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Acute and Chronic Effects of Exercise in Adults with Down Syndrome

Dissertação com vista à obtenção do grau de Doutor em Motricidade Humana na especialidade de Ciências da Motricidade

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Abstract

This thesis intended to explore both the acute and chronic physiological adaptations of persons with Down syndrome (DS) in response to exercise. Specifically, in terms of acute exercise responses, we aimed at investigating the submaximal exercise capacity and cardiac autonomic function of adults with and without DS. Subsequently, we determined whether 12 weeks of exercise training were effective in improving exercise capacity (economy and peak oxygen uptake – VO_{2peak}) and autonomic function both in adults with and without DS. Overall, we found that the submaximal exercise capacity of adults with DS was characterized by poor walking economy, but appropriate VO_2 kinetics. As importantly, these individuals demonstrated reduced cardiac responsiveness to changes in the sympathovagal balance resulting from submaximal dynamic exercise. Findings also indicated a breakdown in their fractal scaling properties of heart rate dynamics that was transversal to resting, exercise and post-exercise recovery conditions. Moreover, compared to nondisabled participants, adults with DS showed reduced cardiodeceleration during recovery from peak exercise intensities. Finally, in general terms, a combined exercise regimen resulted in gains of similar magnitude between participants with and without DS for submaximal exercise capacity and cardiac autonomic function. In conclusion, this thesis provides evidence that persons with DS have reduced submaximal exercise capacity and that this is paired by disturbed autonomic function. Nevertheless, these results also indicate that exercise training is an effective intervention for improving their physiological function in similar magnitude as in adults without DS.

Key words: Exercise physiology; Down syndrome; oxygen uptake; exercise economy; oxygen kinetics; functional capacity; autonomic function; heart rate variability; training; cardiovascular fitness.

Resumo

Esta tese pretendeu explorar as adaptações fisiológicas agudas e crónicas de indivíduos com Trissomia 21 (T21) na resposta ao exercício físico. Em concreto, no domínio das adaptações agudas, compararam-se adultos com e sem T21 para variáveis inerentes à capacidade submáxima de exercício e de função autonómica. Seguidamente, estudou-se a eficácia de um programa de 12 semanas de exercício físico estruturado quanto à melhoria da função fisiológica de pessoas com e sem T21 (economia de esforço, consumo de oxigénio de pico [VO_{2pico}] e função autonómica). Verificou-se que, embora a sua capacidade submáxima de exercício se caracterizasse por uma pobre economia de esforço, os adultos com T21 demonstraram uma cinética do VO_2 comparável aos participantes sem T21. Os participantes com T21 também apresentaram uma atenuação no padrão de resposta cronotrópica face a modificações no equilíbrio simpatovagal decorrentes do exercício. Os resultados indicaram ainda um colapso das propriedades fractais do controlo da frequência cardíaca em situação de repouso, exercício e recuperação. Com igual relevância, os participantes com T21 expressaram um défice de recuperação cronotrópica após o esforço de pico. Finalmente, uma intervenção de 12 semanas de exercício físico combinado resultou em ganhos fisiológicos comparáveis entre adultos com e sem T21. Em conclusão, a presente dissertação apresenta evidências de uma pobre capacidade submáxima de exercício em adultos com T21. Mais ainda, os nossos achados sugerem que esta população apresenta sinais de uma possível disautonomia que é exacerbada pelo estímulo agudo do exercício físico. Apesar disto, uma intervenção pelo exercício físico estruturado parece exercer um impacto positivo e de igual magnitude na função fisiológica de indivíduos com e sem T21.

Palavras-chave: Fisiologia do exercício; Trissomia 21; consumo de oxigénio; economia de esforço; cinética de oxigénio; capacidade funcional; função autonómica; variabilidade da frequência cardíaca; treino; condicionamento cardiovascular.

Publications

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Chapter 3

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1. Prolegomen

1.1. Introduction

Since Down described the condition that today bears his name (Down 1866), a multitude of publications on this subject have permeated the medical literature. In 1980, Puschel and Steinberg conducted an intensive literature search and compiled more than 6000 articles relating to various aspects of Down syndrome (DS). This enormous and growing information explosion reflects the progress that has been made in the field of DS, the most common of the genetic conditions associated with intellectual disability (ID) (Arbuzova 1998).

There has been a shift in the way DS people are seen from incompetence to competence, and an increase in the understanding of their health and cognitive abilities. In the 19th and early 20th centuries – indeed until past the middle of the 20th century – people with DS were all regarded as intellectually disabled, largely falling into the moderate and rather more severe ranges of ID. Institutionalization for people with DS was the norm in western society. Now, there is a general understanding that people with DS have a range of ability levels, from severe ID to low average abilities. Increasingly, individuals with average, and occasionally above average intelligence are seen (Brown 2006). For these individuals, the label of ID is increasingly inappropriate (Bown and Brown 2003). Whatever their intellectual functioning, people with DS have diverse abilities and this has resulted in most individuals worldwide taking an active role in the societies in which they live (Brown 2006).

Many of the changes that have occurred within the field of DS have resulted from research in medicine and in the biological sciences. These developments have influenced the way that intervention is carried out, resulting in the correction of a number of common biological defects experienced by children with DS: congenital heart disease, gastro-esophagic reflux, recurrent otitis, obstructive sleep apnea, lower respiratory tract infections, thyroid dysfunction and pre-senile dementia of Alzheimer's type (Cohen 1999). In this sense, there has been the possibility of opening the door to further learning and development. But there are other aspects that are critically important, and to a very large degree follow these changes in bodily health (Brown 2006).

To obtain the full benefits of improvement in health, the individual is dependent on other changes of a societal and educational nature. Early intervention, which has developed into a refined educational art over the past 50 yr, is now seen as an important means of providing the necessary stimulation to enable young people with DS, to maintain early developmental stages alongside their peers. But there are other important changes (Forlin 2005). In the field of quality of life, which is now playing an increasingly important role in ID, some groups have been involved more than others. Research in quality of life in ID field was first promoted with the most vocal and capable individuals, namely, adults who were young and had mild disabilities; the old and young who were multiply disadvantaged have had to wait in terms of quality of life practice (Schalock et al. 2002; Krykou 2005).

In many countries the large institutions have given way to community care, and most DS people now live with their families. People with DS need individual attention, care from parents, brothers and sisters, normal friends, and not to be regarded as different in the social society and therefore excluded. Unfortunately, to date, many countries have been less successful in moving the concept of inclusion forward effectively in terms of a continuous education (Forlin 2005). Despite this, some individuals with DS make progress through the secondary cycle of education, and there are reports of a number of these adults that go on to tertiary education including college courses, certificates and diplomas and experience in universities (Getzel and Wehman 2005).

Life expectancy for persons with DS has increased dramatically since the early work of Record and Smith (1955). From 1942-1952, they reported that less than 50% of infants survived the 1st yr; by age five, only about 40% were still alive. In contrast, more recent studies (Baird and Sadovnick 1987; Baird and Sadovnick 1989; McGrother and Marshall 1990; Leonard et al. 2000) found out that over 80% of children with DS are still alive by the age of five and approximately 44% survive to the age of 60 (Baird and Sadovnick 1988). This improved survival probably reflects more effective treatment of the above mentioned common causes of death, as well as a changing attitude toward the neonatal care of such infants (Declining Mortality 1990). With these developments there are new challenges, just as there are challenges with the increasing number of older people worldwide. These

challenges in relation to people with DS have the same or similar challenges to the rest of the population. But even when similar, there are often differences – mental health issues such as depression and dementia, along with other issues such as an atypical development that predisposes to asthenic hypotonia, a sedentary life style and obesity (Pueschel and Solga 1992; Rubin et al. 1998; Stanish and Draheim 2005).

An individual with DS can participate in most types of physical activity. Overall, this is a healthy population that enjoys the social aspects of physical activity. With over 80 clinical characteristics studied in individuals with DS, the physical characteristics most related to exercise are: muscle hypotonicity (muscles that have the ability to be stretched far beyond normal limits), hypermobility of the joints or ligamentous laxity (increased flexibility of their joints associated with increased susceptibility to subluxation and dislocation), mild to moderate obesity - greater among adult women then adult men (Rimmer et al. 1992), underdeveloped respiratory and cardiovascular system, short stature (short legs and arms in relation to torso), and poor balance and perceptual difficulties (Winnick 1995). Often, hypotonia and hypermobility are associated with scoliosis - 50% prevalence (Diamond et al. 1981), dislocated hips – 1 to 4% prevalence (Aprin et al. 1985), flat pronated feet – 90% prevalence (Diamond et al. 1981), forward head (Miller et al. 1986), atlanto-occipital instability – 8.5% prevalence (Powers et al. 1994) atlantoaxial instability - 9 to 30% prevalence (Alvarez and Rubin 1986; Jagjivan et al. 1988; Pueschel and Scola 1987). Due to its high prevalence, atlantoaxial instability is a serious concern regarding individuals with DS, being defined as an atlanto-dens interval $\geq$ 5 mm. This severe cervical disorder may predispose to vertebrae subluxation with subsequent spinal cord injury, being an absolute contraindication for exercise participation when diagnosed by radiological techniques (Pueschel 1998). Pathophysiological concerns associated with DS in relation to exercise include congenital heart disease, cardiac abnormalities in adulthood, leukaemia, gastrointestinal disorders and Alzheimer's disease. Other pathophysiological factors to consider in relation to exercise capacity are: thyroid hormonal disease – frequently Hashimoto's disease, abnormal energy expenditure and substrate utilization, impaired sympathetic response to exercise and also macrocytic anemia (Pitetti et al. 1992).

Exercise and physical fitness are considered important for health and well-being (Cooper et al. 1976; Powell et al. 1987; Shepard 1985). It is recognized that exercise training can promote an increased work capacity, decreased body fatness, and improved blood lipid profile (Froelicher et al. 1980; Pollock and Wilmore 1990). Exercise and physical fitness also decrease the risk of developing heart disease and may actually improve life expectancy (Paffenbarger et al. 1986). Furthermore, physical activity may have positive implications on job performance (Blair et al. 1981). Exercise and physical activity are no less important for individuals with ID (Fernhall 1993).

DS implies certain singularities in the broad spectrum of ID. DS individuals present a lower functional capacity than their age matched peers, with or without ID. Such differences are particularly evident in specific physiological deviations, such as lower values for: (1) peak work capacity, (2) peak oxygen uptake, (3) peak minute ventilation, (4) peak heart rate, and (5) peak respiratory exchange ratio (Fernhall et al. 1996). According to these authors, such deviations are possibly related to both a reduction in sympathetic drive, and a deficit in parasympathetic withdrawal that apparently limit their chronotropic response to exercise.

1.2. Main purposes

This thesis intends to contribute to a better understanding of the physiological responses to exercise in adults with DS. This is relevant because, despite being the most common human aneuplody (Canfield et al. 2006), DS remains poorly characterized in terms of exercise physiology (both acute and chronic adaptations). For this reason, our first aim was to review the existent literature on exercise capacity of persons with DS. From a practical standpoint, this approach allowed us to define specific goals to be achieved in the present work.

Considering that poor economy of locomotion has been suggested to limit the functional ability of persons with DS (Black et al. 2007) and the literature is particularly scarce on this issue, our main purpose was to provide further insight into the physiology of DS at light to moderate exercise intensities. Within this context, we first intended to explore whether adults with DS showed reduced walking economy, in response to several treadmill walking speeds and grades, compared to

participants without disabilities. Subsequently, we aimed at analyzing their on- and off- oxygen uptake (VO_2) transients to determine if their reduced exercise performance would result from delayed VO_2 kinetics.

As importantly, because there is compelling evidence that persons with DS exhibit reduced vagal withdrawal while responding to exercise (Figueroa et al. 2005; Heffernan et al. 2005), we further explored this issue. Accordingly, we investigated whether adults with DS showed disturbed cardiac autonomic function during a submaximal walk of moderate intensity. Nonlinear heart rate variability (HRV) analysis was also used to unravel the effects of DS on the fractal properties of R-R interval fluctuations at rest, during submaximal exercise and recovery. This study intended not only to determine whether adults with DS showed a breakdown in fractal scaling properties of HRV, but also to investigate the direction of such collapse (excessive order vs complete randomness). Finally, due to the adequacy of heart rate recovery as an index of vagal tone reactivation (Cole et al. 1999; Androne et al. 2003; Pierpont and Voth 2004), we explored if adults with DS showed delayed cardiodeceleration after maximal exercise.

Despite their importance, conclusions derived from acute physiological testing only allow the diagnosis of deviations from "normal" physiological function. Ultimately, this information may be used to design exercise training interventions aimed at improving specific physiological insufficiencies or limitations. For this reason, after defining a solid diagnostic framework, we also intended to explore whether a 12-week structured exercise intervention would be effective in improving both the submaximal exercise capacity and cardiac autonomic function of persons with DS As a secondary purpose, to investigate the suitability of the conventional exercise training guidelines for this population, we compared if the training effect was of similar magnitude between individuals with and without DS.

1.3. Structure

Overall, this thesis is divided in four chapters: chapter 2 - review of the literature on exercise capacity of persons with DS; chapter 3 - cardiorespiratory and metabolic function in persons with DS;

chapter 4 - cardiac autonomic function in persons with DS; chapter 5 - synopsis. Chapter 2 reviews the existent literature on the causes, effects and management of reduced exercise capacity in persons with DS. In this chapter, the reader may become familiarized with the major physiological determinants of exercise performance. Additionally, special attention is given to the detrimental effects of DS on each of these determinants. As importantly, this chapter also focuses on the relationship between physiological limitations and disturbed performance on functional tasks of daily living. Finally, it describes the limited body of research on the management of poor exercise capacity in DS.

Chapter 3 includes five experimental designs concerned with cardiorespiratory and metabolic data. The first study corresponds to the validation of a treadmill exercise protocol which was used in all ensuing research. The following two studies are concerned with walking economy in persons with DS. Here, we compared the walking economy of adults with and without DS at different walking speeds and grades. Then, study number four presents data on the VO_2 on- and off-kinetics of persons with and without DS in response to moderate exercise. Finally, this chapter includes a study on the effects of a combined exercise regimen on improving the submaximal and peak exercise capacity of persons with and without DS.

Similarly to chapter 3, chapter 4 is further divided into five experimental designs; however, the main focus of chapter 4 is on data for cardiac autonomic function. In the first study, the reader may find an exploratory design on the comparison between the sensitivity of two different HRV spectral approaches to the effects of exercise. Then, study number two provides evidence that DS is associated with atypical changes in the sympathovagal balance from rest to moderate exercise. Subsequently, study number three compares the R-R interval fractal scaling properties between persons with and without DS under different physiological conditions (rest, exercise and recovery). Data on the effects of DS on vagal tone reactivation following peak exercise intensities are then presented in study number 4. Finally, the effects of exercise training on the cardiac autonomic function of persons with DS are described in the last study of chapter 4.

