Alternative Strategies To Improve The Beneficial Effects Of Exercise Throughout Life

Dietary And Physiological Aspects

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‘It’s not that I’m so smart,

it’s just that I stay with problems longer.’

Albert Einstein

Alternative Strategies To Improve The Beneficial Effects Of Exercise Throughout Life: Dietary And Physiological Aspects

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Table of contents

General introduction

Societal challenges of aging populations

Aging is defined as a series of morphological and functional changes that take place over time [1]. The term also refers to the deterioration of biological functions after an organism has attained its maximum reproductive potential [2, 3].

Nowadays, extended lifetime and life expectancy can be seen as a success story for public health policies and socioeconomic development. However, it also challenges societies to adapt in order to maximize health and functional capacity of older people as well as ensure their social participation and security [3].

Life expectancy is defined as the average total number of years that a human is expected to live. Differently, life span is the maximum number of years that a human can live. While the human life span has remained unchanged for the past 100,000 years at ~125 years, life expectancy has sensibly increased (~27 years during the last century), especially in Western countries [4]. The substantial improvement of life expectancy and extension of the length of time being in optimal health (health span) [5] occurred mainly due to the elimination of most infectious childhood diseases, better hygiene, and the implementation of antibiotics and vaccines [6].

Because aging is positively related to the homeostatic imbalance and incidence of pathology, death remains the ultimate consequence of aging [7]. Therefore, the prevention of pathology is the key to increased health span during the aging process [6].

The major theories of aging such as the free radical theory [8], the immunologic theory [9], the inflammation theory [10] and the mitochondrial theory [11] are all specific of a particular cause of aging. These theories provide useful and important insights for the understanding of physiological changes occurring with aging. They simultaneously define key target physiological deficiencies to be addressed by preventive and therapeutic interventions to improve the aging process. In particular, preventive and therapeutic interventions for ameliorating the aging process are focused on lowering the risk for pandemic pathologies and deficiencies such as the metabolic syndrome (MetS), type 2 diabetes mellitus, sarcopenia and cardiovascular dysfunctions [12-14].

Metabolic malfunction accelerates the aging process

According to the World Health Organization (WHO) MetS and its components are a major public health challenge of the twenty-first century [15, 16]. It is estimated that around 20-25% of the population in the world fulfill the criteria for MetS. These people are three times as likely to have a heart attack or stroke compared to people without the syndrome. Additionally, the risk for developing type 2 diabetes is fivefold greater [15]. MetS is characterized by a group of metabolic risk factors. The first agreement about the definition of MetS was drawn up during a meeting organized by the International Diabetes Federation (IDF) in 2005. As stated in their guidelines [17] the major contributing factor is central obesity measured by waist circumference and Body Mass Index (BMI). In addition to central obesity, other factors that have to be considered for the diagnosis of MetS involve the estimation of triglycerides, reduced high-density lipoprotein (HDL) cholesterol, blood pressure, or fasting plasma glucose [15]. The majority of patients are obese, older, sedentary, inactive and insulin-resistant. Determining factors are body weight, genetics, aging, lifestyle and excess caloric intake [16].

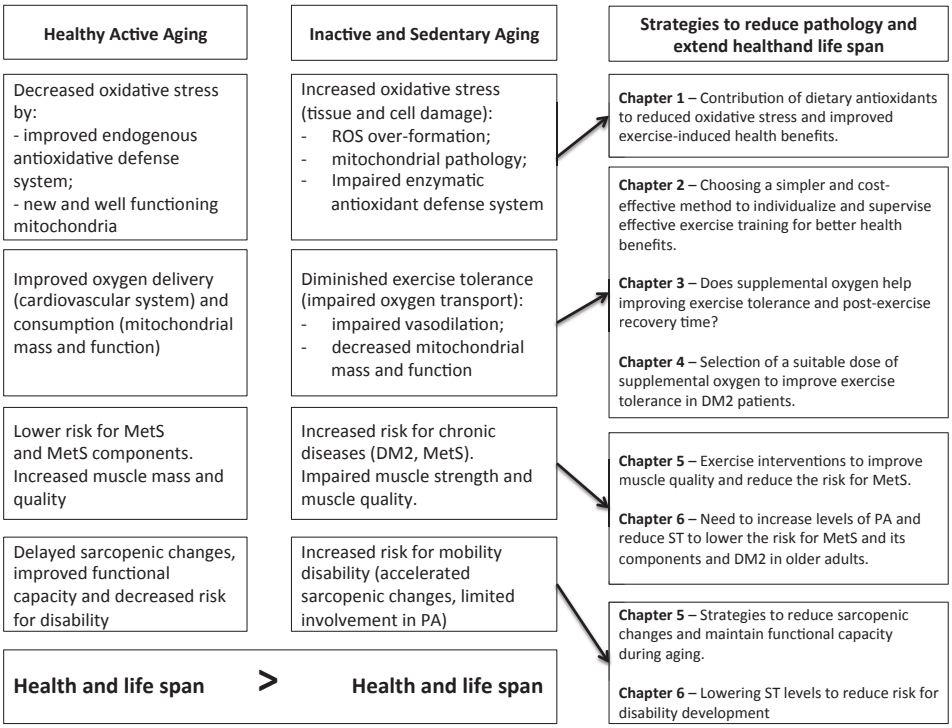
Metabolic syndrome was defined in accordance with the harmonized criteria recommended in the 2009 Joint Interim Statement from multiple scientific associations [17], as the presence of 3 or more components, including (1) abdominal obesity (men: waist circumference ≥ 102cm or women: waist circumference ≥ 88cm); (2) hypertension (systolic blood pressure (SBP) ≥ 130mmHg and/or diastolic blood pressure (DBP) ≥ 85mmHg) or use of antihypertensive medication and a history of physician-diagnosed hypertension; (3) decreased HDL-C cholesterol level (men: HDL-C <40 mg/dL (<2.2 mmol/L) and women: <50 mg/dL (<2.8 mmol/L)) or use of HDL-C-raising medication; (4) elevated triglycerides levels (triglycerides ≥ 150 mg/dL (≥ 8.4 mmol/L)) or use of triglyceride-lowering medication and (5) elevated plasma fasting blood glucose level (glucose ≥ 100 mg/dL (≥ 5.6 mmol/L)) or use of glucose-controlling medication.

Besides the increased risk for type 2 diabetes, presence of MetS leads to an increased risk of cardiovascular dysfunctions, in the form of coronary or peripheral atherosclerosis and heart failure [15].

The aging process is accelerated when metabolic and cardiovascular diseases are present, subsequently increasing the risk for developing a functional decline/disability at an earlier stage in life [13]. Many predisposing age-related conditions, such as obesity, insulin resistance, inflammation and sarcopenia [15] contribute to the increased prevalence of metabolic syndrome and subsequently result in an increased risk for disability [13], a higher

morbidity rate, increased health care costs [12, 18] and a shortened lifespan compared with the general population [2]. Given the worldwide growing pandemic of MetS [15], there is an urgent need to develop more effective strategies that prevent or reverse aforementioned premature aging process [13].

Figure INTRO.1 Alternative strategies to improve beneficial effects of exercise training during impaired aging process.



MetS- metabolic syndrome; ROS- reactive oxygen species; DM2- type 2 diabetes; PA-physical activity; ST- sedentary time.

Oxidative stress, sarcopenia and functional capacity decline increase risk for pathology during aging

In agreement with the free radical theory of aging, reactive oxygen species (ROS), generated as by products of biological oxidations, induce casual and cumulative oxidative damage to macromolecules resulting in cellular dysfunction with age and eventually cell death [11]. Mitochondria, besides being the 'body's power station' for human's functioning, are involved

in the aging process [19]. In particular, these organelles are considered the main intracellular source of reactive oxygen species (ROS) production e.g. superoxide anion (O_2^-), as well as the major target of free radical attack [20]. ROS produced by the mitochondrial respiratory chain damage mitochondrial constituents, including proteins, lipids, and mitochondrial DNA (mtDNA) [21]. Progressive accumulation of oxidant-induced somatic mutations in mtDNA during an individual's lifetime leads to a deterioration in the bioenergetic function of mitochondria [22]. Although this process takes place in almost every organ system, research shows that the level of the oxidative stress also plays a role in the sarcopenic changes and, as such, accelerates functional decline during the aging process [23]. Interestingly, a number of studies showed added value of dietary antioxidant supplements in supporting health benefits by preventing deleterious oxidative stress [24-26]. However, the optimal doses, type and timing of these supplements in relation with the exercise stimulus are equivocal and inadequate antioxidant interventions may even be counter-productive [27]. Therefore, strategies for extending life span, functional capacity and quality of life should be focused on reducing the risk for developing MetS and its components, preventing increased oxidative stress levels and reducing a decline in mass and function of both skeletal muscle and its mitochondria.

Strategies to reduce risk for pathology and disability while aging

Several modifiable factors such as involvement in physical activity, reducing levels of sedentary behavior [28] and adequate nutritional strategies [29, 30] have been shown to contribute extending health span and are likely to support successful aging [13].

In particular, there is a vast body of evidence showing that the involvement in physical activity and reduced levels of sedentary behavior are crucial for preventing the development of chronic diseases e.g. MetS and its components [31, 32], sarcopenia [33], type 2 diabetes [34, 35] and cardiovascular dysfunction [36, 37].

In particular, endurance and resistance type of training interventions are highly recommended by health professionals for its health benefits, including improved aerobic capacity ($\dot{V}O_{2\ peak}$), muscle quality, muscle strength [14] and overall physical condition [28]. These effects are induced by adaptive mechanisms in response to regular training on tissue and cellular levels. These adaptive mechanisms include e.g. improved metabolic and mechanical function of skeletal muscle [33], cardiovascular and respiratory function, insulin sensitivity, glucose uptake [38, 39], improved efficiency of endogenous enzymatic antioxidant defense system

[39], and increased mitochondrial biogenesis [20, 34].

For practical, logistic as well as physiological reasons, the implementation of exercise and lifestyle intervention programs aimed at preventing aforementioned pathologies remains suboptimal. In particular, the lack of individualized and supervised exercise programs [40], low exercise tolerance/performance, low participation levels in physical activity [18] caused by advanced pathologies of the pulmonary and peripheral cardiovascular systems [41, 42] are major obstacles for effective health-interventions. As such, there is a clear need to develop more (cost-) effective and health-beneficial lifestyle intervention programs for aging populations [13].

Outline of the thesis

Although it is well appreciated that cognitive, behavioral and societal factors may be equally important, the present dissertation will mainly focus on the physiological aspects of the exercise-based life style interventions aiming to improve its effects, and hence antagonize the aging process.

Improved longevity and quality of life are negatively related to the levels of pathology during the aging process. Accordingly, Figure INTRO.1 summarizes the most important life-style related pathologies during the aging process, which are more pronounced in a sedentary and unhealthy aging population **(chapter 6)** than in healthy individuals. In particular, sedentary behavior and inactivity increase the risk for chronic diseases (MetS and type 2 diabetes) and its components. Subsequently, inactivity and sedentary lifestyle increase the risk for disability and earlier death. We presented a number of potentially useful strategies to improve the effectiveness of exercise, and hence to more effectively reduce the pathologies of faster aging (Figure INTRO.1). In particular, this dissertation discusses dietary interventions and the impact of sedentary behavior/inactivity on the main pathologies during aging e.g. oxidative stress, sarcopenia, MetS and its components **(chapter 1, chapter 5, chapter 6)**. Additionally, more cost-effective approaches **(chapter 2)** and alternative methods **(chapter 3, chapter 4)** will improve the individual approach and involvement of sizable aging populations with poor exercise tolerance in physical activity. The results of this dissertation emphasize that more active and healthier aging individuals will live longer and higher quality life.

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PART 1

Do dietary antioxidant supplements help improve effects of exercise training?

Oxidative stress is one of the major theories of accelerated aging process [1]. Exercise training is one of the best ways to improve the endogenic antioxidative defense system and to prevent deleterious levels of oxidative stress [2]. However, it is still controversial whether adding supplementation with dietary antioxidants to exercise interventions may still safely improve these effects of exercise [3, 4], and hence eventually expand health span. Therefore, this part discusses effects of antioxidant supplementation in conjunction with endurance training as a potential alternative aid in healthy aging.

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CHAPTER 1

Dietary Antioxidants as Modifiers of Physiologic Adaptations to Exercise

Adjusted from:
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Abstract

Adaptive responses to exercise training are crucial in maintaining physiological homeostasis and health span. Exercise-induced aerobic bioenergetic reactions in mitochondria and cytosol increase production of reactive oxygen species (ROS), where excess of ROS can be scavenged by enzymatic as well as non-enzymatic antioxidants to protect against deleterious oxidative stress. Free radicals, however, have recently been recognized as crucial signaling agents that promote adaptive mechanisms to exercise training, such as mitochondrial biogenesis, antioxidant (AO) enzyme activity defense system upregulation, insulin sensitivity, and glucose uptake in skeletal muscle. Commonly used non-enzymatic AO supplements, such as vitamins C and E, α-lipoic acid, and polyphenols, in combination with exercise training, have been proposed as ways to prevent exercise-induced oxidative stress and hence improve adaptation responses to endurance training. Preclinical and clinical studies to date have shown inconsistent results indicating either positive or negative effects of endurance training combined with different blends of AO supplements (mostly vitamins C and E and α-lipoic acid) on redox status, mitochondrial biogenesis pathways, and insulin sensitivity. Preclinical reports on exercise training combined with resveratrol, however, have shown consistent positive effects on exercise performance, mitochondrial biogenesis, and insulin sensitivity, with clinical trials reporting mixed effects. Relevant clinical studies have been few and have used inconsistent results and methodology (types of compounds, combinations, and supplementation time). The future studies should investigate the effects of specific antioxidants and other popular supplements, such as α-lipoic acid and resveratrol, on training effects in humans. Of particular importance are older adults who may be at higher risk of age-related increased oxidative stress, an impaired AO enzyme defense system, and comorbidities such as hypertension, insulin resistance, and diabetes.

Key words: Exercise Training, Dietary Antioxidants, Mitochondrial Biogenesis, Redox Status, Glucose Metabolism.

Introduction

Exercise training is highly recommended by health professionals for its many health benefits, including improved aerobic capacity, muscle strength, peak oxygen uptake (VO_{2peak}), and overall physical condition [15]. These effects are induced by adaptive responses to regular training, including improved insulin sensitivity and glucose uptake [57], improved efficiency of enzymatic antioxidant (AO) defense system [2], and increased mitochondrial biogenesis [51]. Exercise-induced reactive oxygen species (ROS) are one of the signaling agents for inducing these biologic exercise-training adaptations [23]. In preclinical models, improved efficiency of the enzymatic AO defense system by regular exercise protects cells against oxidative damage and maintains physiological homeostasis [51].

Although exercise-induced ROS production is an important signaling pathway to induce biological adaptations to training, ROS over production could also have a deleterious impact on cells and tissues, i.e., lipid and protein peroxidation [70]. Therefore, some experts suggested consuming more dietary AOs and AO-containing supplements to mitigate the ROS production that can cause excess oxidative stress during and after exercise [9, 36, 47, 65]. The belief that dietary supplements are helpful, or at least safe, when used in conjunction with an exercise program, however, has recently been questioned. For example, a recent study of Ristow et al., found that supplementation with vitamin C (1000mg/day) and E (400IU/day) blunted some of the beneficial effects of exercise, such as improved insulin sensitivity, mitochondrial biogenesis level, and AO enzyme activity, in 19 untrained and 20 pre-trained healthy young individuals, and that exercise alone produced a better outcome. In contrast with the findings of Ristow et al., previous studies have shown no blunting effects of AO supplementation on changes in aerobic capacity (VO_{2peak}) [16, 74], mitochondrial biogenesis [74], and insulin sensitivity [74]. At present, it is unclear whether AO supplements enhance or attenuate exercise training adaptive biological responses in either healthy adults or lower-functioning older adults. The purpose of this chapter, therefore, is to review relevant studies that have examined the effects of exercise alone and combined with AO supplementation on basic training adaptations (redox status, mitochondrial biogenesis, and glucose metabolism) in both animals and humans.

Literature search

Searching PubMed for manuscripts reporting effects of AO supplements on biological adaptation mechanisms to exercise training, we used combinations of the following keywords: *exercise training*, *antioxidants*, *ROS signaling*, *vitamins*, *animal studies, clinical studies, resveratrol, redox status, superoxide dismutase (SOD)*, *glutathione peroxidase (GPx)*, *catalase (CAT)*, *oxidative stress*, *ROS*, *mitochondrial biogenesis*, *peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α)*, *metabolism*, *glucose*, *insulin*, and *insulin resistance*. The goal of this literature search was to find relevant publications that will improve our understanding of the beneficial, neutral, or adverse effects of AO supplements on biologic adaptation responses in combination with endurance exercise training. From these manuscripts, we selected studies that examined and compared exercise-training effects with and without administration of commonly used AO supplements (vitamins C and E, α-lipoic acid, coenzyme-Q10, carotene, resveratrol). Controversy around biological pathways of mitochondrial biogenesis, enzymatic AO activity, and glucose metabolism narrowed our search to only those studies that investigated an effect of exercise training combined with non-enzymatic AOs and training alone on adaptation response to exercise. Therefore, we included all available key markers of redox status (cytosolic superoxide dismutase (SOD1), mitochondrial superoxide dismutase (SOD2), GPx, and CAT), mitochondrial biogenesis (PGC-1α), and glucose metabolism (adenosine monophosphate (AMP)-activated protein kinase (AMPK), glucose transporter 4 (GLUT4), and insulin sensitivity). Using these criteria, we identified only a few preclinical and clinical studies. We therefore included all AO supplement compounds and combinations, endurance exercise training duration and types, and animal and human models. The key criterion for manuscript inclusion was a comparison between endurance- exercise training alone and exercise training combined with AO supplementation. According to these criteria 24 studies were included.

Normal adaptations of antioxidant enzymes, mitochondrial biogenesis, and broad metabolism to exercise training

Basic biological mechanisms during exercise

ROS, within physiological concentration, are important signaling molecules that regulate growth, proliferation, and differentiation, and are responsible for some key adaptations to

exercise training at the tissue and cellular levels, e.g. AO enzyme regulation [2, 31], mitochondrial biogenesis [51], and skeletal muscle hypertrophy [20].

During exercise, oxidative homeostasis is maintained by a network of AO defense mechanisms capable of producing other less-reactive species or neutralizing reactive oxygen metabolites, i.e., SOD, GPx, CAT, and thioredoxin reductase (TRX). SOD, for instance, promotes the dismutation of superoxide radicals ($O_2^{\bullet}$-) and forms hydrogen peroxide and oxygen. GPx utilizes glutathione (GSH) as a reducing equivalent for hydrogen peroxide (H_2O_2) to form oxidized GSH and water in mitochondria and cytosol. Additionally, CAT converts H_2O_2 to water and oxygen [47]. I*n vitro* studies showed that ROS (formation induced by the pro-oxidant herbicide paraquat) induce upregulation of the AO enzymes (SOD, GPx, and CAT) activity in myotubes [12]. This mechanism maintains the oxidant-AO homeostasis during a skeletal muscle contraction in animal models and humans [34, 50, 62].

Adaptation of enzymatic antioxidant mechanisms to exercise training

Because prolonged exercise results in an increased production of oxidants in skeletal muscle, and hence regular activation of enzymatic AO-using mechanisms, endurance exercise training induces adaptations resulting in upregulation of AO enzyme activity in skeletal muscle, i.e., SOD1, SOD2, GPx, and CAT [18, 24, 26, 27, 31, 33, 48]. Endurance exercise training increases total SOD activity in highly oxidative type I (the soleus) and IIa (red gastrocnemius) skeletal muscle fibers [48]. Longer and more intensive endurance training promotes a greater increase in both cytosolic and mitochondrial GPx activity in oxidative skeletal muscle (type I and IIa) fibers. Endurance training also upregulates CAT activity in peroxisomes and mitochondria in highly oxidative muscles [48].

Exercise-induced oxidative stress and the mitochondrial biogenesis mechanism

Endurance training does not result in parallel increases in both oxidant and AO enzyme activity [22]. The AO enzyme activity of SOD, GPx, and CAT generally increase and ROS concentrations decline during normal exercise training [48]. The mismatch seems to have an important beneficial role in exercise training adaptations, e.g., mitochondrial biogenesis (15]/mitohormesis [53]. ROS stimulate the mitochondrial biogenesis cascade in response to endurance exercise training, i.e., chronic muscle contractions [15]. The newly formed mitochondria are known to be highly efficient and produce fewer ROS for the same amount of produced adenosine triphosphate (ATP) [43]. Regular exercise training increases expression

of proteins involved in mitochondrial biogenesis, i.e., PGC-1α, nuclear respiratory factor 1 (NRF-1), and mitochondrial transcription factor A (Tfam) (Fig. 1). PGC-1α is an important transcriptional coactivator of nuclear genes encoding mitochondrial proteins, whilst Tfam regulates the expression of mitochondrial DNA [58]. For example, expression of PGC-1α in skeletal muscle was significantly increased following 4 weeks of endurance exercise training [53], indicating a skeletal muscle contraction-stimulated mechanism of mitochondrial biogenesis. Mitochondria are also one of the main sources of ROS, which are products of oxidative lipid and glucose metabolism during muscle contraction [53]. However, the mitochondria are not the only sources of ROS during muscle contraction. For example, it has recently been shown that muscle contraction increases superoxide activity in cytosol, with a delayed increase in mitochondria. Therefore, it has been proposed that nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidases are the potential sources for superoxide generation [46]. Accordingly, ROS production (level of H_2O_2) was previously shown to increase in isolated mitochondria after acute muscle contraction in comparison with rested skeletal muscle biopsy sample [69].

Skeletal muscle contractions at high intensity increase the AMP/ATP ratio and the Ca_{2+} flux, thus causing upregulation of the gene expression and post-translational modification of PGC-1α by the activation of AMPK, Ca2+/calmodulin-dependent kinase (CaMK), and calcineurin A. Co-activation of PGC-1α activates nuclear respiratory factors (NRF-1 and -2), which promote the expression of Tfam that directly stimulates mitochondrial DNA replication and transcription [28, 45]. Therefore, it consequently enhances mitochondrial biogenesis, which results in greater oxygen consumption [28].

Exercise-induced ROS production as a signaling pathway in insulin sensitivity

Endurance exercise training has been shown to improve insulin sensitivity and to enhance both insulin-stimulated and non-insulin mediated glucose uptake in skeletal muscle [74]. The exercise-induced enhancement in insulin-stimulated glucose disposal by skeletal muscle occurs as a result of increased protein expression of hexokinase 2 (HK2) and GLUT4 [74]. Muscle contraction-stimulated AMPK plays a central role in increased expression of GLUT4 and, hence, the regulation of glucose homeostasis in response to exercise. A muscle-contraction increase in AMPK activity has been correlated with GLUT4 translocation and also with non-insulin glucose transport in skeletal muscle [74]. Moreover, evidence indicates that exercise-induced reactive oxygen and nitrogen species (RONS) production plays an important

role in regulating signaling pathways. Nitric oxide (NO), essential for the formation of reactive nitrogen species (RNS), therefore acts as a stimulator for exercise-mediated skeletal muscle glucose uptake, showing a possible mechanism of enhanced insulin sensitivity in response to exercise training [74]. As mentioned earlier, the muscle contraction cascade leads to aRONS-stimulated increase in expression of PGC-1α, which is an insulin-sensitivity regulator [38]. The latter signaling role of RONS emphasizes its importance in insulin-sensitizing mechanisms and glycemic control [53].

The current section shows the crucial role of ROS and RONS in basic adaptation mechanisms to exercise training. While AO supplements may optimize the training effects by protecting against exercise-induced ROS overproduction, overdosed supplementation may impair exercise training's beneficial adaptation effects.

Dietary antioxidants: daily usage and supplementation

Dietary AO supplements, such as vitamins, α-lipoic acid, coenzyme-Q10, and resveratrol, are commonly used by more than half of the adults in the United States to maintain health and extend life span. Vitamin C and E supplements are the most commonly used and combined dietary supplements among older adults (65 to 74 years in age) [49].

The water-soluble ascorbic acid has been suggested as a very effective donor AO [10]. Moreover, the fat-soluble vitamin E (mostly α-tocopherol) as an AO can scavenge lipid radicals [68, 71]. Oxidized vitamin E can be transformed again to an unoxidized form by other soluble AOs such as vitamin C. Therefore, that mechanism prevents vitamin E radicals from accumulating and inducing excess lipid peroxidation. The combination of vitamin C and E supplements, therefore, is most often used to potentially prevent oxidative stress and has been speculated to slow down the aging processes [68, 72].

According to dietary reference intakes (tolerable upper-intake levels) up to 2000 mg and 1000 mg of vitamin C and E, respectively, was established as the maximum level of daily nutrient intake that is likely to pose no risk of adverse effects [39]. Some preclinical studies have shown that high doses of vitamins C and E may even have pro-oxidative properties and increase deleterious oxidative stress [29, 44]. For example, in some cases increased levels of oxidative stress led to a lens impairment that might have contributed to developed age-related cataract [75]. Supplementation of diets with smaller doses (500 mg per day of vitamin C), however, resulted in a significant increase in ascorbate levels in the plasma and, hence, decreased oxidative stress, compared with the placebo [6].

While even smaller doses of AOs may lead to decreased ROS production, it is unknown whether decreased levels of free radicals may impair ROS-driven adaptations to exercise training, e.g., enzymatic AO activity, mitochondrial biogenesis, and upregulated glucose metabolism. To our knowledge, there is not enough information on safe and effective dosing and interaction of vitamins and other supplements with exercise training in clinical studies.

