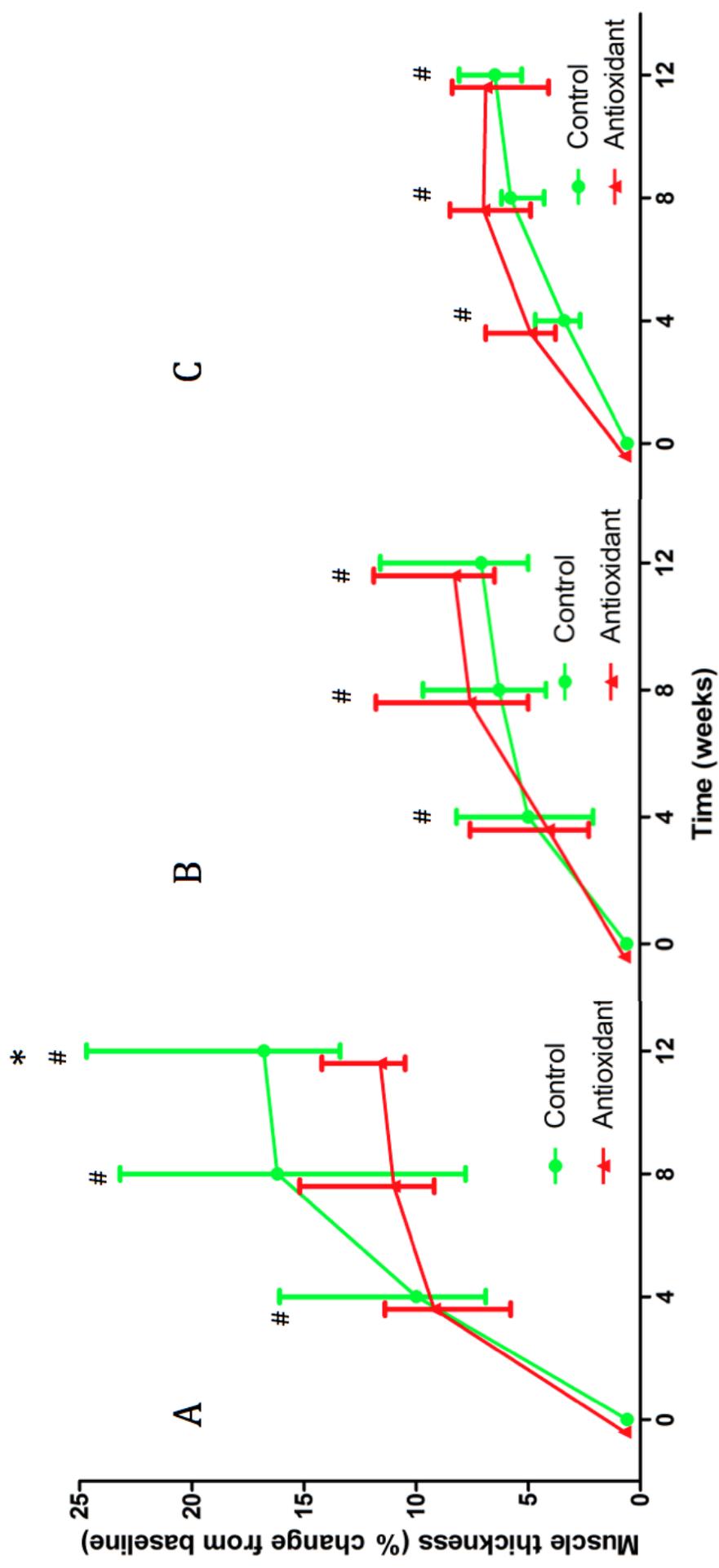
Figure 3
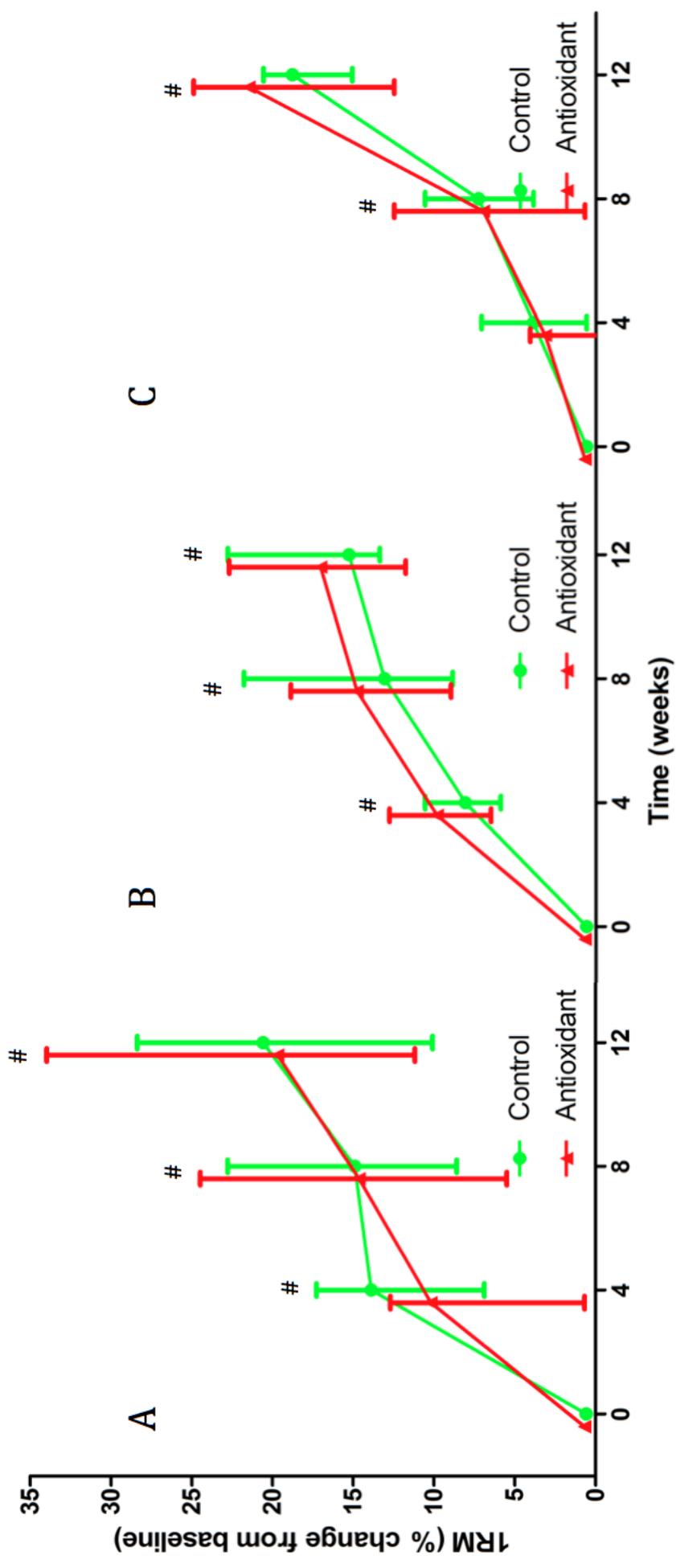
PART 3:

APPENDIX

CONTENTS:

Thomas Bjørnsen

University of Agder

May, 2013

APPENDIX 1

UNIVERSITETET I AGDER

Invitasjon til trenings- og forskningsprosjekt ved Universitetet i Agder:

MANN 60 - 80 ÅR SOM VIL BLI STERK?

Vil du være med, øk muskelmassen din og bli sterk, samtidig som du har det moro i et treningsmiljø under veiledning av fagfolk med høy kompetanse! (alt er gratis)

Hvis svaret er JA, og du oppfyller kriteriene nedenfor, er du aktuell deltaker for et trenings- og forskningsprosjekt ved Universitetet i Agder, Fakultet for helse- og idrettsfag.

Kriterier

- Vi søker menn mellom 60 – 80 år
- Du må ikke ha trent regelmessig styrketrening de siste 6 måneder før studien starter (oppstart medio august)

Trening/testing:

Treningen foregår tre ganger i uken over 12 uker i eget lokale på Spicheren Treningssenter og muskelstyrke testes i forkant og etterkant av denne tidsperioden. Styrketrening blir av veldig mange eksperter ansett som den viktigste treningen for eldre. Samtidig har forskning vist at inntak av antioksidanter har en positiv helseeffekt ved trening. Derfor ønsker vi også å se på en eventuell effekt av antioksidanttilskudd på styrketreningen.

Du inviteres til informasjonsmøte:

Onsdag 08. august 2012 klokken 18:00 i Auditorium B1 007 på Universitetet i Agder (skilt viser veien fra hovedinngangen).

Er det noe du lurer på?

Spørsmål rettes til universitetslektor Ken Joar Hetlelid, tlf. 38 14 21 34 eller epost ken.j.hetlelid@uia.no, eller mastergradsstudenter: Thomas Bjørnsen, mob. 986 19 299, Svein Salvesen, mob. 476 42 399.

