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PhD COURSE IN BIOMEDICAL AND BIOTECHNOLOGICAL SCIENCES

XXX Cycle

"FUNCTIONAL EVALUATION OF EXERCISE TOLERANCE AND OXIDATIVE METABOLISM IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS."

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INDEX

STUDY II EXERCISE TOLERANCE IN PATIENTS WITH LATE ONSET POMPE DISEASE ON ENZYME REPLACEMENT THERAPY: EFFECT OF EXERCISE TRAINING

ABSTRACT

The main topic of my research project was the non-invasive evaluation of oxidative metabolism and exercise tolerance in pathological conditions. Within a translational approach, methods developed in the exercise physiology laboratory were applied on patients, with the aim of investigating the main mechanisms responsible for exercise intolerance. In the investigated patients, as in patients affected by many chronic diseases, exercise intolerance represents one of the main components of the clinical picture, and it significantly affects the patients' quality of life and often their prognosis. Physicians need to identify and quantify precisely the impairment of exercise tolerance, to ascribe it to specific mechanisms, in order to implement with efficacy therapeutic and rehabilitative interventions, mainly centered around exercise prescription. In order to satisfy this need, the proposed methods of functional evaluation need to be non-invasive, in order to be repeated serially without causing too much inconvenience to the patients, allowing to appreciate the course of the disease as a function of time, also following therapeutic or rehabilitative interventions.
In particular, in the present thesis I performed an evaluation of both peripheral (skeletal muscles) as well as central (cardiovascular O_2 delivery) factors, in order to evaluate, through an integrative approach, the effects of pathological conditions on skeletal muscle oxidative metabolism and on physiological adaptations to exercise.

In the first chapter, a general introduction is given about exercise physiology, functional capacity evaluation and exercise tolerance.

The second chapter describes the two main studies I worked on. In the first study, spinal cord injured (SCI) patients have been testes for exercise tolerance, evaluating differences between patients in relation to lesion level, and comparing results with a control group. We evaluated oxidative metabolism, muscle recruitment and biomechanics of propulsion in order to gain a better knowledge of the mechanisms behind skeletal muscle function in patients with spinal cord injury. We found that maximal performance (peak velocity, peak stroke frequency, peak $\dot{V}O_2$, peak HR) was linearly related to lesion level, and significantly different between tetraplegic patients (T) and control subjects (CTRL), but not between paraplegics (P) and CTRL. Also, if examined at same velocity, no substantial differences were observed between SCI patients and CTRL. Interestingly, O_2 cost of wheelchair propulsion was not significantly different in T and P *vs.* CTRL, when examined at the same velocity, despite the impairment in SCI patients in cardiovascular function, neuromuscolar activation and muscle oxidative metabolism, and the differences in propulsion patterns.
In the second study, the population studied were patients with Pompe disease, a rare inherited muscular disorder, caused by a deficiency in acid alpha-glucosidase (GAA), an enzyme that hydrolyzes lysosomal glycogen. We evaluated oxidative metabolism, taking into consideration both central factors (cardiovascular function) as well as peripheral factors (muscle O_2 fractional extraction with NIRS). Moreover, in this study the evaluation of exercise was performed, within a randomized crossover protocol, before and after 6 months of intervention with a specifically designed exercise training program, consisting of articular mobility exercises, aerobic training (performed on a bike), strength training (with elastic bands) and stretching exercises. Training was performed alone or with the association of an hyperproteic diet. A control period of 6 months (no intervention) was also performed. The 3-year study is not completed yet. An

analysis of preliminary results indicated a very good compliance to the exercise and exercise + diet intervention. Apart from a positive effect of the exercise and exercise + diet interventions on the quality of life of the patients, the effects on the investigated physiological variables seems to be relatively minor, with some exceptions. A subgroup analysis may confirm the hypothesis of positive effects of the intervention in the less impaired patients. Pompe's disease is indeed characterized by a wide range of disease severity. A likely conclusion could be that, in general, the exercise and exercise + diet intervention show an efficacy in preventive the natural evolution of the disease towards a progressive worsening of the clinical picture as a function of time.

CHAPTER I

GENERAL INTRODUCTION

List of abbreviations

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
Cao_2	arterial oxygen content
CO2	Carbon dioxide
CPET	Cardio Pulmonary Exercise Testing
Cr	Creatine
Cvo_2	mixed venous oxygen content
H2O	Water
Hb	Hemoglobin
HR	Heart rate
La-	Lactate
Mb	Myoglobin
MET	Metabolic Equivalent of Task
PCr	Phosphocreatine
Pi	Inorganic phosphate
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
SV	Stroke volume
VAT	Ventilatory anaerobic threshold
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E$	Ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake

Exercise physiology

Every physical activity, from running to rising from a chair, depends from the ability of muscle fibers to convert chemical energy from substrates into mechanical work. As it is well known, in all human tissues, comprehending skeletal muscle, the energy to conduct work derives from the hydrolysis of the high-energy molecule Adenosine triphosphate (ATP), which can be defined as the "molecular unit of currency" of intracellular energy transfer, in the reaction within the muscle's fibers contractile system. The main "problem" with skeletal muscle bioenergetics is that, in the presence of an ATP hydrolysis which during exercise can increase very rapidly by hundreds-folds with respect to the resting condition, ATP concentration can decrease only by a small percentage without incurring in fatigue and ultimately to exhaustion. In order to prevent this, ATP hydrolysis must by precisely matched, both in terms of time and amount, by ATP re-synthesis. Skeletal muscle fibers are substantially unique, within the human body, in the sense that they possess three main mechanisms through which ATP can be re-synthesized starting from ADP and Pi.

The energetics of muscle contraction is explained in **Figure 1.1**:

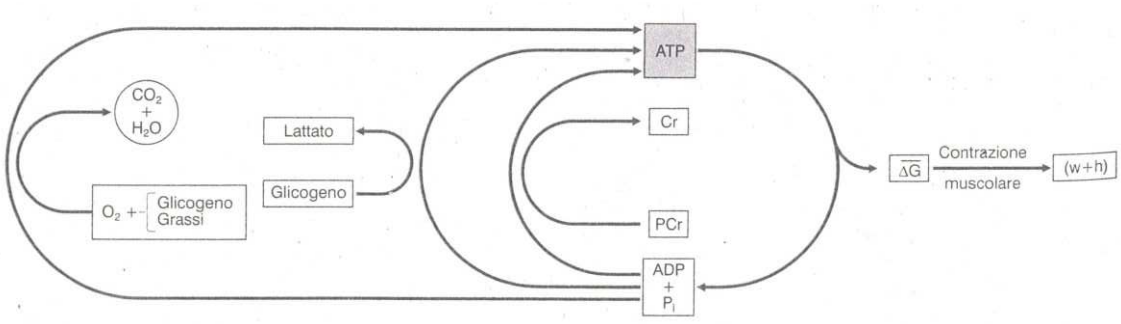

Figure 1.1. The energetics of muscle contraction. PCr, phosphocreatine; Cr, creatine; Pi, inorganic phosphate. The hydrolysis of ATP produces energy (ΔG), converted into mechanical work (w) and heat (h) by the muscle's fibers contractile system. ATP is then resynthesized by the indicated processes. From *di Prampero, La locomozione umana su terra, in acqua, in aria.*

The three main mechanisms from which energy is derived to resynthesize ATP, starting from ADP and Pi, are the following:

1) PCr splitting (Lohman's reaction), in which the high-energy phosphocreatine (PCr) molecule breaks down a high energy phosphate bond with the release of energy, which is utilized to re-synthetize ATP.
2) Anaerobic glycolysis, in which ATP derives from the glycolytic process even in the absence of O_2, the end product being lactate, or more precisely lactic acid.
3) Oxidative phosphorylation, in which the energy deriving from the oxidation of glucidic and lipidic substrates (proteins oxidation does not contribute significantly to ATP resynthesis during exercise) in the mitochondria is utilized to re-phosphorylate ADP in the mitochondrial respiratory chain (in the process termed "oxidative phosphorylation"), with the critical intervention of O2 as the terminal acceptor of electrons in the chain.

A fourth mechanism, based on the adenylate kinase (ADK) contributes only during very intense "supramaximal" exercise. In fact, ADK is an enzyme that catalyzes the conversion of two molecules of ADP to AMP (Adenosine monophosphate) and ATP, providing energy for muscle contraction.

In the following Figure, a schematic representation of the maximal power (expressed in watt/kg of body mass) sustainable by a-human subject on the basis of the three mechanisms responsible for ATP resynthesis (phosphocreatine hydrolysis, anaerobic glycolysis, oxidative phosphorylation) is presented as a function of the time of exercise. The Figure explain why, from a functional point

of view, 3 mechanisms are available and are utilized by skeletal muscle fibers in order to re-synthesize ATP.

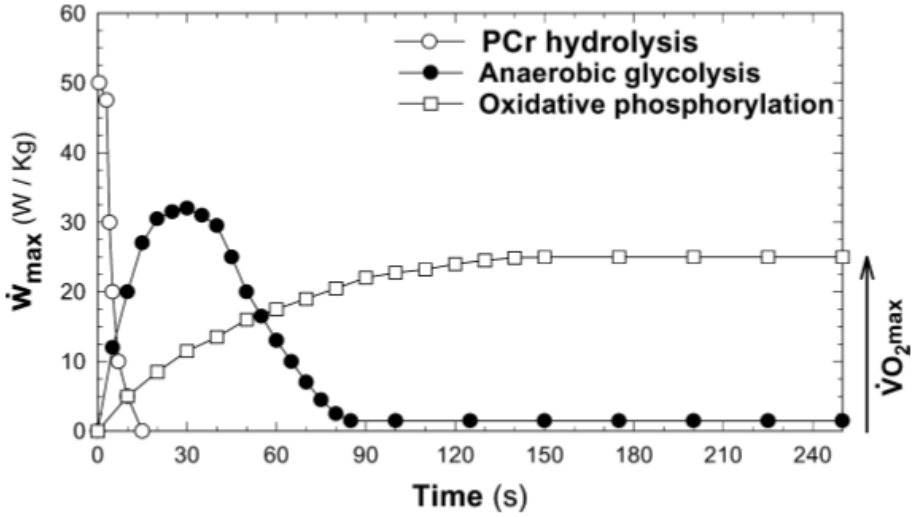

Figure 1.2. The three mechanisms of ATP resynthesis: phosphocreatine (PCr) hydrolysis, anaerobic glycolysis and oxidative metabolism. From *Grassi, 2003*

Energy from phosphocreatine hydrolysis is immediately available, and the maximal power of this mechanism is reached in a fraction of a second, but the mechanism rapidly fatigues and reaches a value close to zero in less than 10 s. At the other hand, oxidative phosphorylation has a relatively lower maximal power, represented by the $\dot{V}O_{2max}$ of the subject (in this example, ~25 W per kg), that is ~72 ml O_2 kg^{-1} min^{-1} above resting), which can be sustained for relatively longer periods of time (for a few minutes at an exercise intensity corresponding to $\dot{V}O_{2max}$). This mechanism is significantly slower in getting into action: about 2 min are needed before its asymptotic value is reached. The characteristics of the third mechanism (anaerobic glycolysis) are intermediate between those of the other two mechanisms, with its peak power reached for exercise durations corresponding to 40-60 seconds.[1]

In order to reach the respiratory chain of skeletal muscles mitochondria, O_2 has to cover a long pathway, which starts from ambient air. Pulmonary and alveolar ventilation bring O_2 from ambient air to the pulmonary alveoli. From there, O_2 crosses by diffusion the alveolar-capillary barrier, reaching capillary blood. The systemic circulation pumps the O_2 (mainly bound to hemoglobin in blood) to the peripheral vessels and to skeletal muscles capillaries, where a second process of diffusion takes place and brings O_2 inside the muscle fiber and, ultimately, inside mitochondria for the process of oxidative phosphorylation. Carbon dioxide (CO_2) produced by mitochondria follows the opposite pathway, through venous and pulmonary circulations, in order to reach the alveolar space and to be exhaled in expired air.

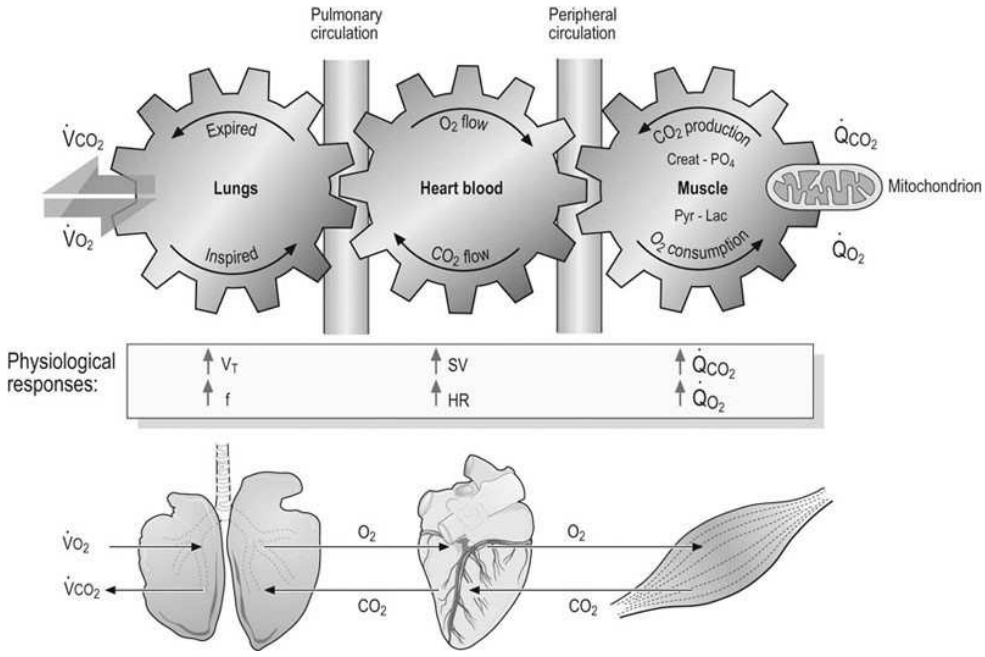

Figure 1.3. Illustration of the pathway for oxygen from the atmosphere to its site of utilization within muscle mitochondria. *Wassermann, 1996*

Figure 1.3 shows that the flux of O_2 from ambient air to mitochondria and the flux of CO_2 in the opposite direction require a tight coupling between respiratory, cardiovascular and skeletal muscles oxidative functions.
The maximal flux of O_2 from ambient air to mitochondria is represented by the maximal O_2 uptake ($\dot{V}O_{2max}$) variable presented in **Figure 1.2**, corresponding to the maximal mechanical power sustainable by oxidative phosphorylation. It can be expressed in L min^{-1} or normalized for body weight and expressed in ml O_2 Kg^{-1} min^{-1}. The maximal oxygen uptake ($\dot{V}O_{2max}$) reflects the maximal ability of a subject to take in, transport and use oxygen, defining his functional aerobic capacity.

$\dot{V}O_{2max}$, or maximal aerobic power, is one of the most important variables of functional evaluation of oxidative metabolism during exercise, and is the result of the integrated maximal responses of the respiratory, cardiovascular and muscular oxidative.
Determination of $\dot{V}O_{2max}$ is usually performed by measuring $\dot{V}O_2$ at the mouth of the subject (indirect calorimetry) during an incremental test, in other words a test in which the work rate (or the speed of a treadmill) is progressively increased. As a first approximation, $\dot{V}O_2$ increases linearly as a function of time, according to a $\dot{V}O_2$ vs. work rate relationship which is substantially independent from age, fitness level, health status (with some notable exceptions, see study n. 2 below). The increase in $\dot{V}O_2$ as a function of work rate usually occurs up to a certain work rate, widely different between subjects, at which $\dot{V}O_2$ typically does not increase further, having reached its "maximal" value. This plateau in $\dot{V}O_2$ has traditionally been used as the best evidence of $\dot{V}O_{2max}$. By definition, the $\dot{V}O_{2max}$ corresponds to the maximal mechanical power which can be sustained for relatively prolonged

(a few minutes) periods of time. For exercises of longer duration, the scenario gets more complicated (see the "threshold" concepts discussed below).

In healthy people, a plateau in $\dot{V}O_2$ occurs near maximal exercise. This plateau in $\dot{V}O_2$ has traditionally been used as the best evidence of $\dot{V}O_{2max}$.

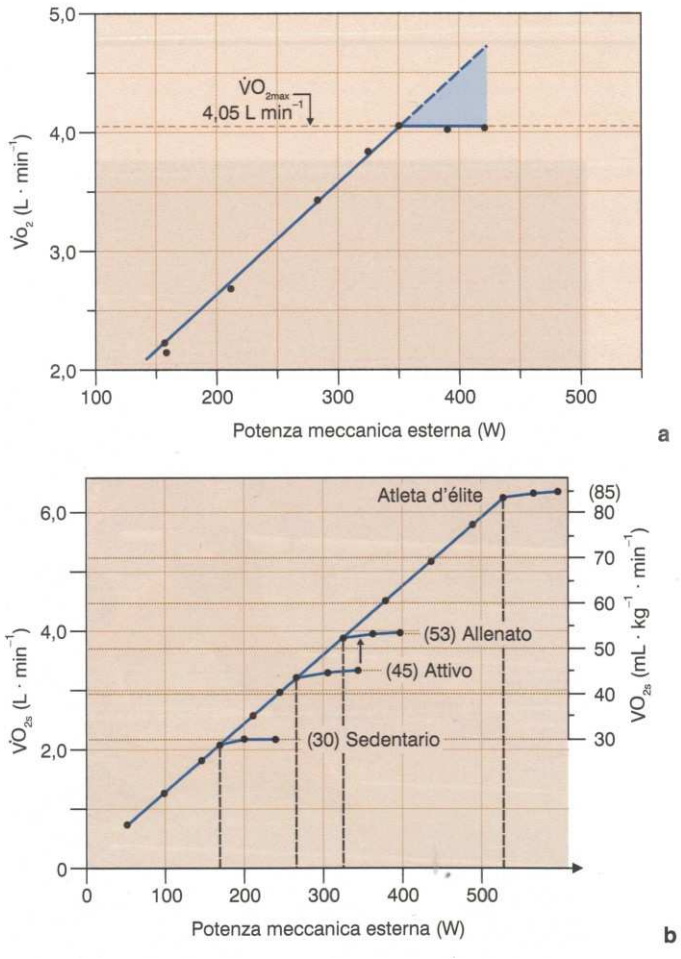

Figure 1.4. A) Steady state of $\dot{V}O_2$ (L min^{-1}) as a function of mechanical power in watts (W) during an exercise on a cycle ergometer in a well-trained subject. $\dot{V}O_{2max}$ has been reached at 350 W and equals 4.05 L min^{-1}. B) $\dot{V}O_{2max}$ expressed in L min^{-1} on the left y axis and in ml kg^{-1} min^{-1} on the right axis, as a function of mechanical power (W) in sedentary, normal active, trained subjects or élite athletes. From *di Prampero, La locomozione umana su terra, in acqua, in aria. Fatti e teorie.*

However, in clinical testing, a clear plateau may often not be achieved before the subject reaches exhaustion. Consequently, peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$), that is the $\dot{V}O_2$ determined at peak exercise, or at exhaustion, independently from the presence of a plateau, is often used as an estimation of $\dot{V}O_{2max}$.[2, 3]

Recently, Poole et al. [4] pointed out that, while the achievement of $\dot{V}o_{2max}$ is not a concern in young, healthy subjects with experience of performing exercise until voluntary exhaustion, not only patients but also naïve subjects to exercise testing and less motivated subjects may stop exercising before their real $\dot{V}O_{2max}$ is reached. Thus, in these circumstances $\dot{V}O_{2peak}$ could represent an underestimation of the "real" $\dot{V}O_{2max}$. Therefore, they suggested a new method to obtain a valid measurement of $\dot{V}O_{2max}$ (**Figure 1.5**): the incorporation of a second, constant work rate test performed at ~110% of the work rate achieved on the initial ramp test to evaluate the achievement of the classic $\dot{V}O_{2max}$-work rate plateau that is the unambiguous validation of $\dot{V}O_{2max}$. The wide utility of this procedure has been established for children, adults of varying fitness, obese individuals, and patient populations.

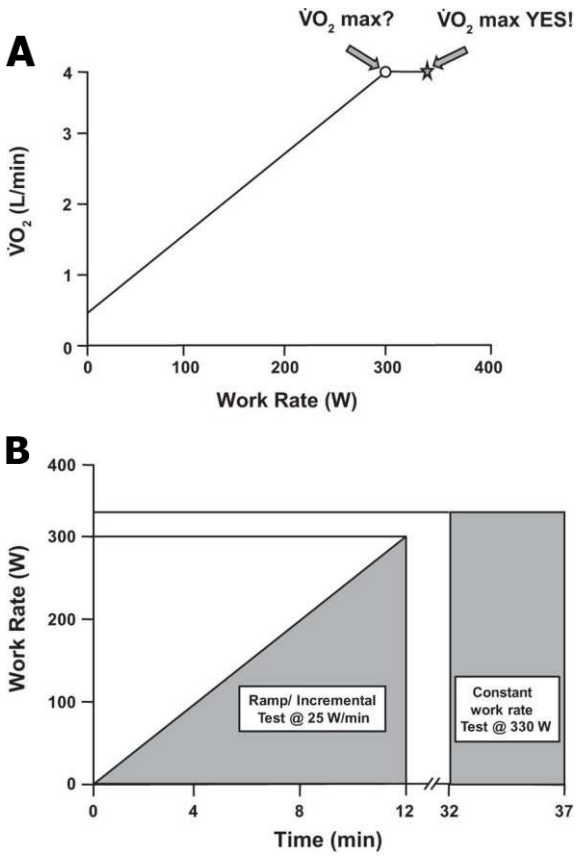

Figure 1.5. A) Validation that the ramp $\dot{V}O_2$ (○) was indeed $\dot{V}O_{2max}$ as evidenced by the presence of the $\dot{V}O_2$-work rate plateau (dashed line to star). B) schematic representation of the combination of the ramp test with the subsequent constant-load test (here at 110% maximum ramp power, 330 W). From *Poole et al., 2017*

$\dot{V}O_2$ can increase from a resting value of about 3.5 ml Kg^{-1} min^{-1} (about 250 ml min^{-1} in an average person) to peak $\dot{V}O_2$ values ($\dot{V}O_{2peak}$) about 15 times the resting value (30-50 ml Kg^{-1} min^{-1}). Athletes (**Figure 1.6**) may attain values over 20 times their resting values (up to 80 ml Kg^{-1} min^{-1}).[2, 5]

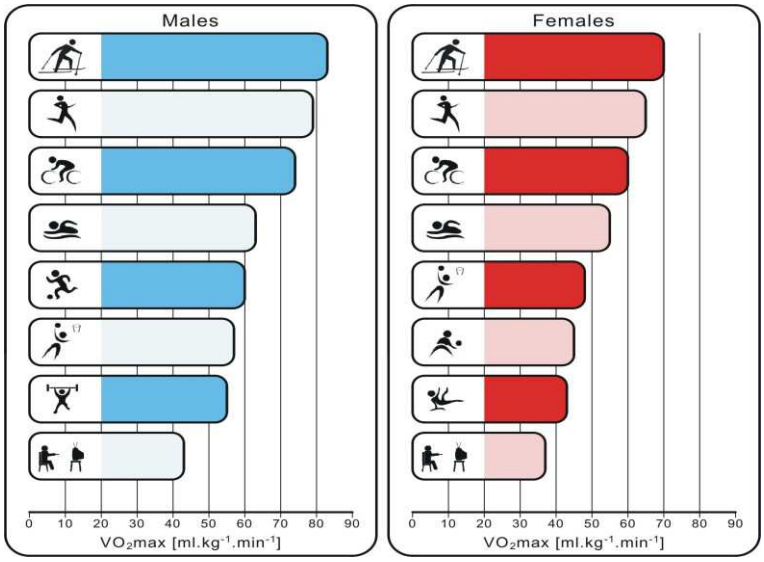

Figure 1.6. Average $\dot{V}O_{2peak}$ values for selected athletes. From *Bernaciková, Physiology.*

During exercises carried out with large muscle masses, in normoxia and in healthy subjects the main determinant of a normal $\dot{V}O_{2peak}$ is the cardiac output, and in particular the SV, whereas peripheral factors (skeletal muscle oxidative metabolism) would play a relatively minor role. The situation may be different in hypoxia, or during exercises with small muscle masses, in which the relative importance of peripheral factors increases. In patients the main limiting factor for $\dot{V}O_{2max}$ often resides in the organ or in the organs mostly affected by the disease: the lungs for patients with pulmonary diseases, the cardiovascular system for patients with cardiovascular diseases, skeletal muscles for patients with primary muscle diseases. This concept cannot be applied rigidly, however: patients with cardiovascular diseases, for example, typically have a reduced level of habitual physical activity, and in these conditions the detraining can significantly affect, for example, also skeletal muscle function. On the other hand, in patients with primary muscle diseases also the cardiovascular system undergoes detraining resulting in an impaired function.

Mean values of $\dot{V}O_{2max}$ expressed as absolute values (Panel A, L min^{-1}) and normalized for kg of body mass (Panel B, ml kg^{-1} min^{-1}), as a function of age and sex (males, red line; females: blue line) are shown in **Figure 1.7**:[6]

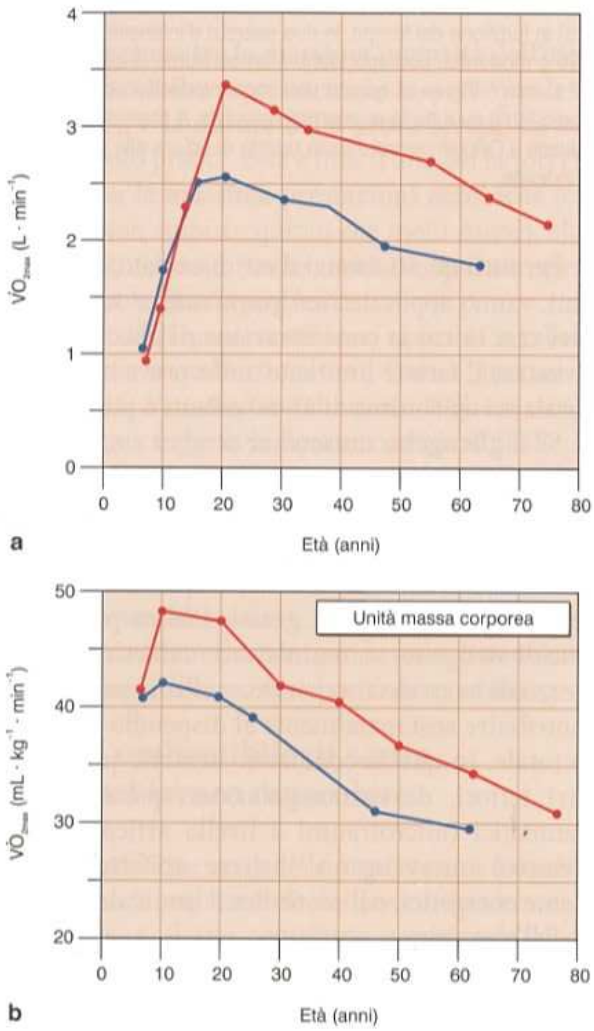

Figure 1.7. Mean values of $\dot{V}O_{2max}$ expressed as absolute values (Panel A, L min^{-1}) and for kg of body mass (Panel B, ml kg^{-1} min^{-1}), as a function of age and sex (males, red line; females: blue line). From *di Prampero PE, La locomozione umana su terra, in acqua, in aria. Fatti e teorie.*

$\dot{V}O_{2peak}$ can be also expressed as percentage of the predicted value (for healthy moderately active subjects), which can be empirically calculated as follows (**Figure 1.8**):[7]

A. Sedentary Men

Cycle factor = 50.72 − 0.372 × A

Step 1. Measure man's weight (W, kg) and height (H, cm) in light clothes without shoes and record age (A, yr)

Step 2. Calculate man's normal (predicted) W in kg as follows:
Normal (predicted) W = 0.79 × H − 60.7

Step 3A. If man's actual W equals normal W:
Predicted peak $\dot{V}o_2$ (ml/min) = actual W × cycle factor

Step 3B. If patient's actual W is less than normal W:
Predicted peak $\dot{V}o_2$ (ml/min) = [(normal W + actual W)/2] × cycle factor

Step 3C. If patient's actual W exceeds normal W:
Predicted peak $\dot{V}o_2$ (ml/min) = (normal W × cycle factor) + 6 × (actual W − normal W)

Step 4. If treadmill is used rather than cycle:
Multiply predicted $\dot{V}o_2$ by 1.11

B. Sedentary Women

Cycle factor = 22.78 − 0.17 × A

Step 1. Measure woman's weight (W, kg) and height (H, cm) in light clothes without shoes and record age (A, yr)

Step 2. Calculate woman's normal (predicted) W in kg as follows:
Normal (predicted) W = 0.65 × H − 42.8

Step 3A. If woman's actual W equals normal W:
Predicted peak $\dot{V}o_2$ (ml/min) = (actual W + 43) × cycle factor

Step 3B. If patient's actual W is less than normal W:
Predicted peak $\dot{V}o_2$ (ml/min) = [(normal W + actual W + 86)/2] × cycle factor

Step 3C. If patient's actual W exceeds normal W:
Predicted peak $\dot{V}o_2$ (ml/min) = [(normal W + 43) × cycle factor] + 6 × (actual W − normal W)

Step 4. If treadmill is used rather than cycle: Multiply predicted peak $\dot{V}o_2$ by 1.11

Figure 1.8. Calculation of predicted $\dot{V}O_{2peak}$ ml^{-1} min^{-1}. From *Wassermann, 1996*

During exercises lasting longer than 5-10 minutes (as it occurs in most activities of everyday's life, both in terms of leisure as in terms of occupational activities) the subjects cannot sustain a work rate corresponding to $\dot{V}O_{2max}$, but only to a percentage of $\dot{V}O_{2max}$. The decrease in $\dot{V}O_{2max}$ is related to the duration of the exercise, according to a function which dictates a monoexponential decrease. The physiological reasons behind this observation are complex, and a detailed discussion of the topic goes well beyond the aims of the present thesis.

The bottom line, however, can be summarized as follows. Anaerobic mechanisms (in particularly anaerobic glycolysis), which as a first approximation are associated with fatigue (mainly as a consequence of the acidosis associated with the accumulation of lactic acid in muscle and blood, as well as to glycogen stores depletion), start to intervene well before $\dot{V}O_{2max}$ is reached. In other words, there is a degree of "superposition" between aerobic and anaerobic metabolism at work rates well below those corresponding to $\dot{V}O_{2max}$. This superposition, in healthy and physically active subjects may start as early as at about 60% of $\dot{V}O_{2max}$. This percentage could be lower in untrained subjects or in patients, and it can be quite higher in athletes. Only exercises in which there is no net contribution of anaerobic mechanisms to the overall energy expenditure can be sustained for prolonged periods of time. It is obvious that the identification of a "threshold" at which the intervention of anaerobic metabolism starts to become significant, would be extremely interesting in terms of a functional evaluation of exercise tolerance.

Several methods are available to identify this "threshold". Also in this respect, a detailed discussion of these concepts goes well beyond the aims of the present thesis. We will simply present one which was utilized in the studies mentioned in the thesis: the "ventilatory threshold" or "gas exchange threshold".[7]

As mentioned above, during an incremental exercise $\dot{V}O_2$ increases linearly as a function of work rate, up to $\dot{V}O_{2max}$. $\dot{V}CO_2$ output, on the other hand, after an initial linear increase (with a slope slightly higher than that of $\dot{V}O_2$, due to the fact that the proportion of sugars utilized as substrates increases progressively as a function of work rate), at a certain work rate presents a sudden and disproportionate increase (with respect to $\dot{V}O_2$). Where does this "excess" CO_2 comes from? The first source is the CO_2 deriving from the dissociation of carbonic acid (H_2CO_3), which in turns derives from the buffering of H^+ (dissociation of lactic acid) by bicarbonate (HCO_3^-) buffers. In other words, the excess $\dot{V}CO_2$ detected during the incremental test (see **Figure 1.9**) is a reflection of the buffering of the lactic acid deriving from anaerobic glycolysis. Thus, the excess $\dot{V}CO_2$ reflects the onset of anaerobic glycolysis.

If the increase of work rate continues, a second "threshold" is identified, in which the excess $\dot{V}CO_2$ is a consequence of the hyperventilation aimed at correcting the metabolic acidosis. In other words: at the first ventilator threshold (gas exchange threshold) buffering of lactic acid occurs. But, if the increase in work rate continues the buffering becomes insufficient, acidosis ensues and the system responds by increasing pulmonary ventilation and thereby $\dot{V}CO_2$.

The functional relevance of the ventilatory thresholds, in terms of defining exercise tolerance, should be obvious, and it has been demonstrated by innumerable studies carried out in many experimental conditions and models.

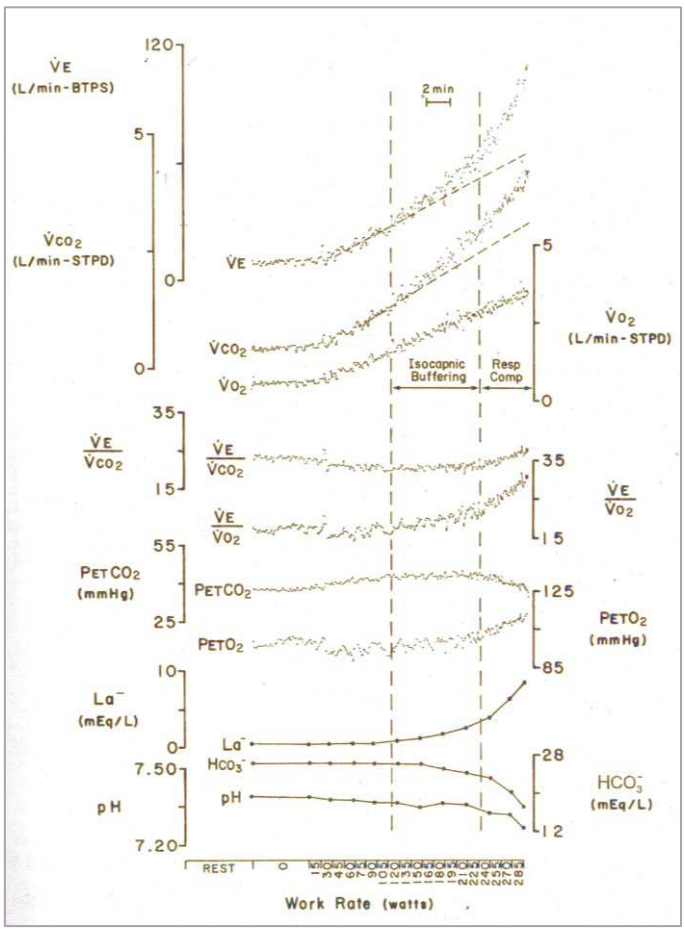

Figure 1.9. Breath-by-breath measurements of $\dot{V}E$, $\dot{V}O_2$ and $\dot{V}CO_2$ during an incremental test on a cycle ergometer. From *Wassermann, 1996.*

Other variables of evaluation of exercise tolerance which have been utilized in the studies mentioned below in this thesis are the following.

- Heart rate at the same work rate. HR increases substantially linearly as a function of work rate, up to a maximal value which substantially depends on the subject's age, and is not associated with training, physical fitness, exercise tolerance, etc. Since the maximal heart rate is unchanged after training, but the work rate (and $\dot{V}O_{2max}$) may substantially increase, then the slope of the HR vs. work rate must decrease with training. The slope decreases in the presence of an enhanced exercise tolerance, or it

increases following in the presence of an impaired exercise tolerance. In other words, for the same work rate a higher HR indicates a reduced exercise tolerance, whereas lower heart rate indicates an increased exercise tolerance.

- When fatigue ensues during a constant work rate exercise, $\dot{V}O_2$ increases. In other words, oxidative metabolism becomes less efficient [8]: more O_2 per unit of time is consumed to perform the same work rate. Thus, analysis of VO_2 after a few minutes of constant work rate exercise can be considered a tool of functional evaluation of fatigue.

Commonly, energy cost of physical activities is expressed in Metabolic Equivalent of Task (MET). It is a widely used physiological concept, representing a simple procedure for expressing energy cost of physical activities as multiples of resting metabolic rate (RMR).
It is a reference metabolic rate, defined as "the quantity of oxygen [per unit of time] consumed by the body from inspired air under basal conditions"[9], or "…the resting metabolic rate, that is, the amount of oxygen consumed at rest, sitting quietly in a chair",[10] and is equal 3.5 ml O_2 Kg^{-1} min^{-1}.[2, 10, 11]

In terms of energy expenditure, MET is also defined as the ratio of work metabolic rate to a standard RMR of 1 kcal (or 4.184 kJ) kg^{-1} h^{-1}:[12, 13]

$$1\ MET = 1\ kcal\ kg^{-1}\ h^{-1} = 4.184\ kj\ kg^{-1}\ h^{-1} = 1.16W\ kg^{-1}$$

For example, the energy expenditure of a 60Kg person watching television for one hour can be estimated as following:

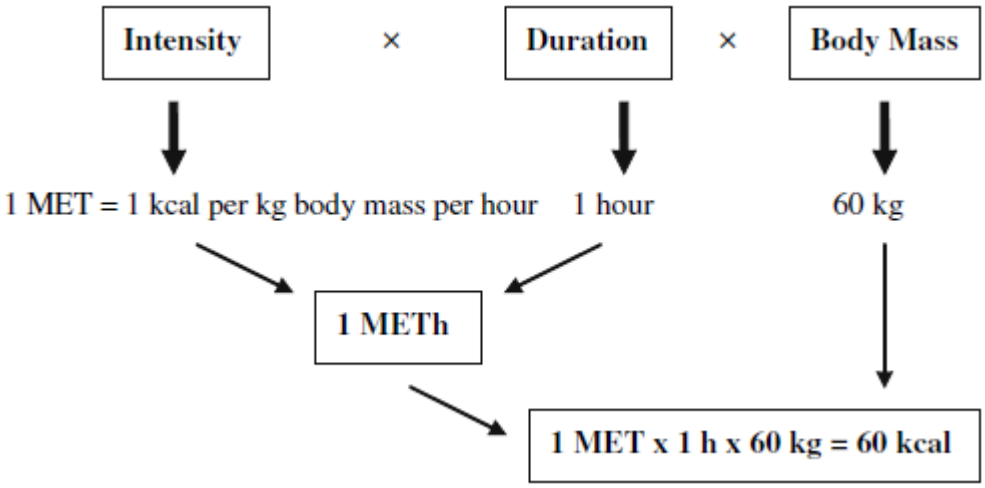

Figure 1.10. Energy expenditure (in kcals or MET hours) as a function of absolute intensity, duration and frequency of physical activity. From *Lagerros and Lagiou, 2007.*

The ratio of CO_2 output to the O_2 uptake ($\dot{V}CO_2$ / $\dot{V}O_2$) is called gas exchange ratio or respiratory exchange ratio (RER), and is determined by the fuels used for metabolic processes. A RER of <1 indicates a mixture of carbohydrates with fat (RER ≈ 0.7) or protein (RER ≈ 0.8), while a RER of 1 indicates metabolism primarily of carbohydrates. RER increases during exercise due to either a wider use of carbohydrates, the buffered lactic acid or hyperventilation (usually towards the end of exercise).[2]

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing (CPET) is a highly sensitive, non-invasive stress test, to evaluate exercise capacity and predict outcome, either in healthy populations as well as patients. It provides assessment of the exercise responses involving the pulmonary, cardiovascular, haematopoietic, neuropsychological, and skeletal muscle systems. CPET is widely used in clinical conditions to evaluate exercise intolerance and for objective determination of functional capacity and impairment.[2]

CPET allows to evaluate both submaximal and peak exercise responses, providing relevant information for clinical decision making. In fact, overall health status correlates better with exercise tolerance than with resting measurements.[14]

CPET involves measurements of respiratory $\dot{V}O_2$, $\dot{V}CO_2$, and ventilatory measures during an exercise test.