Increased oxidative stress may also be deleterious in the aging process and, therefore, influence cognitive function. There is evidence that AO supplements may protect or improve cognitive function in adults aged 65 years and older [19]. Supplementation with multivitamins and vitamins C, E, and β-carotene, for example, was suggested to protect the brain from oxidative damage and delay an onset of cognitive decline [11, 35]. Results from a large, randomized, placebo-controlled trial, however, show that daily multivitamin supplementation had no benefit on delaying cognitive decline in adults aged 65 years and older after more than a decade of treatment intervention and follow-up [19]. Lin et al, found that none of the included trials reported any benefit from supplements on cognitive function in subjects aged 69 to 95 with mild to moderate dementia [35]. Impairments of cognitive function associated with intake of AO supplements are not reported in older adults. Future clinical studies will aim to investigate the potentially beneficial effects of AO supplements on cognitive function in nutrient-deficient older adults.

Is there evidence that antioxidant supplementation can improve or blunt beneficial exercise effects in animals and humans?

Undoubtedly, the damaging effects of excess ROS concentrations may include decreased muscular functionality, histological changes, and muscular soreness, and may attenuate exercise performance [38]. This has been the rationale for consuming large quantities of non-enzymatic AO supplements, e.g., vitamins C and E and α-lipoic acid. It also has led to research into whether non-enzymatic AO supplementation could prevent ROS' damaging effects during exercise and thereby enhance endurance exercise performance [38] in animals and humans. In contrast to the initial protective hypothesis, preclinical studies and some recent human studies have reported controversial results on the blunting effect of non-enzymatic AO supplementation on exercise endurance training [73]. Our focus will be mainly on enzymes and proteins that regulate redox biology (SOD, GPx, and CAT) and metabolism (GLUT4 and PGC-1α). We included *in vivo* animal and human studies on the effect of exercise training combined with non-enzymatic AOs only in skeletal muscle.

Exercise training and antioxidant supplements in preclinical studies

Redox status

Exercise training alone induces upregulation of the main AO enzymes activities (SOD, GPx, and CAT) in an animal model in comparison with untrained animals. Table 1.1 contains preclinical studies of the effect of exercise training combined with AO supplementation, i.e., vitamins C and E and α-lipoic acid [1, 16, 23, 31, 37, 64]. The results showed either no additional [23, 31, 32, 54, 64] or a blunting effect [16, 37] on the main AO enzyme activities.

Authors suggested that the effect of exercise training combined with AOs may depend on the dosage, combination, and duration of AO supplementation, and the type of exercise training [1]. Mostly used supplement combinations were vitamins C and E [23, 31, 54], but also vitamin C and α-lipoic acid [64] and vitamin C alone [16, 37]. Interestingly, the dosages between those studies that reported no or a blunting effect did not differ. Composition of AO supplementation may also have a different AO potential, where the induced effect can be different, e.g., between specific combinations and individual use [1]. For example, α-lipoic acid increases vitamin E uptake in skeletal muscle that can amplify the overall AO effect [1]. The studies that reported no additional effect of AO used a combination of vitamins E and C/α-lipoic acid [23, 31, 64]. Remarkably, the results of Meier et al., and Gomez-Cabrera et al., on a blunting effect of AOs on redox status used vitamin C supplementation alone [16, 37]. According to results of Gomez-Cabrera et al., Vitamin C supplementation combined with exercise training can also impair redox status in rats [16]. The supplementation durations differed between the studies, but this does not seem to be a key factor in blunted expression of SOD and GPx, as studies showing no effect used longer, alike, or shorter time periods [23, 31, 54, 64].

The reported significant reduction of AO enzyme activities does not seem to be induced by the duration or dosage of supplementation and training modalities. The only common factor of these two studies (Meier et al., and Gomez-Cabrera et al.,) out of the six listed in Table 1.1 (preclinical studies) is that in these experiments supplementation with vitamin C alone was used in comparison with the other reported studies. Meier et al., combined coenzyme Q10 and 1% N-acetyl-cysteine with vitamin C supplementation. However, it is unknown whether this combination had an additional blunting effect in comparison with the effect of vitamin C alone [37]. This warrants future studies on impact of AO supplementation using various clamped dosages, intervention durations and training modalities. The future studies will

improve our understanding of the supplement combinations and its impact on training-induced redox status changes.

Mitochondrial biogenesis mechanism in response to exercise training combined with antioxidant supplementation

Mitochondrial biogenesis is the essential adaptive response mechanism to endurance training and mitochondrial content in muscle is a crucial determinant of endurance capacity [1, 51]. Recent preclinical studies have shown either no additional [1, 23, 64] or a blunting effect of exercise training +AO combination on protein expression of PGC-1α [16, 37]. Expression of PGC-1α was blunted in studies where vitamin C supplementation alone was combined with endurance exercise training in animals. Gomez-Cabrera et al. suggested that vitamin C supplementation during endurance training blunts one of the mitochondrial biogenesis paths, i.e., PGC-1α—NRF-1—TFAM—cytochrome C [16]. Additionally, endurance training combined with vitamin C supplementation in rats blunted an improvement in running time in comparison with training alone (26.5% versus 186.7%, respectively), whereas there was no difference in $\dot{V}O_{2\ peak}$ [16]. This is in agreement with the statement that endurance capacity depends on mitochondrial content in skeletal muscle and that $\dot{V}O_{2\ peak}$ is also dependent on cardiovascular training adaptations [16]. While Meier et al. reported no differences in running times, found no differences in the peak power exercise tests performed at the end of the training period, in accordance with Gomez-Cabrera [37].

Moderate ROS levels are crucial for signaling pathways of mitochondrial biogenesis [1, 37]. Decreased expression of PGC-1α, therefore, is associated with reduced ROS-stimulated mitochondrial biogenesis. Supplementation with vitamin C decreased ROS levels, preventing enzymatic AO activity [37]. Moreover, the studies of Meier et al., and Gomez-Cabrera are in agreement with the statement associating blunted expression of PGC-1αwith decreased activity of AO enzymes (SOD, GPx, CAT), the primary endogenic AO defense system [16].

Metabolic response to exercise training combined with antioxidant supplementation in animal skeletal muscle

Increased expression of GLUT4 is one of major adaptive responses to endurance exercise in skeletal muscle [23]. Additionally, studies on endurance training adaptations have shown that expression of GLUT4 is also redox-sensitive [37]. Mitochondrial biogenesis and upregulated

expression of GLUT4 are mediated by the PGC-1α protein, which increases in response to acute as well as endurance exercise training [37, 51].

Although studies on exercise training combined with AO supplements showed inconsistent results regarding redox status and mitochondrial biogenesis pathway, studies on the metabolic response show no difference in GLUT4 expression in response to exercise training with or without administered AOs.

Only two studies investigated both expressions of PGC-1α and GLUT4 in response to exercise training combined with AO supplements. Meier et al., found blunted PGC-1αexpression and no effect on GLUT4 expression [37]. On the other hand, Higashida et al. reported no effect of the exercise training +AO combination on expression of PGC-1α and GLUT4 [23].

Taken together, animal studies have shown consistent results regarding the upregulating effect of exercise training alone on adaptive mitochondrial biogenesis and insulin sensitivity and glycemic regulation. Conversely, results on exercise training +AO were equivocal, and its potential suppressing effect on exercise training may depend on the dose, type of AO composition, and length of time.

Exercise training combined with administration of antioxidant supplements in healthy humans

Redox status

Recent studies have addressed the effects of AO supplementation on the exercise training adaptations in healthy humans [4, 45, 53, 66, 74]. Ristow et al. and Gomez-Cabrera et al. have reported recently that AO supplementation can decrease training efficiency and prevents specific cellular adaptations of exercise training in healthy humans, e.g., mitochondrial biogenesis [16, 53]. Recent clinical trials suggest that various AOs that directly inhibit ROS production may ameliorate the benefits of exercise that depend on ROS signaling [16, 53] or have no different effect from exercise training alone [66, 73].

In Table 1.2 (clinical studies), we present relevant double-blind placebo-controlled studies that investigated enzymatic AO defense activity during endurance exercise training combined with vitamin C and E supplementation in young healthy adults.

Ristow et al. have studied the effect of a 4-week endurance training regimen combined with vitamin supplementation or placebo on training adaptation in 19 untrained and 20 pre-trained

healthy men. They reported a significant effect of vitamins C (1000g) and E (400 IU) supplementation in blocking exercise training -induced expression of AO enzyme mRNAs (SOD1 and GPx1) for the entire cohort of trained and untrained subjects in comparison with no-supplemented groups [53]. The findings are in agreement with the study results of Gomez-Cabrera et al., and Braakhuis et al., where activity of SOD, GPx, and CAT [4] decreased in response to 8 weeks [16] and 9 weeks [4] of endurance training combined with antioxidants in untrained and trained subjects, respectively. In response to endurance and eccentric training in combination with vitamin C and E supplementation, there was no change in CAT activity in comparison with placebo [53, 66]. These results seem to indicate consistently that consumption of these amounts of vitamin C and E supplements blunts SOD and GPx activities in humans [4]. Therefore, this may be blocking exercise-dependent production of the ROS [4, 16] essential for hormetic stimulation of adaptive mechanisms to exercise training, e.g., the PGC-1α pathway of mitochondrial biogenesis. Variability in levels of significance may be training type- and duration-related.

Mitochondrial biogenesis

Available study results have been presented on the effects of endurance exercise training with vitamin C and E supplementation on PGC-1α protein expression, the mitochondrial biogenesis co-activator (Table 1.2). Paulsen et al., have shown that vitamin C and E supplementation blunted any rise in muscle cytosolic PGC-1α levels during an 11-week endurance exercise training program. By contrast, PGC-1α mRNA increased only in the vitamin C and E supplementation group, and nuclear PGC-1α protein levels were unchanged in both groups. The authors explain that muscle biopsies were collected 2 to 4 days after the last training session, and they reflect no immediate activation, nuclear translocation of PGC-1α, or gene expression during exercise [45]. Yfanti et al. have shown no effect of either the training or the supplementation on the basal mRNA expression of PGC-1α [73]. Ristow et al. reported that an exercise-related induction of PGC-1α expression in skeletal muscle was strongly blunted following 4 weeks of exercise training in comparison with the non-AO group [53]. These study results coincide with the opinion that prolonged exercise training (4 to 11 weeks) combined with vitamin C and E supplementation blunt ROS-stimulated cellular primary pathways of mitochondrial biogenesis that seem to block an increase in exercise performance. Yfanti et al. who observed no increase in PGC-1α expression, speculate that the result may be

induced by different training modalities and the applied dose of vitamin C of 500 mg [74] versus 1000 mg in the rest of the relevant studies [16, 45, 53].

Metabolic response to exercise training combined with antioxidant supplements

Exercise endurance training has been shown to improve not only exercise performance, but also other health-related functions, such as muscular glucose uptake, insulin sensitivity maintenance, and insulin-resistance improvement [53]. Animal studies have shown that overexpression of skeletal muscle PGC-1α increases insulin-stimulated glucose disposal in both healthy and insulin-resistant rats. The insulin-sensitizing effects of endurance exercise training seem to be blunted by decreased ROS production induced by vitamin C and E supplementation [53]. Ristow et al. have shown a significant effect of vitamin C and E supplementation on the blockage of an exercise training -induced improvement of insulin sensitivity, i.e. decreased expression of PGC-1α. It was determined by glucose infusion rates ($p<0.001$) in comparison with placebo (Table 1.4). While this is controversial and has not been conducted in other human studies, it is in line with some animal studies [64].

In contrast to the latter, Yfanti et al. have shown that vitamin C and E supplementation before a 12-week training program had no effect on insulin-stimulated glucose uptake in skeletal muscle in comparison with placebo [74]. The increased insulin sensitivity was associated with corresponding increases in total protein kinase B (Akt), GLUT4, and HK2 in skeletal muscle in both supplementation and placebo groups, without significant differences [74]. The discrepancy between those two studies may be dependent on the dose-response of the AO supplements, as the vitamin C dose was lower (500 mg) [73] where there was no effect. There is no sufficient scientific evidence, however, of impaired insulin-sensitivity improvement in response to endurance training combined with vitamin C and E supplements. This warrants more studies on the AMPK—PGC-1α—GLUT4 pathway in response to endurance exercise training with AO supplementation in human subjects. Future studies are important because the AOs are often combined with exercise endurance in healthy and insulin-resistant subjects. Dose-response studies of vitamin C and E supplements during exercise training would contribute to recommendations on effective exercise performance improvements in athletic as well as clinical settings.

In conclusion, evidence exists in both animal and human studies that exercise-induced ROS play a crucial role in stimulating the signaling pathways for enzymatic AO activity (SOD, GPx, and CAT), mitochondrial biogenesis (expression of PGC-1α), and glucose metabolism

(insulin sensitivity (GLUT4 expression) and glucose uptake). Moreover, adaptation to regular moderate ROS production during exercise training allows for more effective enzymatic ROS scavenging, increases mitochondrial oxidative capacity and its efficiency (new, more efficient mitochondria) and, hence, prevents deleterious overproduction of ROS. Study results are unclear about a potential suppressive role of AO supplements in impairing these exercise training -induced adaptive effects.

High consumption of AOs in middle-aged and older adults may impair desired goals to improve metabolic status (obesity, lipid profile, insulin resistance, poor physical condition). Additionally, Higashida et al. (23] have suggested that the separate reports of Ristow et al. [53] and Gomez-Cabrera et al. [16], describing a suppressive role of AO supplements on exercise training, have to be treated with caution because of its methodology. On the other hand, Gomez-Cabrera et al. [17] contrast with Higashida et al. [23] Therefore, the unknown effects of supplemental composition, dose, and time duration on adaptive training effects necessitate further studies to establish safe AO supplement types, doses, and composition without suppressing exercise training's beneficial effects.

Resveratrol as an "alternative antioxidant" and its role as an antioxidant in exercise training adaptation in preclinical and clinical studies

Resveratrol is a polyphenolic and fat-soluble compound present mainly in grapes [7]. To date, there have been a number of *in vitro* and preclinical [25, 56, 60, 61], but also clinical studies investigating resveratrol's AO features [14, 41, 42, 52]. Resveratrol has a short biological half-life (8 to 14 minutes) [67], labile properties, and rapid metabolism and elimination. Because of its poor water solubility and instability, it converts to a less active cis form, and its beneficial AO impact is not fully understood [67].

Resveratrol as an antioxidant and gene regulator

Resveratrol has been studied as an AO supplement that decreases deleterious amounts of ROS, but also as a stimulant of energetic cellular sirtuin 1 (SIRT1)- and AMPK-dependent pathways [38], both important for PGC1α activation. Study results, however, are equivocal perhaps because of resveratrol's poor bioavailability and solubility [3]. There is some evidence of *in vitro* results showing improved availability and uptake of resveratrol by composing it chemically with liposomal carriers. Vanaja et al., for example, found that liposomal forms of resveratrol improved its antioxidative properties and, hence, decreased

oxidative damage in isolated leukocytes [67]. Therefore, this could potentially improve resveratrol's bioavailability and AO effects in preclinical and clinical studies, but further studies are needed. Despite the controversies regarding the bioavailability and delivery of resveratrol, spectacular improvements on metabolism and performance induced by resveratrol supplementation have been documented (mostly in animal studies) [22, 30, 40, 52].

Effects of exercise training combined with resveratrol in preclinical and human studies

While a number of studies have focused on resveratrol's effects on exercise training results in animal models (Table 1.7) [8, 22, 30, 40, 52], we are only aware of two studies that investigated a synergistic effect of combined exercise training with resveratrol supplementation in humans [14, 41].

Preclinical studies

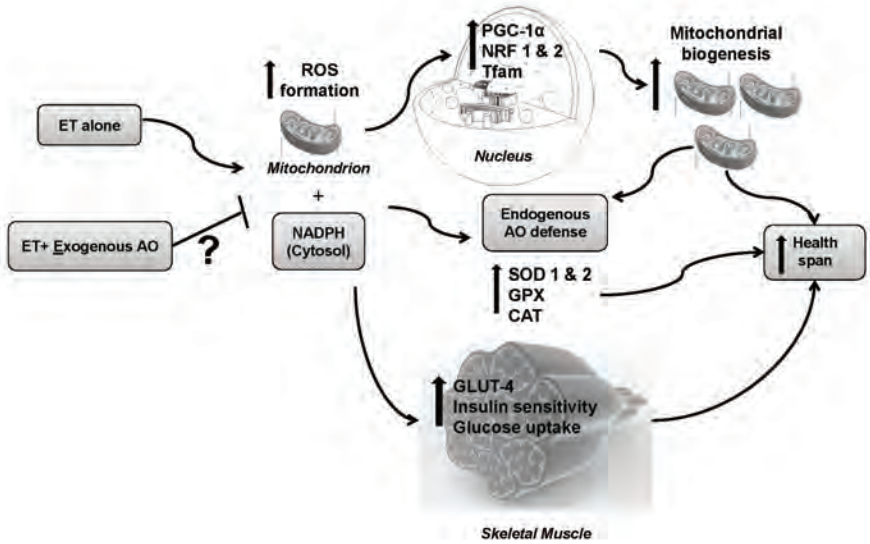
Hart et al. speculated that resveratrol may have induced post-training improvements in aerobic capacity by simultaneously activating different molecular pathways related to mitochondrial function [22]. Indeed, some authors reported improved $\dot{V}O_{2\ peak}$ and running time in response to resveratrol combined with exercise training [22, 40]. Other authors reported increased expression of PGC-1α and, hence, improved mitochondrial biogenesis [8, 22]. In accordance with an improved PGC-1α signaling pathway, an enhanced metabolic adaptive response was also reported by increased GLUT4 expression, insulin sensitivity, and glucose uptake [40].

Additionally, two studies of the same group (Hart et al., 2014 and Hart et al., 2013) have investigated the effects of resveratrol in conjunction with exercise training on running distance in low capacity runner (LCR) and high capacity runner (HCR) rats, respectively. Interestingly, resveratrol supplementation combined with exercise training reduced running distance in LCR [21] and improved running distance in HCR rats [22]. To explain this inconsistency, it was suggested that in some models resveratrol supplementation induces different results in obese prone and obese resistant rats and its effects could be influenced by metabolic status [21].

Taken together, the authors speculate that these synergistic effects may manifest as a result of providing resveratrol when cellular energy demand is high but also in rats with high levels of performance. From these basic and preclinical studies, it seems that this potential additional effect of resveratrol on training effects provides the mechanistic action through which

exercise training combined with resveratrol supplementation (REX) may be a feasible and efficacious intervention for maintaining functional status in humans.

Figure 1.1. Adequate production level of reactive oxygen species (ROS) as a signaling agent in adaptation response to exercise training (ET). This simplified scheme shows a clear potential blunting effect of antioxidant (AO) supplements on biological mechanisms when combined with exercise training.



Human studies

Contrary to the aforementioned animal results on improved exercise performance following REX, one human study has shown a 45% larger improvement of $\dot{V}O_{2\ peak}$ in response to placebo after 8 weeks of high intensity training in comparison with resveratrol-supplemented subjects (Table 1.8) [14]. The latter and other human study of Olesen et al., consistently reported that exercise training combined with resveratrol supplementation had no additional effect on PGC-1α, SIRT1, and AMPK-induced energetic mechanisms as compared to a placebo group [14, 41]. Additionally, Scribbans et al. have also reported no additional effects of resveratrol supplementation on exercise performance and speculated that resveratrol supplementation may have a blunting effect on skeletal muscle gene expression of PGC-1α, SIRT1, and SOD2 [59]. Moreover, Gliemann et al. have reported that resveratrol might have blunted a beneficial effect of exercise training on lipid profile. They have shown that REX eliminated the training effect on *low-density lipoprotein (LDL), total cholesterol/high-density lipoprotein (TC/HDL) ratio,* and TGs [14].

Contrary to animal study results, resveratrol did not improve exercise performance in healthy older adults. Interestingly, resveratrol has been shown to impair the observed exercise training -induced improvements in lipid profile. Moreover, resveratrol impaired a beneficial effect of the exercise training -induced improvement in mean arterial pressure (MAP) [14] and the muscle protein expression of vascular endothelial growth factor (VEGF) [13].

Given the inconsistency of existing animal data on REX and the few human studies indicating impairments in exercise performance and abolished exercise-induced lipid profile improvement following resveratrol supplementation, we suggest that this isolated human report of Gliemann et al., should not be a barrier to further investigation of resveratrol as a potential aid in improving exercise training effects in healthy subjects and in a clinical setting (hypertension, poor physical activity, reduced oxidative capacity) [14]. Moreover, other authors are also critical about the reported negative effects of resveratrol and have questioned Gliemann's methodology and analyses [5, 63].

Conclusion

The review investigated basic biological adaptive mechanisms to exercise training in preclinical and clinical studies in response to exercise training combined with commonly used AO supplements. Most of the relevant animal and human studies showed neither additional nor adverse effects of exercise training combined with AO supplements on redox status,

mitochondrial biogenesis, and glucose metabolism. Only a few animal and human studies have shown a blunting effect of exercise training combined with vitamin AOs on adaptive responses to exercise training in healthy subjects. The reason for this inconsistency could possibly lie in different AO compositions, doses, duration time of supplementation, and exercise training modalities. Additionally, the reported blunting effects of AO on training results could also be misleading by chosen methodology of study protocols. Moreover, it is difficult to speculate about adverse or positive clinical effects of resveratrol because of a narrow body of evidence and because experiments were conducted in only healthy subjects. To date, a blunting influence of AO supplementation during exercise training in healthy humans remains speculative. The lack of strong evidence indicating adverse and/or positive effects and the unclear methods of AO administration in combination with exercise training, however, warrant future studies in healthy aging populations and individuals with chronic health conditions such as sarcopenia, hypertension, and poor physical aerobic condition, for whom training adaptations are crucial for health improvement.

Future directions

The review highlights studies on AO supplements' adverse blunting effects on mitochondrial biogenesis pathways and suggests ROS-induced impaired signaling mechanisms. Enzymatic AO activity seems to be an efficient and effective defense against oxidative stress during exercise training in healthy young and middle-aged adults. Therefore, future double-blinded placebo-controlled studies should focus on investigating a potential positive or negative effect of AO supplements on key exercise training adaptation mechanisms in deficient older adults. Such studies may evaluate exercise responses for relevant biologic factors such as mitochondrial biogenesis, insulin, skeletal muscle glucose uptake, and redox status. Though speculative, adverse and/or positive effects of AO supplementation on exercise training results may depend on doses, compounds, combinations, and endurance training modalities. To address this hypothesis, dose-response clinical trials are needed to determine safe and training-efficient supplementation strategies combined with exercise training in both healthy and unhealthy older adults.

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Table 1.1. Redox status in response to exercise training alone (ET) and training combined with antioxidant supplements (ET+AO) in animal skeletal muscle. The reverse transcription polymerase chain reaction technique was used to measure AO enzyme activities (SOD, GPx, CAT).

Study	Species	Age	Non-enzymatic AO dose/day	Duration and type of training (tr)	Response to ET vs. sedentary: redox	Effect of ET+AO on AO enzyme activity
Preclinical studies						
Leeuwenburgh et al. [34]	Wistar rats	5 months	A-tocopherol 0.2%, ascorbic acid 0.5%, B-carotene 0.015%	19 months; forced running on a running wheel	Increased cytosolic superoxide dismutase (SOD1) and mitochondrial superoxide dismutase (SOD2) activity; increased glutathione peroxidase (GPx) activity; no effect on CAT in skeletal muscle	No effect on SOD1, SOD2, GPx, catalase (CAT) in skeletal muscle
Rosa et al. [56]	Male mice	3 months	Vitamin (Vit) E 10 mg/kg; vit C 10 mg/kg	10 days	Increased SOD2 activity in red blood cells	No effect on SOD2 in red blood cells
Higashida et al. [22]	Male Wistar rats		Vit E 150 mg/kg; vit C 750 mg/kg	9 days supplements/3 days tr; 8 weeks supplements/3 weeks tr	Increased SOD1 and SOD2 activity in skeletal muscle	No effect on SOD1 and SOD2 in skeletal muscle
Strobel et al. [67]	Male Wistar	10 weeks	Vit E 1000 IU; 1.6 g of a-lipoic acid/kg diet	4 days/week (90 minutes/day), 14 weeks	Decreased SOD2 activity; no effect on GPx in skeletal muscle	No effect on SOD2, GPx in skeletal muscle
Meier et al. [40]	Mice		Vit C 12 mg/l, Q10 12 mg/l; N-acetyl-cysteine 1%	4 weeks endurance	SOD1 expression increased (mRNA expression) in skeletal muscle	Decreased SOD1 expression (mRNA expression) in skeletal muscle
Gomez-Cabrera et al. [17]	Wistar rats	3 months	Vit C 0.24 mg/cm^2	Endurance 3 and 6 wks	Increased SOD and GPx activity (mRNA expression) in skeletal muscle	Decreased SOD2 and GPx activity (mRNA expression) in skeletal muscle

Table 1.2. Redox status in response to exercise training alone (ET) and training combined with antioxidant supplements (ET+AO) in human skeletal muscle. The reverse transcription polymerase chain reaction technique was used to measure AO enzyme activities (SOD, GPx, CAT).

Study	Species	Age	Non-enzymatic AO dose/day	Duration and type of training (tr)	Response to ET vs. sedentary: redox	Effect of ET+AO on AO enzyme activity
				Clinical Studies		
Ristow et al. [55]	19 untrained and 20 trained healthy humans	Untrained, 26.7±4.34; trained, 25.40±2.15	Vitamin (Vit) C 1000 mg; vit E 400 IU	Endurance 5 days/4 weeks	Increased SOD1 and 2, GPx, CAT mRNA expression in skeletal muscle	Decreased SOD1 and SOD2, GPx, CAT mRNA expression in skeletal muscle
Braakhuis et al. [5]	23 trained humans	31±8 yrs	Vit C 1000 mg	Endurance, high-intensity training, 2-3 days/3 weeks	No effect of ET on SOD, GPx, CAT activity (enzyme activity levels in erythrocytes)	Decreased SOD post-exercise; decreased CAT in erythrocytes
Theodorou et al. [69]	Humans; 14 vitamins and 14 placebo	Vitamins, 25.6±1.2; placebo, 26.2±1.5	11 weeks; Vit C 1000 mg; vit E 400 IU	Eccentric training 2 days/4 weeks	Decreased CAT activity in red blood cells	No change in CAT in red blood cells

Table 1.3. Mitochondrial biogenesis pathways' stimulation in response to exercise training alone (ET) and exercise training combined with antioxidant supplements (ET+AO) in animal skeletal muscle, comparing effects on peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) signaling pathways.