APPENDIX 2

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK sør-øst	Tor Even Svanes	22845521	12.06.2012	2010/1352

Deres dato: Deres referanse:

22.05.2012

Vår referanse må oppgis ved alle henvendelser

Gøran Paulsen☐

Norges Idrettshøyskole

Postboks 4014

Ullevål Stadion 0806 Oslo

2010/1352 Hvordan påvirker antioksidanttilskudd treningseffekt

Forskningsansvarlig: Norges Idrettshøyskole

Prosjektleder: Gøran Paulsen☐

Vi viser til søknad om prosjektendring datert 22.05.2012 for ovennevnte forskningsprosjekt. Søknaden er behandlet av leder for REK sør-øst på fullmakt, med hjemmel i helseforskningsloven § 11.

Endringen består i at man ønsker å legge til to studiegrupper i prosjektet. Den første gruppen vil være på ca. 60 deltakere i alderen 60-80 år, som vil inngå i styrketreningsarmen av studien. Den andre gruppen vil være på ca. 40 utrente deltakere, som vil inngå i utholdenhetstreningsarmen av studien. Det vil ikke tas muskelbiopsi fra disse kohortene. Prosjektendringen består videre av en utvidelse av prosjektperioden, frem til 2013. Sveinung Berntsen ved Universitetet i Agder går inn i studien som prosjektmedarbeider.

Formålet med endringen er å se på effekten av antioksidanttilskudd i mer differensierte grupper enn det som allerede er inkludert i studien.

Komiteen har ingen forskningsetiske innvendinger til selve designet i den utvidede delen av studien, og registrerer at det blant annet anføres rutiner for klinisk screening av deltakerne før inklusjon som en del av endringsprotokollen. Komiteen legger derfor til grunn at det finnes klare og gode beredskapsrutiner i forsøkene, da det nå skal inkluderes både eldre og mindre

trente personer til prosjektet.

Vedtak

Prosjektendringen godkjennes.

Tillatelsen er gitt under forutsetning av at prosjektendringen gjennomføres slik det er beskrevet i prosjektendringsmeldingen og endringsprotokoll, og de bestemmelser som følger av helseforskningsloven med forskrifter.

Tillatelsen gjelder til 31.12.2013. Opplysningene skal deretter slettes eller anonymiseres, senest innen et halvt år fra denne dato. Prosjektet skal sende sluttmelding på eget skjema, jf. helseforskningsloven § 12, senest et halvt år etter prosjektslutt.

Klageadgang

Du kan klage på komiteens vedtak, jf. forvaltningslovens § 28 flg. Klagen sendes til REK sør-øst. Klagefristen er tre uker fra du mottar dette brevet. Dersom vedtaket opprettholdes av REK sør-øst, sendes klagen videre til Den nasjonale forskningsetiske komité for medisin og helsefag for endelig vurdering.

Med vennlig hilsen

Arvid Heiberg☐prof. dr.med☐leder REK sør-øst C

Kopi til: *Norges Idrettshøyskole v/Tom Atle Bakke: tom.atle.bakke@nih.no*

Tor Even Svanes seniorrådgiver

APPENDIX 3

Forespørsel om deltakelse i forskningsprosjektet:
Hvordan påvirker antioksidanttilskudd effekt av styrketrening på eldre?

Bakgrunn og hensikt

Antioksidanter, både de produsert av cellene selv og de vi får via maten vi spiser (f.eks. vitamin C og E), er viktige for at kroppens celler skal fungere og være motstandsdyktige mot ulike former for stress. Ved aldring kan det oppstå en tilstand av vedvarende stress i cellene. Dette kan motvirkes med både trening og kanskje ved økt inntak av antioksidanter. Denne studien har til hensikt å undersøke kombinasjonen av styrketrening og antioksidanttilskudd, samt effekten av styrketrening og Smartfish-drikk. Smartfish har lagd et produkt som inneholder spesielt ferske, marine fettsyrer, samt naturlige antioksidanter fra frukt-juice (se http://www.smartfish.no/).

Er du en frisk mann mellom 60 og 80 år og bedriver ikke regelmessig styrketrening, kan du delta som forsøksperson i denne studien.

Denne studien er et samarbeidsprosjekt mellom Norges idrettshøgskole (Oslo), Universitet i Agder (Kristiansand) og Sørlandet sykehus (SSHF).

Hva innebærer prosjektet?

