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Improving exercise tolerance in healthy young and older adults: possible role and mechanism of action of Strength training

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TABLE OF CONTENTS

TABLE OF CONTENTS **2**

BACKGROUND **3**

PHYSICAL ACTIVITY GUIDELINES 3

SEDENTARY LIFESTYLE 4

THE RIGHT DOSE OVER THE LIFESPAN 6

EXERCISE TOLERANCE AND THE POWER-DURATION MODEL: 9

HOW INTENSITY CAN LIMIT QUANTITY. 9

THE TWO DIFFERENT EXERCISE PARADIGMS 11

LOSS OF EFFICIENCY 16

INTRODUCTION **25**

PURPOSES AND RESEARCH QUESTIONS **28**

Study one (chapter 1) *28*

Study two (chapter 2) *28*

CHAPTER 1 ..... **29**

"EXCESS VO2": HOW STRENGTH TRAINING AFFECTS METABOLIC EFFICIENCY IN YOUNG AND OLDER ADULTS **29**

INTRODUCTION 29

METHODS 31

RESULTS 39

DISCUSSION 42

CONCLUSIONS 49

CHAPTER 2 ..... **57**

VO2 SLOW COMPONENT: HOW STRENGTH TRAINING AFFECTS METABOLIC STABILITY AND EXERCISE TOLERANCE **57**

INTRODUCTION 57

METHODS 60

RESULTS 71

DISCUSSION 73

CONCLUSIONS 81

Background

Physical Activity guidelines

Evidence of a health benefit for physical activity is seen throughout the life course. In children, effects are predominantly seen in amelioration of risk factors for disease, avoidance of weight gain, achieving a high peak bone mass, and mental well- being[1] [2]. In adults, protection is conferred against the diseases themselves – including cardiovascular disease, cancer, type II diabetes – and obesity. Physical activity also promotes musculoskeletal health and mental health and well-being.[3] The health benefits are even more pronounced in older adults and are particularly important because the diseases involved – most notably osteoporosis, circulatory diseases and depression – affect an older person's ability to maintain an independent lifestyle[1]. All-cause mortality is reduced by regularly engaging in physical activity; this is also the case when an individual increases physical activity by changing from a sedentary lifestyle or a lifestyle with insufficient levels of physical activity to one that achieves the recommended physical activity levels[2]. The physical activity guidelines help people understand the right levels of physical activity and how to make it a part of their regular routine[4].

Since 1978 American College of Sports Medicine has published Position Stands to provide evidence-based recommendations to health and fitness professionals in the development of individualized exercise prescriptions for all stages of life. The position stands are also elaborated for variety of conditions and special populations[4]. The ACSM recommends that children and youth up to 17 years should accumulate at least 60 min of moderate to vigorous intensity physical

activity daily. Amounts of physical activity greater than 60 min have been proven to provide additional health benefits [5]. The focus of these activities should be on developing fundamental movement skills but muscle strengthening, flexibility and bone strengthening activities should also be included 3 times a week.

Regarding adults, they are invited to engage in moderate intensity cardiorespiratory exercise training for ≥ 30 min per day on ≥ 5 times a week for a total of ≥ 150 min a week or, alternatively, vigorous-intensity cardiorespiratory exercise training for 20 min per day on ≥ 3 times a week for a total of ≥ 75 min a week, or a combination of moderate and vigorous intensity exercise to achieve a total energy expenditure of ≥ 500–1000 MET·min·week [3]. On 2–3 times a week, adults should also perform resistance exercises for each of the major muscle groups, and exercise that involve balance, agility, and coordination. Crucial to maintaining joint range of movement, completing a series of flexibility exercises for each of the major muscle–tendon groups (a total of 60 s per exercise) on 2 times a week is recommended [3]. Older adults are also encouraged to participate in ≥ 30 min per day of moderate-intensity aerobic exercise 3 to 5 times a week for a total 150 min a week and supplement with resistance, flexibility, and balance training ≥2 times a week [6 5].

Sedentary lifestyle

Although there is a strong general consensus on the importance of physical activity to maintain health and wellness, people do not engage in the adequate quantity of physical activity for good health [7]. The proportion of adolescents not achieving 60 min per day was equal to or greater than 80% in 56 (53%) of 105

countries in boys, and 100 (95%) of 105 countries for girls [8]. The frequency of adults inactivity is on average 30% and varies greatly between World Health Organisation (WHO) regions (figure 1): 27.5% of people are inactive in Africa, 43.3% in the Americas, 43.2% in the eastern Mediterranean, 34.8% in Europe, 17·0% in southeast Asia, and 33.7% in the western Pacific. Women are more inactive (33.9%) than are men (27.9%). Only 64.1% of adults report walking for at least 10 min consecutively on 5 or more days per week [9].

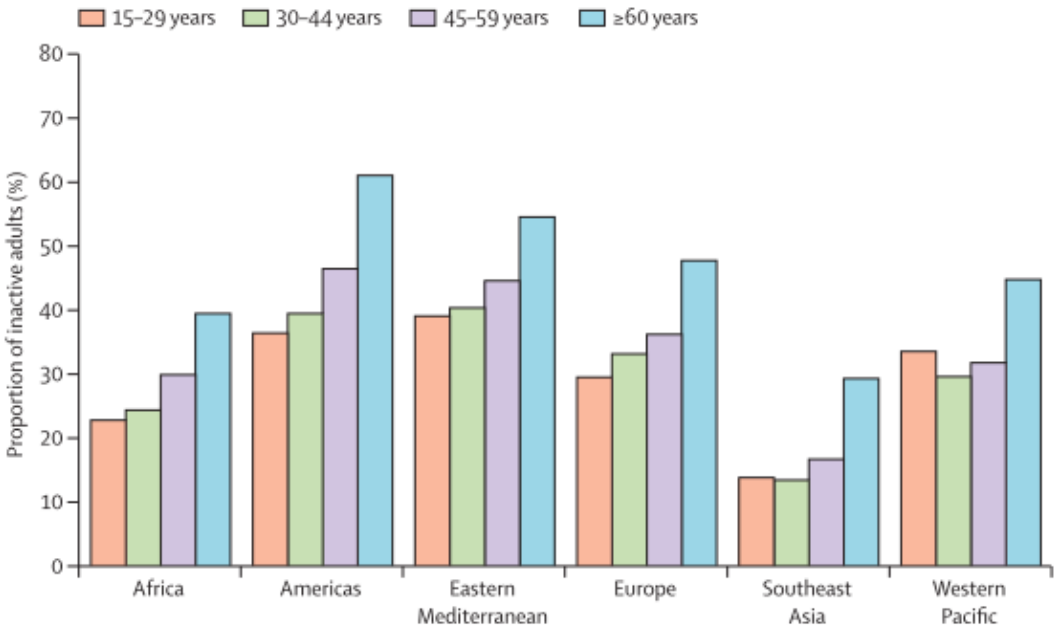

Figure 1. P.C. Hallal et al. 2012. *Global physical activity levels: surveillance progress, pitfalls, and prospects.* Physical inactivity in age groups by WHO region.

Since the industrial revolution, the development of new technologies has enabled people to reduce the amount of physical labour needed to accomplish many tasks in their daily lives. As the availability of new technologies has increased, the reduced physical labour and human energy expenditure have impacted many aspects of the lives of more and more people [10] . Although the technological

revolution has been of great benefit to many populations throughout the world, it has come at a major cost in terms of the contribution of physical inactivity to the worldwide epidemic of non-communicable diseases [11].

In 2009, physical inactivity was identified as the fourth leading risk factor just ahead of being obese or being overweight for non- communicable diseases [12]. 6-10% of all deaths from non communicable diseases worldwide can be attributed to a physical inactivity, and the percentage is even higher for specific diseases [11]. In 2007, 5,3–5,7 million deaths globally from non- communicable diseases could have theoretically been prevented if people who were inactive had instead been sufficiently active [13].

The right dose over the lifespan

ACSM position stands, based on scientific literature, report that the total amount of physical activity is the most critical element for health promotion [14] and that . the recommended levels of physical activity can be achieved in a number of ways including accumulating activity in shorter bouts [3]. Shorter bouts of activity offer an easier starting point to those who are sedentary, and it can be an easier way of incorporating exercise into everyday activities rather than undertaking more structured exercise that involves joining sports clubs or classes [15]. In order for physical activity recommendations to be effective for public health improvement, it is important to promote levels of activity that are effective but at the same time realistic and achievable [15].Current studies indicate significant changes in undertaking physical activity, resulting from certain life events and going through

life cycles. However, this field is poorly studied and explained. Researchers show that one of the reasons may be various social, physical, or psychological events in life [16] or experiencing a certain cycle of life characterized by significant traumas/stages. Growing past childhood to adolescence, from school to work, retiring, starting a family, parental responsibilities those events change lives and behavior of the people (including behavior related to participation in physical activity) [17] [18]. Allender et al. distinguished five such turns, that is, employment status, residence, physical status, relationships, and family structure[19]. Depending on accompanying conditions, for example, age, sex, nationality, or type of change they can have a positive or a negative influence on participation in PA.

According to WHO, still nearly one-quarter of the people in the world (23.3%) do not meet the level of activity necessary to maintain health [9].

Among groups of a particular risk, there are professionally active and unemployed people aged 50–64 years without children who do not meet WHO recommendations (45.3 and 50.4%). As reported by studies, over a half of elderly people (52%) are not engaged in leisure time physical activity (LTPA) [8] despite the fact that, objectively perceiving, the possibilities of being more physically active after retirement are higher. As many as 47.6% of retirees do not realize the proper dose of physical activity necessary to maintain health [20]. Aging and related health problems and functional limitations (not observed at younger ages 55–57) can have a relationship with the physiological inability to undertake some forms of activity such us daily activities [21]. However, it is emphasized that these physical activity recommendations are a minimum. Above this minimum effective dose of exercise, there is a dose-dependent relationship between physical activity

and prevention of chronic disease in all ages. Therefore, further health benefits can be achieved by increasing physical activity levels, in terms of intensity, duration and overall volume, beyond these minimum levels [14]. Current guidelines promote the benefits of both moderate and vigorous activities, leaving individuals to choose their activity patterns according to their preferences and abilities. However, emerging evidence indicates that independent of the volume of activity, engaging in some vigorous activity may be beneficial to health and that 2 min of moderate activity may not be equivalent to 1 min of vigorous physical activity [22]. Indeed unconnected of energy expenditure, vigorous physical activity is more efficient than moderate activity in inducing cardiorespiratory and metabolic fitness [23],which is a stronger predictor of morbidity and mortality than activity [24]. Lack of time has been proven to be the major barrier to physical activity [25]. Because vigorous activity is more time efficient in achieving health benefits than moderate activity, promoting vigorous activity might be particularly fruitful for those for whom insufficient time is a major barrier. Previous research indicates that it is possible to encourage overweight middle-aged men and women with a range of chronic health problems to safely participate in vigorous activity in the form of high-intensity interval training [26]. Although vigorous exercise is associated with an increased risk of cardiovascular events and sudden death, the absolute risk of death is extremely low (1 sudden death per 1.5 million episodes of vigorous exertion). Therefore, in future activity guidelines, it may be reasonable to encourage wider consideration of vigorous activities for those who are capable of doing so by including statements such as “If you can, enjoy some regular vigorous-intensity activity for extra health and fitness benefits” [27].

Other studies [28] [29] have reported significant benefits of vigorous activity in reducing mortality. In this middle to older adult population, these findings suggest that even small amounts of vigorous activity may supplement the benefits of moderate activity alone. They found that independent of activity volume, activity intensity was associated with reduced risk of coronary heart disease and all cause mortality in men [30]. Other clinical and epidemiologic studies have also found that, adjusted for activity amount, higher proportions of vigorous activity are beneficial for cardioprotection [31], metabolic health, and maintenance of physical function in older adults [32]. A potential explanation for the positive effect of engaging in some vigorous activity on longevity might be that high-intensity activities lead to more physiologic adaptations, which improve function and capacity.

Exercise tolerance and the power-duration model:

how intensity can limit quantity

Exercise tolerance is the ability to produce the required level of force/power for an adequate time to accomplish a certain task. This is an instrumental to obtain the heath benefits associated with physical training. Therefore is an important component of physical function and mobility and it increases with the individual fitness level [33].

The inability to tolerate an adequate amount of exercise may be a major mechanism in contributing to functional decline; a key landmark on the pathway from independence to “disability”, and to a significant decline in life quality. A

substantial reduction in exercise tolerance is recognized to limit mobility, particularly in sedentary people and older adults, and therefore represents a barrier to gain many of the well-studied exercise benefits [6].

While in the young and in the young adult populations exercise intolerance can limit the amount of specific physical activities (e.g. running, cycling, walking, etc.), in the elderly, it limits the ability to perform the activities necessary to everyday life, especially getting out of bed, dressing, personal hygiene, eating, walking and leisure activities underline the passage from a state of independence to that of dependence [34]. Exercise "intolerance" can be defined as the inability to sustain a given effort in terms of relative intensity and it seems to be mainly related to a reduction of aerobic capacity but is also related to the specific body response at different exercise intensities [6]. It is a common experience that running, cycling, or swimming at a relatively fast yet comfortable pace can be continued for a considerable period without undue fatigue. However, even slightly increasing the pace substantially increases the perceived effort and dramatically reduces the tolerable duration of exercise. Such "critical" intensity of exercise represents the point at which a participant is unwilling or unable to continue a physical task and in the last decades, has been referred to as the point of 'exhaustion', 'the limit of tolerance', or the point of 'fatigue'. This event occurs when there is an increase of oxygen consumption at the same training load variation [35]. In a whole-body exercise, exercise intolerance is caused by muscle fatigue (reduction in muscle force) and decrease efficiency of contraction (the ratio of mechanical energy to metabolic energy output).

The two different exercise paradigms

A decrease of efficiency of contraction can be observed in two different exercise paradigms: constant power exercise (CWR) and incremental or ramp exercise, suggests that the mechanism(s) governing oxygen consumption (VO_2) may be the same in both exercise paradigms.

CWR

In all activities of daily living there are a lot of challenges in which the energy demands of the working muscles might quickly go from rest to substantial loads. It is important to study the dynamic responses of pulmonary gas exchange in order to understand how the metabolic pathways adapted to these continuous challenges [36].

The pulmonary VO_2 response following the onset of exercise has been well characterized [37]

- Phase I: at the onset of CWR exercise, there is an early rapid increase that started with the first breath. This phase represents the O_2 exchange associated with the initial elevation of cardiac output and pulmonary blood flow;
- Phase II: an exponential increase in VO_2 with a time constant of some 20-45 sec in healthy adults, drives VO_2 to the actual or towards the initially anticipated steady state values. This phase called “primary component” reflects the arrival at the lung of venous blood draining the exercising muscles and reflects the kinetics of O_2 consumption in the exercising muscles;

- Phase III: represents the steady state values.

A lot of studies demonstrated that VO_2 profile changes at different energy domains (figure 2)

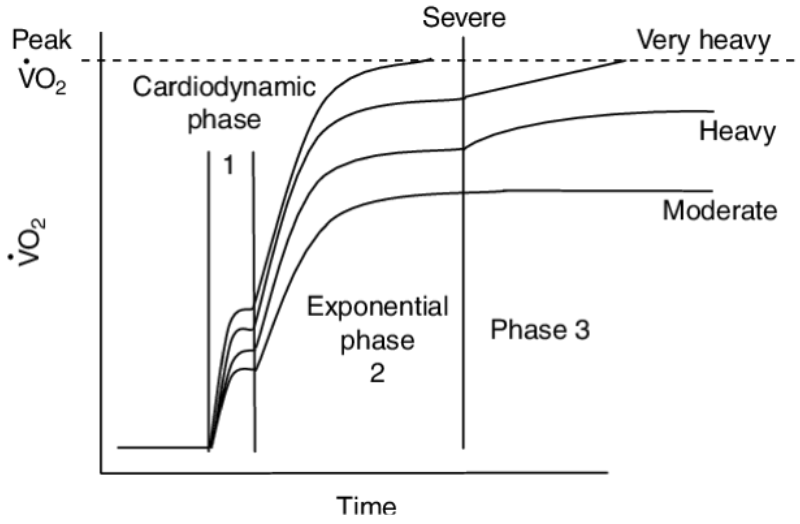

Figure 2. A. M. Jones et al., 2005. *Oxygen uptake kinetics in sport, exercise and medicine.* Schematic representation of VO_2 kinetics in response to different work-rate regions: below ventilatory threshold (<VT, moderate exercise), above ventilator threshold but below VO_{2peak} (>VT, heavy and severe exercise) and above VO_{2peak} (>VO_{2peak}, severe exercise). Each curve has a Phase 1 and a phase 2 and, in addition, the >VT figure has a discernible "slow component" (Phase 3). The dash-dot line in the > VT domain is an extension of the Phase 2 response. For the >VO_{2peak} exercise, the dotted line represents the theoretical value of VO_2 if it could extend beyond the upper limit to meet all the metabolic demands through aerobic metabolism under the assumption that it would continue along its apparent exponential path.

- In moderate exercise (below GET or LT), work rate can be sustained for about long periods and there is not a metabolic acidosis. A steady state is usually attained in about 3 min in young and health adults.