Study	Species	Age	Non-enzymatic AO dose/day	Duration and type of training (tr)	Response to ET vs. sedentary: PGC-1α	Additional effect of AO PGC-1α
Preclinical Studies						
Higashida et al. [22]	Male Wistar rats		Vitamin (Vit) E 150 mg/kg; vit C 750 mg/kg	9 days supplements (suppl)/3 days tr; 8 weeks (wks) suppl/3 wks tr	2- to 3-fold increased expression in skeletal muscle	No effect PGC-1α expression in skeletal muscle
Strobel et al. [67]	Male Wistar	10 wks	Vit E 1000 IU; a-lipoic acid/kg diet 1.6 g	4 days/wk (90 minutes/day), 14 wks	Increased PGC-1α mRNA and protein in skeletal muscle	No effect on PGC-1α mRNA and protein content in skeletal muscle
Abadi et al. [1]	Mice		Chow diet vit E 1000 IU; a-lipoic acid 0.1%	8 wks endurance (treadmill)	Increased PGC-1α protein in skeletal muscle	No effect on PGC-1α protein content in skeletal muscle
Meier et al. [40]	Mice		Vit C 12 mg/l, Q10 12mg/l; N-acetyl-cysteine 1%	4 wks endurance	Increased expression in skeletal muscle	Blunted PGC-1α expression in skeletal muscle
Gomez-Cabrera et al. [17]	Wistar rats	3 months	Vit C 0.24 mg/cm^2	Endurance 3 and 6 wks	Increased PGC-1α expression (and Tfam) in skeletal muscle	Blunted PGC-1α expression (and Tfam) in skeletal muscle

Table 1.4. Mitochondrial biogenesis pathways' stimulation in response to exercise training alone (ET) and exercise training combined with antioxidant supplements (ET+AO) in human skeletal muscle, comparing effects on peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) signaling pathways.

Study	Species	Age	Non-enzymatic AO dose/day	Duration and type of training (tr)	Response to ET vs. sedentary: PGC-1α	Additional effect of AO PGC-1α
Clinical Studies						
Paulsen et al. [48]	Human trained	29 AO, 31 Placebo group	Vitamin (Vit) C 1000 mg; vit E 235 IU	Endurance 11 weeks	Increased cytosolic PGC-1α in skeletal muscle	Blunted
Ristow et al. [55]	Human trained and untrained	Untrained, 26.7±4.34; trained, 25.40±2.15	Vit C 1000 mg; vit E 400 IU	Endurance 4 weeks	Increased PGC-1α RNA in skeletal muscle	Blunted
Yfanti et al. [76]	Human untrained	29±1	Vit C 500 mg; vit E 400 IU	Endurance 12 weeks	No difference in PGC-1α mRNA expression in skeletal muscle	No difference in PGC-1α mRNA expression in skeletal muscle

Table 1.5. Metabolic response to exercise training alone (ET) and exercise training with administered antioxidant supplements (ET+AO) in animal skeletal muscle, comparing effects on glucose transporter 4 (GLUT4) expression.

Study	Species	Age	Non-enzymatic AO dose/day	Duration and type of training (tr)	Response to ET vs. sedentary: GLUT4	Additional effect of AO on GLUT4 expression
Preclinical Studies						
Higashida et al. [22]	Male Wistar rats		Vitamin (Vit) E 150 mg/kg; vit C 750 mg/kg	9 days supplements (suppl)/3 days tr; 8 weeks suppl/3 weeks tr	Increased expression of GLUT4, insulin responsiveness of glucose transport in skeletal muscle	No effect
Meier et al. [40]	Mice		Vit C 12 mg/l; Q10 12 mg/l; N-acetyl-cysteine 1%	4 weeks endurance	Increased GLUT4 expression in skeletal muscle	No effect

Table 1.6. Metabolic response to exercise training alone (ET) and exercise training with administered antioxidant supplements (ET+AO) in human skeletal muscle, comparing effects on glucose transporter 4 (GLUT4) expression.

Study	Species	Age	Non-enzymatic AO dose/day	Duration and type of training (tr)	Response to ET vs. sedentary: GLUT4	Additional effect of AO on GLUT4 expression
Clinical Studies						
Ristow et al. [55]	Human	Untrained, 26.7±4.34; trained, 25.40±2.15	Vitamin (Vit) C 1000 mg; vit E 400 IU	Endurance 4 weeks	Improved insulin sensitivity in skeletal muscle	Inhibition of insulin sensitivity improvement in skeletal muscle
Yfanti et al. [76]	Human	29±1	Vit C 500 mg; vit E 400 IU	Endurance 12 weeks	Increased GLUT4 expression in skeletal muscle	No additional effect on GLUT4 expression (Placebo vs. AO) in skeletal muscle

Table 1.7. Findings from preclinical studies of combined effects of resveratrol and physical exercise in animals, comparing exercise training alone to exercise training combined with resveratrol supplementation (REX).

Study	Species	Age	Dosage of resveratrol	Duration and type of training	Response to ET vs. sedentary	Response to REX
Murase et al. [43]	Male senescence-accelerated mice	13 weeks	0.2% of a diet	12 weeks	Soleus and *extensor digitorum longus* (EDL): increased muscle weight, tetanic contraction force; reduced glucose, triglycerides (TGs), insulin; reduced serum and muscle (EDL) thiobarbituric acid reactive substances (TBARS); increased PGC-1α and GLUT4; increased VO_2 and lipid oxidation	Soleus and EDL: increased muscle weight, tetanic contraction force; reduced glucose, TGs, insulin; reduced serum and muscle (EDL) TBARS; increased PGC-1α and GLUT4; increased VO_2 and lipid oxidation
Dolinsky et al. [9]	Male Wistar rats	10 weeks	4g/kg diet	12 weeks	Increased tetanic contraction force in *transverse abdominal muscle (TAM)* and soleus muscle; reduced plasma TGs	No additional effect of resveratrol on tetanic muscle contraction in TAM and soleus; reduced plasma TGs; increased fat oxidation/decreased glucose oxidation (decreased respiratory exchange ratio); increased PGC-1α/tubulin ratio
Hart et al. [21]	Male high-capacity runner rats	13 months	100 mg/kg body mass (BM)	12 weeks	Increased running distance; increased VO_{2peak}; increased Tfam, PGC-1α	Increased running distance; $VO_{2peak,}$ Tfam, PGC-1α
Kim et al. [32]	Mice injected with kainate (KA)		40 mg/kg BM	6 weeks swimming; 60 minutes/day; 5 days/week	Decreased body weight and lactate threshold; decreased SOD and CAT in combination with ET+KA, but higher than with KA alone at rest	Reduced seizures and increased SOD and CAT (same as control)
Ringholm et al. [54]	Mice	3 months	4 g/kg diet	12 months running wheel	No effect on area under curve (AUC) for blood glucose; increased *vascular endothelial growth factor (VEGF)*	Increased AUC; increased VEGF
Ryan et al. [57]	Mice	3-5; 26-28 months	0.05% in diet	3 days isometric exercise	Increased activity of SOD, GPx, CAT	SOD: no additional effect; increased GPx and CAT activity

Table 1.8. Findings from clinical studies on combined effects of resveratrol and exercise training in humans, comparing exercise training alone to exercise training combined with resveratrol supplementation (REX).

Study	Species	Age	Training	Resveratrol dosage	Response to ET vs. sedentary	Response to REX
Olesen et al. [44]	Men	65±1 years	8-week endurance	250 mg	Increased PGC-1α mRNA; increased exercise performance; reduced TNFa mRNA	PGC-1α: no extra effect; performance (one-legged knee-extensor): no additional effect of resveratrol; no effect of resveratrol on adenosine monophosphate (AMP)-activated protein kinase (AMPK) and sirtuin 1 (SIRT1); resveratrol impaired training-induced reduction of TNFa mRNA
Gliemann et al. [15]	Men	65±1 years	8 weeks of high-intensity training	250 mg	Greater increase in VO_{2peak}; reduced mean arterial pressure; reduced low-density lipoprotein (LDL), total cholesterol/high-density lipoprotein (TC/HDL) ratio, and triglyceride concentrations (TGs) in blood	45% lower VO_{2peak} than in REX; no additional reduction in mean arterial pressure, only in placebo; REX abolished the training effect on LDL, TC/HDL, and TGs; no effect of REX on SIRT1
Gliemann et al. [14]	Men	65±1 years	8 weeks of high-intensity training	250 mg	~20% increase in capillary to fiber ratio (C:F), an increase in the muscle protein expression of vascular endothelial growth factor (VEGF).	No increase in C:F ratio and VEGF protein expression.

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PART 2

Acute effects of modified oxygen conditions during exercise

Exercise training is effective to improve health span during the aging process. However, many older adults cannot be involved in standard exercise training interventions caused by very low exercise tolerance [1]. Therefore, this part presents a potential alternative approach to the nutritional (antioxidant) interventions that may be applied to improve the effects of exercise in deficient elderly populations. Acute modulation of oxygen content in ambient air is a safe and well-accepted ergogenic aid during exercise training [2-4]. However, there are still conflicting data as to whether manipulation of the oxygen content of the inspired air during exercise would be beneficial for exercise tolerance improvement. Several studies in diabetes [5-7] and COPD [8, 9] patients have shown added therapeutic value of acute hypoxic and hyperoxic exercise, respectively. However, it is still unclear whether exercise tolerance is limited mainly by oxygen delivery (cardiovascular dysfunction) and/or consumption (mitochondrial mass and function). Additional oxygen availability may acutely improve oxygen delivery for better exercise tolerance in 'oxygen-delivery-limited' older adults. Therefore, part 2 of this thesis discusses a potential role of hyperoxic exercise to detect oxygen delivery limitations and determine effective doses of hyperoxic air to improve exercise tolerance and to be applied in future tailored training interventions. Additionally, challenging hypoxic conditions were applied to assess sensitivity of potentially useful measuring devices to monitor the individual response to hypo or hyperoxic exercise conditions.

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The experimental setup for the FiO_2 study during the measurement

Legend:

1. One of the gas cylinders with a mixture – oxygen in nitrogen (14%/21%/35% of O_2).
2. A laptop registering a bioimpedance signal through 6 electrodes placed on the upper body of a subject (heart rate, cardiac output, stroke volume).
3. A laptop registering a signal from the near-infrared spectroscopy (NIRS) device placed on a dominant (right) thigh.
4. A computer registering the spiro-ergometry data.
5. A cycle-ergometer

6. Air from the cylinder flew to a Douglas bag where a subject was breathing the air misxture form.
7. Another laptop to note the baseline data and from during the measurement (blood pressure and cycling frequency).
8. A manual blood pressure measuring device used before, during and after the measurement.
9. Hanger for an air tube leading from the cylinder to a Douglas bag.
10. The valve system: a subject could breathe the exact air mixture from the bag and breathe out in to the laboratory environment without mixing.

CHAPTER 2

Heart-rate variability threshold as an alternative method for spiro-ergometry testing: a validation study

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Submitted

Abstract

Although spiro-ergometry is the established 'gold standard' for determination of the second ventilatory threshold (VT2), it is a costly and rather time-consuming method. Previous studies suggest that assessing the second anaerobic threshold (AT2) on the basis of heart rate variability (HRV) during exercise may be a more cost-effective and non-invasive manner. Appropriate validation studies, however, are still lacking.

Aim: To test the reliability and sensitivity of the second HRV threshold (HRVT2) assessment method against VT2 in healthy young adults.

Methods: Eleven healthy, moderately-trained subjects underwent three incremental exercise tests. Ventilation, oxygen uptake ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$) and HRV were measured continuously. Exercise testing was performed in three oxygen (FiO_2) conditions of inspired air (14%, 21% and 35% of oxygen). Participants and assessors were blinded to the FiO_2 conditions. Two research teams assessed VT2s and HRVT2s independently from each other. Agreement between HRV and VT2 was determined by Bland and Altman analysis. Differences between the 3 conditions were determined by a General Linear Model for repeated measures.

Results: Mean workloads corresponding to VT2 and HRVT2 in hypoxia were respectively 19±17 % ($p=0.01$) and 15±15% ($p=0.1$) lower in comparison with hyperoxic conditions. Bland and Altman analysis showed low estimation bias (2.2%) and acceptably precise 95% limits of agreement for workload -15.7% to 20.1%.

Conclusion: HRV-based VT2 assessment has clinically acceptable agreement with VT2 and showed appropriate sensitivity to the manipulated inspiratory oxygen. In accordance with good agreement, HRV-based VT2 assessment has potential applications for exercise monitoring and altitude training purposes.

Keywords

Exercise, Maximal Testing, Cost-Effective, Healthy Subjects, Hypoxia, Hyperoxia

Introduction

The second anaerobic threshold (AT2) is frequently used to design and monitor exercise training programs for healthy people, athletes and patients with chronic diseases (9, 17, 26). Specifically, properly determined AT2 (~90% HRmax) is crucial for establishing appropriate training workloads and intensity needed to effectively improve exercise performance and to prevent injuries and overtraining (11). However, the 'gold standard' assessments of AT2 can only be determined through costly, time-consuming spiro-ergometry testing often in combination with invasive methods such as multiple blood samples of lactate concentrations [La] (5, 6).

Recently, heart-rate variability threshold (HRVT) was introduced as a non-invasive, cost-effective and more straightforward method than the currently practiced 'gold standard' to assess AT1 and AT2 in an individual (4). The method has also been proposed to design and monitor individualized training programs in athletes and patients undergoing cardiac and pulmonary rehabilitation (12). However, appropriate validation and sensitivity analyses to detect changes in individual training status are still lacking.

The HRVT represents a transition point between lower and higher activity of the sympathetic nervous system (SNS), which is strongly correlated with an increase in [La], catecholamine concentrations and minute ventilation (Ve) that occurs at the AT1(10).

One of the most commonly used methods for determining HRVT is the root mean square of the successive differences (HRVT-RMSSD) (19). Another method is based on the Poincaré plotting technique, from which the instantaneous variability of beat-to-beat data is derived by means of SD1 (HRVT- SD1) (22) (25).

In healthy subjects, Sales et al. reported strong correlations between the HRVT1 and the 'gold standard' AT1 assessments (HRVT-RMSSD r=0.91 and HRVT-SD1 r=0.93) (22). So far, most comparative studies have used single measurements to investigate agreement between these two AT determination methods (6, 10, 22). These group-based validation studies, however, do not provide information on the sensitivity of HRVT method to detect e.g. changes in an individual's AT. Furthermore, there is evidence that determination of AT2 is more reproducible than AT1 in trained cyclists (27). Therefore, a comparison of VT2 and HRVT2 was selected for the purpose of this validation study. To test the sensitivity of HRVT for detecting an acute change in physiological stress, we propose a new validation method based on an acute shift in AT2 by

manipulating inspiratory oxygen fraction (FiO_2). For a given workload SNS activity level will be increased during hypoxic exercise (29), and lower under hyperoxic conditions (14). In accordance, AT2 should be reached at a lower workload during hypoxic as opposed to a normoxic or hyperoxic exercise stress test.

To our knowledge, this is the first study to validate the sensitivity of HRVT2 assessment relative to that of AT2 on the basis of the breath-by-breath gas analysis method during multiple incremental exercise tests under decreased and increased FiO_2 conditions.

In accordance, we hypothesized that HRVT2-based workload is strongly associated with the spiro-ergometry VT2 based workload during incremental exercise test performed under both low, normal and high FiO_2 conditions. In addition, we hypothesized that a shift in HRVT2 is associated with a shift in VT2.

Methods

Subjects

A total of 11 (8 males / 3 females) healthy and medium-trained young adults volunteered to participate in this study. The study protocol was approved by the regional Medical Ethics Committee of the Erasmus University Medical Centre in Rotterdam, the Netherlands (MEC; number: 2012-128; (NTR3777)). All participants provided written informed consent.

Experimental design

Subjects completed 3 incremental exercise tests until exhaustion on a the same cycle ergometer (Jaeger ER800, CareFusion, Houten, The Netherlands) on different days. Each test was performed under either hypoxic (14.04%O_2), normoxic (21.2%O_2) or hyperoxic (35.08%O_2) conditions. The tests were separated by at least 72 hours and were performed in a randomized order at the same time of the day. Randomization occurred by drawing opaque sealed envelopes containing the order of the three FiO_2 conditions. Both temperature (21^{o}C) and humidity (35-45%) in the laboratory were held constant. Subjects were asked to refrain from strenuous exercise on the day before the test. Furthermore, they were asked to refrain from caffeinated drinks at least 12 hours before the exercise test.

Testing procedures

Incremental exercise test

All the exercise tests were performed at the Clinical Exercise Performance Laboratory (CEPL) from the Erasmus University Medical Center in Rotterdam, the Netherlands in presence of an exercise physiologist and a sports physician. All subjects were scheduled to perform a symptom limited exercise stress test. This standard ramp test started with a 2 min. rest period followed by 4 min. of unloaded cycling as warming-up. Next, the workload increased with a slope of 1.8 watt/6 sec (men) or a 1.2 watt/6 sec. (women). Subjects were instructed to cycle until exhaustion with a pedal frequency of 60-80 rpm. The loaded phase was terminated when pedaling frequency dropped below 60 rpm. A 5 min. unloaded recovery phase ended the exercise stress test. During warming-up, workload and recovery phase of the exercise protocol, $\dot{V}O_2$, $\dot{V}CO_2$, minute ventilation (Ve), (Oxycon Pro, Carefusion, Houten, The Netherlands) were monitored. Peak oxygen uptake, workload, heart rate and respiratory exchange ratio (RER) were documented. Immediately after each exercise test subjects were asked to rate their perceived exertion on a scale from 6 to 20 using the Borg scale (2).

Table 2.1. Subjects' characteristics.

N=11	**Hypoxia (N=11)**	**Normoxia (N=11)**	**Hyperoxia (N=9)**
	Mean ±SD	**Mean ±SD**	**Mean ±SD**
Age (yrs)		23.6±2.2	
Weight (kg)		69.1±9.8	
Height (cm)		177.9±8.2	
BMI (kg/m^2)		22.0±1.7	
VO_{2peak} (ml/kg/min)	46.8±5.3	54.6±7.0	54.2±6.7
W_{max} (Watt)	289±48	324±54	336±55
Borg score	16.4±0.7	16.3±0.8	16.2±0.8

Heart rate and heart rate variability

Beat-to beat Heart rate (HR) was measured continuously using an HR monitor (Zephyr BioHarness^{TM} 3, Annapolis, USA). The real-time HR data were recorded on an external server

(through a laptop and Bluetooth2.0 connection) with dedicated software (AppSolute Training, ADTS, 1.2 beta version, Vital BV, Hazerswoude-Rijndijk, the Netherlands). Heart rate variability (HRV) was quantified every 30 seconds by first calculating the root mean square of successive differences of R-R intervals (RMSSD), then dividing this by the average R-R interval over the corresponding 30 second period, to calculate normalized RMSSD (nRMSSD).

Medical gasses

According to legal requirements in the Netherlands, each test was performed using medically certified oxygen/nitrogen mixtures stored in 50 L 200 bar gas cylinders (Linde Gas Benelux/BOC Morden, London, UK). The 3 different air mixtures were blinded to the subject and inspired through a Douglas bag (20 liter) connected to an oro-nasal 7400 Vmask^{TM} and a 2730 2-way Y-shape^{TM} non-rebreathing valve (Hans Rudolph, inc. Kansas, USA).

Data analysis

Determination of the 2nd VT

Obtained values of $\dot{V}O_2$, $\dot{V}CO_2$, RER and (Ve) were averaged for each 30 seconds time window. VT2 was determined independently by 2 sports physicians, both having more than 5 years of experience with clinical assessment of CPET. All tests results were encoded and both assessors were completely blinded to the 3 different FiO_2 test conditions. For the present study, VT2 was defined by the second nonlinear increase of the $\dot{V}e/\dot{V}CO_2$ curve (21). When VT2s differed between the 2 assessors, consensus on VT2 was obtained by reviewing each other's assessment. Ventilatory threshold was expressed as workload (Watt) level corresponding with VT2.

Determination of 2nd heart rate variability threshold

HRVT2 was determined by 2 independent researchers also blinded to the different FiO_2 test conditions. The values of nRMSSD were displayed every 30 seconds. HRVT2 was determined by visual inspection. It was defined as the breakpoint representing the start of a substantial increase in nRMSSD, after having reached a minimum value (Figure 2.2). The threshold was expressed as workload (Watt) corresponding with the HRVT2. Inflection points (HRVT2) that could not be determined visually be the independent assessors were excluded from the analysis.

Statistical analysis

The subjects were characterized by descriptive analysis of the data (mean±SD). Bland and Altman plot analysis was used for comparing workload values obtained from the 2 AT assessment methods (agreement between VT2 and HRVT2). The obtained AT2s under the 3 FiO_2 conditions were compared using a General Linear Model with repeated measures. For all statistical tests, P values less than 0.05 were considered as significant. Bonferroni adjustment was applied accordingly (IBM SPSS Statistics version 20).

Results

Table 2.1 presents subjects' characteristics. Participants were moderately trained ($\dot{V}O_{2\ peak}$ 54.6±7.0 ml/min/kg). A total of two HRVT2 inflection points during hyperoxic exercise could not be determined and were excluded from our analysis. Mean workloads corresponding to VT2 and HRVT2 (Table 2.2) in hypoxia were respectively 19±17 % (p=0.01) and 15±15% (p=0.1) lower in comparison with hyperoxic conditions. The mean (± SD) workload of the VT2 methods was 242.4 ± 42.8W, compared with 246.7 ± 49W for the HRVT2 method. Figure 2.1 shows the Bland and Altman plots with the bias and 95% limits of agreement (LoA). The LoA for workload (W) were -15.7% to 20.1%. Although HRVT2 gives an acceptably precise agreement of 2.2 % with VT2, the positive bias (systematic error) occurred in 17 out of 31 cases.

Table 2.2. Comparison of the mean AT2s between three different oxygen conditions.

Conditions	Mean ±SD (Watt)	p-value
VT2 Normoxia vs VT2 Hypoxia	251±45 vs. 218.6±39.0	0.3
VT2 Normoxia vs VT2 Hyperoxia	251±45 vs. 258±37	1.0
VT2 Hypoxia vs VT2 Hyperoxia	**219±39 vs. 258±37**	**0.01**
HRVT2 Normoxia vs HRVT2 Hypoxia	244±44 vs. 223±49	1.0
HRVT2 Normoxia vs HRVT2 Hyperoxia	244±44 vs. 263±48	1.0
HRVT2 Hypoxia vs HRVT2 Hyperoxia	223±49 vs. 263±48	0.1

Discussion

To the best of our knowledge, this is the first study that investigated the level of agreement between 2 independent methods for assessing AT2 based on spiro-ergometry (VT2) and HRV (HRVT2) under different FiO_2 conditions. The main finding is that the agreement between VT2 and HRVT2 was acceptably precise. Additionally, the VT method showed significant sensitivity for detecting a shift in AT2 when comparing hypoxic with hyperoxic exercise conditions. Although statistically not significant, a similar shift was also present in our HRVT method. The latter finding indicates that an acutely and externally induced shift in physiological stress results in a simultaneous shift of ventilatory threshold as well as HRVT2. Therefore, the outcome of the present study supports our hypothesis that the proposed HRVT2 method can serve as an appropriate alternative for detecting VT2 without having to use costly spiro-ergometry equipment.

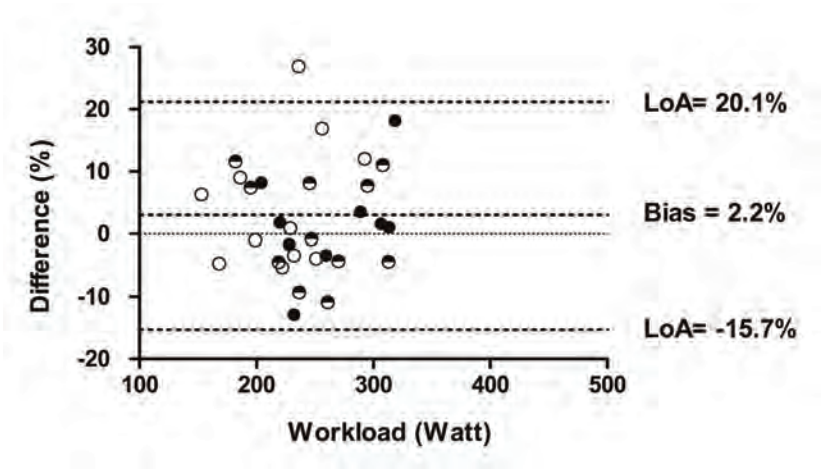
Comparison with other studies on agreement between VT2 and HRVT2

Our Bland and Altman concordance analysis shows an acceptably precise agreement between the VT 2 and HRVT 2 assessment methods. These results are in line with other studies (4, 5, 20). For example, Quinart et al. also reported a good agreement between VT2 and HRVT2 (VT2 expressed in Watts). They investigated 20 obese adolescents (14 girls and 6 boys) who underwent two mixed protocols (combining exercise stages of 3 min duration at the start of the test, and then 1 min duration) before and after a 9-month exercise intervention program (20). Despite the fact that their Bland and Altman analysis demonstrated a low estimation bias and overall good agreement between the two measures (20), their mixed protocol was regarded a study limitation potentially impairing their VT2 accuracy (20). In accordance with their recommendations, we performed linear incremental exercise tests till exhaustion, allowing for optimal detection of VT2 through spiro-ergometry. Although our Bland and Altman analysis shows sufficient agreement between VT2 and HRVT2, future studies using different exercise loading protocols are probably warranted to establish the best protocol for assessing HRVT2.