Dette er et dobbelt blindet, randomisert, kontrollert studie, som betyr at verken du eller forskerne du kommer i kontakt med vet om du inntar vitamin C og E, Smartfish eller placebo ("lure-drikk/-piller"). Du vil innta drikken og piller før og etter trening, samt morgen og kveld de dagene du ikke trener.

I de første ukene av prosjektet vil vi måle din fysiske styrke, muskeltykkelse i lår og armer (v.h.a. et ultralydapparat) og total muskelmasse (v.h.a. en DXA-maskin som sender ut to

røntgenstråler). Det vil tas en blodprøve og kondisjonen din testes på en tredemølle. Du vil også gjennomgå en legesjekk, som inkluderer undersøkelser av hjertet. Kostholdsregistrering og aktivitetsregistrering vil også bli gjennomført. Tester og undersøkelser gjøres ved Spicheren (ved Universitet i Agder) og Sørlandet Sykehus.

Først etter at alle tester og undersøkelser er gjennomført, vil du bli tilfeldig valgt til en av de tre gruppene, innlæringsøkter gjennomføres, og du vil begynne på treningsprogrammet. Du skal trene 3 økter per uke. Treningsperioden varer i 12 uker (40 økter). Styrketreningen består av tradisjonelle øvelser for hele kroppen. Under hver trening skriver du ned hva du gjør (hvor tunge vekter du benytter etc.). Du vil få god veiledning på starten av treningsprogrammet, slik at du lærer deg alle øvelsene godt, og du vil få tett oppfølging underveis i treningsperioden. Det vil være faste tider du kommer og trener sammen med andre deltakere.

Underveis i treningsperioden vil vi gjenta noen av styrketestene og måle muskeltykkelsen din. Etter treningsperioden vil alle testene og undersøkelser gjentas.

Mulige fordeler og ulemper ved å delta i prosjektet
Fordeler:

- Du får en gratis legesjekk.
- Du får bl.a. målt og testet styrken og muskelmassen din.
- Du vil følge et treningsprogram som er laget for at du skal bli sterkere, komme i bedre form og få bedre helse. Du vil få kyndig oppfølging under hele treningsperioden.

Ulemper:

- Du vil bruke tid på tester, undersøkelser og trening.
- Vekttrening medfører risiko for skader. Risikoen for skader anses som lav.
- Noen tester og undersøkelser kan oppleves som anstrengende og ubehagelige.
- Målingen av muskelmassen gir en liten stråledose (tilsvarende en interkontinental flyreise).
- Blodprøver fra en vene i albueområdet kan oppleves som ubehagelig, og det medfører en risiko for infeksjoner. Risikoen for infeksjoner anses som svært lav.

Hva skjer med prøvene og informasjonen om deg?

Alle i prosjektet oppgir sitt navn og telefonnummer. Deretter får alle et unikt nummer som skrives opp ved navnet på navnelisten. Denne listen oppbevares nedlåst i arkivskap godkjent for oppbevaring av personopplysninger. Resultatene fra tester og undersøkelser påføres kun nummeret ditt. Når prosjektet er ferdig slettes navnelisten. Dataene vil da være helt anonyme.

Statens legemiddelverk og kontrollmyndigheter i inn- og utland kan få utlevert studieopplysninger og gis innsyn i relevante deler av din journal. Formålet er å kontrollere at studieopplysningene stemmer overens med tilsvarende opplysninger i din journal. Alle som får innsyn i informasjon om deg har taushetsplikt.

Frivillig deltakelse

Det er frivillig å delta i prosjektet. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta, uten at dette vil få noen negative konsekvenser for deg. Dersom du ønsker å delta, undertegner du samtykkeerklæringen på siste side.

Etikk

Prosjektet har vært fremlagt for Regional komité for medisinsk og helsefaglig forskningsetikk, helseregion sør-øst, som har godkjent prosjektet. Studien er også godkjent av legemiddelverket.

Kontaktinformasjon

Dersom du senere ønsker å trekke deg eller har spørsmål om prosjektet kan du kontakte personene på listen under. Dersom du mener du er påført en skade av deltakelse i prosjektet kan du også henvende deg til personene under som da vil sørge for at du får hjelp/behandling for dette:

| Thomas Bjørnsen | thomas.bjornsen@hotmail.com | 98 61 92 99 |
| Svein Salvesen | svein.salvesen47@gmail.com | 47 64 23 99 |

Ytterligere informasjon om studien finnes i kapittel *A* – *utdypende forklaring av hva studien innebærer.*

Ytterligere informasjon om biobank, personvern og forsikring finnes i kapittel B – *Personvern, biobank, økonomi og forsikring.*

Samtykkeerklæring for underskrift følger etter kapittel B.