The final chapter of this thesis presents a synopsis of the main findings of the preceding chapters, discusses the clinical implications of the results obtained and suggests topics for further research.

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2. Down syndrome and exercise capacity: a review of the literature.

2.1. Reduced exercise capacity in persons with Down syndrome: cause, effect and management

2.1.2. Abstract

Persons with Down syndrome (DS) have reduced peak and submaximal exercise capacity. Because ambulation is one predictor of survival among adults with DS, a review of the current knowledge of the causes, effects and management of reduced exercise capacity in these individuals would be important. Available data suggests that reduced exercise capacity in persons with DS results from an interaction between low peak oxygen uptake (VO_{2peak}) and poor exercise economy. Of several possible explanations, chronotropic incompetence has been shown to be the primary cause of low VO_{2peak} in DS. In contrast, poor exercise economy is apparently dependent on disturbed gait kinetics and kinematics resulting from joint laxity and muscle hypotonia. Importantly, there is enough evidence to suggest that such low levels of physical fitness (reduced exercise capacity and muscle strength) limit the ability of adults with DS to perform functional tasks of daily living. Consequently, clinical management of reduced exercise capacity in DS seems important to ensure that these individuals remain productive and healthy throughout their lives. However, few prospective studies have examined the effects of structured exercise training in this population. Existent data suggests that exercise training is beneficial for improving exercise capacity and physiological function in persons with DS. This paper reviews the current knowledge of the causes, effects and management of reduced exercise capacity in DS. This review is limited to the acute and chronic responses to submaximal and peak exercise intensities because data on supramaximal exercise capacity of persons with DS has been shown to be unreliable.

2.1.3. Key words

Down syndrome, peak exercise capacity, exercise economy, ventilatory threshold, exercise training.

2.1.4. Introduction

Down syndrome (DS) is a chromosomal disorder occurring in about 1 per 650 to 1000 live births and there is also a marked increase in prevalence with advanced maternal age (Parker et al. 2010; Dolk et al. 2005; Canfield et al. 2006; Irving et al. 2008; Morris and Alberman 2009). In Europe, DS accounts for 8% of all registered cases of congenital anomalies and it is the most common cause of intellectual disability (ID) (Dolk et al. 2005; De Walle and Cornel 1995; Leonard et al. 2000).

DS is characterized by altered psychomotor development and an increased risk of concomitant congenital defects and organic disorders, such as congenital heart and gastrointestinal defects, celiac disease and hypothyroidism (Roizen and Patterson 2003). Although life expectancy is still low, recently there has been a substantial increase in life expectancy of persons with DS. Yang et al reported an average increase of 1.7 y of age at death per year studied from 1983-1997 (Yang et al. 2002). This has been due mainly to the successful surgical treatment of congenital heart disease and the improved treatment of congenital anomalies of the gastrointestinal tract (Leonard et al. 2000; Day et al. 2005; Kortenhorst et al. 2005). Average life expectancy of persons with DS has therefore increased into the late 50s and a person with DS who lived to age of 83 has been reported in the literature (Glasson et al. 2002; Chicoine and McGuire 1997).

Preventive health care has also contributed to improved overall outcome and quality of life in persons with DS (Roizen and Patterson 2003). Furthermore, improved educational services and greater social acceptance of people with disabilities in the community have led to deinstitutionalization of people with DS (Brown 2006). Given that ambulation is one important predictor of survival among adults with DS, this ever increasing number of community-dwelling individuals may well benefit from structured exercise interventions to remain productive and healthy throughout their lives (Eyman and Call 1991). This notion becomes even more relevant when considering the ubiquitous nature of reduced exercise capacity in persons with DS (Fernhall et al. 1989; Pitetti et al. 1992a; Fernhall et al. 1996; Fernhall and Pitetti 2001; Baynard et al. 2008; Mendonca et al. 2010b). Accordingly, it is important to provide an etiological basis for such low levels of exercise capacity, as ultimately this will improve the clinical management of health-related physical fitness in persons with DS from

childhood to adulthood. Also, the effects of reduced exercise capacity on the ability of persons with DS to perform functional tasks (i.e., tasks of daily living) needs investigation as this may be helpful in designing specific exercise interventions. Thus, the purpose of this paper is to provide a comprehensive review of the current knowledge of the causes, effects and management of reduced exercise capacity in DS. Because data on the supramaximal exercise capacity of persons with DS is scarce and unreliable, this review is limited to the acute and chronic responses to submaximal and peak exercise intensities in these individuals (Guerra et al. 2009).

2.1.5. Causes for reduced exercise capacity in Down syndrome

The most important physiological factors related to exercise capacity and work performance in humans include peak oxygen uptake (VO_{2peak}), exercise economy and the ventilatory threshold (VT) (Jones 2006; Joyner and Coyle 2008). The finite rate of adjustment of oxidative phosphorylation to sudden increases and decreases in energy demand (i.e., VO_2 kinetics) is also important in minimizing the magnitude of the O_2 deficit and debt which can influence work performance (Delorey et al. 2007). There is considerable evidence to support the clinical significance of all these factors as they are commonly altered in several pathological conditions (i.e., coronary artery disease, myopatic heart disease, valvular heart disease, congenital heart disease, peripheral arterial disease; diabetes, anemia, obesity, obstructive lung disease and restrictive lung disease) (Wasserman et al. 2005). Interestingly, healthy persons with DS (free from congenital or atherosclerotic heart disease, respiratory disease or endocrine disorders) share common features with most of these pathological conditions and this has been shown to limit their work performance during simple tasks, including treadmill exercise (Fernhall and Pitetti 2001).

2.1.5.1. Peak oxygen uptake in persons with Down syndrome

The upper limit of aerobic metabolism that can be maintained during exercise is termed VO_{2peak} (Joyner 1991; Joyner 1993; Coyle 1995; Basset and Howley 2000). This is usually achieved during exercise using relatively large muscle mass (i.e., treadmill exercise) and represents the integrative ability of the heart to generate a high cardiac output, total body hemoglobin, high muscle blood flow and muscle O_2 extraction, and in some cases the ability of the lungs to oxygenate the blood

(Basset and Howley 2000; Mitchell et al. 1958; Kanstrup and Eckbloom 1984; Rowel 1986; Dempsey 1986; Saltin and Strange 1992).

All previous studies on peak exercise capacity of individuals with DS have consistently found a lower VO_{2peak}, a shorter time to exhaustion and a lower peak work rate in these individuals compared to age-matched controls (Fernhall et al. 1989; Pitetti et al. 1992a; Fernhall et al. 1996; Fernhall and Pitetti 2001; Baynard et al. 2008; Fernhall and Tymeson 1987; Pitetti et al. 1988). Fernhall et al first reported VO_{2peak} values of 26 $mL.kg^{-1}.min^{-1}$ for adolescents with DS, and peak heart rates of 170 bpm (Fernhall et al. 1989; Fernhall and Tymeson 1987). This is substantially different from the typical values attained by nondisabled adolescents during peak exercise testing (VO_{2peak}: 50-52 $mL.kg^{-1}.min^{-1}$; heart rate: 195-205 bpm) (Roland 1997). Pitetti et al also obtained similar results in both their studies, showing that adults with DS exhibited VO_{2peak} values from 22-24 $mL.kg^{-1}.min^{-1}$ compared with 30-35 $mL.kg^{-1}.min^{-1}$ for their peers without DS (Pitetti et al. 1992a; Pitetti et al. 1988). The peak heart rates were also lower for the groups with DS (157-159 bpm) compared with those without DS (178-186 bpm). The reason for the low VO_{2peak} and peak heart rates in DS was, at the time, difficult to ascertain and some investigators believed that lack of motivation, and therefore lack of valid peak effort, could explain the findings (Seidi et al. 1987; Lavay et al. 1990). In contrast, others argued that DS might be associated with true chronotropic incompetence due to attenuated adrenergic responsiveness during exercise (Fernhall et al. 1989; Fernhall 1993; Fernhall et al. 1990; Eberhard et al. 1991). According to this hypothesis, chronotropic incompetence would limit peak cardiac output and consequently produce a lower VO_{2peak} in these individuals. Finally, it was also suggested that obesity, which is highly prevalent in persons with DS, could explain their reduced exercise capacity (Fernhall 1993; Rubin et al. 1998; Fernhall and Tymeson 1988; Pitetti et al. 1993; Pitetti and Tan 1990).

With the purpose of exploring the "lack of motivation" hypothesis, Fernhall et al tested the validity and reliability of treadmill peak exercise testing (graded exercise testing – GXT) in adolescents with DS (Fernhall et al. 1990). The authors used objective criteria to identify valid peak efforts such as a plateau in VO_2 (less than a 150 $mL.min^{-1}$ increase) with an increase in work rate, or a plateau in heart rate (less than a 2 bpm increase) with increase in work rate concomitant with a

respiratory exchange ratio > 1.0. Results indicated that, under these conditions (treadmill exercise testing and use of objective criteria for peak effort determination), peak exercise testing in persons with DS was as valid and reliable as in individuals without ID (reliability coefficient of 0.94). Consequently, these findings argued against the "lack of motivation" hypothesis and suggested a true physiological limitation during acute exercise in persons with DS.

The "chronotropic incompetence" hypothesis was subsequently explored in a study that compared the differences in cardiorespiratory capacity between adults with DS and those with other ID (Fernhall et al. 1996). The authors found that the lower peak heart rates of the subjects with DS explained their lower levels of aerobic capacity. Results were therefore in support of this hypothesis and further corroborated the notion that limited cardiac output at peak exercise is the likely explanation of the low work capacity in individuals with DS. This led to speculations of possible relationships between attenuated sympathetic response to exercise (i.e., autonomic dysfunction), chronotropic incompetence and low VO_{2peak} in this population.

In another study, it was shown that peak heart rates in persons with DS were approximately 30 bpm lower than those predicted by the formula 220 minus age (Fernhall et al. 2001). However, a follow-up study by Guerra et al more decisively showed that individuals with DS likely exhibit true chronotropic incompetence during exercise (Guerra et al. 2003). In this study, the chronotropic response index (CRI) of adults with DS was compared to that of nondisabled controls. The CRI is a submaximal exercise variable derived from the relative relation between heart rate and metabolic reserve. The CRI , in contrast to peak heart rate, is independent of effort, motivation, age, resting heart rate and physical fitness (Lauer et al. 1996; Wilkoff and Miller 1992). Not only did participants with DS show lower CRI values than controls, but they also exhibited values similar to those reported in nondisabled populations with true chronotropic incompetence (i.e., coronary heart disease and heart failure) (CRI < 0.9) (Lauer et al. 1996; Hinkle et al. 1972; Ellestad and Wan 1975; Keteyian et al. 1999).

The association between chronotropic incompetence and DS was subsequently confirmed by several reports showing significant differences between peak heart rates attained by these individuals and those of without DS (Baynard et al. 2008; Mendonca et al. 2010b; Baynard et al. 2004a; Mendonca et al. 2010a; Bricout et al. 2008; Fernhall et al. 2009; Fernhall and Otterstetter 2003; Baynard et al. 2004b; Mendonca et al. 2009; Mendonca et al. 2011). Taken together, all these studies point towards strong evidence supporting the "chronotropic incompetence" hypothesis as one of the primary causes for reduced VO_{2peak} in persons with DS. The exact mechanism for this phenomenon is not completely understood, but there is strong evidence for blunted adrenergic responsiveness in persons with DS (Eberhard et al. 1991; Bricout et al. 2008; Fernhall et al. 2009). This may also be related to altered autonomic function, as vagal withdrawal during exercise and spontaneous baroreceptor function have been shown to be reduced in individuals with DS (Figueroa et al. 2005; Heffernan et al. 2005; Iellamo et al. 2005; Agiovlasitis et al. 2010). The most likely candidate is reduced catecholamine response to maximal exercise (Eberhard et al. 1991; Fernhall et al. 2009). Fernhall et al recently showed that individuals with DS exhibited only minor norepinephrine increases and no change in epinephrine in response to peak treadmill exercise (Fernhall et al. 2009). Since the change in both epinephrine and norepinephrine were related to both peak heart rate and peak exercise capacity, and it has been shown that changes in catecholamines are largely responsible for increases in heart rate at exercise intensities above the VT, it is highly likely that the reduced ability to produce catecholamines is the major cause of chronotropic incompetence in persons with DS (Kjaer 1998).

Arguments against the "obesity" hypothesis first resulted from the work of Fernhall et al that found no significant relationships between reduced VO_{2peak} and select anthropometric variables (body size and body mass) (Fernhall et al. 1996). These findings thus suggested some degree of independence between peak exercise capacity and morphologic features typical of DS (lower height and increased body mass). Subsequently, it was shown that obesity was not related to the attenuated hemodynamic response of persons with DS during 3 autonomic provocative maneuvers (peak exercise, cold pressure test, isometric handgrip) (Fernhall and Otterstetter 2003). This was later confirmed by Figueroa et al who also found no association between impaired cardiac autonomic

regulation during isometric handgrip and obesity in adults with DS (Figueroa et al. 2005). Furthermore, in contrast to individuals without DS, obesity has no effect on maximal heart rate and little effect on VO_{2peak} in persons with DS (Fernhall et al. 2003). Consequently, to the best of our knowledge, there is no scientific evidence supporting the "obesity" hypothesis as a main cause of low VO_{2peak} in persons with DS.

Considering the dependency of VO_{2peak} on ventilatory capacity, it seems possible that the reduced peak exercise capacity in DS corresponds to reductions in ventilatory function. In agreement, it has been reported that persons with DS typically exhibit low peak exercise minute ventilation (Fernhall et al. 1996; Fernhall and Pitetti 2001; Fernhall et al. 1990; Fernhall et al. 2001). The lack of ability to achieve high rates of ventilation by individuals with DS could result from reduced airway size, small nasal passages, and large tongue, potentially making it more difficult to breathe during exercise. However, Fernhall and Pitetti reported that the ventilation of subjects with DS was found to be appropriate for their VO_2 during peak exercise (Fernhall and Pitetti 2001). This suggested that the reduced ventilation does not account for the low VO_{2peak} values in this population. Finally, it was also speculated that altered mitochondrial function in DS could impact muscle function during endurance exercise (Baynard et al. 2008). This would be manifested by a delayed rate of VO_2 increase in response to exercise and contribute to low VO_{2peak} in individuals with DS. However, this is not supported by recent findings of preserved VO_2 kinetics in adults with DS while responding to exercise (Mendonca et al. 2010a).