In the current study, VT2 based on the spiro-ergometry data was used as a ‘gold standard’ and compared to the HRVT2 results. The bias (2.2%), mean absolute difference (7.2%) and limits of agreement (-15.7% to 20.1%) do not confirm a perfect agreement between those 2 methods in

comparison to other authors (4, 20). However, it has been shown previously that an inter-observer error in VT assessments on the basis of ventilatory equivalents for oxygen was ~15% (7). In contrast, Sinclair et al. and others have shown that the inter-observer error between assessed VT based on the V-slope method data ranged between 5.6 - 8.1%, which was considered clinically acceptable (24). In the present study, 22 cases out of 31 (71%) showed a difference ≤8.1%. Therefore, the level of error in other spiro-ergometry VT assessments suggests that acceptably precise HRVT2 results may support relevance of the HRVT concept to replace costly VT measurements in a clinical setting.

Figure 2.1 Bland-Altman scatter plot.



Empty circles – hypoxia; half-filled circles – normoxia; filled circles – hyperoxia; LoA – limit of agreement.

Sensitivity of VT and HRVT

As far as we know, only 1 study previously investigated the sensitivity of the HRVT method to detect training adaptations (13). Exercise training modifies anthropometric features, improves cardio-respiratory variables and lowers activity of the sympathetic nervous system (SNS) during sub-maximal exercise (22). In accordance, Quinart et al. were able to observe improvement in training status. In particular, they observed similarly increased workload corresponding to VT2 (~17.5%) and HRVT2 (~15%). The present study extends on their results by showing a rather

similar difference in workload capacity for VT2 when manipulating FiO_2 conditions. Higher FiO_2 (hyperoxia) decreases and lower FiO_2 (hypoxia) increases the activity of SNS during exercise (14, 29). In comparison with a medium or long-term training intervention study, our approach was intended as a more direct method to test the sensitivity of the HRVT assessment method in response to a change of physiological stress. Although our shift in VT2 under hypoxic and hyperoxic conditions is well in line with previous reports (23)., the HRVT2 method appears less sensitive as the shift in HRVT2 did not reach statistical significance. The latter may also be explained by a type II error as we had to exclude two HRVT measurements under hyperoxic conditions due to difficulties to determine a clear inflection point. The lack of statistical difference between normoxia and hypoxia may also be caused by limited statistical power in our study. Another explanation, in line with the high standard deviations of our measurements, is the recently described variation in the individual response to hypoxia/hyperoxia. The latter may have further reduced the contrast and explain why our study protocol was unable to detect a significant change in VT2 or HRVT2 (Figure 2.2 A, B and C) when comparing normoxia with hypoxia and hyperoxia (8, 15, 18).

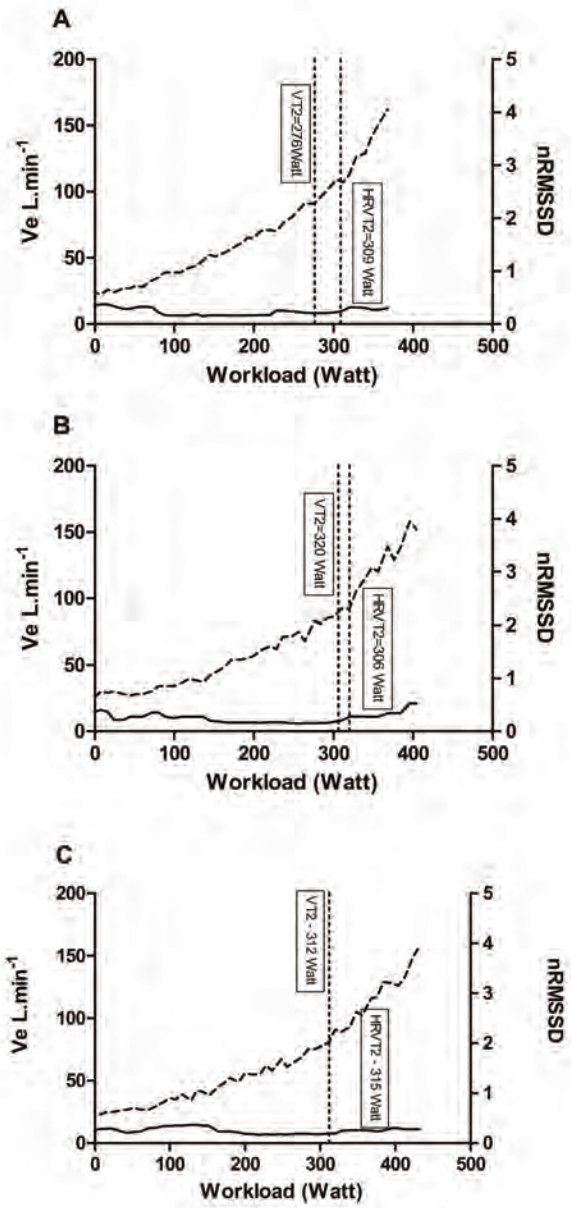
Choice and accuracy and methodological aspects of the HRVT assessment methods

In the present study, an upward inflection in the time-domain HRV measure nRMSSD (RMSSD: RRI ratio) was used to determine HRVT2 as opposed to frequency domain measures (3, 4). By comparison, frequency domain measures should not be applied to a time window of less than 10 times the lower bound of the frequency band (i.e. 67 seconds for a high-frequency lower bound of 0.15 Hz) (1).

Therefore, the present study employed the time-domain measure nRMSSD (RMSSD: RRI ratio), utilizing 30s windows, which was perhaps the simplest to perform (1).

HRV measurements are sensitive to the autonomic nervous system disturbances in pathologies (e.g. Parkinson's disease, diabetes-related autonomic neuropathy (28) and to exercise performance improvement. Especially, during exercise the HRV signal can also be influenced by non-neural factors (16). In particular, the non-neural influences of respiration on the sino-atrial node, particularly during exercise at and above VT2, likely contribute to the upward inflection of

Figure 2.2 Example of assessed AT2 based on minute ventilation and nRMSSD data sets under hypoxic (A), normoxic (B) and hyperoxic (C) conditions.



nRMSSD (1). This makes the time-domain method practically useful as a proxy measure for VT2 detection.

Study limitations

The current study has some limitations. In particular, regarding the identification of HRVT2, a clear upward inflection point could not always be identified. Therefore, 2 measurements were excluded, as both of the 2 independent assessors could not detect an inflection point corresponding to HRVT2.

The currently used, and as far we know, best available VT and HRV methods both require a visual assessment of inflection points in both ventilation and HRV data. As there is no gold standard for determining VT, either of the methods may have introduced some level of bias or error. Although computationally simple and achieving a high temporal resolution, time-domain HRV measures such as nRMSSD have not previously been validated for determining VT2. Previously, frequency-domain measures such as the product of HF power and HF frequency (3, 4) have been employed. Future research could be directed towards refining the application of time-domain or alternative HRV analysis to estimate ventilation thresholds.

Conclusion

In the present study it was shown that there is acceptably precise agreement between the VT2 and HRVT2 assessment methods to detect a shift in ventilatory threshold under different FiO_2 conditions. This opens up new avenues for a cost-efficient and non-invasive HRVT method that could be easily applied to determine, monitor and individualize the appropriate workload intensity in different types of aerobic exercise training modalities and potentially also for altitude training purposes.

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CHAPTER 3

Oxygen delivery is not a limiting factor during post-exercise recovery in healthy young adults

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Submitted

Abstract

It is still equivocal whether oxygen uptake recovery kinetics are limited by oxygen delivery and can be improved by increased availability of oxygen. Given its potential application for optimising an exercise-based rehabilitation program, the present study aimed to investigate whether combined non-invasive measurements of muscle and pulmonary oxygen uptake kinetics can be used to assess oxygen delivery limitations in healthy subjects.

Methods: Sixteen healthy young adults performed three sub-maximal exercise tests (6 min at 40% W_{max}) under hypoxic (14%O_2), normoxic (21%O_2) and hyperoxic (35%O_2) conditions on separate days in randomized order. Both Pulmonary $\dot{V}O_2$ and near infra red spectroscopy (NIRS) based Tissue Saturation Index (TSI) offset kinetics were calculated using mono-exponential curve fitting models.

Results: Time constant τ of $\dot{V}O_2$ offset kinetics under hypoxic (44.9±7.3s) conditions were significantly larger than τ of the offset kinetics under normoxia (37.9±8.2s) and hyperoxia (37.0±6.0). TSI mean response time (MRT) of the offset kinetics under hypoxic conditions (25.5±13.0s) was significantly slower than under normoxic (15.0±7.7) and hyperoxic (13.0±7.3) conditions. Overall, in our healthy subjects, τ values of $\dot{V}O_2$ were significantly larger than MRT TSI (p=0.0001) under all three oxygen conditions. Hyperoxia had no effect on baseline, steady-state and amplitude of $\dot{V}O_2$, TSI or total blood volume (tHb) values in comparison with normoxic conditions. NIRS and spirometry-based recovery kinetics were both sensitive to detect oxygen delivery limitations during hypoxic exercise conditions.

Conclusion: Since oxygen uptake kinetics of healthy subjects do not benefit from supplementary oxygen, our results indicate that performing acute submaximal exercise bouts under normoxic and hyperoxic conditions may assist in detecting oxygen delivery limitations in skeletal muscle of e.g. cardiovascular or pulmonary compromised patients with exercise intolerance. The latter information can potentially be used to discriminate on forehand between responders and non-responders before prescribing hyperoxic exercise training.

Keywords

Exercise, Oxygen Uptake, NIRS, TSI, Hypoxia, Hyperoxia

Introduction

The rate of change of pulmonary oxygen uptake ($\dot{V}O_2$) following an acute bout of submaximal exercise, also defined as $\dot{V}O_2$ offset kinetics, reflect the ability of an individual to recover from exercise [1, 2]. Changes in cardiac output as well as the balance between oxygen delivery and utilization in activated muscle tissue are the main contributing factors influencing oxygen uptake kinetics. In accordance, slower recovery kinetics in patients with an impaired cardiovascular function appear to represent an oxygen delivery limitation [3], either due to impaired blood flow and/or endothelial dysfunction in skeletal muscle [3, 4]. Despite the fact that over the past decennium the application of oxygen uptake kinetics has gained more interest from the field of clinical exercise physiology [2], $\dot{V}O_2$ off-set kinetics alone still provide insufficient information regarding the limiting factor determining exercise tolerance or intolerance. From the theoretical point of view that physiology is aimed at balancing oxygen delivery and consumption in skeletal muscle, it has been suggested that when lowering the inspiratory oxygen fraction (FiO_2) during submaximal exercise, oxygen delivery is the rate-limiting step in recovering from a submaximal exercise bout [5]. In comparison with assessing $\dot{V}O_2$ kinetics at the onset of exercise, the offset oxygen kinetics appear a more sensitive measureto detect oxygen delivery limitations under hypoxic conditions [6]. However, the few available studies have been inconclusive. Some research suggests that exercise tolerance and $\dot{V}O_2$ onset kinetics can be improved by increased oxygen availability through a higher inspiratory fraction of oxygen (FiO_2 =30%) [7], whilst others did not show any improvement [8, 9]. Despite these equivocal findings, these studies suggest that manipulating FiO_2 might be a useful strategy to determine whether oxygen delivery is a performance-limiting factor.

In summary, the offset kinetics following a submaximal bout of exercise under both hypoxic, normoxic and hyperoxic conditions has the potential to serve as a diagnostic tool to assess skeletal muscle oxygen delivery dysfunction in an individual. Simultaneous measurements of muscle tissue oxygenation (SmO_2) derived from spatially resolved near infrared spectroscopy (NIRS) can be used as a measure of fractional oxygen extraction. In this way, the relationship between oxygen delivery and consumption can be determined at the skeletal muscle level, exposing the peripheral contributors to slowing or speeding of pulmonary oxygen kinetics. Speeding of muscle oxygenation kinetics in the presence of, for instance, hyperoxia will indicate

improved O_2 delivery relative to utilization, whilst the opposite can be achieved by lowering O_2 delivery under hypoxic conditions. Given its potential application for designing an exercise-based rehabilitation program, the present study aimed to investigate whether combined non-invasive measurements of muscle and pulmonary oxygen uptake kinetics can be used to assess oxygen delivery limitations in healthy subjects. Based on the available research, we hypothesized *a priori* that in moderately trained subjects hyperoxia would improve oxygen uptake recovery kinetics, while hypoxia would do the opposite.

Methods

Subjects

Sixteen healthy, young adults (BMI: 22.0 ± 1.5kg/m^2, (22±2 yrs) were recruited through social media at Erasmus University Medical Centre in Rotterdam, the Netherlands and agreed to participate in the study (Table 1). The study protocol, which was a sub study of a larger clinical trial on optimization of exercise therapy in type 2 diabetes patients, was approved by the regional Medical Ethics Committee of the Erasmus University Medical Centre in Rotterdam, the Netherlands (MEC; number: 2012-128; and registered at the Dutch Trial Registry number: NTR3777.

Experimental protocol

Subjects visited the Clinical Exercise Performance Laboratory (CEPL) four times. An interview, physical examination and all exercise tests (1 maximal + 3 submaximal tests) were at the Erasmus University Medical Center in Rotterdam, the Netherlands within a time frame of 4 weeks. During the first appointment a sports physician performed an interview and physical examination. To assess maximal workload (W_{max}) and maximal oxygen uptake ($\dot{V}O_{2\ peak}$) subjects were asked to perform a standard incremental exercise test on a cycle ergometer (protocols: ramp 120 (2 Watt/10 seconds) for women and a ramp 180 (3 Watt/second) for men). Perceived exertion level after the incremental exercise test was rated using a Borg Scale [10].

Blinding procedure

During the next three visits (with 7 days washout periods) participants underwent a sub-maximal exercise test under various inspiratory fractions of oxygen (FiO_2) 14%, 21% and 35% O_2 (BOC Morden, London, UK). The subjects were blinded to the randomized order of FiO_2 during the submaximal tests by drawing an opaque sealed envelope. The sub-maximal exercise test protocol was as follows: 10 minutes of rest, 3 minutes of unloaded cycling, 6 minutes of cycling at 40% of their W_{max} and 5 minutes of recovery. Subjects were instructed to maintain cadence between 60 and 80 revolutions per minute (rpm).

Table 3.1. Subjects' characteristics

n = 16 (10 male and 6 female)	**Mean ± SD**
Age (years)	22.3 ± 2.4
Weight (kg)	77.4 ± 11.9
Height (cm)	183 ± 9
BMI (kg/m²)	23.0 ± 2.3
$\dot{V}O_{2\ peak}$ **(ml/min/kg)**	45.6 ± 8.8
Wmax (Watt)	315.2 ± 63.0
ATT (mm)	6.1 ± 3

BMI - Body Mass Index; $\dot{V}O_{2\ peak}$ maximum oxygen uptake; ATT adipose tissue thickness;

Medical gasses

Each test was performed under either mixture of 14%, 21% and 35% of oxygen in nitrogen - 50 liter cylinders (BOC Morden, London, UK). The control oxygen conditions (21% of oxygen in nitrogen) were ordered and prepared in the EMC and delivered to the CEPL by the internal medical gasses distributor (Linde Gas, The Netherlands). The air was inspired from a cylinder through a Douglas bag (20 liter) connected to an oro-nasal 7400 Vmask™ and a 2730 2-way Y-shape™ non-rebreathing two-way valve (Hans Rudolph, inc. Kansas, USA).

Respiratory gas measurements

The analysis of oxygen uptake ($\dot{V}O_2$) and production of carbon dioxide ($\dot{V}CO_2$) levels were continuously measured through a metabolic cart (Oxycon Pro, Jaeger, Mannheim, Germany).

NIRS measurements

The methodology of the NIRS measurement procedures as well as data collection of an absolute measure of tissue oxygen saturation (tissue saturation index (TSI), have been described elsewhere [11]. Given the thickness of the subcutaneous adipose tissue may confound the NIRS signal amplitude, the skinfold thickness was measured and reported. In particular, a skinfold measured with a calliper was divided by 2 to give adipose tissue thickness (ATT) [11].

Absolute values

The methodology of calculating all $\dot{V}O_2$ and TSI absolute values (amplitude, baseline and steady-state) were described in detail in a reproducibility study of Niemeijer et al. [11].

Pulmonary VO_2 kinetics

Fitting of mono-exponential curves of onset and offset oxygen uptake kinetics was performed in Python 2.7 (Python Software Foundation), in order to calculate the time constant and increase in oxygen uptake. Two formulas were used for offset kinetics, as described before [12].

$$\dot{V}O_2\ (t) = \dot{V}O_{2\ \text{steady state}} - B * (1 - e^{-(t - Td)/\tau})$$

B = $\dot{V}O_2$-amplitude during exercise (ml/min), Td = time delay (s) and τ = time constant tau (s).

NIRS kinetics analysis

Time constants (τ) of recovery were calculated by fitting the TSI data to a first-order (mono-exponential) model using the non-linear least squares method (Python 2.7, Python Software Foundation). Additionally, the mean response time (MRT) was calculated as the sum of tau and time delay (MRT = τ + Td). Considering better reproducibility, we used MRT TSI for the kinetics comparisons with tau $\dot{V}O_2$. The coefficient of determination (r^2) was applied to

determine how well the fitted mono-exponential curve approximated the real data points. Additionally, r^2 ranges from 0 to 1 with 1 as an indicator for a line that perfectly fits the real data. The methodological details of the recovery TSI kinetics are available elsewhere [11].

Statistical analysis

Subject' characteristics were expressed as mean±SD. The obtained results under the three oxygen conditions were compared using a General Linear Model with repeated measures (IBM SPSS Statistics version 20). Level of significance was set at $p < 0.05$. The Bonferroni post-hoc analysis was used in multiple comparisons.

Results

VO_2 kinetics

Curve fitting level was sufficiently accurate for hypoxia (r^2=0.89±0.07), normoxia (r^2=0.92±0.04) and hyperoxia (r^2=0.92±0.04). The τ of $\dot{V}O_2$ offset kinetics under hypoxic conditions was respectively 7±9 and 8±11 seconds larger than τ of the offset kinetics under normoxia (p=0.02) and hyperoxia (p=0.04) (Table 3.3). However, there was no significant difference in τ between normoxic and hyperoxic conditions (Table 3.3).

TSI kinetics

Monoexponential curve fitting was sufficiently accurate for hypoxia (r^2=0.97±0.02), normoxia (r^2=0.93±0.06) and hyperoxia (r^2=0.90±0.11). The MRT of TSI offset kinetics under hypoxic conditions was respectively 10±11 and 12±13 seconds larger than τ of the offset kinetics under normoxia (p=0.007) and hyperoxia (p=0.008) (Table 3.3). Hyperoxic conditions did not speed the offset kinetics. The τ values of $\dot{V}O_2$ were significantly larger than MRT of TSI (p=0.0001) under the oxygen conditions (Table 3.3).

Absolute baseline and steady-state values of VO_2 and TSI

The absolute steady-state values of $\dot{V}O_2$ were not different in normoxia ($\dot{V}O_2$ p=1.0) and hyperoxia ($\dot{V}O_2$ p=1.0) than in hypoxia. Only the absolute steady-state values of TSI were significantly different in hypoxia compared with normoxia (p=0.0001) and hyperoxia (p=0.003).

TSI amplitude values were significantly different in between normoxia and hyperoxia (p=0.01) and normoxia and hypoxia (p=0.001) (Table 3.2). Additionally, there was no difference (p=1.00) in amplitude of total blood volume in the muscle (tHb) between the FiO_2 conditions.

Discussion

In the present study we investigated whether higher FiO_2 can be used to determine a musculoskeletal oxygen delivery limitation during recovery following a constant-load submaximal bout of exercise. In contrast with our hypothesis, the main finding of this study was that the higher FiO_2 conditions did not accelerate the recovery kinetics and that oxygen delivery during normoxic and submaximal exercise was not a limiting factor in healthy and moderately trained participants.

Oxygen uptake and muscle oxygenation offset kinetics under hyperoxic conditions

To date, it is still equivocal whether additional oxygen availability in inspired air can be beneficial in improving oxygen-delivery-dependent exercise tolerance. The effect of the higher FiO_2 on the recovery kinetics rate may both depend on cardiovascular function [9] and individual sensitivity to manipulated FiO_2 conditions [17]. Although cardiovascular compromised patients may show improved exercise tolerance from extra oxygen [18], it is still challenging to differentiate responders from non-responders. Although the present study was performed in healthy subjects without any known cardiovascular impairments, intra-individual comparisons and differences in recovery kinetics between hypoxic and normoxic conditions suggest that manipulating FiO2 can be used to test whom potentially may benefit from an increased FiO2 during submaximal exercise conditions.

Opposite to our original hypothesis, we did not find beneficial effects of hyperoxia on neither $\dot{V}O_2$ or muscle oxygenation offset kinetics (Table 3.3). As such, our results extend on the study by Macdonald et al. who performed submaximal exercise test below the ventilatory threshold in normoxia and hyperoxia (70% O_2) and reported no additional benefits of hyperoxia on $\dot{V}O_2$ offset kinetics [8]. Both our and their results indicate that during submaximal exercise muscle oxygen delivery is not a limiting factor in healthy subjects. This raises the question whether other subjects with impairments in cardiovascular and/or pulmonary oxygen delivery might improve their exercise tolerance by additional oxygen availability in inspired air during submaximal

exercise. For example, Payen et al. reported that hyperoxic air accelerated recovery time by 36% in COPD patients with diminished oxygen delivery function[19]. Following the same line of thought, Kemps et al. have suggested that faster offset kinetics were associated with better exercise tolerance in CHF patients with peripheral O_2 delivery deficit [20]. In accordance, both cardiovascular and pulmonary compromised patients could potentially benefit from testing submaximal recovery oxygen kinetics under normoxic and hyperoxic exercise conditions to assess whether hyperoxia could reduce the mismatch between oxygen delivery and consumption.

Despite previous work by Grassi et al. suggests that muscle oxygen kinetics are closely related to pulmonary oxygen uptake kinetics [21]. The reported results on slower kinetics of both muscle oxygenation and pulmonary $\dot{V}O_2$ under hypoxic conditions than under normoxic and hyperoxic conditions in the present study extend on these results (Table 3.3). As it also has been reported that muscle oxygenation rate has similar recovery kinetics as PCr following a submaximal exercise bout [22], recovery kinetics assessment using NIRS onlycan potentially be a more straight forward and cost-effective method.

Regarding the peripheral oxygen delivery limitations, Belardinelly et al. have shown that the $\dot{V}O_2$ and muscle oxygenation recovery kinetics in CHF patients were slower than in healthy subjects. Interestingly, the authors reported no difference in MRT of $\dot{V}O_2$ and muscle oxygenation kinetics speed in CHF patients (0% difference) while these kinetics measures were clearly different in healthy subjects (48% difference). The authors try to explain this by suggesting that muscle blood flow was reduced in CHF patients because of the peripheral cardiovascular dysfunction i.e. inability to increase blood flow in peripheral arteries to meet oxygen delivery needs [4]. The latter could be a potential explanation for our results as our healthy subjects without oxygen delivery limitations have shown a significant difference between tau $\dot{V}O_2$ and the MRT TSI recovery kinetics under hypoxia (20±16s), normoxia (23±10s) and hyperoxia (24±9s). Additionally, MRT has been reported as more reproducible for muscle oxygenation kinetics than tau [16].

Table 3.2. Mean baseline (rest) and steady-state absolute values of $\dot{V}O_2$ and TSI under hypoxic, normoxic and hyperoxic conditions.

	Hypoxia	Normoxia	Hyperoxia	P-value
			Baseline	
$\dot{V}O_2$ (ml/kg/min)	2.9±0.4	3.0±0.5*	3.2±0.5**	0.06*; 0.8**; 1.0***
TSI (%)	67.2±5.3	68.4±4.8*	70.8±7.8**	0.8*; 0.3**; 0.6***
tHb (µM_cm)	118.6±38.9	115.1±39.1*	115.5±37.8**	0.4*; 0.68**; 1.0***
			Steady-state	
$\dot{V}O_2$ (ml/kg/min)	20±3.1	19±2.9*	18.9±2.9**	1.0*; 1.0**; 1.0***
TSI (%)	**60.3±7.6**	**65.4±6***	**66.1±8.9****	**0.0001*; 0.003****; 1.0***
tHb (µM_cm)	109.7±36.4	106.4±36.1*	106.7±34.8**	0.28*; 0.56**; 1.0***
			Amplitude of Recovery	
$\dot{V}O_2$ (ml/kg/min)	15.6±2.4	15.3±2.4*	15.4±2.4**	1.0*; 1.0**; 1.0***
TSI (%)	**9.7±5.0**	**6.4±4.1***	**5.8±2.7****	**0.001*; 0.01****; 1.0***
tHb (µM_cm)	-8.8±4.5	-8.7±4.4*	-8.7±5.9**	1.0*; 1.0**; 1.0***

Amount of '*' assigns the statistical difference between FiO_2 conditions (*-hypoxia with normoxia; **-hypoxia with hyperoxia and ***-normoxia with hyperoxia) to a p-value.

Taken together, these results suggest that recovery kinetics following a moderate-intensity bout of exercise under hyperoxic conditions were not limited by oxygen delivery in young and healthy subjects. Using both the $\dot{V}O_2$ and muscle oxygenation recovery kinetics measurements under increased FiO_2 may help detecting cardiovascular oxygen delivery limitations in deficient subjects. Acutely improved muscle oxygenation and $\dot{V}O_2$ kinetics may be used to decide on the potential benefit of additional oxygen concentrations during exercise training in subjects with oxygen delivery limitations.

Oxygen uptake and muscle oxygenation offset kinetics under hypoxic conditions

In order to investigate the sensitivity of NIRS and $\dot{V}O_2$ measurements we investigated offset kinetics of $\dot{V}O_2$ and TSI under hypoxic conditions as a model to artificially induce an oxygen delivery limitation in healthy subjects. In line with previous work in this area [1] we found slower oxygen uptake and muscle oxygenation kinetics (Table 3.3). Slower recovery kinetics under lower FiO_2 conditions may suggest a blunted compensatory hyperemic vasodilatory response (decreased blood volume) [4] in older/diseased subjects [23]. In particular, subjects with more severe circulatory dysfunction had slower recovery $\dot{V}O_2$ kinetics [4]. In our study there was no difference in the amplitude of the total volume of blood (tHb) between the FiO_2 conditions. This suggests that there was no need for compensatory vasodilation to increase oxygen delivery under hypoxic conditions.