Nowadays, the mostly used systems utilize breath-by-breath analysis techniques because they provide the best measures of the metabolic response to exercise. Oxygen and carbon dioxide gas analyzers are usually incorporated in a "metabolic cart" designed specifically for functional testing (**Figure 1.11**).

Figure 1.11. Metabolic cart for CPET connected to the mask during an incremental test at the cycle ergometer.

Many different protocols are used for functional testing: The purpose of the test and the functional abilities of the tested subject/patient, determine the choice of protocol.

Also, different instruments can be used to exercise, such as treadmill, cycle ergometer, hand ergometer, rollers system, etc. In **table 1.1** the main differences between the two probably mostly used:

Variable	Cycle	Treadmill
Peak oxygen content (PVO_2)	Lower	Higher
Work rate measurement	Yes	No
Blood gas collection	Easier	More difficult
Noise and artefacts	Less	More
Safety	Safer	Less safe?
Weight bearing in obese subjects	Less	More
Degree of leg muscle training	Less	More
More appropriate for	Patients	Active normal subjects

Adapted from ATS/ACCP Statement on Cardiopulmonary Exercise Testing.[1]

Table 1.1 Exercise equipment: cycle ergometry vs treadmill. From *Albouaini et al, 2007*

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AIM OF THE THESIS

The aim of the thesis is the non-invasive evaluation of oxidative metabolism and exercise tolerance in patients. In particular, an evaluation of both peripheral (skeletal muscles) as well as central (cardiovascular O_2 delivery) factors has been performed. These evaluations can be useful in determining not only exercise tolerance but also quality of life in healthy as well as pathological populations.

In the first study, spinal cord injured patients have been testes for exercise tolerance, evaluating differences between patients in relation to lesion level, and comparing results with a control group. We evaluated oxidative metabolism, muscle recruitment and biomechanics of propulsion in order to gain a better knowledge of the mechanisms behind skeletal muscle function in patients with spinal cord injury.

In the second study, we evaluated oxidative metabolism in patients with Pompe Disease. Moreover, in this study the evaluation of exercise tolerance has been associated with mid-term intervention (6 months) consisting in aerobic and strength training eventually associated with a diet, to evaluate possible positive effects on the outcome of the pathology.

In short, the aim of the studies was to evaluate, through an integrative approach, the effects of pathological conditions on skeletal muscle oxidative metabolism and on physiological adaptations to exercise.

CHAPTER II

STUDY I O_2 COST OF WHEELCHAIR PROPULSION AND PROPULSION STRATEGY IN TETRAPLEGIC AND PARAPLEGIC PATIENTS.

Abstract

Objective To evaluate the O_2 cost of wheelchair propulsion and propulsion strategy in patients with spinal cord injury (SCI) in relation to lesion level. **Setting:** Exercise physiology laboratory, physical medicine and rehabilitation hospital. **Participants:** Sixteen patients with chronic SCI (9 paraplegics, P; 7 tetraplegics, T) and nine control (CTRL) subjects. **Interventions:** Measurements of O_2 cost of wheelchair propulsion (by indirect calorimetry), heart rate (HR), propulsion strategy (by video-kinematic analysis) and surface Electromyography during exercises on an ergometer with no resistance set on rollers, at a self-selected habitual speed and at the maximal sustainable speed (MS). CTRL performed 3 exercises: one at the MS for T, one at the MS for P, and one at their own MS. **Main Outcome Measures:** Pulmonary O_2 uptake ($\dot{V}O_2$); O_2 cost of wheelchair propulsion; propulsion strategy; HR; propulsion velocity; muscle activation. **Results:** Peak $\dot{V}O_2$, peak HR, velocity and stroke frequency at MS were linearly related with lesion level (higher level, greater impairment); values in T were lower than in P or CTRL, whereas no differences were observed between P and CTRL. Propulsion pattern was different in the patients (particularly in T) *vs.* CTRL. At the same velocity, the O_2 cost of wheelchair progression was not different in T or in P *vs.* CTRL. Muscle activation has shown to be heterogeneous in T, and relatively similar in P and CTRL. **Conclusions:** Despite the different neuromuscular activation and propulsion pattern, the impaired skeletal muscle oxidative metabolism and cardiovascular function, in T and P oxidative economy (oxidative energy spent per unit of distance) during wheelchair propulsion was not impaired. Maximal performance was impaired in T, but not in P.

List of abbreviations

ARC	Arcing
ASIA	American Spinal Injury Association
BB	Biceps brachii
CTRL	Control subjects
CTRL-MS	Controls' trial at their own maximal sustainable speed
CTRL-P	Controls' trial at the mean speed sustained by P during MS
CTRL-T	Controls' trial at the mean speed sustained by T during MS
DA	Deltoid anterior
DL	Double loop
DM	Detoid medial
DP	Deltoid posterior
HR	Heart rate
MS	Maximal sustainable speed
P	Paraplegic patients
PM	Pectoralis major
RMS	Root Mean Square
SA	Serratur anterior
SC	Semicircular
SCI	Spinal cord injury
sEMG	Surface Electromyography
SL	Single loop
SSP	Supra Spinatus
SSS	Self-selected speed
T	Tetraplegic patients
TB	Triceps brachii

$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E$	Ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_2peak$	Peak oxygen uptake

Introduction

Spinal cord injury (SCI) is a severe condition leading to impairments of lower and at times upper limbs movements, as well as (in patients with lesions above T6) of cardiovascular-and autonomic nervous system functions. The higher the level, and the more complete the lesion, more serious is the loss in function. Apart from the effects deriving from profound skeletal muscle deconditioning and atrophy, as well as sensibility deficits [1-3], in patients with high lesion levels the sympathetic stimulation to the myocardium and vasomotor function may be impaired, leading to a reduced maximal cardiac output and to a reduced capacity to redistribute blood according to peripheral needs, and thereby impairing the overall capacity to transport O_2 to the working muscles. In fact, as a consequence of autonomic nervous system malfunction, the sympathetic hypoactivity results in low heart rate, low blood pressure, orthostatic hypotension, loss of diurnal fluctuation of blood pressure and disturbed reflex control. A consequence of interruption of control of the sympathetic spinal cord centers by central nervous system is autonomic dysreflexia: a severe imbalanced reflex to noxious stimuli below the level of neurological injury, producing alterations in cardiac rhythm and vasomotor, pilomotor and sudomotor activity. Also, distension and manipulation of the urinary bladder frequently cause autonomic dysreflexia. [2-5]. Moreover, higher values for norepinephrine and epinephrine were observed following sympathetic hyperstimulation [6]. All these factors lead to massive vasoconstriction and arterial hypertension, and can result in cerebral bleeding, pulmonary edema, myocardial infarction, seizures and death [3, 4, 7, 8]. Thermoregulatory functions in patients with SCI may also be impaired, most likely caused by impairment of the autonomic and somatic nervous systems, and therefore a disrupt control of skin blood flow and sweating below the level of

lesion. This results in symptoms of hyperthermia, and in worst cases heat syncope and stroke. [3, 9-11]. Beyond the capacity of force generation [12], also skeletal muscle oxidative metabolism can be severely impaired in SCI patients [13]. As a consequence of all this, aerobic function and exercise tolerance during prolonged exercise are usually significantly impaired in SCI patients [2-4, 14-16]. Peak $\dot{V}O_2$ values are indeed known to be substantially lower in these patients than in age-matched able-bodied subjects, and the decreased $\dot{V}O_2$peak is known to be related to lesion level, being more pronounced in the presence of higher lesions [1-3, 17]. Apart from the lesion level, other factors may influence muscle function and exercise tolerance in SCI patients, such as sex, age, time since injury and regular participation in physical activity [2, 16, 18-22]. In any case, the reduced exercise tolerance significantly contributes to the reduced quality of life and exposes the patients to the cardiovascular and metabolic consequences of inactivity and deconditioning [12, 15, 21, 23, 24].

SCI patients are mostly forced to use wheelchairs for locomotion. Wheelchair propulsion is known to be inefficient compared to other forms of locomotion. In fact, gross efficiency of wheelchair propulsion in SCI patients corresponds to 2-10% [25-28], up to 12% in studies using racing wheelchairs and trained athletes [29]. This inefficiency could be related not only to muscle recruitment limitations and the other impairments present in patients with SCI, but also to intrinsic biomechanical characteristics of wheelchair propulsion, possibly related to factors such as stroke frequency [30], propulsion pattern [31], and experience with the use of wheelchairs [32]. Independently from the cause, however, a reduced efficiency is inevitably associated with a reduced exercise tolerance [33]. A better knowledge of these aspects would be useful in the development of rehabilitative interventions.

Upon such premises, in the present study we evaluated the O_2 cost of wheelchair propulsion in paraplegic (P) and tetraplegic (T) SCI patients. We decided to investigate only the contribution of aerobic metabolism to energy expenditure, since during prolonged exercise oxidative metabolism represents by far the most important contributor to the energy yield, and is the only one which can be directly and non-invasively investigated. We decided to evaluate the patients during wheelchair propulsion on a roller ergometer with no resistance set on the rollers, in order to evaluate an everyday type of locomotion. A group of able-bodied controls and lower-limb amputees experienced in the use of wheelchairs acted as controls. We hypothesize a different propulsion pattern and a higher O_2 cost of wheelchair propulsion in P and T patients versus the controls.

Materials and methods

Subjects

Sixteen patients with complete and incomplete chronic (at least 1-year post-injury) spinal cord injury (SCI) and ten control subjects (CTRL) took part in this study. The patients were full-time manual wheelchair users, with full or partial use of their arms and hands. The patients were recruited among those followed in the Department of Rehabilitation Medicine of the Physical Medicine and Rehabilitation Institute "Gervasutta", Udine, Italy. Inclusion criteria were male sex, diagnosis of SCI, clinical stability and experience in manual wheelchair usage.

Some characteristics of the patients and CTRL are given in **Table 2.1**. Body mass index (BMI) was calculated as body mass (in kg) divided by height^2 (kg m^{-2}). No significant difference in BMI was observed between groups. For tetraplegic patients (T) the lesion level was from C4 to C8. In all T the SCI recognized a traumatic cause. In 3 P patients the lesion was above T6. For paraplegic patients (P) the lesion level was from D4 to D12. In 7 out of 9 P patients the cause of SCI was traumatic. The American Spinal Injury Association (ASIA) injury level (category A-D) [34] is also reported in the Table. In all P the ASIA level was A. No differences in the number of years elapsed since SCI was observed between T and P. About 2/3 of T and 50% of P participated regularly in sport events (such as table tennis, wheelchair basket, wheelchair rugby and hand bike) approximately 3-5 hours weekly.

TABLE 2.1. Subject characteristics of the three groups: tetraplegics (T), paraplegics (P), controls (C).

subject	*age (yr.)*	*wheight (Kg)*	*height (m)*	*BMI (Kg/m²)*	*athlete*	*physical characteristic**	*ASIA*	*etiology*	*years after lesion*
T1	31	52	1.8	16.05	no	SCI C4	A	traumatic	15
T2	39	68	1.75	22.2	yes	SCI C5	C	traumatic	15
T3	56	78	1.86	22.55	yes	SCI C5	C	traumatic	37
T4	43	64	1.78	22.2	yes	SCI C6	A	traumatic	23
T5	39	62	1.73	20.72	yes	SCI C6	A	traumatic	17
T6	27	65	1.8	20.06	no	SCI C7	C	traumatic	7
T7	46	80	1.77	25.54	yes	SCI C8	D	traumatic	6
mean	***40.1 ±9.6***	***67 ±9.6***	***1.78 ±0.04***	***21.5 ±2.9***					***17.1 ±10.5***
P1	33	83	1.8	25.62	no	SCI D4	A	epidural hematoma	30
P2	29	70	1.76	22.6	no	SCI D5	A	traumatic	7
P3	48	82	1.76	26.47	no	SCI D5	A	traumatic	11
P4	33	72	1.9	19.94	no	SCI D7	A	neoformation	1
P5	36	69	1.82	20.83	yes	SCI D7	A	traumatic	14
P6	35	68	1.8	20.99	yes	SCI D8	A	traumatic	12
P7	24	112	1.8	34.52	yes	SCI D10	A	traumatic	3
P8	41	82	1.88	23.2	yes	SCI D12	A	traumatic	16
P9	45	72	1.82	21.74	yes	SCI D12	A	traumatic	12
mean	***36 ±7.6***	***78.9 ±13.8***	***1.82 ±0.05***	***24 ±4.5***					***11.8 ±8.5***
C1	21	76	1.81	23.2	no	AB			
C2	25	90	1.88	25.46	no	AB			
C3	21	61	1.75	19.92	no	AB			
C4	47	83	1.9	22.99	yes	AMP			
C5	35	83	1.9	22.99	yes	AMP			
C6	44	85	1.74	28.08	no	PAT			
C7	38	60	1.72	20.3	no	PAT			
C8	37	75	1.8	23.15	no	AB			
C9	32	100	2	25	yes	AMP			
C10	24	70	1.8	21.6	yes	PAT			
mean	***32.4 ±9.4***	***78.3 ±12.5***	***1.8 ±0.1***	***23.3 ±2.5***					

NOTE: for each group, mean values are expressed ±SD.
*Physical characteristic: *SCI= spinal cord injury; AB=able-bodied; AMP=amputee; PAT=polytrauma (C6), Guillain-Barré syndrome (C7), osteosarcoma (C10)

Exclusion criteria were signs or symptoms of diabetes or of any major cardiovascular, respiratory or orthopaedic disease contraindicating or significantly interfering with the tests. Patients were treated with oxybutynin, analgesic and muscle relaxants. No patients or CTRL were treated with beta-blockers.
CTRL were either physical therapists experienced in wheelchair usage, lower limbs amputees or patients with pathological conditions leading to regular wheelchair use in daily and/or sport activities. All amputees participated regularly in sport events.
The participants provided signed consent statements, after being fully informed about the purposes and testing procedures of the investigation, which were approved by the ethics committee of the Physical Medicine and Rehabilitation Institute "Gervasutta", Udine, Italy, where all experiments were performed. All procedures were in accordance with the recommendations set forth in the Helsinki Declaration of 1975, as revised in 2013.

Exercise protocol

Subjects came to the laboratory in the morning, and were allowed time to gain familiarity with the investigators and the experimental arrangement, were carefully instructed about the procedures, and were familiarized with the protocol using short practice runs. All pushing trials were performed on a computerized roller system (Ergo 1, STI - 001, Sti Engineering srl, San Daniele del Friuli (UD), Italy) with no braking resistance. The system consists of two metal rollers mounted on a metal base. The front wheels of the wheelchair were fixed to the metal base and the back of the wheelchair was fixed with straps on the rear frame of the roller system, in order to minimize movements of the wheelchair during the pushing trials. Velocity was recorded through the computer connected

to the roller system. All SCI subjects used their own everyday wheelchairs during the test. For CTRL subjects, a standard everyday wheelchair of the right measure for each participant was used.

After a stationary start, SCI subjects performed two 4-min exercises: the first one at the self-selected speed (SSS), and after 2 minutes of recovery (or when heart rate [HR] was back to resting values) subjects were asked to perform a maximum effort trial at their maximal sustainable speed (MS).

CTRL subjects performed three 4-min exercises: one at the mean speed sustained by T during MS (CTRL-T), one at the mean speed sustained by P during MS (CTRL-P), and one at their own maximal sustainable speed (CTRL-MS). Experiments in CTRL were carried out after those in T or P.

Measurements and analyses

Pulmonary ventilation, O_2 uptake ($\dot{V}O_2$) and CO_2 output ($\dot{V}CO_2$) were determined by means of a portable metabolic unit (MedGraphics VO2000, St. Paul, MN, USA), which provides a 3-breath average of variables through telemetric transmission. This system has been validated in previous studies [35, 36]. Prior to testing, the VO2000 was calibrated according to the manufacturer's instructions, which consist in performing an auto-calibration routine that is run through the software. It uses room air calibration of the O_2 and CO_2 analyzers as well as an auto-calibration procedure for the pneumotachometer. The current software program utilized with the VO2000 does not allow a two-point calibration using known gas volumes or manual calibration of the pneumotachometer with a calibration syringe. The device employs a patented flow meter, which uses a proportional sampling valve and a 3-breath average for the measurement of $\dot{V}O_2$, $\dot{V}CO_2$, and

$\dot{V}E$. The flow device is connected to a neoprene facemask (with a silicone adapter) that covers the subject's mouth and nose (**Figures 2.1, 2.2**).

Figure 1.1. Neoprene rubber mask connected to gas analyzer

Figure 1.2. Metabolimeter unit (MedGraphics VO2000, St. Paul, MN, USA) and *MedGraphics Breeze Suite Software*

Mean values over each minute were then calculated. The O_2 cost of wheelchair propulsion, expressed as aerobic energy expenditure ($\dot{V}O_2$ above rest per unit of body mass) per unit of covered distance, was calculated as net $\dot{V}O_2$ ($\dot{V}O_2$ above resting) divided by velocity. Aerobic energy expenditure was also expressed in joules by assuming a respiratory exchange ratio 0.96 [37].

Heart rate (HR) was recorded by means of a portable heart rate monitor (Polar S810, Polar Electro Oy, Kempele, Finland) and data were analyzed with *Polar ProTrainer5 software*. HR values during the tests were averaged over 1-min periods. Mean values of variables determined during the last minute at maximal speed were taken as "peak" values.

A video camera (Nikon J1 MODEL, 1 NIKKOR VR 10 – 30mm) recorded data during the entire test for the kinematic two-dimensional analysis, at a sampling rate of 60 frames per second. The camera was placed on a tripod on the right side of the subject, at a height of 0.5 meters from the floor, and at a distance of 3 meters from the participant [38]. A calibration frame was used for spatial reference.

Three anatomical landmarks were identified with white markers and secured with Velcro stripes to a black skin-tight shirt (**Figure 2.3**): one on the acromioclavicular joint, one on lateral epicondyle approximating elbow joint axis, and one on the ulnar styloid process as suggested in previous studies [38].

Figure 2.3. Markers positioning for kinematic analysis.

Next, using a video analysis software (Kinovea v 0.8.24), a cartesian coordinate system was created, using the hub of the wheel as origin of the axis, and 6 complete stroke cycles have been analyzed during the last 10 seconds of every minute (**Figure 2.4**).

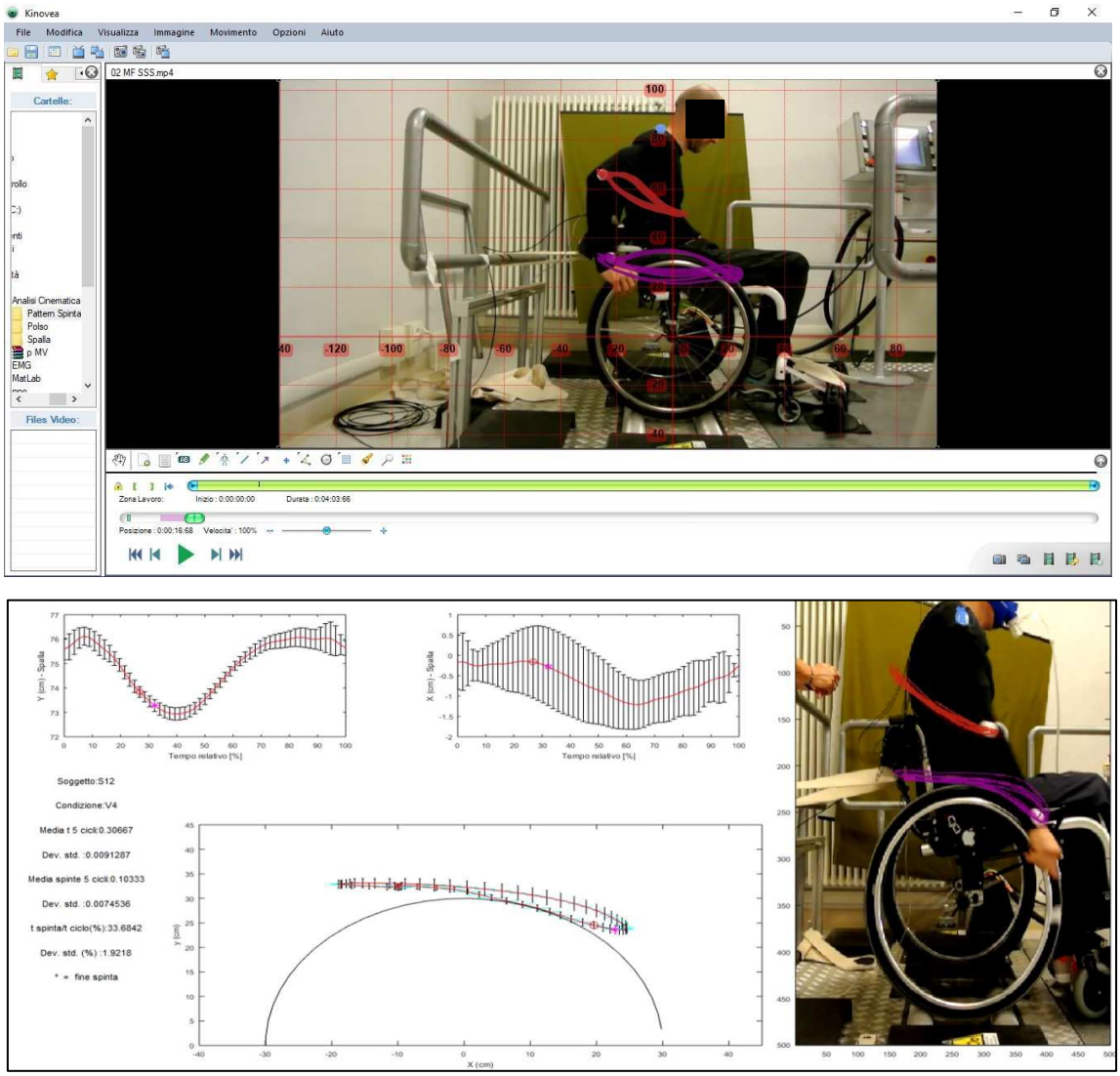

Figure 2.4. Kinematic data analysis using *Kinovea video analysis software* and *Matlab.*

A computer program was created (Matlab, Mathworks Inc., MA, USA) to display all pattern plots through a digital filtering process, in order to smooth the data and hence remove high-frequency noise induced by the digitizing process [39].

Pattern plots were displayed at random to four researchers who classified them into one of the four types previously defined [40-43]: single loop (SL), identified by the hand rising above the handrim during the recovery phase; double loop (DL), identified by the hand rising first above the handrim and then crossing over and dropping under the handrim during the recovery phase; semicircular (SC), identified by the hand falling under the handrim during the recovery phase; arcing (ARC), identified by the hand following an arc along the handrim during the recovery phase (**Figure 2.5**).

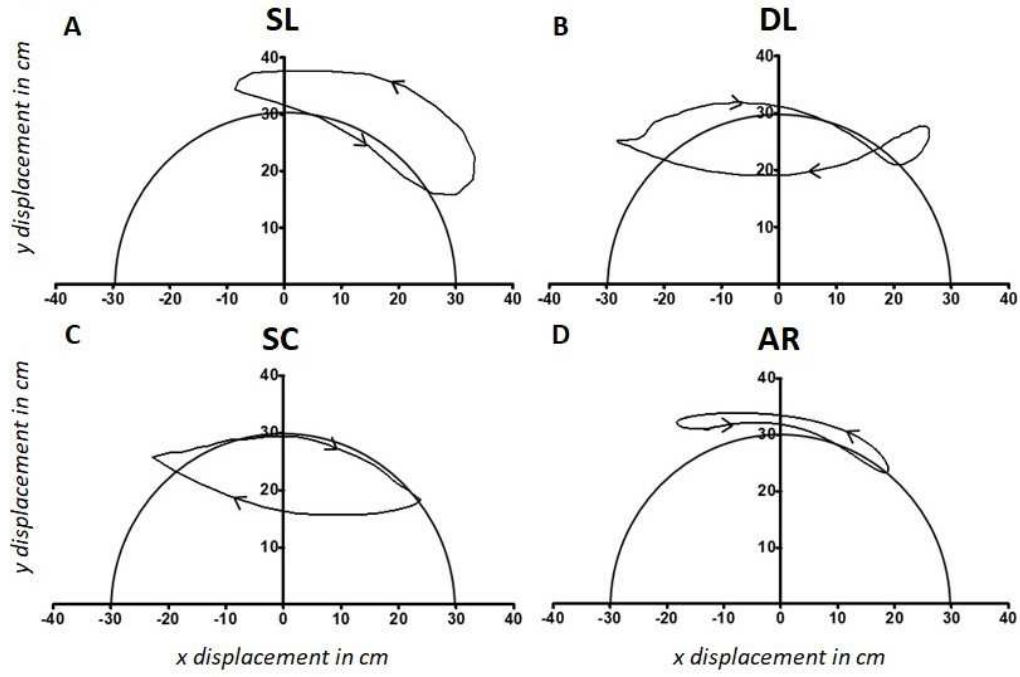

Figure 2.5. Four classic propulsion patterns identified from the hand motions of manual wheelchair users: (A) single loop (SL); (B) double loop (DL); (C) semicircular (SC); (D) arcing (ARC). The origin of the axis corresponds to the hub of the wheel. The dashed line is the path followed by the hand, and the arrows indicate the direction the hand moves.

A triaxial accelerometer (Biopac Systems Inc., Santa Barbara, CA, USA) was placed on subject's wrist and secured with tape to record forearm movements in three orthogonal directions, and synchronized with EMG to define stroke frequency and stroke cycles.

During the entire test, eight channels of surface Electromyography (sEMG) were recorded according to SENIAM (surface EMG for non-invasive assessment of muscles) protocol.[44]
Pre-gelled disposable Ag/AgCl electrodes were placed with fixed electrode distance of 20 mm on the following muscles (**Figure 2.6, 2.7**):

- Deltoid anterior (DA);
- Deltoid medial (DM);
- Deltoid posterior (DP);
- Triceps brachii (TB);
- Biceps brachii (BB);
- Pectoralis major (PM);
- Serratus anterior (SA);
- Supraspinatus (SSP).

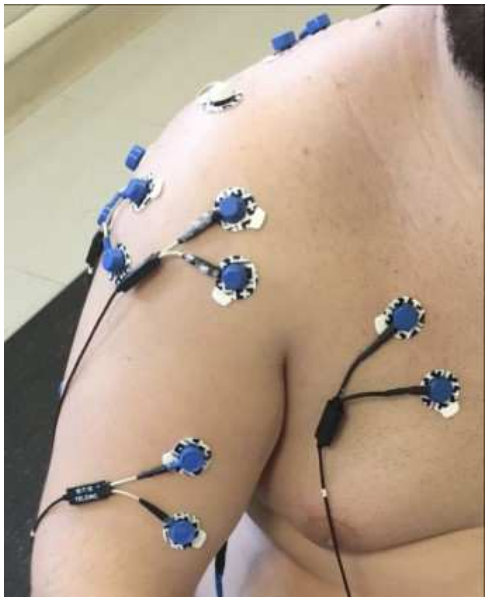

Figure 2.6. Positioning of sEMG electrodes.

Figure 2.7. Pre-gelled disposable Ag/AgCl electrodes

The sEMG signal has been recorded with BIOPAC System MP100 (Biopac Systems Inc., Santa Barbara, CA, USA) and analyzed using *AcqKnowledge* software version 3.7.2 (**Figures 2.8, 2.9**).

Figure 2.8. BIOPAC System MP100 (Biopac Systems Inc., Santa Barbara, CA, USA)

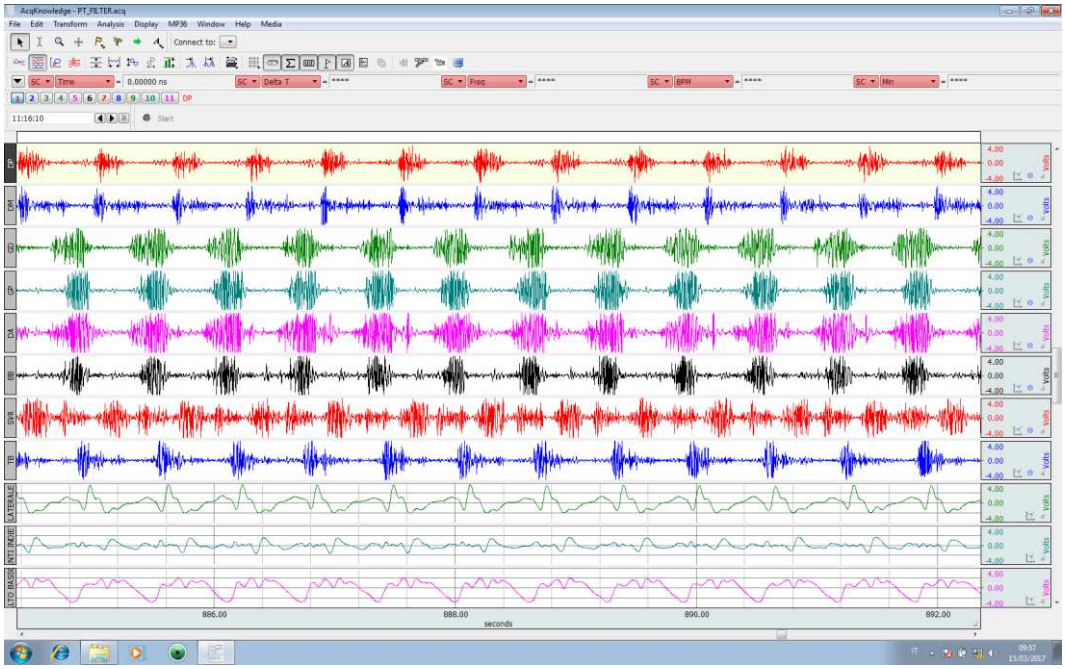

Figure 2.9. sEMG signal acquired using AcqKnowledge software version 3.7.2 and BIOPAC System MP100 (Biopac Systems Inc., Santa Barbara, CA, USA)

The EMG signals were collected during the entire test, but only the first 10 and the last 10 strokes of every minutes have been taken into consideration for the EMG analysis. EMG was used to calculate onset and offset timing of muscle activation. Stroke cycles have been defined through the synchronization with the triaxial accelerometer. Next, through signaling processing, Root Mean Square (RMS) values have been calculated.

Statistical analyses

Results were expressed as mean values ± standard deviation (SD). Comparisons between two groups were performed by two-sided Student's t-test. Comparisons between more than two groups were performed by one-way ANOVA; a Bonferroni's post-hoc test was used when significant differences emerged at ANOVA. Data fitting by linear regressions was performed by the least-squared-residuals method. The level of significance was set at $P<0.05$. Statistical analyses were performed by a software package (GraphPad Prism v. 5.0, GraphPad, CA, USA).

Results

Individual values during MS of velocity of wheelchair propulsion, $\dot{V}O_{2peak}$ and HR_{peak} obtained in T and in P are reported as a function of lesion level in **Figure 2.10**. A statistically significant linear relationship was observed for all variables. In other words, with higher lesion levels lower values of velocity, $\dot{V}O_{2peak}$ and HR_{peak} were found.

The horizontal dashed lines in the Figure represent the mean values obtained in T, P and CTRL. Velocity was significantly higher in CTRL (7.2 ± 2.1 km h^{-1}) and in P (7.0 ± 1.5) than in T (4.0 ± 1.2); no significant differences were observed between CTRL and P. The same conclusions can be drawn for $\dot{V}O_{2peak}$ (22.4 ± 6.7 ml kg^{-1} min^{-1}, 19.0 ± 5.9 and 10.6 ± 3.9, respectively, in CTRL, P and T) and HR_{peak} (167.1 ± 13.5 beat min^{-1}, 161.3 ± 12.7 and 117.5 ± 25.4, respectively, in CTRL, P and T).

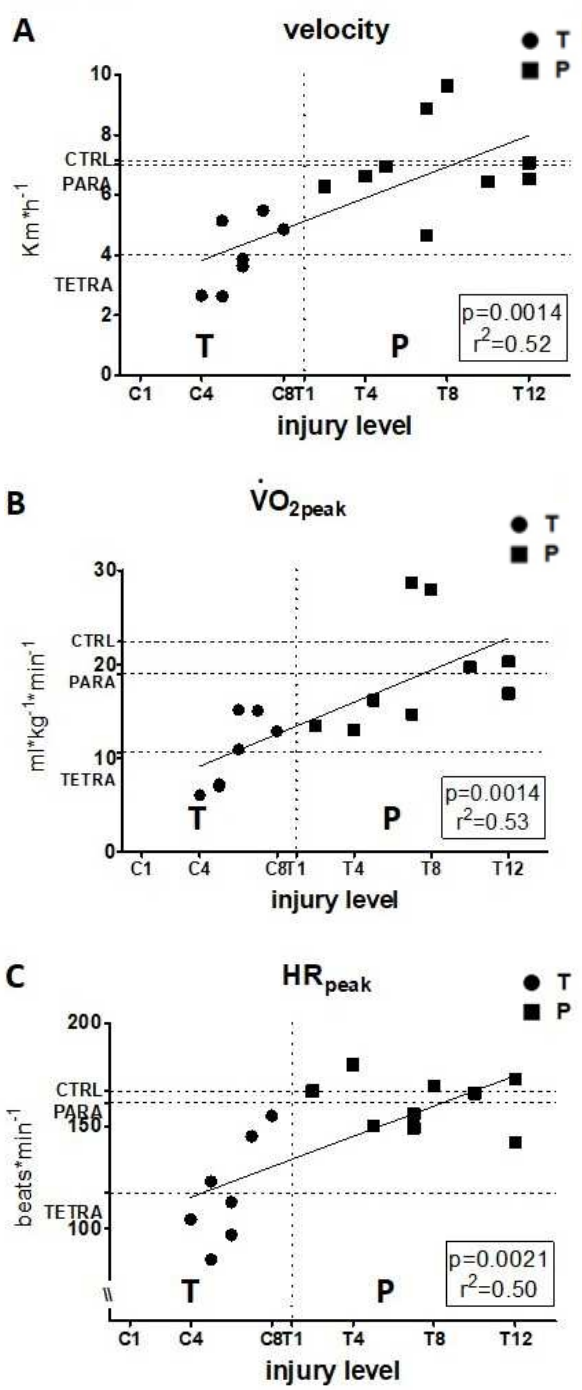

Figure 2.10. Data as a function of injury level: (A) Velocity (km/h); (B) V̇O2peak (mL kg^{-1} min^{-1}); (C) HRpeak (beats/min). Horizontal dashed lines indicate mean values for T, P and CTRL. The dotted vertical line divides the patients into T and P.

On the other hand, no significant relationship was observed between the peak O_2 cost of wheelchair propulsion and lesion level (**Figure 2.11**). In the Figure, the peak O_2 cost is expressed as J kg^{-1} m^{-1} (left y axis) and as ml O_2 kg^{-1} m^{-1} (right y axis). From the Figure it appears that, with the possible exception of the two T patients with the highest lesion levels, the peak O_2 cost was independent of lesion level. Mean values (indicated by the horizontal dashed lines) were not significantly different between the 3 groups.

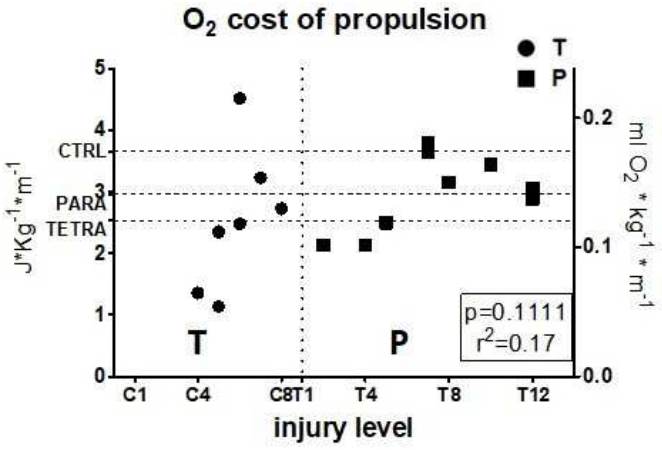

Figure 2.11. O_2 cost of wheelchair propulsion expressed as J kg^{-1} m^{-1} (left y axis) and as ml O2 kg^{-1} m^{-1} (right y axis) at peak velocity as a function of injury level. Horizontal dashed lines indicate mean values for T, P and CTRL. Dotted vertical line divides the patients into T and P.