- In heavy exercise (above GET or LT), can be identified a metabolic acidosis, with an increase in lactate and H^+. Steady state in gas exchange is reached with a certain delay, about 10-15 min, bringing to a reduced work efficiency and causing the appearance of the "VO_2 slow component" ($VO2_{sc}$). The exercise is sustained using a greater amount of muscle glycogen and a higher ventilatory response.
- In severe exercise, metabolic acidosis increase inexorably as the exercise continues: a profile signalling ensuing fatigue. The VO_2 gradually increases and the steady state is never achieved, rather reaching the VO_{2peak} values; since there is no more balance between production and removal of lactate, blood and muscle lactate and H progressively increase and muscle phosphocreatine (PCr) is progressively depleted. Here we can note a reduction in work efficiency, which plays a fundamental role in determining the exercise.

Whereas the VO_2 response below GET is described by a mono-exponential function, the VO_2 response above GET, has been shown to better described by bi-exponential processes which represents the amplitude of the VO_{2sc}. This suggests an intensity-dependent loss of muscle efficiency as high intensity exercise proceeds. In conclusion, during CWR at higher power output, the VO_2 causes VO_2 excess and quickly brings the subjects to VO_{2peak}. In addiction the subject progressively decreases the power output in order to oppose the development of VO_{2SC}. In the field of physiology, the study of VO_{2sc} is of interest to enhance basic understanding of muscle energetics, metabolic control and the determinants of the

efficiency of skeletal muscle contraction. Moreover, the study of VO_{2sc} is of importance because it is related to the progressive loss of muscle homeostasis and the development of fatigue.

RAMP

At the and of the 20th century there is the development of a new exercise protocol, the ramp. This test is characterized by a linear and continuous increase in work rate. In this protocol, the specific non steady state conditions provides information on the ability of the aerobic system to adjust to continuously changing in metabolic demand [36]. During the initial phase of ramp test, despite the linear increase in work rate, the pulmonary VO_2 response lags the metabolic demand by a time interval, i.e. mean response time (MRT). The MRT is quantified as the time interval between the onset of the ramp and the intersection of the extrapolation of the baseline VO_2 and the backwards extrapolation of the linear VO_2/time relationship below the GET [38]. Following this initial delay, VO_2 increases linearly with time (t) and work rate (W). Below the GET, the linear phase of the VO_2 response to ramp exercise has the following equation: y= ax+b, where a is the relationship between + VO_2 and work rate ($\Delta VO_2/\Delta W$) and b is the y-intercept or VO_2 at baseline work rate determined from the linear VO_2/W relationship. The $\Delta VO_2/\Delta W$ represents an expression of delta efficiency. This parameter used to quantify mechanical muscle efficiency, is consequently one of the main parameters observed in endurance events. Above GET, also the anaerobic metabolism is involved leading to a slow VO_2 response as similar as VO_{2sc} in constant work rate (figure 3). One study observed that the VO_2/W relationship in three different ramps, i.e ramp 15, ramp 30 and ramp 60, the slope of upper portion ((S_2) 12.5 ± 0.4; 10.5 ± 0.3 and 8.7 ± 0.2 mL min Watts

respectively) was significantly higher compared with the lower portion ((S1) 9.9 ± 0.2; 9.9 ± 0.2; 8.4 ± 0.3 mL min Watts respectively). This suggests that in high intensity exercise the VO_2/W relationship is lost; the upward deflection in the VO_2/W, due to a delayed additional increase in VO_2 is related to a drop in mechanical muscle efficiency. It has been observed that endurance athletes shown an attenuation on the rise of VO_2 above GET and that high intensity training may reduced slope (especially the upper portion of the VO/W relationship, S_2). This suggests that includes muscular adaptations, improving mechanical muscle efficiency and reducing $\Delta VO_2/\Delta W$ predominantly above GET. In conclusion, the upward deflection in the VO_2/W appears to be related to the VO_{2sc} which occurs during constant work rate, highlighting that the physiological mechanisms to explain this phenomenon are similar.

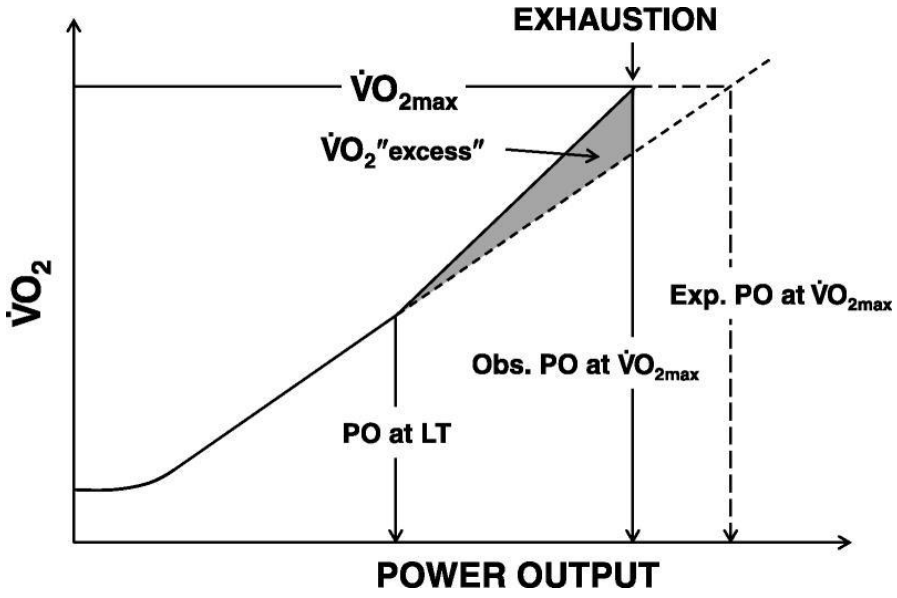

Figure 3. A. M. Jones et al., 2005. *Oxygen uptake kinetics in sport, exercise and medicine.* Vo_2 response during incremental ramp test when intensity exercise is above lactate threshold (LT). The relationship between power output and VO_2 is linear until LT; than linearity is lost and the Vo2 response is greater in compared with power output leading the subject to reach early the Vo_{2ma} and reducing exercise tolerance. The upwards curvilinear deflection leads to excess VO_2 caused by a drop in mechanical efficiency.

Loss of efficiency

During constant power exercise at an exercise intensity above the lactate threshold (in the heavy-intensity domain), a slowly developing increase in VO_2 over time can be observed that has been termed the slow component of VO_2 kinetics (VO_{2sc}). In addition, during incremental exercise, in the heavy-intensity domain, an increase of VO_2 for a given increase power output can be observed that is in excess compared to that observed at lower power outputs (excessVO_2) [39] [38]. As a consequence, the subject reaches maximal oxygen consumption (VO_{2peak}) at a lower power output compared with a situation in which no excess VO_2 is seen [39].

The above described phenomena, i.e. VO_{2sc} and excessVO_2, have a common denominator: the "loss of efficiency". The factors responsible of are still debate [34]. Yet it is well understood that >85% originates from the locomotory muscles [40]. Those suggest that the inefficiency is related to an increased energy demand in the muscles. The loss of efficiency could be caused by: *i)* a progressive *recruitment*, as a function of time, of less-efficient type II fibers [36] *ii) fatigue* or *iii)* by a complex interaction of *both* processes.

i) *Recruitment hypothesis*: a number of studies have tested the hypothesis that a progressive recruitment of low-efficiency, glycolytic, type II muscle fibers is the main mechanism responsible for the reduced efficiency at exercise intensities above the lactate threshold [39]. However, attempts to quantify the involvement of specific muscle fibers in coincidence with the occurrence of either VO_2sc or excessVO_2 have been hampered by the poor temporal and spatial resolution of the available techniques (i.e. Electromyography, Magnetic Resonance Imaging) leading to conflicting results [41].

ii) *Fatigue hypothesis*: as an alternative hypothesis, the genesis of the reduced efficiency has been attributed to fatigue within type I, oxidative muscle fibers [42].Fatigue itself causes an increase in the ATP and/or O_2 cost of exercise during exercise due to the accumulation of metabolites or ions in fatiguing muscle that affect Ca^{2+} dynamics, troponin sensitivity to Ca^{2+} and the contraction force of the cross-bridges attachment [43]. This phenomenon could explain the occurrence of a VO_2sc and excessVO_2 independently from a sequential recruitment of different fibers.

iii) *Recruitment-Fatigue hypothesis*: an alternative or complementary explanation is that VO_{2sc} and excessVO_2 arise due to the combined effects of fatigue on the initially recruited fibers (both type I and II fibers) and the recruitment of new, less efficient fibers. Type I fibers might be increasingly activated at a high contraction velocity that is sub-optimal in terms of their force production. This would require the activation of additional motor units (mainly type II fibers that due to the higher recovery cost, require increasing energy (ATP) and O_2 over time) [39].

In summary prolonged exercise tolerance can be defined as the level of physical exertion an individual can achieve or endure before exhaustion.

Strength training and its the possible role in young and older adults

Strength Training is a type of exercise known for increasing muscular strength, power, speed, hypertrophy, endurance, balance, coordination, motor performance, and for reducing the percentage of body fat [44] .Nowadays it is well known that regular exercise has the important function in promoting physical fitness, desirable body weight and cardiorespiratory health. However, in the past the overwhelming emphasis was on aerobic activity with little encouragement for resistance training which has been underestimated. Resistance training has been recommended by national health organizations, such as the American College of Sports Medicine (ACSM), American Heart Association and the American Association for Cardiovascular and Pulmonary Rehabilitation [5 45] for numerous performance enhancing benefits for the maintenance and improvement of health and performance. The main benefits of strength training are:

i) *Reversing muscle loss*: brief sessions (e.g. 12 to 20 total exercise sets) of training can increase muscle mass in adults of all ages through the 10th decade of life. Lean weight can gain of about 1.4 kg following approximately 3 months of resistance training.
ii) *Recharging resting metabolism*: training stimulates increased muscle protein turnover and actually has a dual impact on resting metabolic rate, since the greater muscle mass necessitates more energy at rest for ongoing

tissue maintenance. Research has shown significant increases in resting metabolic rate (about 7%) [46] after several weeks of resistance training.

iii) *Reducing body fat:* resistance training is recommended in the management of obesity and metabolic disorders, since may improve insulin sensitivity and glycemic control [44].

iv) *Improving Cardiovascular Health:* the reported findings related to cardiovascular benefits of strength training included three main factors: resting blood pressure (e.g. subject who trained twice a week significantly reduced systolic and diastolic blood pressure by 3.2 and 1.4 mmHg, respectively), blood lipid profiles (e.g. ST may increase HDL cholesterol by 8% to 21%, decrease LDL cholesterol by 13% to 23% and reduce triglycerides by 11% to 18%) and vascular condition [47].

v) *Increasing bone mineral density (BMD):* strength training seems to be positively correlated with high. BMD in both young and older adults and seems to have a more potent effect on bone density than other types of physical activity such as aerobic and weight bearing exercise. For examples, some studies reported an improvement in BMD of 3.2% compared with control group after strength training. Nevertheless the evidence shown different changes in BMD, because BMD change is related to different responses in different bones [48].

vi) *Reversing aging factors:* some researches have evaluated the effects of strength training on muscle mitochondrial content and function, and have reported that circuit of strength exercise can increase both the mitochondrial content and the oxidative capacity of muscle tissue. Thus it seems that this

typology of training can slow or counteract mitochondrial deterioration that typically occurs with aging [49].

After illustrating the benefits associated with strength training, it is equally important to understand the adaptive responses of the organism that can be divided in morphological and neural adaptations.

i) *Morphological adaptations:* Skeletal muscle adapts to exercise in various and specific ways. Skeletal muscle can enlarge when subjected to stress demanding greater force production. Fiber-type and architectural transitions can occur, ultimately increasing force, power and endurance capacity. In short, training induces the following adaptations [50].

ii) *Changes in whole muscle size:* after a few months of regular strength training there is an increase in muscle size. However, it is influenced to some factors such as muscle group, sex and age. For example, older adults show a smaller increase in muscle size than young adults.

iii) *Muscle fibre hypertrophy:* long period of strength training increases cross sectional area (CSA) which is the result of fibre hypertrophy. This facilitates the increase in the contractile material (number of cross bridges) arranged in parallel and increases force production. Muscle growth occurs in both oxidative and glycolitic fibres. However, it has been found preferential hypertrophy of type II fibre with training; for example one study showed that fibre II area increased 33% whereas fibre I increased 27% after 6 months of strength training. Nevertheless, during a detraining period, a rapid atrophy occurs, suggesting a greater plasticity of this fibres.

iv) *Other structural changes:* strength training increases the number of

myofibrils, the density of the sarcoplasm, sarcoplasm reticulum, T tubules and sodium-potassium ATPase pump activity.

Neural adaptations: The nervous system is extremely important for modulating acute exercise performance and the subsequent training adaptations. When maximal force and power is desired from muscle, all available motor units must be activated. This generally results from an increase in:

i) *Recruitment:* refers to voluntary activation of motor units during effort. Motor units are recruited in an orderly progression based on the "size principle", i.e. they are recruited in succession from smaller (slow twitch or type I) to larger (fast twich or type II). Small units are recruited first for more intricate control and larger units are recruited later to supply substantial force for high intensity contractions. The activation threshold is the most critical determinant of motor unit recruitment; force and power production may vary greatly because most muscles contain a range of type I and type II motor units. These last are recruited at high force, power or speed requirement. A training adaptation that takes place is that the level of muscle mass activation may decrease when muscle sizes increases. A larger muscle does not require as much neural activation to lift standard weight as it did before the growth took place (because the muscle fibers themselves are larger and stronger and therefore require less stimuli to produce a certain level of force).

ii) *Rate of firing:* refers to the number of times per second a motor unit discharge. Firing rate is affected by the nerve's conduction velocity as

conduction velocities are higher in type II motor units. Conduction velocity tends to be higher in power athletes compared with endurance athletes possibly due to a larger contingent of type II motor units in power athletes. A positive correlation exists between the amount of force produced and firing rate which is enhanced with anaerobic training. In fact, resistance training induced an improve in maximal firing frequency in both young and elderly individuals. Moreover, improving high firing rates is important in order to increase the rate of force development. The latest is considered the most important functional benefit induced by resistance training, since it plays an important role in the ability to perform rapid and forceful movements, both in highly athletes as well as elderly individual who need to control unexpected perturbations in postural balance [51].

iii) *Synchronization of firing:* occurs when two or more motor units fire at fixed time intervals. It is thought that motor synchronization may be advantageous for bursts of strength or power needed in a short period of time. The motor units of strength athletes appear to exhibit greater synchronization than untrained individuals [52] and some studies has been shown greater motor unit synchronization following strength training.

Strength training for the numerous reasons above mentioned can be a good way, never evaluated, to clarify mechanism of loss of efficiency and quantify usefulness (impact on exercise tolerance).

In older people, a growing fast population, who experience a physiological progressive reduction of VO_{2peak}, the identification of specific and adequate

interventions to attenuate age-associated loss of exercise tolerance is paramount. Typically, fat mass increases with age and peaks around age 60–75 years [53] whereas muscle mass and strength start to decline progressively with a more accelerated loss after the age of 60 [54]. Observational studies indicate that approximately 1% of muscle mass is lost per year after the fourth decade of life [55]. Aging is associated with a decline in a variety of neural, hormonal, and environmental trophic signals to muscle. The reduction in physical activity, hormonal changes, pro-inflammatory state, malnutrition, loss of alpha-motor units in the central nervous system, and altered gene expression accelerate the loss of muscle mass and mass-specific strength [56]. Resistance training has already been reported to be important for the prevention of sarcopenia and balance in the elderly and because of its potential to increase fat free mass, which is associated with an increase in resting metabolic rate among the elderly. Activities that promote strength combined with coordination and balance are particularly valuable with advancing aging, as underlined ACSM, for maintaining capacity to perform common activities of daily living and, in particular, for reducing the risk of falling and of being seriously injured. Position Stand of American College of Sport Medicine (ACSM) [3] for older adults, recommended both multiple- and single-joint exercises (free weights and machines) with 60-80% of 1 RM for 2-3 d/wk. Several interventional studies investigated the effects of ST on physical function in older adults. While the rate of possible negative side effects and contraindications is very low if the dose is adapted to the patient [57] muscle mass and cross sectional diameter of the muscle can be increased through training at an intensity corresponding to 60% to 85% of the individual maximum voluntary strength. Hypertrophy of both type I fibers and type II fibers is responsible for the

improvement. In addition, the rate of force development can also be improved, provided that training intensity is above 85% of the individual maximum voluntary strength [57]. Elderly people over 70 years of age seem to have a faster muscle atrophy, caused by the reduction size of the fast fibers, in particular of the type IIb fiber (fast twitch fatigable) [58]. The reduction in the maximum force affects especially the lower limbs [54], caused mostly by a reduction of spontaneous physical activity. With advancing age they run into motor units recruitment problems mainly due to type II fiber atrophy. In fact, in biopsies there is a greater decrease in type II fibres (20-50%) compared to type I fibers (1-20%) for those >/=60 years. Making a general assessment the decrease of both types I and II fibers is around 50% in the ninety-year-olds compared to the twenties [59]. Lexell ae al. describe a significant loss of thickness and in particular of the number of fibers, especially of type II fibers, in the quadriceps [60]. As well as strength there is a loss of power as increasing age. From 65 to 85 years there is an annual loss of power and the ability to recruit fibers by 3.5% [46]. The alteration of muscle parameters affects other parameters related to functional efficiency enough that the loss of strength seems to cause 35% of the loss of aerobic fitness [61]. Only 8 weeks of strength training shown to be related to an increase in strength and walking speed in subjects up to 90 years of age.