2.1.5.2. Ventilatory threshold in persons with Down syndrome

The ability to exercise for long periods at high fractions of the VO_{2peak} is an important determinant of work performance (Sjodin and Svedenhag 1985; Scrimegeour et al. 1986; Costill et al. 1973; Maughan and Leiper 1983). The self-selected fractional utilization of the VO_{2peak} during endurance exercise is linked to the VT (Coyle 1995; Jones and Carter 2000). Therefore, the VT is often used as a submaximal index of aerobic exercise capacity and it has been described as the exercise intensity at which minute ventilation increases at a disproportional rate compared to the increase in VO_2 (Wasserman et al. 2005; Mahon and Cheatham 2002; McArdle et al. 2001). For

clinical purposes, the VT has been used in a variety of populations because it does not necessitate peak exercise effort (Hebestreit et al. 2000; Ohuchi et al. 1996; Reybrouck et al. 2001; Washington et al. 1988; Climstein et al. 1993).

The literature on the VT of persons with DS is very limited with only 2 previous studies available, making it very difficult to infer possible relationships between this physiological variable and poor exercise capacity in this population (Baynard et al. 2004b; Mendonca et al. 2010a). The lack of research on this topic is probably related to the difficulty in detecting VT in most individuals with DS. Baynard et al showed that, although detection rates approached 100% in adolescents with ID without DS, the VT was only detectable in ~ 60% of those with DS (Baynard et al. 2004b). They also showed that the minute ventilation over time method yielded the best detection rate in adolescents with DS. Interestingly, it was found that the VT (expressed as a percentage of VO_{2peak}) of participants with DS did not differ from that of ID controls without DS (DS: 65-70% vs controls with ID: 58-61%). Recent research conducted by Mendonca et al also found similar fractional utilization of VO_{2peak} at the VT between adults with DS and nondisabled controls of similar age, sex and body mass index (DS: 67.5%; nondisabled controls: 63.3%) (Mendonca et al. 2010a). Several investigations agree that the VT occurs between 52-57% of VO_{2peak} for nondisabled girls and boys between the ages of 11-20 y (Climstein et al. 1993; Rhodes et al. 1997). Younger children (7-12 y) exhibit higher relative VT, at 68-75% of VO_{2peak} (Climstein et al. 1993). For adults, the VT typically occurs at 50-70% VO_{2peak} although it can be as high as 80-85% VO_{2peak} in highly trained individuals (Sjodin and Svedenhag 1985). Thus, persons with DS seem to have a relatively normal VT when expressed as a percentage of VO_{2peak}. Consequently, it is unlikely that their limited exercise capacity results from low VT. Nevertheless, this needs to be further investigated and actual measurements of blood lactate concentration during graded exercise would be valuable to help clarifying this issue.

2.1.5.3. Exercise Economy in persons with Down syndrome

Another important factor that contributes to endurance exercise capacity has been termed "exercise economy" (Joyner and Coyle 2008). Economy refers to how much speed or power can be generated for a given level of VO_2 ($mL.kg^{-1}.min^{-1}$) during activities such as walking, running or

cycling. Exercise economy reflects the interaction of numerous factors including muscle morphology, elastic elements and joint mechanics in the efficient transfer of ATP to mechanical speed. Good exercise economy is manifested by lower fractional utilization of VO_{2peak} at a given speed and, consequently, in a reduction in glycogen utilization and less reliance on O_2-independent metabolism leading to attenuated metabolic acidosis (Jones 2006). Even though VO_{2peak} is very important for high level performances, the individual workload attained at peak exercise is a function of both VO_{2peak} and exercise economy (Jones 2006). This led some authors to explore whether exercise economy of persons with DS further exacerbates the amount of physical work they can perform because of their low VO_{2peak}. The main hypothesis was that, due to several anatomical and functional characteristics of DS (i.e., joint laxity, muscle hypotonia, gait instability), these individuals would present lower walking economy than nondisabled controls during exercise (Kubo and Ulrich 2006; Smith and Ulrich 2008).

Although there are few studies on submaximal exercise capacity of persons with DS, recent research has provided important information on this issue. Mendonca et al first reported similar walking economy between adults with and without DS during horizontal treadmill exercise (Mendonca et al. 2009). However, in this study, the submaximal treadmill speed was selected on the basis of previous findings showing that adults with DS prefer very low walking speeds (~ 2.5 $km.h^{-1}$). Because the O_2 cost of locomotion ($mL.kg^{-1}.km^{-1}$) displays a U-shape when analyzed as a function of walking speed, this might have placed the nondisabled participants at greater disadvantage compared to those with DS, thus limiting subsequent interpretations of the data. This suggested that the use of a wider range of walking speeds would be necessary to draw meaningful conclusions about walking economy in DS. While exploring this issue, Agiovlasitis et al showed an upward shift in the U-shaped O_2 cost of locomotion of adults with DS compared to that of nondisabled controls (Agiovlasitis et al. 2009). Importantly, these results were obtained when comparing both groups at similar dimensionless Froude number walking speeds (dissipating the effects of shorter legs in participants with DS). Moreover, when expressing walking economy as a function of body mass (VO_2/kg), it was also found that the VO_2 increased more steeply with increases in walking speed in adults with DS than in controls

(Agiovlasitis et al. 2009). This was later confirmed by Mendonca et al. (2010b). These authors further explored differences in the delta VO_2 response to positive changes in walking grade between adults with and without DS. They found that increases in treadmill grade at constant speed yielded similar delta VO_2 between groups, thus suggesting that individuals with DS responded as efficiently as nondisabled controls to increments in walking grade. Consequently, there is enough evidence in the literature to suggest that, not only is the VO_{2peak} of persons with DS substantially reduced, these individuals also exhibit low exercise economy at speeds faster than their preferred walking speed which further compromises their exercise capacity. Of several candidate biomechanical variables (kinematic and kinetic) possibly contributing to the lower walking economy in DS, dynamic balance has been shown to be particularly challenging to these individuals (Agiovlasitis et al. 2009; Black et al. 2007). Accordingly, this may well implicate higher metabolic cost at faster walking speeds. The use of higher levels of muscle stiffness and angular impulse (forcing) by persons with DS when walking on a treadmill may also contribute to higher energy expenditure during positive variations in walking speed (Ulrich et al. 2004).

In summary, the existent research indicates that persons with DS show lower relative VO_{2peak} than controls without disabilities from childhood to adulthood. It is highly likely that this is due to reduced catecholamine response to dynamic exercise which is manifested by chronotropic incompetence and limited cardiac output at peak exercise intensities. As reported by Baynard et al., this is different from that seen in individuals with ID without DS in whom the relative VO_{2peak} is similar to that of nondisabled controls across all age groups (Baynard et al. 2008). One possible contributor to the low peak aerobic capacity across all ages in persons with DS is reduced physical activity levels; however, there is compelling evidence to support a true physiological limitation to exercise performance in these individuals (i.e., chronotropic incompetence). As importantly, adults with DS also show poor physiological response to submaximal exercise (i.e., reduced exercise economy) and this further aggravates their limited exercise capacity. Because disturbances in dynamic balance during locomotion have been shown to occur in DS at a young age, it is possible that reduced exercise economy may also be manifested by children and adolescents with DS (Kubo and Ulrich

2006). Therefore, otherwise healthy persons with DS exhibit a clinical profile characterized by reduced exercise capacity at all ages. Among several possible causes, including limited exercise opportunities, there is enough evidence to support an altered physiological response to exercise performed at different intensity domains in this population.

2.1.6. Effects of reduced exercise capacity in Down syndrome

Neuromuscular strength and exercise capacity are important prerequisites for many activities of daily living, including tasks such as eating, dressing, rising from a chair, and walking. These factors are essential in maintaining independence and are important measures of functional ability (Cowley et al. 2010). As mentioned, individuals with DS are significantly weaker and have lower VO_{2peak} than those with and without other forms of ID at all stages of life (Baynard et al. 2008; Croce et al. 1996; Pitetti and Boneh 1995; Angelopoulou et al. 1999; Pitetti et al. 1992b). Such findings suggest that compromised basic function may be one of the main effects of reduced exercise capacity in this population (Pitetti and Boneh 1995; Carmeli et al. 2002a; Carmeli et al. 2002b; Carmeli et al. 2004). Exercise capacity is also related to vocational performance in adults with ID and individuals removed from competitive employment show a decrease in adaptive skill functioning and quality of life (Beasley 1982; Croce and Horvat 1992; Horvat and Croce 1995; Kober and Eggleton 2005; Stephens et al. 2005).

Even though it would seem reasonable to expect some association between low physical fitness (reduced exercise capacity and/or muscle weakness) and poor performance in tasks of daily living in persons with DS, only recently was this issue appropriately explored. Cowley et al analyzed relationships between 3 timed tasks of daily living (chair rise, gait speed and stair ascent and descent), age and physical fitness (VO_{2peak}, knee extensor and flexor strength) (Cowley et al. 2010). Findings indicated that knee extensor strength was the most influential variable in predicting timed task performance, followed by VO_{2peak}. In contrast, age was not a significant predictor of timed task performance in this population. Interestingly, the fact that VO_{2peak} predicted functional ability (chair rise and stair ascent) in a group of young adults with DS contrasts with findings in adults of similar age without DS. Such relationships have only been shown to exist in nondisabled adults of 65 y and

older (Alexander et al. 2003; Arnett et al. 2008). This led the authors to speculate that functional ability and independency of individuals with DS could become greatly affected if their trajectory of VO_{2peak} decline with age would be similar or worse to that of people without DS. This was relevant because an increased rate of biological aging had been previously shown to occur in DS (~ twofold compared to nondisabled subjects) (Nakamura and Tanaka 1998). However, Baynard et al showed that VO_{2peak} does not decline in these individuals after the age of 16 y (Baynard et al. 2008). Consequently, in contrast to that hypothesized by Cowley et al. (2010), the relationship between low VO_{2peak} and poor functional ability may possibly span all developmental stages of persons with DS and therefore, be independent of the aging process. Accordingly, such relationship may be inherent to DS itself and may not be affected by or predisposed to premature aging. The lack of association between age and performance on functional tasks of daily living, as described by Cowley et al. (2010), further supports this contention (Croce et al. 1996). Although the exact reason for the lack of VO_{2peak} decline with age in DS is not known, it is feasible that if this population has such low physical activity levels starting at a young age, any further reduction in physical activity will not manifest itself in an age-associated reduction in VO_{2peak}. Another possibility is that, they may just stay active enough to maintain enough functionality to meet the demands of daily living while avoiding substantial decrements in their VO_{2peak} as they age (Baynard et al. 2008). Therefore, functional ability, in part mediated by exercise capacity, may well limit long-term employment and independence in this population, resulting in decreased community integration, increased need for services and support, and decreased quality of life.

Although little empirical data support this notion, children with DS exhibit fewer episodes of high intensity physical activity compared to their nondisabled peers (Whitt-Glover et al. 2006). Physical activity patterns may be influenced by the energy expenditure during a certain activity. Thus, reduced exercise economy over a wide range of walking speeds may possibly provide a partial explanation for the high levels of sedentary behavior reported in this population (~ 79%) (Mendonca et al. 2010b; Agiovlasitis et al. 2009; Stanish and Draheim 2005). Considering that, under normal circumstances, physical activity accounts for between 15 and 30% of a person's total daily energy

expenditure this may also contribute to the high prevalence of overweight reported in DS (males: 45%; females: 56%) (Rubin et al. 1998; Starling et al. 1998). To gain a more comprehensive understanding on this topic, future research that assesses the relationship between these variables in DS would prove invaluable. Restricted opportunities to participate in exercise programs taking place at a community setting may also provide partial explanation for these findings (Carmeli et al. 2002a). Even though such programs have been shown to be effective to improve muscle function in persons with DS, there is limited research on this topic (Shields et al. 2008).

2.1.7. Management of reduced exercise capacity in Down syndrome

It is important for persons with DS to maintain their functional status so that they can lead healthy and satisfying lives without being institutionalized, especially as they age. Exercise training interventions targeted at children with DS are of particular importance because early intervention may have greater effects over the life span. Nevertheless, to the best of our knowledge, few prospective studies have examined the effects of structured exercise training in persons with DS and their findings are somewhat contradictory. Millar et al observed increases in work capacity but no changes in the VO_{2peak} of 14 adolescents with DS after a 10-wk, 3 d/wk aerobic exercise program (Millar et al. 1993). These findings were subsequently confirmed by Varela et al after a 16-wk rowing program performed 3 d/wk (Varela et al. 2001). This led the authors to speculate that the capacity for improving aerobic functioning in DS might be limited. Nevertheless, both studies reported gains in exercise endurance after training; however, they were unable to provide a definite explanation for these findings. More recently, Tsimaras et al were the first to document a significant increase in the VO_{2peak} of adults with DS after an aerobic training regimen consisting of 3 sessions/wk for 12 wk (Tsimaras et al. 2003). Similar findings were also reported after 12 wk of a combined exercise program in adults with DS and after 28 wk of multi-ergometer aerobic conditioning (Mendonca et al. 2011; Rimmer et al. 2004; Mendonca and Pereira 2009). Given these latest reports, there is evidence that adults with DS respond positively to structured exercise training, particularly under regimens of combined aerobic and resistance training and this is further supported by a meta-analysis by Dodd and Shields (2005). It is difficult to determine why some studies found increases in VO_{2peak} with training while others did not.

Tsimaras et al suggest that it may be due to inadequate monitoring of intensity in the studies that failed to show increases in VO_{2peak} (Tsimaras et al. 2003). Most of the studies mention difficulties in motivating the subjects to keep exercising; and none reported the success rates of maintaining the required exercise intensity.

There are only 5 trials that investigated whether persons with DS have the capacity to improve their levels of muscle strength in response to progressive resistance training. Three of these trials included progressive resistance training and 2 used a combined exercise program (Mendonca et al. 2011; Shields et al. 2008; Rimmer et al. 2004; Davis 1987; Weber and French 1988). Overall, findings indicate that programs prescribed for a frequency of 3 days/wk improve the upper- and lower-limb muscle strength of persons with DS (Rimmer et al. 2004; Davis 1987; Weber and French 1988). In contrast, training regimens of lower frequency (2 days/wk) are apparently associated with gains in muscle endurance (Mendonca et al. 2011; Shields et al. 2008). Even though this might seem important, none of these studies analyzed possible relationships between gains in muscle strength or endurance and increased VO_{2peak} in persons with DS after training.

Considering that the limitations to exercise capacity in DS result not only from reduced VO_{2peak}, but also from compromised exercise economy, there were still some relevant and unanswered questions about the potential benefits of exercise training in this population. While exploring these issues, Mendonca et al recently found significant improvements in walking economy (ranging from 6.6 to 10.4 %) after combined exercise training in adults with DS (Mendonca et al. 2011). Interestingly, it was also shown that this improved walking economy explained the gains seen in the work capacity (GXT time to exhaustion) of these individuals after training. Consequently, although not measured in their studies, the gains in work capacity reported by Millar et al and Varela et al were most likely due to improved exercise economy after training (Millar et al. 1993; Varela et al. 2001).