Group mean (± SD) data (calculated every minute) of velocity of wheelchair propulsion (panel A), $\dot{V}O_2$ (panel B), HR (panel C), obtained in T and P during the 4 minutes at self-selected speed (SSS) and the subsequent 4 minutes of maximal speed (MS) are shown in **Figure 2.12**. For CTRL, the data obtained at the same velocity of MS in T (CTRL-T) and in P (CTRL-P) are also shown in the Figure (dashed lines), together with the data obtained for CTRL at their own MS. For the statistical analyses, a single x ±SD value was calculated for the different variables during the 4 minutes of SSS and MS.

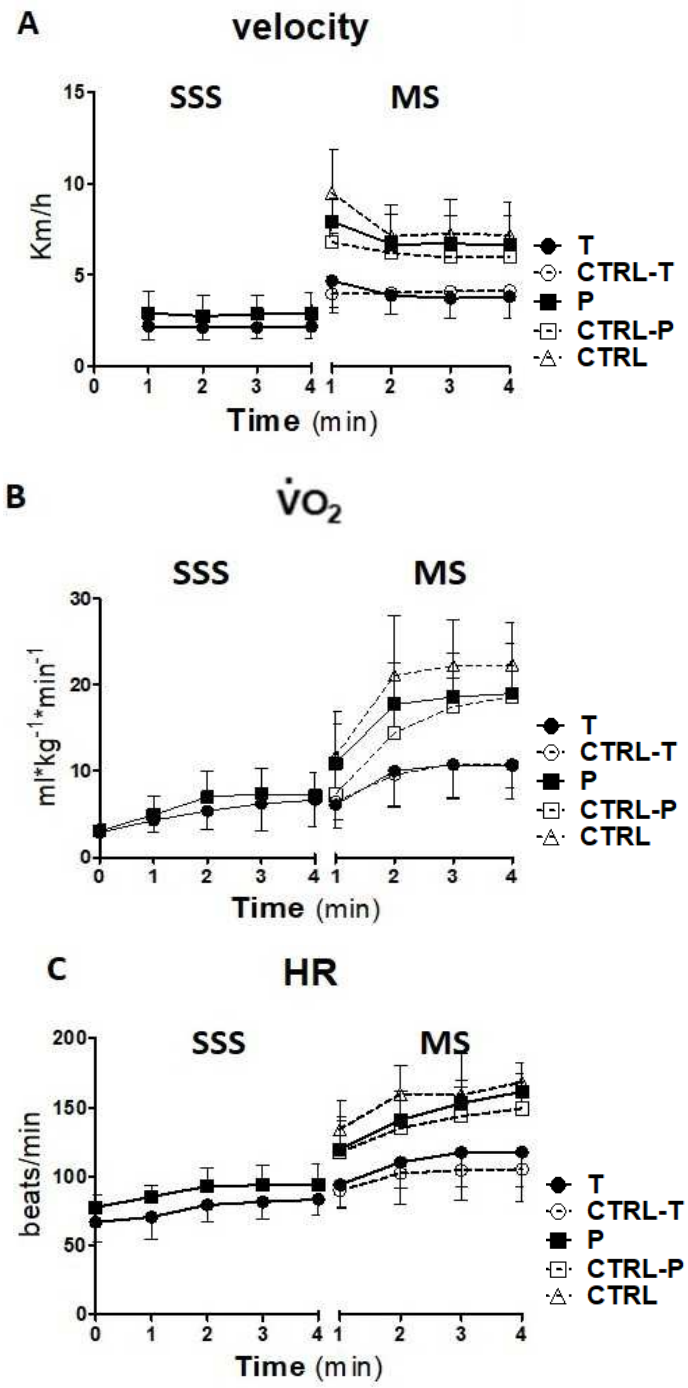

Figure 2.12. Group mean (±SD) data, calculated every minute as a function of time during the 4 minutes at self-selected speed (SSS) and the subsequent 4 minutes of maximal speed (MS): (A) velocity (km h^{-1}); (B) $\dot{V}O_2$ (mL kg^{-1} min^{-1}); (C) HR (beats min^{-1}). Full symbols and plain lines indicate T (circles) and P (squares) mean values in every minute of exercise, while empty symbols and dashed lines indicate CTRL mean values.

The Figure shows that during SSS all variables were not significantly different in T and in P. Panel A also demonstrates that the experimental protocol was successfully followed: MS in T and in P were substantially identical to those in CTRL-T and CTRL-P, respectively. In both T and P velocities in MS were significantly higher than those in SSS. As a consequence of this, in T and in P also $\dot{V}O_2$ and HR values were higher in MS vs. SSS; in T the difference did not reach statistical difference. During MS, velocity, $\dot{V}O_2$ and HR were higher in CTRL vs. T, and in P vs. T, whereas no significant differences were observed between CTRL and P.

Homologous data of those described in Figure 2.11 are shown in **Figure 2.13** for the O_2 cost of wheelchair propulsion. Interestingly, once matched for velocity the O_2 cost (aerobic energy expenditure per unit of covered distance) was not significantly different in T vs. CTRL-T, nor in P vs. CTRL-P.

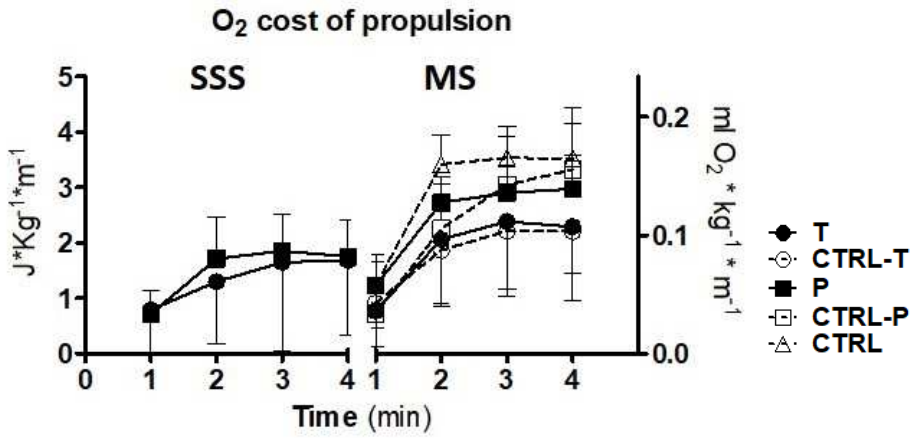

Figure 2.13. Group mean (±SD) data (calculated every minute) of O_2 cost of wheelchair propulsion expressed as J kg^{-1} m^{-1} (left y axis) and as ml O_2 kg^{-1} m^{-1} (right y axis) as a function of time during the 4 minutes at self-selected speed (SSS) and the subsequent 4 minutes of maximal speed (MS). Full symbols and plain lines indicate T (circles) and P (squares) mean values in every minute of exercise, while empty symbols and dashed lines indicate CTRL mean values.

Stroke frequency data are presented in **Figure 2.14** as a function of lesion level and as group mean values as a function of time. Also in this Figure the horizontal dashed lines indicate the mean values in the 3 groups. Panel A shows that data were linearly related with lesion level; values in T were significantly lower than those in P or in CTRL; no statistically significant difference was found between the last two groups. Panel B shows that by matching the velocity in CTRL-T to that of T (MS), and in CTRL-P to that of P (MS) also stroke frequency was not significantly different. Similarly, to the variables previously described, no differences were observed between T and P at SSS.

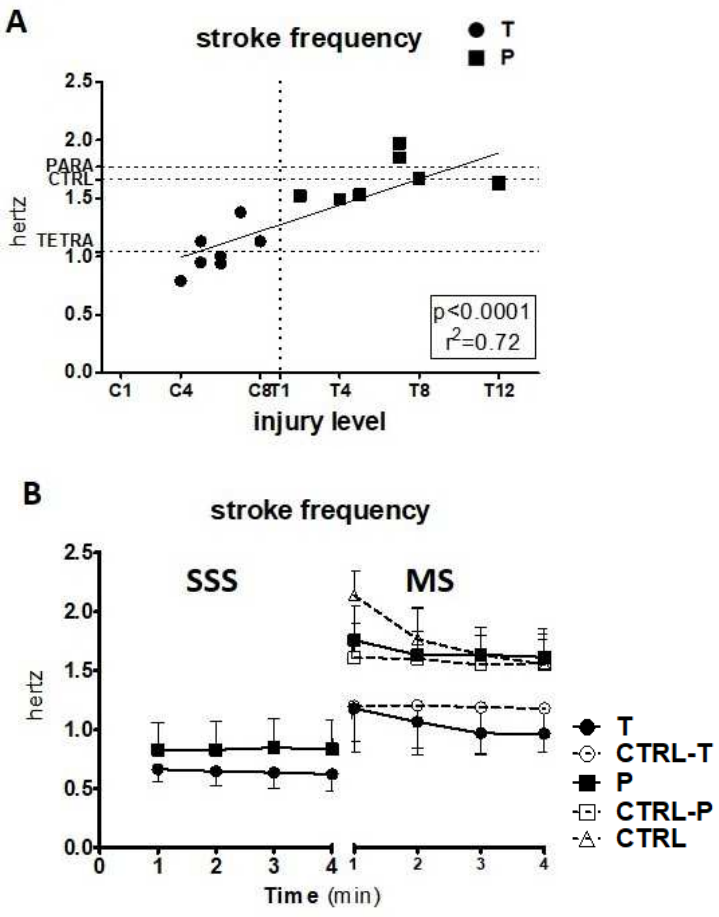

Figure 2.14. (A) Mean stroke frequency data as a function of lesion level; (B) group mean (±SD) data (calculated every minute) as a function of time.

Stroke frequency as a function of velocity is shown in **Figure 2.15**. Data are linearly related, showing that patients who use lower stroke frequencies reach lower velocities.

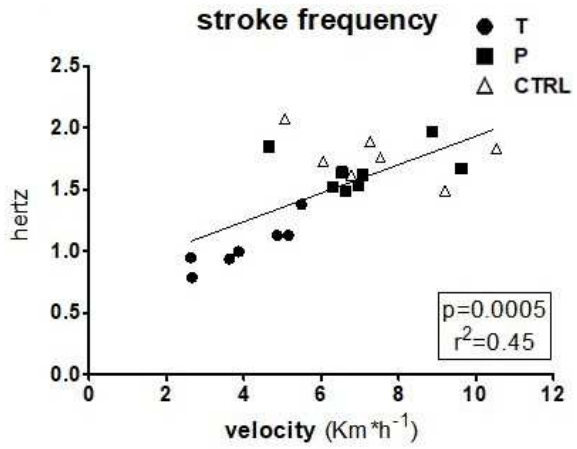

Figure 2.15. Mean stroke frequency data as a function of velocity in patients and controls.

Figure 2.16 shows that at SSS propulsion patterns were very similar in the two groups: patients of both groups used predominantly DL and SC, whereas only a minority of patients of both groups used AR.

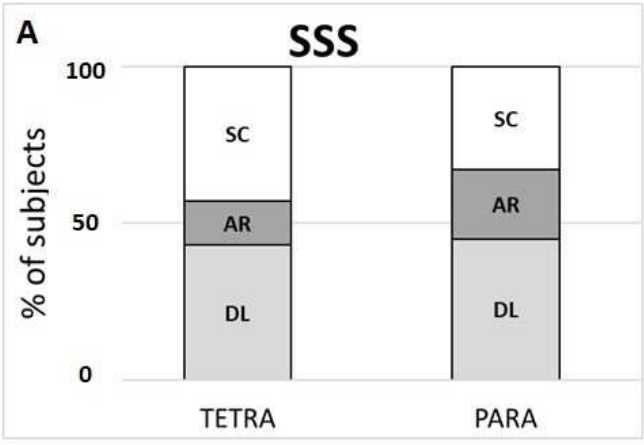

Figure 2.16. Pattern distribution in % in T and in P at

However, when examined at MS (**Figure 2.17**), the propulsion pattern in T was very different from that of P, with a much greater use of DL by T.

Also, when comparing patients and CTRL at the same velocity, we observed different patterns between T at MS and CTRL at same velocity, with a much greater use of DL by T and of AR by CTRL (only one CTRL utilized DL and one SL). Less pronounced differences were observed between P at MS and CTRL at same velocity (only one CTRL utilized SC).

At their own MS, neither patients nor CTRL used SC. Both P and CTRL used only AR and SL contrarily to T, who mainly chose DL.

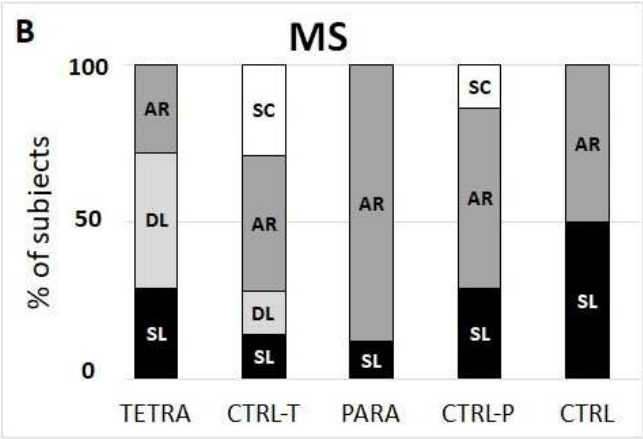

Figure 2.17. Pattern distribution in % in T, P and CTRL at MS.

Amplitude of muscle activation expressed in mV (mean values of the first 10 and last 10 strokes in the 4 minutes) recorded by sEMG, as a function of time (expressed in % of stroke cycle duration) is shown in the next Figures. The muscles mainly involved in the pushing phase are shown in panels on the left-side (biceps brachii, deltoid anterior, pectoralis major and serratus anterior) plus the triceps brachii from the left panel. The three remaining muscles shown in the panels on the right-side (deltoid posterior, deltoid medial, supraspinatus) are the

muscles mainly involved in the recovery phase. The push phase corresponded approximately to 20-35% of the stroke cycle, and the recovery phase to 65-80%. Mean values of muscle activation timing during SSS (blue line) and MS (green line) in T (Panel A) and in P (Panel B) are displayed in **Figure 2.18**.

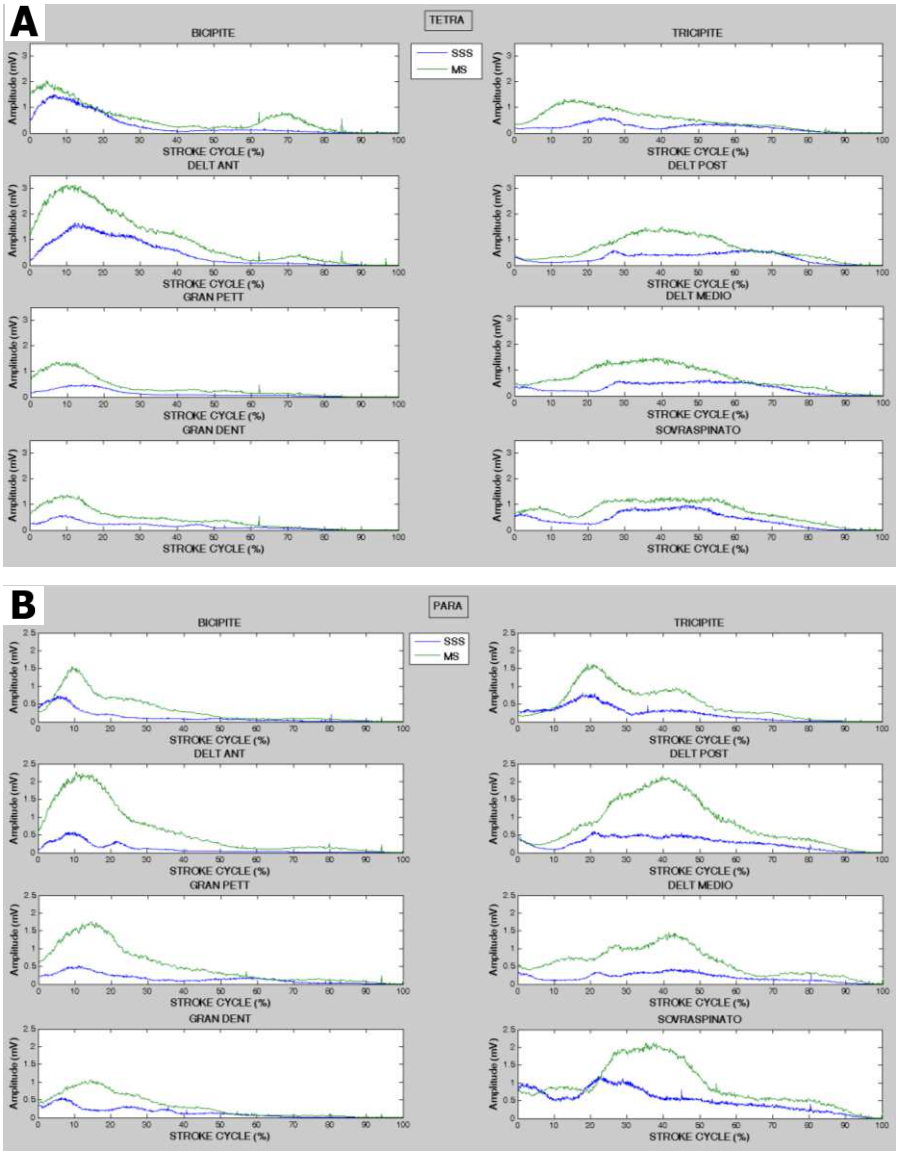

Figure 2.18. Mean values of muscle activation (mV) in T patients at SSS and at MS (A) and in P patients at SSS and MS (B).

In the T group, muscle recruitment order is as follows: BB, DA, PM, SA, TB, DM, DP and SSP. The BB and TB are active during almost the whole push phase: the BB is mainly involved in the first part, and the TB during the second part of the push phase. DA and PM are the muscles mainly involved in the push phase, with a peak at about 15% of the stroke cycle. Interestingly, the DA is activated until about 50% of the stroke cycle. During the recovery phase, the active muscles are DP, DM and SSP, with a light activation of the TB.

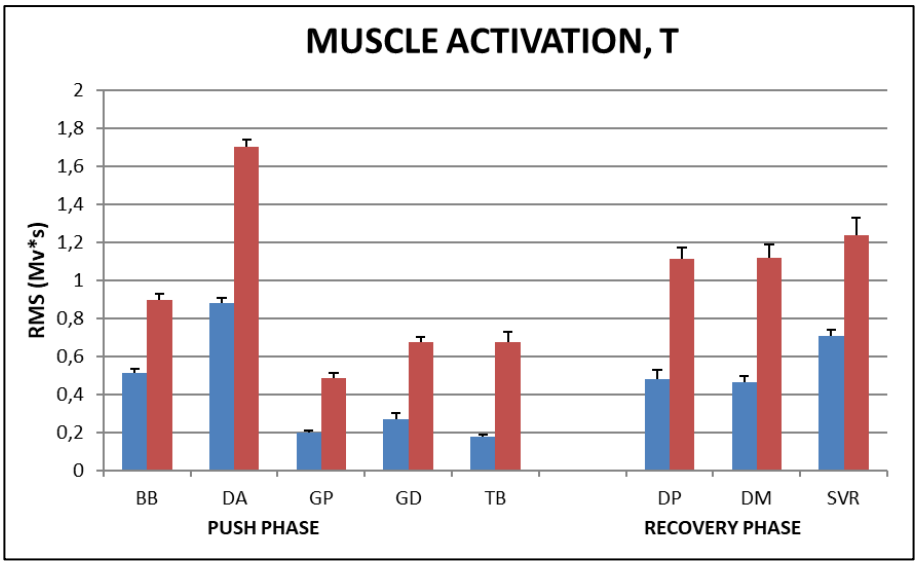

Figure 2.19. Muscle activation in T, during SSS (blue) and during MS (red).

A major activation can be observed for all muscles during MS compared to SSS. Except for DA, recovery muscles develop a higher activation compared to the pushing muscles during MS. The DA is the muscle with the greater activation, in synergy with BB and TB, and also the one that absorbs the energy before starting the recovery phase, in synergy with the DM, due to a compensatory mechanism of the functional deficit of the TB, the trunk and hand muscles.

In the P group, muscle activation order appears slightly different compared to T. In fact, muscles are recruited as follows: BB, DA, SA, PM, TB, DP, DM and SSP. Similarly to T, the BB and TB are active during almost the whole push phase, and the BB is mainly involved in the first part, while the TB during the second part of the push phase. DA and PM are the muscles mainly contributing to the push, having an activation peak around the 12-15% of the stroke cycle. During the recovery phase, the DP is the most active, together with DM, SSP and a partial activation of the TB as observed in T.

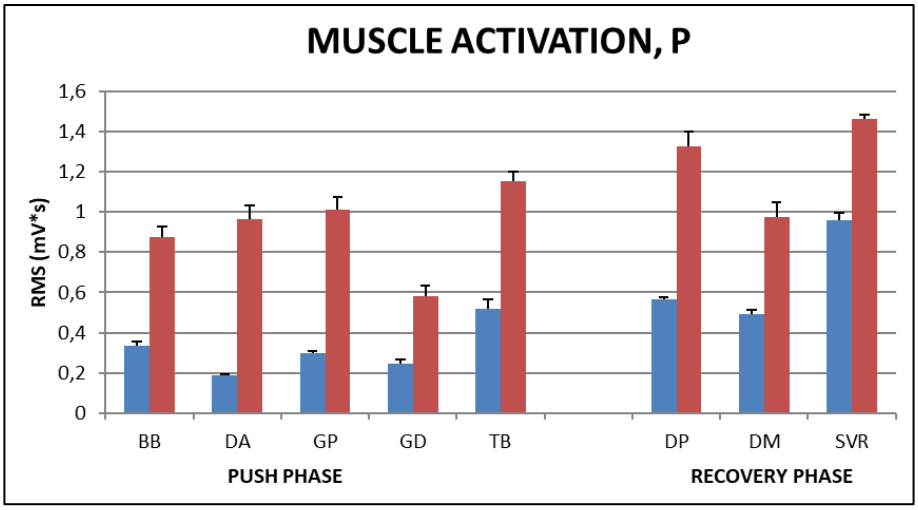

Figure 2.20. Muscle activation in P, during SSS (blue) and during MS (red).

As expected, also in P a major activation can be observed for all muscles during MS compared to SSS. Recovery muscles develop a higher activation compared to the pushing muscles during both SSS as well as MS. During the recovery phase, muscles alternate to produce and absorb energy: shoulder extension muscles (DM and DP) and elbow flexor (BB) absorb the energy at the beginning of the recovery, to decelerate the arm, and simultaneously the DP has to accelerate the arm backwards, performing a double task.

Figures 2.21 and **2.22** show muscle activation timing respectively in T and in P patients at SSS (Panel A) and at MS (Panel B). Activation pattern was heterogeneous in the T group, mostly at SSS. The push phase corresponded to ~35% of stroke cycle at SSS and to ~30% at MS.

Similarly to T, also in P some differences can be observed between patients at SSS, but timing of activation at MS was more similar between patients if compared with T at MS. Also in P, the push phase corresponded to ~35% of stroke cycle at SSS and to ~30% at MS.

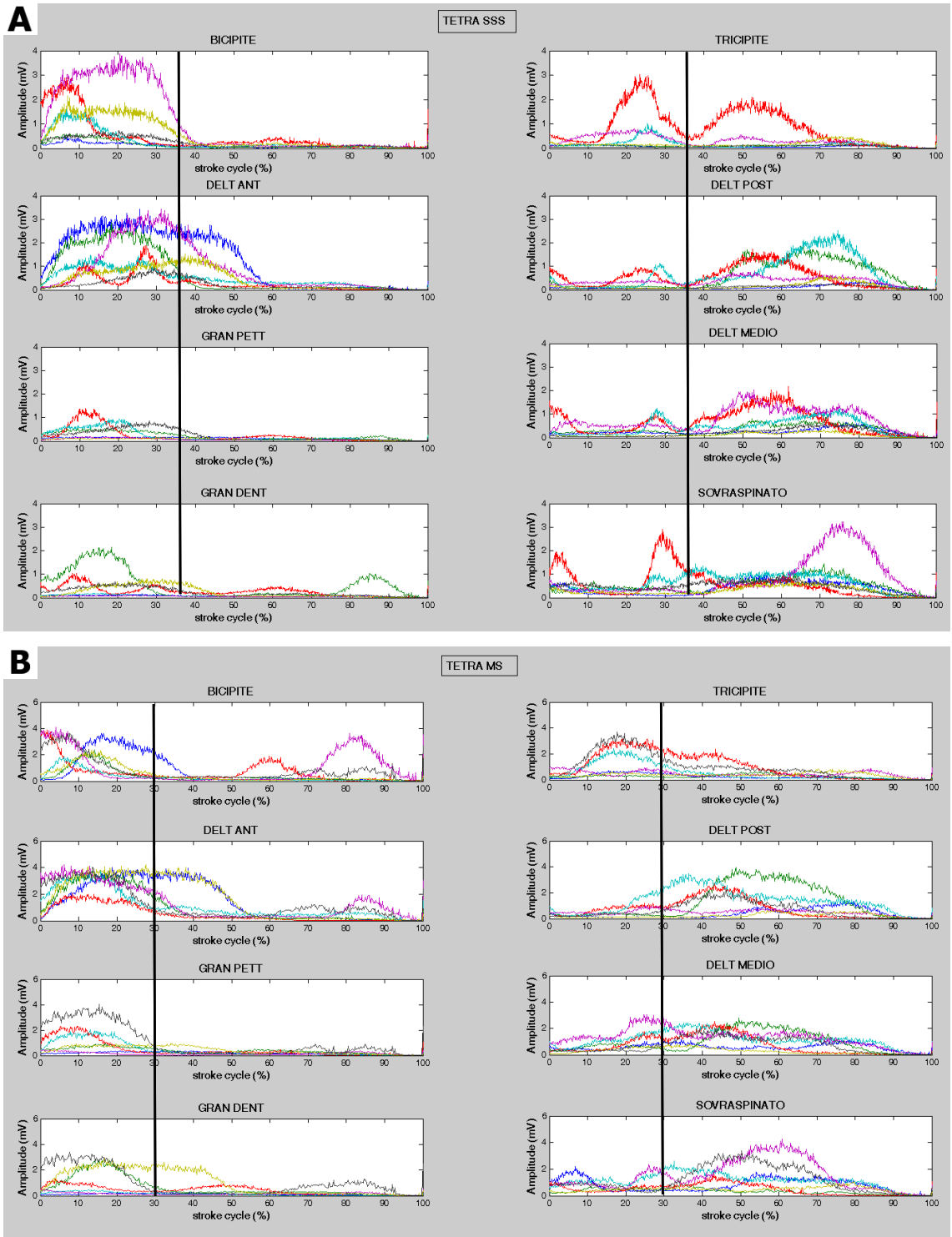

Figure 2.21. Muscle activation (mV) in T patients at SSS (A) and at MS (B).

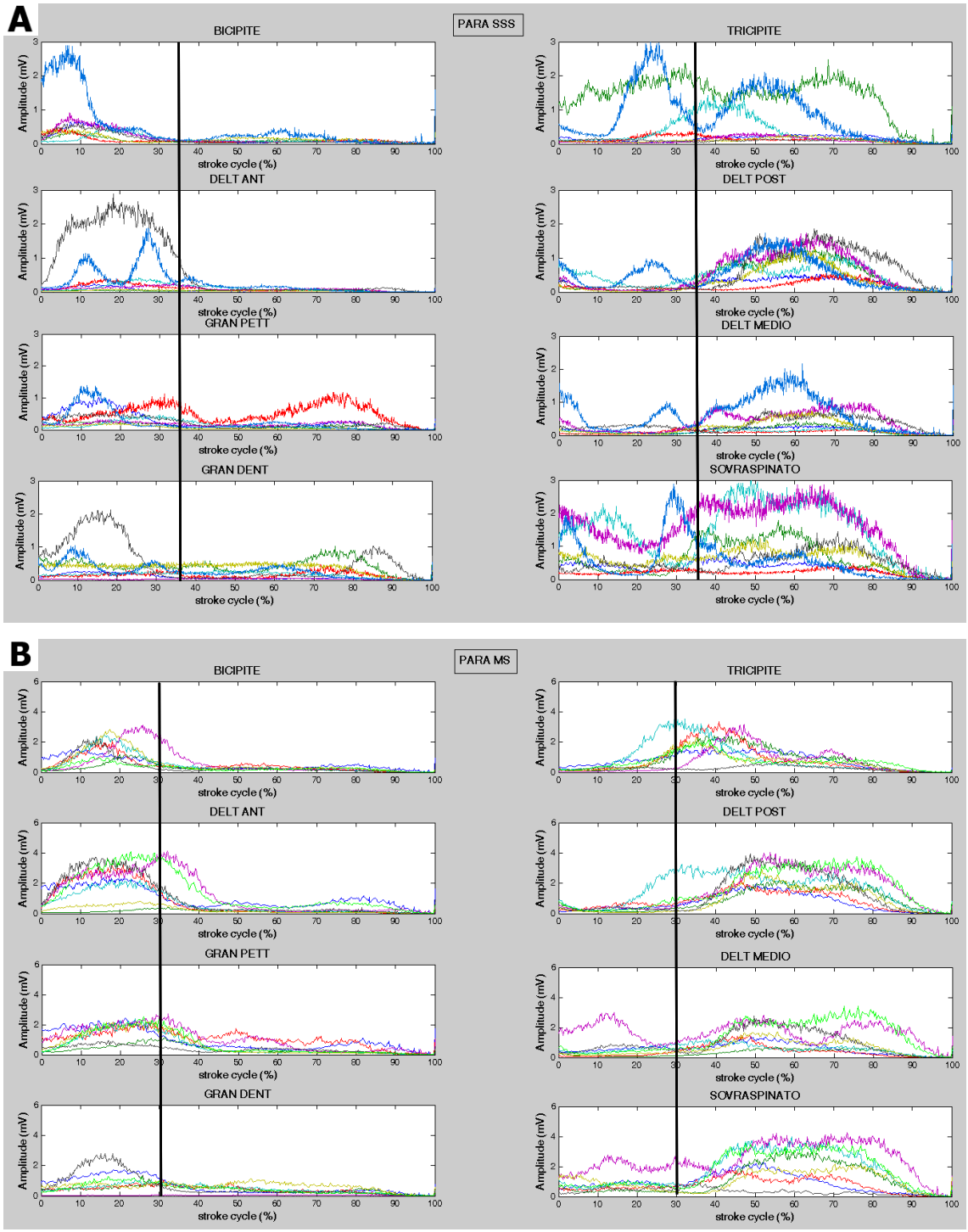

Figure 2.22. Muscle activation (mV) in P patients at SSS (A) and at MS (B).

When comparing T and P patients (respectively blue and red line) to CTRL (green line) at same velocity (**Figure 2.23**), we observed differences in muscle activation timing both between T and CTRL as well as between P and CTRL.

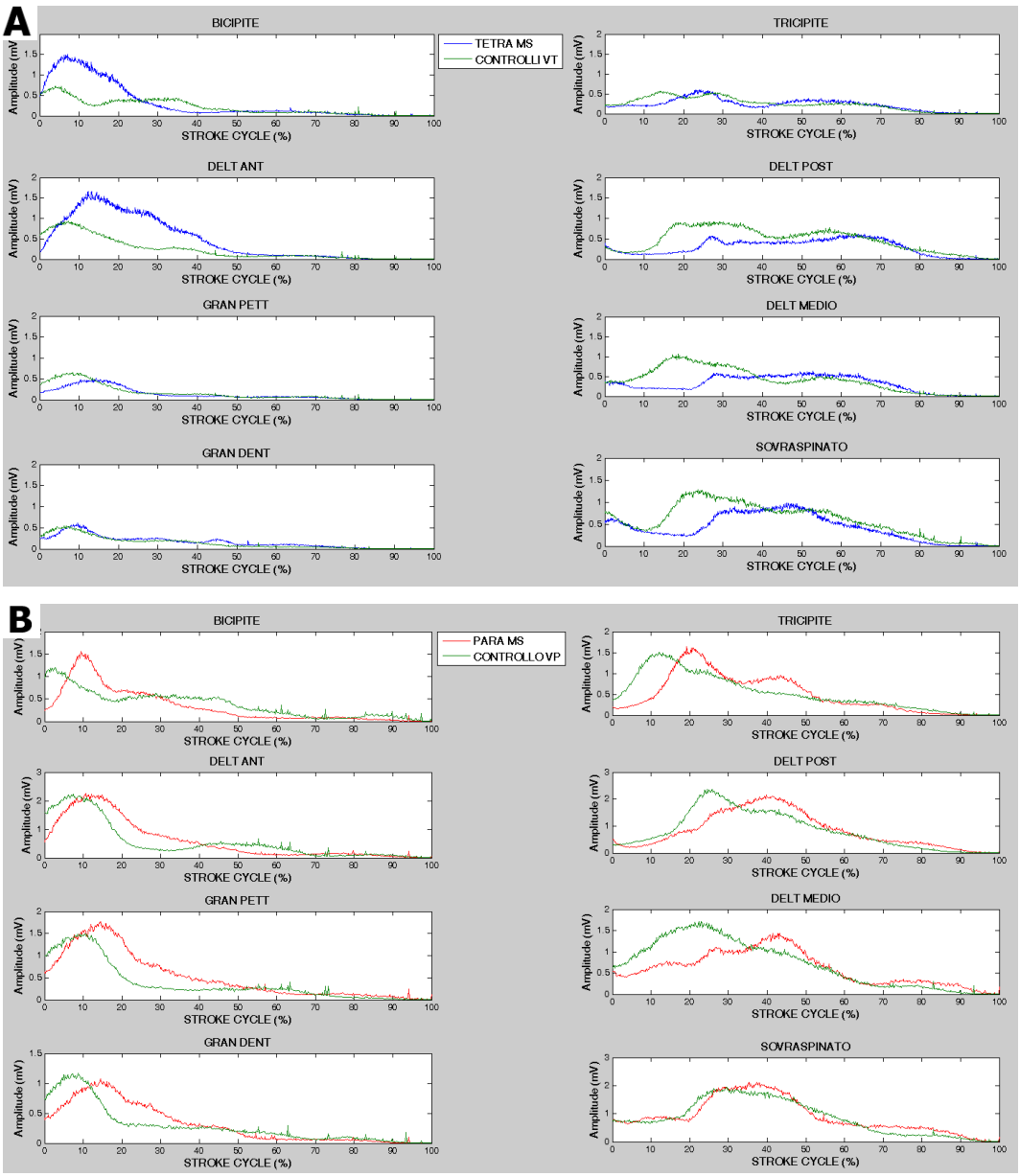

Figure 2.23. Mean values of muscle activation (mV) in T at MS vs. CTRL at CTRL-T (A) and in P at MS vs. CTRL at CTRL-P (B).

Muscle activation patterns in CTRL subjects at CTRL-T (Panel A) and CTRL-P (Panel B) are shown in **Figure 2.24**.

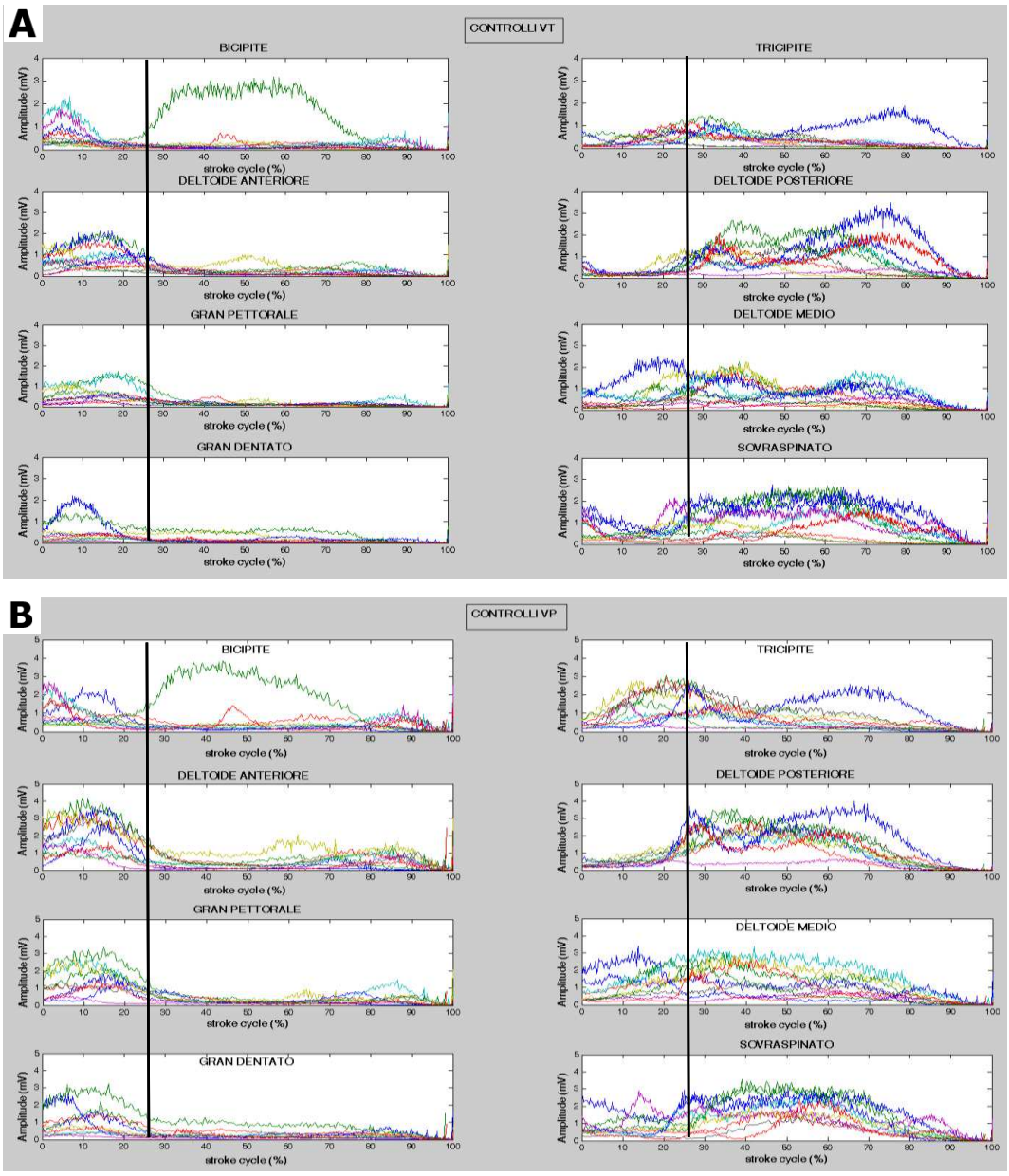

Figure 2.24. Muscle activation (mV) in CTRL at CTRL-T (A) and at CTRL-P (B).