Introduction

The capacity to perform physical exercise of prolonged duration is essential to sustain independently, effectively and at an adequate speed, the movement tasks related to work, leisure and activities of daily living [62]. Physical exercise has well documented effects on maintenance and promotion of overall health throughout the life span. Therefore, the ability to perform exercise, at adequate intensity and for an adequate duration, has indirect effects on the quality of life and is instrumental to health promotion and long-term health maintenance [15].

Exercise intolerance is defined as the inability to produce force or power adequate to accomplish a task. In a whole-body exercise, exercise intolerance is caused by muscle fatigue (reduction in muscle force) and decrease efficiency of contraction (the ratio of mechanical energy to metabolic energy output) [35].

A decrease of efficiency of contraction can be observed in two different exercise paradigms: constant power exercise and incremental exercise, suggests that the mechanism(s) governing oxygen consumption (VO_2) may be the same in both exercise paradigms [35]. During constant power exercise at an exercise intensity above the lactate threshold (in the heavy-intensity domain), a slowly developing increase in VO_2 over time can be observed that has been termed the slow component of VO_2 kinetics (VO_{2sc}). In addition, during incremental exercise, in the heavy-intensity domain, an increase of VO_2 for a given increase power output can be observed that is in excess compared to that observed at lower power outputs (excessVO_2)[39 63]. As a consequence, the subject

reaches maximal oxygen consumption (VO_2peak) at a lower power output compared with a situation in which no excess VO_2 is seen [39 63]. The above described phenomena, i.e. VO_{2sc} and excess VO_2, that have a common denominator in the "reduced efficiency", could be caused by: *i)* a progressive recruitment, as a function of time, of less-efficient type II fibers [36] *ii)* fatigue or *iii)* by a complex interaction of both processes.

Increase exercise efficiency is essential to sustain independently, effectively and at adequate speed the daily movement tasks and to acquire the dose-dependant health benefits of an active lifestyle. Exercise tolerance reflects the body's capacity to transport and utilise oxygen and as such it is a valid and synthetic index of overall health. It also dictates the amount of exercise or exercise «dose» that a person is able to acuire to mantain and improve both function and health throughout life, in a dose-dependant virtous cycle. Any interventions able to elicits a positive adaptive response to better maintain efficiency over time during both incremental and CWR exercises may be especially important.

Interventions show to be necessary for a growing fast population such as the elderly. Resolving the mechanistic bases of fatigue is central, in the older adult population to the identification of specific, effective and applicable countermeasures to attenuate exercise intolerance and to improve health and quality of life. The possible role of strength training in attenuating the excessVO_2 has never been evaluated in the elderly.

Strength training intervention, by increasing maximal force, reducing the recruitment of high-threshold motor units to sustain power output at a given exercise intensity. If the recruitment of high-threshold glycolytic motor units be the main responsible for the

genesis of the excess VO_2, and efficiency loss, strength training can reduce its amplitude while enhancing exercise tolerance. Exercise tolerance can be regarded relevant for exercise prescription and sport performance both. In older people strength training intervention can be therefore considered the only mode of training, regardless oxidative metabolism effects, because is dose adapted and has low contraindication for untrained/ low fitness people. It can be an applicable countermeasure to attenuate exercise intolerance and to improve health and quality of life.

Purposes and Research Questions

The research questions asked in this thesis have been separated in three questions, as listed below:

Study one (chapter 1)

Can the exercise tolerance at an intensity of exercise above the anaerobic threshold during a ramp incremental exercise be improved by a training intervention with the aim to improve the muscle contractile efficiency in healthy young and older adults?

Study two (chapter 2)

Can the exercise tolerance at an intensity of exercise above the anaerobic threshold during a constant work rate exercise be improved by a training intervention with the aim to improve the muscle contractile efficiency in healthy young and older adults?

Chapter 1

"Excess VO2": how strength training affects metabolic efficiency in young and older adults

Introduction

During ramp incremental cycle exercise to exhaustion, there is a linear increase in VO_2 relative to the mechanical power output (PO), with a functional gain ($\Delta VO2/\Delta PO$) that varies between 8 and 12 $ml \cdot min^{-1} \cdot W^{-1}$ [38]. A homogeneous linear relationship is often assumed across the whole exercise intensity spectrum. However, when the exercise exceeds a critical intensity, the rise in VO_2 as a function of work rate displays an increased slope [38,64] that justifies the description of VO_2/PO relationship as a double-linear as opposed to a single-linear function [38]. The development of this so called "excess VO_2" [64] in the heavy-intensity domain of incremental exercise entails a progressive loss of efficiency. The more pronounced the excess VO_2 is, the earlier VO_{2peak} will be reached and task failure will ensue, in turn causing a reduction/impairment of exercise tolerance [35].

The excess VO_2 appearing in the heavy-intensity domain of an incremental exercise has been considered related to the slow component of oxygen consumption occurring during constant-work rate exercise above the lactate threshold [35]. For this reason, it has been suggested that similar physiological mechanisms, intrinsic to the working muscles [39], may underpin the loss of efficiency that characterises the heavy-intensity domain in both exercise paradigms [35,38]. A progressive increase in fatigue of type I fibers, that is

associated with the increase the ATP cost of contraction and of the O_2 cost of ATP resynthesize [43] and/or a progressive increase in the recruitment of intrinsically less efficient type II fibers [39] have been proposed as putative causes of the loss of efficiency; however, the relative role of fatigue and recruitment and their possible interaction remain to be fully elucidated [35].

Importantly, aerobic training interventions have been shown to contribute to a reduction or elimination of the loss of efficiency during ramp incremental [37] and constant work rate exercises [39]. Additionally, the slow component of oxygen consumption tends to be relatively small in endurance trained athletes [39]. A reduction of muscle fatigue, thanks to enhanced muscle blood flow [65], improved mitochondrial biogenesis [66] and muscle oxidative capacity [37] and reduced O_2 cost of ATP production/ATP cost of contraction [39,67], has been proposed to explain the improvement of muscle efficiency associated with endurance training.

On the contrary, a possible role of an intervention affecting muscle recruitment pattern in reducing the loss of efficiency in the heavy-intensity domain of exercise has never been evaluated. During an isometric exercise the increase in relative force production is obtained through the recruitment an increasing percentage of the muscle motor units, starting from type I and progressively including larger type II motor units [68]. Similarly, a progressive recruitment of motor units has been observed in the *vastus lateralis* over a ramp incremental exercise; furthermore, a "threshold" phenomenon has been observed (*i.e.* an increased slope of the root mean square of the surface EMG as a function of power output), supporting the idea that higher-threshold motor units are recruited to sustain the increase in the mechanical power output above a specific relative

exercise intensity [69–71]. In this context, it may be conceived that an intervention, such as strength training, that is able to increase maximal muscle force, would reduce the recruitment of high-threshold motor units to sustain a given absolute power output [72]. In turn, if the recruitment of higher order motor units plays a role in the genesis of the excess VO_2, then a strength training intervention should be able to affect it improving exercise tolerance and performance in the young population.

The study of the excess VO_2 and of possible intervention that that could reduce it, might be useful not only in young adults but also in older adults to increase exercise tolerance, daily mobility and quality of life. Ageing is indeed associated with a progressive reduction in the ability to produce force due to a selective atrophy and de-recruitment of type II fibres and a loss of efficiency in type I fibres. A specific training intervention that aims at improving fibres recruitment (both type I and II) might be somewhat beneficial in older adults to affect exercise tolerance. Thus, the present study tested the hypothesis that strength training, by increasing maximal force and reducing the recruitment of high-threshold motor units to sustain a given absolute exercise in the heavy-intensity domain, would reduce the excess VO_2 in young and older adults males.

Methods

Participants

16 healthy young males (mean ± SD: age 26 ± 3 years, height 1.76 ± 0.67 m; body mass 74 ± 11 kg) and 21 healthy older adults males (mean ± SD: age 68 ± 4 years, height 1.72 ± 6.22m; body mass 75 ± 10 kg) took part of this study. Young and old participants were randomly assigned to perform either 4 weeks of strength training (intervention group) or to maintain their normal lifestyle (control group). Inclusion criteria were:

healthy young (18-35 years old) male and healthy older adults (> 65 years old) male that had not been involved in any structured exercise training program for at least 6 months. Exclusion criteria were: being an athlete/well trained individual undergoing regular strength training, smokers, BMI >30, medical conditions that are known to affect cardiovascular or metabolic response to exercise or the use of medications that can interfere with the ability perform exercise or with the physiological response to exercise or increase the risk of exercise-related injuries. Participants provided written informed consent to participate in the study that was conducted with permission of the Ethical Committee of the University of

Verona and in accordance with the Declaration of Helsinki.

Testing

After medical clearance, within 3 days before and after the 4-week training period, all participants completed the following tests:

i) A maximal ramp-incremental exercise test to the limit of tolerance on a cycle ergometer;

ii) A one repetition maximum (1RM) tests in the weight room [45];

iii) An isometric strength test on a force platform (only for young group).

All tests were conducted in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity) at a similar time of the day. Participants were asked to avoid heavy exercise and caffeinated/alcoholic beverages the day before each test. A resting period of 24 h was imposed between each test.

Ramp incremental test: Each participant performed a ramp incremental test on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL)

consisting of a 5-min baseline cycling at 20 W, followed by ether a 25-$W \cdot min^{-1}$ or 20-$W \cdot min^{-1}$ increase in PO, for young and older adults respectively until volitional exhaustion. Participants were asked to cycle in the range of 70-90 rpm and the same self-selected cadence was used for both pre and post tests. The accepted criteria for maximal effort were: (*i)* a plateau in the VO_2 response; (*ii)* a respiratory exchange ratio (R_{peak}) >1.1; and (*iii)* a peak heart rate (HR_{peak}) > 90% of the predicted maximum based on age [73].

Breath-by-breath pulmonary gas exchange and ventilation were continuously measured using a metabolic cart (Quark B^2, Cosmed, Italy) [73].

Muscle oxygenation and deoxygenation ([HHb]) were evaluated using a quantitative NIRS system (Oxiplex TSTM, ISS, Champaign, USA). After shaving, cleaning and drying of the skin area, the NIRS probe was longitudinally positioned on the belly of the *vastus lateralis* (VL) muscle ~15 cm above the patella and attached to the skin with a bi-adhesive tape. The probe was secured with elastic bandages around the thigh. The apparatus was calibrated before each test after a warm-up of at least 30 minutes as per manufacturer recommendations. A comprehensive description of this method has been previously reported by Fontana et al. [73].

In the young adults' group, surface electromyography (EMG) of the left *vastus lateralis* muscle was continuously recorded by means of a wireless system (Wave wireless EMG, Cometa, Milan, Italy). A pair of surface electrodes (Blue sensor, Ambu®, Ballerup, Denmark) was attached to the skin with a 3-cm inter-electrode distance. The electrodes were placed longitudinally with respect to the underlying muscle fibers arrangement and located according to the recommendations by Surface EMG for Non-

Invasive Assessment of Muscles (SENIAM). Before electrode application, the skin was shaved and cleaned with alcohol in order to minimize impedance. The skin was marked using non-permanent ink in order to place the electrodes on the same site on the two tests (pre and post training) thus reducing the variability associated with day-to-day differences in EMG electrodes placement. The EMG transmitter connected to the electrodes was well secured with adhesive tape to avoid movement-induced artifacts and the EMG signal was checked prior each test. Raw EMG signals were pre-amplified (gain 375, bandwidth 10–500 Hz) and digitized at a sampling rate of 2 kHz (Wave wireless EMG, Cometa, Milan, Italy).

1RM test: after familiarization (see below), 1RM was determined directly for two lower-body exercises (Squat and Deadlift) and one upper-body exercise (Bench Press) as the maximum resistance that could be lifted once throughout the full range of motion (determined in the unweight position) maintaining a correct execution form. Before attempting a 1RM, participants performed a standard warm up as per ACSM guidelines [74]. Then, a series of 3-5 single repetitions with increasing loads was performed until failure to complete one movement with correct form over the full range of motion [74].

Isometric strength test: in young participants all isometric contractions were performed on a custom-built isometric rack that allowed the bar to be fixed at any height above the floor. The isometric rack was placed over a force plate (Advanced Mechanical Technologies, Newton, MA), which sampled at 600 hz. All participants performed a minimum of two familiarization-testing sessions one week before the initiation of the actual study to ensure that maximal isometric attempts were completed. A standardized warm-up based upon previous literature was utilized [75]. The position for each isometric

pull was established before each trial with the use of a goniometry to ensure a knee angle of 140±5° with the barbell placed at the mid-thigh position (i.e. the position that allows the highest force generation during a whole-body exercise [76]). Once the position was established, participants were strapped to the bar in order to avoid any movement. With each trial, participants were instructed to pull as hard and as fast as possible. Each participant performed four isometric mid-thigh pulls separate by a 3-minutes recovery between trials. The best attempt was used for further analysis.

Data analysis

Ramp incremental test: Gas exchange threshold (GET), respiratory compensation point (RCP), peak VO_2 (VO_{2peak}) and peak PO (PO_{peak}) were determined as previously describe [77]. Briefly, VO_{2peak} was determined as the highest VO_2 obtained over a 30s interval and PO_{peak} was defined as the highest mechanical power output achieved at termination of the RI exercise. GET and RCP were estimated by visual inspection from gas exchange variables by three blinded expert reviewers [78].

The slope of the relationship between the change in VO_2 for a given change in power output (Δ_{VO2}/Δ_{PO}) was modeled using a double linear fit (OriginPro 2016, Origin Lab Corp, Massachusetts, USA) by considering the first linear function (Δ_{VO2}/Δ_{PO}_$slope_1$) from the region of the start of the ramp up to the deflection point in the VO_2 signal (*i.e.*, the point at which the VO_2 response starts to display a steeper slope). The second linear function (Δ_{VO2}/Δ_{PO}_$slope_2$) was considered from the deflection point up to the point at which VO_2 reached its peak or just before any visible plateau in VO_2 [71]. The following piece-wise double-linear regression was fitted minimizing the residual sum of squares:

$f = \text{if}\,(x < BP,\ g(x),\ h(x))$

$g(x) = i_1 + (s_1 \cdot x)$

$i_2 = i_1 + (s_1 \cdot BP)$

$h(x) = i_2 + (s_2 \cdot (x - BP))$

fit f to y

where f is the double-linear function, x is power output and y is VO_2, BP is the power output coordinate corresponding to the interception of the two regression lines, i_1 and i_2 are the intercepts of the first and second linear function respectively and s_1 and s_2 are the slopes.

The NIRS-derived [HHb] deflection point ($[HHb]_{BP}$) and the plateau in the [HHb] signal ($[HHb]_{plateau}$; indicative of the upper limit in VO_2 extraction in the observed muscle during the incremental test [73]) were identified by fitting the individual values of [HHb] corresponding to the incremental portion of the RI exercise as a function of power output. A piece-wise 'double-linear' model was used to characterize this response as detailed in Bellotti, 2013 [79]. Thereafter, $[HHb]_{BP}$ was determined as the power output coordinate corresponding to the interception of the two identified regression lines.

The raw EMG signals were rectified and smoothed using a fourth-order band-pass Butterworth digital filter with a frequency range set between 20 and 500 Hz. A one-second average of the root mean square (RMS) was calculated from the raw signal and was used as an index of the total muscle activation [80,81]. For each participant, a resting and a peak RMS value were identified respectively as the average of the last 60-seconds at 20W before ramp initiation and the highest value reached during the incremental portion of the exercise (with resting value equal to 0 and peak value equal to 100). Based

on these normalized values the $\%RMS \cdot W^{-1}$ slope was determined using a piece-wise 'double-linear' model as explained before regarding VO_2 and [HHb] data fitting. EMG signal was analyzed using custom-made programs written in MATLAB software (MathWorks Inc., Natick, MA).

Finally, the power output, the VO_2 (after left-shifting the VO_2 signal to account for the mean response time [82]) and the $\%VO_{2peak}$ corresponding to the break points in RMS and [HHb] signals during the ramp incremental exercises were also calculated.

Training program

Before the beginning of the study, all the young and older adults participants took part of a 2-week familiarization period, which consisted of 6 strength-training sessions on non-consecutive days and performed with no overload to avoid any possible adaptations that could interfere with the main sub-sequent intervention. During this period, each participant received close supervision and instruction on proper exercise technique and training principles.

Subjects in the intervention group trained in a weight room 3 times per week (90 min each session) on non-consecutive days. The young and older adults trained for 4 weeks and performed a total of 12 training session. All training session were supervised and instructed by a qualified strength coach with an instructor/participants ratio of 1/4. The training exercises (three fundamentals whole-body exercises and two complementary exercises) were performed with Olympic barbell and plates (Eleiko, Sweden) in a power rack. As *per* ACSM guideline for novice lifters, the load modulation over time was conducted using a linear model that implies a decreasing of training volume while increasing intensity [74]. Training characteristics are detailed in table 1.