Finally, until recently, it was not known whether the relative gains in physical fitness of people with DS paralleled those seen in nondisabled individuals. Mendonca et al showed that a group of adults with DS improved their physical fitness to a similar magnitude as adults without disabilities

after 12 wk of combined aerobic and resistance training (Mendonca et al. 2011). Overall, this represents an important practical implication for the management of reduced exercise capacity in persons with DS. Specifically, since adults with DS respond to combined exercise training as those without disabilities, this supports that conventional exercise prescription guidelines, as those recommended by the American College of Sports Medicine, are well suited for this population (ACSM 2009).

2.1.8. Conclusions

Reduced exercise capacity in persons with DS represents a significant problem since low fitness and activity levels have been associated with reduced survival rates in this population (Eyman and Call 1991). Although several other factors may also be implicated, physiological differences such as chronotropic incompetence and low levels of muscle strength and muscle hypotonicity are known to contribute to the problem. Fernhall et al suggested that the inability to reach expected maximal heart rates limits cardiac output of individuals with DS and subsequently limits their VO_{2peak} (Fernhall et al. 1996). The reason for these low maximal heart rates is apparently related to a combination between reduced adrenergic responsiveness and blunted vagal withdrawal during exercise (Fernhall et al. 2009; Figueroa et al. 2005). Although low levels of muscle strength and hypotonicity have been associated to low VO_{2peak}, they may also be a plausible cause of reduced exercise economy in this population. In contrast, even though the literature on this topic is scarce, the VT of persons with DS does not seem to limit their ability to respond appropriately to endurance exercise.

Reduced levels of muscle strength and low VO_{2peak} have been shown to be determinants of poor performance in tasks of daily living in persons with DS (Cowley et al. 2010). The relationship between these variables is not affected by the aging process in these individuals and therefore, spans all stages of their development. As a consequence, exercise training interventions targeted at children with DS are of particular importance.

Finally, the effect of exercise training has been shown to be beneficial for improving exercise capacity and physiological function in persons with DS (Mendonca et al. 2011; Tsimaras et al. 2003;

Rimmer et al. 2004; Mendonca and Pereira 2009; Dodd and Shields 2005). The literature suggests that a combined aerobic and resistance exercise program may have a larger impact on physical fitness than aerobic exercise alone in people with DS. This is supported by improvements seen in both VO_{2peak} and exercise economy resulting from combined exercise regimens (Mendonca et al. 2011; Rimmer et al. 2004). However, although there is enough evidence to suggest that programs designed to improve exercise capacity can be beneficial for people with DS, it is not known whether gains in physical fitness can be retained by this population over time because no study included a follow-up phase. The effect that a participant's age, sex, or concurrent health problem might have on program outcomes or on person's ability to participate in these programs also remains largely unknown. There is also a need to determine implications for longer term exercise training regimens and to further explore the effectiveness of increased opportunities to participate in exercise programs taking place in a community setting starting at a young age. These issues need to be addressed in future studies.

2.1.9. References

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3. Analysis of cardiorespiratory and metabolic data:

Validation of a treadmill exercise protocol.

Submaximal exercise capacity of adults with Down syndrome.

3.1. Between-day variability of net and gross oxygen uptake during graded treadmill walking: effects of different walking intensities on the reliability of locomotion economy.

3.1.2. Abstract

Background: Although of clinical relevance, there are few studies conducted on the reliability of walking economy.

Objective: This study intended to determine if walking economy reproducibility increases as a function of walking intensity and if there is any advantage in expressing walking economy as net versus gross VO_2 for reproducibility purposes.

Research design and Methods: Sixteen participants (9 males, 7 females; age 22.3 ± 4.3 yr) performed resting, submaximal and maximal protocols on two different days under identical circumstances within a 7-day period. The submaximal protocol consisted of five 5-min walks (4 $km.h^{-1}$) at treadmill grades of 0, 2.5, 5.0, 7.5 and 10%.

Results: Findings indicate that increments of 2.5% in treadmill grade were effective in increasing gross and net VO_2 over walks. Reliability of both measures increased as a function of walking relative intensity, reporting intraclass correlation coefficients ranging from 0.89-0.94 and 0.87-0.91 and mean coefficients of variation (CV) from 7.3-3.6% and 8.8-4.4%, respectively. There were no significant differences between the CV of gross and net VO_2 across the spectrum of walking relative intensities.

Conclusions: In conclusion, there is no advantage of expressing walking economy as net VO_2 versus gross VO_2 for reproducibility purposes, and a single treadmill testing session at a constant speed of 4 $km.h^{-1}$ is reliable for estimating group and individual walking economy, particularly at higher percent grades.

3.1.3. Key Words

Net oxygen uptake, gross oxygen uptake, walking economy, reliability, exercise, oxygen uptake

3.1.4. Introduction

Locomotor economy, defined as the oxygen uptake (VO_2) of walking or running at a given submaximal speed, is an important determinant of overall physical stress during locomotion (Morgan et al. 2002). A number of variables have been identified that could influence locomotor economy, including circadian variation, footwear, training status and treadmill accommodation (Morgan et al. 1991). As most daily activities are of submaximal intensity and involve self-ambulation, the level of within-subject variability in walking economy is critical to an understanding of the efficacy of a treatment or the presence of a training effect (Unnithan et al. 1995).

Several studies designed to determine the reproducibility of oxygen uptake during treadmill running reported an intraindividual variation between 1.5 and 5%, indicating that within-subject results are relatively stable (Brisswalter and Legros 1994; Morgan et al. 1987; Morgan et al. 1994b, Morgan et al. 1991, Pereira and Freedson 1997; Pereira et al. 1994; Saunders et al. 2004). On the other hand, few studies have been performed on determining the variation of walking economy, with the vast majority focusing on children and special populations (Astrand 1960; Morgan et al. 2002; Tseh et al. 2000; Keefer et al. 2005; Dobrovolny et al. 2003). A coefficient of variation (CV) in VO_2 of about 15% was found in one study in which 42 healthy women, 20-62 years old, walked horizontally on a treadmill at 5 $km.h^{-1}$ (Astrand 1960). More recently, by measuring the day-to-day variation in VO_2 and energy expenditure in 20 female adolescents (16.1-18.8 years old) walking at a flat 5 $km.h^{-1}$ treadmill speed, Wergel-Kolmert and Wholfart (1999) reported CV of 6.4, 8.1 and 5.7% for relative VO_2, absolute VO_2 and energy expenditure, respectively. Therefore, apparently, walking economy is a less reliable measure than running economy, as it is probably affected by psychological factors that influence VO_2 for less strenuous work output. Supporting this view, it has been reported that the reliability of running economy increases for horizontal running speeds eliciting higher relative intensities (Pereira and Freedson 1997; Pereira et al. 1994). As in previous studies walking economy reliability was explored as a function of a single treadmill workload, it is not known if its reproducibility depends on relative intensity. Therefore, it is possible that, as treadmill walking workload is increased, the intraindividual variation in walking economy will concomitantly decrease.

Of similar importance, most previous studies on locomotion economy have neglected to distinguish gross VO_2 (the total O_2 consumed while walking) and net VO_2 (the increased O_2 consumption while walking above the resting oxygen rate) (Bowen et al. 1998; Duffy et al. 1996; Felici 1997; Wergell-Kolmert and Wohlfart 1999). While the net VO_2 is more directly related to walking economy, most early studies used measures of gross VO_2 (Waters and Mulroy 1999; Ralston 1958; Waters et al. 1988). In recent years, net measures have been recommended (Baker et al. 2001; Schwartz et al. 2005) with several groups reporting locomotion economy as a function of net VO_2 (Brehm et al. 2007; Unnithan et al. 1996; Unnithan et al. 1999). It has been suggested by Baker et al. (1999) that net VO_2 is more a stable parameter as it is less dependent on walking speed than gross VO_2 and, therefore, more suitable for reliable walking economy assessments. However, to date, the only study that compared the intra-subject variability of net with gross VO_2 concluded that there was more variability in the former than in the latter (Brehm et al. 2007). Unfortunately, as the authors did not control for absolute or relative walking intensities it is not clear if there are advantages of expressing walking economy as a function of one or the other. Furthermore, it is not known if the reliability of net or gross VO_2 increases with walking relative intensity. Clarification of both these issues would be particularly important to the understanding of the relevance of including a resting protocol on walking economy measurements, and also to clarify if the reliability of walking economy assessments can be further improved by manipulating absolute and relative work intensities. Thus, one of the purposes of this study was to investigate if walking economy reproducibility increases as a function of walking relative intensity. Additionally, we intended to determine if there was any advantage in expressing walking economy as net versus gross VO_2 in terms of reproducibility of the measurements. For determining the between-day reproducibility of relative exercise intensities and to analyse possible relationships with the reliability of walking economy, we also measured the variability associated with maximal treadmill exercise performance.

3.1.5. Methods

3.1.5.1. Participants

A total of 16 participants, 9 male and 7 female physical education students volunteered to take part in the study (22.3 ± 4.3 years old). Participant characteristics are presented in table 3.1. All participants were experienced treadmill walkers and runners and they all were similarly active, accumulating nine-hours of physical activity per week as part of their academic work. Medical histories were obtained through direct interviews and exclusion criteria were as follows: 1) history of thyroid or cardiovascular disease, 2) history of diabetes or other metabolic disease that might affect outcome measures, 3) heart rate altering medications, 4) smoking, 5) pulmonary or respiratory disorders, including asthma and 6) orthopaedic injury preventing successful completion of the exercise protocol. After thorough explanation of the study protocol to participants, and after having been shown the equipment, written informed consent was attained. This study was approved by the university institutional review board.

3.1.5.2. Measurements

All subjects were tested in a postprandial state, approximately 2-4 hours after their last meal. Participants refrained from exercise 24 hours before testing and caffeine ingestion on testing days. Participants were advised to wear the same footwear during all visits and all testing was completed within a 7-day period. The days of testing consisted of: (1) a standardized body composition assessment, (2) a resting protocol, (3) a continuous submaximal steady-state exercise protocol, and (4) a maximal graded exercise protocol. Testing was carried out in the laboratory with an environmental temperature between 21 ºC and 24ºC and a relative humidity between 44 and 56%. In an attempt to control for possible circadian variations in submaximal walking economy, the measurements were performed between 07.00 and 11.00 h at approximately the same time for each individual. A minimum of 2 days between each test was instigated in order to prevent any possible residual fatigue between sessions.

Body mass was measured at both visits using a calibrated digital scale, and height was measured using a stadiometer (Secca 770, Hamburg, Germany - standing digital scale/height rod attached). Body mass index (BMI) was calculated by dividing the participants' mass in kilograms by the square of their height in metres.

Expired gas measurements were made using a computerized on-line breath-by-breath system (Quark b^2, Cosmed® Srl-Italy), which was calibrated before each test with a known volume and with known gas concentrations. Heart rate data were obtained by means of a Polar RS 800 G3 heart rate monitor (Polar Sports Ltd, Kempele, Finland).

Table 3.1 Descriptive characteristics of the participants.

	Age (yr)	**Body mass (kg) visit 1**	**Body mass (kg) visit 2**	**Height (cm)**	**BMI (kg/m^2)**
Males (n = 9)	23.4 ± 5.0	71.4 ± 8.6	71.3 ± 8.4	175.9 ± 3.5	23.1 ± 2.7
Females (n = 7)	20.5 ± 1.9	56.3 ± 3.7	56.5 ± 3.7	164.0 ± 2.5	20.9 ± 1.8

Values are mean ± SD.

Abbreviations: BMI, body mass index.

3.1.5.3. Testing protocols

For calculation of test-retest reliability the walking economy test was completed twice within a 7-day period. Each test was conducted after a 15-min resting period in the sitting position to stabilize circulation and breathing. Resting VO_2 was obtained during the last 5 min of the resting period. Walking economy was determined by measuring submaximal VO_2 on a motorised treadmill (h/p/cosmos® mercury med 4.0). The protocol involved continuous walking at a constant speed of 4 km.h^{-1} at five different treadmill grades (0, 2.5, 5.0, 7.5 and 10%), for 5 min each. The submaximal treadmill speed was selected on the basis of the results reported by Cavagna et al. (1976), who found that at, 4 km.h^{-1}, the work done at each step to lift the center of mass of the body equals the work done to increase its foreword speed. According to these authors, the total mechanical energy is at this speed at a minimum, as is the energy cost.

On-line VO_2 uptake measurements were taken during the submaximal protocol. The VO_2 data were displayed as 30-s averages. The mean of the last 2 min of each 5-min walk was used as the participants' submaximal steady-state VO_2 ($mL.min^{-1}$) and used for subsequent analysis (Whipp 1971). Walking economy was expressed both as gross and net VO_2. Net VO_2 was determined by subtracting the resting steady-state VO_2 from the gross steady state VO_2, obtained during the last 2 min of the walk. Additionally, groups submaximal relative work intensities were determined as percentages of VO_{2max} (fractional utilization – FU).

3.1.5.4. Maximal protocol

VO_{2max} was determined by means of a continuous incremental test to volitional exhaustion commencing immediately after the fifth submaximal walk at both testing sessions. For this purpose, treadmill grade was increased from 10 to 12.5 % while maintaining a speed of 4 $km.h^{-1}$ for an additional minute. From this point, grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every minute until exhaustion. The test was terminated when the subject reached exhaustion and grasped the hand rails of the treadmill. As in previous studies, the VO_2 data were displayed in 20-s averages. Data were then examined to determine if VO_{2max} had been attained according to the following criteria (McArdle et al. 2001): 1) attainment of the age-predicted maximum heart rate, 2) RER $\geq$ 1.15, and 3) a plateau or decrease in VO_2. If one of the first two and the 3rd criterion were not achieved, the subject was required to repeat both the submaximal and maximal protocols after a recovery period ranging from 2-7 days. This was implemented for one female participant whose VO_{2peak} of the first visit was not lower than the VO_{2max} attained at the second.

3.1.5.5. Statistical analysis

Standard descriptive statistics (mean and standard deviation) were used to summarize the data. Resting, submaximal and maximal data were studied using analysis of variance with two way repeated measures to test for visit and treadmill grade effects. To further characterize subjects' between-day variability in resting, submaximal and maximal physiological responses, intraclass correlations coefficients (ICC) for gross VO_2, net VO_2, minute ventilation (Ve), heart rate (HR), respiratory exchange ratio (RER) and FU were calculated across the two visits. This latter method of analysis was

employed to provide an index of reliability sensitive to changes in both order and magnitude of the repeated measurements (Vincent 1995).