The push phase corresponded to ~25% of stroke cycle in both conditions, being ~10% shorter compared to patients. If examined at their own MS, push phase in CTRL was even shorter than those observed at CTRL-T and CTRL-P, being ~20% of stroke cycle as shown in **Figure 2.25**.

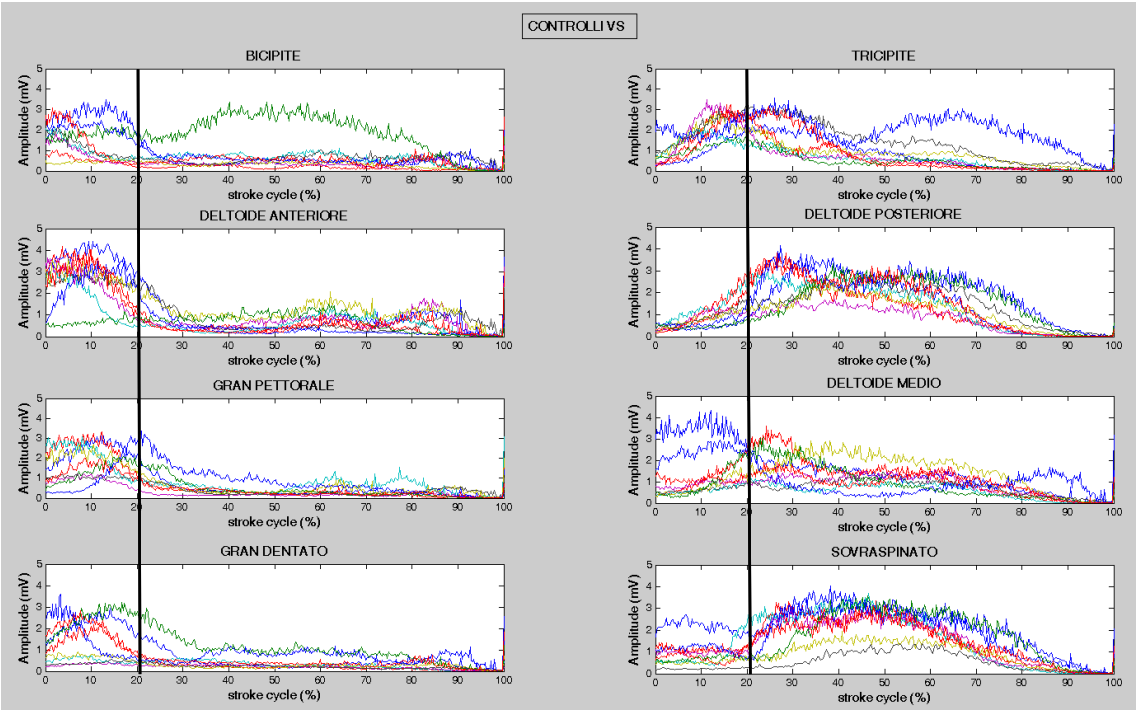

Figure 2.25. Muscle activation (mV) in CTRL at their own MS.

Mean values of muscle activation during the stroke cycle are shown in **Figure 2.26** and support the results previously described in terms of activation timing.

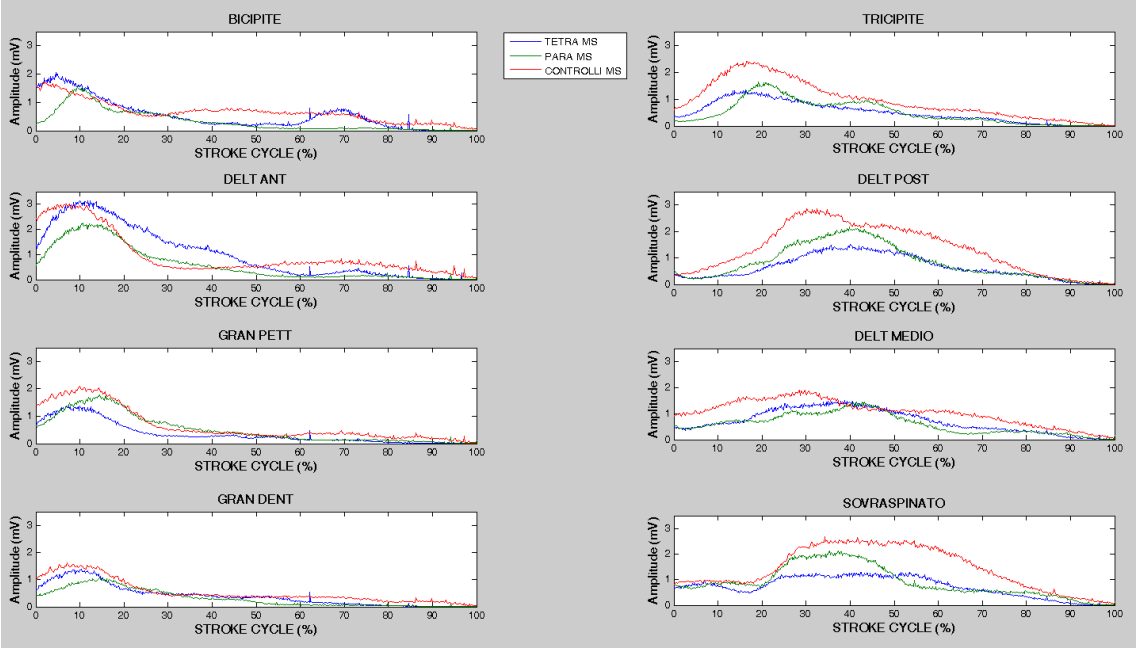

Figure 2.26. Mean values of muscle activation (mV) in CTRL subjects at CTRL-T, at CTRL-P and at their own MS.

In **Figure 2.27**, we can observe that at higher velocities, muscle recruitment trend doesn't change. Nevertheless, also in CTRL, similarly to patients, recovery muscles are the mostly active, and all the muscles show a higher activation during higher velocities. Also, during the pushing phase, the DA is the muscle with a higher activity, followed by TB, BB, PM and SA.

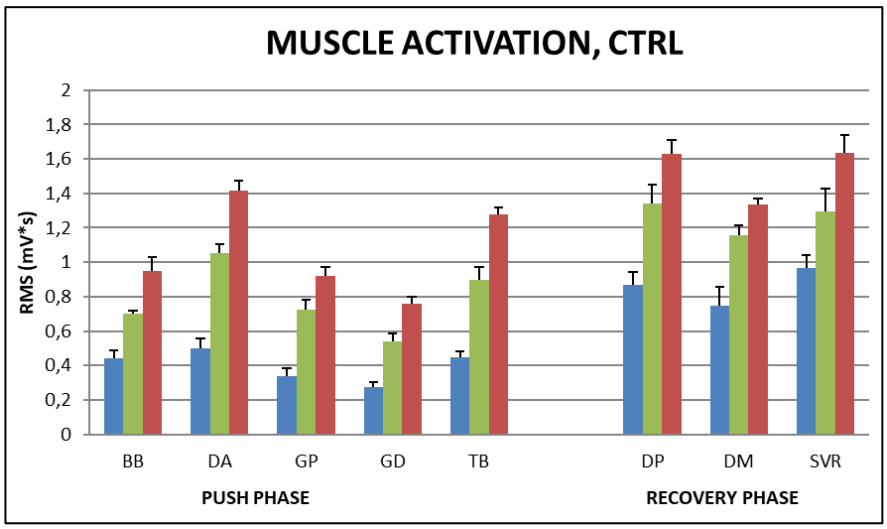

Figure 2.27. Muscle activation in CTRL, at CTRL-T velocity (blue), CTRL-P velocity (green) and at their own MS (red).

Furthermore, we analized the activation trend during the 4 minutes of every trial. Muscle activation as a function of time during the 4-min test is shown in the next Figures.

Figure 2.28 shows mean values of RMS (Mv s^{-1}) as a function of time during the 4 minutes of every trial at MS, comparing T, P and CTRL: No significant differences have been observed in the trend of muscle contraction during the 4-min trials, except for the fact that CTRL seem to maintain the activation constant or with a slightly increase during the 4 minutes.

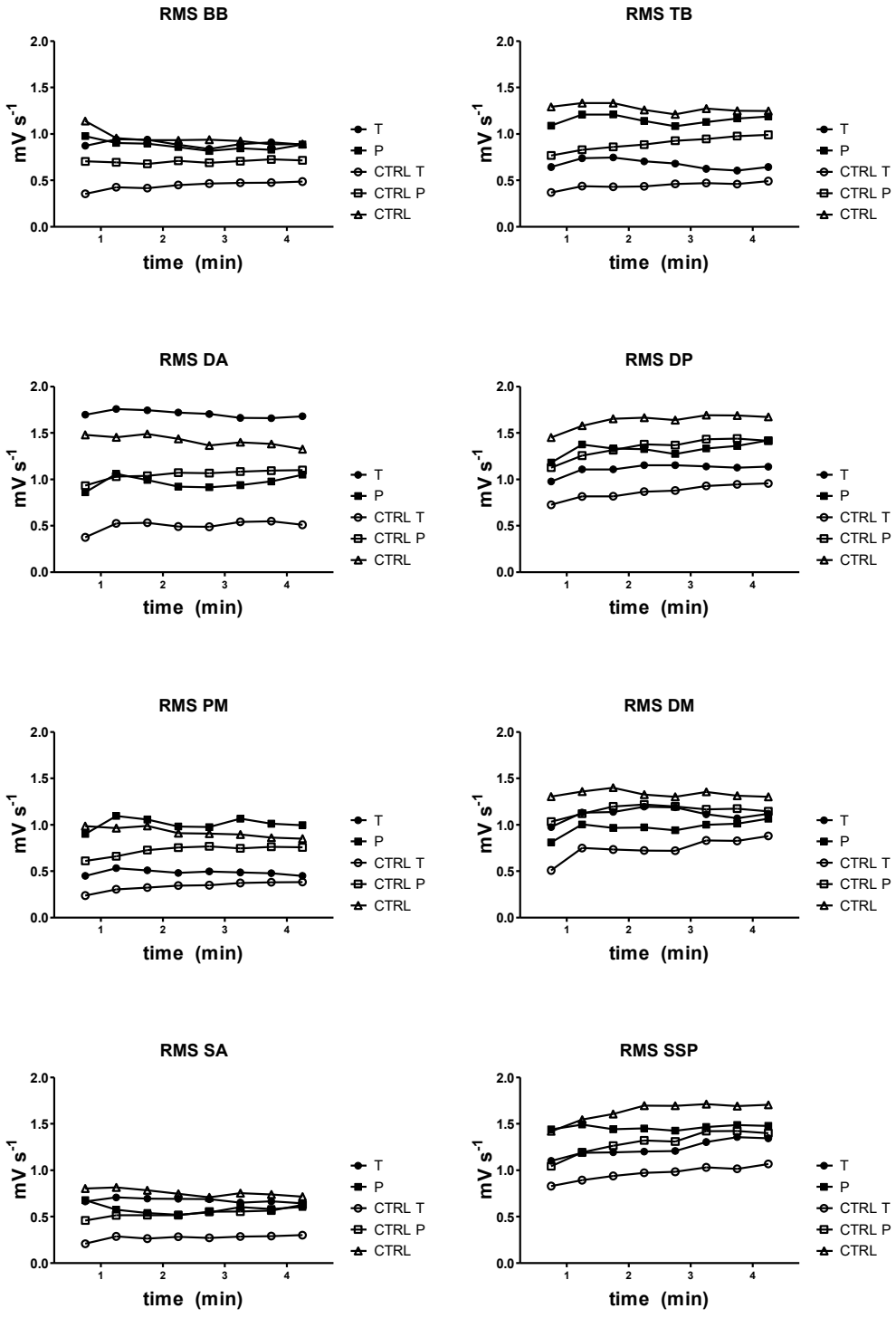

Figure 2.28. Muscle activation in T, P and CTRL as a function of time, during the 4-min trial at MS.

As an example, activation of BB during the 4-min trial are shown in **Figure 2.29**, comparing SSS and MS in T and in P, and CTRL at their three velocities.

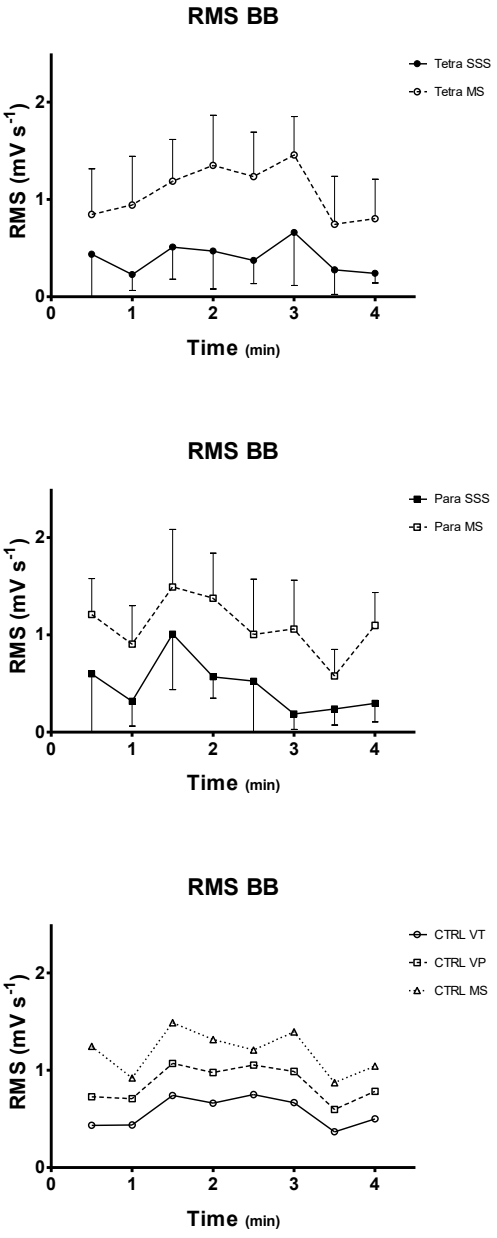

Figure 2.29. Activation of BB in T, P and CTRL.

It is evident, that a higher velocity generally corresponds to a higher muscle activation. Interestingly, if comparing patients to CTRL at the same velocity, the results clearly change: while T have a higher muscle activation in comparison to CTRL-T, data for P and CTRL-P are quite similar.

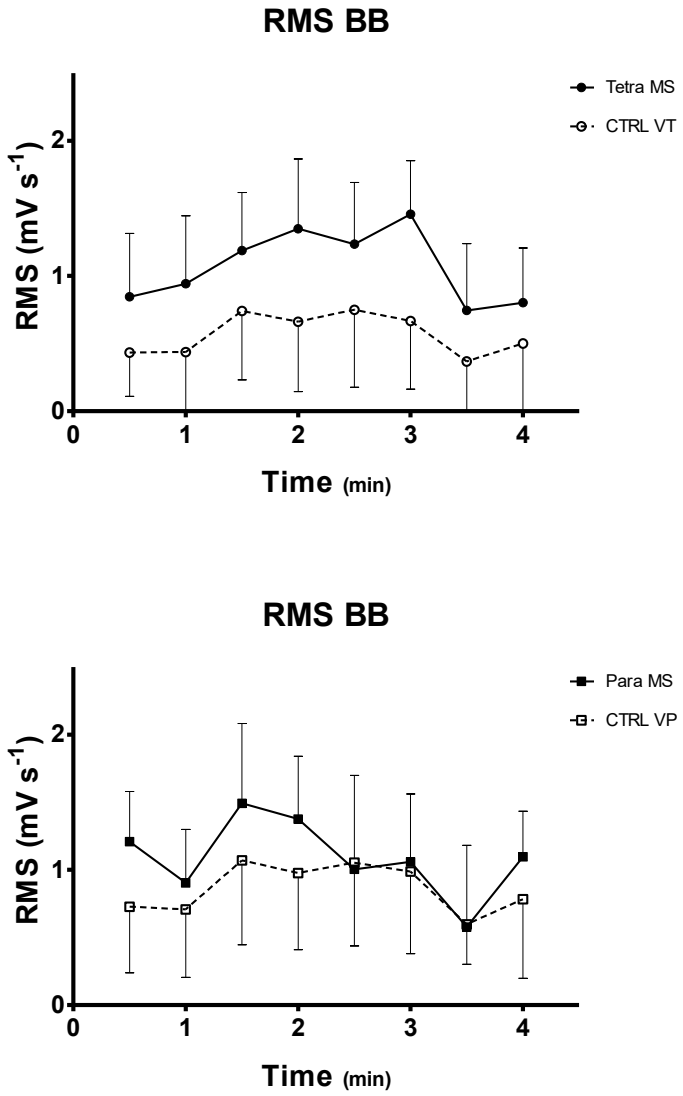

Figure 2.29. Activation of BB in T, P and CTRL.

Discussion

The main observation of the present study was that, when investigated at the same velocity, the O_2 cost of wheelchair propulsion was not significantly different in T and P SCI patients compared to CTRL. This occurred despite the very different propulsion pattern in the patients (particularly in T) *versus* that observed in CTRL. In other words, despite the SCI and the presumably impaired activation pattern of the muscle of the upper limbs, which translated into an altered propulsion pattern, and despite the impaired cardiovascular (in patients with lesions above T6) and muscular adjustments to exercise which are known to occur in these patients [3, 14-16, 21], the O_2 cost of propulsion was unaltered compared to the controls. This remarkable observation implies that the altered activation and propulsion patterns in T and P SCI patients, together with the cardiovascular and muscular impairments, do not significantly affect the oxidative economy (O_2 cost) of wheelchair propulsion. Besides being of interest from a basic science point of view, these findings are of interest in terms of exercise prescription and the functional evaluation in these patients.

It should be remembered that in the present study no resistance was put on the rollers of the ergometer, in order to simulate as closely as possibly the propulsion during activities of everyday life. The rollers system offered a rolling resistance similar to that on natural surfaces; this resistance is made up by several factors, including the mass of the subject and of the wheelchair, the weight distribution between the front casters and the rear wheels, the size and type of tires and the surface on which the wheelchair is used [45]. As a note of caution, therefore, it should be recognized the above-mentioned observations could apply only to the

adopted exercise protocol, and not to exercise protocols in which increasing resistances are applied to the rollers.

In the present study, only oxidative metabolism was investigated. An evaluation of the contribution of the other bioenergetics mechanisms (anaerobic glycolysis-glycogenolysis, phosphocreatine splitting) would require assumptions, invasive measurements, or measurement protocols incompatible with wheelchair propulsion. A throughout discussion of these concepts goes beyond the purposes of the present study. In any case, instead of the "energy cost" of propulsion in the present study we discuss only of the "O_2 cost". This intrinsic limitation should be relatively minor, however, since relatively long (4 minutes) exercises were investigated, in which the contribution of oxidative metabolism to the overall energy expenditure is overwhelming.

The unchanged O_2 cost of wheelchair propulsion observed in T or P SCI patients does not obviously mean that SCI did not affect performance during wheelchair propulsion. Maximal velocity of propulsion (as well as VO_2peak and HRpeak) were indeed, as expected [3, 24, 46-48], linearly related to lesion level. In other words, higher lesion levels were associated with more pronounced impairments.

All T and P patients completed the 4 minutes at their maximal speed, which was significantly higher in P than in T, whereas it was not significantly different between P and CTRL. The self-selected speed, which was presumably close to the speed normally utilized by the patients during everyday life, was not different in T and P. In T the self-selected speed was about 50% of the patients' maximal speed, whereas in P this percentage was about 40%. This suggests that both T and P, at their self-selected speed, have a significant functional reserve. It is also noteworthy that the maximal performance in P was not significantly different from that in CTRL.

The results obtained by Goosey et al. [30] indicate that in SCI patients the freely chosen stroke frequency is the more economical. Thus, in the present study the patients were free to utilize their preferred stroke frequency. The peak values of this variable were also linearly related to the lesion level: higher lesion levels were associated with a lower peak stroke frequency. Moreover, peak stroke frequency was linearly related to peak velocity, with an r^2 value of about 0.50. Interestingly, data for CTRL lie very close to the regression line drawn for the patients. In other words, peak stroke frequency was presumably an important determinant of peak velocity, and the inability of T to reach elevated peak stroke frequencies presumably contributes significantly to their impaired peak performance.

During wheelchair propulsion different propulsion patterns can be chosen. In fact, during the pushing phase of the cycle the hand must follow the handrim, whereas during the recovery different patterns can be chosen. Four main pushing patterns have been described [22, 38, 40-43, 49]: single loop (SL), identified by the hand rising above the handrim during the recovery phase; double loop (DL), identified by the hand rising first above the handrim and then crossing over and dropping under the handrim during the recovery phase; semicircular (SC), identified by the hand falling under the handrim during the recovery phase; arcing (ARC), identified by the hand following an arc along the handrim during the recovery phase. Previous studies related to these patterns show controversial results, and no firm conclusions can be made in terms of which patterns are preferentially utilized by able-bodied subjects and by T or P SCI patients [22, 38, 40-43, 49]. Confounding factors related to methodological differences between studies (speed, slope, type of wheelchair, resistance applied to the wheels, preferred or imposed cadence, previous experience in wheelchair propulsion by the control

subjects, residual muscle function in patients, etc.) could explain, at least in part, these controversial results. Previous experience in wheelchair propulsion has shown to be related with a higher mechanical efficiency and lower energy expenditure at same propulsion velocities, in comparison with novice wheelchair users [32, 50, 51]. In previous studies [38, 40] SC pattern has been found to be related with a reduced push frequency and more time spent during the push phase in relation to the recovery phase. This would lead to a more efficient propulsion, and this pattern is therefore recommended by clinicians for everyday propulsion. Clinical guidelines [52] suggest indeed the use of SC pattern because of better biomechanics (the hand follows an elliptical pattern with no sudden changes in hand direction and extra hand movements). Nevertheless, during our trials, only at lower velocities (i.e. during SSS) a minority of patients used the SC pattern. Only a minority of CTRL subjects utilized SC at the higher speeds (see below). Controversial results have been described about the AR pattern: on one side AR is assumed to be inefficient [38, 49] due to abrupt switch between push and recovery phases. Nevertheless, in a study performed on inexperienced able-bodied subjects, the AR technique has shown the highest mechanical efficiency, and SC the lowest, regardless of velocity [31]. Moreover, Richter et al. (2007) [42] observed that ARC became the most frequently utilized pattern when subjects were pushing uphill during an incremental test on a treadmill, hypothesizing it to be the most biomechanically efficient pattern. In the present study patients and controls were free to choose their own pattern of propulsion. The relative distributions of the adopted patterns were widely heterogeneous in all investigated groups. During self-selected speed, the distribution of patterns in T and P was quite similar: SC, DL and a minority of AR. During maximal speed (maximal for T and P) the distribution of patterns in the two groups of patients was quite different: about equal percentages of AR, DL and SL in T, a great

majority of AR and a small minority of SL in P. CTRL (but not patients), showed also a minority of subjects using SC. In general terms, in CTRL the distribution of patterns among subjects was very different from that observed in T, and relatively similar to that observed in P. The present study is underpowered to determine the presence of relationships between propulsion patterns, the O_2 cost of propulsion and other variables. In any case, in the present study the subjects were free to choose propulsion characteristics such as velocity, stroke frequency and pattern.

Differences have been observed between groups and between patients in the same group, presumably due to a major impairment in upper limbs' muscle recruitment in patients with higher lesion levels, and due to the different pushing strategy (stroke frequency, pattern and velocity) chosen by the patients.

The EMG analysis showed results similar to those present in the literature.[27, 53-59] In general, recovery muscles showed a higher activation in comparison to push muscles: In fact, the recovery phase is a complex task, where a high muscle activation is requested to decelerate the arm and produce power to accelerate it backwards, performing a double task.

There seems to be a higher muscle activation in terms of RMS in patients rather than in CTRL if compared at same velocity, in particular for BB, TB, DA and DP. That could be due to a compensatory mechanism to the motor control deficit of the trunk in T and P patients, that inevitably reflects on the upper limbs muscles.[53, 55-57, 59]

In T, the activation of the BB has shown to be higher than in P and CTRL, and that implies a co-contraction of BB and TB during the push phase between 10-30% of the stroke cycle. This probably happens due to the lack in motor control of the trunk and the functional deficit of the TB. In fact, the first part of the push

phase, can be used to push the wheel, but also to put the trunk in an upright position in order to gain stability. For the same reason, also at the end of the push phase, and at the beginning of the recovery one, we can observe a co-contraction of DM, DA, DP and SSP.
In P, we observed an advanced recruitment of the DP as described in the literature [53-56] probably due to the compensatory mechanism previously described. The activation of the BB resulted higher and started later if compared to CTRL, almost causing the simultaneous activation of BB and TB, probably due to a lack in trunk control, using arms to put the trunk in an upright position and to gain stability. For the same reason, the activation of the DM during the recovery, is in co-contraction with the TB, DP and SSP.

Study limitations

Some limitations of the present study should be recognized. The tests were performed on the patients' everyday wheelchair, and on standard wheelchairs for CTRL. Wheelchairs of SCI were slightly different from one to another in terms of settings and weight, thus leading to a possible confounding factor. However, the use of standardized tools could require wheelchair users to modify their natural propulsion technique, which we wanted to avoid.

The use of an instrumented wheel (not utilized in the present study) could be of interest for future studies, in order to measure the forces applied on the handrim and therefore calculate the mechanical efficiency.

Assignment of pattern type by the evaluating researchers was not always unanimous: in some cases, difficulties were encountered in distinguishing the recovery loop shape between SL and ARC, or between DL and ARC. Also,

sometimes the pattern observed seemed to be a hybrid between two patterns as previously observed also by Koontz et al. [41].

Moreover, it is to clarify if the RMS values obtained from the analysis of the sEMG signal, are comparable between subjects.

Conclusions

In conclusion, despite the presence of significantly different propulsion patterns, particularly between CTRL and T, in the present study the O_2 cost of wheelchair propulsion was not significantly different in T and P *vs.* CTRL, when examined at the same velocity. In other words, despite the presumably different neuromuscular activation, the impaired skeletal muscle oxidative metabolism and cardiovascular function, in T and P oxidative economy during wheelchair propulsion was not impaired. Maximal performance (peak velocity, peak stroke frequency, peak HR, peak $\dot{V}O_2$) was linearly related to lesion level (lower values with higher lesions), and the values in T were lower than in P or CTRL, whereas no significant differences were observed between P and CTRL. In other words, maximal performance during wheelchair propulsion was impaired in T, but not in P. Similarly to previous results, also muscle activation seems to be similar between P and CTRL, and much more different between T and CTRL.

Acknowledgements

Firstly, thanks to all who took part this study. Thanks to all the co-authors and to all the physicians, technicians and students for their experimental assistance and data analysis: Prof. Bruno Grassi, Dr. Emiliana Bizzarini, Dr. Mirco Floreani, Dr. Rosmary Blanco, Dr. Alberto Collodel, Dr. Alessandro Poletto, and Luca Gasparotto.

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STUDY II EXERCISE TOLERANCE IN PATIENTS WITH LATE ONSET POMPE DISEASE ON ENZYME REPLACEMENT THERAPY: EFFECT OF EXERCISE TRAINING

The present study won a 3 years grant from the *Ministero della Salute Italiana* ("Bando della ricerca finalizzata, giovani ricercatori 2011-2012"). Thanks to a collaboration between clinicians, dieticians, neurologists and physiologists, a multidisciplinary approach characterizes this study.

This study belongs to a collaboration between 4 different Units: The *Regional Coordinator Centre for Rare Diseases of the Academic Hospital of Udine*, the *Exercise Physiology Laboratory of the University of Udine*, the *Neuromuscular Unit of the Carlo Besta Institute* in Milan and *Istituto di Bioimmagini e Fisiologia Molecolare, Centro Nazionale delle Ricerche* in Segrate, Milano.

The tests started in January 2015 and the study is still ongoing. Therefore, in this thesis, only the preliminary results will be reported.

Abstract

Background Late onset Pompe disease (LOPD) is a progressive neuromuscular disorder which can lead to important disability and impaired quality of life. The specific enzyme replacement therapy (ERT) has limited long-term effect on improving muscle functions. Functional evaluation of exercise tolerance is a tested method to evaluate patient's clinical outcome. Before the introduction of ERT, the positive effects of diet and exercise training had been demonstrated in LOPD, but the effect of both interventions is still debated and poorly known. **Aim of the study** The aim of this study is to evaluate if exercise training (EX), alone or in combination with diet therapy (EX+DI), could improve exercise tolerance, motor functions and quality of life in LOPD patients chronically treated with ERT. **Materials and methods** A cross-over study was designed. LOPD patients were selected from two different clinical units in Udine and Milan. Participants were assigned to a sequence of two treatments (exercise or exercise and diet) and one control period, each lasting 6 months, followed by a 13-week wash-out. Exercise tolerance test, muscular strength, 6-minute walking test, serum muscular enzymes (AST, ALT, CPK, LDH), pulmonary function tests, body composition and quality of life (SF-36 questionnaire) were evaluated before and after each period. **Preliminary Results** The study is still ongoing. Thirteen patients took part in the study: at present nine of them performed the tests pre-post CTRL. Four patients performed the tests pre-post EX, and six patients pre-post EX+DI. Two patients are on EX treatment, and four patients are on EX+DI treatment. Preliminary results are showing a good home-training compliance of ~75%, and results of the functional evaluation show some positive effects of EX and particularly of EX+DI intervention. **Conclusions** Preliminary data suggest some ~~a~~ positive effects of exercise training and hyperproteic diet in LOPD patients

under chronic ERT. However, further information will be gained from the analysis of all the data available at the end of the study.

List of abbreviations

6MWT	6-minute walking test
ALT	Alanine aminotranferase
AMD	Acid maltase deficiency
AST	Aspartate aminotransferase
BIA	Body Impedance Assessment
BMI	Body mass index
CK	Creatine kinase
CO2	Carbon dioxide
CPET	Cardio Pulmonary Exercise Testing
CTRL	Control period
CWR	Constant work rate
ECG	Electrocardiography
ERT	Enzyme replacement therapy
EX	Exercise treatment
EX+DI	Exercise + Diet treatment
FEV1	forced expiratory volume during the first second
FVC	Forced vital capaciy
GAA	Acid alfa-glucosidase
GET	Gas exchange threshold
GSDII	Glycogen storage disease type II
Hb	Hemoglobin
HR	Heart rate
IOPD	Infantile-onset Pompe disease
LOPD	Late-onset Pompe disease
Mb	Myoglobin

MVC	maximal voluntary contraction
NIV	Non-invasive nocturnal ventilation
O_2	Oxygen
RER	Respiratory exchange ratio
RMR	Resting metabolic rate
RPE	Rate of perceived exertion
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E$	Ventilation
VL	Vastus lateralis
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
VT	Tidal Volume

Introduction

Pompe disease is a rare lysosomal storage disorder. It is caused by an acid alfa-glucosidase (GAA) deficiency, which normally breaks down the glycogen inside the lysosomes. It is therefore also known as acid maltase deficiency (AMD) or glycogen storage disease type II (GSDII). The enzyme deficiency results in glycogen accumulation in autophagic vacuoles and lysosomes of multiple tissues, especially in skeletal, respiratory and cardiac muscles, leading to a progressive invalidating disease, leading to organ failure and/or death. [1, 2]
The pathogenesis is characterized by lysosomal enlargement, organellar rupture, leakage of acid hydrolases into the cytoplasm, and autophagic buildup, which are postulated to cause aberrant mitochondrial architecture, impairments in vesicular trafficking, and degradation of myofibrils, thereby underlying muscular fatigue and muscle weakness in GSDII.[3]

It is inherited in an autosomal recessive manner (**Figure 3.1**), and has an estimated incidence of one on 40.000 in the US and Holland, one on 50.000 in China and one on 146.000 in Australia.[4]

This deficiency is caused by a gene mutation on the long arm of chromosome 17.[5] More than 450 mutations have been discovered.[3]

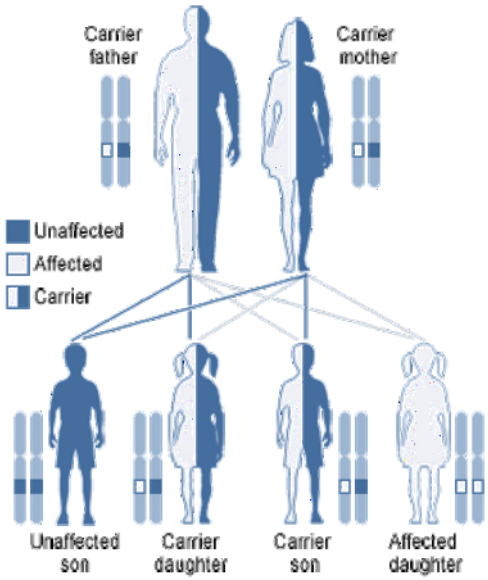

Figure 3.1. Autosomal Recessive Inheritance *(U.S. National Library of Medicine)*

Clinical Features

Symptoms can manifest at any age. Two main types of Pompe disease have been described, differing in severity and the age at which they appear.

The classic form of infantile-onset Pompe disease (IOPD), usually presents in patients within the first months of life, and is caused by a complete deficiency of GAA.[6] Infants typically experience muscle weakness (myopathy), poor muscle tone (hypotonia), and very few movements, not achieving motor skills such as rolling over, sitting or standing (see **Figure 3.2**). Also, an enlarged liver (hepatomegaly) and tongue are typically present, as well as heart defects (hypertrophic cardiomyopathy) leading to cardiac failure. Due to feeding difficulties, affected infants may also fail to gain weight and grow as expected. If untreated, this form of Pompe disease leads to death due to heart failure within the first year of life.

Figure 3.2. Muscle weakness in infants with IOPD.

The non-classic form of IOPD presents in the first year of life with a slower progression, and less severe cardiomyopathy.[7] In the literature, it has also been classified with the infantile or childhood forms.[1]

A partial deficiency of GAA, leads to a milder phenotype, named late-onset Pompe disease (LOPD). It mainly involves skeletal and respiratory muscles, while heart is mostly not affected. First symptoms are muscle weakness and cramps, difficulties in participating in sports, climbing stairs and rising from a lying position. Patients affected by LOPD experience progressive muscle weakness, especially in the legs and the trunk, and breathing muscles.[8] Mobility and respiratory difficulties do not proceed at the same pace: patients might need ventilation at night while still being able to walk, and others with a normal pulmonary function might become wheelchair dependent. As the disorder progresses, breathing problems can lead to respiratory failure.[4]

Diagnosis

Recognizing Pompe disease can be challenging, as signs and symptoms are similar to those of other diseases and disorders. As a result, Pompe disease may often not be readily considered during the clinical evaluation and significant diagnostic delays are common.[8] The diagnostic process may start from the clinical evaluation, or from a genetic test in case of familiar screening, or from the neonatal screening, where available.
The laboratory blood exams usually show increased muscle enzyme: creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotranferase (ALT), lactate dehydrogenase.[1]

The signs that should bring a physician to the suspect of a juvenile form is a patient with an age between 2 and 18 years presenting with muscular weakness, or motor abnormalities, without a cardiac involvement, with possibly recurrent respiratory infections.

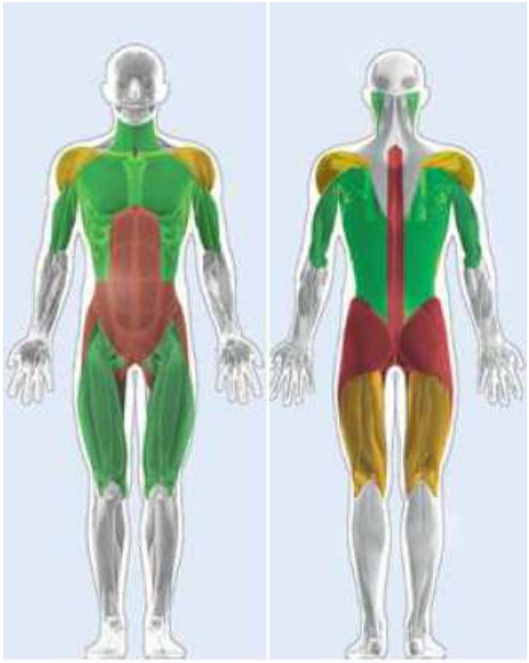

Figure 3.3. Muscle involvement in Late-onset Pompe Disease: red, severe; yellow, moderate; green, mild; gray, null or minimal.

The main clinical features of the adult forms are the consequences of the skeletal muscle involvement and of the respiratory muscle involvement and includes: proximal lower limbs and paraspinal muscle weakness, exercise intolerance, back pain, respiratory distress, sleep apnea, exertional dyspnea, recurrent respiratory infections. Respiratory disturbances can cause headache and somnolence. In some cases, the disease presents suddenly with a severe acute respiratory failure.[9]

Instrumental exams, such as electromyography and nerve conduction studies, are not specific but can help in the diagnosis, showing myopathic features. Also,

skeletal muscle biopsy can be useful. However, the diagnosis of Pompe disease should always be confirmed by enzymatic essays or molecular analysis. The differential diagnosis should include other muscular disorders such as muscular dystrophies, inflammatory myopathies, and other congenital storage disorders.[10]

Enzyme Replacement Therapy

Since 2006 an Enzyme replacement therapy (ERT) with recombinant human GAA (Myozyme®) is available. Although it alters the progression of Pompe disease, the effectiveness of the drug depends on repeated and lifelong intravenous infusion of the enzyme, increasing the risk of an immune response and drug resistance. Furthermore, fast-twitch fibers (oxidative/glycolytic and glycolytic, IIa/IIx/IIb) appear more refractory to ERT than slow-twitch fibers (oxidative, I), due to different mechanism such as differential perfusion characteristics between fibers.[3]
However, different authors observed that the positive effects are evident within the first year of treatment, but they seem to have a limited long-term effect on improving muscle functions.[11-13] The limitation of the current available therapy stimulate research on more effective treatments.