Table 3.3. Mean τ values of $\dot{V}O_2$ and TSI offset kinetics under hypoxic, normoxic and hyperoxic conditions.

	Hypoxia	Normoxia	Hyperoxia	P-value
			Offset kinetics	
τ $\dot{V}O_2$ (s)	44.9±7.3	37.9±8.2*	37.0±6.0**	**0.02*; 0.04****; 1.0***
MRT $_{TSI}$ **(s)**	25.5±13.0	15.0±7.7*	13.0±7.3**	**0.007*; 0.008****; 0.8***

Amount of '*' assigns the statistical difference between FiO_2 conditions (*-hypoxia with normoxia; **-hypoxia with hyperoxia and ***-normoxia with hyperoxia) to a p-value.

These results suggest that hypoxia may induce higher oxygen consumption demand in skeletal muscle despite low exercise intensity (table 3.2). However, a higher TSI amplitude could also be induced by higher relative intensity at steady-state under hypoxic conditions (RER=1.02±0.1) in comparision with normoxia (0.96±0.1) and hyperoxia (0.92±0.1).

Taken together, slower TSI and $\dot{V}O_2$ recovery kinetics under hypoxic conditions show good sensitivity of NIRS and spiro-ergometry measurements and the kinetics assessment methods as the lack of sensitivity to hypoxia would suggest an unreliable detection of oxygen delivery limitation under hyperoxic conditions.

In summary, the present study design extends on previous studies by applying both hypoxic and hyperoxic conditions in assessing oxygen uptake recovery kinetics. The slower recovery kinetics during hypoxia suggest good sensitivity of $\dot{V}O_2$ and TSI kinetics measurement methods. On the other hand, no improvement in the recovery kinetics on both pulmonary ($\dot{V}O_2$) and muscle (TSI) levels under higher FiO_2 indicates that healthy medium-trained subjects have no oxygen delivery limitation during submaximal exercise under normoxic condtions and will probably show no benefit of hyperoxic exercise training

As we were only able to study healthy young subjects, our results warrant future studies in CHF, type 2 diabetes and COPD patients. Furthermore, because of the interpersonal-response variability to hyperoxic conditions, it stills needs to be seen whether observations following acute exposure to different levels of hyperoxia can be translated or be used to predict the medium and long-term response to hyperoxic exercise training.

Conclusion

Since oxygen uptake kinetics of healthy subjects do not benefit from supplementary oxygen, the present study indicates that performing acute submaximal exercise bouts under normoxic and hyperoxic conditions may assist in detecting oxygen delivery limitations in skeletal muscle of e.g. cardiovascular or pulmonary compromised patients with exercise intolerance. The latter information can potentially be used to discriminate on forehand between responders and non-responders before prescribing hyperoxic exercise training.

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CHAPTER 4

Hyperoxia increases arterial oxygen pressure during exercise in type 2 diabetes patients: a feasibility study

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Abstract

Objective: The study investigated the feasibility and potential outcome measures during acute hyperoxic in type 2 diabetes patients (DM2).

Methods: Eleven DM2 patients (7 men and 4 women) were included in the study. The patients cycled (30 min at 20% W_{max}) while breathing 3 different supplemental oxygen flows (SOF, 5, 10, 15 L/min). During hyperoxic exercise arterial blood gases and intra-arterial blood pressure measurements were obtained.

Results: Arterial pO_2 levels increased significantly (ANOVA, $p<0.05$) with SOF: 13.9±1.2 (0 L/min); 18.5±1.5 (5L/min); 21.7±1.7 (10 L/min); 24.0±2.3 (15 L/min). HR (HR) and pH increased significantly after terminating administration of hyperoxic air.

Conclusions: A SOF of 15 L/min appears to be more effective than 5 or 10 L/min. Moreover, HR, blood pressure, blood lactate, pH are not recommended as primary outcome measures.

Keywords

Diabetes Type 2, Hyperoxia, Exercise, Oxygen, Dose.

Introduction

Breathing a hyperoxic gas mixture has been shown to acutely enhance power output (W) by 8 -13% [1-6], increase oxygen uptake ($\dot{V}O_2$) by 10-14% [2, 3, 6-10], decrease blood lactate level [11] and lower perceived exertion [7] during aerobic type of exercise. Both healthy subjects and COPD patients show improved exercise performance with hyperoxia [12-14]. These findings suggest that certain other clinical populations with impaired cardiovascular and./or -pulmonary fitness levels, might benefit from exercise under hyperoxic conditions as well. Patients with type 2 diabetes (DM2) might be good candidates for hyperoxic exercise training as previous research indicated that DM2 patients frequently have a reduced diffusion capacity of the lungs (8-25%), inversely related to blood glucose levels as well as duration and severity of DM2 [15-17]. Pathophysiological mechanisms explaining the impaired pulmonary function may be micro-angiopathy, chronic inflammation and autonomic neuropathy [16, 18] resulting in a diminished alveolar micro-vascular reserve [15, 17, 19-21]. Impaired alveolar gas exchange in DM2 patients has been shown to correlate with a lower $\dot{V}O_2$ and workload capacity during aerobic type of exercise [22].

Although beneficial effects of exercise under hyperoxic conditions have been reported for different types of chronic disease populations [1, 4, 8, 23-26], experimental data on an effective dose of hyperoxic air during exercise in DM2 patients are still lacking. Despite the ongoing debate on oxygen transport and consumption [50], increased oxygen availability in arterial blood may improve intracellular transport and uptake of active muscle tissue, and subsequently improve exercise performance. In accordance, the aim of the present feasibility study was to establish an effective dose of supplemental oxygen in deconditioned DM2 population as a basis to guide and optimise future hyperoxic exercise training protocols.

Methods

Subjects

Eleven patients diagnosed with DM2 for at least 2 years and not taking anti-hypertensive medication were screened and included at the outpatient clinic at Erasmus University Medical Center in Rotterdam, the Netherlands. The characteristics of the eligible patients are presented in Table 4.1. Out of 22 screened patients, a total of six patients were not willing to participate in the hyperoxic exercise intervention following the maximal exercise test. Four patients were excluded from the hyperoxic experiment because it was not possible to introduce an intra-arterial catheter in the radial artery. One patient was excluded from our study because of

abnormally high lactate levels during exercise and was diagnosed with mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome afterwards. Baseline characteristics of excluded patients were not different from the experimental group. Included subjects gave their informed consent to participate in the study, approved by the medical ethical committee of the Erasmus University Medical Center in Rotterdam (ISRCTN number: NTR2299).

Table 4.1. Subject characteristics

(n=11)	Mean±SD
Sex (male: female)	7:4
Age (yr)	56.3±6.3
T2D duration (yr)	10.5±6.6
Weight (kg)	87.7±16.5
Length (cm)	171.1±11.0
BMI (kg/m^2)	30.1±6.1
Abdominal circumference (cm)	100±13
Fat percentage* (%)	33.9±9.1
Fasting glucose (mmol/L)	11.3±3.0
HbA1c (%)	8.3±1.3

* based on skinfold measurements (Durnin & Womersly) (Durnin & Womersley, 1969) No significant differences ($p<0.05$)

Procedures

Prior to the hyperoxic exercise session all subjects performed a maximal exercise test on a cycle ergometer (Jaeger ER800) using an incremental workload (1.85 Watt/6 s for men, 1.2 Watt/6s for women). The oxygen uptake ($\dot{V}O_2$) (Oxycon Pro, Viasys, Houten, Netherlands) and HR (HR) (Polar wear-link, Finland) were measured continuously. A second visit was scheduled within 1-3 wks following a maximal exercise test. During the second visit subjects performed a hyperoxic exercise session. The bout consisted of 25 min of submaximal cycling at 20% of the maximal workload capacity (W_{max}). The workload was chosen to ensure that the subjects reach steady-state, based on the assumption that the anaerobic threshold is at least 40% W_{max} in DM2 patients. After calibration, beat-to-beat blood pressure was obtained through a percutaneous intra-articular catheter in the radial artery of the non-dominant hand

[51]. Data were registered in a computer and analysed using specialized software (Beat scope, Finapres Medical Systems, Amsterdam, the Netherlands). The intra-articular catheter was inserted following the Allen's test. It was performed to minimize the risk of ischemia of the hand. The exercise protocol consisted of 6 phases: 5 min of rest without supplemental oxygen flow (SOF), 10 min without SOF, 5 min with 5 L/min SOF, 5 min with 10 L/min SOF, 5 min with 15 L/min SOF, 5 min without SOF (figure 4.1). Stage duration of 5 min was chosen to reach steady-state during, at least, the last 2 min of each stage [27, 28]. The last stage was added to assess the effect of cessation of SOF.

The SOF was administered directly into a face mask (without a reservoir bag, Teleflex Inc. Hudson RCI adult Multi-Vent air entrainment mask), allowing the inhalation of room air to meet the subjects' ventilatory demands. In our study we chose to dose oxygen as a fixed flow quantity, instead of a fixed inspirational fraction with a maximum of 15 L/min. This design was chosen to match the possibilities of the standard facilities for supplemental oxygen available in most primary and secondary healthcare settings. During the last min of each phase arterial blood gas and the rate of perceived exertion (Borg score) [29] was obtained.

Statistical Analysis

An independent sample T-test was used to analyse the baseline characteristics and maximal exercise test. We used a single factor ANOVA with repeated measures to compare the means of the given variables during different hyperoxic exercise phases. Differences with a p-value <0.05 were considered as significant. The Bonferroni adjustment was applied. Data were presented as mean ± SD.

Results

Participants

The characteristics of the included participants are presented in Table 4.1.

Maximal exercise test

The average maximal oxygen uptake ($\dot{V}O_{2\ peak}$) of the subjects was 1.83±0.59 L/min. The mean $\dot{V}O_{2\ peak}$ was on average ~24% ($p<0.05$) below the average of a healthy population of the same age, weight, length and sex based on the regression equations by Fairbarn et al. [30]. Maximal HR was not significantly different form the predicted values according to the

Tanaka regression equation [31]. The results of the maximal exercise test are presented in Table 4.2.

Hyperoxic exercise: blood gas analysis

All included subjects were able to complete 25 min of submaximal hyperoxic exercise. Arterial pO_2 levels (Figure 4.2a) did not change immediately after starting the exercise (T1-2). The arterial pO_2 increased significantly with increased SOF (T2-T5) in a dose-dependent manner and returned to baseline after cessation of the SOF administration (T5-T6). The arterial pCO_2 (Figure 4.2b) did not change significantly in response to exercise under different SOF. There was a decreasing trend of the pH (Figure 4.2c) at the onset of the exercise bout (T1-T2), while pH increased significantly (p-value) during the last step when the supplemental oxygen flow administration was stopped (T5-T6).

Hyperoxic exercise: HR, blood pressure and rate of perceived exertion

The HR (Figure 4.3a) increased significantly after the onset of the exercise bout (T1-T2), and remained unchanged during the SOF phases (T2-T5) and increased after stopping the SOF (T5-T6). After an initial increase of systolic and diastolic blood pressure after starting the exercise (T1-2), systolic, diastolic and mean arterial blood pressure (Figure 4.3b-d) did not change during the SOF. Furthermore, the rate pressure product (Figure 4.3e) showed a significant increase after stopping the SOF (T5-T6). The rate of perceived exertion (Figure 4.3f) increased with the start of exercise (T1-2) and did not increase significantly during exercise (T2-T6).

Discussion

We tested the feasibility of hyperoxic exercise and dose-response in deconditioned type 2 diabetes patients. The main finding of this feasibility study was that exercise under a supplemental oxygen flow of 15 L/min increased pO_2 more effectively than lower doses (5 and 10 L/min) in DM2 patients.

Technical feasibility

From a technical perspective our results demonstrate that supplemental oxygen, applied with a standard open facemask (5-15 L/min), results in significant increases in arterial pO_2 levels during exercise. Higher pO_2 at increased SOF (i.e. 5, 10 and 15 L/min) suggests a dose-

Table 4.2. Maximal exercise test (n=11)

	Maximal	Predicted	% predicted	AT	Ratio AT/max.
Load (watt)	145.5±61.9	182.1±74.3†	84±24*	85.1±38.5	0.58±0.1*
Load/weight (watt/kg)	1.70±0.7	2.52±0.82	69±23*	0.99±0.4	0.58±0.1*
$\dot{V}O_2$ (ml/min)	1830±593	2499±773†	76±21*	1334±354	0.75±0.1*
$\dot{V}O_2$ (ml/min.kg)	21.4±7.0	28.9±8.8	76±24*	15.5±4.0	0.75±0.1*
HR (bpm)	155±18	169±5‡	92±10	128±17	0.83±0.1*
RER	1.09±0.1			0.92±0.1	0.85±0.1*
SBP (mmHg)	180±30				
DBP (mmHg)	79±12				
RPE (Borg)	15.8±2.8				

AT: anaerobic threshold using a V-slope method; SBP: systolic blood pressure; DBP: diastolic blood pressure; * Significant difference ($p<0.05$)

† Using the Fairbarn & Wasserman equations (Fairbarn et al., 1994; Wasserman & Whipp, 1975)

‡ Using the Tanaka equation (Tanaka et al., 2001)

dependent effect. The pO_2 levels obtained from the radial artery during hyperoxic exercise in the present study (24.0±2.3 kPa) were comparable with the arterial pO_2 levels measured by Plet et al. in healthy subjects. Administration of 55% of oxygen improved maximal oxygen uptake by 12% during cycle-ergometry in comparison with normoxic exercise [9]. Other studies investigating the influence of hyperoxia during exercise found slightly higher pO_2 levels of approximately 40 kPa obtained from the femoral artery with an inspired oxygen fraction of 60% [32-34]. Taken together, our data show that supplemental oxygen applied during submaximal exercise via a standard open face mask increases arterial pO_2 levels. Additional oxygen availability could compensate for the diminished diffusion capacity, endothelial function and low aerobic capacity seen in most deconditioned DM2 patients [15, 17, 19-21]. The latter suggests that a hyperoxic training study in deconditioned DM2 patients could be a potential solution in a medical fitness centre, since no special equipment is needed other than an open facemask and standard gas cylinders with O_2. However, before investigating training effects under hyperoxic conditions, this warrants further controlled trials on cardiovascular and pulmonary function in DM2 patients. The results will improve

our understanding on whether additional oxygen during exercise may improve oxidative metabolism in populations such as DM2 with deficient cardiovascular and respiratory function.

Patient recruitment and study population

Despite the invasive nature of our study and the fact that use of antihypertensive medication was an exclusion criterion for the present study, the majority (76%) of the eligible DM2 patients that were approached in our outpatient clinic were willing to participate in our feasibility study. Although, a training study requires a more long-term commitment, the willingness to participate in our feasibility study indicates that it might be possible to recruit a sufficient and representative proportion of subjects for a randomized clinical trial on the medium-term effects of hyperoxic exercise training.

In accordance with previous studies [35-38], the mean $\dot{V}O_{2\ peak}$ of the investigated patient sample is well below the average of the healthy population, even when corrected for a high BMI. High HbA1c and fasting glucose levels showed that our deconditioned and overweight subjects had poorly regulated DM2. As such, the present study population may not be representative for the general, well-controlled DM2 population. Long-term adherence has been reported to vary substantially (10-80%) in conventional exercise programs for DM2 patients [39-44]. However, effects of hyperoxic exercise training in other deconditioned patient populations, with a reduced alveolar and capillary diffusion capacity [12-14], showed the anticipated increase in exercise capacity. Less perceived exercise intensity and improvement of exercise performance will also motivate deconditioned, overweight and poorly regulated patients with DM2 to adhere to hyperoxic exercise training.

Potential outcome measures

In contrast with previous hyperoxic exercise studies [8, 9, 33, 45-47], we observed no change in HR, blood pressure or rate of perceived exertion during exercise while increasing the supplemental oxygen flow during exercise. However, after stopping administration of SOF we observed a significant increase in HR and rate pressure product (HR * systolic blood pressure (SBP)). The cardiovascular response during phase 6 indicates that hyperoxia lowers the cardiovascular burden during submaximal steady state exercise in deconditioned patients with DM2. A number of physiological mechanisms might explain why, in comparison with previous hyperoxic exercise studies, SOF did not lower HR and systemic blood pressure

during phase 3-5 in our experimental set up. First, it is possible that even at an exercise intensity of 20% $\dot{V}O_2$peak, our deconditioned patients were not completely in a steady- state condition during phase 3-5. Second, in comparison with previous hyperoxic exercise studies, the absolute exercise intensity may have been too low to cause a significant drop in HR, blood pressure or the rate of perceived exertion (Borg score). Third, the arterial wall stiffening in combination with the diabetes-related endothelial dysfunction may have impaired a normal vascular response to hyperoxia [48, 49].

Limitations of the study

Unfortunately, for medical ethical reasons (invasive study) it was difficult to add a healthy control group or different oxygen conditions. Because of this limitation we can only speculate about the physiological reason for this abnormal response to hyperoxic exercise. Arterial blood gas collection (arterial blood withdrawal) was vastly limited because of impaired structure of arterial walls in the DM2 patients. Nevertheless, the present feasibility study suggests that HR, blood pressure and rate of perceived exertion may not be suitable primary outcome measures for a hyperoxic training study in unfit DM2 patients. Instead, direct assessment of the $\dot{V}O_{2\ peak}$ or potentially the use of $\dot{V}O_2$ and/or NIRS kinetics (see chapter 3) should be considered in a hyperoxic training study to monitor and document change in exercise performance.

Conclusions

Based on arterial pO_2 measurements, a supplemental oxygen flow of 15 L/min appears sufficient to compensate for impaired alveolar and capillary oxygen transport and/or consumption in deconditioned DM2 patients. Based on this feasibility study and the results presented in chapter 3, we propose to first investigate acute effects of various inspiratory oxygen fractions on the cardio-respiratory system and speed of oxygen uptake or NIRS kinetics. This will improve our understanding on potential exercise performance enhancement benefits of supplementary oxygen. This would warrant future studies to investigate the medium-and long-term benefits of hyperoxic exercise training in patients with DM2.

Table 4.3. Hyperoxic exercise session

	T1	T2	T3	T4	T5	T6
pO_2 (kPa)	13.4±1.1	13.9±1.2	18.5±1.5[a]	21.7±1,7[a]	24.0±2.3[a]	13.5±1.5
Oxygen content (mmol/L)	8.3±1.0	8.3±0.9	8.3±1.0	8.6±1.2	8.4±1.0	8.2±1.1
pCO_2 (kPa)	4.9±0.5	5.1±0.3	5.1±0.4	5.1±0.4	5.0±0.5	4.9±0.5
pH	7.40±0.0	7.39±0.0	7.40±0.01	7.40±0.02	7.40±0.03	7.41±0.02[b]
Lactate (mmol/L)	1.96±0.8	2.36±0.9	2.21±1.01	2.13±1.09	2.00±1.09	2.03±1.10
Heart rate (bpm)	86±11[c]	102±13	102±13	102±12	106±12	110±13[c]
Systolic blood pressure (mmHg)	159.4±19.5[d]	192.6±31.7	186.1±30.0	186.0±29.7	183.3±33.5	187.1±27.3
Diastolic blood pressure (mmHg)	85.6±10.2	90.5±11.0	88.9±11.1	89.1±9.9	89.0±11.7	89.7±10.1
Rate pressure product	13626±2483[e]	19722±4569	19106±4000	19062±4115	19471±5112	20627±4137[e]
Mean arterial pressure (mmHg)	110.2±12.4	124.5±17.1	121.3±16.6	121.4±15.6	120.5±18.2	122.2±15.0
Rate of perceived exertion (/20)	6.0±0.0[f]	9.8±2.0	10.8±2.6	11.6±3.3	12.3±4.3	13.1±4.9

[a] T3-5 are significantly different from T1,T2 and T6, and each other ($p<0.05$).

[b] T6 is significantly higher than T2 and T3 ($p<0.05$).

[c] T1 is significantly lower than T2-6 . T6 is significantly higher than T1, T3, and T4 ($p<0.05$).

[d] T1 is significantly lower than T2, T3 and T6 ($p<0.05$).

[e] T1 is significantly lower than T2-6. T6 is significantly higher than T1 and T3 ($p<0.05$).

[f] T1 is significantly lower T2-T6 ($p<0.05$).

Figure 4.1. Hyperoxic exercise protocol. ABG = arterial blood gas, RPE = rate of perceived exertion (Borg score).

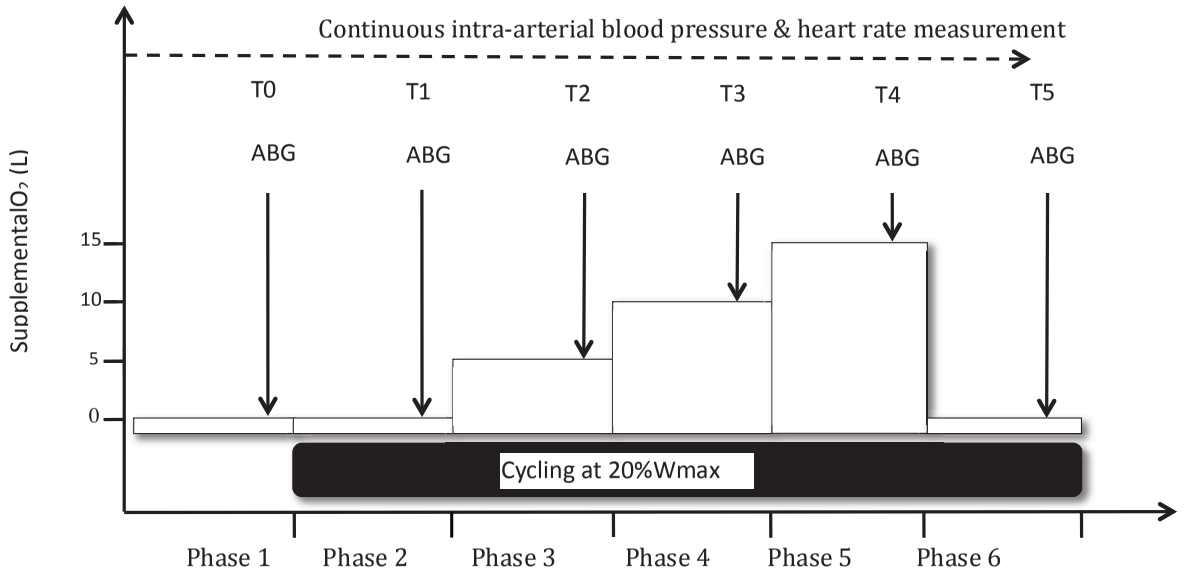
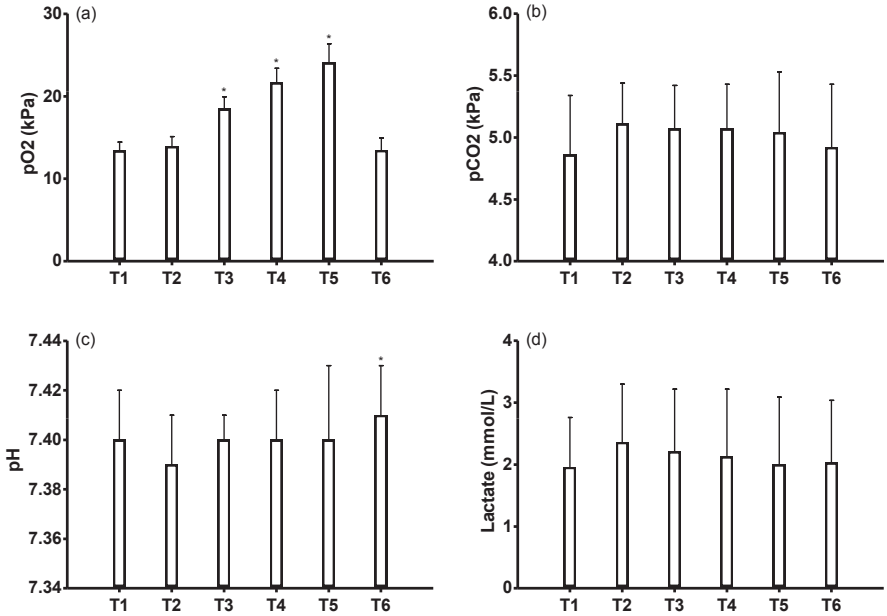
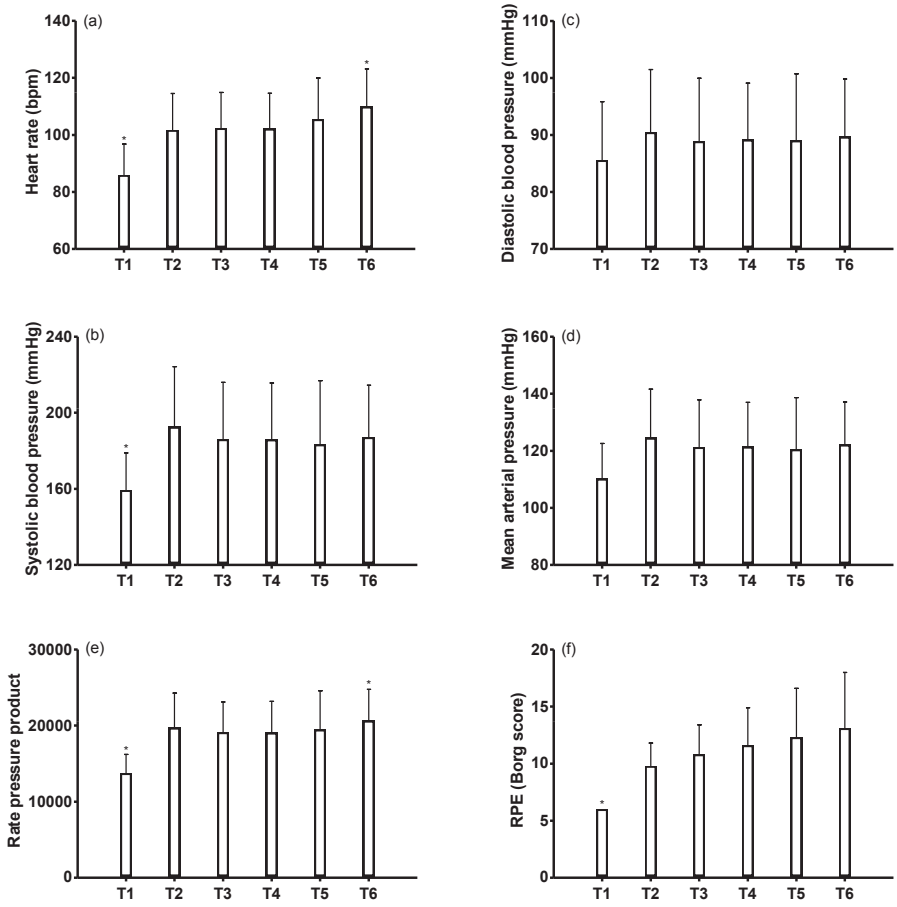


Figure 4.2. Blood gas analysis



(a) pO_2 levels during hyperoxic exercise. * T3-5 are significantly different from T1, T2 and T6, and each other ($p<0.05$). (b) pCO_2 levels during hyperoxic exercise. No significant changes ($p<0.05$). (c) pH levels during hyperoxic exercise. * T6 is significantly higher than T2 and T3 ($p<0.05$). (d) Lactate levels during hyperoxic exercise. No significant changes ($p<0.05$)

Figure 4.3. Cardiovascular response and rate of perceived exertion



(a) HR during hyperoxic exercise. * T1 is significantly lower than T2-6. T6 is significantly higher than T1, T3, and T4 ($p<0.05$). (b) Systolic blood pressure during hyperoxic exercise. * T1 is significantly lower than T2, T3 and T6 ($p<0.05$). (c) Diastolic blood pressure during hyperoxic exercise. No significant changes ($p<0.05$). (d) Mean arterial blood pressure during hyperoxic exercise. No significant changes ($p<0.05$). (e) Rate-pressure product during hyperoxic exercise. * T1 is significantly lower than T2-6. T6 is significantly higher than T1 and T3 ($p<0.05$). (f) Rate of perceived exertion (Borg score) during hyperoxic exercise. * T1 is significantly lower T2-T6 ($p<0.05$).