The stability of steady-state submaximal measurements was also assessed by calculating the coefficients of variation (CV). The CV in gross VO_2, net VO_2, Ve, HR, RER and FU for each subject were calculated as the ratio of standard deviation to the mean of the two submaximal trials using the steady-state time period. The CV were subsequently multiplied by 100 and expressed as a percentage of the absolute value. The same analysis was conducted on the data collected from the resting and maximal exercise protocol. Subsequently, a multivariate analysis of variance was computed to investigate the existence of differences between the CV of gross and net VO_2 at each treadmill grade. All statistical calculations were computed using SPSS version 16.0 and a significance level of 0.05 was used.

3.1.6. Results

3.1.6.1. Resting analysis

There was no change in body mass for males or females over the two visits ($p > 0.05$) (table 3.1). The repeated measures ANOVA reported no resting differences between visits for VO_2, Ve, HR or RER ($p > 0.05$). However, while resting VO_2 was moderately reproducible (ICC of 0.68; $p < 0.05$) and considerably variable (CV of 12.9 ± 3.0%), Ve and RER were not reliable ($p > 0.05$). HR was found to be the resting measurement with highest reliability (ICC of 0.88) and least variation (CV of 6.7 ± 1.6%) between visits (table 3.2).

Table 3.2 Reproducibility of resting cardiorespiratory variables.

Variable	Visit 1	Visit 2	ICC	CV (%)
VO_2 ($mL.min^{-1}$)	231.4 ± 63.0	225.4 ± 63.1	0.68*	12.9 ± 3.0
Ve ($L.min^{-1}$)	8.6 ± 1.5	8.8 ± 1.7	0.51	9.9 ± 2.5
HR (bpm)	73.1 ± 15.8	70.4 ± 13.1	0.88**	6.7 ± 1.6
RER	0.83 ± 0.08	0.83 ± 0.10	-0.36	9.2 ± 2.5

Values are mean ± SD.

Abbreviations: ICC, intraclass correlation coefficient; CV, coefficient of variation; VO_2, oxygen uptake; Ve, minute ventilation, HR, heart rate; RER, respiratory exchange ratio.

*$p < 0.05$; ** $p < 0.0001$.

3.1.6.2. Submaximal walking economy

There was a significant increase in gross VO_2, net VO_2, Ve and HR with treadmill grade (table 3.3). Although RER values did not change with increasing treadmill grade from 0 to 2.5%, or from 5 to 7.5 and 10%, there were significant differences between the former two and the last three grades ($p < 0.05$). One other variable, FU, was calculated across the five treadmill walking grades and it increased as a function of treadmill walking grade ($p < 0.0001$) (table 3.3).

As shown in table 3.4, repeated measures ANOVA yielded no significant differences between visit 1 and visit 2 for gross VO_2, net VO2, Ve, HR, RER or FU across the five treadmill grades ($p > 0.05$). With the exception of RER, ICC analyses revealed significant between-day relationships for all other variables at each treadmill grade. The highest ICC values for gross and net VO_2 were obtained at 10% (0.94 and 0.91, $p < 0.0001$) and the lowest at 0% treadmill grade (0.89 and 0.87, $p < 0.0001$). The ICC for Ve progressively increased from 0% (0.77, $p < 0.01$) to the highest value, registered at 7.5% treadmill grade (0.93, $p < 0.0001$). Although similar between 0 and 2.5% (0.93, $p < 0.0001$), ICC values for HR progressively increased from 2.5 to 10% treadmill grade (0.96, $p < 0.0001$). While similar between 2.5 and 5% grades (0.78, $p < 0.01$), the ICC values for FU generally increased from 0% (0.73, $p < 0.01$) to 10% treadmill grade (0.86, $p < 0.0001$).

Table 3.3 Cardiorespiratory and metabolic variables at submaximal walking grades.

	VO_2 gross ($mL.min^{-1}$)	**VO_2 net ($mL.min^{-1}$)**	**Ve ($L.min^{-1}$)**	**HR (bpm)**	**RER**	**FU (%)**
0%	656.7 ± 117.5*	428.5 ± 76.2*	18.1 ± 2.8*	87.8 ± 15.9*	0.79 ± 0.04	22.9 ± 4.0*
2.5%	813.8 ± 146.3*	585.5 ± 103.4*	21.7 ± 2.9*	93.7 ± 16.4*	0.83 ± 0.03	28.3 ± 4.7*
5%	963.9 ± 166.5*	735.6 ± 123.6*	25.7 ± 3.6*	100.5 ± 17.6*	0.85 ± 0.03#§	33.6 ± 5.8*
7.5%	1145.5 ± 195.2*	917.2 ± 152.3*	30.3 ± 4.0*	108.3 ± 18.1*	0.87 ± 0.03#§	40.0 ± 7.1*
10%	1294.0 ± 207.7*	1065.7 ± 164.5*	33.3 ± 4.5*	115.5 ± 19.7*	0.86 ± 0.03 #§	45.2 ± 7.9*

Values are mean ± SD.

Abbreviations: VO_2, oxygen uptake; Ve, minute ventilation; HR, heart rate; RER, respiratory exchange ratio; EE, energy expenditure; FU, fractional utilization.

*All values are different at $p < 0.0001$; # (RER 10%; RER 7.5%; RER 5%) > RER 0%, $p < 0.0001$; § (RER 10%; RER 7.5%; RER 5%) > RER 2.5%, $p < 0.05$.

Mean between-day CV for gross and net VO_2 over visits was ~4-7% and ~4-9%, respectively. The CV of gross and net VO_2 between visits were lowest at 10% (3.6 and 4.4%) and highest at 0% grade (7.3 and 8.8%). For HR and FU, the CV were also lowest at 10% (1.0 and 5.9%) and highest at 0% grade (7.5 and 11.2%), respectively. Similarly, the highest CV for Ve and RER were obtained at 0% grade (7.7 and 7.0%) and the lowest at 10% grade (5.8% and 2.9%) (table 3.4). MANOVA reported no differences between the CV of gross and net VO_2 at each treadmill grade: 0% ($F = 0.75$, $p > 0.05$), 2.5% ($F = 1.15$, $p > 0.05$), 5% ($F = 0.21$, $p > 0.05$), 7.5% ($F = 0.64$, $p > 0.05$) and 10% ($F = 0.48$, $p > 0.05$).

Table 3.4 Reproducibility of cardiorespiratory and metabolic variables at submaximal walking grades.

Variable	Visit 1	Visit 2	ICC	CV (%)
0% grade				
VO_2 gross ($mL.min^{-1}$)	663.4 ± 129.7	650.0 ± 118.0	0.89*	7.3 ± 1.3
VO_2 net ($mL.min^{-1}$)	432.2 ± 86.8	424.7 ± 74.6	0.87*	8.8 ± 1.8
Ve ($L.min^{-1}$)	18.3 ± 3.0	17.8 ± 3.1	0.77**	7.7 ± 1.6
HR (bpm)	89.7 ± 18.0	85.8 ± 14.8	0.93*	7.5 ± 1.2
RER	0.79 ± 0.05	0.80 ± 0.06	0.14	7.0 ± 0.8
FU (%)	23.5 ± 4.0	22.3 ± 4.9	0.73**	11.2 ± 2.4
2.5% grade				
VO_2 gross ($mL.min^{-1}$)	802.8 ± 149.4	824.8 ± 153.2	0.93*	6.6 ± 1.1
VO_2 net ($mL.min^{-1}$)	571.5 ± 104.5	599.4 ± 117.1	0.85*	8.4 ± 1.5
Ve ($L.min^{-1}$)	21.5 ± 3.4	21.9 ± 3.0	0.84**	5.3 ± 1.3
HR (bpm)	94.7 ± 18.0	92.8 ± 15.8	0.93*	5.4 ± 0.9
RER	0.82 ± 0.04	0.84 ± 0.04	0.34	4.4 ± 0.7
FU (%)	28.4 ± 4.6	28.2 ± 5.7	0.78**	7.8 ± 2.0
5% grade				
VO_2 gross ($mL.min^{-1}$)	961.6 ± 168.3	966.2 ± 178.8	0.91*	6.1 ± 1.0
VO_2 net ($mL.min^{-1}$)	730.31 ± 122.9	740.9 ± 141.1	0.85*	6.9 ± 1.4
Ve ($L.min^{-1}$)	25.8 ± 3.7	25.5 ± 3.8	0.89*	4.8 ± 1.2
HR (bpm)	102.0 ± 20.3	99.0 ± 15.8	0.94*	3.9 ± 0.9
RER	0.85 ± 0.04	0.86 ± 0.04	0.27	3.7 ± 0.6
FU (%)	34.1 ± 5.6	33.1 ± 7.1	0.78**	7.7 ± 1.8
7.5% grade				
VO_2 gross ($mL.min^{-1}$)	1127.2 ± 178.9	1163.8 ± 226.1	0.91*	4.7 ± 1.1
VO_2 net ($mL.min^{-1}$)	895.8 ± 136.0	938.4 ± 197.7	0.79**	6.5 ± 1.5
Ve ($L.min^{-1}$)	30.4 ± 4.5	30.2 ± 3.7	0.93*	4.3 ± 1.3
HR (bpm)	109.5 ± 20.0	107.1 ± 17.0	0.95*	2.3 ± 0.9
RER	0.87 ± 0.05	0.87 ± 0.04	0.37	3.2 ± 0.7
FU (%)	40.1 ± 7.0	40.0 ± 8.6	0.79**	7.4 ± 2.0
10% grade				
VO_2 gross ($mL.min^{-1}$)	1281.6 ± 197.5	1306.4 ± 229.1	0.94*	3.6 ± 1.3
VO_2 net ($mL.min^{-1}$)	1053.1 ± 155.2	1081.1 ± 186.5	0.91*	4.4 ± 1.8
Ve ($L.min^{-1}$)	33.5 ± 4.7	33.0 ± 4.8	0.90*	5.8 ± 1.6
HR (bpm)	116.8 ± 20.6	114.3 ± 17.4	0.96*	1.0 ± 1.0
RER	0.86 ± 0.04	0.86 ± 0.04	0.34	2.9 ± 0.8
FU (%)	45.6 ± 7.4	45.0 ± 9.5	0.86*	5.9 ± 2.4

Values are mean ± SD.

Abbreviations: ICC, intraclass correlation coefficient; CV, coefficient of variation; VO_2, oxygen uptake; Ve, minute ventilation, HR, heart rate; RER, respiratory exchange ratio; EE, energy expenditure; FU, fractional utilization. * $p < 0.0001$; ** $p < 0.01$.

3.1.6.3. Maximal VO_2 analysis

As reported in table 3.5 there was no visit effect for VO_2, Ve, HR or RER at maximal exercise ($p > 0.05$). VO_2 and Ve exhibited the highest between-day ICC (0.92, $p < 0.0001$). While maximal RER was not reliable (0.31, $p > 0.05$), HR was highly reliable (0.90, $p < 0.001$) between visits. HR had the lowest between-day CV (1.4%) and Ve the highest one (11.0%). The CV for VO_{2max} was 8.6% from day to day and that obtained for RER was 5.9%.

Table 3.5 Reproducibility of cardiorespiratory variables at maximal exercise.

Variable	Visit 1	Visit 2	ICC	CV (%)
VO_2 ($mL.min^{-1}$)	2892.1 ± 695.8	3044.7 ± 819.5	0.92*	8.6 ± 1.6
Ve ($L.min^{-1}$)	111.5 ± 36.8	114.3 ± 38.7	0.92*	11.0 ± 3.0
HR (bpm)	191.7 ± 10.1	190.2 ± 7.5	0.90*	1.4 ± 0.4
RER	1.22 ± 0.12	1.23 ± 0.13	0.31	5.9 ± 1.8

Values are mean ± SD.

Abbreviations: ICC, intraclass correlation coefficient; CV, coefficient of variation; VO_2, oxygen uptake; Ve, minute ventilation, HR, heart rate; RER, respiratory exchange ratio. *$p < 0.0001$.

3.1.7. Discussion

The main finding of the present study is that the reproducibility of net VO_2 is similar to that of gross VO_2 across a spectrum of five different walking relative intensities. Additionally, we found that the reliability of both measures increases as a function of treadmill grade and walking relative intensity.

Our results do not confirm the hypothesis previously advanced by Baker et al. (2001) nor do they support the findings of Brehm et al. (2007). Baker et al. (2001) proposed that net VO_2 would reduce the variability of walking economy measurements. However we did not find higher ICC values for net VO_2 in comparison to gross VO_2 for five different relative walking intensities. Brehm et al. (2007) had previously found higher intra-subject variability in net VO_2 when compared to gross VO_2.

The present study does not agree with that of Brehm et al. (2007) once we observed no significant differences between the CV of net and gross VO_2 for each treadmill workload. Of relevance, Brehm et al. tested their participants using a submaximal protocol that included 5-min of over-ground walk with the subjects being asked to walk their usual self-selected speed. Hence, it can be hypothesized that the higher degree of net VO_2 variability in the Brehm et al. (2007) study might be related to differences in walking speed as an additional source of subjects' between-day variation. One other important potential source of error in net VO_2 measurements is related to variations in resting VO_2 between days. As in Brehm et al. (2007) study, we also did not apply the standard conditions for the measurements of resting energy expenditure, namely abstinence from food for 8-12 hours (Haugen et al. 2003). It is possible that the CV of net VO_2 across relative walking intensities could have been further reduced if standard conditions for resting VO_2 had been respected. However, it does not seem viable to apply such rigorous conditions in clinical walking economy assessments.

Another important finding was that, at a constant speed of 4 $km.h^{-1}$, the mean values of walking economy were similar over different treadmill grades between visits (Fig. 3.1 and Fig. 3.2). These results are in accordance with those of prior studies assessing treadmill flat walk performance on healthy female adolescents (Wergel-Kolmert and Wohlfart 1999), healthy children (Unithan et al. 1995; Keefer et al. 2000), children with cerebral palsy (Keefer et al. 2005) and stroke patients (Dobrolvny et al. 2003). However, we obtained ICC ranging from 0.89-0.94 for the different treadmill grades which are higher than those of 0.83, 0.78 and 0.89 reported by Wergel-Kolmert and Wohlfart (1999), Keefer et al. (2005) and Dobrolvny et al. (2003), respectively. Therefore, walking economy reliability is apparently improved by graded treadmill walking in comparison to horizontal walking. Supporting this assumption, the ICC values in this study increased from the horizontal walk to a 10% treadmill grade. These findings are associated to the effect of treadmill grade on the subject's relative walking intensity. As it is well known, the stability of submaximal performance increases with exercise intensity and this is associated with a reduction of psychological influence over the physiological exercise responses (Crews 1992).