Physical activity & ERT

Several studies demonstrated that aerobic and strength training have remarkable positive effects on multiple organ systems, and its practice is therefore suggested in several chronic diseases such as cardiovascular diseases [14], cancer [15],

hepatitis C [16] and HIV.[17] Therefore, exercising can be of benefits also for LOPD patients, improving muscle strength and motor function.

However, even if physical activity enhances blood flow to working muscle, it seems not to improve drug uptake during ERT or cause a reduction in glycogen content.[3] Anyhow, endurance and strength training have been demonstrated to be beneficial as an adjunctive therapy to ERT in Pompe.[18-23]

Aims of the study

Exercise training, alone or in combination with hyperproteic diet, could improve exercise tolerance, motor function and muscle strength in LOPD patients on chronic ERT treatment. Specifically, aerobic exercise could increase the use of fatty acids as an alternative source of energy, thus reducing proteolysis, autophagy and muscle destruction.[21] Moreover, exercise training could counteract the general deconditioning typical of chronic diseases as well as the chronic inflammatory condition associated with inactivity.[24]

The aim of this study is to investigate if exercise training, alone or in combination with an hyperproteic and low carbohydrate diet, can improve exercise tolerance and motor functions in LOPD patients chronically treated with ERT, decreasing glycogen accumulation and protein catabolism in muscles. Functional evaluation of exercise tolerance by utilizing methods and approaches developed in the exercise physiology laboratory could be useful in order to assess the clinical outcome of several chronic diseases, including LOPD.

Materials and methods

Subjects

This study belongs to a collaboration between 4 different Units: The *Regional Coordinator Centre for Rare Diseases of the Academic Hospital of Udine*, the *Exercise Physiology Laboratory of the University of Udine*, the *Neuromuscular Unit of the Carlo Besta Institute* in Milan and *Istituto di Bioimmagini e Fisiologia Molecolare, Centro Nazionale delle Ricerche* in Segrate, Milano.
Different professional figures are involved, including physicians expert in Pompe disease, neurologists, physiologist with experience in exercise functional studies, dietician and nurses.
Patients were recruited from the 2 different clinical centers in Udine and Milan.

Inclusion criteria were:
1. age ≥18 years;
2. diagnosis of LOPD confirmed by enzymatic test and/or genetic analysis;
3. enzyme replacement therapy for at least two years.

Exclusion criteria were:
1. significant cardiovascular disease;
2. wheelchair bound;
3. severe respiratory insufficiency;
4. pregnancy.

Patients who already performed regular physical activity or were on diet therapy were also excluded.

Thirteen subjects took part in the study. Main characteristics are given in **Table 3.1**:

subject	*gender*	*age at study entry (yr.)*	*weight (Kg)*	*height (cm)*	*BMI (Kg/m^2)*	*age at diagnosis*	*years on ERT*	*comorbidities*	*ventilatory support*
UD01	M	19	71	187	20.3	3	9	none	none
UD02	M	48	106	190	29.4	41	6	hypertension	none
UD03	F	49	45	164	16.7	29	6	osteoporosis	none
UD04	M	55	105	183	31.4	35	9	hypertension	NIV
UD05	F	46	78	168	27.6	25	9	hypertension; asthma	NIV
UD06	F	42	59	163	22.2	29	8	none	NIV
UD07	F	45	40.5	162	15.4	16	10	osteoporosis; hemorrhoids; dyspepsia	NIV
MI01	F	71	54.5	163	20.5	66	3	gastritis	none
MI02	M	71	64	180	19.8	67	4	removal of vocal cord cancer; surgical for prostatic hypertrophy	NIV
MI03	F	45	74.8	165	27.5	41	5	discal hernia L4-L5	none
MI04	F	56	45	168	15.9	37	6	high blood pressure; depressive syndrome	NIV
MI05	F	59	63.6	153	27.2	54	5	none	NIV
MI06	M	51	58.5	165	21.5	45	6	none	NIV
mean±SD		***50.5±13.3***	***66.5±20.7***	***170.1±11.2***	***22.7±5.4***	***25.0±12.6***	***8.1±1.6***		
NOTE: NIV = non invasive nocturnal ventilation									

Table 3.1. Subject characteristics. UD, patients enrolled in Udine. MI, patients enrolled in Milano.

Experimental Design

The study was designed as a partially blinded crossover study. Blinding is applied to researcher working at laboratory assays.

Participants were assigned to a sequence of two treatments: training (EX) or training in association with an hyperproteic diet (EX+DI), and one control (CTRL) period (free diet, no exercise training). Each period lasted 6 months and was followed by a 13-weeks wash-out. Patients continued ERT during the whole study period, at the usual dosage (20 mg kg^{-1}).

Training was performed at home, and consisted of 4 sessions/week of about 1 hour per session. A personalized training program was developed following a functional evaluation of each patient. Each session included:

- 10-15 min. warm-up, joint mobility exercises;
- 15 min. strength training with very moderate loads of the main muscle groups, carried out by elastic bands;
- 30 min. constant work-rate exercise on a cycle ergometer at ~70% of the patient's maximal heart rate;
- 5-10 min. stretching.

During the visit at the exercise physiology laboratory, patients received the elastic bands and accessories, as well as all necessary instructions to carry out the training at home. Cycle ergometer with a HR sensor was shipped to each patient's domicile.

Every patient received the following booklet with exercise instruction:

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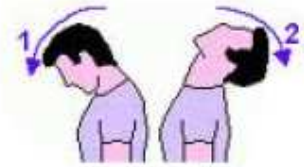
PROGRAMMA DI

TRAINING DOMICILIARE

RISCALDAMENTO

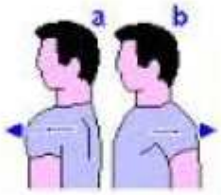
COLLO



10 (5+5) piegamenti testa alternando avanti e indietro

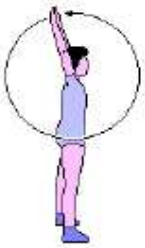
10 (5+5) piegamenti testa alternando destra e sinistra

SPALLE



10 (5+5) spinte spalle alternando avanti e indietro

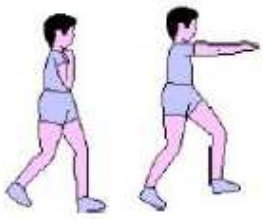
10 rotazioni spalle verso dietro



5 circonduzioni braccia avanti + 5 indietro

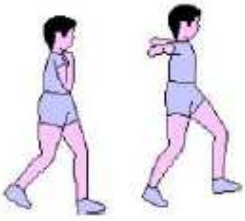
5 slanci braccia alto/basso

BRACCIA



5 distensioni braccia avanti

5 distensioni braccia in alto



5 distensioni braccia di lato

GAMBE

10 (5+5) flessioni gamba in avanti

10 (5+5) flessioni gamba di lato

10 (5+5) inclinazioni busto alternando destra/sinistra

10 (5+5) rotazioni busto alternando destra/sinistra

ALLENAMENTO FORZA

Nelle pagine seguenti troverete le indicazioni per svolgere gli esercizi per l'allenamento della forza. Seguite con attenzione le indicazioni e cercate di rispettare sia il **numero di ripetizioni e serie** per ogni esercizio, sia la **durata delle pause**.

Per aumentare la resistenza dell'elastico (= più fatica!) basterà accorciarlo. Al contrario, se l'esercizio vi sembra troppo impegnativo, allungate l'elastico.

Laddove indicato, utilizzate come indicato gli accessori forniti.

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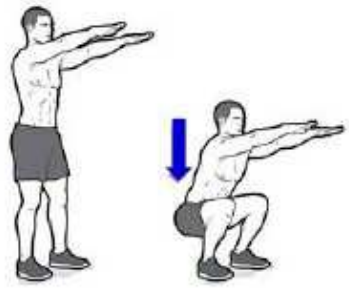
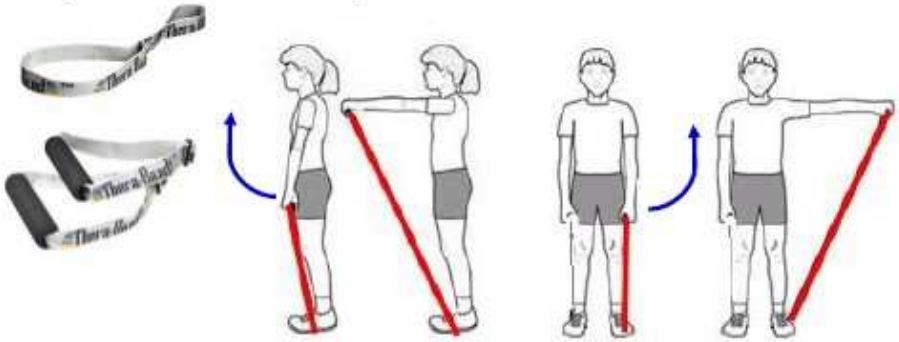
Ausilio di fissaggio flessibile e sicuro (permette svariati esercizi che altrimenti sarebbero possibili solo in coppia)

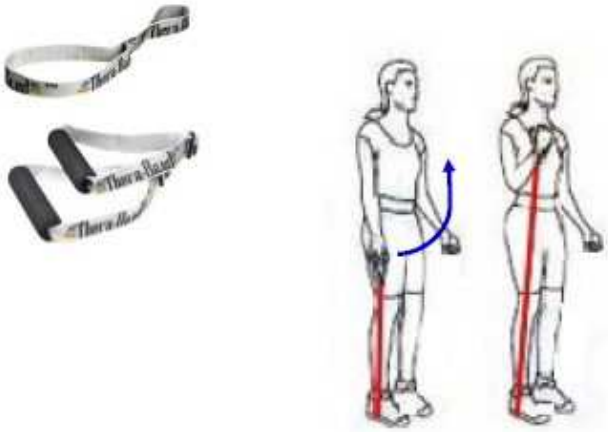
ESERCIZIO	*RIPETIZIONI*	*SERIE*	*PAUSA*
1) **SQUAT**	5	4	30''



2) **ALZATE FRONTALI/LATERALI**	10+10	3	30''



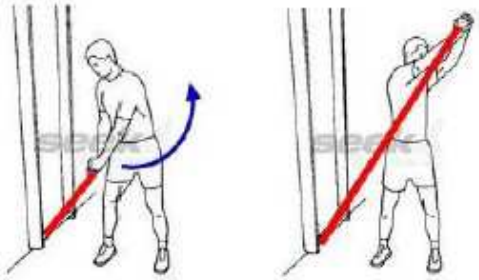
3) **ARM CURL**	10+10	3	30''



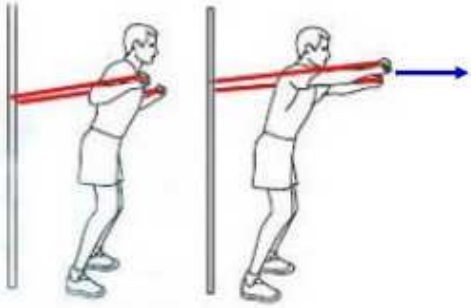
4) **ADDUTTORI** 10+10 3 30''

5) **ABDUTTORI** 10+10 3 30''

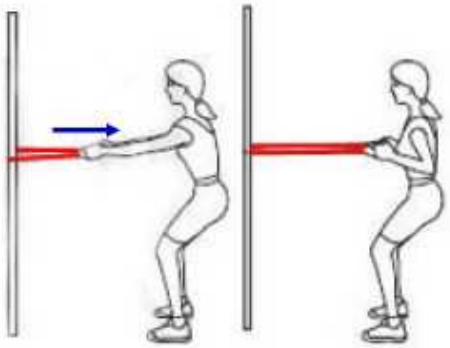
6) **TORSIONI DEL BUSTO** 10+10 3 30''



7) **PRESS (pettorali)** 10 3 30''



8) **REMATE** 10 3 30''



ALLENAMENTO AEROBICO

(CYCLETTE)

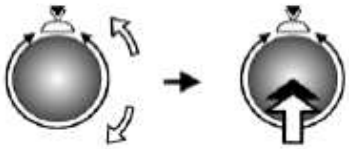
1. Indossare la fascia cardio

2. Inserire la spina ed accendere la bici
   (tasto ON/OFF sulla base posteriore)

3. Verificare che la CareCard sia inserita nel lettore (in alto, sotto al computer della bici)

4. Allenamento

Premere il tasto MENU



Utilizzando la rotella sulla destra selezionare:

- **Avvio**
- **Ergo_memo**
- **Progr. su card: 1**

Quando sul display si visualizzano i dettagli del programma selezionato, premere ancora una volta la rotella per confermare.

Iniziare l'allenamento.

5. Quando l'allenamento è completato, sul display compare la scritta "fine programma" e contemporaneamente si sente una melodia.

Se per qualsiasi motivo siete costretti ad interrompere l'allenamento in anticipo, **PREMERE IL TASTO MENU**  prima di spegnere la macchina.

6. Spegnere la cyclette (tasto ON/OFF sulla base posteriore) e staccare la spina

STRETCHING

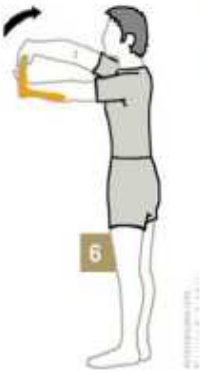
1.Alzare ed abbassare le spalle **10 volte**

2.Ruotare il busto verso destra e verso sinistra

10 volte

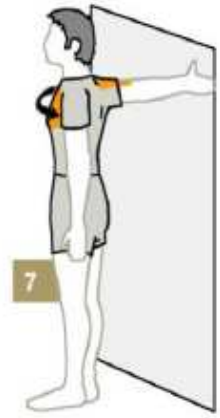
3.Inclinare il busto a destra afferrando il gomito sinistro e viceversa **30''**

4.Incrociare le dita e tendere le braccia sopra la testa, palmi delle mani verso l'alto **30''**



5.Portare una mano sulla spalla opposta, ed esercitare un po' di pressione **30''**

6.Con il braccio teso, tirare delicatamente le dita verso di sé, con il palmo rivolto in avanti **30''**



7.Con il braccio teso verso l'esterno e la mano appoggiata al muro, ruotare il tronco verso il lato opposto **30''**

8.Appoggiare le mani poco al di sopra dei glutei, e portare indietro i gomiti **30''**

9.Piegare le gambe e spingere verso il basso **30''**

10. Nella stessa posizione, portare il busto in avanti **30''**

11. Con una gamba distesa, piegare l'altra e portare il piede oltre la gamba, ruotando il torso in direzione opposta **30''**

12. Accovacciati sui talloni, piegare la schiena in avanti **30''**

13. Piegare il tronco verso una delle gambe cercando di afferrare il piede **30''**

14. Afferrare la parte posteriore del piede con la mano tirandolo lentamente verso i glutei. **30''**

Patients' compliance was monitored, and low compliance risk was minimized through weekly calls or emails. Also, a training diary was completed during the entire intervention period in order to evaluate compliance to training at the end of the intervention.

The hyperproteic diet was tailored by a specialist dietician to each patient, according to the usual calorie intake, and is composed of 25-30% protein, 30-35% carbohydrate and 35-40% fat (**Figure 3.4**). Compliance was monitored through a 3-day food diary every month.

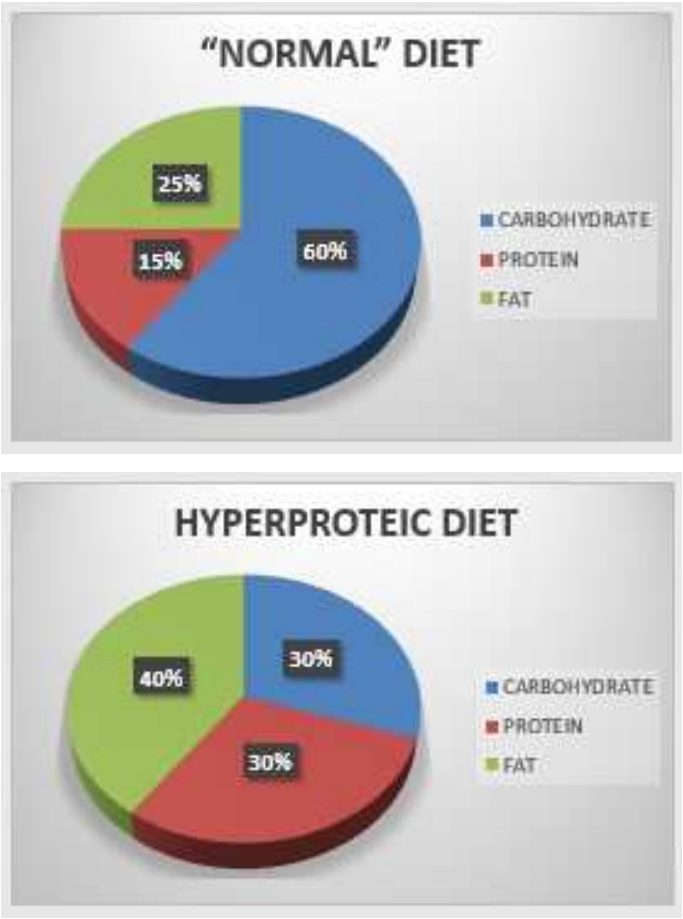


Figure 3.4. Comparison between a "normal diet" and the hyperproteic diet.

Measurements and exercise protocol

Before and after each period, the effectiveness of interventions was verified through functional evaluation tests, measuring:

- body mass index, body composition by Body Impedance Assessment (BIA)
- pulmonary function (spirometry)
- pulmonary gas exchange during an incremental test on a bike ergometer
- maximal aerobic power
- kinetic of physiological parameters during submaximal constant work-rate exercises
- heart rate
- strength (by an isometric maximal voluntary contraction, MVC)
- time to exhaustion
- motor function (6-minute walking test, 6MWT)

Body mass was measured with a manual weighing scale (Seca, Germany).

Height was measured on a standardized wall-mounted height board. Body mass index (BMI) was then calculated.

Body composition (i.e. fat-free mass and total skeletal muscle mass) was assessed by bioelectric impedance analysis (BIA) performed by a tetrapolar BIA device (BIA 101, Akern, Florence, Italy), in accordance with the conventional standard technique [25]: 4 electrodes were fixed to the skin on the wrist and ankle (**Figure 3.5**).

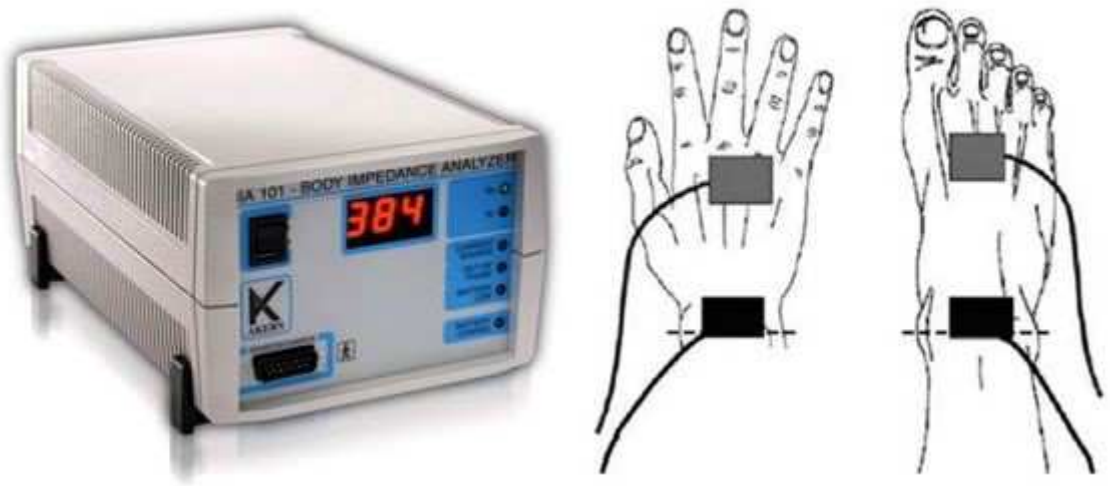


Figure 3.5. Body composition measurement by tetrapolar BIA device (BIA 101, Akern, Florence, Italy)

Data were then analyzed using the software provided by the manufacturer (BodyGram™ Version 1.31, Akern Bioresearch, Florence, Italy) as shown in **Figure 3.6**.

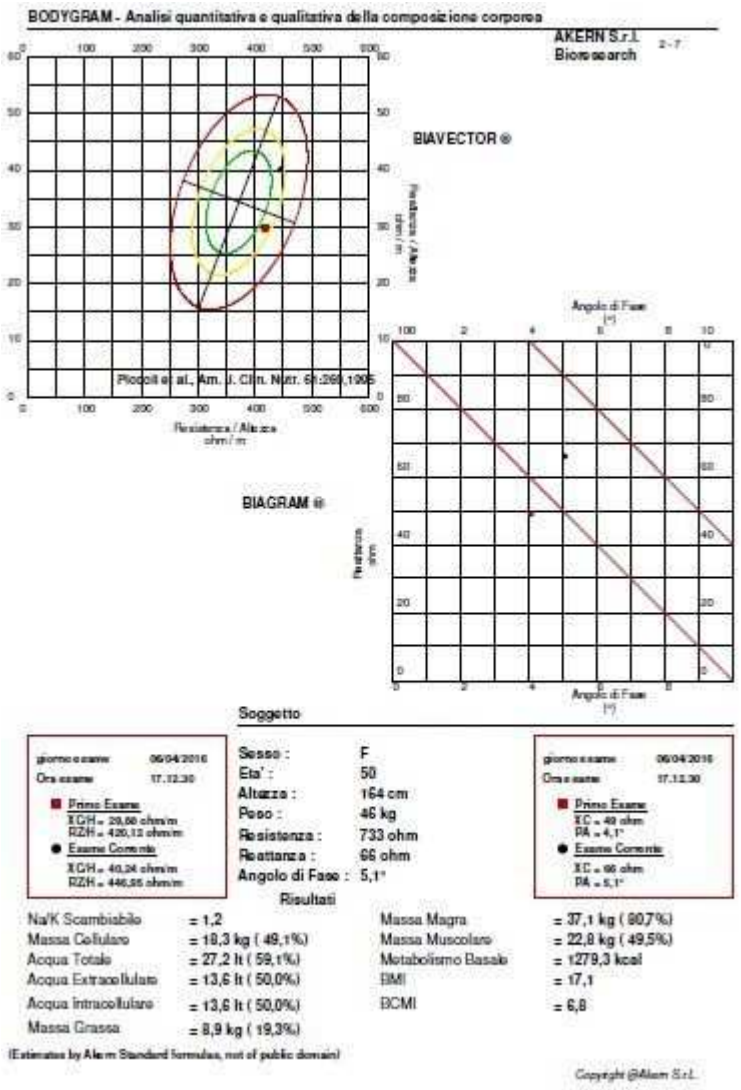


Figure 3.6. BIA data analysis by BodyGram™ Version 1.31 (Akern Bioresearch, Florence, Italy)

Also, thickness of skin and subcutaneous adipose layer on the vastus lateralis of the right leg was measured, using a real-time B-mode ultrasound apparatus (Esaote Biomedica AU3 Partner, Florence, Italy) with a 7.5 MHz linear-array probe, as shown in **Figure 3.7**.

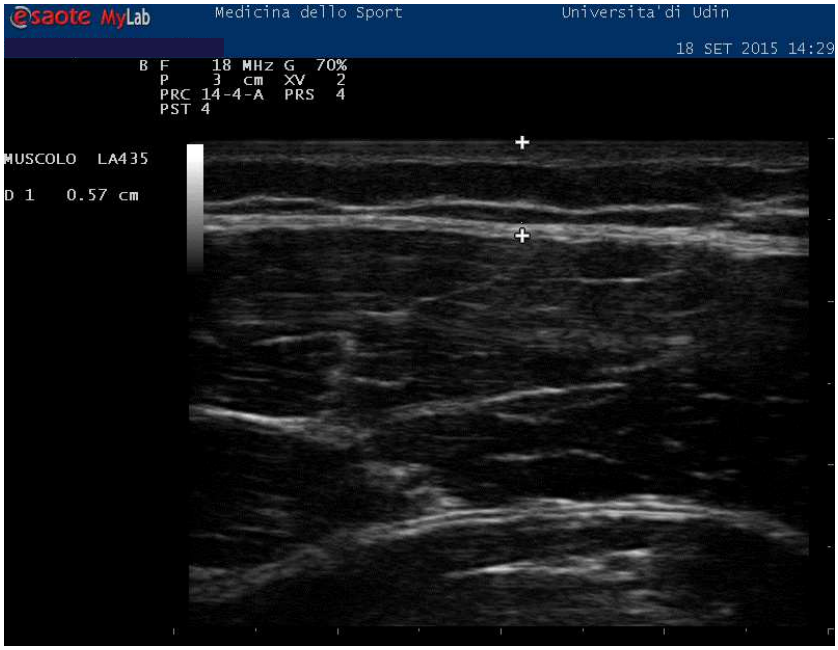


Figure 3.7. Ultrasound image of the vastus lateralis muscle.

Pulmonary function was measured by spirometry both in sitting and supine conditions. Forced vital capacity (FVC) and forced expiratory volume during the first second of the forced expiration (FEV1) have been analyzed as absolute values and as % of reference values for age and weight.

Furthermore, we evaluated the postural drop, i.e. the change in FVC upon assuming the supine position.[26-31]

ΔFVC was calculated as follows:

$$(FVC_{sitting}-FVC_{supine})/FVC_{sitting} * 100\%$$

In fact, while several studies have shown that ERT has positive effects on skeletal muscle function, the effects of ERT on lung function especially in supine position seem to be less pronounced.[32-37] We also calculated the Tiffeneau Index, and compared it to the expected value of 0.8.

Muscular strength of the right lower limb (knee angle at 110°) and right upper limb (elbow angle at 90°) was measured during an isometric maximal voluntary contraction (MVC), in both flexion and extension using the TSD121C isometric dynamometer (Biopac Systems Inc., Santa Barbara, CA, USA) as shown in **Figure 3.8**. Force analog output was sampled at a frequency of 1 kHz, and was acquired with BIOPAC System MP100 (Biopac Systems Inc., Santa Barbara, CA, USA) using *AcqKnowledge* software version 3.7.2. Then, peak values of every trial were identified.

Figure 3.8. The TSD121C isometric dynamometer (Biopac Systems Inc., Santa Barbara, CA, USA). By *www.biopac.com*

An 8-minutes very-low intensity constant work rate exercise was performed on a cycle ergometer (Monark Ergomedic 839E by Monark Exercise AB, Vansbro, Sweden), immediately followed by an incremental exercise (+5W or 10W every minute) until exhaustion. For all variables, values determined at voluntary exhaustion were considered "peak" values. Time to exhaustion was taken as an index of performance. Pulmonary ventilation (VE), tidal volume (VT), ventilatory frequency (BR), O_2 uptake ($\dot{V}O_2$), and CO_2 output ($\dot{V}CO_2$) were determined on a breath-by-breath basis by means of a metabolic cart (CPET, COSMED Inc., Rome, Italy). Calibration of O_2 and CO_2 analyzers was performed before each experiment by utilizing gas mixtures of known composition.

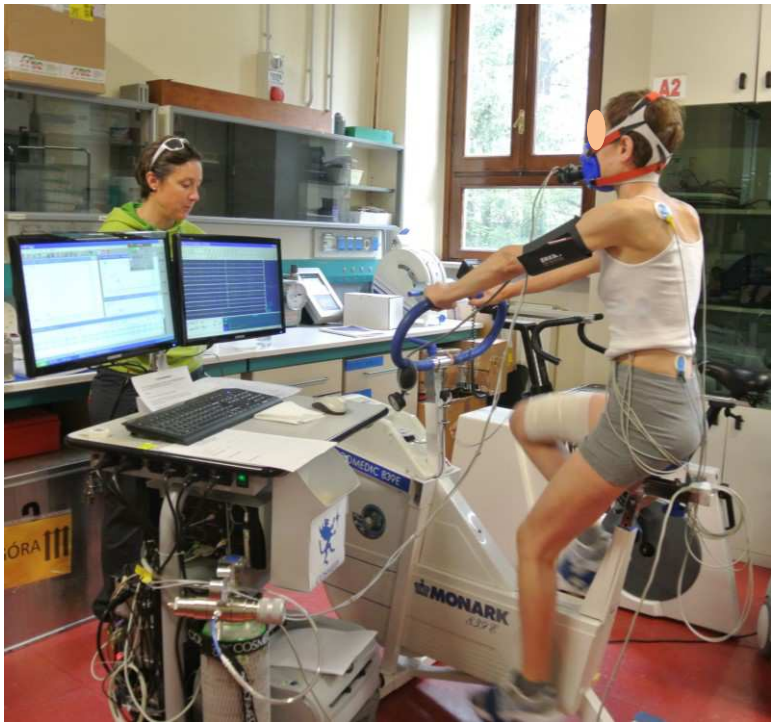


Figure 3.9. Incremental test on the cycle ergometer.

Expiratory flow measurements were performed by a turbine flow meter, calibrated before each experiment by a 3-liter syringe. The respiratory exchange ratio (RER) was calculated as $\dot{V}CO_2/\dot{V}O_2$. The gas exchange threshold (GET) was determined for each subject by the V-slope method [38] on pulmonary $\dot{V}O_2$ and $\dot{V}CO_2$ data, averaged every 10s. Heart rate (HR) was determined by electrocardiography (ECG), and data were recorded during the entire test.

At the end of every minute during the test on the cycle ergometer, subjects were asked to evaluate the perceived exertion by using the Borg CR-10 Scale [39] with the 0 value meaning "nothing at all" to 10 value meaning "extremely strong".

Quality of life was evaluated using the validated, standardized Italian version of the Short Form (36) Health Survey (SF-36). The SF-36 questionnaire is a 36-item, patient-reported survey of patient health, measuring patient's quality-of-life, and is widely used. It takes in consideration 8 different domains:

- PF=Physical functioning;
- RP=Role physical;
- BP= Freedom from pain;
- GH=General health perceptions;
- VT=Vitality;
- SF=Social role functioning;
- RE=Role emotional;
- MH=Mental health.

Furthermore, two general physical and mental health scores are obtained from the analysis of the data:

- PCS=Standardized physical component scale;
- MCS=Standardized mental component scale.

SF-36 questionnaire was administered before and after each intervention.

Moreover, neurologic functional tests, serum muscular enzymes (AST, ALT, CPK, LDH), GSGC scores (Gait, Stairs, Gower, Chair) have been performed in the hospital.

The study was approved by the local Ethical Committee both in Udine and in Milan. Participants were informed about the aims and methods of the investigation and gave their written, informed consent. The experiments concerning the functional evaluation were carried out at the Exercise Physiology Laboratory of the University of Udine and at the Istituto di Bioimmagini e Fisiologia Molecolare, Centro Nazionale delle Ricerche in Segrate, Milano. All tests were performed under continuous medical supervision and following standard safety procedures. All procedures were in accordance with the recommendations set forth in the Helsinki Declaration of 1975, as revised in 2013.

Statistical Analysis

Results were expressed as mean values ± standard deviation (SD). Comparisons between two groups were performed by two-sided Student's t-test. Comparisons between more than two groups were performed by one-way ANOVA; a Bonferroni's post-hoc test was used when significant differences emerged at ANOVA. Data fitting by linear regressions was performed by the least-squared-residuals method. The level of significance was set at $P<0.05$. Statistical analyses were performed by a software package (GraphPad Prism v. 5.0, GraphPad, CA, USA).

Results

Table 3.2 shows interventions carried out until October 2017.

	patient nr.	1st int.	2nd int.	3rd int.
UDINE	1	CTRL	EX	EX+DI
	2	CTRL	EX+DI	(EX)
	3	CTRL	EX	EX+DI
	4	CTRL	-	-
	5	CTRL	EX	-
	6	EX	(EX+DI)	
	7	EX	(EX+DI)	
MILANO	8	CTRL	EX+DI	(EX)
	9	CTRL	-	-
	10	CTRL	EX	EX+DI
	11	CTRL	EX	EX+DI
	12	(EX+DI)		
	13	(EX+DI)		

Table 3.2. Intervention carried out until October 2017. Interventions indicated in brackets, were in progress in October 2017.

In Udine, seven patients took part in the study: five of them performed the tests pre-post CTRL. Two patients performed the tests pre-post EX, and three patients pre-post EX+DI. One patient will finish the EX period in December 2017, and two patients just began in October 2017 the EX+DI period.

In Milano, 6 patients took part in the study: four performed the tests pre-post CTRL, two patients pre-post EX and three patients performed the tests pre-post

EX+DI. One patient is on EX period, and two patients are finishing the EX+DI period, and will perform the EX period after a 13-week wash-out.

Therefore, on October 2017, 8 interventions still need to be completed in the next months.

In Udine, three patients were not able to perform the exercise on the cycle ergometer during the functional evaluation tests at the Exercise Physiology Laboratory: one after the CTRL period, two before the EX period, while one was not able to perform the exercise on the ergometer during all the three visits at the Exercise Physiology Laboratory (**Table 3.3**).

		CTRL		EX		EX+DI	
	patient nr.	pre	post	pre	post	pre	post
UDINE	1	OK	OK	OK	OK	OK	OK
	2	OK	OK	OK	dec-17	OK	OK
	3	OK	OK	OK	OK	OK	OK
	4	OK	OK	-	-	-	-
	5	OK	NO	NO	OK	-	-
	6	-	-	NO	OK	OK	
	7	-	-	NO	NO	NO	
MILANO	8	OK	OK			OK	OK
	9	OK	OK	-	-	-	-
	10	OK	OK	OK	OK		
	11	OK	OK	OK	OK		
	12					OK	nov-17
	13					OK	nov-17

Table 3.3. Ability of exercise testing on cycle ergometer in patients.

Training compliance

We evaluated the compliance to the home training program, and observed a compliance of 75% on average (**Figure 3.10**), which can be considered very good: Only very few patients showed a quite low training compliance.

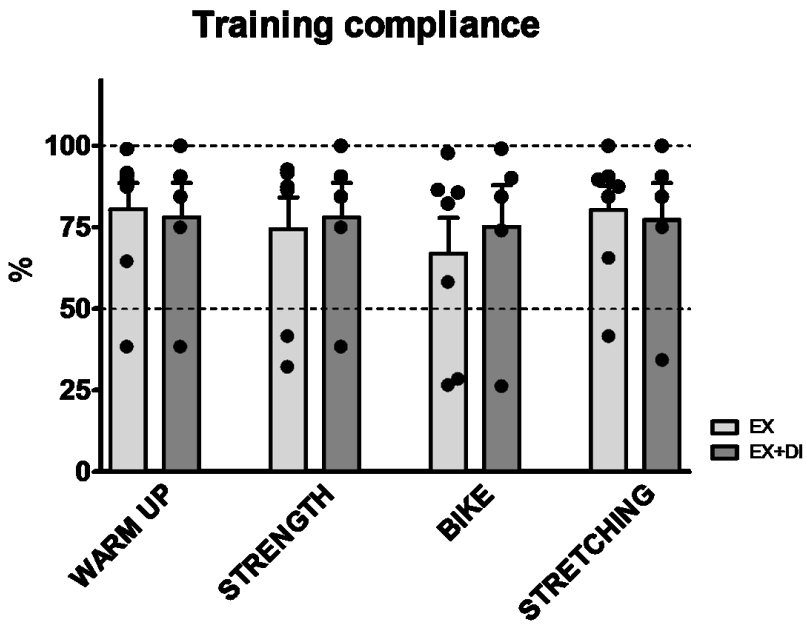


Figure 3.10. Training compliance during EX and EX+DI interventions, expressed as a % of the expected value (100% = 4 session/week; Warm up, strength, bike, stretching).

Constant work rate exercise

After neglecting the initial phase of adjustment (first 3 minutes of exercise), $\dot{V}O_2$ and HR kinetics during constant work rate (CWR) exercise have been evaluated before and after every intervention. As an example, $\dot{V}O_2$ and HR kinetics of a patient are shown respectively in **Figures 3.11, 3.12**.

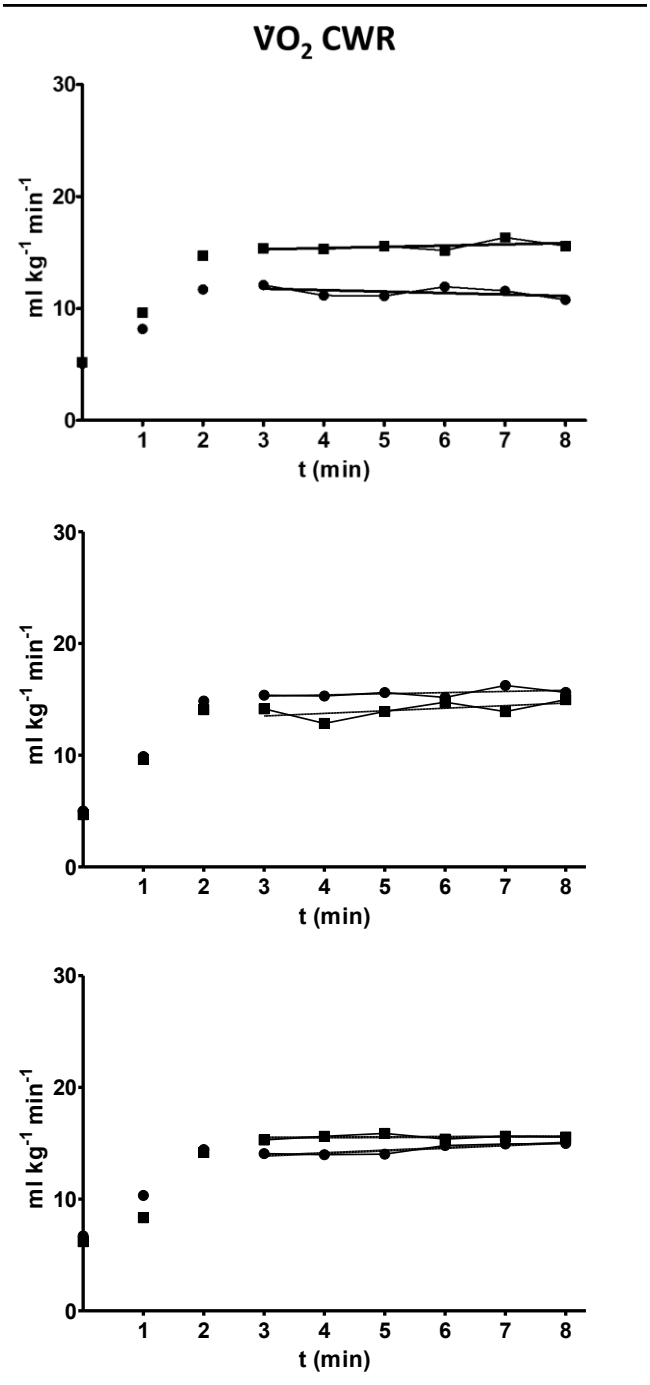


Figure 3.11. Heart rete kinetic during constant work rate, before (●) and after (■) CTRL, EX and EX+DI.