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PART 3

Impact of lack of PA and sedentary behavior in elderly persons on muscle mass loss and risk for metabolic syndrome

Part 3 of this thesis discusses the consequences of the lack of physical activity and increased sedentary behavior. This part emphasizes the magnitude of the problem of the increasingly higher risk for the metabolic syndrome and mobility disability in older age. As discussed in the previous two parts, we present data to support the notion that alternative approaches are warranted to improve the effectiveness and access to physical activity interventions. More effective physical activity interventions will target the main pathologies of the aging process, improve quality of life and expand health and life span.

CHAPTER 5

The Role of Muscle Mass, Muscle Quality, and Body Composition in Risk for the Metabolic Syndrome and Functional Decline in Older Adults

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Abstract

Age-related body composition changes include both loss of muscle mass (sarcopenia) and increase in fat mass, which jointly contribute to a decline in metabolic functions. Muscle quality is positively related to functional capacity and a lower risk for the development of the metabolic syndrome in aging populations. Muscle quality and muscle strength have become more reliable measures of functional capacity and mobility disability than muscle mass quantity. Recent reports also suggest that excess fat mass in older adults may impair muscle quality and that combination of excess body fat and muscle loss, a condition termed sarcopenic obesity, has even greater consequences on the muscle architecture and function than age-related muscle loss alone. A current challenge for clinicians and researchers is to develop interventions that will help decrease fat mass levels and maintain good muscle quality and strength levels in high risk older adults.

Keywords

Sarcopenia, Metabolic Syndrome, Functional Capacity, Aging.

Introduction

Skeletal muscle is metabolically one of the most active tissue in the human body [1] . Due to the combination of aging and corresponding reduction in activity levels, muscle mass loss progresses faster, leading to disability in older age [2-4]. This age-related muscle loss (sarcopenia) diminishes the metabolic (e.g. glucose regulation) and mechanical (e.g. muscle strength and functional capacity) function of the skeletal muscle [5-8]. There is a growing body of evidence that indicates that the overall quantity of muscle mass may be less important than muscle quality (muscle strength/muscle mass unit) for the metabolic and mechanical function of skeletal muscle [9-12]. Therefore, muscle quality (muscle strength/muscle mass unit) has been suggested to be a more important treatment target than muscle mass [11, 13, 14].

There is also a growing body of evidence, which suggests that excess weight or obesity may attenuate the structure of skeletal muscle and its function. Obesity dramatically increases the risk of functional decline and mobility disability in older adults. Unfortunately, the combination of unhealthy weight gain and reduction in lean body mass that occurs during aging can directly contribute to impairments in functional capacity [15]. Moreover, the co-occurrence of excess adipose tissue and low muscle mass can lead to a high risk condition termed sarcopenic obesity [16, 17]. Some studies, however, suggest a protective function of higher levels of fat mass in older adults [18, 19].

The progressive reduction of the structural and mechanical function of skeletal muscle during aging can ultimately lead to impairments in the functional capacity [15] and contribute to metabolic disease conditions (e.g., metabolic syndrome) [20]. Moreover, impairments in functional capacity and associated reductions in physical activity can increase risk for metabolic disease conditions (e.g., metabolic syndrome) and disability in older adults [20]. Thus, high functional capacity, associated with healthy skeletal muscle function, is crucial in older age and important to be maintained throughout one's life.

The complexity of the association between age-related body composition changes with reductions in functional capacity and metabolic function during aging warrants further investigation. The purpose of this review is to summarize the association between muscle quality and muscle strength with body composition, functional capacity, and the metabolic syndrome in obese and non-obese aging populations. The first section describes the impact of aging on changes in body composition. The next section describes the relationship between muscle quality and unhealthy weight gain during aging. Subsequently, we review the relationship between muscle quality, muscle strength, muscle mass and functional capacity in

older adults. This relationship is distinguished between obese and non-obese individuals. Finally, the last section focuses on the relationship between muscle quality, muscle strength, muscle mass, and the metabolic syndrome in older adults.

Effect of aging on body composition

Aging is associated with unhealthy changes in body composition (e.g. muscle loss and increased fat mass) that contribute to a decline in function and significantly increases the risk for disability even when body weight remains stable [21-23]. These changes [24] are associated with increased fat depots within the muscle as well as a loss in muscle strength [24] and muscle power [25]. Age-related changes in muscle composition appear to be even more pronounced in obese individuals [9, 26], and the combination of muscle loss and fat gain may act synergistically to increase risk of physical disability in obese older adults [21-23]. This is of concern as the rise in the prevalence of obesity has been particularly dramatic among older adults who now represent thirty-three percent of older American adults (age > 60 years) [27] . Moreover, the combination of muscle loss and fat gain may act synergistically to increase risk of physical disability in older adults [27-29].

In addition to obesity, sedentary lifestyle appears to contribute to the unhealthy body composition changes that promote age related functional decline [30-32]. Emerging evidence suggests high levels of sedentary behavior (sitting) contributes to lipid accumulation [33-35], metabolic impairments [36], and loss of muscle mass during aging [37], all of which strongly contribute to functional decline [20, 28, 38, 39]. Recent systematic reviews and meta-analyses have found a particularly strong relationship between sedentary time with type 2 diabetes [40] , functional decline [41], and increased risk of all-cause mortality and functional decline in both middle-age and older adults [40, 42]. Inactivity decreases whole skeletal muscle contractile performance, which can lead to reductions in functional capacity [43] . Thus, the detrimental metabolic effects of prolonged sedentary behavior are likely related to the dramatic reduction in the activity of large muscle groups, which are responsible for up to 80% of the body's blood glucose disposal [44-46]. These findings are of concern as the majority of middle-age adults in affluent countries spend over half their waking day (~8-9 hours) engaged in sedentary pursuits [44, 47].

The specific biological mechanism through which obesity and aging contribute to muscle degradation and functional decline is not currently known. Accumulating evidence suggests that reductions in mitochondrial (Mt) function have a pivotal role in the pathogenesis of muscle degradation and age-related functional decline [48, 49], though these relationships

have primarily been found in pre-clinical models and cross-sectional studies. Emerging findings have also highlighted the importance of the intracellular quality control process of autophagy in the maintenance of healthy muscle and regulation of body lipid stores [50, 51]. In line with this, both aging and obesity are characterized by a reduction in efficiency of cellular maintenance, repair, and turnover mechanisms, resulting in the accumulation of lipids and damaged biomolecules and organelles [52-55]. Once accumulated, excess body fat can also adversely affect autophagy through direct effects on the autophagic machinery [56, 57], or through increased production of ROS primarily from damaged mitochondria [48, 49]. The complex role of the autophagic-lysosome system in contributing to changes in Mt function, body weight, muscle composition and physical function during aging, however, has not been well studied in humans.

Relationship between muscle quality and obesity

A number of studies from the Health ABC cohort have studied the relationship between the level of adipose tissue and muscle quality [9, 11, 58-66]. Initially, Reed et al. [60] studied the relationship between body weight and muscle quality in 100 men and 117 women aged 65 and over. The authors [60] observed that higher body weight is related to higher muscle quality in both older men and women. Despite this, it is difficult to determine whether these individuals were in a healthier metabolic state since the body composition distribution was not available. With prospective data from 728 men and 783 women whose BMI was measured at regular intervals from the age of 15 until the age of 60-64, Cooper et al. [61] showed that those located above the 80th percentile of muscle quality had significantly less fat than those located below the 20th percentile. They also observed that significant BMI gain in their lives (regardless of the period), increased their risk to have low muscle quality [61]. More specifically, it seems that becoming obese after the age of 40 increases the probability of having low muscle quality around 60-64 years [61]. Villareal et al. [62, 63] showed in people aged on average 70 years that obesity (BMI 30 > 38%, respectively) was significantly associated with lower values of muscle quality.

Segal et al. [64], however, observed no significant difference in muscle quality between different subgroups of BMI (<30, 30-35 and >35) in 161 men and women (52, 5%) with a mean age of 55 years. Koster et al. [9]• showed in 1129 men and 1178 women aged between 70 to 79 years that leg muscle quality differs depending on the percentage of fat mass. Thus, in men and in women, a higher percentage of fat mass is an important contributor to low muscle quality in the lower extremity. In the same cohort, Newman et al. [11] showed that

this relationship is quadratic. Leg muscle quality seems optimal for a body fat percentage between 16 and 22% in men and between 26 and 34% in women. However, when following older adults during 7 years and dividing this cohort in quartile of body fat, men in the highest quartile of fat also had the slowest decline in muscle quality [9]. These results can be interpreted in two ways: (1) fat mass has a protective effect, or (2) muscle quality of those in the highest quartile of fat mass decreased slower because it was lower at the beginning.

Otherwise, Peterson et al. [65] showed that muscle quality is inversely related to the amount of subcutaneous fat in men and women. In addition, after 12 weeks of resistance training, this relationship remained unchanged [65]. Additionally, both Goodpaster et al. [59] and Cawthon et al. [58] showed that the knee extensor muscle quality was significantly associated with muscle density (which is considered an indicator of extracellular lipid infiltration in muscle) [66] , but not with subcutaneous or inter-muscular fat (between muscles) in men and women. The authors found that muscle density explained 9 and 11% of the variance in muscle quality in men and women, respectively [59] . However, it is important to note that all these studies used different adiposity indicators (BMI, body fat percentage, fat subcutaneous and muscle density). Overall, these results suggest that a high level of adiposity (regardless of the index used) is associated with low muscle quality. Nevertheless, the results of Koster et al. [9] raise interesting questions about the potentially protective role of fat.

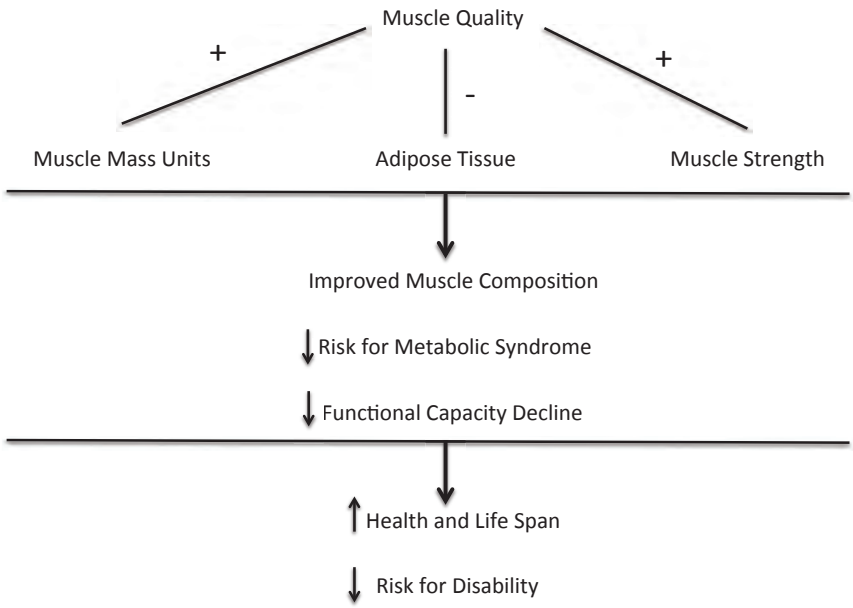
Muscle quality, muscle mass and muscle strength (obese and non-obese) and functional capacity in aging

In recent years, it has been shown that larger muscle mass only is not sufficient to maintain functional capacity in older age [10, 58, 67]. During aging, the process of muscle mass and muscle strength loss is well defined as sarcopenia [68]. It has been proposed that sarcopenia is diagnosed based on a low whole-body or appendicular muscle mass in combination with poor physical functioning [12, 68].

Although sarcopenia is associated with increased risk of physical disability and poor quality of life [68, 69], a number of studies to date showed that muscle composition (muscle quality) rather than muscle mass is related to better physical function [10, 58]. In particular, several factors might influence the muscle composition e.g. architecture and fiber type (fiber size and number of type I and type II fibers), fat micro- and macro-infiltration, fibrosis (increase in collagen) and neurological modulation of contraction [10, 70]. Moreover, a number of reports have found that muscle strength is related to functional capacity in older adults [13, 71-73]. However, it is inconclusive how much increased body fat content (obesity) worsens the

relationship between the metabolic, mechanical properties of skeletal muscle mass and poses higher risk on functional capacity and future disability in older adults.

Figure 5.1. Importance of high muscle quality and low adipose tissue levels in improving life and health span in older adults.



Relationship between muscle quality, muscle mass and muscle strength and functional capacity in non-obese older adults

In geriatrics, muscle quality is considered to be a clinically relevant determinant of physical performance and disability level in older adults [74]. Kennis at al. reported that the level of muscle mass in middle-aged individuals did not contribute to the longitudinal changes in muscle quality based on basic strength and only barely contributed to the longitudinal changes in muscle quality based on velocity-dependent muscle strength [74]. Therefore, baseline muscle strength is an important determinant of the decline in muscle quality based with advancing age [10, 74].

Additionally, during aging, the number of functioning motor units (muscle work efficiency) decreases, with an accelerated loss of fast motor units (type 2b fibers) [10]. Reinnervation of the denerved fibers by the remaining motor units ends in a net conversion of fast type II

muscle fibers into slow type I fibers. This conversion compromises the strength-generating capacity of muscle and functional capacity. Impairment of muscle quality may also be accelerated by higher fat mass content [10, 70, 71].

A relationship between muscle quality, muscle mass and muscle strength and functional capacity in obese people

The relationship between muscle quality and physical function is influenced by: level of muscle mass, the degree of obesity (BMI) and age [70]. Impaired physical function can be caused by several factors such as high fat mass and low muscle strength [6]. In particular, individuals with both high fat mass and low muscle strength would have greater impairments in their physical function than individuals with obesity or strength loss alone [6].

Age-related muscle mass loss is typically accompanied by an increase in fat mass [70]. As a result, bodyweight in men and women might be quite stable in middle age, or slightly increased even though the relative amount of body fat actually increases in comparison with lean tissue [10]. This is an important step in the development of sarcopenic obesity [75]. It is suggested that mechanisms for sarcopenic obesity overlap with those proposed for age-related sarcopenia [70]. Net contracting mass might be smaller than estimated because of infiltration of skeletal muscle with fat and connective tissue (myosteatosis) [70, 76]. Moreover, abnormal protein synthesis rates and anabolic resistance to exercise are especially evident in sarcopenic obesity. In obesity, pro-inflammatory cells in fat may contribute to development of sarcopenia [70].

The increased presence of enhanced reactive oxygen species and chronic inflammation related to an increased fatty-acid load could also result in mitochondrial damage in skeletal muscle [76] . It has been shown that individuals with low muscle strength are approximately two times more likely to be obese compared to those with normal strength after adjusting for age, gender and body weight [70]. Additionally, muscles that chronically function in the higher range of their capacity spectrum might be less energy efficient and can lead to accumulated muscle damage [10]. In particular, the energy expenditure, oxygen consumption and muscle force required for an obese person are higher than those required by a normal-weight individual. Taken together, co-existing obesity and muscle quality impairment can act synergistically in developing the risk for multiple functional deficits [70]. Obese individuals with large low muscle strength and high body mass are at higher risk of being disabled in the future [10, 12, 70].

Relationship between muscle mass, muscle quality and metabolic syndrome

Obesity, particularly abdominal obesity, has been widely recognized as a predisposing factor to cardiovascular diseases (CVD) and the metabolic syndrome [77]. Approximately 53% of older adults currently have the metabolic syndrome as compared to 18% of adults below age 40 [78] . Independently of abdominal obesity, aging has been shown to be associated with decreased muscle mass [79]. Some estimates indicate that 25 to 50% of adults aged 65 and older have low levels of muscle mass [80]. However, it has been also suggested that low muscle mass may contribute to metabolic complications and cardiovascular disease (CVD) in older adults [14, 16, 80, 81].

One potential explanation for these findings is that skeletal muscle atrophy is fundamental to the metabolic alterations related with physical inactivity and the reduction of energy expenditure, leading to insulin resistance [82]. In addition, a recent epidemiological study suggests that low muscle mass is closely related to impairments in glucose homeostasis, underscoring the potential additive and negative effects of low muscle mass when combined with obesity on glucose regulation and insulin resistance [83].

To our knowledge, the limited research that has been conducted on the effect of low muscle mass-obesity on metabolic risk factors and CVD in older adults has produced mixed findings [3, 17, 84-87]. Two studies [5, 84] indicate that low muscle mass-obesity has no particular deleterious impact on metabolic risk factors and CVD in Caucasian postmenopausal women. In contrast, Prado et al. [17] in their review and Lu et al. [85] showed that a low muscle mass and a high fat mass increased CVD risk in elderly Asian people. Yet another study by Castaneda et al. suggest there may be health benefits associated with low muscle mass-obesity, as they showed that elderly Caucasian with low muscle mass and obesity have less diabetic risk (glucose and insulin level) than elderly Caucasians with obesity and normal muscle mass [86]. Goulet et al. observed also a better insulinemic profile in obese postmenopausal women [88]. Baumgartner et al. reported that elderly people with obesity and low muscle mass have less metabolic syndrome but higher type II diabetes [87]. Lebon et al. concluded recently that a lower muscle mass/body weight is not detrimental of insulin sensitivity even after adjusted for visceral fat mass in sedentary postmenopausal women [89]. Stephen et al. showed that low muscle mass when combined with obesity induced a modest increase in the risk of CVD in community dwelling older men and women aged over 65 years old, and that this relationship seems mediated mostly by muscle strength and muscle quality [3]. It is currently unknown whether excess fat mass alone radically increases the risk for the

metabolic syndrome and it components, however, it is clear that good muscle quality is of high importance for obese subjects.

In addition, the loss of muscle mass, strength and quality are observed with aging [90]. Low muscle strength occurs in 60 % of adult aged 60 and older [6]. Poor muscle quality (muscle strength/muscle mas or muscle strength/ body weight) has been shown to be associated with poor cardio-respiratory function [91], a decline in mobility [12, 92], incident disability [93] and mortality [71, 75]. There is some evidence of a direct association between the metabolic syndrome and insulin resistance with low muscle quality [2, 8]; intramuscular adipose tissue (IMAT) [4, 59] and high blood pressure [4].

Based on these previous observations, the combined effect of low muscle strength and/or low muscle quality with obesity would be predicted to contribute to a worse metabolic profile compared to either condition alone. These individuals represent 7.6 to 11.1% of older adults [3, 94]. Moreover, it has been demonstrated that both abdominal obesity and low muscular strength are characterized by high circulating levels of pro-inflammatory cytokines which are recognized as risk factors for CVD [16]. Interestingly, a recent review reported higher muscle strength in metabolically healthy but obese individuals despite lower skeletal muscle mass [18], suggesting a better muscle quality (muscle strength per unit of muscle mass) in these individuals. This statement is supported by the findings of Karelis et al. [7] and Atlantis *et al.*, who showed that higher levels of insulin sensitivity are associated with higher levels of muscle quality [14]. However, Senechal et al recently showed that when combined with abdominal obesity, dynapenia has been associated with more metabolic alterations in adults 50 yrs of age and older than dynapenia or obesity alone [95]. These conclusions have been confirmed by Barbat-Artigas et al. who recently showed that dynapenic (low muscle strength /body weight) and obese postmenopausal Caucasian women (mean age: 60±5 yrs) had a better metabolic profile and a better insulinemic profile than non-dynapenic obese postmenopausal women [96, 97].

Conclusion

Muscle quality and muscle strength are directly related to the levels of body composition changes and functional capacity during aging. Age-related decline in muscle quality may be amplified by excess adiposity, however, some studies suggest fat mass may provide a protective aspect. Thus, the relation of fat mass with muscle quality and functional decline during aging is still equivocal and needs more investigation. Additionally, high levels of adipose tissue and low muscle quality are associated with higher risk of disability and

development of the metabolic syndrome and its components in older age. As summarized in Figure 5.1, high levels of muscle quality are strongly related to higher functional capacity, lower risk for the metabolic syndrome, and hence health-span in aging populations.

Future Directions

Future studies should focus on developing interventions that will help decrease fat mass levels and maintain muscle quality levels. In particular, a growing body of evidence suggest strength training should be an integral part of interventions for sarcopenic older adults and those who are at risk for the metabolic syndrome. Additionally, short bursts of high-intensity interval training interventions are suggested for subjects with poor fitness levels as a time-efficient alternative to endurance training. Individualization, effectiveness, and combinations of these training methods need to be further investigated in older adults with the metabolic syndrome and sarcopenic populations.

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CHAPTER 6

Sedentary Time is Associated with the Metabolic Syndrome in Older Adults with Mobility Limitations - The LIFE Study

Adjusted from:
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Abstract

Background: Epidemiological and objective studies report an association between sedentary time and lower risk of the metabolic syndrome (MetS) and its risk factors in young and middle-age adults. To date, there is a lack of objective data on the association between sedentary time and MetS among older adults.

Methods: The association between objectively measured sedentary time (accelerometry) with MetS and MetS components was examined in a large sample of older adults with mobility limitations (N = 1198; mean age = 78.7 ± 5.3 years) enrolled in the Lifestyle Interventions and Independence for Elders (LIFE) study. Participants were divided into tertiles according to percentage of daily sedentary time, and the relation between sedentary time with MetS and MetS components was examined after adjusting for age, sex, ethnicity and BMI.

Results: Participants in the highest sedentary time tertile had significantly higher odds of MetS (OR=1.54) (95% CI 1.13 to 2.11) in comparison with participants in the lowest tertile (p=0.03). Participants in the highest sedentary time tertile had larger waist circumference (p=0.0001) and lower HDL-C (p=0.0003) than participants in the lowest sedentary time tertile.

Conclusions: Sedentary time was strongly related to higher odds of MetS. These results, based on objectively measured sedentary time, suggest that sedentary time may represent an important risk factor for the development of MetS in older adults with high likelihood for disability.

Keywords

Aging, Accelerometry, Glucose, Disability, Waist Circumference.

Introduction

The metabolic syndrome (MetS) is a combination of interrelated risk factors of metabolic origin that has become more prevalent in an aging population. Over half (53%) of adults aged 60 years or older in the United States currently have MetS, whereas less than 20% of adults below the age of 40 have MetS [1]. This is of concern as MetS has been found to directly promote the development of cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2) [2]. As older adults represent the fastest growing segment of the population [3], improvement of clinical management and preventive strategies directed at MetS [4] in this high risk population is crucial.

With increasing age, a larger percentage of adults live a sedentary lifestyle, as defined by not meeting minimum level of activity recommended in current guidelines. For example, the prevalence of sedentary time is ~39% in adults between 30 and 44, ~46% in adults between 45 and 59 years, and 60% in adults age 60 and older [6]. Moreover, only a small percentage of older adults 60 years and older, 20% and 12% of older men and women respectively [5], are meeting current physical activity guidelines [7, 8].

Traditionally, it has been thought that individuals are protected from cardio-metabolic disease conditions if they meet recommended levels of physical activity, regardless of their activity levels throughout the remainder of the day [9-11]. Recent studies have indicated, however, that meeting the nationally recommended levels of PA does not prevent cardio-metabolic disease if individuals engage in high levels of sedentary time throughout the day. For example, Matthews et al. found that meeting the recommended amounts of PA failed to mitigate cardio-metabolic health risks among American adults aged 50-71 years who reported television viewing for more than 7 hours per day. Specifically, these individuals were found to have a 50% greater risk of all-cause mortality and a two-fold greater risk of cardiovascular disease compared to those who spent <1hour/day on television viewing after adjusting for moderate to vigorous PA (MVPA) [4].

To our knowledge, only one study has examined the association of sedentary time with MetS, using objective measures (i.e., accelerometry) in middle age adults. In this cross-sectional study, which included 521 healthy middle-age Japanese participants, activity levels were measured for 7 days, and the association between activity levels with MetS was evaluated [12]. The key finding of this study was that sedentary time was independently associated with the higher risk for MetS in middle-age adults (30 – 64 years), regardless of whether or not they engaged in recommended levels of physical activity [12].