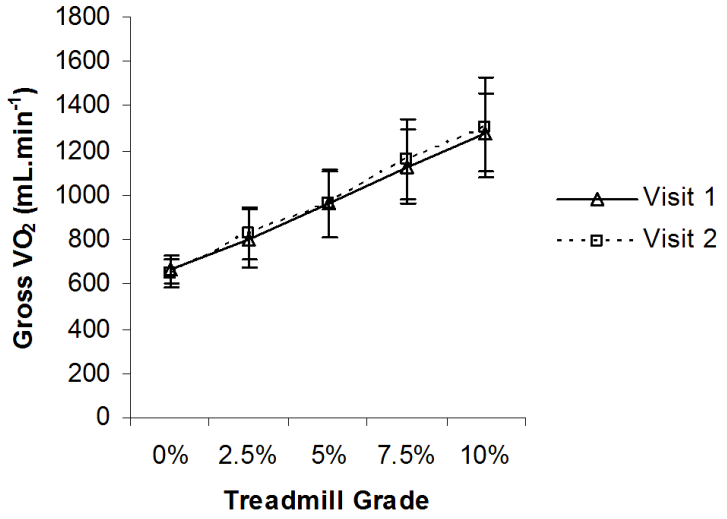

Fig. 3.1 Between-day variability of gross oxygen uptake (VO_2) up five treadmill gradients at a constant speed of 4 $km.h^{-1}$; ($p > 0.05$).

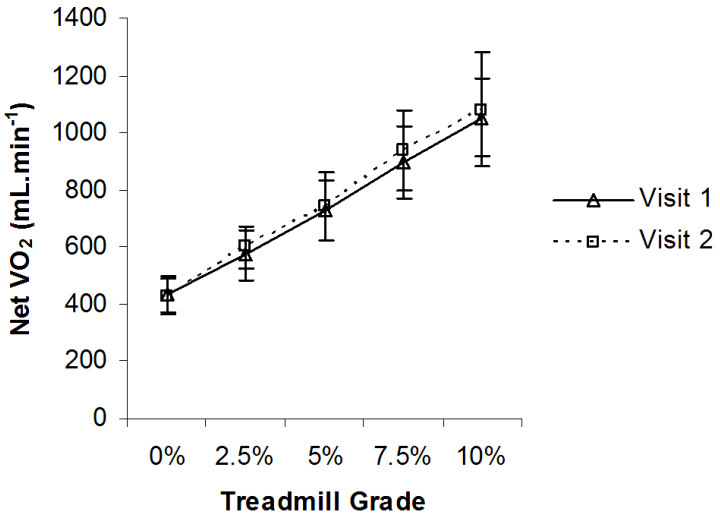

Fig. 3.2 Between-day variability of net oxygen uptake (VO_2) up five treadmill gradients at a constant speed of 4 $km.h^{-1}$; ($p > 0.05$).

We found little between-day variation in walking economy, ranging from 7.3% for gross VO_2 and 8.8% for net VO_2 at horizontal treadmill walking to 3.6% and 4.4% at a grade of 10%, respectively. The subjects' body mass was similar over visits, therefore, any variation in the walking economy would be unlikely to be attributable to fluctuation in body mass. Wergel-Kolmert and

Wohlfart (1999) had previously reported a CV of 8.1% in absolute VO_2 for female adolescents during a 5 $km.h^{-1}$ treadmill horizontal walk for two consecutive days, which is close to the CV of 7.9% obtained in present study during treadmill horizontal walking. Interestingly, the CV obtained at the 10% graded treadmill walk lies within the 1.5-5% interval generally reported for running economy designs (Brisswalter and Legros 1994; Morgan et al. 1987; Morgan et al. 1994b, Morgan et al. 1991, Pereira and Freedson 1997; Pereira et al. 1994; Saunders et al. 2004) substantiating the positive contribution of treadmill grading for stable assessments of walking economy.

Four further variables were investigated in order to assess their suitability as indices of submaximal intensity stability: respiratory exchange ratio, minute ventilation heart rate and fractional utilization. Respiratory exchange ratio data generated from this study are in agreement with that reported by Unnithan et al. (1995), confirming its low level of reliability (ICC of 14-37%) and thus, reflecting the multitude of substrate and ventilatory factors that influence this parameter. Reliability of minute ventilation increased with walking intensity and these results agree with those of Armstrong and Costill, (1985) and Davies et al. (1970). Additionally we obtained CV values ranging from 5.8-7.7% which are close to the values of 4.3% and 7.3% reported by Morgan et al. (1994a) and Saunders et al. (2004), respectively. Therefore, while the respiratory exchange ratio should not be regarded as a reliable submaximal index, minute ventilation can be viewed as a stable measure during graded treadmill walk at higher intensities.

There were no differences and little variation (1.0-7.5%) in heart rate response between visits and a high between-day ICC ranging from 0.93-0.96 was reported for this measurement. This implies that heart rate is a highly reliable submaximal parameter, particularly at higher walking intensities, which has significant implications for exercise prescription. The ICC for fractional utilization increased with treadmill walking grade, varying from 0.73 to 0.86, while between-day variation ranged from 5.9 to 11.2%. As for HR, these findings are relevant since exercise intensities are also frequently prescribed, for both clinical and sporting purposes, based upon percentages of the VO_{2max}.

At maximal exercise, the within-subject variation in VO_2 (8.6%) and minute ventilation (11.0%) was higher than those obtained for the submaximal protocol, opposed to heart rate which had very little between-visits variation (1.4%). However, there were no between-day differences for VO_{2max}, maximal minute ventilation or maximal heart rate and these measurements were highly reliable (ICC ~0.90) indicating a stability of order and magnitude between visits. Our results corroborate previous studies examining the reproducibility of maximal exercise testing in healthy subjects which have found similar CV for VO_{2max} (8.4%) and maximal minute ventilation (12%) (Garrard and Emmons 1986). On the other hand, as for submaximal treadmill walking, the respiratory exchange ratio had a low ICC at maximal exercise, confirming its unsuitability for individual assessments across different exercise intensities.

In conclusion, there is no advantage of expressing walking economy as net VO_2 versus gross VO_2 for reproducibility purposes, and a single treadmill testing session at a constant speed of 4 $km.h^{-1}$ is reliable for estimating group and individual walking economy in young healthy adults, particularly at higher percent grades.

3.1.8. References

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3.2. Walking economy in male adults with Down syndrome

3.2.1 Abstract

The purpose of this study was to investigate walking economy in response to steady-state locomotion in adult males with Down syndrome (DS) and in healthy controls. Twelve participants with DS (34.5±7.0 yr) and 11 non-disabled controls (34.3±8.7 yr) performed submaximal (0% grade, 2.5 $km.h^{-1}$ for 8 min) and maximal treadmill tests with metabolic and heart-rate measurements. For submaximal walking, submaximal oxygen uptake (VO_2) (9.1 vs 9.5 $mL.kg^{-1}.min^{-1}$), net VO_2 (5.9 vs 5.4 $mL.kg^{-1}.min^{-1}$) were not different between the groups ($p > .05$). However, oxygen-pulse (6.6 vs 8.6 mL/beat) was lower and relative work intensity (44.6 vs 19.9% of max) was higher in individuals with DS compared to controls ($p < 0.05$). Findings indicate similar walking economy between groups. Nevertheless, participants with DS exercised at lower submaximal oxygen-pulse and higher percentage of VO_{2peak}.. Therefore, despite similar walking economy, participants with DS have lower cardiorespiratory function than controls for a given steady-state treadmill speed.

3.2.3. Key Words:

Down syndrome, walking economy, functional capacity, exercise

3.2.4. Introduction

Individuals with Down syndrome (DS) have low aerobic capacity (Fernhall et al. 1996; Fernhall et al. 2001) and low levels of muscle strength (Guerra et al. 2000; Pitetti and Boneh 1995). This may contribute to the perception of low levels of physical activity in this population (Fernhall and Unnithan 2002). Although little empirical data support this notion, children with DS exhibit fewer episodes of high intensity physical activity compared to their non-disabled peers (Whitt-Glover, O'Neill and Stettler 2006). Physical activity patterns may also be influenced by the energy expenditure during a certain activity. However, it is unknown if differential energy expenditure may explain differences between individuals with and without DS.

Walking economy is represented by the energy requirements to perform a pre-determined amount of work, or the energy requirements to sustain a given submaximal walking intensity (Morgan and Craib 1992). Individuals with a good walking economy use less oxygen than others with poor walking economy at the same steady-state exercise condition (Thomas et al. 1999).

Walking economy is affected by atypical stride patterns that are characterized by frequent muscle co-contractions, isometric contractions against gravity, jerky movements and instability which is associated to an unbalance between generation of energy at one joint and absorption at another (Winter 1990). On the contrary, longer stride lengths (Cavanagh and Williams 1982) and lower peak vertical force at foot strike are typically more economical (Cavanagh et al. 1977). Gait stability is an important issue for people with DS, as their upright locomotion is affected by low muscle tone and ligamentous laxity. Individuals with DS present increased fluctuation of ankle movement during the walking cycle, lower walking velocities, shorter steps, wider strides and more time spent in both stance and double support (Parker et al. 1985; Smith and Ulrich 2008), all of which can probably modify their overall energy consumption during ambulation.

Individuals with DS also have other comorbidities known to affect the gait pattern in healthy adults including: (1) sedentary life style (Stanish and Draheim 2005), (2) obesity (Rubin et al. 1998) and (3) neurophysiological changes. Sedentary non-disabled adults adopt a more cautious walking

style than active adults, exhibiting shorter step lengths and slower step velocities (Rosengren et al. 1998). Obese persons walk slower (Spyropoulos et al. 1991) with wider step width and higher energetic cost (Browing and Kram 2005). Decreased proprioception, vision, tactile sensation and strength are known to contribute to the adoption of a walking pattern selected to enhance stability (Menz et al. 2003), but which is also less economical.

As some of the most common features of DS are known to affect their biomechanics of walking and as most daily activities are of submaximal intensity, the physiological evaluation of submaximal walking economy is relevant in these individuals. The purpose of the present study is to compare walking economy between individuals with DS and controls without disabilities in response to a standardized steady-state submaximal walk. We hypothesized that adults with DS would have lower walking economy compared to non-disabled healthy controls.

3.2.5. Methods

3.2.5.1. Participants

Twenty-three Caucasian healthy male participants (12 with DS, 11 controls without disabilities), aged 21 to 49 yr, were included in the present study. Descriptive statistics are presented in table 3.6. All participants with DS had medical approval for physical activity participation from their personal physicians. Those with congenital heart disease, ambulatory, musculoskeletal, visual, or auditory problems were not included in the study. Written informed consent was obtained from the participants' legal guardian. Other inclusion criteria were considered: (1) absence of involvement in any formal exercise endurance training for at least six months, and (2) male gender.

The control group was selected based on the following: (1) healthy medical status, (2) non-smoking condition, (3) gender match with the DS participants, (4) age match with the DS participants, (5) absence of involvement in any formal exercise endurance training for at least six months, (6) previous familiarization with treadmill walking, and (7) agreement with the testing procedures confirmed by signature of the informed written consent. None of the participants had any medication known to affect the exercise response.

Table 3.6 Descriptive data of participants with DS and of controls without intellectual disabilities.

Variable	Participants with DS (n=12)	Controls (n=11)
Age (yr)	34.5 ± 7.0	34.3 ± 8.7
Height (cm)	155.4 ± 9.5	175.3 ± 6.4*
Body mass (kg)	66.9 ± 8.9	73.0 ± 11.9
BMI (kg/m^2)	27.9 ± 4.6	23.6 ± 2.6*
BSA (m^2)	1.6 ± 0.1	1.8 ± 0.2*

Values are mean ± SD.

Abbreviations: BMI, body mass index; BSA, body surface area. * Participants with DS differ from controls ($p < 0.05$).

The participants with DS were recruited from a vocational center for individuals with intellectual disabilities. They all lived at home and were bussed to the center daily. Most of the vocational activities at this center site involved light physical work for 5 to 6 hours, 5 days a week. Control participants were recruited from the local and university communities. This study was approved by the University's internal review board.

3.2.5.2. Study Design

Participants with DS were familiarized with the treadmill exercise tests before data collection. The familiarization period consisted of two visits per week for 12 weeks, for 30 min per session and included flat and graded treadmill walks at 2.5 and 4.0 $km.h^{-1}$, respectively. After the familiarization period, each individual could perform each test on a motorised treadmill (h/p/cosmos mercury med 4.0, Nussdorf-Traunstein, Germany) without hand-rail support. This period was particularly important because, with task-oriented treadmill practice, individuals with DS improve their movement biomechanics, producing gait stiffness and impulses more similar to that of their peers without disabilities (Smith et al. 2007). The familiarization period allowed for minimizing baseline differences in biomechanical factors that could affect locomotor economy. Subjects in the comparison group were already accustomed to treadmill walking and running, so there were no familiarization sessions for this group.

All participants were tested in a single occasion and in a postprandial state, approximately 4 hours after their last meal during the early morning period. They were asked to refrain from exercise and caffeine ingestion during the 24 hours preceding testing. The day of testing consisted of: (1) a standardized anthropometric assessment, (2) a resting protocol, (3) a submaximal steady-state exercise protocol, and (4) a maximal graded exercise protocol.

Body mass was measured using a calibrated digital scale, and height was measured using a stadiometer (Secca 770, Hamburg, Germany - standing digital scale/height rod attached). Body mass index (BMI) was calculated by dividing the participants' mass in kilograms by the square of their height in meters. The Dubois and Dubois (1916) formula was used for body surface area calculations. The resting cardiopulmonary data were used in combination with those obtained from the submaximal test to determine the net oxygen uptake (VO_2) of both groups. Thus the net VO_2 was calculated by subtracting the resting steady-state VO_2 to the submaximal exercise steady-state VO_2. As similar values were found in both groups, we expressed submaximal VO_2 as a function of body mass. As recommended by Pollock, (1977) the data collected from the maximal exercise protocol were used to express the groups relative submaximal work intensity as a percentage of VO_{2peak}. The efficiency of the cardiovascular system during submaximal locomotion and peak exercise was evaluated with the oxygen pulse (VO_2/Heart rate) (Goran et al. 2000). This parameter is calculated by dividing VO_2 by the simultaneously measured heart rate. Its values are dependent on the stroke volume and the difference between the arterial and mixed venous blood O_2 content. An exercise response with a higher VO_2/Heart rate than predicted indicates a better than average cardiorespiratory function (Wasserman et al. 2005a). Submaximal and peak O_2 pulse were defined as VO_2 divided by heart rate during both exercise conditions and were expressed as milliliters per beat. VO_2 was measured via a computerized on-line breath-by-breath system (Quark b^2, Cosmed, Rome, Srl-Italy), which was calibrated before each test with a known volume and with known gas concentrations.