Statistics

After assumptions verification (*i.e.*, outliers, normality, homogeneity of variance and covariance and sphericity, tested respectively using studentized residuals analysis, Q-Q plot, Levene's test, Box's test and Mauchly's test), a two-way mixed ANOVA (2x2; BW) was performed to assess whether differences existed between independent groups (between-subjects factor: intervention *vs.* control) over time (within-subjects factor: pre *vs.* post) in the measured statistics. In addition, a three-way mixed ANOVA (4x2x2; BBW) was performed to assess any statistical difference between RCP, PO_{BP}, $[HHb]_{BP}$ and EMG_{BP} (break points) (measured in Watt, VO_2 and $\%VO_{2peak}$), pre-and-post training (time) within and between the two groups (groups). For both ANOVAs, the F-statistic for both higher and lower order effects, were interpreted using the Greenhouse-Geisser correction [83] and, whether significant, pairwise comparisons were performed to detect any intra and inter-factor differences. The adjusted α level for every pairwise comparison was calculated using Student-Newman-Keuls's method [84]. The required sample size was calculated based on an expected effect size estimation (medium effect size) on the primary dependent variable of interest (the absolute change in the VO_{2SC} amplitude), using G-power package (http://gpower.hhu.de) and ensureing $1-\beta >80\%$.

Data are presented as means ± SD. 95% Confidence intervals around mean differences (95% ΔCIs [lower limit, upper limit]) and effect sizes of those differences (Cohen's *d,* ranked as trivial (0-0.19), small (0.20-0.49), medium (0.50-0.79) and large (0.80 and grater) [85]) are also reported as objective and standardized measures of magnitude of

effects and as alternative metrics of meaningfulness [86]. In effect size calculation, the SD in the control group at baseline, was used to standardize the mean difference for each contrast [83].

Regarding regression analyses (*i.e.*, double linear fitting procedures) the goodness of fit was assessed using the residual sum of squares (representing the degree of inaccuracy in the fitting), the model sum of squares (representing the improvement in prediction resulting from using a double-linear model rather than a straight line) and the R^2 (interpreted as the proportion of improvement using a double-linear model).

All statistical analyses were performed using STATA (Version 14, Texas, USA) and α was set in advance at the 0.05 level; statistical significance was accepted when $p < \alpha$.

Results

For all participants in both age groups adherence to the training program was 100%. Volume (number of repetitions) and average intensity (% 1RM) performed as a function of weeks are displayed in table 1.

Changes in the morphological variables (Body mass and BMI) are represented in table 2 and functional ($HR_{peak,}$ VO_{2peak}, $R_{peak,}$ PO_{peak}, GET and RCP) statistics as a function of group and time are represented in table 4.

A shown in table 3, the ability to produce force increased significantly in the strength training group. Young adults significantly improved both 1RM and isometric strength tests. Older adults significantly improved the individual 1RM. No significant changes were observed in the young and older adults control groups.

In the young subjects, VO_{2peak} and PO_{peak} did not change compared to pre-training intervention (Δ=-1%, p=0,52 ; Δ=5%, p=0,98). In contrast, in older subjects, strength training induced a significant increase in both PO_{peak}, (Δ=7%, p=0.006) and VO_{2peak} (Δ=9%, p=0.05).

During the ramp incremental exercise, a significant excess VO_2 ($ml \cdot min^{-1}$) was present in young adults before training in the intervention group in 87% on the participants (7 out of 8), with Δ_{VO2}/Δ_{PO}_$slope_2$ being significantly higher than Δ_{VO2}/Δ_{PO}_$slope_1$ (11.4±1.1 vs 8.1±0.6 $ml \cdot min \cdot W^{-1}$) (ΔCI around the mean difference [0.73, 5.15], d = 1.5, p = 0.02). This difference in the slopes disappeared after strength training (8.1.4±2.1 vs 8.2±1.1 $ml \cdot min \cdot W^{-1}$) (ΔCI [-2.96, 2.97], d = 0.01, p = 0.99). In the older adults, no excess VO_2 ($ml \cdot min^{-1}$) was present before training (Δ_{VO2}/Δ_{PO}_$slope_1$ 9.7±1.4 vs Δ_{VO2}/Δ_{PO}_$slope_2$ 9.4±3.8 $ml \cdot min \cdot W^{-1}$) (ΔCI [-0.87, 3.03], d = -0.08 , p = 0.24) with only 3 participants having a $slope_2$ higher than the $slope_1$. After training, 10 out of 11 older adults presented an excess VO_2 ($ml \cdot min^{-1}$) (Δ_{VO2}/Δ_{PO}_$slope_1$ 8.8±1.3 vs Δ_{VO2}/Δ_{PO}_$slope_2$ 11.5±3.7 $ml \cdot min \cdot W^{-1}$) (ΔCI [-0.48, 4.28], d = 0.7, p = 0.02). In addiction, Δ_{VO2}/Δ_{PO}_$slope_1$ was significantly decreased (Δ=-20%, p= 004) following strength training in the older adults. (table 5) In control young adults participants, the excess VO_2 was present before in all participants (8 out of 8) (ΔCI [1.67, 5.53], d = 2.4, p =0.003) and after the control period (ΔCI [0.64, 4.16], d = 1.7, p = 0.01). In control old participants, the excess VO_2 was present before only on 3 out of 10 subjects (p=0.24) (as per the intervention group) but after 5 weeks the excess VO_2 did not change and was still present in 3 out of 10 subjects (p=0.02). (table 5, figure 1-2.).

Δ_{VO2}/Δ_{PO} BP did not change after strength training in both groups (young and older adults) and in both conditions (training and control groups) (table 5).

In the incremental portion of the exercise, [HHb] increased linearly as a function of workload up to a deflection point (detected in 100% of participants), where the rate of increase was reduced, often resembling a plateau (figure 1-2). The $[HHb]_{BP}$ (expressed in $mL \cdot min^{-1}$ or in watt), the [HHb]_slope and the plateau in the [HHb] signal did not change significantly after strength training or after the control period in both groups (table 5).

For the young adults the RMS increased from initial values during the ramp incremental exercise up to a deflection point (EMG_{BP}) (Figure 1.). A significant difference in the Δ_{RMS}/Δ_{W} was present before training in the intervention group in 87% on the participants (7 out of 8), with Δ_{RMS}/Δ_{PO}-$slope_2$ being significantly higher than Δ_{RMS}/Δ_{PO}-$slope_1$ (ΔCI [0.11, 0.22], d = 2.9, p = 0.01). This difference in the slopes 2 disappeared after strength training (ΔCI [-0.02, 0.14], d = 0.70, p = 0.13). In control participants, a difference in Δ_{RMS}/Δ_{PO}_slopes was present before in 75% on the participants (6 out of 8) (ΔCIs [0.06, 0.22], d = 1.9, p = 0.01) and remained so after the control period (ΔCIs [0.07, 0.19], d =2.3, p =0.03) (table 2 and figure 1). Finally, the EMG_{BP} did not change after training in both groups (table 5 and figure 1).

No significant differences existed between the RCP, the VO_{2BP}, the $[HHb]_{BP}$ and the EMG_{BP} measured in watt, in VO_2 or in % VO_{2peak}, in both age groups and between training conditions (control vs. intervention), pre-and-post training as shown by: *i)* nonsignificant three-way interactions (breakpoints*time*group; all Fs < 0.30, ds < 0.03, ps > 0.91); *ii)* non significant two-way interactions (breakpoints*time, breakpoint*group, time*group; all Fs < 0.36, ds < 0.06, ps > 0.40) (table 5).

Discussion

The present study tested the hypothesis that strength training, by increasing maximal force and affecting the recruitment of high-threshold motor units to sustain a given exercise intensity, would reduce the excess VO_2 observed during a ramp incremental exercise in young and older adults males.

The primary finding of this investigation was that a strength training program significantly improved the ability to produce force in young healthy individuals (*i.e.* 1RM, IPF and IPRFD) and in older individuals (*i.e.* 1RM).

In agreement with our hypotheses, these changes in young adults were associated with *i)* a significant and large reduction in the magnitude of the excess VO_2 (measured as a 29% reduction in the Δ_{VO2}/Δ_{PO}-slope$_2$) accompanied by a change in the PO_{peak} by an average of 16±10 watt (5±3%) and a 89% reduction of the $PO_{deficit}$; *ii)* A temporal-associated large reduction in the intensity of muscle activation during a ramp incremental exercise (measured as a 25% reduction in Δ_{RMS}/Δ_{PO}-slope$_2$) and *iii)* with no changes in the GET, RCP, VO_{2peak} or any of the [HHb] indexes.

On the other hand, contrary to our hypothesis, older adults didn't show an excess VO2 before training. In addition, 4 weeks of strength training, the older group was characterized by an excess VO_2 appearance, caused by a significant reduction in the Δ_{VO2}/Δ_{PO}_slope$_1$ (- 20%). Additionally, in the training group, a significant improvement in VO_{2peak} was observed for older adults with a concomitant increase in GET and RCP both expressed in $Lmin^{-1}$ and in Watt. While no changes in any of the [HHb] indexes occurred.

This is the first study indicating a potential role of strength training in the reduction of the observed excess VO_2 during a ramp incremental cycling exercise in young adults. In addition, this is the first study that investigates the effect of strength training on the excess VO2 in the elderly.

In the young group and in agreement with previous reports [38,64], the present study observed a disproportional rise in VO_2 in relation to power output at exercise intensities exceeding the anaerobic threshold (excess VO_2). This excess VO_2 could be caused by the same progressive recruitment of less-efficient type II fibers that occurs during a constant work rate exercises [35,38,39] denoting the so called slow component. A positive correlation between the mechanical muscle inefficiency during a step transition exercise and the percentage distribution of fibers types has been previously reported [87]. In that study, participants with higher proportion of type II fibers have a greater inefficiency of muscle contraction than those with a lower proportion of type II fibers [87]. This "recruitment hypothesis" is also supported by previous studies showing a close relationship between the type of active fibers in the *vastus lateralis* and the intensity of exercise during a ramp incremental exercises such that at about 40% VO_{2peak} type I fibers are almost exclusively recruited, whereas at about 60% VO_{2peak} both type I and type II fibers are activated. Additionally, above 75% VO_{2peak} a predominance of type II fibers recruitment is observed [88,89].

Based on our baseline results of a steeper and progressive increase in the Δ_{RMS}/Δ_{PO} relationship after the appearance of a threshold response (EMG_{BP}), a significant increase in the recruitment of motor units containing presumably less efficient type II fibers occurs at exercise intensities above approximately 75% of VO_{2peak} in the young group. This rapid

and steeper increase in RMS observed as a function of power output during a ramp incremental exercise above the anaerobic threshold would represent the point at which an increased contribution from fast twitch motor units occurs to maintain the required energy supply for muscle contraction [71,89]. This phenomenon may occur as a result of a change in the pattern of motor unit recruitment from predominantly slow twitch motor units to fast twitch motor units [69,71].

Based on the absence of the excess VO2 at baseline in the elderly, we speculate that older adults are characterized by a different recruitment pattern and that muscle fiber composition might play a role in the observed "efficiency" in older adults compared with young adults.

Biological aging is associated with declines in the muscle mass, strength performance, and cardiorespiratory fitness resulting in an impaired capacity of elderly performing daily activities [90] .To counteract the age affect on neuromuscular ability, strength training in elderly populations is the most effective strategy to improve neuromuscular functions and consequently to maintain the functional capacity during aging [90].

Of an interesting note, aging is characterised by a substantial loss of both thickness and number of muscle fibers, in particular type II muscle fibers [60]. Due to this acceleration of the age-related selective atrophy of type II muscle fibers,[91] older adults have significantly more type I muscle fibers than younger adults. For this reason, older adults are therefore characterized by a specific "neuromuscular economy" and therefore, it is plausible that an increased reliance on type I fibers (caused by the inability to recruit type II fibers to sustain power) in untrained older adults may be associated with the "ability" to maintain a constant and proportional VO_2/PO relationship even above the anaerobic

threshold during incremental exercise to exhaustion. On the contrary, strength training is associated with a specific hypertrophy and by an improved recruitment on type II muscle fibers [90] counteracting the typical reduced neuromuscular activity (due to a more sedentary lifestyle and/or functional dis- ability) and/or impaired neuronal input that characterized the age-related decline in skeletal muscle function.

In young adults, five weeks of strength training resulted in an attenuation of both the excess VO_2 and the increase in the RMS signal as a function of the mechanical power output, observed during a ramp incremental exercise, suggesting that the mechanism(s) responsible for this training-induced enhancement in the mechanical efficiency of muscle contraction might also be those involved in strength gain [52]. Following strength training there are specific fiber-type changes that might be involved in the observed attenuation of the excess VO_2. A reduction in myosin heavy chain IIb and an increase in myosin heavy chain IIa (mirroring a change in fiber type composition, with a reduction in the percentage of type IIb fibers and an increase in the percentage of type IIa fibers [92]) have been observed during the early phase of strength training [93]. In addition to this adaptations, in older adults there might be also a shift in metabolism to more aerobic fiber type recruitment during exercise, that would reduce the metabolic cost related to muscle tension and enhance contractile efficiency [94] therefore reducing the slope of the VO2/PO relationship before GET.

In the present study, the extent of strength adaptation in elderly people is comparable to that in younger people. Both age group showed strength gains (20-25%) at a similar or greater magnitude compared with those observed in other studies [95, 96,97,98,97]. In Holviala et al.[97] 21 weeks of strength training resulted in similar strength

gains in young and elderly men (20%). In another study, [99] using a similar strength training regime, demonstrated strength enhancements of similar magnitudes after 21 weeks of strength training (22%). It is interesting to note that the same strength adaptations observed in the present study occurred in a shorter period of time than in the abovementioned studies (4 vs. 21 weeks [98, ,97,99]).

The measure for structural adaptation in elderly persons is the same as in young people: strength specific training for several weeks to months has been found beneficial in this setting. An increase in muscle strength (both muscle volume and neuromuscular adaptations) has been shown in elderly men and women after a training period of 6 to 9 weeks. According with the result of this study compared with the baseline level, this effect even seems more pronounced in elderly people than in younger ones. In the literature, a rapid increase in strength has been observed especially during the first few weeks—depending on the baseline [57].This is due to neural adaptation mechanisms in the sense of improved acquisition and frequency of motor skills. In addition, an increased efficiency of the motor units resulted in elderly people tolerating submaximal loads for a longer duration—for example, during strength specific training [57].

Furthermore, considering the relative short intervention period, it is conceivable that a training induced adaptation affected the neural mechanisms involved in the skeletal muscle contraction [100,101] including: muscle recruitment [102], motor units discharge rate [103], motoneurons excitability [104] and motoneurons inhibitory stimuli (e.g. Renshaw's cells activity [94,105]. In fact, the following adaptations have been described following strength training in both young and older adults: *i)* less muscle is required to lift a given absolute load [103]; *ii)* a lower neural stimulus is required to evoke the same absolute response [51,94,106]

and *iii)* a lower presynaptic inhibition on motoneurons (evoking a different firing pattern, higher in its maximal nature and lower in its sub-maximal nature) [94,105]. Therefore, based on the above adaptations after strength training, it is plausible that the same force requirement may induce less and different muscle recruitment and a lower firing rate from motoneurons. In doing so, the fatigue effect on muscle caused by fibers recruitment and by the discharge rate on myofibrils, could be reduced improving the contraction efficiency of the muscle itself. In addition, in the older adults group strength training may also be responsible for a recovered ability to recruit type II muscle fibers [90] that may increase their contribution towards force production in the heavy-intensity domain of an incremental cycling exercise. This could explain the "re-appearance" of the excess VO_2 that is typically observed in healthy controls under the above exercise paradigm.

From a health benefits perspective, the reduction in the oxygen excess associated with higher intensities of exercise in young adults and/or in athletes is an important training adaptation that improves exercise tolerance and/or metabolic efficiency toward high intensities of exercise. On the contrary, being more efficient at lower exercise intensities (more similar to the activities of the daily living) might be beneficial in the older adults to improve life quality and independence.

A reduction in the magnitude of the excess VO_2 in response to endurance training has been previously reported[37]. Several adaptive changes within the muscles after endurance training might contribute to the observed reduction in the excess VO_2. For example, improvements in muscle blood flow distribution[37], increased muscle oxidative capacity [107] and mitochondrial biogenesis [64], as well as a change in the ATP production/utilization per unit of generated power output [67] are the putative mechanisms.

Although this information is important, no previous study has investigated the effect of strength training in the reduction of the excess VO_2 during ramp incremental exercise in young and old healthy subjects. Unlike the results from endurance training studies, the findings from this 4-week strength training intervention indicate no changes in metabolic responses to cycling exercise, as no differences were observed in the upper limit of aerobic power (VO_{2peak}) or in the indexes that set the exercise intensities boundaries (*i.e.*, GET and RCP) for the young group. On the contrary, older adults significantly improved VO_{2peak} after strength training suggesting different metabolic adaptations as per the young participants. Previous studies reported no change in the maximal oxygen consumption after strength training [57] with the majority of those reporting an overall impairment of the cardiovascular function after strength training. This result is in accordance with novel studies supporting the role of strength adaptations in enhancing the ability of skeletal muscle to generate energy via oxidative metabolism and therefore to improve cardiorespiratory fitness [57].

Lastly, in both groups, the [HHb] kinetic response during exercise did not change after training, suggesting that the balance between microvascular blood flow and muscle O_2 utilization were not affected by this strength training intervention. Overall, these results indicate that changes in the patter of muscle fiber recruitment, rather than "metabolic" adaptations following strength training, were mainly responsible for the reduction in the oxygen excess observed at higher intensities of exercise at least in the young group. On the contrary, in the elderly, both a metabolic (improved VO_{2peak}) and functional (increased force) were responsible for the appearance of the excess VO_2

underling a plausible better efficiency in recruitment type I fibers and an improved ability to recruit type II fibers.