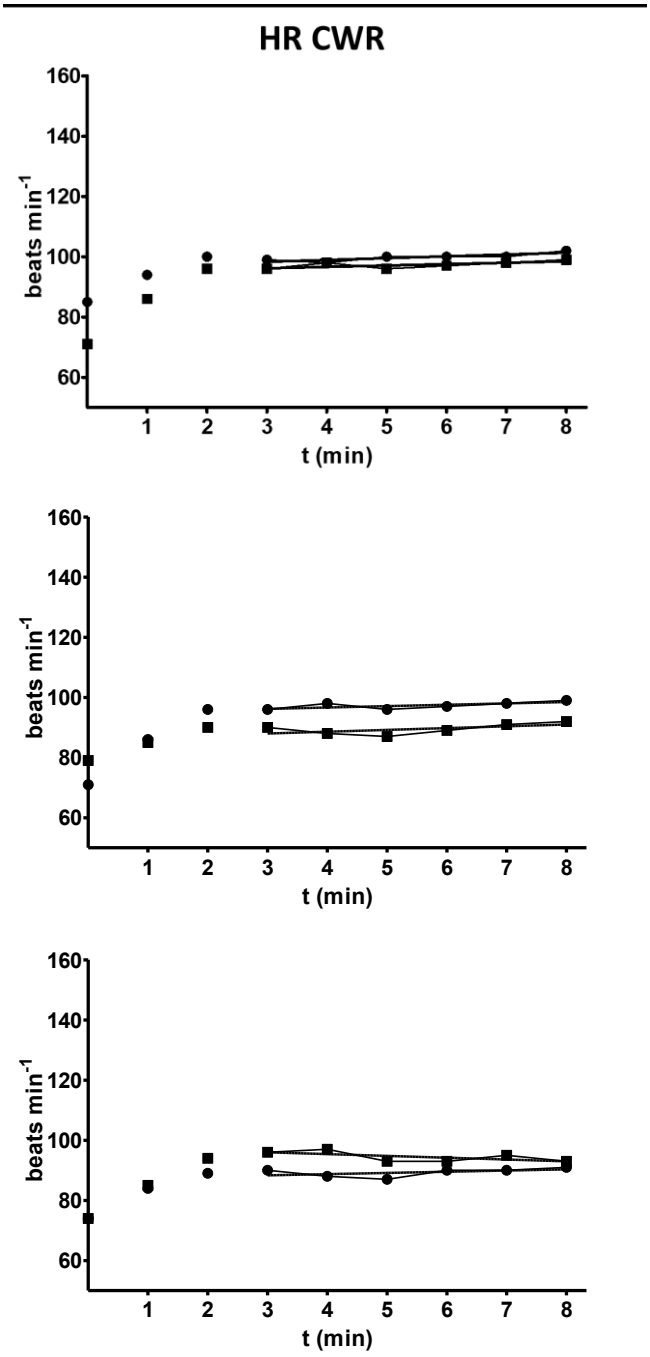


Figure 3.12. Heart rete kinetic during constant work rate, before (●) and after (■) CTRL, EX and EX+DI.

Data were analyzed starting from the third minute of exercise. A linear regression of data was performed on the experimental points. No significant differences in the slope values of $\dot{V}O_2$ and HR were observed before and after any intervention (**Figure 3.13**).

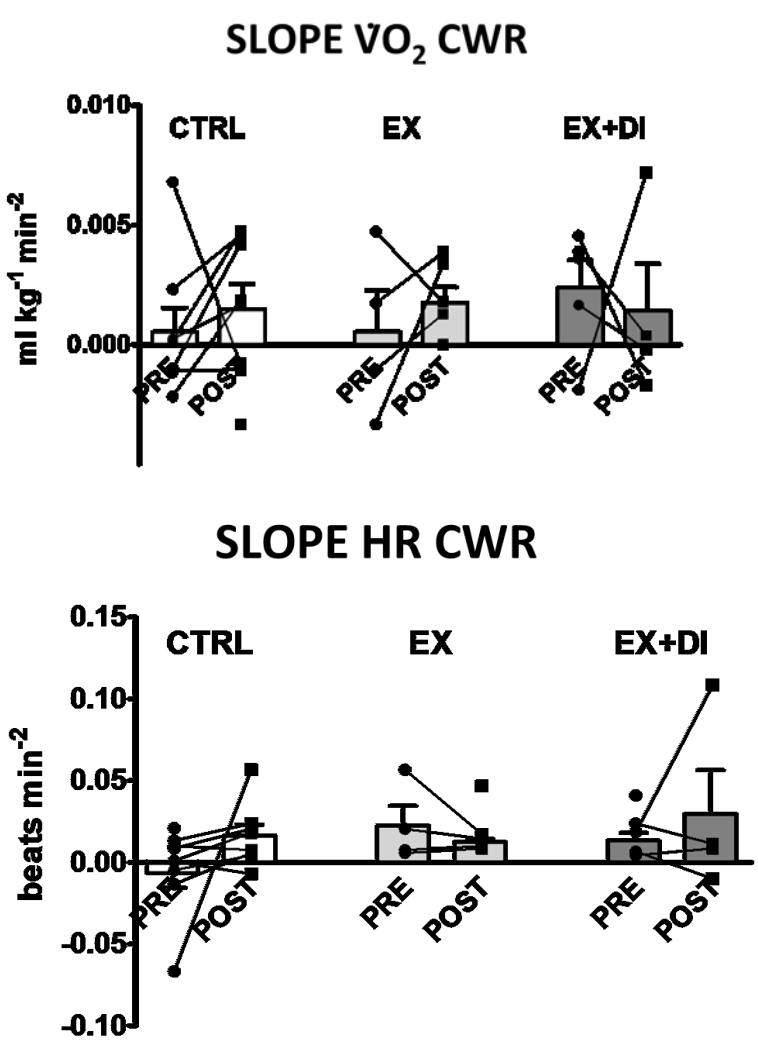


Figure 3.13. Slopes of $\dot{V}O_2$ and HR kinetics during CWR.

Values of $\dot{V}O_2$, HR and RER at the end of CWR were not significantly different before and after the interventions, as shown in **Figure 3.14**.

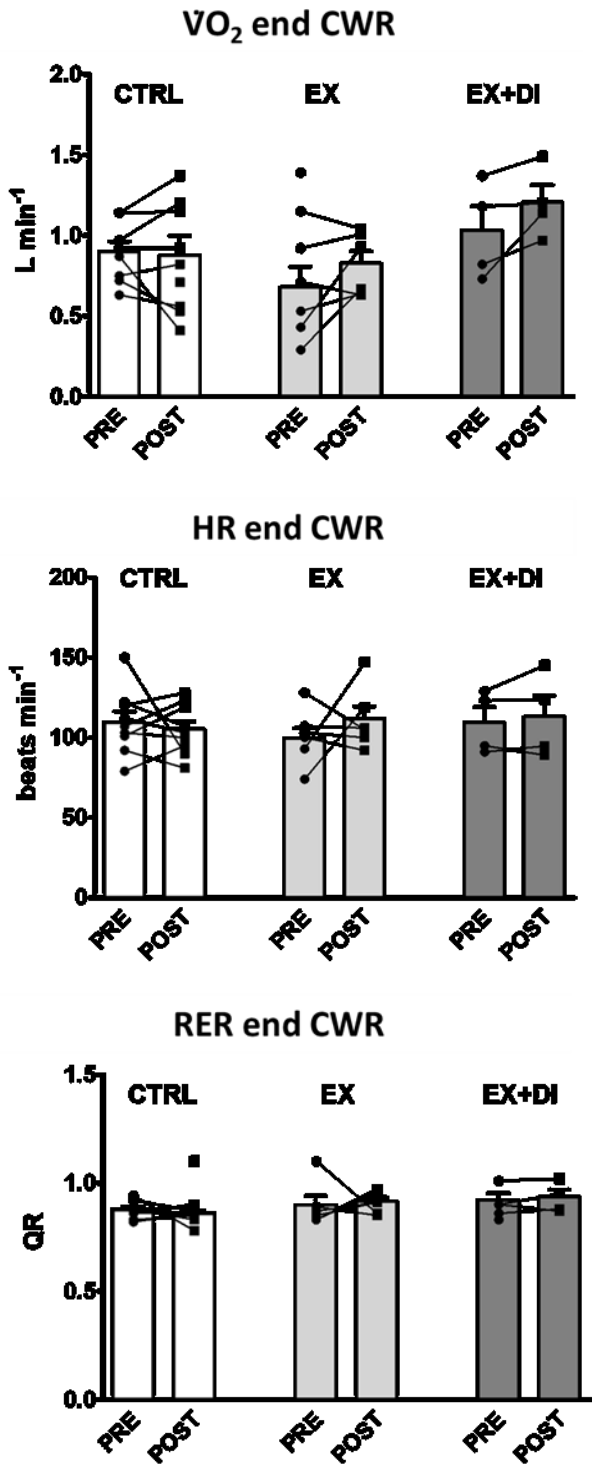


Figure 3.14. $\dot{V}O_2$, HR and RER values at end CWR.

Incremental exercise

Peak values of $\dot{V}O_2$ (expressed as L min-1 and as ml kg-1 min-1), HR and RER at the end of the exercise (corresponding to the inability of the patient to sustain the effort) are shown in **Figure 3.15, 3.16**:

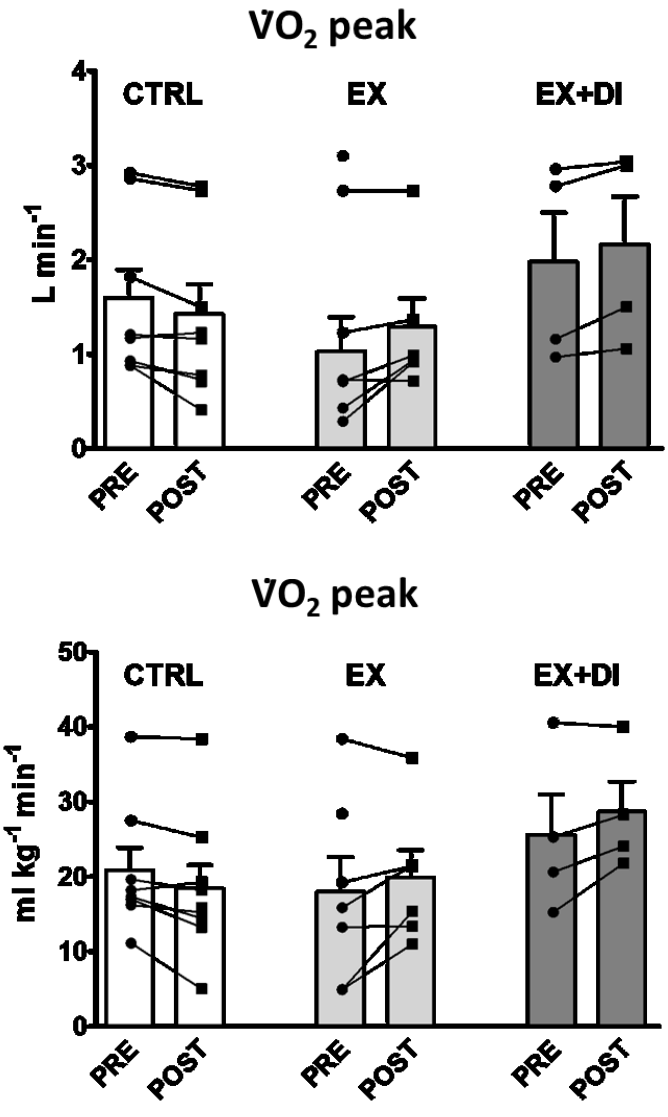


Figure 3.15. $\dot{V}O_2$ values at the end of exercise.

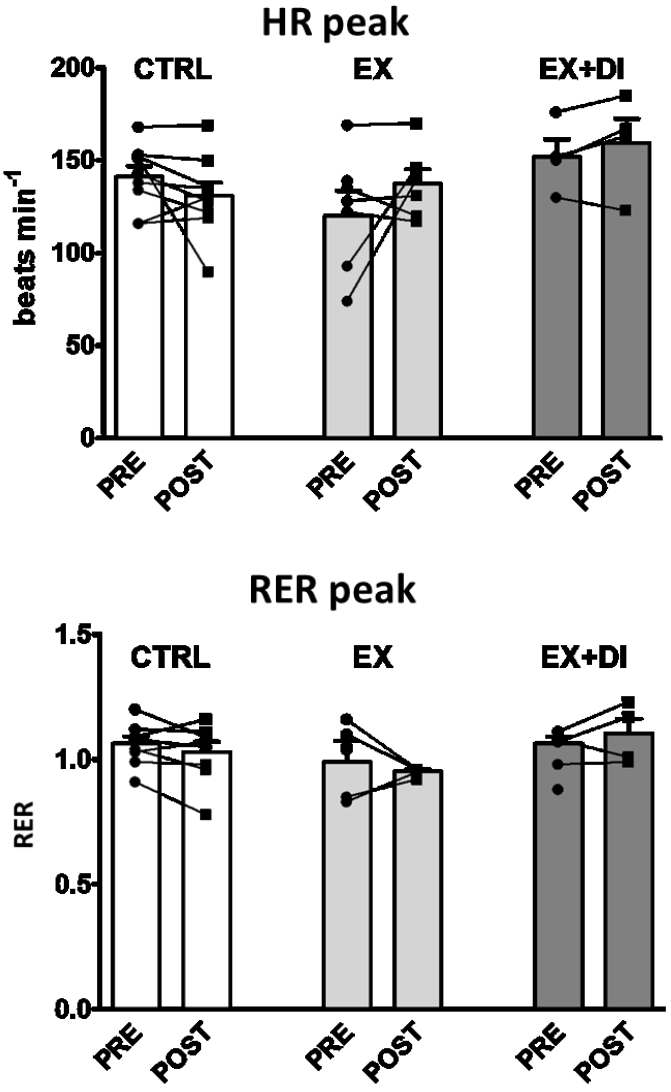


Figure 3.16. HR and RER values at the end of exercise.

No significant differences were observed before and after any intervention, even though there seems to be an increase in $\dot{V}O_2$ peak, both after EX as well as after EX+DI.

No significant differences were observed before and after any intervention. However, $\dot{V}O_{2peak}$ values showed a tendency to decrease in CTRL, and a tendency to increase in EX and in EX+DI. If confirmed upon completion of the study, these data may be of interest, suggesting that the two interventions could revert the progressive decrease in $\dot{V}O_2$ as a function of time observed in the absence of any intervention.

Using the calculation to predict $\dot{V}O_{2peak}$ (ml^{-1} min^{-1}) suggested by Wassermann[40] and described in the introduction of this thesis, we observed that mean values corresponded to 75.9%, ranging from a minimum of 55% to a maximum of 102%.

A similar trend was observed for the Peak power output reached at the end of exercise and Time to exhaustion, which were similar or slightly lower before and after 6 months of no intervention (CTRL), and tended to be higher after both interventions (**Figures 3.17, 3.18**).

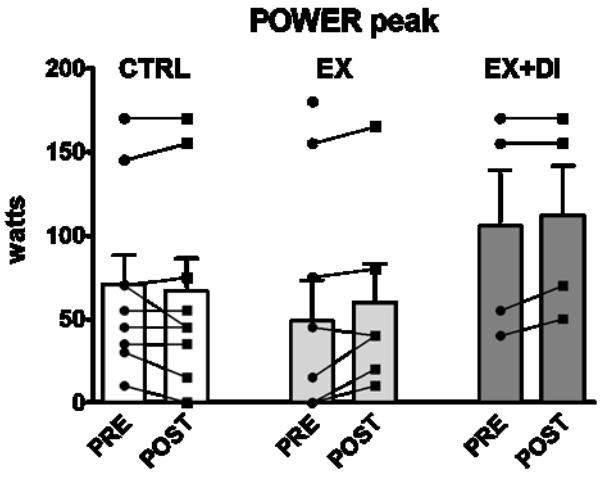


Figure 3.17. Peak power (watts) at the end of exercise.

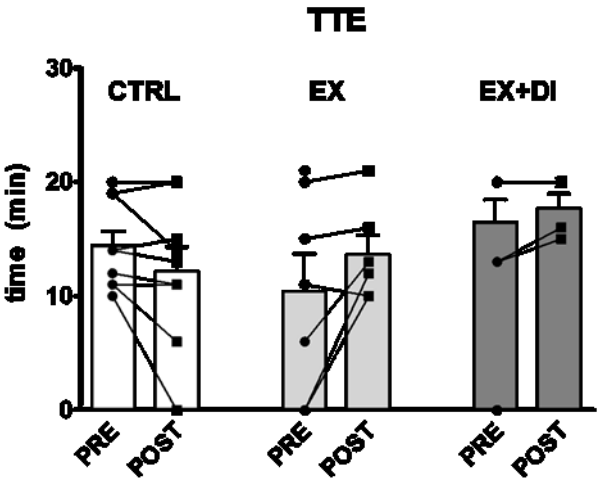


Figure 3.18. Time to exhaustion (watts).

We also evaluated $\dot{V}O_2$ as a function of power output during the incremental exercise test, and compared the results with the expected values for healthy

population, as suggested by Åstrand.[41] Individual data are shown in **Figure 3.19**.

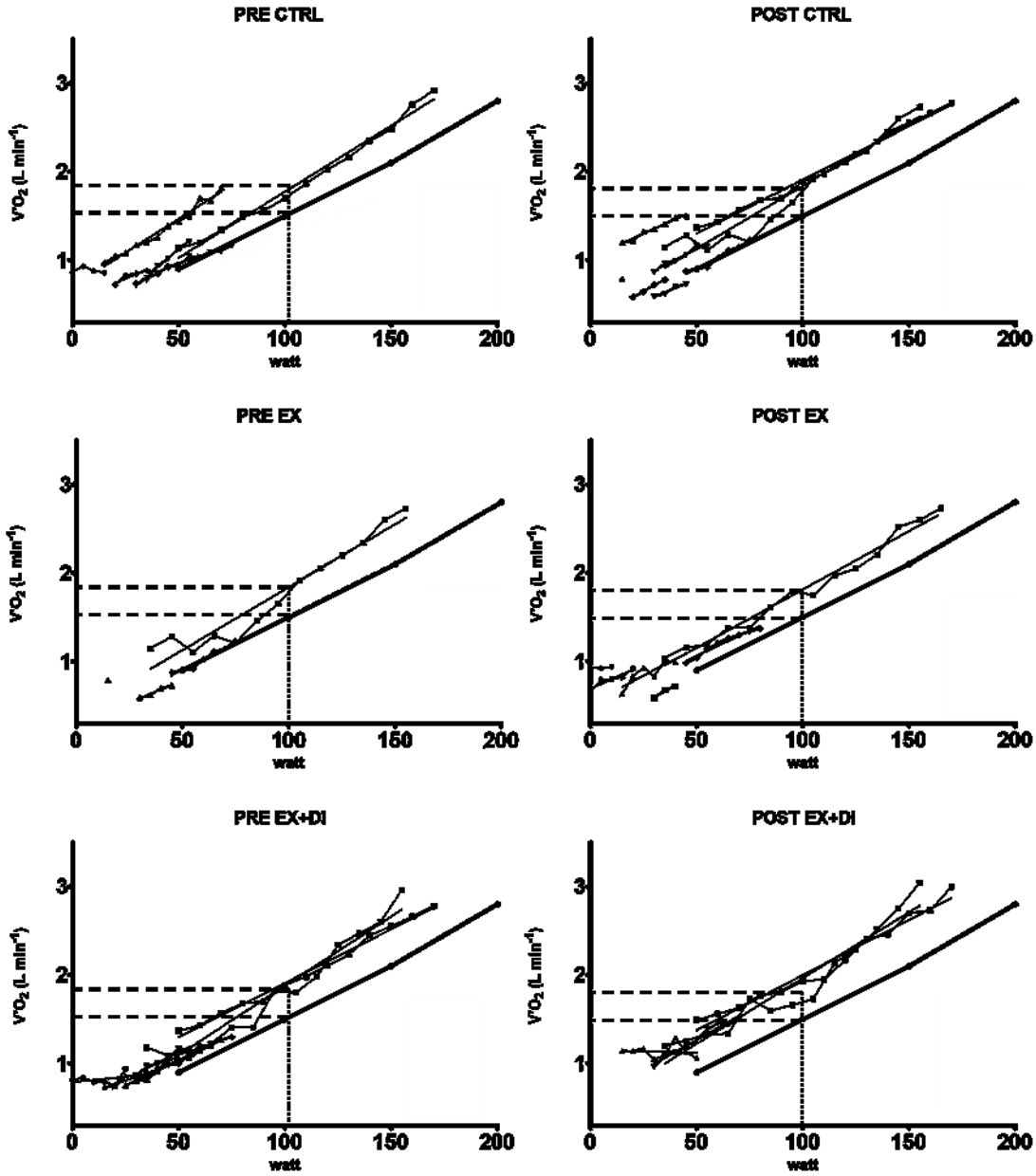


Figure 3.19. $\dot{V}O_2$ in patients (thinner lines) as a function of power output during the incremental exercise test, compared to expected values as suggested by Åstrand (thicker line).

Interestingly, in all conditions the values obtained in the patients were on the left of the regression line drawn for the reference values of healthy subjects. In other words, for the same work rate values were higher (higher O_2 cost of exercise) in the patients. Values were very similar after versus before any intervention. In other words, EX or EX+DI di not correct the higher O_2 cost of exercise observed in the patients. In all conditions the slopes of the individual regression lines (**Figure 3.20**) were not different compared to the reference value.

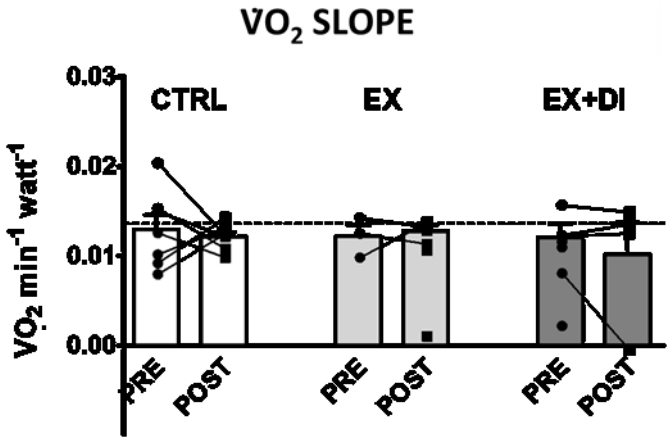


Figure 3.20. $\dot{V}O_2$ slope in patients compared to expected values suggested by Åstrand (dashed line).

Rate of Perceived Exertion

Perceived exertion was evaluated by using the Borg CR-10 Scale at the end of every minute of exercise. At the end of CWR, RPE tended to be higher after the 6-months CTRL period, and lower after both EX and EX+DI interventions, although the observed differences did not reach statistical significance. No difference was found comparing data recorded at the end of exercise (**Figure 3.21**).

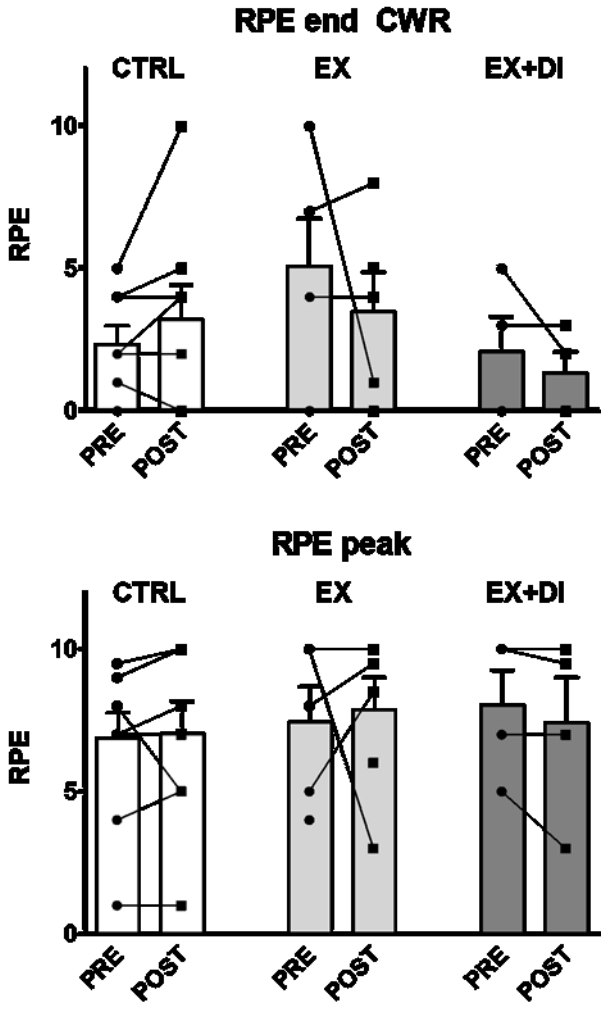


Figure 3.21. RPE at the end of CWR and at the end of exercise (peak).

Spirometry

FVC and FEV1 data obtained during the spirometry test, respectively in sitting and supine position, are shown in **Figure 3.22**, expressed as % of reference values.

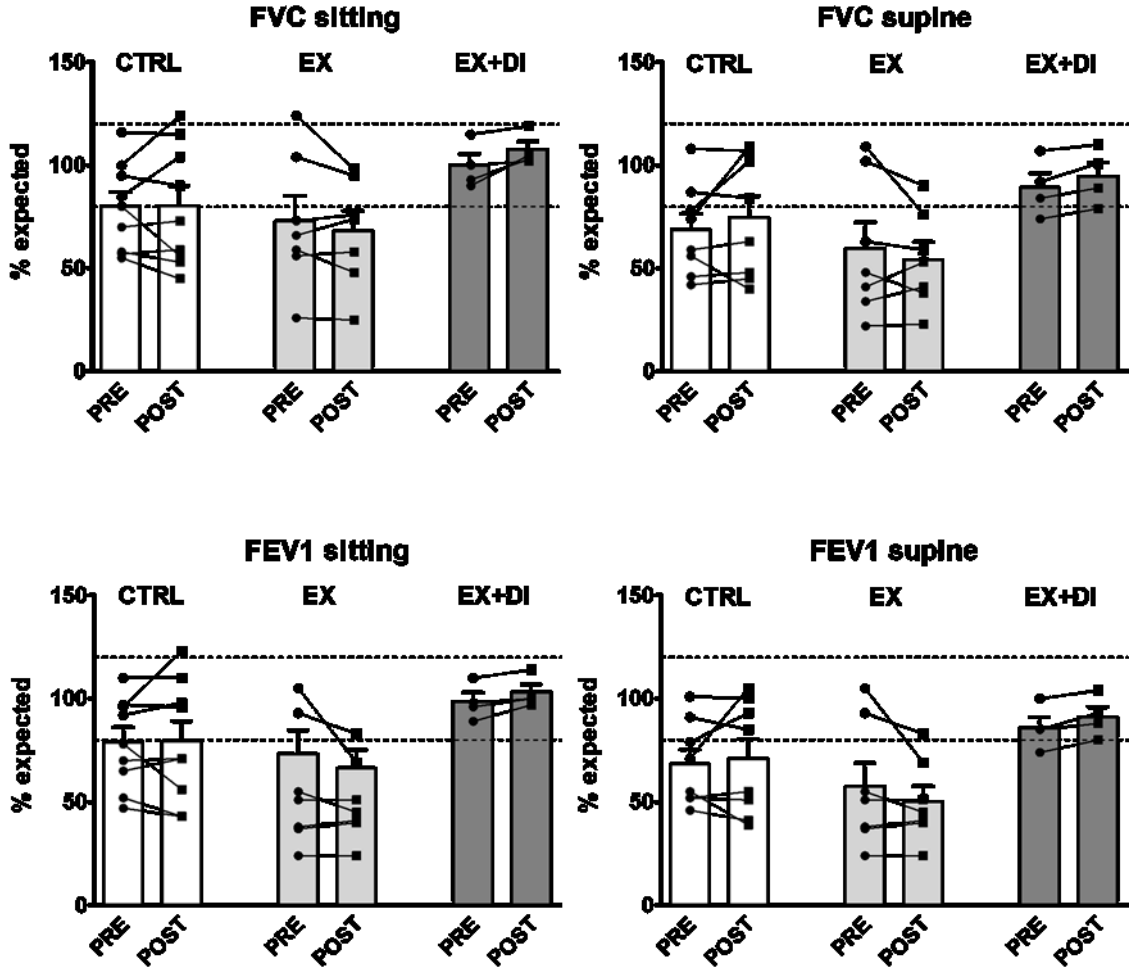


Figure 3.22. FVC and FEV1 values expressed as a % of reference values for age, sex and BMI.

All patients with FVC values below 80% of the expected value, with the exception of one, were treated with non-invasive ventilation (NIV) during the night.
No significant changes were observed in after versus before any of the experimental conditions.

We also calculated the FEV1/FVC ratio, i.e. the Tiffeneau Index (**Figure 3.23**). Mean values in patients corresponded to about 0.8, indicating the absence of any obstructive pathology.

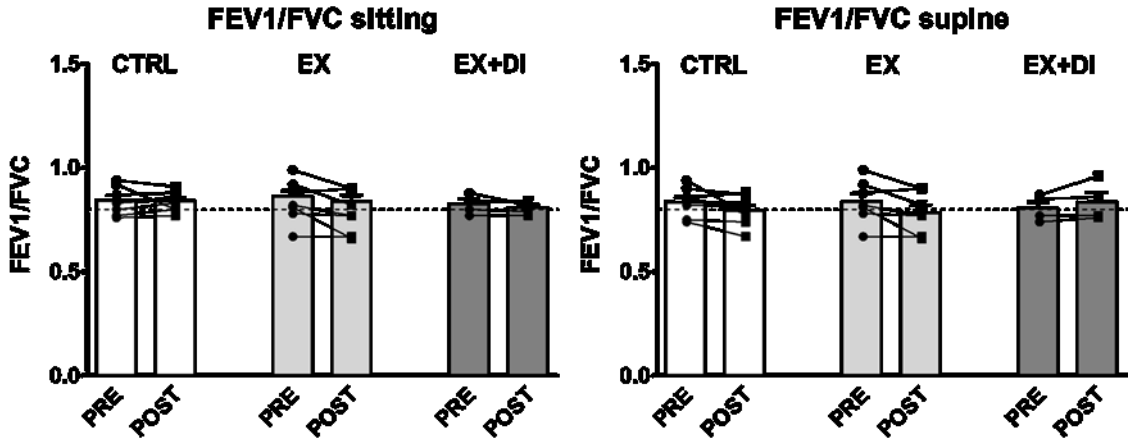


Figure 3.23. Ratio between FEV1/FVC, i.e. the Tiffeneau Index.

Furthermore, we evaluated the postural drop (ΔFVC), i.e. the change in vital capacity upon assuming the supine position. Normally, ΔFVC lies around 5%. A ΔFVC greater than 20% is thought to reflect diaphragmatic weakness.
As shown in **Figure 3.24**, although most patients had a postural drop greater than the normal value, only two subjects showed clear signs of diaphragmatic weakness. No significant differences were observed after versus before any of the interventions.

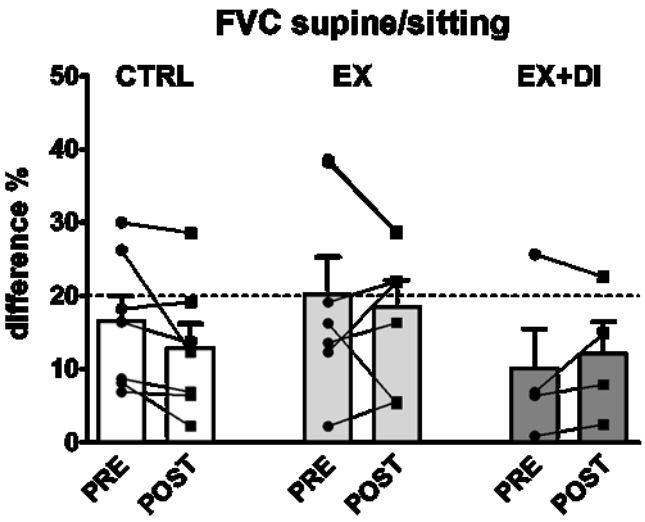


Figure 3.24. Postural drop between sitting and supine position. Expected value of 20% is identified by a dashed line.

Strength

Values of MVC in lower and upper limbs, measured by performing an isometric maximal voluntary contraction, are shown in **Figure 3.25**. No significant differences were in after versus before any of the investigated conditions.

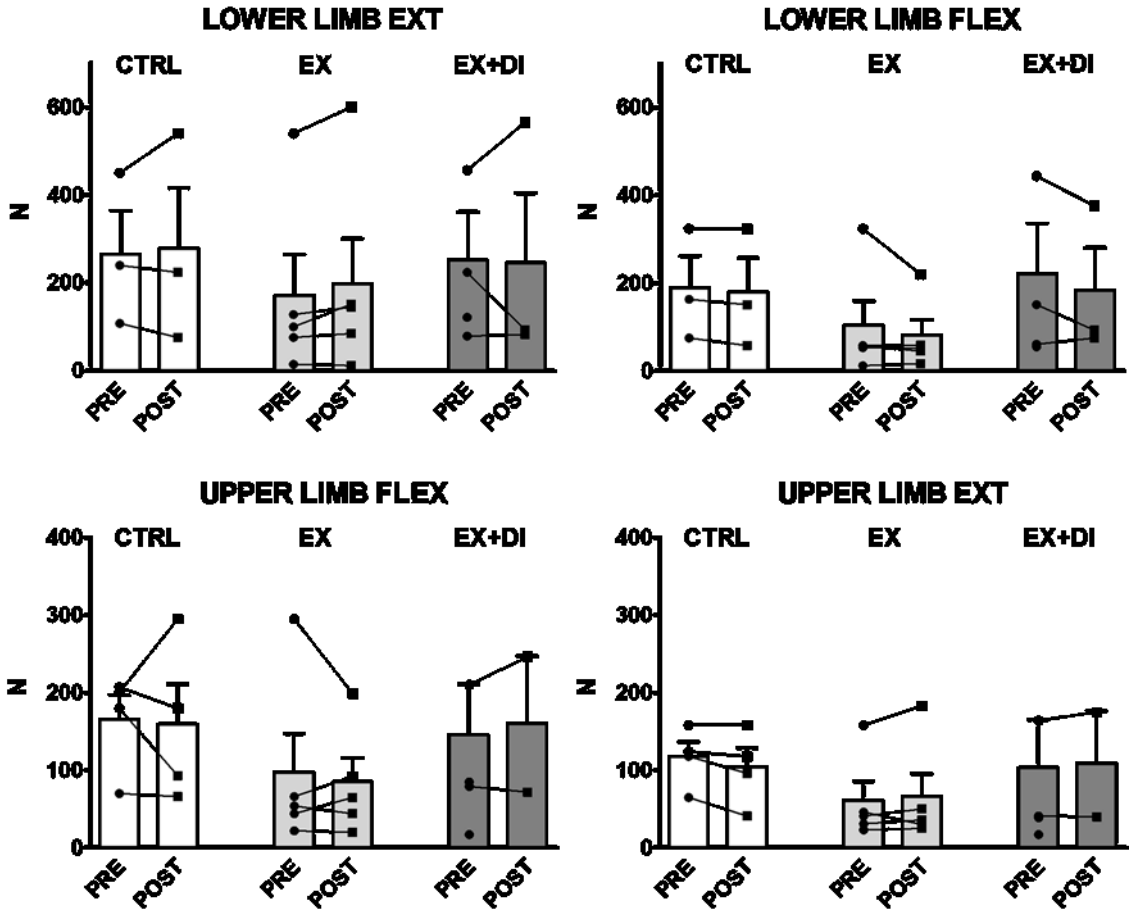


Figure 3.25. MVC in lower and upper limbs, in flexion and extension.

Furthermore, we evaluated the ratio between flexor and extensor muscles in the lower limbs, and extensor and flexor muscles in the upper limbs. In both cases the expected ratio is 2/3, corresponding to a value of 0.67. The mean values

observed in the present study, in all conditions, are pretty close to this value, as shown in **Figure 3.26**. Values in after were not significantly different compared to values in before in all experimental conditions.

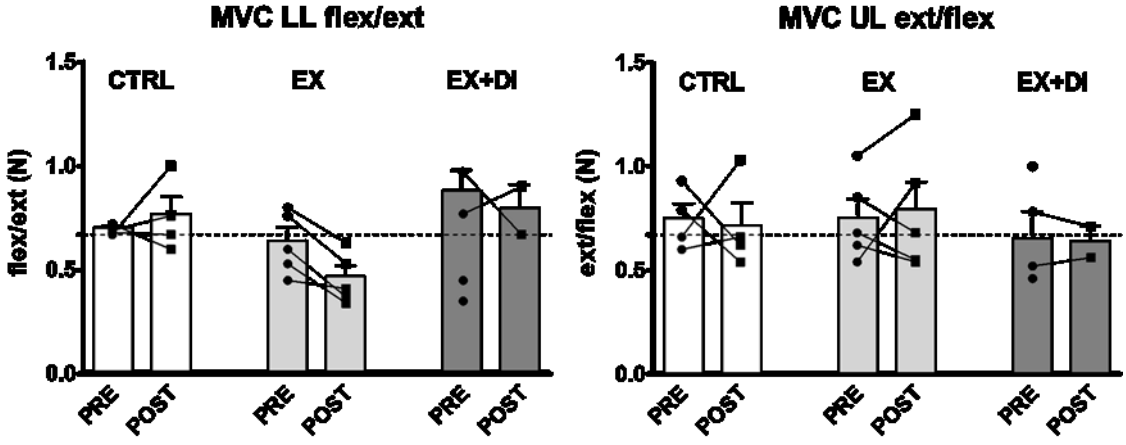


Figure 3.26. Ratio between flexor and extensor muscles in the lower limbs, and extensor and flexor muscles in the upper limbs.