3.2.5.3. Protocols

Resting and submaximal exercise protocol

The participants resting VO_2 was obtained during a 5-min period, following a quiet rest of 10 min in the seated position (Whipp 1971). Thereafter, participants walked at 0% grade, 2.5 $km.h^{-1}$ for 8 min. The 8-min period of data collection represented both a submaximal exercise test, and a general warm up for the subsequent graded exercise test. The submaximal treadmill speed was selected on the basis of the results reported by Smith and Ulrich (2008), who found that adults with DS prefer walking velocities of approximately 2.5 $km.h^{-1}$.

The VO_2 data were displayed as 30 s averages. The mean of the last 3 min of the 5-min resting period was defined as the participants resting steady-state VO_2. For the submaximal exercise test, the mean of the last 5 min of the 8-min walk was also defined as the participants submaximal steady-state VO_2 (Whipp 1971). The submaximal exercise net VO_2 was determined by subtracting the resting steady-state VO_2 to the submaximal steady state VO_2, obtained during the last 5-min of the slow walk.

Graded maximal exercise protocol

Peak VO_2 was assessed using a continuous treadmill walking protocol. The selected protocol has been shown to be both a valid and reliable measure of maximal cardiorespiratory fitness in both individuals with DS and control participants without disabilities (Fernhall et al. 1990). Participants walked at a speed of 4.0 $km.h^{-1}$ for 2 min. The grade was then increased by 2.5% every 2 min until a grade of 12.5% was reached. From this point, grade was held constant, whereas speed was increased 1.6 $km.h^{-1}$ every minute until exhaustion.

The VO_2 data were displayed in 20 s averages. A valid VO_{2peak} was defined as the highest value obtained during the last stage of exercise with a RER over 1.0 (Fernhall and Otterstetter 2003; Fernhall and Pitetti 2001). Heart rate was monitored throughout the exercise test by a Polar heart rate monitor (Polar, Kempele, Finland).

3.2.5.4. Statistical Analyses

Descriptive statistics were calculated for all variables. Group data were compared using a multivariate analysis of variance, and statistical significance was set at *p* less than 0.05 throughout. All statistics were performed using SPSS version 16.0 for Windows. Data are displayed as mean ± SD.

3.2.6. Results

The resting, submaximal and graded exercise data are presented in table 3.7. Participants with DS had a lower resting VO_2 and O_2 pulse in comparison to controls ($p < 0.05$), but similar resting heart rates ($p > 0.05$). There were no differences between groups for submaximal heart rate, submaximal VO_2, net submaximal VO_2, minute ventilation, BSA adjusted minute ventilation and RER values ($p > 0.05$). However, in comparison with the controls, participants with DS exhibited lower submaximal O_2 pulse ($p < 0.05$). Participants with DS also exercised at a higher percentage of their VO_{2peak} compared to controls ($p < 0.05$). As shown in table 3.7, both groups attained similar RER values at peak exercise. However, the control participants exhibited a significantly higher VO_{2peak}, peak O_2 pulse and peak heart rate than individuals with DS ($p < 0.05$).

Table 3.7 Resting, submaximal and peak cardiopulmonary data of participants with Down syndrome (DS) and of controls without intellectual disabilities.

Variable	Participants with DS (n=12)	Controls (n=11)
Resting protocol		
HR_{rest} (bpm)	69.5 ± 12.5	74.0 ± 17.6
VO_{2rest} ($mL.kg^{-1}.min^{-1}$)	3.1 ± 0.8	4.0 ± 0.7*
O_2 $pulse_{rest}$ (mL/beat)	3.0 ± 0.6	4.0 ± 0.9*
Submaximal protocol		
HR_{submax} (bpm)	90.6 ± 14.7	80.7 ± 13.9
$VO_{2submax}$ ($mL.kg^{-1}.min^{-1}$)	9.1 ± 2.6	9.5 ± 1.1
Net $VO_{2submax}$ ($mL.kg^{-1}.min^{-1}$)	5.9 ± 2.3	5.4 ± 1.0
Abs. pulmonary minute $ventilation_{submax}$ (L/min)	17.1 ± 4.2	19.7 ± 3.0
BSA pulmonary minute $ventilation_{submax}$ ($L.min^{-1}.m^2$)	10.6 ± 2.7	10.8 ± 2.1
O_2 $pulse_{submax}$ (mL/beat)	6.6 ± 1.3	8.6 ± 1.2*
RER_{Submax}	0.91 ± 0.10	0.85 ± 0.05
$VO_{2reserve}$ (%)	44.6 ± 11.6	19.9 ± 9.3*
Graded exercise protocol		
VO_{2peak} ($mL.kg^{-1}.min^{-1}$)	20.4 ± 4.5	47.6 ± 11.8*
O_2 $pulse_{peak}$ (mL/beat)	9.7 ± 2.2	19.3 ± 5.6*
RER_{peak}	1.3 ± 0.1	1.3 ± 0.1
HR_{peak} (bpm)	141.7 ± 19.9	178.9 ± 7.8

Values are means ± SD.

Abbreviations: HR_{rest}, resting heart rate; VO_{2rest}, resting oxygen consumption; O_2 $pulse_{rest}$, resting oxygen pulse; HR_{submax}, submaximal heart rate; $VO_{2submax}$, submaximal steady-state oxygen consumption; Net $VO_{2submax}$, submaximal net oxygen consumption; Abs. pulmonary minute $ventilation_{submax}$, submaximal absolute pulmonary minute ventilation; BSA pulmonary minute $ventilation_{submax}$, submaximal body surface area adjusted pulmonary minute ventilation; O_2 $pulse_{submax}$, submaximal oxygen pulse; RER_{submax}, submaximal respiratory exchange ratio; $VO_{2reserve}$, reserve of oxygen consumption; VO_{2peak}, peak oxygen consumption; O_2 $pulse_{peak}$, peak oxygen pulse; RER_{peak}, peak respiratory exchange ratio; HR_{peak}, peak heart rate. *Participants with DS differ from controls ($p < 0.05$).

3.2.7. Discussion

The main finding of the present study was that, after a long period of familiarization to treadmill walking, individuals with DS exhibited similar steady-state VO_2 during a 2.5 $km.h^{-1}$ flat walk as healthy controls. These results were sustained even after adjusting for net VO_2, which dissipates the effects of group differences in resting metabolic requirements. Therefore, contradicting our hypothesis, the standard walking economy physiological parameters did not differ between DS and

control participants. On the other hand we found a 13.3% lower submaximal O_2 pulse in individuals with DS in comparison to the controls for the same treadmill speed. Apparently, these results are not limited to submaximal exercise intensities, as they persisted across resting (15%) and peak exercise conditions (50%). In fact, lower O_2 pulse values as the ones obtained for the participants with DS in our study usually suggest underlying heart disease (Wasserman et al. 2005b). Even in the absence of congenital heart disease and in asymptomatic states, persons with DS exhibit aberrations in heart and valve morphology (Rosenquist et al. 1974; Ammirati et al. 1991). Thus, there may be some degree of stroke volume compromise in the heart of otherwise healthy adults with DS, potentially explaining the low O_2 pulse in our study.

We found no group differences for submaximal RER, a parameter which reflects substrate utilization and that generally increases with exercise intensity (Christensen and Hansen 1939; Friedlander et al. 1997). These findings are relevant when considering that participants with DS walked at higher relative submaximal intensities than the controls. Therefore, for a given steady-state submaximal treadmill speed, individuals with DS work at higher relative submaximal intensities than controls while metabolizing approximately the same proportion of fat and carbohydrate (Bergman and Brooks 1999).

This study has several limitations. Generalizations of our findings are compromised by our small sample size and by having excluded female participants from the present experimental design. Further research should be conducted to explore this issue on a larger group of DS individuals including the females. Another limitation of the present study is that neither external nor internal work was assessed across our experimental design. This would have required having to take into account variables such as the displacement of the centre of mass (COM), of body segments relative to COM, co-excitation of antagonist muscles responsible for variable amounts of positive and negative work, elastic energy storage and release, tendon stiffness, among others, which was not possible in the present study. Nevertheless our results are relevant because, despite previous extensive characterization of peak exercise capacity of persons with DS (Fernhall et al. 1996; Guerra et al. 2000; Fernhall et al. 1990; Fernhall and Otterstetter 2003), it was unknown if their submaximal physiological

responses differed from those of non-disabled peers. We found that, while having similar walking economy as healthy controls, individuals with DS exhibit lower levels of submaximal cardiorespiratory efficiency. As most daily living activities vary between light to moderate intensities, it is important to design specific exercise interventions aimed at improving the submaximal aerobic capacity of these individuals.

3.2.7.1. Conclusions

In summary, we found that, after a long period of treadmill familiarization walking economy of adult individuals with DS does not differ from that of non-disabled paired matched controls in response to an acute steady-state submaximal exercise bout. However, individuals with DS have a lower submaximal cardiorespiratory function than controls. Finally, in spite of having similar walking economy as controls, individuals with DS exercise at a higher percentage of VO_{2peak} at a 2.5 $km.h^{-1}$ speed on a treadmill flat surface. Ultimately, our findings reflect that despite exercising at similar steady-state submaximal VO_2 as controls, individuals with DS are further compromised by their lower cardiorespiratory efficiency and poor peak exercise capacity, substantiating the need to increase their physical fitness levels by recommending well validated exercise prescriptions.

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3.3. Walking economy of adults with Down syndrome

3.3.1. Abstract

This study intended to investigate walking economy (WE) in response to different treadmill speeds and grades in adults with Down syndrome (DS) and in non-disabled controls. 18 participants (14 males; 4 females) with DS (33.6±7.6 yr) and 16 non-disabled (12 males, 4 females) controls (33.3±8.0 yr) performed submaximal (2.5 $km.h^{-1}$ and 4 $km.h^{-1}$ at 0% grade; 4 $km.h^{-1}$ at 2.5% and 5% grade, for 5 min each) and maximal treadmill tests with metabolic and heart rate measurements. Oxygen uptake (VO_2) was not different between groups at rest or during the slowest treadmill speed. However, at faster speeds and increased grades, adults with DS presented lower WE than controls ($p < 0.0001$). Subsequent analyses revealed that, despite showing higher delta VO_2 response to the selected speed increments ($p < 0.0001$), individuals with DS produced similar VO_2 increase as controls to grade variations. Therefore, adults with DS exhibit lower WE than non-disabled controls at a speed faster than their preferred walking speed. Additionally, in comparison to controls, individuals with DS show a greater change in energy expenditure with a change in walking speed. In conclusion, lower WE in individuals with DS is mainly related to their inability to adapt efficiently to positive variations in walking speed.

3.3.2. Key Words:

Energy expenditure, locomotion, functional capacity, exercise

3.3.3. Introduction

Numerous studies have shown that individuals with Down syndrome (DS) have diminished work capacity, concomitant with reduced peak oxygen consumption (VO_{2peak}) (Fernhall et al. 1996; Fernhall and Pitetti 2001) and low levels of muscle strength (Guerra et al. 2000; Pitetti and Boneh 1995). It has also been suggested that their low levels of spontaneous physical activity, compared to their non-disabled peers (Fernhall and Unnithan 2002), may be due to inefficient activity performance as a result of both anatomical and functional characteristics (Smith et al. 2007). Joint laxity and muscle hypotonia of persons with DS contributes to reduced gait stability (Kubo and Ulrich 2006) compromising their general mobility while eliciting a higher energy cost for upright locomotion. Individuals with DS exhibit disturbed gait kinematics, particularly when facing an imposed novel task (ie, walking on a treadmill). Under these circumstances, their gait kinematics is characterized by a preference for slower walking speeds and production of wider step width, paralleled by more frequent strides of shorter length (Ulrich et al. 2004; Smith et al. 2007). This is concomitant with a more flexed walking posture (Parker et al. 1986) that is accompanied by higher dynamic instability of body segments both within and between gait cycles (Buzzi and Ulrich 2006); increased time spent on double-limb support (Smith and Ulrich 2008) and reduced time of single-limb support (Parker et al. 1986). As a consequence, individuals with DS are likely to have higher metabolic cost at a given walking speed (Black et al. 2007).

Only one study has previously compared walking economy of adults with DS with that of non-disabled controls (Mendonca et al. 2009). There were no group differences in submaximal VO_2 during a single low speed walking task on a treadmill flat surface, after a long period of familiarization. These findings are consistent with the work of Smith et al. (Smith et al. 2007) showing that, with sufficient practice, individuals with DS learn to put less energy into each step, particularly at the slowest walking speeds, becoming more efficient without modifying stiffness as much as non-disabled controls. However, it is not known if this is sustained for faster walking speeds (ie, those traditionally reported as preferred walking speeds for non-disabled controls) or graded treadmill locomotion. As some of the most common features of DS are known to affect their biomechanics of walking and as most daily

activities are of submaximal intensity, the physiological evaluation of submaximal walking economy is relevant in these individuals. In persons with DS, increased walking speed is known to result in both higher levels of lower limb stiffness and increased instability at the thigh, which could increase energy expenditure (Buzzi and Ulrich 2006). Consequently, it is possible that adults with DS exhibit lower walking economy than non-disabled controls at higher treadmill walking speeds and grades. This is important since an affected walking economy may partially explain why they show a preference for slow walking speed (Smith and Ulrich 2008). Thus, the purpose of this study was to compare walking economy between individuals with DS and non-disabled controls in response to different treadmill submaximal speeds and grades. We hypothesized that adults with DS would present lower walking economy than non-disabled controls at higher treadmill walking speeds and grades.

3.3.4. Methods

3.3.4.1. Participants

A total of 34 Caucasian healthy participants (18 with DS [14 males; 4 females], 16 controls without disabilities [12 males; 4 females]), aged 18 to 50 yr, were included in the present study. Descriptive statistics are presented in table 3.8. All participants with DS had medical approval for physical activity participation from their personal physicians. Those with congenital heart disease, ambulatory, musculoskeletal, visual, or auditory problems were not included in the study. Written informed consent was obtained from the participants' legal guardian, and verbal assent was obtained from the participants. In addition, none of the participants were involved in a formal exercise training program for at least six months, and most were sedentary or moderately active.

The control group was selected based on the following: (1) healthy medical status, (2) non-smoking condition, (3) gender match with the DS participants, (4) age match with the DS participants, (5) absence of involvement in any formal exercise endurance training for at least six months, (6) previous familiarization with treadmill walking, and (7) agreement with the testing procedures confirmed by signature of the informed written consent. None of the participants had any medication known to affect the exercise response.

Table 3.8 Descriptive data of participants with DS and of non-disabled controls.