Conclusions

In conclusion, data from this study suggest that the training-induced in young adults a decrease in the magnitude of the non-linearity in the Δ_{VO2}/Δ_{PO} relationship above the anaerobic threshold can be at least partly explained by the observed increase in the ability to produce force after strength training, which results in an improvement of muscle work efficiency and leads to an enhancement in metabolic stability during cycling incremental exercise.

On the contrary, in the elderly strength training may be responsible for a recovered ability to recruit type II muscle fibers that may increase their contribution towards force production in the heavy-intensity domain of an incremental cycling exercise. This could explain the "re-appearance" of the excess VO_2 that is typically observed in young healthy under the above exercise paradigm. While further studies are warranted to identify a direct cause-effect relationship between changes in muscle force and Δ_{VO2}/Δ_{PO} relationship, one practical consequence of the above findings is that the overall effect of strength training on aerobic exercise tolerance in older adults could be minimal. In fact, while ST is associated with a ~10% increase in VO_{2peak} the concomitant increase in muscle inefficiency offsets the benefits of the improved aerobic capacity. Further studies are required to clarify the overall impact of ST on aerobic metabolism and exercise tolerance and the consequent implications for exercise prescription in older adults.

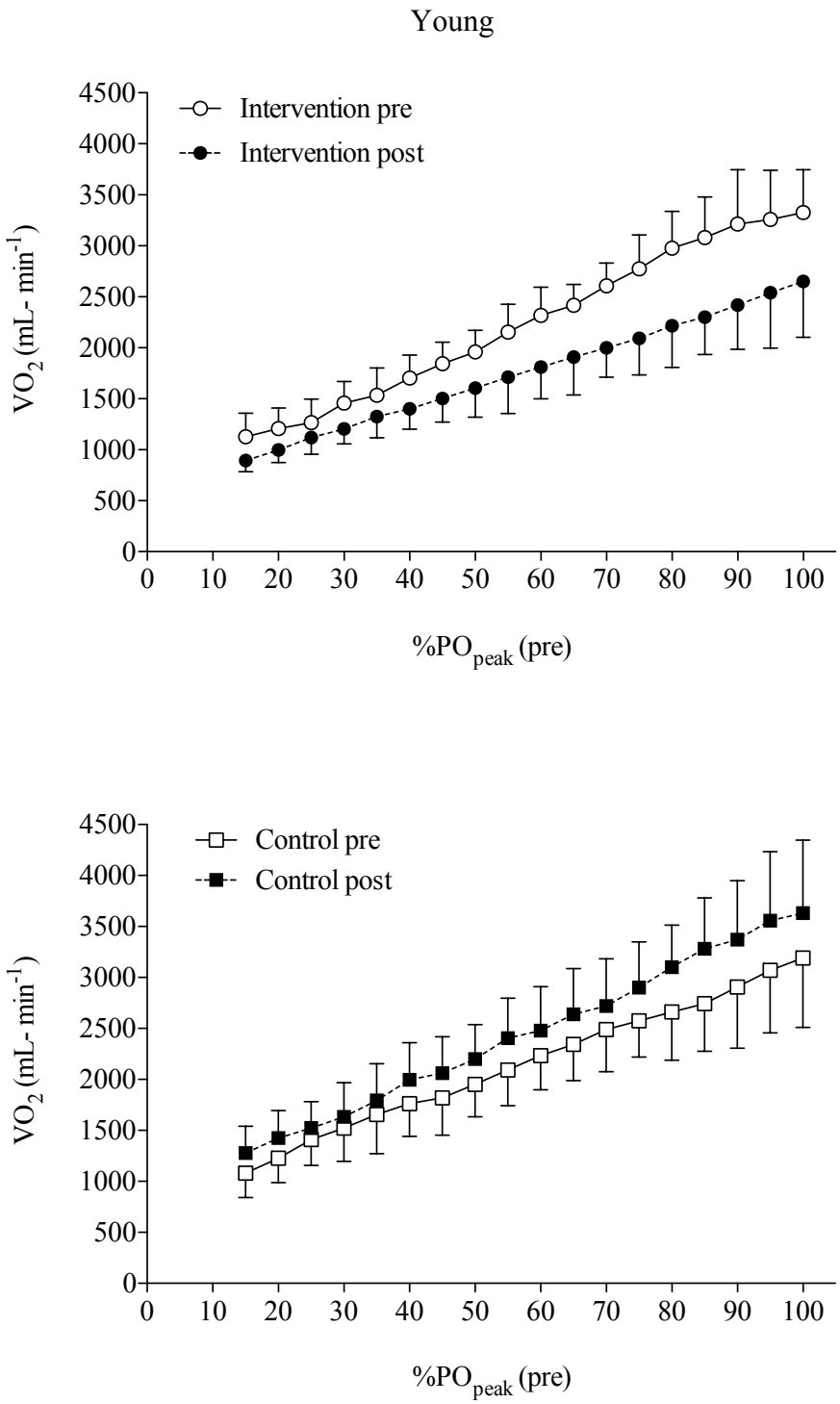

Figure 1: Young adults groups' average data have been calculated based on the results of the fitting procedures and displayed for VO_2 ($ml \cdot min^{-1}$) as a function of power output (watt). Data are presented for the pre (white circles) and the post (grey circles) condition and for the intervention (left column) and the control group (right column).

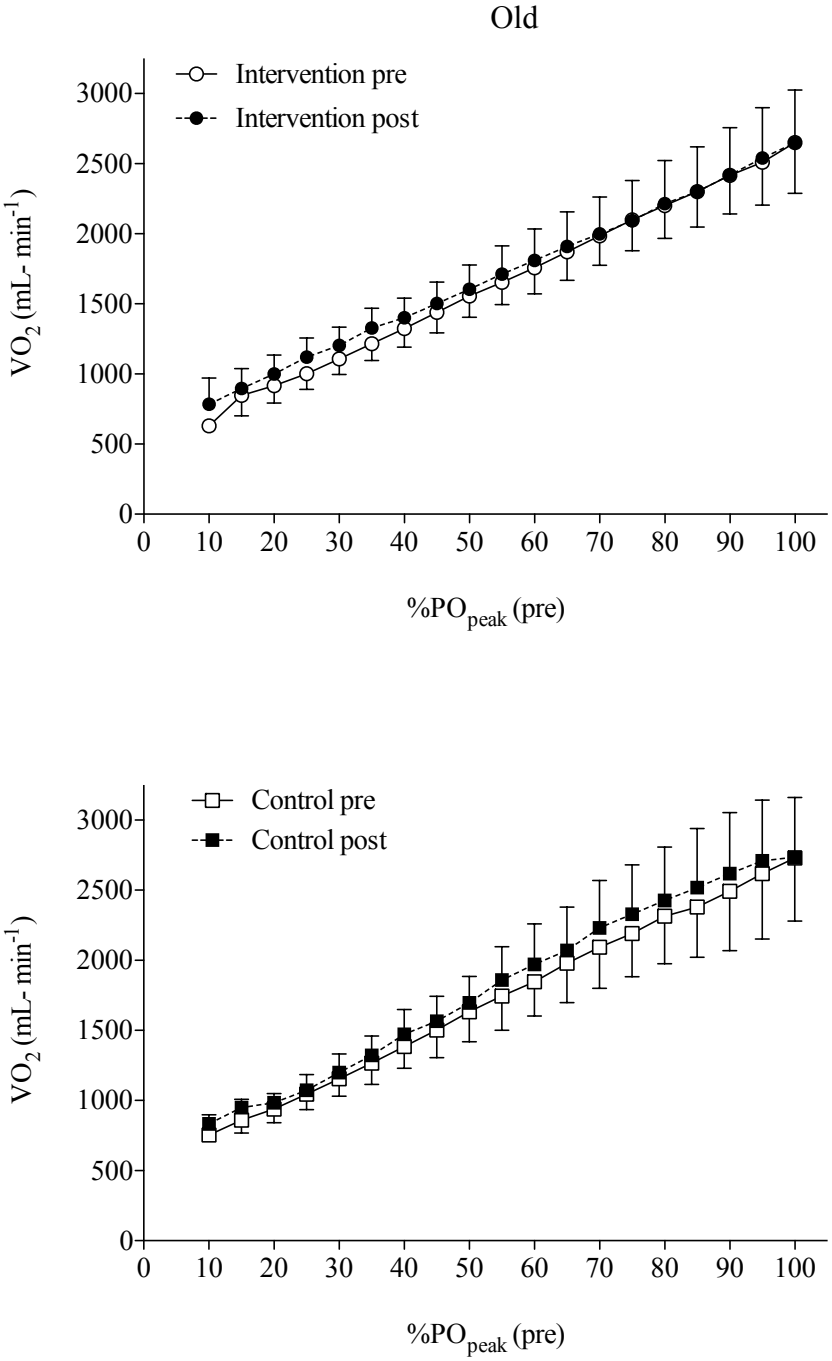

Figure 2: Older adults groups' average data have been calculated based on the results of the fitting procedures and displayed for VO_2 ($ml \cdot min^{-1}$) as a function of power output (watt). Data are presented for the pre (white circles) and the post (grey circles) condition and for the intervention (left column) and the control group (right column).

Table 1. Participants' morphological characteristics as a function of group and time. Data are presented as mean ± SD.

	Young				Old			
	Intervention group		Control group		Intervention group		Control group	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Age (yrs)	24±2	24±2	28±2	28±2	67±4	67±4	68±4	68±4
Height (cm)	177±8	177±8	176±6	176±6	172±6	172±6	171±6	171±6
Body mass (kg)	72.7±15.2	73.6±13.9	76.0±5.6	75.9±5.7	75±10.4	75±10.2	75±10.2	75±10
BMI ($Kg \cdot m^{-2}$)	23.1±3.5	23.4±3	24.6±1.8	24.6±1.8	25±3	25±2.9	25.5±3.1	25.4±3.0

Table 2. Daily training regimen characteristics and average daily volume (kg) and relative intensity (% 1RM) are displayed as a function of the training weeks.

	Week 1-2 rep x set@RI			**Week 3** rep x set@RI			**Week 4** rep x set@RI			**Week 5** rep x set@RI		
	A	B	C	A	B	C	A	B	C	A	B	C
1.**Squat**	8x4@65%	8x4@65%	4x6@75%	4x6@75%	2x2@80% 2x2@85% 1x2@90% 1x3@95%	4x4@80%	3x4@90%	7x3@65% 5x3@70%	5x5@75%	4x1@80% 3x2@83% 2x3@87% 3x2@83%	3x2@80% 3x3@87% 3x2@83%	2x2@85% 2x2@87% 1x4@95% 3x1@85%
2.Bench	8x4@65%	8x4@65%	4x6@75%	4x6@75%	2x2@80% 2x2@85% 1x2@90% 1x3@95%	4x4@80%	3x4@90%	7x3@65% 5x3@70%	5x5@75%	4x1@80% 3x2@83% 2x3@87% 3x2@83%	3x2@80% 3x3@87% 3x2@83%	2x2@85% 2x2@87% 1x4@95% 3x1@85%
3.Deadlift	8x4@65%	8x4@65%	4x6@75%	4x6@75%	2x2@80% 2x2@85% 1x2@90% 1x3@95%	4x4@80%	3x4@90%	7x3@65% 5x3@70%	5x5@75%	4x1@80% 3x2@83% 2x3@87% 3x2@83%	3x2@80% 3x3@87% 3x2@83%	2x2@85% 2x2@87% 1x4@95% 3x1@85%
4.Pull ups	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM
5.Push Press	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM
Total Volume	148	148	124	124	91	100	88	160	127	118	115	97
Average Intensity	65%	65%	75%	75%	87.5%	80%	90%	67.5%	75%	83%	83%	89%

Table 3. Force is presented as a function of group for young and older adults. Data are presented as mean ± SD.

	Young				Old			
	Intervention group		Control group		Intervention group		Control group	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1RM Squat (kg)	99±19	112±19*	104±17	103±16	58±10	71±9*	49±10	53±12*
1RM Bench Press (kg)	90±14	90±12*	79±20	82±15	39±5	45±5*	37±5	37±7
1RM Deadlift (kg	100±22	111±22*	110±9	110±12	85±16	104±14*	101±22	104±24*

Note: *p* is the probability value associated with the observed mean difference; * indicate a significant difference with respect to the pre training condition ($p < 0.05$).

Table 4. Participants' morphological and physiological characteristics as a function of group and time for young and older adults. Data are presented as mean ± SD

	Young				Old			
	Intervention		Control		Intervention		Control	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
VO_{2peak} ($L \cdot min^{-1}$)	3.31±0.40	3.29±0.44	3.86±0.56	3.71±0.68	2.63±0.32	2.87±0.43*	2.78±0.44	2.87±0.5
PO_{peak} (watt)	309±30	325±42	346±37	352±52	224±48	239±54*	248±43	243±37
F_{peak} (N)	394±42	371±52	379±18	337±17	425±85	463±75	448±89	454±59
HR_{peak} (bpm)	185±5	186±6	187±5	185±4	153±11	152±17	157±11	153±12
HR_{peak} %	97±3	96±5	99±2	98±1	96±6	95±10	98±6	96±8
GET ($L \cdot min^{-1}$)	1.90±0.2	1.84±0.2*	2.31±0.4	2.27±0.4	1.60±0.2	1.88±0.2*	1.70±0.3	1.63±0.8
GET % VO_{2peak}	57±5	56±6	60±10	62±10	60.6±5.4	66.4±11.8	62.3±5.8	56.8±7.4*
RCP ($L \cdot min^{-1}$)	2.51±0.3	2.42±0.3	2.90±0.5	2.84±0.5	1.91±0.2	2.25±0.3*	2.26±0.4	2.19±0.4
RCP % VO_{2peak}	75±6	73±6	75±9	77±12	72.8±10.0	78.8±6.8*	81.2±8.2	76.4±8.4

Note: VO_{2peak}: peak oxygen consumption; PO_{peak}: peak power output; GET: gas exchange threshold; RCP: respiratory compensation point Δ_{CIs}: confidence. * indicate a significant difference with respect to the pre training condition ($p < 0.05$).

Table 5. $\Delta VO_2/\Delta W$, and [HHb] variables are presented as a function of group for young and older adults .Data are presented as mean ±SD.

		Young						Old					
		Intervention Group			Control Group			Intervention Group			Control Group		
		Pre	Post	p	Pre	Post	p	Pre	Post	p	Pre	Post	p
$\Delta VO_2/\Delta W$	$Slope_1$ ($ml \cdot min^{-1} \cdot W^{-1}$)	8.1±0.6	8.1±1.1	0.94	8.0±1.0	7.5±0.9	0.28	9.7±1.4	8.8±1.3	**0.04**	9.3±1.5	10.2±1.1	1.68
	$Slope_2$ ($ml \cdot min^{-1} \cdot W^{-1}$)	11.4±1.0	8.2±2.1*	**0.04**	11.6±2.1	10.0±1.8	0.13	9.4±3.4	11.5±3.7	0.15	8.3±2.2	7.8±2.7	0.40
	BP (W)	210±37	211±49	0.96	247±40	251±49	0.83	172±56	168±56	0.7	170±49	192±41	0.25
	BP ($L \cdot min^{-1}$)	2.64±0.51	2.52±0.46	0.63	2.72±0.40	2.76±0.52	0.86	2.11±0.38	2.15±0.42	0.9	2.09±0.44	2.39±0.52	0.1
	Plateau (mMol)	33.4±12.1	36.3±12.8	0.65	31.2±14.0	33.1±8.5	0.74	32.4±19.8	38±14	0.46	35±23	42.9±17	0.15
	BP (W)	215±37	222±41	0.72	256±40	251±52	0.83	161±41	158±43	0.84	134±32	140±39	0.65
	BP ($L \cdot min^{-1}$)	2.69±0.34	2.51±0.39	0.34	2.83±0.45	2.72±0.43	0.76	2.05±0.33	2.06±0.49	0.15	1.8±0.25	1.93±0.43	0.46
	BP ($\%VO_{2peak}$)	81±10	76±12	0.38	73±12	73±11	0.99	78±13	72±14	0.28	66±10	67±13	0.83

Note: $\Delta VO_2/\Delta W$: relationship between oxygen consumption and mechanical power output; [HHb]: deoxyhemoglobin; *p* is the probability value associated with the observed mean difference; * indicate a significant difference with respect to the pre training condition ($p < 0.05$)

Chapter 2

VO2 Slow Component: how strength training affects metabolic stability and exercise tolerance

Introduction

The VO_2 slow component (VO_{2SC}) represents an increase in oxygen consumption (VO_2) during constant work rate exercise that projects above the expected VO_2 steady-state for a given workload. This VO_{2SC} occurs when exercise is performed in the heavy (above lactate threshold or the gas exchange threshold (GET)) [39] or even in the very-heavy/severe (above the respiratory compensation point (RCP) or critical power (CP)) [77] intensity domains. Depending on the intensity at which the exercise is performed, the magnitude of the slow component can account for ~25% of the total increase in VO_2 above the pre-exercise baseline [34], or even rise until VO_{2peak} is reached and exercise tolerance is impaired [39].