6-Minute Walking Test

Results obtained during the 6MWT performed by the patients before and after the interventions, didn't show any significant difference for all experimental conditions, as shown in **Figure 3.27**.

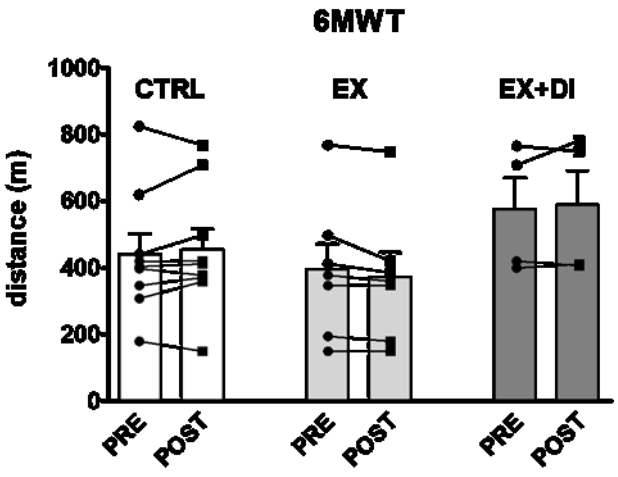


Figure 3.27. Results of the 6MWT.

Quality of life - SF-36 Questionnaire

In **Figure 3.28** we can observe that median scores of almost all the domains, as well as the Physical and Mental component scales, remained unchanged before and after CTRL. Only General health perception and Mental health showed a slight increase in the scoring.

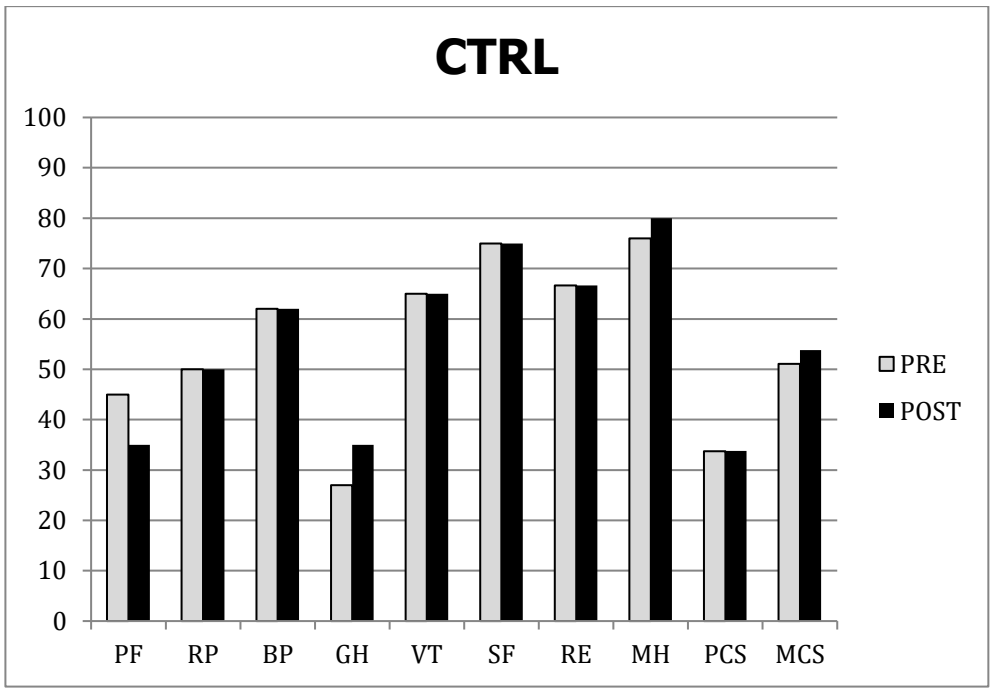


Figure 3.28. SF-36 questionnaire results, before and after CTRL. PF=Physical functioning; RP=Role physical; BP= Freedom from pain; GH=General health perception; VT=Vitality; SF=Social role functioning; RE=Role emotional; MH=Mental health; PCS=Standardized physical component scale; MCS=Standardized mental component scale.

On the other side, after the EX intervention (**Figure 3.29**), we observed an increase in Role physical and Freedom from pain scores for the Physical health domains, and a moderate increase in Social role functioning for the Mental health domains. Physical and Mental component scales remained unchanged.

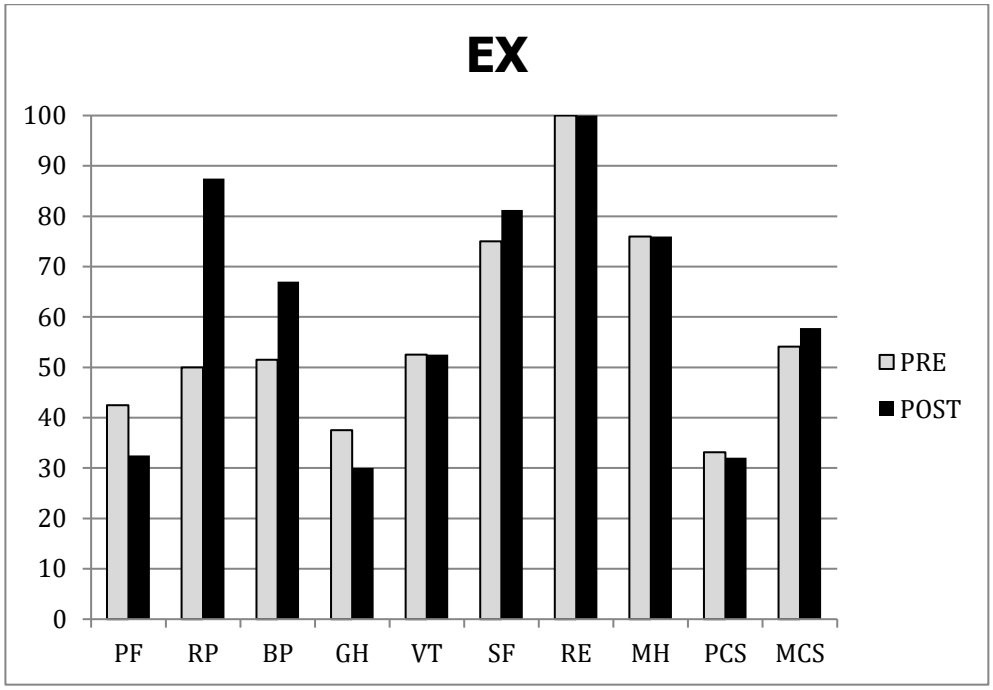


Figure 3.29. SF-36 questionnaire results, before and after EX. PF=Physical functioning; RP=Role physical; BP= Freedom from pain; GH=General health perceptions; VT=Vitality; SF=Social role functioning; RE=Role emotional; MH=Mental health; PCS=Standardized physical component scale; MCS=Standardized mental component scale.

Major changes can be observed following the EX+DI intervention (**Figure 3.30**): in fact, all Physical health domains improved, except for Role physical, which remained unchanged. All the four Mental health domains improved after the intervention. Results in single domains are confirmed by Physical and Mental component scales scores, both clearly improved after the intervention.

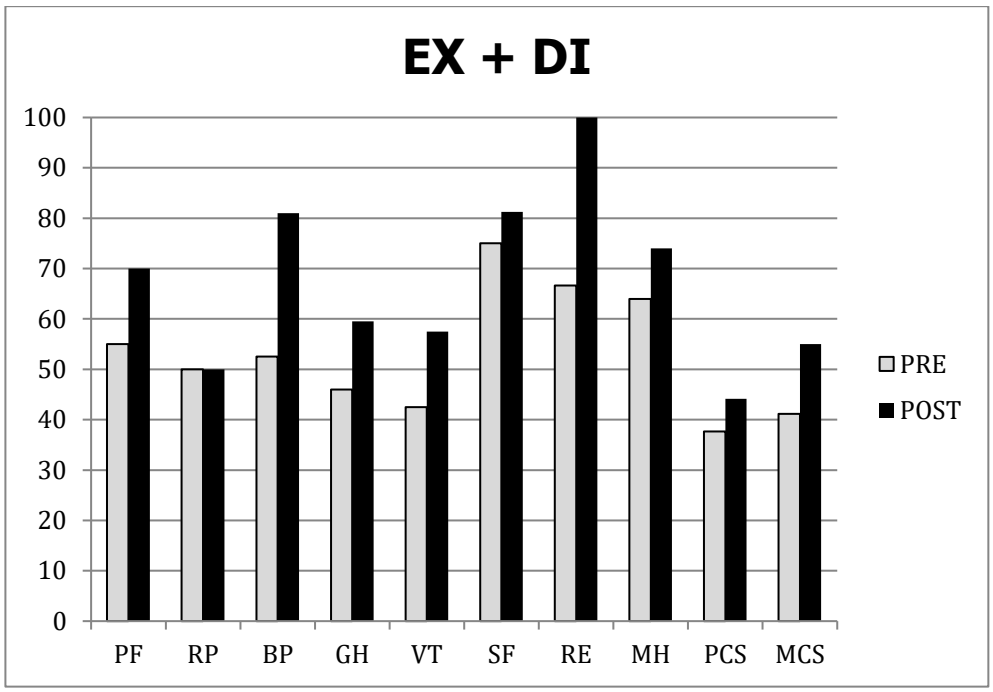


Figure 3.30. SF-36 questionnaire results, before and after EX+DI. PF=Physical functioning; RP=Role physical; BP= Freedom from pain; GH=General health perceptions; VT=Vitality; SF=Social role functioning; RE=Role emotional; MH=Mental health; PCS=Standardized physical component scale; MCS=Standardized mental component scale.

These results suggest that patients on ERT have an improved quality of life, in both physical and mental component, following a 6-month training and diet program.

Discussion

Although our data are incomplete, and the study is still ongoing, some general considerations can be elicited from our preliminary data.

The compliance with the diet was in some cases poor and perceived as difficult. On the other hand, the compliance with the training program was very good. This demonstrates that in LOPD on ERT long-term (6 months in the present study) training interventions are feasible.
The exercise and the exercise + diet interventions determined, on average, a significant positive effect in the sensation of well-being and in the scores evaluating the domains related to the quality of life.
The intervention did not have significant effects on spirometry data. Although 8 (corresponding to 60%) of our patients require assisted ventilation at night, no significant impairments of spirometry values were observed. A postural drop, reflecting diaphragm weakness, was observed only in 2 patients. The exercise and the exercise + diet interventions did not significantly change any of the investigated variables.

The analysis of measurements related to exercise tolerance are somewhat less clear and straightforward. Although the preliminary data need to be confirmed by the experiments on the remaining patients, some insights can still be derived.

A significant inter-patients variability of data was observed. In fact, as an example, $\dot{V}O_{2peak}$ mean value was 1.6 L min^{-1}, ranging from a minimum of 0.9 L min^{-1} to a maximum of 3.1 l min^{-1}, and corresponding to 55% to 102% the predicted values for age, sex and body mass. This is not surprising, considering

that the functional and clinical impairments in LOPD patients are known to vary widely from patient to patient. This factor makes more difficult to demonstrate statistically significant differences.

As expected, $\dot{V}O_{2peak}$ values were on average substantially lower than those observed in healthy control subjects. Whereas in the control condition $\dot{V}O_{2peak}$ showed a tendency to decrease during the 6-months, patients (LOPD are usually characterized by a progressive functional impairment as a function of time), in the exercise and exercise + diet condition, a tendency towards an improvement was observed. If confirmed by the remaining experiments, these observations could have a substantial significance: superimposed on ERT, exercise and exercise + diet not only interrupt the progressive decline in aerobic function, but can obtain a slight improvement.

The tendency towards an improvement after exercise and exercise + diet was described also for other variables, such as the RPE at the end of the constant work rate exercise, peak power reached at the end of the incremental test and time to exhaustion.

On the other hand, an impairment of oxidative metabolism which was not corrected by any intervention was the higher O_2 cost of exercise, which is a consequence of a reduced efficiency of oxidative metabolism. For the same VO_{2peak}, an increased O_2 cost of exercise is inevitably associated to a reduced peak work rate [42], thereby leading to a reduced exercise tolerance. In the LOPD patients the negative effects of the higher O_2 cost of exercise on exercise tolerance are further aggravated by the significantly lower than normal VO_{2peak}

values. In any case, this significant functional impairment was not corrected or even alleviated by the exercise and by the exercise + diet interventions.

In conclusion, the intervention proposed has shown to be feasible and the patients reported an improvement in quality of life following training, to the point that some of them requested a new training program after finishing the study. The results suggest a general improvement after the intervention, contrasting the worsening, due to the progressive characteristic of the disease.

Further information will be gained by the analysis of the data not available at the moment.

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CONCLUSIONS

As mentioned in the Introduction, exercise tolerance (that is the capacity to sustain physical exercise) is an important determinant of the clinical picture of many chronic diseases, and it significantly affects the patients' quality of life. Moreover, for several chronic diseases it has been demonstrated that variables evaluating exercise tolerance represent very important prognostic factors. From what mentioned above it should be evident that the possibility to evaluate exercise tolerance precisely, non-invasively and quantitatively would be of utmost relevance for clinicians. The possibility to identify the organ(s), the system(s) or the mechanism(s) mainly responsible for the functional impairment would represent another extremely useful information, also for the definition of specific therapeutic and/or rehabilitative interventions. In order to do all this, the proposed methods need to be non-invasive, in order to be performed serially on the same patient and to allow an evaluation of the evolution of the disease, and they also need to be relatively simple to perform, in order to allow for their dissemination.

Within this *scenario*, in recent years methods originally developed in the exercise physiology laboratory were "taken to the patient", according to a translational approach, and applied in the evaluation in chronic diseases. The two works presented in the present thesis represent examples of this approach.

In the first study we evaluated some physiological adaptations to exercise in patients with spinal cord injuries (paraplegics and tetraplegics ~~with incomplete lesions~~). A lot of work has been carried out on these patients over the years, and our main focus was to evaluate the O_2 cost of wheelchair propulsion. No

resistance was set on the wheels of the wheelchair, in order to simulate everyday's utilization. The most relevant observation was that, when investigated at the same absolute speed of control subjects (physiotherapists, amputees or subjects with pathologies expert in the use of a wheelchair), paraplegic and tetraplegic patients show an O_2 cost (pulmonary O_2 uptake divided by propulsion speed, in other words pulmonary O_2 uptake per unit of covered distance) which is not significantly different from that of the controls. This occurs despite profound differences in the pattern of muscle activation (as demonstrated by electromyography) and propulsion pattern of the wheelchair push (as demonstrated by video analysis). The O_2 cost of exercise is an important determinant of exercise tolerance. For the same maximal aerobic power, a higher O_2 cost of exercise is inevitably associated with an impaired maximal performance. Our study demonstrated that the O_2 cost of exercise does not represent a significant limiting factor for performance in paraplegic and tetraplegic patients. Despite their documented neuromuscular impairment, a sort of a "chronic training" allows these patients to be as "efficient", at the same absolute speed, compared to controls. On the other hand, in our study we confirmed other impairments previously described in these patients, such as a reduced maximal propulsion speed, a reduced maximal aerobic power, a reduced maximal heart rate. We also observed differences in muscle activation as well as in preferred pushing strategy. This last point, together with the data of the O_2 cost of propulsion, suggest that patients developed their own best strategy to push in an as efficient way as possible.

The second study was performed on patients with late onset Pompe disease (LOPD). This disease is one of few genetic diseases for which a specific treatment is available, with the administration of the "missing enzyme" GAA (acid alfa-

glucosidase) obtained with recombinant human GAA. Many patients have been chronically treated with enzyme replacement therapy (ERT) for several years. The problem is that after a year of treatment, the initial positive effects of ERT seem to wane. The idea behind the study was to see if the adoption of a structured and supervised program of home-based exercise training, associated or not with an hyperproteic diet, would prevent or delay the loss of efficacy of ERT in LOPD. As mentioned in the thesis, the 3-year study is still ongoing, and only preliminary results were available for this thesis. Two clinical centers have been involved. Each intervention (control, exercise, exercise + diet) lasted for 6 months; adequate wash-out periods were observed between interventions. The preliminary results show a very good compliance by the patients to the proposed interventions. Several aspects related the quality of life improved versus the control condition. For some variables related to exercise tolerance and oxidative metabolism an improvement seems to be present with the interventions. On the other hand, no effects on muscle force and spirometry values (LOPD is characterized by an impairment of respiratory muscles as well) were observed. These findings need to be confirmed by the analysis of data of the last patients undergoing the interventions. From the preliminary data it is not clear yet if the superposition of the hyperproteic diet to the exercise intervention enhanced or not the effects on oxidative performance during exercise obtained by exercise training alone.

PUBLICATIONS

O_2 cost of wheelchair propulsion and propulsion strategy in tetraplegic and paraplegic patients.

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The authors declare no conflict of interest.

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ABSTRACT

Objective To evaluate the O_2 cost of wheelchair propulsion and propulsion strategy in patients with spinal cord injury (SCI) in relation to lesion level.

Design: Case series, functional evaluation.

Setting: Exercise physiology laboratory, physical medicine and rehabilitation hospital.

Participants: Sixteen patients with chronic SCI (9 paraplegics, P; 7 tetraplegics, T) and nine control (CTRL) subjects.

Interventions: Measurements of O_2 cost of wheelchair propulsion (by indirect calorimetry), heart rate (HR), propulsion strategy (by video-kinematic analysis) during exercises on an ergometer with no resistance set on rollers, at a self-selected habitual speed and at the maximal sustainable speed (MS). CTRL performed 3 exercises: one at the MS for T, one at the MS for P, and one at their own MS.

Main Outcome Measures: Pulmonary O_2 uptake ($\dot{V}O_2$); O_2 cost of wheelchair propulsion; propulsion strategy; HR; propulsion velocity.

Results: Peak $\dot{V}O_2$, peak HR, velocity and stroke frequency at MS were linearly related with lesion level (higher level, greater impairment); values in T were lower than in P or CTRL, whereas no differences were observed between P and CTRL. Propulsion pattern was different in the patients (particularly in T) *vs.* CTRL. At the same velocity, the O_2 cost of wheelchair progression was not different in T or in P *vs.* CTRL.

Conclusions: Despite the different neuromuscular activation and propulsion pattern, the impaired skeletal muscle oxidative metabolism and cardiovascular function, in T and P oxidative economy (oxidative energy spent per unit of distance) during wheelchair propulsion was not impaired. Maximal performance was impaired in T, but not in P.

Key Words Spinal cord injury; wheelchair O_2 cost; wheelchair propulsion strategy.

Abbreviations

AR	Arcing pattern
ASIA	American Spinal Injury Association
CTRL	Control subjects
CTRL-T	Controls' trial at the mean speed sustained by T during MS
CTRL-P	Controls' trial at the mean speed sustained by P during MS
CTRL-MS	Controls' trial at their own maximal sustainable speed
DL	Double loop pattern
HR	Heart rate
HR_{peak}	Peak heart rate
MS	Maximal sustainable speed
P	Paraplegic patients
SC	Semicircular pattern
SCI	Spinal cord injury
SL	Single loop pattern
SSS	Self-selected speed
T	Tetraplegic patients
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}E$	Ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_{2peak}$	Peak oxygen consumption

Introduction

Spinal cord injury (SCI) is a severe condition leading to impairments of lower and at times upper limbs movements, as well as (in patients with lesions above T6) of cardiovascular and autonomic nervous system functions. The higher the level, and the more complete the lesion, more serious is the loss in function. Apart from the effects deriving from profound skeletal muscle deconditioning and atrophy, as well as deficits in sensibility and thermoregulatory capacity[1-4], in patients with high lesion levels the sympathetic stimulation to the myocardium and vasomotor function may be impaired. This leads to a reduced maximal cardiac output and capacity to redistribute blood according to peripheral needs, impairing the overall capacity to transport O_2 to the working muscles. Beyond the capacity of force generation[4], also skeletal muscle oxidative metabolism can be severely impaired in SCI patients[5]. This usually results in an impaired aerobic function and exercise tolerance during prolonged exercise.[3-9] Peak O_2 uptake ($\dot{V}O_{2peak}$), index of maximal aerobic function, is substantially lower in these patients than in age-matched able-bodied subjects, and the decreased $\dot{V}O_{2peak}$ is known to be related to lesion level, being more pronounced in higher lesions.[2-4,10] Other factors may influence muscle function and exercise tolerance in SCI patients, such as sex, age, time since injury and regular participation in physical activity.[3,8,11-15] The reduced exercise tolerance negatively affects the patients' quality of life and exposes them to the cardiovascular and metabolic consequences of inactivity and deconditioning.[1,7,14,16,17]

SCI patients are mostly forced to use wheelchairs, which may be inefficient compared to other forms of locomotion.[18-22] This inefficiency could be related to impairments such as muscle recruitment limitations in patients with SCI, but also to intrinsic biomechanical characteristics

of wheelchair propulsion, possibly related to factors such as stroke frequency[23], propulsion pattern[24], and experience.[25] A reduced efficiency is inevitably associated with a reduced exercise tolerance.[26] A better knowledge of these aspects would be useful in the development of rehabilitative interventions.

Upon such premises, in the present study we evaluated the O_2 cost of wheelchair propulsion in paraplegic (P) and tetraplegic (T) SCI patients. We investigated only the contribution of aerobic metabolism to energy expenditure, since during prolonged exercise it represents by far the most important contributor to the energy yield, and is the only one which can be directly and non-invasively investigated. We evaluated the patients during wheelchair propulsion on a roller ergometer with no resistance set on the rollers, to evaluate an everyday type of locomotion. A group of able-bodied controls and lower limbs amputees experienced in the use of wheelchairs acted as controls. We hypothesize a different propulsion pattern and a higher O_2 cost of wheelchair propulsion in P and T patients versus the controls.

Methods

Subjects

Sixteen patients with complete and incomplete chronic (at least 1-year post-injury) spinal cord injury (SCI) and ten control subjects (CTRL) took part in this study. The patients were full-time manual wheelchair users, with full or partial use of their arms and hands. Inclusion criteria were male sex, diagnosis of SCI, clinical stability and experience in manual wheelchair usage.

126 Some characteristics of the patients and CTRL are given in **Table 1**. No significant differences
127 in body mass index (BMI) (kg m^{-2}) was observed between groups. In tetraplegics (T) the lesion
128 level was from C4 to C8, in paraplegics (P) from D4 to D12. In 3 P patients the lesion was above
129 T6. The neurological deficits resulting from SCI, quantified according to the International
130 Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) published by the
131 American Spinal Injury Association (ASIA)[27], time since injury and regular participation in
132 physical activity (approximately 3-5 hours weekly) are also reported in the Table.
133 Exclusion criteria were signs or symptoms of diabetes or of any major cardiovascular,
134 respiratory or orthopaedic disease contraindicating or significantly interfering with the tests.
135 Patients were treated with oxybutynin, analgesic and muscle relaxants. No patients or CTRL
136 were treated with beta-blockers.
137 CTRL were physical therapists, lower limbs amputees or patients with pathological conditions,
138 all experienced in wheelchair usage in daily and/or sport activities.
139 The participants provided signed consent statements, after being fully informed about the
140 purposes and testing procedures of the investigation, which were approved by the ethics
141 committee of the Hospital where experiments were performed. All procedures were in
142 accordance with the recommendations set forth in the Helsinki Declaration of 1975, revised in
143 2013.
144
145 *Exercise protocol*
146
147 Subjects were allowed time to gain familiarity with the investigators and the experimental
148 arrangement, were carefully instructed about the procedures, and were familiarized with the
149 protocol using short practice runs. All pushing trials were performed on a computerized roller
150 system[a], with no braking resistance. The system consists of two metal rollers mounted on a

metal base. The front wheels of the wheelchair were fixed to the metal base and the back of the wheelchair was fixed with straps on the rear frame of the roller system, to minimize movements of the wheelchair during the pushing trials. Velocity was recorded through the computer connected to the roller system. All SCI subjects used their own everyday wheelchairs during the test. For CTRL subjects, a standard everyday wheelchair of the right measure for each participant was used.

After a stationary start, SCI subjects performed two 4-min exercises: the first one at the self-selected speed (SSS), and after 2 minutes of recovery (or when heart rate (HR) was back to resting values) subjects were asked to perform a maximum effort trial at their maximal sustainable speed (MS).

CTRL subjects performed three 4-min exercises: one at the mean speed sustained by T during MS (CTRL-T), one at the mean speed sustained by P during MS (CTRL-P), and one at their own maximal sustainable speed (CTRL-MS). Experiments in CTRL were carried out after those in T or P.

Measurements and analyses

Pulmonary ventilation, O_2 uptake ($\dot{V}O_2$) and CO_2 output ($\dot{V}CO_2$) were determined by means of a portable metabolic unit[b], which provides a 3-breath average of variables through telemetric transmission. This system has been validated in previous studies.[28,29] Prior to testing, the instrument was calibrated according to the manufacturer's instructions. Mean values over each minute were calculated. The O_2 cost of wheelchair propulsion, expressed as aerobic energy expenditure ($\dot{V}O_2$ above rest per unit of body mass) per unit of covered distance, was calculated as net $\dot{V}O_2$ divided by velocity. Aerobic energy expenditure was also expressed in joules by assuming a respiratory exchange ratio 0.96.[30]

HR was recorded by means of a portable heart rate monitor.[c] HR values were averaged over 1-min periods. Mean values of variables determined during the last minute at maximal speed were taken as "peak" values.

A video camera[d] recorded data at a sampling rate of 60 frames per second for the kinematic two-dimensional analysis. The camera was placed on a tripod on the right side of the subject, at a height of 0.5 meters from the floor, and at a distance of 3 meters from the participant. Three anatomical landmarks were identified: acromioclavicular joint, lateral epicondyle approximating elbow joint axis and ulnar styloid process.[31]

A triaxial accelerometer[e] was placed on subject's wrist and secured with tape to record forearm movements in three orthogonal directions, and synchronized with EMG to define stroke frequency and stroke cycles.

A calibration frame was used for spatial reference. Next, using a video analysis software[f], a cartesian coordinate system was created, using the hub of the wheel as origin of the axis, and 6 complete stroke cycles were analyzed during the last 10 seconds of every minute.

A computer program was created[g] to display all pattern plots through a digital filtering process.[32] Pattern plots were displayed at random to four researchers who classified them into one of the four types previously defined[33-36]: single loop (SL), identified by the hand rising above the handrim during the recovery phase; double loop (DL), identified by the hand rising first above the handrim and then crossing over and dropping under the handrim during the recovery phase; semicircular (SC), identified by the hand falling under the handrim during the recovery phase; arcing (AR), identified by the hand following an arc along the handrim during the recovery phase (**Figure 1**).

Statistical analyses

Results were expressed as mean values ± standard deviation (SD). Comparisons between two groups were performed by two-sided Student's t-test. Comparisons between more than two groups were performed by one-way ANOVA; a Bonferroni's post-hoc test was used when significant differences emerged at ANOVA. Data fitting by linear regressions was performed by the least-squared-residuals method. The level of significance was set at $P<0.05$. Statistical analyses were performed by a software package.[h]

Results

Individual values of velocity of wheelchair propulsion during MS, VO_{2peak} and HR_{peak} obtained in T and in P are reported as a function of lesion level in **Figure 2**. A statistically significant linear relationship was observed: with higher lesion levels, lower values of velocity, $\dot{V}O_{2peak}$ and HR_{peak} were found. The horizontal dashed lines represent the mean values obtained in T, P and CTRL. Velocity was significantly higher in CTRL (7.2±2.1 km h^{-1}) and in P (7.0±1.5) than in T (4.0±1.2); no significant differences were observed between CTRL and P. The same conclusions can be drawn for $\dot{V}O_{2peak}$ (22.4±6.7 ml kg^{-1} min^{-1}, 19.0±5.9 and 10.6±3.9, respectively, in CTRL, P and T) and HR_{peak} (167.1±13.5 beat min^{-1}, 161.3±12.7 and 117.5±25.4, respectively, in CTRL, P and T).

No significant relationship was observed between the peak O_2 cost of propulsion and lesion level (**Figure 3**). Peak O_2 cost was expressed as J kg^{-1} m^{-1} (left y axis) and as ml O_2 kg^{-1} m^{-1} (right y axis). The peak O_2 cost was independent of lesion level, and mean values were not significantly different between the 3 groups.

Group mean (± SD) data (calculated every minute) of velocity, $\dot{V}O_2$ and HR obtained in T and P during the 4 minutes at SSS and the subsequent 4 minutes at MS are shown in **Figure 4**. For CTRL, the data obtained at the same velocity of MS in T (CTRL-T) and in P (CTRL-P), and at their own MS, are also shown (dashed lines). For the statistical analyses, a single x±SD value was calculated during the 4 minutes of SSS and MS.

During SSS all variables were not significantly different in T and in P. Panel A demonstrates that the experimental protocol was successfully followed: velocities in T and in P were substantially identical to those in CTRL-T and CTRL-P, respectively. In patients, velocity, $\dot{V}O_2$ and HR values in MS were significantly higher *vs*. SSS; in T the difference did not reach statistical difference. During MS, velocity, $\dot{V}O_2$ and HR were higher in CTRL *vs.* T, and in P *vs.* T, whereas no significant differences were observed between CTRL and P.

Homologous data of those described in Figure 4 are shown in **Figure 5** for the O_2 cost of wheelchair propulsion. Once matched for velocity the O_2 cost was not significantly different in T vs. CTRL-T, nor in P vs. CTRL-P.

Stroke frequency data are presented in **Figure 6** as a function of lesion level and as group mean values as a function of time. Data were linearly related with lesion level and values in T were significantly lower than those in P or in CTRL; no statistically significant difference was found between the last two groups. Stroke frequency was not significantly different between CTRL-T and T (MS), nor between CTRL-P and P (MS). No differences were observed between T and P at SSS. Stroke frequency as a function of velocity is shown in **Figure 7**. Data are linearly related: subjects using lower frequencies, reached lower velocities.

Figure 8 shows that at SSS propulsion patterns were very similar in the two groups: patients used predominantly DL and SC, whereas only a minority used AR. When examined at MS, the propulsion pattern in T was very different from that of CTRL-T, with a much greater use of DL by T. Less pronounced differences were observed between P at MS and CTRL-P (only one CTRL utilized SC). Comparing the groups at their own MS, P and CTRL utilized only AR and SL, while T mainly chose DL.

Discussion

The main observation of the present study was that the O_2 cost of wheelchair propulsion was not significantly different in SCI patients compared to CTRL. This occurred despite the very different propulsion pattern in the patients (particularly in T) *versus* that observed in CTRL. In other words, despite the consequences of SCI, such as impaired upper limbs muscles activation, altered propulsion pattern and impaired cardiovascular and muscular adjustments[4,5,7,8,14], the O_2 cost of propulsion was unaltered compared to CTRL. This remarkable observation implies that the impairments observed in SCI do not significantly affect the oxidative economy (oxidative energy expenditure per unit of covered distance) of wheelchair propulsion. Besides being of interest from a basic science point of view, these findings are of interest in terms of exercise prescription and the functional evaluation in these patients.

In the present study no resistance was put on the rollers of the ergometer, in order to closely simulate the propulsion during activities of everyday life. The rollers system offered a rolling resistance similar to that on natural surfaces, which is made up by the mass of the subject and of the wheelchair, the weight distribution between the front casters and the rear wheels, the

size and type of tires and the surface on which the wheelchair is used.[37] As a note of caution it should be recognized the above-mentioned observations could apply only to the adopted exercise protocol, and not to incremental exercise protocols.

Only oxidative metabolism was investigated in the present study. An evaluation of the contribution of the other bioenergetics mechanisms (anaerobic glycolysis-glycogenolysis, phosphocreatine splitting) would require assumptions, invasive measurements, or measurement protocols incompatible with wheelchair propulsion. Instead of the "energy cost" of propulsion we discuss only of the "O_2 cost". This intrinsic limitation should be relatively minor, however, since 4 minutes-exercises were investigated, in which the contribution of oxidative metabolism to the overall energy expenditure is overwhelming.

The unchanged O_2 cost of wheelchair propulsion observed in SCI patients does not obviously mean that SCI did not affect performance during wheelchair propulsion. Maximal velocity of propulsion (as well as $\dot{V}O_{2peak}$ and HR_{peak}) were indeed, as expected[4,9,17,38,39], linearly related to lesion level: higher lesion levels were associated with more pronounced impairments.
All SCI patients completed the 4 minutes at their maximal sustainable speed, which was significantly higher in P than in T, whereas it was not significantly different between P and CTRL. SSS, which was presumably close to the speed utilized during patients' everyday life, was not significantly different in T and P. In T SSS was about 50% of the patients' MS, whereas in P this percentage was about 40%, suggesting that both T and P, at their SSS, have a significant functional reserve. It is also noteworthy that the maximal performance in P was not significantly different from that in CTRL.

In the present study the subjects were free to utilize their preferred stroke frequency, as the freely chosen stroke frequency has shown to be the more economical.[23] Values of this variable were also linearly related to the lesion level and velocity: a lower stroke frequency was associated with higher lesion levels and lower velocities. Data for CTRL lie very close to the regression line drawn for the patients. Thus, stroke frequency was presumably an important determinant of velocity, and the inability of T to reach elevated stroke frequencies presumably contributes significantly to their impaired peak performance.

During wheelchair propulsion four main different propulsion patterns can be chosen, as previously described[15,31,33-36,40] and discussed in Methods. Previous studies show controversial results, and no firm conclusions can be made in terms of which patterns are preferentially utilized by SCI or able-bodied subjects.[15,31,33-36,40] Confounding factors related to methodological differences between studies such as speed, type of wheelchair, preferred or imposed frequency or pattern, residual muscle function in patients and previous experience in wheelchair propulsion by the control subjects, could, at least in part, explain these controversial results.[25,41,42] In previous studies[31,33], SC pattern was related with a reduced push frequency and more time spent during the push phase in relation to the recovery phase, and this pattern is recommended for everyday propulsion by clinical guidelines, because of its better biomechanics.[43] In the present study patients and controls were free to choose their own pattern of propulsion. Only at SSS a minority of patients used the SC pattern; a minority of CTRL utilized SC, but only when performing CTRL-T and CTRL-P trials. Controversial results have been previously described about AR pattern.[24,31,35,40] In our study AR was the mostly used by P and CTRL at MS. During SSS the distribution of patterns in T and P was quite similar: SC, DL and a minority of AR. During MS the distribution of patterns in the two groups of patients was quite

different: about equal percentages of AR, DL and SL in T, a substantial majority of AR and a small minority of SL in P. CTRL at their own MS utilized AR and SL, similarly to P.

The present study is underpowered to determine the presence of relationships between propulsion patterns, the O_2 cost of propulsion and other variables.

Conclusions

In the present study, despite the presence of significantly different propulsion patterns, particularly between CTRL and T, the O_2 cost of wheelchair propulsion was not significantly different in patients *vs.* CTRL. In other words, although the presumably different neuromuscular activation, the impaired skeletal muscle oxidative metabolism and cardiovascular function[4,7,16,17], oxidative economy in T and P during wheelchair propulsion was not impaired. Maximal performance (velocity, stroke frequency, peak HR, peak $\dot{V}O_2$) was linearly related to lesion level (lower values with higher lesions), and the values in T were lower than in P and CTRL, whereas no significant differences were observed between P and CTRL. In other words, maximal performance during wheelchair propulsion was impaired in T, but not in P.

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455 **Suppliers**
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458 a. Ergo 1, STI - 001, Sti Engineering srl, San Daniele del Friuli (UD), Italy
459 b. MedGraphics VO2000, St. Paul, MN, USA
460 c. Polar S810, Polar Electro Oy, Kempele, Finland
461 d. Nikon J1 MODEL, 1 NIKKOR VR 10 – 30mm
462 e. BIOPAC TSD 109, Biopac Systems Inc, Santa Barbara, CA, USA
463 f. Kinovea v 0.8.24
464 g. Matlab, Mathworks Inc., MA, USA
465 h. GraphPad Prism v. 5.0, GraphPad, CA, USA

FIGURE 1. Four classic propulsion patterns identified from the hand motions of manual wheelchair users: A. Single loop (SL), identified by the hand rising above the handrim during the recovery phase; B. Double loop (DL), identified by the hand rising first above the handrim and then crossing over and dropping under the handrim during the recovery phase; C. Semicircular (SC), identified by the hand falling under the handrim during the recovery phase; D. Arcing (AR), identified by the hand following an arc along the handrim during the recovery phase. The origin of the axis corresponds to the hub of the wheel. The dashed line is the path followed by the hand, and the arrows indicate the direction the hand moves.

FIGURE 2. Individual data as a function of injury level: (A) Velocity (km h^{-1}); (B) $\dot{V}O_{2peak}$ (mL kg^{-1} min^{-1}); (C) HR_{peak} (beats min^{-1}). Linear regression lines fitting the data are also shown. Horizontal dashed lines indicate mean values for T, P and CTRL. The dotted vertical line divides the patients into T and P.

FIGURE 3. Individual data of O_2 cost of wheelchair propulsion expressed as J kg^{-1} min^{-1} (left y axis) and as ml O_2 kg^{-1} min^{-1} (right y axis) at peak velocity as a function of injury level. Horizontal dashed lines indicate mean values for T, P and CTRL. The dotted vertical line divides the patients into T and P.

FIGURE 4. Group mean (±SD) data, calculated every minute as a function of time during the 4 minutes at self-selected speed (SSS) and the subsequent 4 minutes of maximal speed (MS): (A) velocity (km h^{-1}); (B) $\dot{V}O_2$ (mL kg^{-1} min^{-1}); (C) HR (beats min^{-1}). Full symbols and plain lines indicate T (circles) and P (squares) mean values in every minute of exercise, while empty symbols and dashed lines indicate CTRL mean values.