The relative contribution that sedentary time, measured objectively, has in relation to the probability of MetS and associated MetS components in an older adult population is not currently known. Based on findings from epidemiological studies and recent clinical trials in middle-aged adults [4, 12], we hypothesized that more time spent being sedentary would be associated with a higher prevalence of MetS and MetS components in older adults with mobility limitations.

Methods

Participants

All participants volunteered to participate in a long-term lifestyle intervention study (minimum 24 months up to 42 months), the Lifestyle Interventions and Independence for Elders (LIFE) study, which tested whether a long-term structured physical activity program is more effective than a health education program (also referred to as a Successful Aging program) in lowering the likelihood for major disability. A total of 1635 sedentary men and women aged 70 to 89 years were recruited across eight clinical sites [13]. Participants were eligible to participate if they had physical limitations, defined as a score on the Short Physical Performance Battery (SPPB) [14] of 9 or below, but were able to walk 400 meters. Detailed descriptions of participant characteristics, as well as inclusion and exclusion criteria, have previously been provided [15]. A key exclusion factor for the LIFE study was self-reported activity, defined as spending less than 20 min/wk in the past month getting regular physical activity and reporting less than 125 min/wk of moderate PA (<125 min/wk).The analytical sample for the present study included LIFE participants with validated baseline accelerometry and laboratory data.

Sedentary and Physical Activity monitoring

The Actigraph tri-axial accelerometer (Model GT3X; Actigraph Inc., Pensacola, FL) was used to objectively measure sedentary and physical activity time. Participants were asked to wear an accelerometer for 7 days immediately following their baseline clinic visit. During the 7-day monitoring period, participants were asked to put the monitor on each morning (after dressing) and remove the monitor just prior to going to bed at night. Sedentary time was recorded only during awake time, as participants did not wear accelerometers while sleeping. The monitor was removed for bathing, showering, or any other activity that might result in exposure to water. Activity was recorded using 1-second epochs, which were added up 60 s epochs [16]. Non-wear time was defined as a 60-minute window of zero counts in all three

axes, allowing for up to two minutes of non-zero counts (cts)<100 in the vertical axis. A cut point of <100 cts/min was used to designate sedentary time, For sedentary time, the vast majority of studies have used a cut-point of <100 counts per min to designate sedentary time with additional checks during data cleaning to identify periods of no-wear time [17]. For the data to be included in this study, participants had to wear the accelerometer during at least 3 consecutive daysfor 10 hours per day in free-living conditions.

MetS classification

MetS was defined in accordance with the harmonized criteria recommended in the 2009 Joint Interim Statement from multiple scientific associations [18], as the presence of 3 or more components, including (1) abdominal obesity (men: waist circumference ≥ 102cm or women: waist circumference ≥ 88cm); (2) hypertension (systolic blood pressure (SBP) ≥ 130mmHg and/or diastolic blood pressure (DBP) ≥ 85mmHg) or use of antihypertensive medication and a history of physician-diagnosed hypertension; (3) decreased HDL-C cholesterol level (men: HDL-C <40 mg/dL and women: <50 mg/dL) or use of HDL-C-raising medication; (4) elevated triglycerides levels (triglycerides ≥ 150 mg/dL) or use of triglyceride-lowering medication and (5) elevated plasma fasting blood glucose level (glucose ≥ 100 mg/dL) or use of glucose-controlling medication.

Baseline blood analysis

Blood samples for assessment of a lipid panel and fasting plasma glucose were collected in the early morning, after a 12-hour fast at the baseline assessment visit. Blood (57.5-69.5 ml) was collected via venipuncture into plain, serum-separation, EDTA-treated, heparin-treated, vacutainers by a trained phlebotomist. Baseline samples were sent to a central diagnostic testing laboratory for a lipid panel (triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol (calculated)) and fasting glucose levels. The baseline data were used to determine the metabolic syndrome and its components in the basis of the standardized criteria, stated above.

Statistical analyses

Demographic and clinical characteristics were summarized across sedentary time tertiles using means and standard deviations for continuous variables and frequencies and percentages for categorical variables. Means were compared across tertiles using one-way analysis of variance, and categorical proportions (including MetS and MetS components) across tertiles were compared using Wald chi-square tests from unadjusted logistic regression models. The odds of classification to MetS or MetS components by standardized sedentary time measures were estimated using logistic regression models adjusted for age, sex, and race/ethnicity. Next, models were repeated with additional adjustment for BMI. All analyses were performed using SAS v 9.3 (SAS Institute, Cary, NC). Statistical tests were performed assuming a Type I error rate of 0.05, and pairwise comparisons across tertiles were deemed significant at a Bonferroni-adjusted p=0.0167 level.

Results

Baseline characteristics of participants

From the total of 1635 participants, 359 participants were excluded due to missing or insufficient accelerometry data and a further 78 participants lacked sufficient information for determining MetS status. The participants excluded from these analyses only differed from the study sample with respect to the Modified Mini-Mental State (3MS) scores (0-100 scale). Specifically, excluded participants had significantly lower 3MS scores (<80) than participants in our study sample (91.5±5.5) [19], which may have affected their ability to adhere to the accelerometry protocol. This led to a sample size of 1198 participants in the present study. The mean (± SD) age of included participants was 78.7 ± 5.3 years, 33.8% were men, and 23.0% were racial/ethnic minorities (nonwhite). Average BMI of the participants was 30.2 ± 6.0 kg/m^2. Participants were divided into tertiles of sedentary time based on mean percent daily sedentary time measured over valid wear days as follows: ≤74.2% lowest tertile, >74.2% but ≤81.1% middle tertile, >81.1% highest tertile. Table 6.1 displays characteristics of participants based on sedentary time levels.

Increased likelihood of MetS and MetS components according to tertiles of sedentary time

Older age in male participants was associated with increased sedentary time (p<0.0001) (Table 1). Participants in the highest sedentary time tertile had a higher prevalence of MetS, approaching significance, (p=0.06) compared to participants in the middle and the lowest tertiles (Table 6.2).

Table 6.2 shows the prevalence of MetS and MetS components (plasma glucose levels, abdominal obesity, HDL-C, TG levels and higher blood pressure) among participants with MetS according to tertiles of sedentary time. The fasting glucose component (fasting glucose ≥ 100 mg/dL) of MetS was significantly higher among participants in the highest sedentary time tertile in comparison with participants the lowest sedentary time tertile (p=0.003) but did not differ from participants in the middle tertile. No significant differences were found across tertiles for the following MetS components: abdominal obesity, HDL-C, triglycerides, and blood pressure.

Table 6.3 shows significantly higher overall odds of MetS (p=0.002) and higher likelihood for abnormal plasma glucose levels (p=0.007) among participants with higher amounts of sedentary time. Specifically, for each standard deviation increase in sedentary time, overall odds of MetS and plasma glucose were 22% and 19% higher, respectively. This logistic regression model was adjusted for sex, age, ethnicity, and BMI according to tertiles of sedentary time.

Table 6.1. Characteristics of the LIFE Study participants according to percentage of sedentary time.

	Lowest Tertile (n= 399) Mean±SD or n (%)	Middle Tertile (n= 400) Mean±SD or n (%)	Highest Tertile (n= 399) Mean±SD or n (%)	p-value across tertiles
Age (yrs)	**77.47±5.0** [b,c]	**78.86±5.5** [a,c]	**79.92±5.1**	**0.0001**
Gender (men)	**90 (22.6)** [b,c]	**124 (31.0)** [a,c]	**191 (47.9)**	**0.0001**
Race (white)	291 (72.9)	312 (78.0)	319 (80.0)	0.66
BMI (kg/m^2)	29.90±5.7	30.65±6.0	30.26±6.4	0.21
Waist Circumference (cm)	**99.56±14.5** [b,c]	**102.54±15.4** [a,c]	**104.41±16.5**	**0.0001**
Plasma glucose (mg/dL)	102.28±21.3	103.73±24.5	105.28±24.7	0.20
HDL Cholesterol (mg/dL)	**63.81±18.2** [b,c]	**61.16±16.4** [a,c]	**58.67±18.7**	**0.0003**
Triglycerides (mg/dL)	118.9±54.2	120.65±50.9	126.27±65.3	0.17

Percentage of sedentary time levels: ≤74.2% lowest tertile, >74.2% but ≤81.1% middle tertile, >81.1% highest tertile. % - percentage of the total number of participants. Superscripts mean significant difference between the lowest tertile (a), the middle (b) and the highest (c) tertiles.

Table 6.2. MetS and MetS components based on percentage of sedentary time tertiles among participants.

	Lowest Tertile (n= 399) n (%)	Middle Tertile (n= 400) n (%)	Highest Tertile (n= 399) n (%)	p-value across tertiles
Metabolic Syndrome	180 (45.1)	200 (50.0)	213 (53.4) [a, b]	0.06
MetS Plasma Glucose	**176 (44.3)** [c]	**198 (49.5)**	**224 (56.2)**	**0.003**
MetS HDL Cholesterol	82 (20.7)	76 (19.1)	100 (25.3)	0.09
MetS Triglycerides	116 (29.2)	111 (28.0)	124 (31.5)	0.56
MetS Abdominal Obesity	287 (72.5)	299 (75.1)	289 (73.0)	0.61
MetS Blood Pressure	315 (79.0)	318 (79.5)	317 (79.5)	0.98

Categorical measures are reported as number (n) and percentage (%) of participants with MetS and MetS components according to sedentary time tertiles. Sedentary time levels: highest tertile: ≤74.2% lowest tertile, >74.2 but ≤81.1% middle tertile, >81.1% highest tertile. Superscripts mean significant difference between the lowest (a), the middle (b) and the highest (c) tertiles.

Table 6.3. Odds ratios of meeting MetS and MetS components per standard deviation change in percentage spent in sedentary time.

	Sedentary Time **With BMI adjustment**			Sedentary Time **Without BMI adjustment**		
	OR	95% CI	P-value	OR	95% CI	P-value
Overall MetS	**1.22**	**(1.07,1.39)**	**0.002**	**1.26**	**(1.12,1.43)**	**0.0002**
Plasma Glucose	**1.19**	**(1.05,1.34)**	**0.007**	**1.22**	**(1.08,1.38)**	**0.002**
HDL-C Cholesterol	1.15	(0.99,1.34)	0.07	**1.17**	**(1.01,1.36)**	**0.04**
Triglycerides	1.10	(0.96,1.26)	0.19	1.12	(0.98,1.29)	0.09
Waist Circumference	1.06	(0.87,1.30)	0.57	1.13	(0.98,1.30)	0.09
Blood Pressure	1.06	(0.91,1.22)	0.47	1.09	(0.94,1.26)	0.27

All models adjusted for age, sex, and race/eth. Additional adjustment for BMI was added where indicated. Odds ratio per standard deviation change in sedentary behavior measure.1SD for % sedentary activity is 8.18%. CI – confidence interval.

Discussion

The present investigation examined whether the amount of time spent in sedentary behavior in functionally limited older adults with mobility limitations was related to MetS and MetS components. The major finding of our investigation is that higher amounts of sedentary time were associated with higher odds of MetS. In addition, we found that higher levels of sedentary time were related to higher prevalence of specific components of MetS, including plasma glucose levels and low HDL-C levels. We also found extremely high levels of sedentary time in this population based on objective accelerometry measurement. Specifically, participants spent ~ 11 hours per day in sedentary activities. Moreover, levels of sedentary time increased with age, and we also observed higher prevalence of MetS with increasing age.

In line with findings in teenagers and middle-age adults, [12, 20] we found that sedentary time was positively associated with occurrence of MetS among older adults with mobility limitations participating in the LIFE study. Specifically, the present study found that participants with the highest odds of MetS spent approximately three more hours per day engaged in sedentary time than participants with the lowest odds (12 hours versus 9 hours of awake time). Our findings suggest that higher amounts of sedentary time may increase the probability of MetS, even among sedentary older adults. Thus, our findings add to the literature by demonstrating a strong positive association between sedentary time and likelihood of MetS in an older adult population with mobility limitations.

Our study extends the previous literature [12, 21] by showing that there is also a strong positive association between sedentary time and plasma glucose levels in older adults with MetS, suggesting that high levels of sedentary time may independently impair regulation of glycemia across all adult populations. Compared to participants with lower levels of sedentary time, participants with higher levels of sedentary time were also observed to have lower levels of HDL-C and increased waist circumference (Table 6.1) which have both been found to increase cardiometabolic dysfunction probability in older adults [22-24]. Our results are in accordance with an epidemiological study conducted by Kim et al, which measured activity levels objectively and reported that prevalence of dyslipidemia in a Japanese population was higher in a more sedentary group compared to a less sedentary group (>5.4hours/day versus <3.5 hours/day of sedentary time)[12].

We report a higher percentage of sedentary time [~85% (12 hours/day) vs. 68% (9 hours/day) of awake time] in this sample of older adults with mobility limitations than what has been reported in middle-age adults [~63% (~9 hours/day) of awake time][25]. The participants in

the current study were specifically recruited because they were not achieving current national recommendations for physical activity.

An important strength of this study is the use of objective measurements to obtain estimates of sedentary time in a large study sample of older adults. Other objective studies investigating the impact of sedentary time on the association with MetS were based on self-report [4, 26] or used smaller study samples in younger and middle-aged adults [12, 25]. Our sample also consisted of older adults with a high prevalence of MetS components, allowing for a more comprehensive evaluation of the connection between sedentary time and likelihood of MetS [15].

The present findings are limited by the cross-sectional nature of our analyses. Another limitation is that sedentary time was measured during relatively brief time periods (i.e., 3 – 7 days). This might have led to an under- or overestimation of actual sedentary time of the participants and associations between sedentary time and physical activity in their daily life. However, given that MetS, sedentary behavior, and functional limitations are quite prevalent in the general population, our results are likely generalizable to most older adults. Finally, a sizable percentage (~22%) of participants were excluded due to lack of accelerometry data, and ~5% lacked sufficient information for determining MetS status.

Conclusion

In conclusion, the results from the present investigation indicate that older adults with mobility limitations spend a large proportion of their time engaged in sedentary behavior, and that time spent being sedentary was strongly related to higher likelihood of MetS. Our findings suggest that sedentary time represents an important risk factor for the development of MetS in older adults with mobility limitations, and thus deserves further investigation. Prospective studies are needed, however, to further investigate the effects that sedentary time has on likelihood of MetS in this population, as well as whether reducing sedentary time in older adults may lower probability of MetS.

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General discussion and main findings

In this dissertation, we investigated alternative strategies to improve the beneficial physiological effects of exercise, extend health and life span and reduce the risk for pathology and disability while aging. Based on a review of the literature **(chapter 5)** and a large cross-sectional study in sedentary elderly people **(chapter 6)** we showed that the levels of sedentary behaviour in aging populations seem to accelerate mobility disability and are likely to contribute to a reduced health and life span. Individually tailored exercise interventions and potentially also dietary co-interventions may counteract or attenuate some of the negative side effects of an inactive and sedentary lifestyle. According to the free radical theory of aging, progressive accumulation of oxidative stress also plays a role in aging-associated sarcopenia. From this perspective it would make sense to use anti-oxidants. However, our extensive literature review, described in **chapter 1**, indicates that some antioxidant nutritional interventions can be counterproductive, whilst others may have a positive effect on exercise performance. In accordance, alternative and more tailor-made strategies to counteract the diminished exercise tolerance and improve the beneficial effects of exercise are warranted. Our literature research shows that such interventions should preferably be tested before being prescribed or advised to an individual with established disease or increased risk for chronic disease and disability**.**

In part 2 of the present thesis, we investigated a novel strategy to test whether an individual subject or patient may be responsive to increased oxygen availability during exercise. Although our novel strategy could only be tested in a population of healthy subjects and patients with type 2 diabetes, the different studies indicate that manipulating inspiratory oxygen fraction (FiO_2) might serve as a validation **(chapter 2)**, a diagnostic **(chapter 3)** and potentially also a therapeutic **(chapter 4)** tool. Measurements of oxygen uptake recovery and muscle tissue reoxygenation kinetics under different FiO_2 conditions can be easily and cost-effectively applied in a clinical setting to assess oxygen delivery dysfunction and determine whether there might be need for supplemental oxygen to improve exercise tolerance in aging populations. Additionally, we also found that assessment of heart rate variability (HRV) during exercise can probably replace costly spiro-ergometry equipment for training design and monitoring to individualize and improve exercise interventions in clinical settings. As our HRV-based decision algorithms have only been tested in a healthy young population, more work is needed to validate its application in elderly and chronic disease populations who might be suffering from cardiac autonomic dysfunction.

Approaching low levels of physical activity, increased risk for chronic diseases and disability with exercise and nutritional interventions

Despite globally increasing levels of consciousness regarding the importance of physical activity and healthy nutritional habits, the metabolic syndrome pandemic keeps on expanding through all ages. The metabolic syndrome and its components lead to development of chronic diseases and increase the risk for disability in older age. Therefore, it is a great challenge for exercise physiologists and nutritionists to improve the effectiveness of exercise interventions regarding training modalities, effective strategies for faster post-exercise recovery and preventing some of the deleterious effects of oxidative stress.

Overall, exercise is beneficial to maintain the physiological homeostasis [1]. Some reports confirm the effectiveness of either endurance[2, 3], strength (muscle quality and strength) [3]. However, combined exercise training interventions to effectively decrease the risk for the metabolic syndrome appear most effectively [4]. Additionally, application of the most suitable exercise training types can target the reduction of the metabolic risk factors, such as insulin resistance, chronic inflammation, and attenuate associated pathologies such as muscle loss (sarcopenia) **(chapter 5).** The combined endurance and strength training interventions occur as the most effective in improving metabolic health and functional capacity during aging **(chapter 5)**. Current reports showed that muscle quality and muscle strength are directly related to the levels of body composition changes (adipose tissue levels) and functional capacity during aging [5]. Age-related decline in muscle quality may be amplified by excess adipose tissue and associated chronic inflammation [6, 7]. However, some studies suggest that fat mass may also have a health-protective function [8]. Additionally, high levels of adipose tissue and low muscle quality are associated with higher risk of disability and development of the metabolic syndrome and its components in older age. Therefore, future studies should focus on developing interventions that will help decrease fat mass levels and maintain muscle quality levels. In particular, a growing body of evidence suggests strength training should be an integral part of interventions for sarcopenic older adults and those who are at risk for the metabolic syndrome. Individualization, effectiveness, and combinations of training methods and modalities need to be further investigated in older adults with the metabolic syndrome and sarcopenic populations **(chapter 5)**.

Both the aging process and an exercise stimulus itself increase the formation rate of reactive oxygen species (ROS). Over-production (oxidative stress) of ROS can be deleterious for structure and function of cells and tissues, especially in frail elderly people **(chapter 1)**. On

the other hand, 'appropriate' formation level of ROS productions induces adaptive mechanisms to obtain beneficial effects of exercise (improved insulin sensitivity increased endogenous, muscle hypertrophy, antioxidative enzymes activity and mitochondrial biogenesis) [9-12]. A number of recent reports debated on potential blunting effects of dietary supplements in conjunction with exercise training [11, 13, 14]. However, available data on the blunting effects of dietary antioxidants in conjunction with endurance training are equivocal. Additionally, **chapter 1** draws attention of high consumption levels of most popular antioxidants (vitamins C and E). Based on the U.S. population the doses are not well regulated. For example, a maximal dose of vitamin C per day equals 3000mg, whereas recent studies using 500mg of vitamin C suggest potential harming prooxidant effects on e.g. risk for cataract [15, 16] and blunting exercise effects e.g. decreased mitochondrial biogenesis [11]. The literature review presented in **chapter 1** showed that there is no strong evidence on adverse or beneficial effects of antioxidant supplements (Vitamins C and E, α -lipoic acid and resveratrol) in conjunction with endurance exercise training. As such, we concluded that there is currently lack of clarity and a need for future studies on the optimal doses and combinations of commonly used antioxidant supplements (vitamins C and E, α-lipoic acid). Additionally, future studies warrant selecting training modalities for the most beneficial physiological effects of dietary antioxidants in conjunction with endurance training in aging population **(chapter 1)**. Controversy, presented in separate reports on healthy subjects, around resveratrol and its potential beneficial function in deficient populations (e.g. to treat sarcopenia and hypertension) warrant future dose-response studies with different training modalities e.g. strength and interval or endurance type of training.

It is well known that recommended levels of physical activity are crucial to lower the risk for the metabolic syndrome, its components and disability progression during the aging process [1]. We showed, using objective methods, that high levels of sedentary time in the awake time are associated with higher risk for the metabolic syndrome and its components. In the study of Matthews et al. it has been shown that despite reported regular physical activity, high levels of sedentary time did not compensate for the higher amount of sedentary time [17]. Additionally, even short bouts of moderate intensity type of exercise appear insufficient to lower the risk for cardiovascular disease when sedentary time levels are high [17]. **Chapter 6** extends on this study by showing that in elderly with mobility limitations even small reductions of sedentary time lower the likelihood of the metabolic syndrome.

In accordance with these scientific data, we recommend to aim for reduced levels of sedentary time and increased levels of light physical activity (e.g. walking, house-keeping, gardening) as the latter may also improve health span and decrease levels of disability in older age.

Cost-effective training individualization and monitoring

The population of older adults is still increasing, which also means that the number of eligible people who may benefit from well-structured exercise interventions is rising as well. Cost-effective and non-invasive exercise testing methods and monitoring to optimize and tailor exercise training interventions for large groups is therefore challenging. In **chapter 2** we showed that the heart rate variability measurements appear to be a good alternative for time-consuming, costly or invasive spiro-ergometry and lactate measurements, which was recently also shown by other authors [18, 19]. Determining anaerobic thresholds is crucial for designing and monitoring endurance training process in order to obtain the best health promoting benefits (improved exercise performance, blood glucose level regulation, fat burning, oxidative enzymes activity). The current study showed acceptably precise agreement between the HRV and spiro-ergometry methods **(chapter 2)**. Although further studies are needed to validate the long-term application in different types of exercise training program for different types of populations, that the present study indicates that the exercise based HRV assessment method of the second AT may have clinical relevance for larger scale applications to optimize the benefits of well-structured exercise programs.

Different FiO_2 conditions as a potential diagnostic and treatment strategy for improved exercise effects in deficient populations

Exercise scientists and physiologists have been looking for non-invasive and easier methods to improve effects of exercise training alone in healthy and patient populations. Number of reports showed effectiveness of exercise training interventions under low (altitude training) [20, 21] and high FiO_2 (hyperoxia) [22, 23] conditions. Hypoxic conditions (even at 580 m [24] challenge the physiological system during a single or multiple bouts of exercise (hyperemic response, decrement in VO_2) and this has been the rationale behind hypoxic training as a method to subsequently improve exercise tolerance (onset and offset oxygen uptake kinetics at sea level [19]. In **chapter 3** we used the challenging effects of hypoxic exercise to test the sensitivity of oxygen uptake and muscle oxygenation methods during the recovery kinetics following a submaximal exercise bout. For example, NIRS measurements can be influenced by interpersonal response levels [20] to different FiO_2 and adipose tissue

thickness [25]. Significantly slower recovery kinetics showed sufficient sensitivity of $\dot{V}O_2$ and NIRS measuring methods to detect potential improvements in the recovery kinetics under hyperoxic conditions. Additionally, other authors showed significant reliability of the NIRS measurements up to an adipose tissue thickness of 16mm [25]. Our subjects in **chapter 3** had an average adipose tissue thickness of 6.1mm, which could cause marginal or no influence on the muscle oxygenation measurements.

In **chapter 3** we demonstrated that hyperoxic conditions (FiO_2= 35%) did not accelerate the recovery oxygen uptake kinetics in healthy subjects without known oxygen delivery limitations. The concept of using both hypoxic and hyperoxic conditions during acute submaximal exercise may therefore be applied as a cost-effective and noninvasive method in a clinical setting for older adults and patients with CHF, COPD and DM2 **(chapter 3)**. In particular, acute hypoxic exercise bout can determine individual sensitivity to the measurement methods. Additionally, acute hyperoxic exercise can establish what level of FiO_2 can improve oxygen delivery deficit (if any) and be further applied in training conditions. In accordance with our findings, future studies should be focused on investigating low and high FiO_2 as a diagnostic strategy for oxygen delivery limitations during acute exercise bout. Subsequently, established effective high FiO_2 levels should be investigated in exercise training in subjects with detected oxygen delivery limitations.

Although our experiments described in **chapter 4** were a feasibility study, they indicate the practical usefulness of such an approach by applying 3 levels of flows of hyperoxic air in a group of advanced-stage and deconditioned type 2 diabetes patients with a high cardiovascular risk profile. Using a set up that can be easily applied in a clinical training center, we found that a flow of 15 L.min-1 increased the arterial oxygen pressure to sufficient high levels in this deconditioned population. Nevertheless, considering the interpersonal variability in dose-response to hyperoxic exercise [20, 26], we suggest to perform a dose-response test for an individual hyperoxic air concentration before designing and prescribing exercise training under hyperoxic conditions. Although future studies are warranted, NIRS or $\dot{V}O_2$ uptake recovery kinetics could have added value as well to find an optimal flow and concentration of oxygen.

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General conclusions

1. There is currently no strong evidence on adverse effects of antioxidant supplements in conjunction with endurance training on exercise adaptation in healthy subjects. However, potential health benefits of dietary antioxidants in subjects with vitamin and cardiovascular deficiencies warrant investigating suitable types, combinations of antioxidants and different training modalities **(chapter 1)**.
2. Non-invasive and cost-effective HRVT2 assessments showed clinically acceptable reliability in comparison with the 'gold standard' VT2 method. The HRVT method may be easier applied in numerous rehabilitation patient groups for individualized training design and monitoring **(chapter 2)**.
3. Hyperoxic conditions did not improve the oxygen uptake kinetics in healthy young adults **(chapter 3)**. However, supplemental oxygen may improve exercise tolerance in subjects with oxygen delivery limitations e.g. type 2 diabetes, chronic heart failure as higher flow of oxygen in inspired air elevates arterial oxygen levels **(chapter 4)**.
4. Increased levels of sedentary time and inactivity are associated with higher likelihood for the metabolic syndrome and its components in older adults **(chapter 6)**. Training interventions focused on reducing adipose tissue levels and improving muscle quality will prevent progression of the metabolic syndrome, sarcopenic changes and disability at earlier age **(chapter 5)**.