Although the mechanisms underpinning the VO_{2SC} are still debated [35], it is proposed that the majority of VO_{2SC} (> 85%) originates from the working muscles [39]. In

fact, recent studies indicated that this extra VO_2 component is related to an increased energy demand in the muscles, caused by a time-dependent (*i.e.*, duration of exercise) and intensity-dependent increase in ATP and/or O_2 cost of force production [35]. Other factors such as a disturbance in muscle metabolites concentration (e.g., an increase in inorganic phosphate and $[H^+]$), cardio-respiratory work, and temperature increase have been recognized as minor contributors to the VO_2 slow component [39].

This reduced efficiency in the oxidative system to perform work could be caused by a progressive recruitment of less-efficient type II fibers [108], by fatigue [43] or by a complex interaction of both [87]. However, the most prevalent hypothesis postulates that a progressive recruitment of less oxidatively efficient and more glycolytic type II muscle fibers is the main mechanism responsible for the VO_{2SC} at exercise intensities above the lactate threshold [39]. In relation to this, a strong positive correlation has been demonstrated between the percent of type II fibers within the working muscles, and the magnitude of VO_{2SC} [87,109].

The increase in exercise tolerance plays a key role, especially in the older adults, in the prevention of such diseases due to its close relationship with many of the chronic diseases and disabilities that largely affect the elderly, such as cardiovascular disease, cancer, type II diabetes, accidental falls, obesity, metabolic syndrome, mental disorders, and musculoskeletal diseases [110-111] . In the elderly people, the reduction of efficiency is aggravated by a decline in muscle strength and mass, that has been linked to physical frailty, falls, functional decline and impaired mobility [112] . Although many factors, including chronic illness, a sedentary lifestyle may contribute to muscle weakness and loss of skeletal-muscle mass in people of advanced age. The gradual decline in skeletal

muscle mass and strength with aging, sarcopenia, is characterized by a reduction in the number of both type I and II muscle fibers and specific type II muscle fiberatrophy [90-113]. Regardless of the mechanisms that are responsible for the development of the VO_2 slow component, several interventions have been shown that it can be attenuated or eliminated (Jones et al. 2011), and most of these interventions involved endurance exercise training [39]. Endurance training may reduce the VO_{2SC} by enhancing muscle blood flow (and/or its distribution) as well as muscle oxidative capacity [37,114], which in turn might reduce fatigue in type I muscle fibers. Interestingly, despite the interest for performing exercise interventions that would help reduce the VO_{2SC} and elucidate its mechanisms, the role of strength training interventions to the VO_{2SC} has never been evaluated. This is surprising as strength training, with the aim to increase maximal force [68], should reduce the recruitment of high-threshold motor units to sustain mechanical power output during cycling exercise at a given exercise intensity [72]. In addition, endurance training improves metabolic hyperemic response and optimizes the matching between local O_2 delivery and utilization, especially in individuals with suboptimal vascular response, such as elderly subjects [115]. Several studies have also shown that strength training can counteract age related impairments [116-49]. The extent of adaptation in elderly people is comparable to that in younger people. Sarcopenic muscle fibers thus do not per se have reduced mechanical muscle function but have a confirmed potential for adapting to strength training. However, elderly people who do strength training is currently low (about 10% to 15%) [96].

If the main mechanism responsible for the VO_{2SC} is the progressive recruitment of high threshold glycolytic motor units, then a strength training intervention should reduce the VO_{2SC} associated to higher intensities of exercise, and thus enhance exercise tolerance.

Therefore, the aim of this study was to evaluate the possible effect of strength training on the VO_{2SC} in healthy young and old individuals. We tested the hypothesis that a 4-week strength training intervention would attenuate the development of the VO_{2SC} and increase exercise tolerance in young and older adults, likely due to a reduction in the recruitment of high-threshold, type II motor units during constant work rate cycling exercise in the heavy intensity domain.

Methods

Participants

16 healthy young males (mean ± SD: age 26 ± 3 years, height 1.76 ± 0.67 m; body mass 74 ± 11kg) and 21 healthy older adults males (mean ± SD: age 68 ± 4 years, height 1.72 ± 6.22m; body mass 75 ± 10 kg) took part of this study. Young participants were randomly assigned to perform either a strength training intervention (intervention group; young adults (#8) and older adults (#11)) or to maintain their normal lifestyle (control group; young adults (#8) and older adults (#10)). Young and older adults participants perform 4 weeks of strength training. Inclusion criteria were: healthy young (18-35 years old) male and healthy older adults (> 65 years old) male that had not been involved in any exercise training program for at least 6 months. Exclusion criteria were: being an athlete/well trained individual undergoing regular strength training, smokers, BMI>30,

medical conditions that are known to affect cardiovascular or metabolic response to exercise or the use of medications that can interfere with the ability perform exercise or with the physiological response to exercise or increase the risk of exercise-related injuries. Participants provided written informed consent to participate in the study that was conducted with permission of the Ethical Committee of the University of Verona and in accordance with the Declaration of Helsinki.

Testing

After medical clearance, within 8 days before and after the 4-week training period, all participants completed the following tests:

i) A maximal ramp-incremental (RI) exercise test to the limit of tolerance on a cycle ergometer;

ii) 3 repetitions of a 10-min constant work rate exercise at an intensity that represented a power output associated to 50% of the difference between the GET and VO_{2peak} (Δ50). Additionally, participants performed 1 additional repetition of the Δ50 exercise at the same relative intensity until exhaustion;

iii) For both young and old groups a one repetition maximum (1RM) test in the weight room [117];

iv) *F*or young adults an additional isometric strength test on a force platform [76].

All tests were conducted in an environmentally controlled laboratory (22-25°C, 55-65% relative humidity) at a similar time of the day. Participants were asked to avoid heavy

exercise and caffeinated/alcoholic beverages the day before each test. A resting period of 24h was imposed between each test.

Ramp incremental exercise: Each participant performed a RI test to exhaustion on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL) consisting of a 4-min baseline cycling at 20 W, followed by ether a 25-$W \cdot min^{-1}$ or 20-$W \cdot min^{-1}$ increase in PO, for young adults and for older adults respectively, until volitional exhaustion [73]. Participants were asked to cycle in the range of 70-80 rpm. The accepted criteria for maximal effort were: (*i)* a plateau in the VO_2 response; (*ii)* a respiratory exchange ratio (R_{peak}) >1.1; and (*iii)* a peak heart rate (HR_{peak}) > 90% of the predicted maximum based on age (REF).

Constant work rate exercises: For the quantification of the VO_{2SC} each participant performed 3 constant work rate bouts exercise on an electromagnetically braked cycle ergometer (Sport Excalibur, Lode, Groningen, NL) consisting of 1 min baseline, 4 min cycling at 20 W followed by a 10 min cycling at an intensity of exercise relative to the individual Δ50. This heavy work rate was chosen because it has been demonstrated that a sizeable slow component can be reliably measured at this intensity, with a steady state being achieved within 10 min in the population studied [118,119]. The constant work rate exercise duration was chosen to allow a sufficient evolution and duration of the VO_{2SC}. One of the 3-Δ50 exercises (random order) was prolonged until reaching the individual limit of tolerance (*i.e.*, time to exhaustion (t_e)). The trial was terminated upon the participant being unable to maintain a pedal cadence of 60 rpm, despite strong verbal encouragement.

During all cycle ergometer exercises, breath-by-breath pulmonary gas exchange and ventilation were continuously measured using a metabolic cart (Quark B^2, Cosmed, Italy) [73]. Muscle oxygenation and deoxygenation ([HHb]) were evaluated during the Δ50 exercise using a quantitative NIRS system (Oxiplex TSTM, ISS, Champaign, USA). After shaving, cleaning and drying of the skin area, the NIRS probe was longitudinally positioned on the belly of the *vastus lateralis* (VL) muscle ~15 cm above the patella and attached to the skin with a bi-adhesive tape. The probe was secured with elastic bandages around the thigh. The apparatus was calibrated on each testing day after a warm-up of at least 30 minutes as per manufacturer recommendations. A comprehensive description of this method has been reported by Murias et al. [115].

During Δ50 exercises, for young adults only, surface electromyography (EMG) activity of the left VL muscle was continuously recorded by means of a wireless system (Wave wireless EMG, Cometa, Milan, Italy) during one of the three laboratory visits (randomly chosen). A pair of surface Ag/AgCl electrodes (Blue sensor, Ambu®, Ballerup, Denmark) was attached to the skin with a 3 cm inter-electrode distance. The electrodes were placed longitudinally with respect to the underlying muscle fibers arrangement and located according to the recommendations by Surface EMG for non-invasive assessment of muscles (SENIAM) [120]. Before electrode application, to minimize inter-electrode resistance, the skin was shaved and cleaned with alcohol in order to minimize impedance. The skin was marked using non-permanent ink in order to place the electrodes on the same site for the two tests (pre and post training) thus reducing the variability associated with day-to-day differences in EMG electrodes placement. The remote controller connected to the electrodes was well secured with adhesive tape to

avoid movement-induced artifacts, and the EMG signal was checked prior each test. Raw EMG signals were pre-amplified (gain 375, bandwidth 10–500 Hz) and digitized at a sampling rate of 2 kHz (Wave wireless EMG, Cometa, Milan, Italy).

In the young group, crank torque was measured independently from the left and right crank arms by strain gauge transducers (Sport Excalibur, Lode, Groningen, NL; peak force 2000 N, <0.5 N resolution and measurement uncertainty of <3%). Instantaneous angular velocity of the crank (rad s^{-1}) was measured every 2° using three independent sensors sampling in series (measurement uncertainty of <3%). Effective force (*i.e.* the propulsive force applied perpendicularly to the crank arm) was determined by the ratio between torque and the constant length of the crank arm (170 mm). Before the experiments began, a classical calibration procedure (with known mass) was performed and a zero adjustment was done before each session.

1RM test (both young and old group): after familiarization (see below), 1RM was determined directly for two lower-body exercises (Squat and Deadlift) and a upper body exercise (Bench Press) as the maximum resistance that could be lifted once throughout the full range of motion maintaining a correct execution form. Before attempting a 1RM, participants performed a standard warm up as per ACSM guidelines [45]. Then, a series of 3-5 single repetitions with increasing loads was performed until failure to complete one movement with correct form over the full range of motion [45].

Isometric strength test (young group only): All isometric contractions were performed on a custom-built isometric rack that allowed the bar to be fixed at any height above the floor. The isometric rack was placed over a force plate (Advanced Mechanical Technologies, Newton, MA), which sampled at 600 hz. All participants performed a

minimum of two familiarization-testing sessions one week before the initiation of the actual study to ensure that maximal isometric attempts were completed. A standardized warm-up based upon previous literature was utilized (12). The position for each isometric pull was established before each trial with the use of a goniometry to ensure a knee angle of 140±5° with the barbell placed at the mid-thigh position (i.e. the position that allows the highest force generation during a whole-body exercise (12)). Once the position was established, participants were strapped to the bar in order to avoid any movement. With each trial, participants were instructed to pull as hard and as fast as possible. Each participant performed four isometric mid-thigh pulls separate by a 3-minutes recovery between trials. The best attempt was used for further analysis.

Data analysis

Ramp incremental test: Gas exchange threshold (GET), respiratory compensation point (RCP), peak VO_2 (VO_{2peak}) and peak PO (PO_{peak}) were determined as previously described [77]. Briefly, VO_{2peak} was determined as the highest VO_2 obtained over a 30s interval and PO_{peak} was defined as the highest mechanical power output achieved at termination of the RI exercise. GET and RCP were estimated by visual inspection from gas exchange variables by three blinded expert reviewers [78].

Constant work rate exercise: VO_2 kinetics was modeled using non-linear least-squares regression (OriginPro 7.5, OriginLab Corp., Northampton, MA, USA). Breath-by-breath VO_2 was filtered for errant breaths (*i.e.* values resulting after sighs, swallows, coughs etc., defined as residing outside of 99% prediction limits) and interpolated to 1 s intervals [22]. Responses from the three exercise transitions were ensemble averaged to improve the signal-to-noise and averaged into 5 s bins for non-linear regression fitting. Previous

studies have concluded that this "extra" oxygen component is of independent and delayed onset with respect of the phase II of the VO_2 kinetics response [34,119] and, additionally, using a fixed fitted interval (e.g., 3-6 min difference) did not accurately estimate the slow component amplitude [119]. Therefore, oxygen uptake kinetics parameters were determined using a two-phase response with independent time delays as follow:

$$VO_2(t) = VO_{2Bsl} + A_p\left(1 - e^{-\frac{t-TD_p}{\tau_p}}\right) + A_s\left(1 - e^{-\frac{t-TD_s}{\tau_s}}\right)$$

where VO_2 (t) is whole body oxygen consumption at time t, VO_{2Bsl} is pre- transition VO_2 , A_p and A_s are the primary and slow component amplitudes respectively, TD_p, TD_s, τ_p and τ_s are their respective time delays and time constants. The first 20 s of data were removed before modeling to reduce the influence of the venous-return component [121].

The NIRS-derived [HHb] data during the Δ50 exercise ([HHb]) were time aligned and averaged to 5-s bins. This [HHb] response has been described to consist of a time delay at the onset of exercise, followed by an exponential-like increase in the signal [115]. The time delay (TD) for the [HHb] data was determined as previously described [122]. The [HHb] data were modeled from the end of the TD-[HHb] with a single exponential model as described as follow:

$$\Delta[HHb](t) = [HHb]_{Bsl} + A_p\left(1 - e^{-\frac{t-TD_p}{\tau_p}}\right)$$

where [HHb] (t) is the [HHb] response at time t, $[HHb]_{Bsl}$ is pre- transition [HHb] value, A_p is the primary amplitude of the response, TD_p, and τ_p are time delays and time constant of the response, respectively.

The second-by-second [HHb] and VO_2 data were normalized for each subject (0–100% of the response). The normalized VO_2 was left shifted by 20 s to account for the phase I-

phase II transition as previously described [22]. Data were further averaged into 5-s bins for statistical comparison of the rate of adjustment for [HHb] and VO_2. Additionally, an overall [HHb]-to-VO_2 ratio for the adjustment during the exercise on-transient was derived for each individual as the average value from 20s until the appearance of the slow component (determined by the individual TD_s resulted from the VO_2 kinetics fitting procedure). The start point was selected to be 20 s to begin beyond the physiological TD-[HHb] derived from NIRS. An end point equal to TD_s was selected to ensure that both the [HHb] and VO_2 signals (for the primary component of the response) had already reached 100% of their amplitudes.

The raw EMG signals were rectified and smoothed using a fourth-order band-pass Butterworth digital filter with a frequency range set between 20 and 500 Hz. The onset and offset of EMG activity were obtained by a mathematical method where the onset of muscle activation was determined as the signal with an amplitude of at least 2 standard deviations beyond the mean EMG value at baseline; this was adopted as threshold criteria for determination of the muscle activation – deactivation dynamics. The root mean square (RMS) was calculated as the 30 s average of the muscle activation phase (excluding the offset dynamic) and was used as an index of the total muscle activation [118]. The mean power frequency (MDF) was used as an indicator of the distribution of the frequency content within the EMG signal [108] and was calculated from the raw EMG signal. All EMG parameters were all expressed as a percentage of the 3rd minute of exercise (comparatively to the onset of the VO_{2SC}). The EMG signal was analyzed using a custom-made program written in MATLAB software (MathWorks Inc., Natick, MA).

Effective force expressed to the pedals (*i.e.* the propulsive force applied perpendicularly to the crank arm) was determined by the ratio between torque and the constant length of the crank arm (170 mm) using a custom-made program written in MATLAB software (MathWorks Inc., Natick, MA). Data was time-aligned to the onset of the constant work rate exercise and expressed as 30 s average from the onset to the end of exercise. The force applied to the pedal during the constant work rate exercise was expressed in percent relative to the peak force recorder during the ramp incremental exercise (F_{peak}). The average force expressed over the whole the step transition (F_{avg}) was calculated as representative of the force required to sustain the applied mechanical load.

Isometric strength test: Based on the vertical force component recorder by the force platform, two isometric variables were calculated. The Isometric Peak Force (IPF) was determined as the highest force value (N) recorder during the isometric strength maximal attempts. Contractile rate of force development (RFD) was defined as the slope of the force–time curve ($\Delta_{force}/\Delta_{time}$) from the onset of contraction up to the IPF. Isometric Peak RFD (IPRFD) was determined as the highest RFD value ($N \cdot s^{-1}$) recorded from the onset of contraction up to the IPF in incrementing time periods of 10 ms. The onset of contraction was defined as the instant when the force signal exceeded the baseline by 7 N [72].

Training program

Before the beginning of the study, all the participants young and older adults, took part of a 2-week familiarization period, which consisted of 6 strength-training sessions on non-consecutive days and performed with no overload to avoid any possible adaptations that could interfere with the main sub-sequent intervention [45]. During this period, each

participant received close supervision and instruction on proper exercise technique and training principles.

Subjects in the intervention groups (both young and old) trained in a weight room 3 times per week (90 min each session) on non-consecutive days. All subjects trained for 4 weeks and performed a total of 15 training sessions. All training session were supervised and instructed by a qualified strength coach with an instructor/participants ratio of 1/4. The training exercises (three fundamentals whole-body exercises and two complementary exercises) were performed with Olympic barbell and plates (Eleiko, Sweden) in a power rack. As *per* ACSM guideline for novice lifters, the load modulation over time was conducted using a linear model that implies a decreasing of training volume while increasing intensity [74]. Training characteristics are detailed in table 1.