Kapittel A- utdypende forklaring av hva studien innebærer

Kriterier for deltakelse
- Alder: 60-80 år
- Kjønn: Mann
- Har ikke drevet systematisk styrketrening de siste 6 mnd. (trening med vekter).
- Ingen kjente sykdommer eller medisinbruk som kan påvirke treningseffekten (ta kontakt om du er usikker på hva dette innebærer).
- Inntar ingen former for kosttilskudd ved prosjektstart.

Tidsskjema – hva skjer og når skjer det?
- Tester av muskelmasse og styrke gjennomføres samt utfylling av spørreskjema gjennomføres første gang i august-september 2012, og andre og siste gang desember 2012.
- Treningsperioden er fra august-september til november-desember 2012.

Mulige fordeler
- Se ovenfor

Mulige bivirkninger
- Se ovenfor

Mulige ubehag/ulemper
- Se ovenfor

Pasientens/studiedeltakerens ansvar
- Å komme til avtalt tid for testing og undersøkelser, samt følge treningsopplegget og innta supplementene.

Kapittel B - Personvern, biobank, økonomi og forsikring

Personvern
Opplysninger som registreres om deg er
- Alder
- Telefonnummer
- Kjønn
- Høyde
- Vekt
- Muskelmasse og -styrke
- Treningshyppighet og varighet (treningsdagbok)

Norges idrettshøgskole ved administrerende direktør er databehandlingsansvarlig.

Biobank
Blodprøvene som blir tatt og informasjonen utledet av dette materialet vil bli lagret i en forskningsbiobank ved Universitetet i Agder. Hvis du sier ja til å delta, gir du også samtykke til at det biologiske materialet og analyseresultater inngår i biobanken. Professor Truls Raastad ved Norges idrettshøgskole er ansvarshavende for forskningsbiobanken. Biobanken planlegges å vare til 2023. Etter dette vil materiale og opplysninger bli destruert og slettet etter interne retningslinjer.

Utlevering av materiale og opplysninger til andre
Nei.

Rett til innsyn og sletting av opplysninger om deg og sletting av prøver
Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har videre rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser, er brukt i vitenskapelige publikasjoner eller om informasjonen er anonymisert (etter at navnelisten er slettet)

Økonomi
Studien er finansiert av midler fra Norges idrettshøgskole, Universitetet i Agder, Smartfish, SSHF og regionalt forskningsfond i Agder.

Forsikring
Norges idrettshøgskole er en statlig institusjon og er således selvassurandør.

Informasjon om utfallet av studien
Forsøkspersoner får utlevert egne resultater og det vil avholdes et informasjonsmøte for forsøkspersonene i etterkant av undersøkelsen. Resultatene fra alle forsøkspersonene vil bli publisert i internasjonale, fagfellevurderte tidsskrift. Resultatene publiseres som gjennomsnitt for flere personer, og det vil ikke være mulig å identifisere enkeltdeltakere gjennom de publiserte resultatene.

Samtykke til deltakelse i studien

Jeg er villig til å delta i studien

(Signert av prosjektdeltaker, dato)

Jeg bekrefter å ha gitt informasjon om studien

(Signert, rolle i studien, dato)

APPENDIX 4

REGISTRERINGSSKJEMA FOR AKTUELLE DELTAKERE
Navn:
Alder:
Telefon:
Mobil:
Adresse:
Personnummer:
E-post:

Har du mulighet for å trene på dagtid (ja/nei)?

Skal du reise bort i løpet av perioden
midten av august til midten av desember (ja/nei)?

Hvor mange dager sammenhengende?

Treningsbakgrunn de siste 6 måneder?

Bruk av medisiner (ja/nei)?
Navn:
For hva:

Røyker daglig?

Kroniske plager som kan begrense deltakelse,
eller ha innvirkning på treningen?

Andre hensyn vedrørende helsestatus?

Vær ærlig for deg selv og ovenfor prosjektet!

APPENDIX 5

Treningsdagbok Uke 1 - økt 1

Dato: _____

Klokkeslett: _____

Økt: _____   8-10 reps

Pausevarighet: _____   1 minutt

Øvelser	Mål	Oppvarming		Sett 1		Sett 2		Sett 3		Sett 4		Omni
	Kg	kg	reps	kg	reps	kg	reps	kg	reps	kg	reps	
Bulgarsk knebøy												
Beinpress 2bens												
Benkpress												
Sittende roing												
Opptrekk												
Stående tåhev												
Franskpress												
Scott curl												
Ryggstrekk												

Kommentarer