Summary

It is certain that the aging process leads to death, but decreasing the levels of pathology throughout life improves the quality of life and extends life span. Therefore, this dissertation focuses on alternative strategies that may contribute to improving the aging process and associated common pathologies. In particular, this work presents exercise as the foundation of healthy aging and discusses potential strategies to improve existing suboptimal interventions to improve the aging process.

The introductory chapter defines the aging process and its main pathologies. The dissertation presents how non-invasive and more cost-effective interventions may ameliorate the aging process.

Chapter 1 addresses the current controversy around the effects of dietary antioxidants in conjunction with endurance training. Based on some pre-clinical and clinical work, reduced formation of reactive oxygen species may blunt health benefits of endurance type of exercise training. However, the evidence is still not robust and number of reports do not show adverse effects of popular dietary antioxidants combined with endurance training. Additionally, it is possible that some dietary supplements (e.g. resveratrol) may improve effects of endurance training in subjects with e.g. vitamin deficiencies and cardiovascular dysfunction. Therefore, the lack of clarity warrants future studies on doses, types and combinations of dietary antioxidants and different exercise training modalities. Next to nutritional strategies, there is need for alternative interventions that will improve effects of exercise non-invasively and cost-effectively.

Chapter 2 presents a validation study comparing 2 methods for the 2nd anaerobic threshold (AT2) during an incremental exercise test. AT2 assessment based on heart rate variability (HRVT) is a non-invasive and more cost-effective method than assessment based on the spiro-ergometry output or a lactate curve. Our results show that HRVT is sufficiently valid and accurate, but its application in rehabilitation patient groups still warrants separate validation studies. Nevertheless, our results indicate that it has potential application for individualized training design and monitoring.

Chapter 3 and 4 describe a novel strategy to improve exercise tolerance by oxygen supplementation during exercise. Using a set up that can be easily applied in a clinical training center, **chapter 4** shows that a 100% oxygen flow of 15 L.min-1 applied through a mask increased the arterial oxygen pressure to sufficient high levels in deconditioned type 2

diabetes patients. This feasibility study shows that higher oxygen availability may improve oxygen delivery to exercising muscles and improve exercise tolerance, if needed. The novel experimental method described in **chapter 3** showed that recovery $\dot{V}O_2$ and muscle oxygenation kinetics following a submaximal bout of exercise under both hypoxic, normoxic and hyperoxic conditions may be useful to test whether supplemental oxygen is beneficial or not. Our results indicate that for submaximal exercise bouts healthy subjects will not benefit from supplementary oxygen. Nevertheless, performing acute exercise bouts under hyperoxic conditions may help detecting oxygen delivery limitations. Additionally, acute exercise tests under hyperoxic conditons will help establishing doses supplemental oxygen for better exercise training effects in deficient subjects e.g. older adults, CHF and COPD patients.

Chapter 5 and 6 address the topics of increased sedentary time, physical activity level decline and its consequences (sarcopenia) in older adults. **Chapter 6** shows higher likelihood for the metabolic syndrome and its components in most sedentary and inactive older adults. In aging populations at risk or with the metabolic syndrome **(chapter 5)** inactivity and sedentary behavior lead to its progression together with the muscle quality loss. In particular, low levels of muscle quality and high levels of adipose tissue are associated with a functional capacity decline and progression of the metabolic syndrome. Taken together, the lack of light, moderate-to-vigorous physical activity and resistance training increase the risk of chronic diseases (type 2 diabetes and cardiovascular disease) and disability at relatively younger age.

Finally, in the general discussion we discuss our new insights and findings and make recommendations for future research and alternative strategies that may prevent aging related pathologies and improve the beneficial effects of exercise throughout life.

Samenvatting

Het ‘leven is eindig’ en ‘ouderdom komt met gebreken’, zijn vaak gehoorde gezegden in de Nederlandse taal. Het is dan ook algemeen geaccepteerd dat het verouderingsproces van ons lichaam uiteindelijk tot de dood leidt. Echter, het reduceren van de snelheid waarmee ons lichaam veroudert vermindert de kans op ziekten en leidt over het algemeen tot een beter kwaliteit van leven en toename van het aantal (gezonde) levensjaren. Gezien de wereldwijde toename van een vergrijzende bevolking richt dit proefschrift zich op alternatieve strategieën die kunnen bijdragen aan de verbetering van het verouderingsproces en de hiermee samenhangende ziekteprocessen. Het uitgangspunt van dit proefschrift berust op de kennis dat voldoende en gestructureerde lichaamsbeweging een goede basis vormt voor een gezond verouderingsproces. Daarnaast worden potentiële strategieën besproken en onderzocht die bestaande beweeginterventieprogramma’s verder zouden kunnen verbeteren.

De inleiding van dit proefschrift definieert de verschillende fysiologische aspecten van het verouderingsproces alsmede de belangrijkste hiemee samenhangende ziekteprocessen. Het hoofdstuk beschrijft hoe in theorie niet-invasieve en meer kosten-effectieve interventies het verouderingsproces aanzienlijk zouden kunnen verbeteren.

Hoofdstuk 1 richt zich vervolgens op de huidige controverse rond de effecten van antioxidanten, zoals hoge doses vitamine C en E, met name wanneer deze worden toegepast in combinatie met duurtraining. Op basis van een aantal pre-klinische en klinische studies is bekend dat een reductie van inspanningsgerelateerde zuurstofradicalen bepaalde gezondheidsvoordelen van duurtraining kunnen verminderen. Echter, het bewijs hiervoor is nog niet geleverd aangezien voorlopige resultaten van een aantal interventiestudies dit tegenspreken. Bovendien zijn er zelfs aanwijzingen dat bepaalde typen voedingssupplementen zoals resveratrol de effecten van duurtraining verbeteren, met name bij patiënten met bijvoorbeeld vitamine tekorten of een slecht functionerend hartvaatstelsel.Gezien alle onduidelijkheid en de populariteit van bepaalde type anti-oxidanten en vitamines om veroudering tegen te gaan, zijn meer studies gerechtvaardigd ten aanzien van dosering, typen, combinaties van antioxidanten, bij de verschillende vormen van fysieke training. Echter, naast onderzoek naar bepaalde voedingsinterventies, is er ook behoefte aan alternatieve interventies die de effectiveit van lichaamsbeweging op het verouderingsproces verder kunnen verbeteren. **Hoofdstuk 2** beschrijft een validatiestudie naar 2 methoden om de 2de anaerobe drempel (AT2) te bepalen tijdens eem oplopend inspanningsprotocol. De 2de

anaerobe drempel wordt gebruikt om de trainingsintensiteit bij een sport- of revalidatieprogramma te bepalen, maar vergt normaliter bepaling van een lactaatcurve dan wel vrij kostbare ademgasanalyse-apparatuur. In dit onderzoek werde de klassieke ademgasanalyse methode vergeleken met een nieuwe methode gebaseerd op hartslagvariabiliteit (HRVT). Onze resultaten laten zien dat de methode op basis van hartslagvariabiliteit bij gezonden afdoende valide en nauwkeurig zijn. Dit opent deuren voor vervolgstudies naar validatie en gebruik voor bepaalde patientenpopulaties waarbij een geindividualiseerd trainingsprogramma dient te worden opgesteld of waarbij de trainingsprogressie dient te worden gemonitord.

In hoofdstuk 3 en 4 wordt vervolgens een aantal nieuwe methodieken gepresenteerd om de meerwaarde van zuurstof suppletie tijdens inspanning vast te stellen. Met behulp van een opstelling die vrij eenvoudig kan worden toegepast in een revalidatiesetting tonen we aan inhalatie van 15 L per min 100% zuurstof toegediend via een masker de zuurstofconcentratie in het slagaderlijke bloed tot een voldoende hoog niveau verhoogd in gedeconditioneerde patiënten met type 2 diabetes. Deze haalbaarheidsstudie suggereert dat een hogere beschikbaarheid van zuurstof de zuurstofspanning in de skeletspier tijdens matig intensieve inspanning verbetert en mogelijk ook de inspanningstolerantie verhoogt. Aan de hand van een nieuwe experimentele methode beschreven in **hoofdstuk 3** tonen we aan dat het herstel van zuurstofopname en spierweefseloxygenatie kinetiek, onder verlaagde, normale en verhoogde zuurstofcondities, nuttig kan zijn om te bepalen of extra toediening van zuurstof bij matig-intensieve inspanning gunstig is of niet. Ofschoon onze resultaten laten zien dat extra zuurstof bij gezonde proefpersonen geen meerwaarde heeft zou deze nieuwe methode mogelijk wel nuttig kunnen zijn bij patienten met een chronische aandiening (COPD, Type 2 diabetes, hartfalen).

Hoofdstuk 5 en 6 gaat vervolgens in op de problematiek van de toename in zittijd, afname van het fysieke activiteitenniveau en de gevolgen daarvan (sarcopenie) bij de ouder wordende mens. De resultaten van een grootschalig onderzoek, beschreven in **hoofdstuk 6** laten zien dat het metabool syndroom en zijn componenten frequenter aanwezig is bij de meeste sedentaire en inactieve groep ouderen. Op basis van de een uitgebreide literatuur studie (**hoofdstuk 5)** wordt duidelijk dat het metabool syndroom, in combinatie met chronische inactiviteit en sedentair gedrag het verlies van spiermassa en spierkracht verder versnellen. Het verlies van functioneel spierweefsel in combinatie met vetstapeling en overgewicht leidt vervolgens weer tot een verdere achteruitgang van de functionele capaciteit en toename van het metabool syndroom. Deze vicieuze cirkel van gebrek aan fysieke inspanning en afname van spiermassa

verhoogt vervolgens het risico op chronische ziekten zoals hart-en vaatziekten en type 2 diabetes en leiden uiteindelijk tot vervroegde invaliditeit.

Aan de hand van de nieuw verkregen inzichten wordt in de algemene discussie ingegaan op de in dit proefschrift gepresenteerde resultaten en worden tenslotte aanbevelingen gedaan voor toekomstig onderzoek naar alternatieve beweeginterventiestrategieën. Slimme en geindividualiseerde beweeginterventiestrategieen zouden vroegtijdige veroudering kunnen voorkomen en de kwaliteit van leven en het zelfstandig functioneren, tot op een hoge leeftijd, kunnen behouden.

Publications

1. **Mankowski RT**, Aubertin-Leheudre M, Beavers DP, Botoseneanu A, Buford TW, Church T, Glynn NW, King AC, Liu C, Manini TM, Marsh AP, McDermott M, Nocera JR, Pahor M, Strotmeyer E, Anton SD; LIFE Research Group.Sedentary time is associated with the metabolic syndrome in older adults at high risk for mobility limitations - The LIFE Study. Exp Gerontol. 2015 Jun 27. pii:S0531-5565(15)30004-8

2. **Mankowski RT**, Anton SD, Aubertin-Leheudre M. The Role of Muscle Mass, Muscle Quality, and Body Composition in Risk for the Metabolic Syndrome and Functional Decline in Older Adults. (2015) Curr Geri Rep. DOI: 10.1007/s13670-015-0132-y Epub ahead of print]

3. **Mankowski RT**, Anton SD, Buford TW, Leeuwenburgh C.Dietary Antioxidants as Modifiers of Physiologic Adaptations to Exercise. Med Sci Sports Exerc. 2015. [Epub ahead of print]

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I obtained a master degree in physiotherapy (3+2 years model) in Poland. However, my interest in Biochemistry and Exercise Physiology began in the first year and stayed as a priority for the rest of my study period (many thanks to my bachelor and master theses promotor, **Prof. Barbara Klapcinska,** for loads of inspiring influences).
During the last two years of my Master's studies in Poland I decided to participate in the Erasmus Life-Long Learning program. I spent 5 months at Palacky University in Olomouc in the Czech Republic. Besides experiencing brilliant price/quality Czech food & beverage goods, I also met magnificent international people and participated in great classes given by **Dr. Carol Leitschuh (USA), Dr. Jeff Walkley (Australia) and Prof. Donald Roberson (USA)**. During that time I improved my English language skills and got more "taste" for international adventures and continuing my education abroad.

After obtaining my Master's degree, I directly applied to be admitted to a PhD trajectory at the same university in Poland. At the same time I chose the Netherlands as my next destination, having an idea of pursuing my Polish PhD in cooperation with a Dutch university. I sent my CV and a motivation letter to a number of Dutch and Belgian universities. Long story, short, my email sent to **Prof. Henk Stam** (my current PhD promotor), which he forwarded to **Dr. Stephan Praet** (my current PhD supervisor), drew the required interest and resulted in a meeting in June 2010. After a very short collaboration period with my Polish supervisor, I gave up the Polish PhD position.

Despite my "madness" for learning and pursuing a PhD degree at Erasmus University Medical Centre, there was unfortunately (now I know that it was fortunate, overall) no paid position available. My only option was to find a supervisor, develop my own project, find some resources to perform the experiments and obviously have a 'side' job to make a living.

Considering the order of my job opportunities, as first, I would like thank TNT Post Office for giving me a chance to experience cycling with 30kg of flyers on snowy bicycle lanes and letting me explore some endless streets of the Hague with the weirdest house numbering, ever. I would also like to thank Albert Heijn, fruit & vegetable industries and cleaning services for refusing my job applications ☺

After 2 weeks of experience as a mailman, I started working as a personal fitness/medical trainer at Get Fit Stay Fit, personal training studio. Here, I would like to thank the owners: **Saskia Steysiger** and **Tjeerd Mouthaan;** for giving me a chance and providing me with personal training clients. Thanks to this brilliant and flexible job I could train my clients at early mornings (starting at 6 AM), so later in the day I could work on designing and pursuing my PhD research and later I gave a few more sessions to my clients in the evening (finishing usually around 11 PM). My clients also appreciated very much my wide availability during weekends. Saturday and Sunday -fun-days drew a lot of interest of about 10-15 clients. We also developed a very close client-trainer relationship. My 'fit people' have done many significant things during my time in the Netherlands.

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Thanks to my personal training job with many international clients and excellent English language skills of the Dutch population I could 'survive' using my English.

However, theoretically and practically it did not sound right to be in a Dutch speaking country without possessing the Dutch language skills. Therefore, shortly after arriving in the Netherlands I signed up for a course of the Dutch language. Long story, short, after 1.5 years I sat the NT2 (Nederlands als tweede taal) exam and received the Dutch language certificate at B1 level. Many thanks to my extremely motivated and enthusiastic teacher, **Merel van Slageren,** and the whole course group who made it fun too. **Merel**, thank you for the pre-exam butt-kick "Robert, jij hebt de tentamens over een maand, maar je bent er nog helemaal niet klaar voor!" I think you were right as I could not speak Dutch at that moment ☺. However, a month of putting my whole mind into *Bob De Bouwer*, Woezel en Pip and *Google Translate* made it real! At this moment, I am so happy that I could write response letters to the Medical Ethical Committee and getting all the benefits and knowledge from Dutch spoken meetings and seminars.

Talking about languages, I would like to thank **Mr. Andrew Jones** for inviting me and helping discover the Toastmasters International, where with pleasure I still master my public speaking and the English language skills. Thanks to "Table Topic" sessions I am sure of satisfying immediate answers to spontaneous questions without having even a clue about an answer.

That part above briefly highlighted my life in The Hague and its impact on obtaining a doctorate degreee, which was and is going on till today. It is time now to get back to the origins of my academic career in Rotterdam.

The whole new research proposal was written under supervision of **Dr. Stephan Praet.** The supervision content and angles exceed the scope of this acknowledgment section. Therefore, I will be brief. Shortly speaking, thank you, **Stephan**, for supervising my research project and for joining me in being creative in looking for devices and research services that we could borrow and use for free (no research funding). Thanks for going through and signing endless response letters to the Medical Ethical Committee (10 months in the process; thank you **Mrs. Serieta Mohkamsing** for helping in the first translations to the Dutch Language). Regarding the application to the Medical Ethical Committee and approving the use of medical gasses in my study, it would be impossible without great help of the Pharmacy Department, especially **Ms. Rianne Zaal**. Getting back to the main topic, thank you, **Stephan**, for helping me to participate in international conferences (USA, UK) regarding scientific development as well as social activities (Finals in Athletics at the Olympic Games in London…still cannot believe

how we got those tickets; Californian treats in Napa Valley and many more). Additionally, many thanks for teaching me how to dine in Rotterdam ☺

In order to summarize it, despite of many limitations regarding my doctorate degree, you are majorly responsible for where I am standing now scientifically and socially. Thanks very very much for putting me in contact with **Prof. Christiaan Leeuwenburgh** from the Institute on Aging at the University of Florida, which at the end resulted in receiving an offer of a postdoctoral position at the University of Florida.

During my PhD trajectory I was extremely eager to gain more research experience abroad. Like I mentioned above, after a positive introduction by **Dr. Stephan Praet** to **Prof. Christiaan Leeuwenburgh**, I spent 3 months in beautiful and WARM Gainesville, working hard at the Institute on Aging and enjoying the surroundings. I would like to thank **Prof. Christiaan Leeuwenburgh** and **Dr. Stephen Anton** for brilliant supervision and producing 2 scientific articles while being in the USA. Thank you, **Christiaan,** for agreeing to become my second promotor, for lending me a bike in Gainesville and for giving me this enormous scientific career opportunity.

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I would like to thank my study participants and my minor students who gave me 'some' numbers to process. Thanks for your flexibility to be able to come to the lab at strangest times (very early morning or very late evening).

Talking about data processing, thank you **Dr. Victor Niemeijer and Jasper Jansen** for the expertise on oxygen uptake kinetics and data processing, respectively.

Big thanks to my first prmotor, **Prof. Henk Stam**, for forwarding my first email to **Dr. Stephan Praet**, admitting me to the department and funding my last 10 month to complete my PhD. Practically, you opened the first door to my success ☺

Finally, I must thank my wonderful wife, **Nadia Mankowski-Zernouf**, for her love, mental (yes, she is a psychologist ☺) & physical support, for brilliant Moroccan/Egyptian dinners and patience for me spending time with my "other wife" (laptop)…these are your words, Honey ☺. Because of you and how much we have done and what we have gone through together, I certainly know what I work for and who I live for…

انا بحبك

Curriculum Vitae

Personal details

Date of birth: 18 July 1986

Place of birth: Cracow

Nationality: Polish

Professional qualifications

July – to date **Research associate (OPS) at the Department on Aging and Geriatric Research at the University of Florida in Gainesville, FL, the United States of America**

April – July 2014 **Visiting research scholar at the Institute on Aging and Geriatric Research at the University of Florida in Gainesville, FL, the United States of America**

Sep 2011 – June 2015 **Ph.D. candidate at the Erasmus University Medical Centre in Rotterdam, Netherlands**
Doctoral dissertation entitled "Alternative strategies to improve the beneficial effects of exercise throughout life: dietary and physiological aspects".

Jan 2011 - Sep 2011 **Pre-doctoral training at the Erasmus University Medical Centre**
Courses: Biostatistics for clinicians and Methodology in clinical research; training in a human physiology laboratory.

Sep 2008 - Jul 2010 **M.Sc. in Physiotherapy** (with honours)
Jerzy Kukuczka Academy of Physical Education in Katowice, Poland.

Feb - Jun 2009 **Erasmus Exchange Program (LLP-Lifelong Learning Program)**
Exchange student at Palacky University in Olomouc, Czech Republic.

Sep 2005 - May 2008 **B.Sc. in Physiotherapy**

Jerzy Kukuczka Academy of Physical Education in Katowice, Poland.

Career summary

July 2015 – to date **Research associate (OPS) at the Department on Aging and Geriatric Research at the University of Florida in Gainesville, FL, the United States of America**

- Study participant screening and follow-up.
- Molecular biology laboratory training (high-resolution respirometry and Western blots)
- Data collection, entry and analyses.
- Literature review, manuscript and research grant preparation.

Apr – July 2014 **Visiting research scholar at the Institute on Aging and Geriatric Research at the University of Florida in Gainesville, FL, the United States of America**

- Active participation in departmental research meetings.
- Attending seminars and symposia.
- Secondary data analysis resulting in a co-authored scientific article.
- Co-authored relevant review article.

Mar 2014 – to date **Independent consultant at Pure&Well – Fitness and Wellness Consultancy**

- Lifestyle and Wellness coaching with individual clients.
- Corporate wellness programmes.
- Consulting in the area of sport science, exercise physiology, medical personal training.

Jan - Dec 2014 **Assignment - Fitness Department Manager at Inprime Corporate Wellness Programs**

- Recruitment of fitness and body&mind professionals for corporate wellness projects.
- Strategic planning for the department of wellness programs including recommendations for budgetary implications.
- Informing upper management of key activities and delivering periodic progress reports.

Sep 2011 - to date **Erasmus University Medical Centre in Rotterdam, Netherlands**
Doctoral Scientific Researcher (hospitality agreement)

- Designing a doctoral research project.
- Leading a research project at the Department of Rehabilitation Medicine and Physical Therapy, subdivision of Sports Medicine.
- Teaching duties – 3rd year medical students *(exercise physiology)*.
- Supervising Master's research projects of medical students.
- Active participation in weekly departmental research meetings.
- Presenting research findings at international scientific seminars and conferences **(conference shortlist upon request)**.

Jan 2011 - to date **Get Fit Stay Fit – Personal Fitness Solutions, The Hague, Netherlands**

Medical Personal Trainer at Get Fit Stay Fit, a personal fitness organisation specialised in serving individual clients and companies.

- Establishing individual health programmes with clients in order to achieve desired goals.
- Leading and supervising training processes.
- Developing long-term professional relationships with clients.

Apr 2012 - to date **Volunteer board member of the Erasmus PhD Association Rotterdam *(EPAR)*, Netherlands**

- Regular meetings with the University Council of the Erasmus University in Rotterdam.
- Co-operation with the National PhD Association *(Dutch: Promovendi Netwerk Nederland - PNN)*.
- Organising events for PhD candidates (educational workshops, professional fairs, awards, social events).

Jun – Aug 2010 **Volunteer counsellor at summer camps for type 1 diabetes children.**
Summer diabetes camps organised by the Polish Diabetes Association in Dzwirzyno, Poland

- Member of the camp staff responsible for daily physical activity and organisation of camp events.
- Involved in medical support and daily education of the diabetes camp participants (diet, physical activity).
- Research data collection (influence of daily physical activity on glycaemic profile in type 1 diabetes children).

Key skills

- **Project design** – Developed a doctoral research project under the supervision of a senior research fellow. This included designing a research proposal and developing intra- and inter-academic co-operation. Supervising medical students in the design and implementation of Master's research projects.
- **Project management –** Had the lead in a scientific research trial. This involves managing administrative and ethical issues and the inclusion of subjects, as well as leading the research team.
- **Analytical skills – Solving methodological and feasibility issues of research trials.** Problem identification, analysing and solving.
- **Communication skills** – An academic teacher, scientific conference speaker as well as a lifestyle & fitness coach. Presentation skills and public speaking. Experienced as a personal trainer in interpersonal communication with individual clients.

- **Organisational skills** – Board member of the Erasmus PhD Association Rotterdam *(EPAR)*. This involves the organisation of informative, educational and social events for PhD candidates (fairs, workshops, social networking meetings) and awards (PhD of The Year Award).
- **Business relationship building –** Developing a long-term relationship with coaching clients with a high level of professional seniority. Setting individual goals leading to desired outcomes and to establishing new challenges for business relationship continuity.
- **Languages** – Speaks two foreign languages, in addition to his native Polish: English (professional and academic environment) and Dutch (obtained B1-level certification after 1.5 years of residency in the Netherlands).

Hobbies

Novel fitness training methods, endurance sports – marathons, MTB, kayaking, Toastmasters International *(public speaking),* travelling.

Erasmus MC
Universitair Medisch Centrum Rotterdam

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student: Robert Mankowski Erasmus MC Department: Rehabilitation Medicine Research School: N/A	PhD period: 2012-2015 Promotor(s): Prof. H.J. Stam; Prof. C. Leeuwenburgh Supervisor: Dr. S.F.E. Praet

1. PhD training

	Year	**Workload (Hours)**
General academic skills		
- Biomedical English Writing and Communication	2014/2015	56
- Scientific Integrity and Data Management Practises	2012 2013	5 8
- Career Development Workshop (PhD Day) (Presentation Skills; Storytelling, Personal Branding, Networking)		
Research skills		
- Biostatistics for Clinicians (NIHES)	2011	20
- Introduction to Clinical Research (NIHES)	2011	20
In-depth courses (e.g. Research school, Medical Training)	2015	50
- MiP School (Mitochondrial Biology)		
Presentations		
- Poster ACSM Orlando	2014	8
- Poster IPE Miami	2014	8
International conferences		
- ACSM San Francisco	2012	40
- Sport Nutrition Conference Oxford	2012	20
- ACSM Orlando	2014	40
- IPE Miami	2014	30

Other		
- Research visit – Institute on Aging, UF, FL, USA	Apr-Jul	700
- Reserch meeting – Department of Rehabilitaion	2014	100
Medicine	2011-2015	
2. Teaching activities		
	Year	**Workload (Hours/ECTS)**
Lecturing		
- Minor class – Rehabilitation and Sport	2012	10
- Minor class – Rehabilitation and Sport	2012	10
- Minor class – Rehabilitation and Sport	2012	10
Supervising practicals and excursions		
- Exercise training design	2013	50
- Exercise training design	2014	50
Supervising Master's theses		
- Medical Student (L. Spraakman)	2013-2014	200
- Exercise Science (S. Stokla)	2014-2015	50
Other		
- Board member of Erasmus PhD Association Rotterdam (EPAR) : organizing a PhD Day at Erasmus University; organizing The PhD of The Year Award.	2012-2013	100
Total		**1485 hours**