Statistics

After assumptions verification (*i.e.*, outliers, normality, homogeneity of variance and covariance and sphericity, tested respectively using studentized residuals analysis, Q-Q plot, Levene's test, Box's test and Mauchly's test), a two-way mixed ANOVA (2x2; BW) was performed to assess whether differences existed between independent groups (between-subjects factor: intervention *vs.* control) over time (within-subjects factor: pre *vs.* post) in the measured statistics. F-statistics for both higher and lower order effects, were interpreted using the Greenhouse-Geisser correction [84] and, when significant, pairwise comparisons were performed to detect any intra and inter-factor differences. The adjusted α level for every pairwise comparison was calculated using Student-Newman-Keuls's method [83]. The required sample size was calculated based on an expected effect size estimation (medium effect size) on the primary dependent variable of interest (the

absolute change in the VO_{2SC} amplitude), using G-power package (http://gpower.hhu.de) and ensuring 1-β >80%. The analyses were repeated twice for both the young and old adults groups.

Data are presented as means ± SD. 95% Confidence intervals around mean differences (95% ΔCIs [lower limit, upper limit]) and effect sizes of those differences (Cohen's *d,* ranked as trivial (0-0.19), small (0.20-0.49), medium (0.50-0.79) and large (0.80 and grater) [85]) are also reported as objective and standardized measures of magnitude of effects and as alternative metrics of meaningfulness [86]. For the effect size calculation, the SD in the control group at baseline, was used to standardize the mean difference for each contrast [83].

Regarding regression analyses (*i.e.*, double exponential model) the goodness of fit was assessed using the residual sum of squares (representing the degree of inaccuracy in the fitting), the model sum of squares (representing the improvement in prediction resulting from using a double-exponential model rather than a single exponential model) and the R^2 (interpreted as the proportion of improvement using a double-exponential model).

In addition, on the young group, to assess any statistical differences over time in the force applied on the pedal and in the EMG signals (*i.e.* iEMG, RMS and MPF) during the Δ50% exercises, a two-tailed z-distance was calculated between the 3rd and the 6th minute of exercises within each signal. A z-distance between two points higher than 1.96 was considered statistically significant at the 0.05 level.

All statistical analyses were performed using STATA (Version 14, Texas, USA) and α was set in advance at the 0.05 level; statistical significance was accepted when $p < \alpha$.

Results

The same samples of participants, young and old, has been previously used for a different study purpose. For this reason, data from the ramp incremental exercises have been already presented in study one. However, here are displayed for descriptive purposes. Participants' characteristics and pre-training exercises statistics are listed in table 1. Changes in the morphological (body mass and BMI) and functional (VO_{2peak}, PO_{peak}, GET, RCP and F_{peak})(Table 4) statistics measured during the ramp incremental exercise as a function of group and time are presented in table 4.

Training program and adherence: Participants successfully completed the strength program with a compliance of 83% (10/12 training sessions) for the young adults and 92% (11/12 training sessions) for the older adults and no injuries were reported. Volume (number of repetitions) and average intensity (% 1RM) performed as a function of weeks are displayed in table 3.

Force: As shown in table 2, the ability to produce force increased significantly in both the intervention groups for 1RM tests (squat: 14±10%, deadlift: 11±5% in the young adults and squat: 21±11%, deadlift: 22±17% and bench press 14±12% in the older adults). In the young adults, isometric peak force (IPF: 19±7%) and the rate of force development measured during the mid-thigh pull test (IPRFD: 42±26%), whereas no significant changes were observed in the control group (average differences: 1±4%). No differences were detected at baseline between all groups in any of the above measured statistics.

VO_2 kinetics: Groups average responses during the Δ50 exercise for the VO_2 kinetics, the [HHb] and the EMG (in the young adults) signals are displayed in figure 6.

VO_2 kinetics parameters at baseline were not different between groups in both the young and older adults (all $d < 0.55$ and all $p > 0.05$; table 6).

Before the strength training intervention, all young adult subjects exhibited a significant slow component (p = 0.001, CI [0.16, 0.36]) (table 6 and figure 1-2). After training a significant reduction of the VO_{2SC} was observed in the intervention group (ΔCI [-606, -351], d = -4.01, p = 0.001) with no difference detected for the control group (ΔCI [-220, 145], d = -0.21, p = 0.68). The VO_{2SC} reduction in the young training group was associated with a 74±56% (8±5 min) increase in the time to reach exhaustion (table 6) while no comparable gain was detected in the control group (3±17% or 0.5±2 min) (table 6). No significant differences between young groups were observed for other VO_2 kinetics parameters post strength training (table 6).

In the older adults, VO_{2SC} ($ml \cdot min^{-1}$) was present before training in training group (p = 0.001, CI [0.16, 0.36]) and in control group (p = 0.001, CI [0.16, 0.4). After training was observed a significant slow component for both the training group (ΔCI [0.02, 0.93], d = 0,32; p= 0.001) and the control group (ΔCI [-2, 0.77], d = - 0,4; p= 0.31) (table 6). The VO_{2SC} reduction in older adults training group was associated with a 32±67% (6±3 min) increase in the time to reach exhaustion (table 6) while no comparable gain was detected in the control group (9±9% or 1.6± 0.7 min) (table 6).

[HHb] kinetics: The overall time course of the [HHb] (as reflected by τ[HHb]) and the calculated TD[HHb] at baseline were similar between young adults groups (all $d < 0.45$ and all $p > 0.35$) and older adults groups (all $d < -0.01$ and all $p > 0.95$) (table 4). No changes in response to training were observed for both statistics in both the intervention and the control groups (table 6).

After 4 weeks of either strength training or control period, τ[HHb], τVO_2 and the [HHb]/VO_2 peak did not change significantly in both young and older adults (table 6).

EMG: In young adults no between-group differences were detected between the 3rd and the 6th minute of exercise in the MPF, iEMG and RMS signals neither before, nor after the 4-week intervention/control period (figure 1). The calculated two-tailed z-distances in the EMG signals did not reach the required threshold (*i.e.* >1.96) to declare statistical differences (all *z*s < 1.96 and all *p*s > 0.05) as a function of time in any conditions.

Discussion

The present study tested the hypothesis that strength training, by increasing maximal force and potentially reducing the recruitment of high-threshold motor units to sustain a given absolute exercise intensity, would reduce the VO_2 slow component observed during a constant work rate exercise in young and older males. The strength training program implemented in the present study was shown to be feasible, and resulted in a high compliance and adherence rate of 83% for the older adults and 92% for the young adults.

The primary finding of this investigation was that a 4-week strength-training program significantly improved the ability to produce force in both populations (*i.e.* 1RM, IPF and IPRFD for the young adults and 1RM for the older adults). This increase in muscle strength is similar to previously published studies [123,124,125] .That result was accompanied by a significant increase in time to exhaustion of 74±56% for the young adults and 32±67% during the constant work rate exercise. This increase is associated with a better exercise tolerance and its positive functional implications.

This is the first study indicating a possible role of strength training in the reduction of the VO_2 slow component observed during a constant work rate cycling exercise in healthy young adults. In addition, this is the first study that investigates the effect of strength training on the VO_2 slow component in the elderly.

Young healthy individuals CWR tests, before training, were characterized by a VO_2 slow component. The increase in time to exhaustion after training, has been connected by a significant reduction in the magnitude of the VO_2 slow component (92±8%) needed to sustain the same absolute exercise intensity after training. Contrariwise, during the CWR tests, older adults showed, at baseline, a significant VO_2 slow component that eventually increased (48±7%) after training.

In the young group, the reduction of VO_2 slow component seems to be caused by a reduction of fast fiber (type II) recruitment, suggested by a significant change of the EMG signal, and not by a change in VO_{2peack} that not vary after strength training. The different fiber recruitment after the increment of strength it seems therefore connected to time of exhaustion enhancement. Older adult subjects were characterised by a significant rise in the VO_2 slow component, with a concomitant increment in both GET, RCP and VO_{2peak} (9%) after training (expressed both in terms of Watt and $L*min^{-1)}$. Due to the absolute change in $VO_{2peak,}$ the VO_2 slow component expressed as relative to VO_{2peak} did not change pre and after training (±10%). In the absence of the EMG data for the older adults' group, we might suppose that, due to an increase in the ability to produce strength, the observed increase in the VO_2 slow component is driven by a better recruitment of type II fibers after strength training.

Biological aging is associated with declines in the muscle mass, strength performance, and cardiorespiratory fitness resulting in an impaired capacity of elderly performing daily activities. To counteract the age effect on neuromuscular ability, strength training in elderly populations is the most effective strategy to improve neuromuscular functions and consequently to maintain the functional capacity during aging. More specifically, age-associated skeletal muscle sarcopenia is generally characterized by reduced muscle mass and strength and is manifested by preferential type II myofiber atrophy, [123, 125] myofiber necrosis and myofiber type grouping, [126] and increased intramuscular tissues. Preferential type II myofiber atrophy at least partially accounts for an accelerated loss of power with age. Previous studies in older adults have shown that the muscle adaptive response to resistance training is characterized by type II muscle fiber hypertrophy and enhanced recruitment [127] [113]. These specific adaptations might explain how the VO_{2SC} was partly mediated by fatigue on already recruited type I fibers before training. Of an interesting note, aging is characterised by a substantial loss of both thickness and number of muscle fibers, in particular type II muscle fibers [60]. Due to this acceleration of the age-related selective atrophy of type II muscle fibers,[91] older adults have significantly more type I muscle fibers than younger adults. For this reason, untrained older adults are therefore characterized by a specific reliance on type I fibers (caused by the inability to recruit type II fibers to sustain power) during aerobic exercise. This specific physiological adaptation can explain a loss in efficiency over type on the already recruited type I fibers associated with the inability to maintain a constant and VO_2 consumption during constant aerobic exercise to exhaustion. In fact, during constant cycling exercise, within the working muscles, the genesis of the VO_{2SC} might be attributed

to fatigue developed on the initially recruited oxidative muscle fibers (i.e. type I) causing a progressive recruitment of different, preferentially type II, fibers [87]. On the contrary, strength training is associated with a specific hypertrophy and by an improved recruitment on type II muscle fibers [90] counteracting the typical reduced neuromuscular activity (due to a more sedentary lifestyle and/or functional dis- ability) and/or impaired neuronal input that characterized the age-related decline in skeletal muscle function. This specific adaptation might explain a rise in the VO_{2SC} after strength training mediated by an increased ability to recruit type II fibers. Assuming no changes in the maximal oxygen consumption, this effect would have increased the size of the VO_{2SC} expressed relative to VO_{2peak}. On the contrary, older adults significantly improved VO_{2peak} after strength training suggesting different metabolic adaptations as per the young participants. Previous studies reported no change in the maximal oxygen consumption after strength training [57] with the majority of those reporting an overall impairment of the cardiovascular function after strength training. This result is in accordance with novel studies supporting the role of strength adaptations in enhancing the ability of skeletal muscle to generate energy via oxidative metabolism and therefore to improve cardiorespiratory fitness [57].

The observed increase in strength after 4-weeks of training for the young adults in the present study, is similar to that reported in previous studies [45,128] and resulted in attenuation the VO_{2SC} during constant work rate cycling exercise. These results suggest that the mechanism(s) responsible for this training-induced enhancement in the mechanical efficiency of muscle contraction might also be those involved in strength gain [52], at least in healthy young participants and therefore in the young model. There are

several adaptations that likely occur after strength training, and that might have induced an improvement in the work efficiency of exercising skeletal muscle, and thus a reduction in the VO_{2SC} in the young group. First, following strength training there are fiber-specific changes that might be involved in the observed attenuation of the VO_{2SC}. A reduction in myosin heavy chain IIb and an increase in myosin heavy chain IIa (mirroring a change in fiber type composition, with a reduction in the percentage of type IIb fibers and an increase in the percentage of type IIa fibers [92]) has been observed during the early phase of strength training [93,94]. These adaptations, along with a shift in metabolism to more aerobic fiber type recruitment during exercise, would reduce the metabolic cost related to muscle tension and enhance contractile efficiency [128]. Finally, the kinetics of force development after strength training can also play a role in altering metabolic demand of the exercising muscle during cycling (4). Specifically, metabolic cost is greater at the beginning of a muscle contraction compared with the maintenance phase of the contraction [129].

The pre-training results indicated a significant development of a VO_2 slow component as a function of time for both groups. The overall pre-training amplitude of the slow component in the present investigation is in line to those previously reported for both young and older adults[39,43,108,130] Although various mechanisms such as increased ventilatory and cardiac work, lactate clearance, stimulation from circulating hormones, and increased temperature have been proposed to contribute to the VO_2 slow component [131], the total contribution from these processes appears to be relatively small [34] and O_2

consumption from the locomotors muscles has been indicated to account for more than 85% of the VO_{2SC} [63]. The most prevalent hypothesis is that the VO_{2SC} might be the result of an increased recruitment of motor units innervating less oxidatively efficient type II fibers, which has been shown to occur at exercise intensities at or above Δ50% [39]). For example, whereas at intensities of about 40% VO_{2peak} type I fibers are almost exclusively recruited, and at intensities of about 60% VO_{2peak} both type I and type II fibers are activated, higher intensities of exercise above 75% VO_{2peak} (which closely resembles the intensity of exercise evaluated in this study) a predominance of type II fibers recruitment is observed [89]. Further support to the idea that increased recruitment of motor units innervating type II fibers is connected to the slow component of VO_2 is provided the strong correlation between the percent of type II fibers and the magnitude of VO_{2SC} (16)).

Reductions in the magnitude of the VO_{2SC} in response to endurance training have been previously reported [39,132]. In fact, the slow component of VO_2 has been shown to be attenuated very rapidly in response to endurance training, with a reduction of about 50% evident just after 2 weeks of training (19), and several adaptive changes within the muscles have been proposed to the observed reduction in the VO_2 slow component [65,67,133]. Although this information is important, this study is the first to investigate the effect of strength training in the change of the VO_{2SC} in young and old individuals.

An additional finding of the present study was an absence of electromyography evidence in young adults, for a progressive recruitment of type II fibers during a heavy exercise bout exhibiting a VO_{2SC} (Figure 1). Additionally, 4-weeks of strength training did not result in a significant change in any of the measured EMG variables collected on the young groups. Despite our initial hypotheses, the results of the present investigation

suggested that an alteration in fiber recruitment was not present in concomitance with the development of the VO_{2SC}. The high variability in the EMG signal, as displayed in figure 1, may partly explain the lack of any statistical significant results before and after training. In addition, the lack of temporal change in the EMG signals cannot rule out a fiber recruitment hypothesis as a mechanistic basis for the slow component development. As discussed by Cannon and colleagues [43], the relatively small shift in recruitment that might occur during the development of the VO_{2SC} and the possible recruitment exchange between fatiguing type I and type II fibers may result in a unchanged EMG data as observed in the present study. Therefore, muscle fiber recruitment patterns may have changed throughout the exercise bout and following strength training in the present study, but these changes may not be identified by the measure of EMG.

Over the past decades various attempts have been made to relate the VO_{2SC} with a progressive recruitment of glycolytic type II muscle fibers during constant work rate exercise, by interpreting the development of the temporal-associated surface electromyography signal. Some authors found and increase in both the integrated electromyogram (iEMG) and in the root mean square (RMS) to be associated to the VO_2 slow component, suggesting a likely recruitment of additional type II fibers [108,114]. However, other authors indicated no relationship between EMG signals and the slow component of oxygen consumption during cycling [43,108]. Therefore, due to these controversial findings, it should be highlighted that the intrinsic limitation in the EMG analysis may have hindered the interpretation of some of the results and conclusions presented in the current study. The technical difficulties of EMG measurement are well recognized, and the reproducibility of EMG remains a topic of discussion [134].

Furthermore, the interpretation of EMG during cycling exercise poses some challenges as the dynamic nature of this movement could have induced some alterations in electrodes positioning and so affected the records of surface EMG [135].

From a health benefits perspective, the reduction in the oxygen excess associated with higher intensities of exercise observed in the present study after strength training is an important training adaptation that significantly improved exercise tolerance in the Youngs. Interestingly, time to exhaustion and therefore exercise tolerance increased after training for both groups. However, this was apparently caused by two different mechanisms in young and old participants. For the young group, exercise tolerance increased by a reduction in the VO_{2SC} with no change in the ceiling of aerobic capacity (VO2peak), therefore delaying the point at which the upper limit of aerobic exercise was reached. On the contrary, for old participants, VO_{2SC} didn't change while an increase in VO_{2peak} was observed. For this reason, older adults were able to sustain exercise for longer time without reducing the VO_{2SC}.

Such extended ability to sustain exercise, although mediated by two different mechanism, might impacts compliance to aerobic activities thus improving the total amount of volume that can be performed at a given intensity of exercise and the total energy expenditure. From this perspective, strength training could significantly impact the ability to protract aerobic exercise for an adequate amount of time and/or to extend the volume of exercise at a given exercise intensity, being a primary training component in health promotion and athletes' development.

Conclusions

In conclusion, data from this study suggest that the training-induced decrease in the magnitude of the VO_2 slow component and the increase in exercise tolerance observed during cycling above the lactate threshold can be at least partly explained by the reported increase in the ability to produce force after strength training in young adults. However, the data presented have indicated no association between the development of the slow component and a shift in EMG signals during heavy cycling exercise. While the complexities of the electromyography measurement may have influenced the ability to elucidate the involvement of a progressive recruitment of type II fibers in the development of the VO_{2SC} further study are warranted in order to better elucidated the possible influence of strength training in affecting muscle fibers recruitment during exercise in the heavy intensity domain in young participants.