FIGURE 5. Group mean (±SD) data (calculated every minute) of O_2 cost of wheelchair propulsion expressed as J kg^{-1} min^{-1} (left y axis) and as ml O_2 kg^{-1} min^{-1} (right y axis) as a function of time during the 4 minutes at self-selected speed (SSS) and the subsequent 4 minutes of maximal speed (MS). Full symbols and lines indicate T (circles) and P (squares) mean values in every minute of exercise, while empty symbols and dashed lines indicate CTRL mean values.

FIGURE 6. (A) Mean stroke frequency data as a function of lesion level; (B) group mean (±SD) data (calculated every minute) as a function of time.

FIGURE 7. Mean stroke frequency data as a function of velocity in patients and controls.

FIGURE 8. (A) Pattern distribution of wheelchair propulsion in % in T and P patients at SSS; (B) pattern distribution in % in T, P and CTRL at MS. See text for further details.

Figure 1.

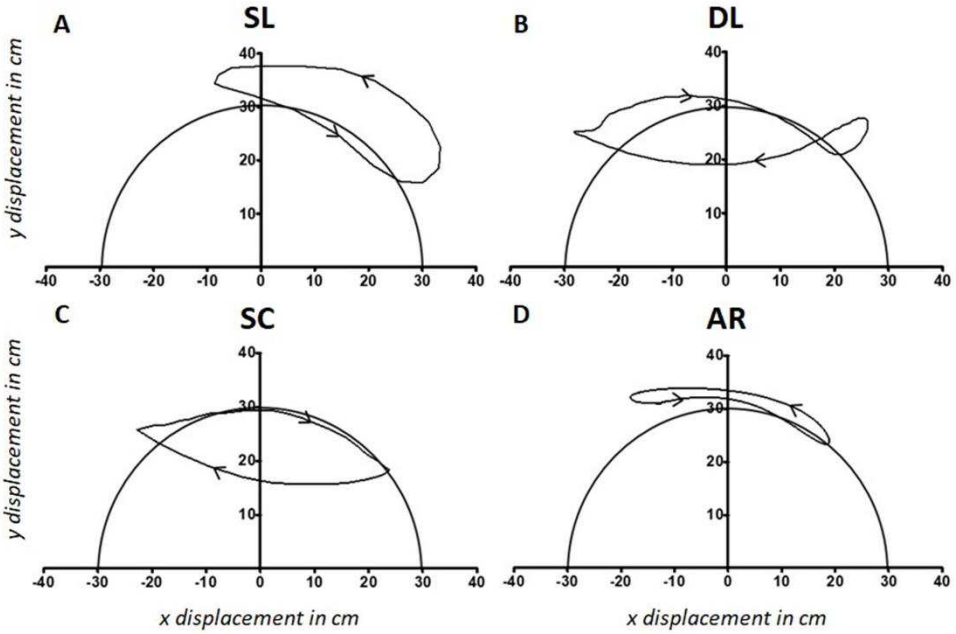

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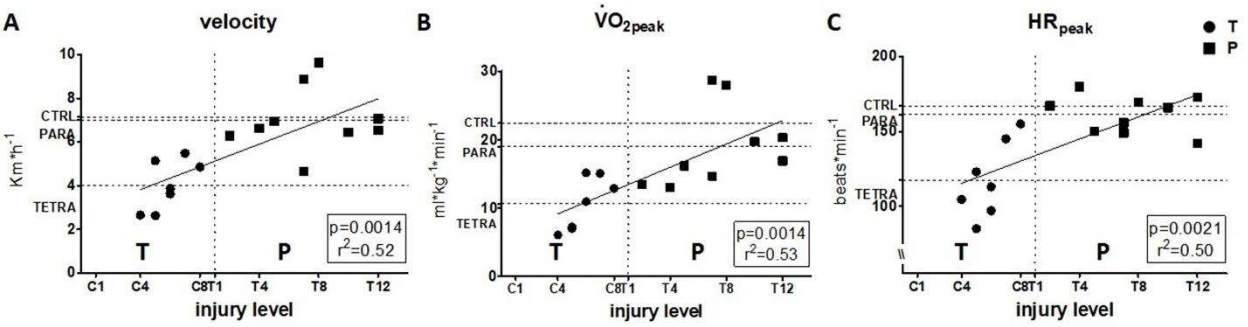
Figure 2.



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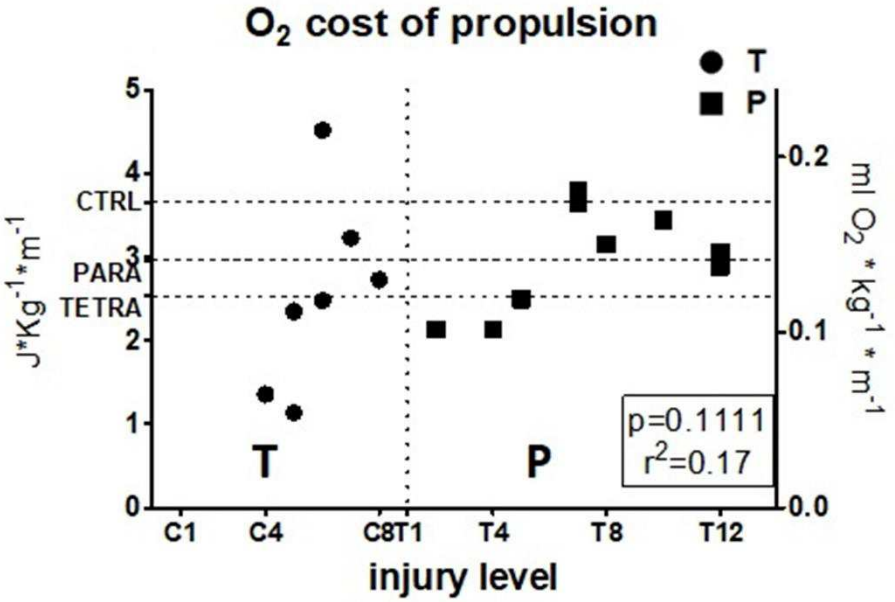

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Figure 4.

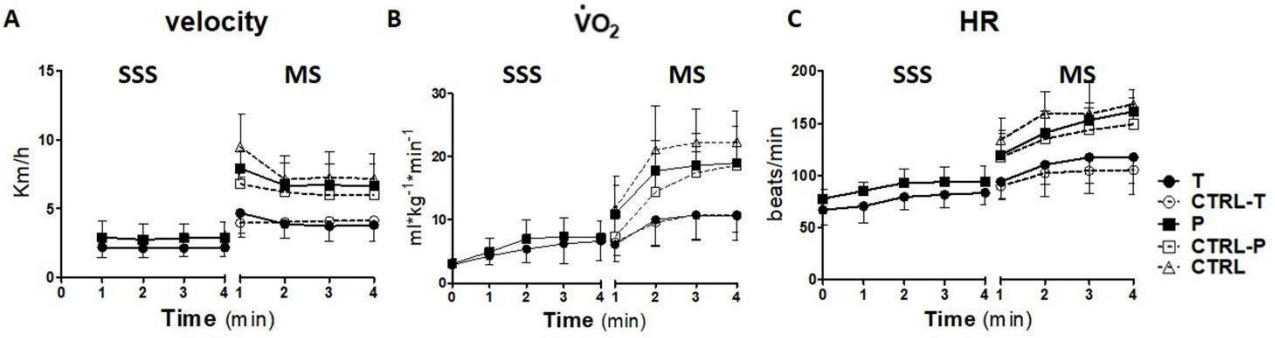

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Figure 5.

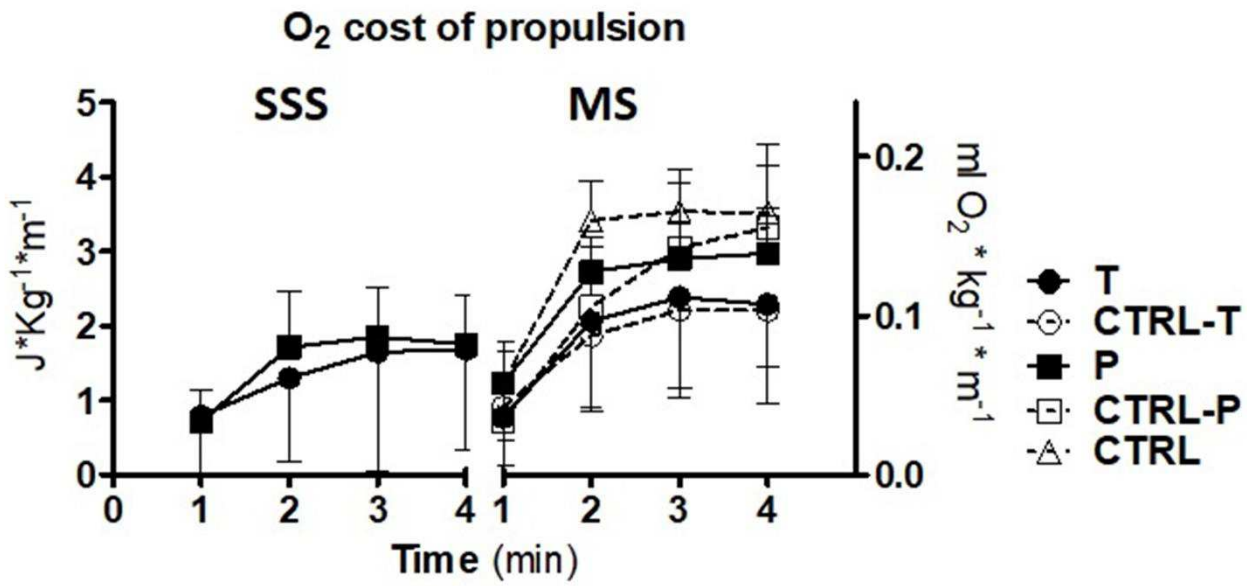

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Figure 6.

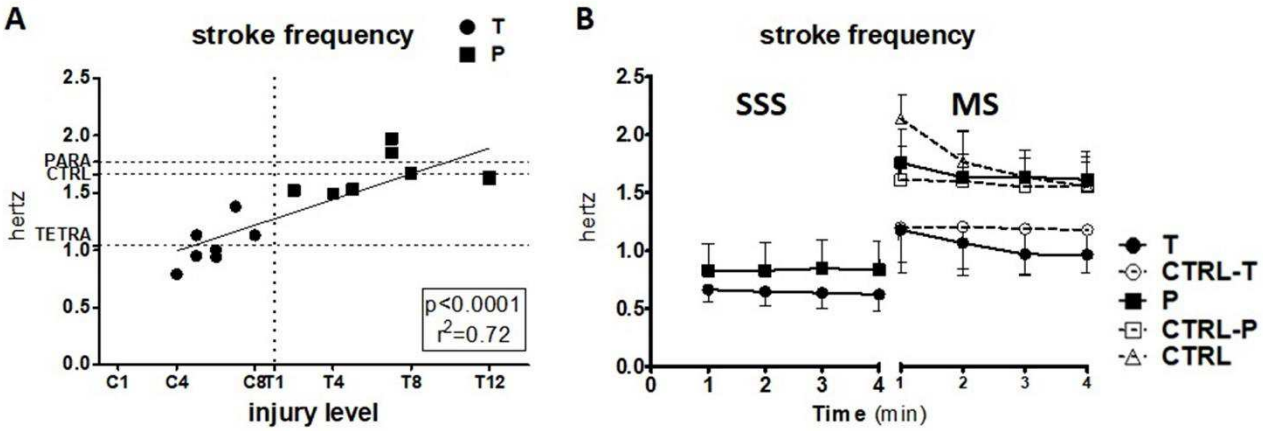

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Figure 7.

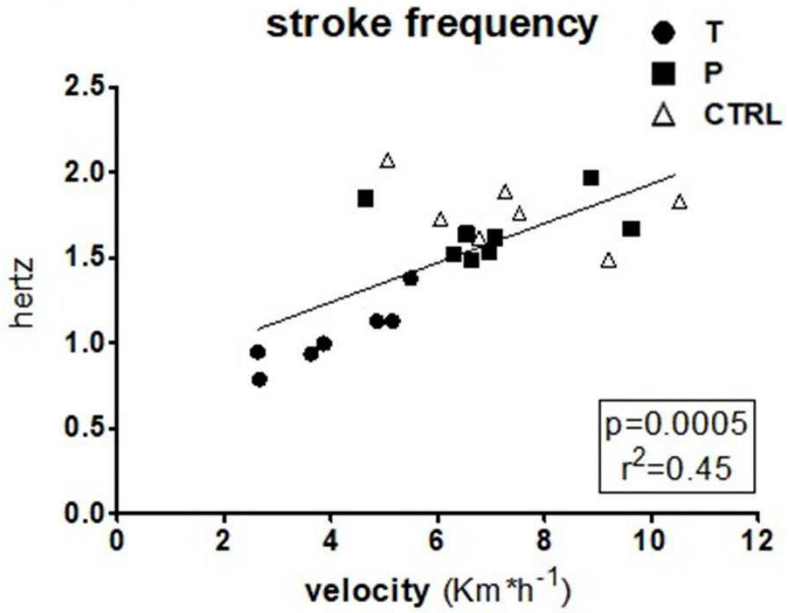

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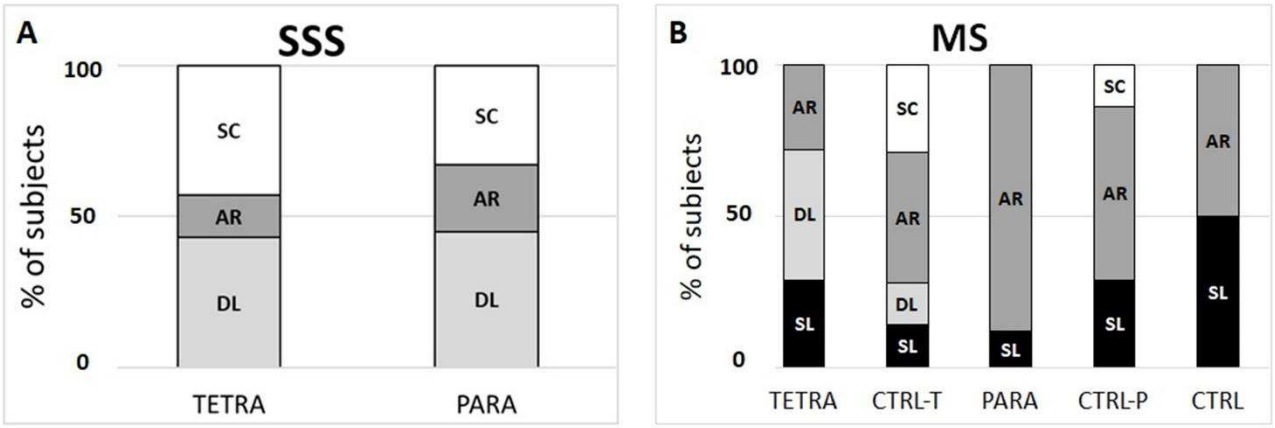
Figure 8.



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513 **TABLE 1. Subject characteristics of the three groups: tetraplegics (T),**

514 **paraplegics (P), controls (C).**

515

subject	*age (yr.)*	*wheight (Kg)*	*height (m)*	*BMI (Kg/m²)*	*athlete*	*physical characteristic**	*ASIA*	*etiology*	*years after lesion*
T1	31	52	1.8	16.05	no	SCI C4	A	traumatic	15

T2	39	68	1.75	22.2	yes	SCI C5	C	traumatic	15
T3	56	78	1.86	22.55	yes	SCI C5	C	traumatic	37
T4	43	64	1.78	22.2	yes	SCI C6	A	traumatic	23
T5	39	62	1.73	20.72	yes	SCI C6	A	traumatic	17
T6	27	65	1.8	20.06	no	SCI C7	C	traumatic	7
T7	46	80	1.77	25.54	yes	SCI C8	D	traumatic	6
mean	***40.1 ±9.6***	***67 ±9.6***	***1.78 ±0.04***	***21.5 ±2.9***					***17.1 ±10.5***
P1	33	83	1.8	25.62	no	SCI D4	A	epidural hematoma	30
P2	29	70	1.76	22.6	no	SCI D5	A	traumatic	7
P3	48	82	1.76	26.47	no	SCI D5	A	traumatic	11
P4	33	72	1.9	19.94	no	SCI D7	A	neoformation	1
P5	36	69	1.82	20.83	yes	SCI D7	A	traumatic	14
P6	35	68	1.8	20.99	yes	SCI D8	A	traumatic	12
P7	24	112	1.8	34.52	yes	SCI D10	A	traumatic	3
P8	41	82	1.88	23.2	yes	SCI D12	A	traumatic	16
P9	45	72	1.82	21.74	yes	SCI D12	A	traumatic	12
mean	***36 ±7.6***	***78.9 ±13.8***	***1.82 ±0.05***	***24 ±4.5***					***11.8 ±8.5***
C1	21	76	1.81	23.2	no	AB			
C2	25	90	1.88	25.46	no	AB			
C3	21	61	1.75	19.92	no	AB			
C4	47	83	1.9	22.99	yes	AMP			
C5	35	83	1.9	22.99	yes	AMP			
C6	44	85	1.74	28.08	no	PAT			
C7	38	60	1.72	20.3	no	PAT			
C8	37	75	1.8	23.15	no	AB			
C9	32	100	2	25	yes	AMP			
C10	24	70	1.8	21.6	yes	PAT			
mean	***32.4 ±9.4***	***78.3 ±12.5***	***1.8 ±0.1***	***23.3 ±2.5***					

NOTE: for each group, mean values are expressed ±SD.

*Physical characteristic: *SCI= spinal cord injury; AB=able-bodied; AMP=amputee; PAT=polytrauma (C6), Guillain-Barré syndrome (C7), osteosarcoma (C10)

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Abstract

The aging process alters upright posture and locomotion control from an automatically processed to a more cortically controlled one. The present study investigated the postural-cognitive dual-task paradigm in young and older adults using functional Near-Infrared Spectroscopy (fNIRS). Twenty healthy participants (10 older adults 72±3y, 10 young adults 23±3y) were assessed in four different postural conditions:1) single-task (ST stance), 2) a cognitive dual-task (DT stance), and the more complex 3) single-task (ST tandem) and 4) dual-task (DT tandem) conditions while monitoring the oxygenation levels of dorsolateral prefrontal cortex (DLPFC). Cognitive task while standing was better performed in the younger adults, who gave more correct answers in both dual-task conditions ($p \leq 0.027$); there was no difference between either age groups in terms of body sway parameters ($p \geq 0.141$) or calculated dual-task effects ($p \geq 0.143$). Cerebral oxygenation values (O_2Hb) increased significantly between the ST stance and ST tandem ($p = 0.033$), and ST stance and DT tandem ($p = 0.031$) whereas no changes were found in deoxygenated (HHb) hemoglobin. Finally, the perceived exertion differed between all conditions ($p \leq 0.003$) except in ST tandem and DT tandem ($p = 0.204$). There was a general lack of age-related changes except the better cognitive performance under motor-cognitive conditions in young compared to older adults. However, the current results point out that DLPFC is influenced more strongly by postural than cognitive load. Future studies should assess the different modalities of cognitive as well as postural load.

Keywords: Postural control, Dual-tasking, Aging, functional near-infrared spectroscopy (fNIRS), Attention

1. Introduction

Aging causes a shift from an automatic to a more cortical control of upright posture and locomotion (Papegaaij, Taube, Baudry, Otten, & Hortobagyi, 2014; Seidler et al., 2010; Zwergal et al., 2012). Older adults show increased and more widespread involvement of cortical areas for postural control compared to young adults; notably in the prefrontal cortex (Holtzer, Epstein, Mahoney, Izzetoglu, & Blumen, 2014; Seidler et al., 2010). These over-activations in older adults have been interpreted as a dedifferentiation of brain activation, or as a compensation for age-related declines in brain structure and function (Seidler et al., 2010). This increase in cortical engagement implies that postural processing is more attention-demanding in this age group compared to their younger counterparts. Further evidence for a more conscious, attention-demanding postural control strategy in older age has been provided indirectly by studies using a posture-cognition dual-task paradigm (Boisgontier et al., 2013; Ruffieux, Keller, Lauber, & Taube, 2015).

In everyday life, it is common to experience situations in which a postural task, (e.g., standing or walking), is performed concurrently with a secondary task. Older adults are particularly prone to dual-task costs in situations where attentional resources are shared between a postural and one (or more) additional tasks. In fact, it has been shown that the costs of performing a postural and a cognitive task concurrently are greater in older adults (Krampe, Schaefer, Lindenberger, & Baltes, 2011; Lindenberger, Marsiske, & Baltes, 2000; Little & Woollacott, 2014; Tsang et al., 2013), implying an increased risk of falls. Indeed, the reduced ability to allocate sufficient attentional resources to postural tasks may account for the high number of falls in the elderly (Chen et al., 1996). Performance in balance and/or locomotor tasks under dual-task conditions has been shown to be a good predictor of falls (Beauchet et al., 2009; Zijlstra, Ufkes, Skelton, Lundin-Olsson, & Zijlstra, 2008).

The prefrontal cortex plays a prominent role in postural, cognitive, and dual-task performance. Studies using functional near-infrared spectroscopy (fNIRS) have shown significant activation in the prefrontal cortex, including the dorsolateral prefrontal cortex (DLPFC), during walking (Holtzer et al., 2011; Holtzer et al., 2015) in response to a perturbation to upright stance (Mihara, Miyai, Hatakenaka, Kubota, & Sakoda, 2008), or during performance of a balance task in a semi-immersive virtual reality environment (Basso Moro et al., 2014; Ferrari et al., 2014). In the latter two studies, prefrontal oxygenation levels were positively correlated with task difficulty. Higher prefrontal oxygenation levels during standing were found in patients with Parkinsonian syndromes compared to healthy older adults (Mahoney et al., 2016). Thus, it has been suggested that the prefrontal cortex is critical for one's ability to selectively allocate (visuospatial) attention (Holtzer et al., 2015; Mihara et al., 2008) and to integrate visual and

proprioceptive information (Ferrari et al., 2014) in order to maintain or regain postural stability. Besides this role in the processing of postural tasks, the DLPFC also plays a key role in working memory tasks (Barbey, Koenigs, & Grafman, 2013; McCarthy et al., 1994; Petrides, 2000). Thus, there is evidence that the prefrontal cortex, particularly DLPFC, is crucial for the performance of postural dual-tasks. Studies using dual-task walking paradigms found increases in prefrontal activity when an attention-demanding task was added to normal walking in young adults, while older adults showed smaller increases (Holtzer et al., 2011) or even deactivations (Beurskens, Helmich, Rein, & Bock, 2014).

To our knowledge, no age-comparative fNIRS study exists to date that has investigated such effects using a standing postural task in combination with a working memory task. Furthermore, in the studies cited above (Beurskens et al., 2014; Holtzer et al., 2011), oxygenation levels were measured during walking alone, or during dual-task walking, but not during single-task performance of the additional task. Therefore, the aim of this study was to investigate the effect of performing a standing and a working memory task alone, and concurrently, on prefrontal activity in young and older adults, and to see whether differences in task performance could be explained by changes in prefrontal cortex activity. For this purpose, we measured behavioral performance as well as prefrontal oxygenation levels using fNIRS during standing, counting backwards, and when the two tasks were combined, in both young and older adults. We hypothesized there would be greater dual-task costs in the older compared to young adults. We further expected an increase in prefrontal activity from the single-task conditions to the dual-task conditions, which was assumed to be less pronounced in the older adults.

2. Methods

All procedures were carried out in accordance with the ethical standards of the 1964 Declaration of Helsinki and were approved by the National Medical Ethics Committee. Written informed consent was obtained from all participants prior to the study and no payment was provided for participation.

2.1 Participants

Older adults were identified and contacted from the clinical database of an existing EU-funded project entitled "Physical Activity and Nutrition for Quality Ageing" (PANGeA). For the purpose of this study, 10 healthy older adults (72.3 ± 3.2 years, height 166.8 ± 8.6 cm, body mass 73.8 ± 11.2 kg, BMI 26.5 ± 3.3 kg/m^2) were randomly selected from a pool of 152 volunteers and recalled to the laboratory for further testing. In addition, 10 healthy young adults (22.6 ± 2.8 years, height 173.4 ± 6.6 cm, body mass 75.3 ± 12.2 kg, BMI 24.9 ± 2.7 kg/m^2) were recruited and randomly selected from the database of the study program Applied Kinesiology (University of Primorska, Slovenia). Exclusion criteria included any history, or current symptoms of: severe acute metabolic, neuromuscular, and cardiovascular diseases, excessive obesity (over 45% fat), infectious diseases, cancer, bleeding, physical exhaustion, mild cognitive impairment or dementia, critical ischemia of the lower limbs, and patients unable to complete all measurement protocols. All participants were right-handed and had normal or corrected-to-normal vision.

2.2 Study design and tasks

This group-design study consisted of a dual-task paradigm that combined two different postural tasks with a cognitive task. The postural tasks consisted in standing as still as possible during normal stance and during tandem stance while focusing on a black point placed at eye-level approximately one meter in front of the participant. In the tandem stance conditions, a force plate (AMTI HE600600-2k, Advanced Mechanical Technology, Inc., Watertown, MA, USA) was used to measure displacements of the center of pressure (COP) in both medio-lateral (m-l) and antero-posterior (a-p) directions. From these values, we calculated sway path, frequency, and amplitude separately for each direction as well as total sway path.

The cognitive task consisted in subtracting serial threes from a randomly chosen 3-digit number between 400 and 500. The number of correct answers was counted for each trial. Participants were instructed to perform as many correct subtractions as possible (i.e., prioritize correctness over speed).

The postural tasks were performed as single-tasks as well as in combination with the cognitive task. Thus, all participants completed four conditions (in random order): normal stance single- (ST stance) and dual-

task (DT stance) as well as tandem stance single- (ST tandem) and dual-task (DT tandem). For the dual-task conditions, no instruction was given regarding task prioritization. Two trials of 60 seconds were performed in each condition. All measurements were carried out in a separate and quiet room to avoid any external disturbances. At the end of each trial, participants were asked to subjectively rate their perceived exertion (0 - 10 Borg scale).

2.3 Cerebrovascular setup

Measurement of prefrontal cortex oxygenation was performed using a single-distance continuous wave fNIRS set-up (Oxymon, Artinis, The Netherlands), using methods described elsewhere (Salvadego et al., 2011). Briefly, this device contained a headset that held a near-infrared emitter with laser light at 780 and 850 nm and a detector pair placed above the left hemisphere, above the DLPFC. Optode spacing was 45 mm corresponding to a depth of 20-25 mm. Following instructions by Holtzer and colleagues (Holtzer et al., 2011), changes in oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin concentrations relative to a 5-second baseline, recorded immediately before the first condition, were calculated for each condition.

2.4 Data analysis

For the behavioral parameters, the average of the two trials of each condition was used for further analysis. Data were analyzed with IBM SPSS Statistics 22.0 software for Windows (SPSS, Inc., Chicago, Ill, USA). Homogeneity of variances and normality of the distribution of the parameters was tested with the Levene's and Shapiro-Wilk's test, respectively. Two-way mixed-design analyses of variance (ANOVA) with age as a between-subject factor (young vs. older adults) and condition as a within-subject factor (single- vs. dual-task) were performed on each behavioral variable separately (i.e., correct numbers for serial threes and COP parameters). Hemodynamic responses were also analyzed using two-way mixed design ANOVAs with the factors age and condition (ST and DT stance and tandem conditions) on oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin variables. Significant main or interaction effects were followed up by Bonferroni-corrected post hoc tests; Student's *t*-tests were run on the differences between the ST and the DT conditions. Additionally, dual-task effects (DTEs) were calculated for all parameters (Marusic et al., 2015) and compared between age groups with Student's *t*-tests. Statistical significance was set at the level of $p < 0.05$.

3. Results

3.1 Baseline characteristics

Table 1 summarizes the participants' characteristics. The independent sample *t*-test showed that participants varied by age ($p < 0.001$), but were well-matched for all other parameters ($p \geq 0.074$). Furthermore, older adults showed no signs of cognitive impairments (MoCA score ≥ 26).

Insert Table 1 approximately here

3.2 Cognitive performance

A significant interaction effect of age and condition (DT stance vs. DT tandem), $F(1,18) = 10.655$, $p = 0.004$, $\eta^2 = 0.372$, was found for the number of correct answers. Young adults gave more correct answers than older adults in DT stance ($p = 0.027$) as well as during DT tandem ($p = 0.003$) condition. Moreover, young adults increased the number of correct answers from the DT stance to the DT tandem condition ($p = 0.002$), whereas there was no difference between conditions in older adults (see Figure 1).

Insert Figure 1 approximately here

3.3 Postural control

Analysis showed no significant main or interaction effects for parameters of COP sway path (total, m-l, and a-p; all $p > 0.141$). Additionally, no significant differences were found between the two groups in the DTE parameters (all $p \geq 0.143$).

Insert Table 2 approximately here

3.4 Hemodynamic changes

Hemodynamic changes for O_2Hb values were entered into a two-way mixed-design ANOVA with age as the two-level between-subject factor, postural conditions (ST and DT stance, and ST and DT tandem) as the four-level repeated within-subject factor and revealed no significant interaction effect (p = 0.898). There was a significant main effect of condition on O_2Hb values (see Figure 2), $F(3,54) = 7.329$, p = 0.002, $\eta^2 = 0.289$. Post hoc tests revealed a significant change from ST stance to ST tandem (p = 0.033), and ST stance to DT tandem (p = 0.031), while there were no significant changes from ST stance to DT stance (p = 0.641) and ST tandem to DT tandem (p = 1.000). Finally, for HHb values, there was no significant interaction (p = 0.890), nor main effect of condition (p = 0.663).

Insert Figure 2 approximately here

3.5 Subjective rating of physical and cognitive load

Friedman's ANOVA revealed a significant difference in the perceived effort between conditions ($\chi^2(3) = 43.006$, $p < 0.001$). Post hoc tests (Wilcoxon) with Bonferroni corrections (p = 0.0125) were significant between all conditions ($p \leq 0.003$) except between ST tandem and DT tandem ($Z = -1.271$, p = 0.204). Additionally, Mann-Whitney U tests revealed no significant differences between the two age groups for all four conditions for the subjective rating of Borg scale ($p \geq 0.063$): no differences were obtained for ST stance (p = 0.676), DT stance (p = 0.507) and ST tandem (p = 0.147), with a non-significant trend for DT tandem (p = 0.063).

Insert Figure 3 approximately here

4. Discussion

This is the first study to investigate a postural-cognitive dual-task paradigm in young and older adults using fNIRS. The study revealed significant increases in O_2Hb in the DLPFC when the difficulty of the postural task was increased (i.e., from normal to tandem stance), whereas there were no changes when cognitive load was added to standing. This was true for both age groups with no significant age differences in oxygenation levels. For the behavioral parameters, no significant differences between age groups or conditions were found except for a slightly better performance in the cognitive task from the single- to the dual-task condition in young adults. Finally, a subjective measure of physical and cognitive load revealed no age-related differences and was found to be significantly different between all conditions expect when cognitive load was added to a tandem postural stance.

Against our expectations, we found no significant DTEs – neither in young nor in older adults – except for the improvements in the subtracting task in the young adults. Accordingly, no differences in prefrontal oxygenation levels were found between the single- and the dual-task conditions or between age groups. Similar findings were reported in a study that compared walking alone to walking while talking (Beurskens et al., 2014). Other studies using similar paradigms, however, found increased oxygenation levels in the prefrontal cortex during walking while talking compared to normal walking in both young and older adults (Holtzer et al., 2011; Holtzer et al., 2015). The discrepancy between these findings and our results could be explained by differences in the attentional demands of the postural tasks used; possibly, the two-legged standing tasks used in the present study were not challenging enough to induce significant effects. It has been shown in a systematic review (Boisgontier et al., 2013) that static standing tasks are not always challenging enough to detect age-related changes in dual-task abilities. Indeed, studies using postural tasks with dynamic surface conditions systematically reported greater dual-task costs in older compared to young adults (Boisgontier et al., 2013). Similarly, it has been shown that walking is more attention-demanding than standing, in both young and older adults, possibly due to the particular demands of generating bilateral, synchronized limb movements (Yogev-Seligmann, Giladi, Gruendlinger, & Hausdorff, 2013). Further support for this interpretation comes from a study that combined standing tasks with a cognitive task and found increases in DLPFC oxygenation during dual-task only in a one-legged standing condition and not during two-legged standing (Fujita, Kasubuchi, Wakata, Hiyamizu, & Morioka, 2016).

Interestingly, one study reported a smaller increase in prefrontal activity from single- to dual-task walking in older compared to young adults (Holtzer et al., 2011) while another study even found a deactivation in older adults in one dual-task condition while in young adults there was no change (Beurskens et al., 2014). This might be somewhat surprising since it has been shown that performing

postural and cognitive tasks alone increases prefrontal activity more in older than in young adults (Seidler et al., 2010). It has been suggested that older adults may underutilize the prefrontal cortex when performing an attention-demanding task while walking (Holtzer et al., 2011). This suggestion is supported by two studies (Manor et al., 2016; Zhou et al., 2015) showing that transcranial direct current stimulation of the prefrontal cortex improves dual-task performance in older adults. In these studies, the costs of performing a working memory task concurrently with standing or walking were significantly reduced when prefrontal activity was facilitated by the stimulation while it had no influence on single-task performances. Another interpretation is that older adults shift processing resources to other brain areas in dual-task situations (Beurskens et al., 2014). Since in the present study, brain activity was measured only within a limited area of the prefrontal cortex, namely DLPFC, we cannot exclude activity changes in other brain areas in the older adults.

There are some methodological limitations that should be taken into consideration when interpreting the present study. Firstly, the fNIRS system allowed us to perform measurements only within a limited area of the prefrontal cortex, namely DLPFC. Future studies could possibly include a multichannel fNIRS device to cover a larger brain area. Secondly, due to the fact that normal aging is also accompanied by declines in executive function, including deficits in processing speed, working memory capacity, and attentional processing (namely in tasks requiring selective and/or divided attention) (Harada, Natelson Love, & Triebel, 2013), other cognitive loads aside from serial threes need to be tested.

In summary, this is the first study to provide an indication that increasing postural demands contributes more to the observed increase in DLPFC activity during a postural dual-task than increasing the cognitive load. The fNIRS measurements showed significantly higher O_2Hb concentration level changes in the DLPFC when postural task difficulty was increased (from ST stance to ST tandem). In contrast, there were no significant increases in O_2Hb concentration between the stance (ST stance) and the dual-task condition (DT stance) nor tandem stance (ST tandem) and the dual-task condition (DT tandem). This suggests that changing the posture from normal stance to tandem stance increases cognitive load, at least in the DLPFC, while adding a serial threes cognitive task to tandem stance does not. Understanding the neural contribution to reduced postural control in aging could help developing novel interventions for elderly to reinforce postural control, especially in challenging dual- and multi-task conditions, and improve their quality of life, including a faster return to premorbid daily functioning.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts to report.

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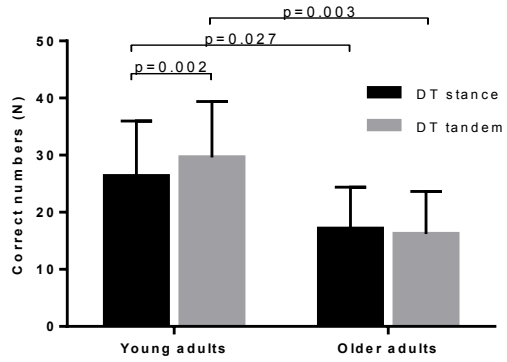

Figure 1: Cognitive performance (serial threes) during DT stance and DT tandem position

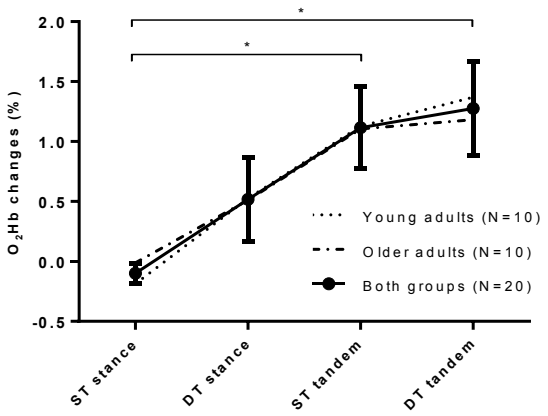

Figure 2. O_2Hb changes between four conditions

Note: no significant differences were observed between the two groups. However, significant changes between conditions are marked with *. The calculation of relative O_2Hb is stated in paragraph "2.3 Cerebrovascular setup".

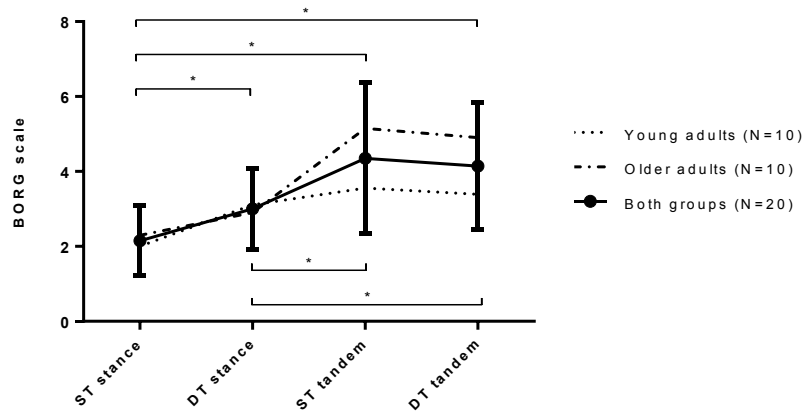

Figure 3. BORG scale of perceived exertion

Note: no significant differences were observed between the two groups. However, significant changes between conditions are marked with *.

Table 1: Participants' characteristics

	Young adults (N=10)	Older adults (N=10)	*p* value
Sex	7 women	6 women	
Age (y)	22.6 ± 2.8	72.3 ± 3.2	< 0.001
Height (cm)	173.4 ± 6.6	166.8 ± 8.6	0.074
Weight (kg)	75.3 ± 12.2	73.8 ± 11.2	0.798
Education duration (y)	14.3 ± 1.6	14.1 ± 2.1	0.828
MoCA score		28.0 ± 1.2	

Note: Data are mean ± SD. MoCA, Montreal Cognitive Assessment.

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