On the contrary, in the elderly, strength training may be responsible for a recovered ability to recruit type II muscle fibers that may increase their contribution towards force production in the heavy-intensity domain during a constant work rate cycling exercise. This could explain the apparent increase in the VO_{2SC} after strength training. While further studies are warranted to identify a direct cause-effect relationship between changes in muscle characteristics and the change in the VO2 slow component, one practical consequence of the above findings is that the overall effect of strength training on aerobic exercise tolerance in older adults could be beneficial due to an increase in the peak oxygen consumption. Further studies are required to clarify the overall impact

of ST on aerobic metabolism and exercise tolerance and the consequent implications for exercise prescription in older adults.

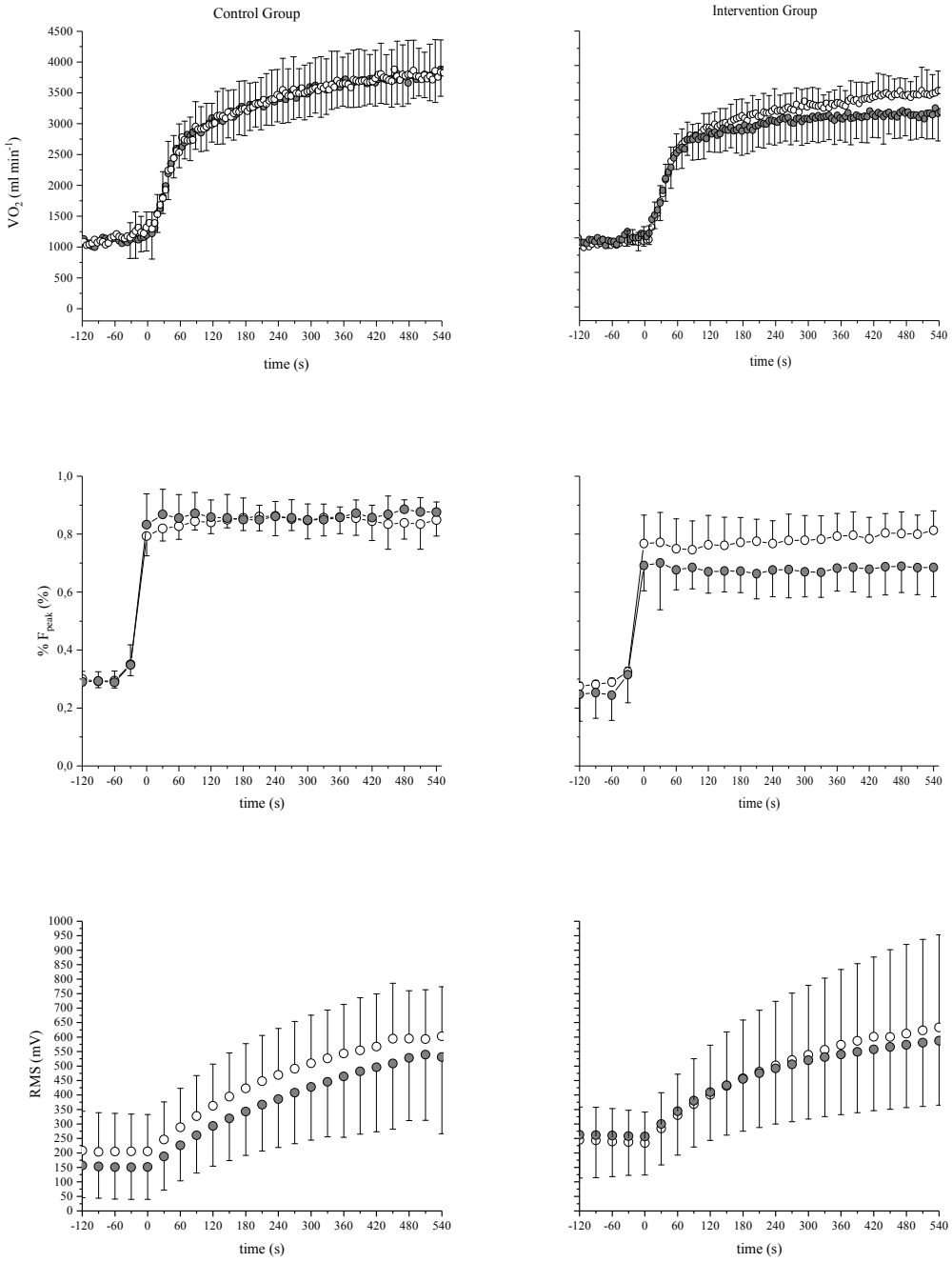

Figure 1: Young adults groups' average data have been calculated based on the results of the fitting procedures and displayed respectively (top to bottom), VO_2 ($ml \cdot min^{-1}$), relative force applied on the pedal with respect to the peak force ($\%F_{peak}$ (%)) and Root mean square (mV) as a function of time (seconds). Data are presented for the pre (white circles) and the post (grey circles) condition and for the control (left column) and the intervention group (right column). Statistical comparisons are detailed in the result section.

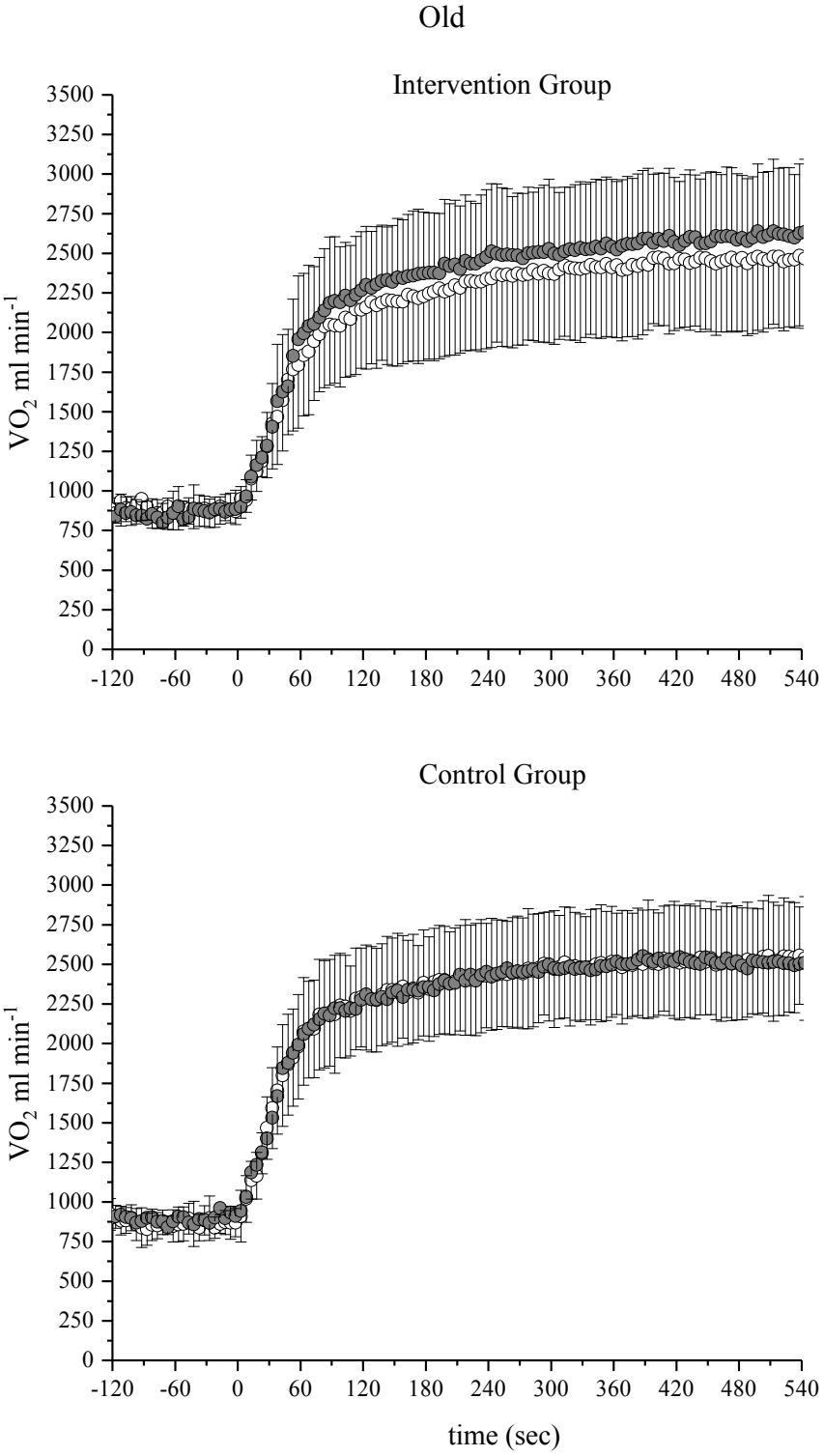

Figure 2: Older adults groups' average data have been calculated based on the results of the fitting procedures and displayed VO_2 ($ml \cdot min^{-1}$) as a function of time (seconds). Data are presented for the pre (white circles) and the post (grey circles) condition and for the control (left column) and the intervention group (right column). Statistical comparisons are detailed in the result section

Table 1. Participants' morphological characteristics as a function of group and time. Data are presented as mean ± SD.

	Young				Old			
	Intervention group		Control group		Intervention group		Control group	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Age (yrs)	24±2	24±2	28±2	28±2	67±4	67±4	68±4	68±4
Height (cm)	177±8	177±8	176±6	176±6	172±6	172±6	171±6	171±6
Body mass (kg)	72.7±15.2	73.6±13.9	76.0±5.6	75.9±5.7	75±10.4	75±10.2	75±10.2	75±10
BMI ($Kg \cdot m^{-2}$)	23.1±3.5	23.4±3	24.6±1.8	24.6±1.8	25±3	25±2.9	25.5±3.1	25.4±3.0

Table 2. Daily training regimen characteristics and average daily volume (kg) and relative intensity (% 1RM) are displayed as a function of the training weeks.

	Week 1-2 rep x set@RI			**Week 3** rep x set@RI			**Week 4** rep x set@RI			**Week 5** rep x set@RI		
	A	B	C	A	B	C	A	B	C	A	B	C
1.**Squat**	8x4@65%	8x4@65%	4x6@75%	4x6@75%	2x2@80% 2x2@85% 1x2@90% 1x3@95%	4x4@80%	3x4@90%	7x3@65% 5x3@70%	5x5@75%	4x1@80% 3x2@83% 2x3@87% 3x2@83%	3x2@80% 3x3@87% 3x2@83%	2x2@85% 2x2@87% 1x4@95% 3x1@85%
2.Bench	8x4@65%	8x4@65%	4x6@75%	4x6@75%	2x2@80% 2x2@85% 1x2@90% 1x3@95%	4x4@80%	3x4@90%	7x3@65% 5x3@70%	5x5@75%	4x1@80% 3x2@83% 2x3@87% 3x2@83%	3x2@80% 3x3@87% 3x2@83%	2x2@85% 2x2@87% 1x4@95% 3x1@85%
3.Deadlift	8x4@65%	8x4@65%	4x6@75%	4x6@75%	2x2@80% 2x2@85% 1x2@90% 1x3@95%	4x4@80%	3x4@90%	7x3@65% 5x3@70%	5x5@75%	4x1@80% 3x2@83% 2x3@87% 3x2@83%	3x2@80% 3x3@87% 3x2@83%	2x2@85% 2x2@87% 1x4@95% 3x1@85%
4.Pull ups	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM	5x4@6RM
5.Push Press	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM	4x8@10RM
Total Volume	148	148	124	124	91	100	88	160	127	118	115	97
Average Intensity	65%	65%	75%	75%	87.5%	80%	90%	67.5%	75%	83%	83%	89%

Table 3. Force is presented as a function of group for young and older adults. Data are presented as mean ± SD.

	Young				Old			
	Intervention group		Control group		Intervention group		Control group	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1RM Squat (kg)	99±19	112±19*	104±17	103±16	58±10	71±9*	49±10	53±12*
1RM Bench Press (kg)	90±14	90±12*	79±20	82±15	39±5	45±5*	37±5	37±7
1RM Deadlift (kg	100±22	111±22*	110±9	110±12	85±16	104±14*	101±22	104±24*

Note: *p* is the probability value associated with the observed mean difference; * indicate a significant difference with respect to the pre training condition ($p < 0.05$).

Table 4. Participants' morphological and physiological characteristics as a function of group and time for young and older adults. Data are presented as mean ± SD

	Young				Old			
	Intervention		Control		Intervention		Control	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
VO_{2peak} ($L \cdot min^{-1}$)	3.31±0.40	3.29±0.44	3.86±0.56	3.71±0.68	2.63±0.32	2.87±0.43*	2.78±0.44	2.87±0.5
PO_{peak} (watt)	309±30	325±42	346±37	352±52	224±48	239±54*	248±43	243±37
F_{peak} (N)	394±42	371±52	379±18	337±17	425±85	463±75	448±89	454±59
HR_{peak} (bpm)	185±5	186±6	187±5	185±4	153±11	152±17	157±11	153±12
HR_{peak} %	97±3	96±5	99±2	98±1	96±6	95±10	98±6	96±8
GET (W)	132±19	135±31	132±19	135±31	113±33	119±39	128±31	109±30*
GET ($L \cdot min^{-1}$)	1.90±0.2	1.84±0.2*	2.31±0.4	2.27±0.4	1.6±0.2	1.88±0.2*	1.7±0.3	1.63±0.8
GET % VO_{2peak}	57±5	56±6	60±10	62±10	60.6±5.4	66.4±11.8	62.3±5.8	56.8±7.4*
RCP (W)	201±2	210±3	243±2	251±5	160±3	176±4*	189±5	168±4
RCP ($L \cdot min^{-1}$)	2.51±0.3	2.42±0.3	2.90±0.5	2.84±0.5	1.91±0.2	2.25±0.3*	2.26±0.4	2.19±0.4
RCP % VO_{2peak}	75±6	73±6	75±9	77±12	72.8±10.0	78.8±6.8*	81.2±8.2	76.4±8.4

Note: VO_{2peak}: peak oxygen consumption; PO_{peak}: peak power output; GET: gas exchange threshold; RCP: respiratory compensation point Δ_{CIs}: confidence. * indicate a significant difference with respect to the pre training condition ($p < 0.05$).

Table 5. Groups' workload Δ50 characteristics. Data are presented as mean ± SD

	Young								Old							
	Intervention				Control				Intervention				Control			
	Pre		Post		Pre		Post		Pre		Post		Pre		Post	
	3'	6'	3'	6'	3'	6'	3'	6'	3'	6'	3'	6'	3'	6'	3'	6'
CWR workload (W)	224±24				254±28				161±38				176±36			
$Watt_{peak}$ %	72.6±2.1		69.5±4.5		73.8±3.3		73.0±7.7		73.46±5.8		73.8±5.7		71.9±6.5		71.0±13.3	
VO_{2peak} %	78.7±2.5		78±3.1		80.1±5.2		79.5±4.9		80.0.±6.1		68.5±3.2		73.4±14.5		72.9±5.3	

Table 6. Groups parameters estimate as a function of time during the Δ50 exercise for the VO2 kinetics, the force applied to the pedal, [HHb] and the EMG responses. Data are presented as mean ± SD.

		Young						Old					
		Intervention Group			Control Group			Intervention Group			Control Group		
		Pre	Post	p	Pre	Post	p	Pre	Post	p	Pre	Post	p
VO_2	Bls ($L \cdot min^{-1}$)	0.96±0.11	1.08±0.14	0.08	1.15±0.19	1.19±0.20	0.69	0.93±0.9	0.91±0.88	0.38	0.92±0.7	0.88±0.1	0.43
	TD_p (s)	12±3	14±6	0.41	11±6	10±5	0.73	8±8	10±7	0.51	8±8	10±5	0.84
	τ_p (s)	26±6	26±3	0.99	20±7	21±6	0.76	32±17	30±13	0.54	23±7	22±9	0.16
	A_p ($L \cdot min^{-1}$)	1.76±0.22	1.55±0.31	0.14	1.88±0.32	1.92±0.36	0.82	1.3±0.31	1.3±0.3	0.86	1.4±0.3	1.6±0.3	0.28
	TD_s (s)	183±41	186±54	0.90	160±54	165±30	0.82	150±46	161±42	0.22	159±34	175±27	0.13
	τ_s (s)	223±35	188±57	0.16	186±43	183±32	0.87	227±166	250±100	0.65	257±160	163±25	0.19
	A_s ($L \cdot min^{-1}$)	0.52±0.17	0.04±0.01	**0.01**	0.71±0.20	0.68±0.13	0.73	0.26±0.14	0.31±1.37	**0.04**	0.29±0.16	0.24±0.14	0.31
	t_e (min)	11±2	19±5	**0.01**	10±2	10±2	0.63	17±4	23±7	**0.002**	16±7	18±6	0.23
[HHb]	τ (s)	10±3	13±4	0.12	10±4	13±6	0.26	14±6	11±4	0.80	10±2	10±1	0.11
	TD (s)	12±3	10±4	0.28	13±2	13±2	0.85	8.3±3	8±3	0.88	8±3	8±1	0.69

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