Variables	**Participants with DS (n=18)**	**Controls (n=16)**
Age (years)	33.6±7.6	33.3±8.0
Height (cm)	154.0±8.7	173.8±6.9*
Body mass (kg)	67.2±9.1	69.9±11.8
BMI (kg/m^2)	28.5±4.3	23.0±2.7*
BSA (m^2)	1.6±0.1	1.8±0.2#
VO_{2peak} ($mL.kg^{-1}.min^{-1}$)	29.1±6.3	46.9±10.6*
HR_{peak} (bpm)	159.0±16.6	185.2±10.9#
RER_{peak}	1.19±0.17	1.37±0.13#
Ve_{peak} ($L.min^{-1}$)	61.0±17.6	123.0±33.2*
BSA Ve_{peak} ($L.min^{-1}.m^2$)	37.9±9.8	68.5±15.9*

Values are means ± SD.

Abbreviations: BMI, body mass index; BSA, body surface area; VO_{2peak}, peak exercise oxygen uptake; HR_{peak}, peak exercise heart rate; RER_{peak}, peak exercise respiratory exchange ratio; Ve_{peak}, peak exercise minute ventilation. * Subjects with DS differ from controls ($p < 0.0001$); # Subjects with DS differ from controls ($p < 0.01$).

The participants with DS were recruited from a vocational center for individuals with intellectual disabilities. They all lived at home and were bussed to the center daily. Most of the vocational activities at this center site involved light physical work for 5 to 6 hours, 5 days a week. Control participants were recruited from the local and university communities. This study was approved by the University's internal review board.

3.3.4.2. Familiarization

Before data collection, each participant with DS was familiarized with the laboratory setting, treadmill protocols, and use of the headgear and face mask. Familiarization periods included a total of 4 sessions distributed over a period of two weeks. Session one involved 2 min of treadmill walking with double hand-rail support at 2.5 $km.h^{-1}$ and 0% grade, followed by 8 min at 4 $km.h^{-1}$ and 2.5% grade. Subsequently, during recovery, treadmill speed and grade were readjusted to 2.5 $km.h^{-1}$ and 0% for two additional minutes. During session 2, participants completed a 2-min horizontal walk at 2.5 $km.h^{-1}$ with single hand-rail support, after which, treadmill speed was increased to 4 $km.h^{-1}$ for 5 min. Speed was then held constant, while grade was increased 2.5% every 2.5 min until a 5% grade was reached. Recovery was structured as described for session 1. Session 3 was generally similar to session 2; however it involved 5-min exercise stages that were completed without hand-rail support. Finally, adding to that performed during session 3, session 4 included the use of the headgear and face mask and additional increments in treadmill grade and speed. Specifically, while speed was held constant, grade was further increased every 2 min by 2.5% until a 12.5% grade was reached. At this point, speed was increased 1.6 $km.h^{-1}$ every minute until exhaustion. Subjects in the comparison group were already accustomed to treadmill walking and running, so there were no familiarization sessions for this group.

3.3.4.3 Measurements

All subjects were tested in a postprandial state, approximately 2-4 hours after their last meal. Participants refrained from exercise 24 hours before testing and caffeine ingestion on testing days. The days of testing consisted of: (1) a standardized body composition assessment, (2) a resting protocol, (3) a continuous submaximal steady-state exercise protocol, and (4) a maximal graded exercise protocol. Testing was carried out in the laboratory with an environmental temperature between 21-24ºC and a relative humidity between 44-56%. In an attempt to control for possible circadian variations in walking economy (Minetti et al. 1993), the measurements were performed between 07.00 and 11.00 h at approximately the same time of day for all individuals.

Body mass was measured using a calibrated digital scale, and height was measured using a stadiometer (Secca 770, Hamburg, Germany - standing digital scale/height rod attached). Body mass index (BMI) was calculated by dividing the participants' mass in kilograms by the square of their height in meters. The Dubois and Dubois (1916) formula was used for body surface area (BSA) calculations. Expired gas measurements were made using a computerized on-line breath-by-breath system (Quark b^2, Cosmed® Srl-Italy), which was calibrated before each test with a known volume and with known gas concentrations. Heart rate data were obtained by means of a Polar RS 800 G3 heart rate monitor (Polar R-R Recorder, Polar Electro, Kempele, Finland).

3.3.4.4 Testing protocols

The participants resting VO_2 was obtained during a 5-min standing period, following a quiet rest of 10 min in the seated position. Cardiorespiratory data were collected while exercising on a motorised treadmill (h/p/cosmos® mercury med 4.0). The protocol involved 5 min of continuous horizontal walking at a constant speed of 2.5 $km.h^{-1}$. Subsequently, speed was increased to 4 $km.h^{-1}$ and held constant at three different treadmill grades (0, 2.5 and 5%), for 5 min each. The submaximal treadmill speeds were selected on the basis of the results reported by Smith and Ulrich (2008), Mendonca et al. (2009) and Cavagna et al. (1976). Accordingly, adults with DS prefer walking velocities of approximately 2.5 $km.h^{-1}$ (Smith and Ulrich 2008) and present similar walking economy as non-disabled controls at this specific treadmill workload (Mendonca et al. 2009). On the other hand, for adults without disabilities, the work done at each step to lift the center of mass of the body equals the work done to increase its foreword speed at 4 $km.h^{-1}$ (Cavagna et al. 1976). Thus, the total mechanical energy at this speed is at a minimum, as is the energy cost.

The VO_2 data were displayed as 30 s averages. The mean of the last 3 min of the 5-min resting period was defined as the participants resting steady-state VO_2. For the submaximal exercise test, the mean of the last 3 min of each 5-min walk was also defined as the participants submaximal steady-state VO_2 (Whipp 1971). Additionally, the submaximal relative work intensities were determined as percentages of VO_{2max} (fractional utilization - FU).

3.3.4.5. Maximal protocol

VO_{2peak} was determined by means of a continuous incremental test to volitional exhaustion commencing immediately after the last submaximal walk. For this purpose, treadmill grade was increased every 2 min by 2.5% until a 12.5% grade was reached. From this point, grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every minute until exhaustion. The test was terminated when the subject reached exhaustion and grasped the hand rails of the treadmill. This protocol has been shown to be both a valid and reliable measure of maximal cardiorespiratory fitness in both individuals with DS and control participants without disabilities (Fernhall et al. 1990). The VO_2 data were displayed in 20-s averages. A valid VO_{2peak} was defined as the highest value obtained during the last stage of exercise with a respiratory exchange ratio (RER) over 1.0 (Fernhall and Otterstetter 2003; Fernhall et al. 1996).

3.3.4.6. Statistical Analyses

Descriptive statistics were calculated for all variables. Before comparing both groups, data were tested for normality and homocedasticity with the Kolmogorov-Smirnov and Levene's tests, respectively. Subsequently, a one-way ANOVA was computed to explore age differences between participants with DS and nondisabled controls. Two one-way MANOVAs were performed to determine group differences among descriptive characteristics, each with related sets of dependent variables. Set 1 included the following anthropometric variables: height, weight, BMI and BSA. Set 2 consisted of peak cardiopulmonary variables, namely: VO_{2peak}, peak heart rate, peak absolute minute ventilation (Ve), peak BSA adjusted minute ventilation (Ve_{BSA}) and peak RER. A two-way ANOVA (group [2] by time [rest, submax 1, submax 2, submax 3, submax 4] with repeated measures, was conducted to determine whether differences existed for VO_2. When a significant interaction was observed, post hoc one-way ANOVAs were used to determine where the difference occurred. We used Bonferroni's adjustment for multiple comparisons for all repeated measures analyses. Because Ve_{BSA} differed between groups through submax 2 to submax 4, and Ve can affect walking economy, we repeated the ANOVAs using Ve_{BSA} as a covariate at each work load to help control for its influences on walking economy. To further explore the differential effects of treadmill speed vs grade on the

participants' walking economy, we calculated delta VO_2 between adjacent steady-state physiological conditions (ie, rest to submax 1 [Δ1 workload], submax 1 to submax 2 [Δ2 workload], submax 2 to submax 3 [Δ3 workload] and submax 3 to submax 4 [Δ4 workload]). A two-way ANOVA with repeated measures (group [2] by delta workload [Δ1 workload, Δ2 workload, Δ3 workload, Δ4 workload]) was conducted on delta VO_2 to compare differences between groups in response to the increments in treadmill workload.

3.3.5. Results

Descriptive and peak exercise data are presented in table 3.8. No differences were observed between groups for age. Significant main effects resulted for both anthropometric (Wilks Lambda = 0.264, F = 20.22, $p < 0.0001$) and peak cardiopulmonary analyses (Wilks Lambda = 0.277, F = 14.64, $p < 0.0001$). Follow up univariate analyses on anthropometric data revealed that, in comparison with controls, participants with DS were shorter (F = 52.91, $p < 0.0001$), had higher BMI (F = 18.98, $p < 0.0001$) and lower BSA (F = 11.65, $p < 0.01$). Furthermore, participants with DS also had lower peak exercise values for VO_2 (F = 36.51, $p < 0.0001$), heart rate (F = 28.81, $p < 0.01$), absolute Ve (F = 47.75, $p < 0.0001$), Ve_{BSA} (F = 46.54, $p < 0.0001$), and RER (F = 12.71, $p < 0.01$).

3.3.5.1. Walking economy

Submaximal VO_2 results are shown in figure 3.3. We obtained a significant group main effect (F = 35.03, $p < 0.0001$), a significant time main effect (F = 549.32, $p < 0.0001$), and a significant group x time interaction (F = 22.99, $p < 0.0001$). Post hoc tests revealed that VO_2 increased as a function of treadmill work loads for both groups ($p < 0.0001$). However, results indicated that adults with DS presented higher VO_2 while walking on a flat and graded treadmill surface (2.5 and 5%) at 4 $km.h^{-1}$ ($p < 0.0001$). On the other hand, there were no between-group differences for resting or submaximal VO_2 at a 2.5 $km.h^{-1}$ treadmill speed. When controlling for the effect of Ve_{BSA}, we found that group differences in submaximal VO_2 remained significant for both 0 and 2.5% graded walks at 4 $km.h^{-1}$ (DS: 15.4 ± 2.0; controls: 11.3 ± 1.0 $mL.kg^{-1}.min^{-1}$, F = 32.42, $p < 0.0001$ and DS: 17.5 ± 2.4; controls: 13.4 ± 1.2 $mL.kg^{-1}.min^{-1}$, F = 9.16, $p < 0.01$, respectively). On the contrary, there were no

group differences in submaximal VO_2 for the 4 km.h^{-1} speed at 5% grade after controlling for the effect of Ve_{BSA} (F = 3.34, p > 0.05).

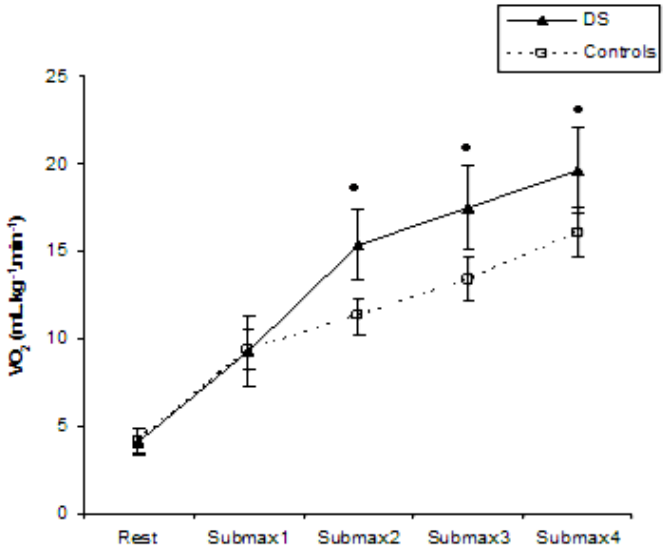

Fig. 3.3 Oxygen uptake (VO_2) at rest and during four different treadmill workloads (Submax1, 2.5 km.h^{-1} - 0% grade; Submax2, 4 km.h^{-1} - 0% grade; Submax3, 4 km.h^{-1} - 2.5% grade; Submax4, 4 km.h^{-1} - 5% grade) in individuals with Down syndrome (DS) and non-disabled controls. Values are means ± SD. * Significant differences between groups (p < 0.0001).

Subsequent analyses were conducted to explore between-group differences on the amplitude of delta VO_2 response to adjacent workloads increments (delta workloads). We found a significant group main effect (F = 29.75, p < 0.0001), a significant delta workload main effect (F = 23.54, p < 0.0001) and a significant group x delta workload interaction (F = 55.46, p < 0.0001). As shown in figure 3.4, compared to controls, participants with DS presented higher delta VO_2 following the increase in treadmill speed from 2.5 to 4 km.h^{-1} (Δ2 workload) (p < 0.0001). On the other hand, both groups had comparable delta VO_2 amplitudes from resting to walking conditions at 2.5 km.h^{-1} (Δ1 WL) and in response to both increments in treadmill grade (Δ3 workload and Δ4 workload). Furthermore, whereas delta VO_2 decreased from Δ1 workload to Δ2 workload for controls (p < 0.0001); adults with DS presented no such change between delta workloads. The opposite was found for the transition from Δ2 workload to Δ3 workload. Results indicated that while delta VO_2 did not differ for control subjects under these conditions, participants with DS decreased their delta VO_2

between adjacent delta workloads ($p < 0.0001$). Finally, there were no between-group differences for delta VO_2 at Δ3 workload and Δ4 workload, or within-group differences from Δ3 workload to Δ4 workload.

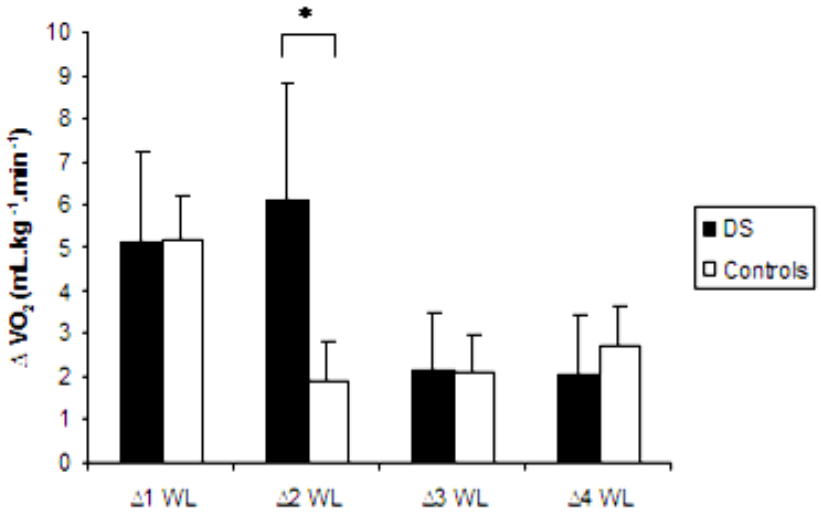

Fig. 3.4 Delta oxygen uptake in response to different delta workloads (WL) on the treadmill in participants with DS and non-disabled controls (rest to 2.5 $km.h^{-1}$ – 0% grade [Δ1 WL], 2.5 $km.h^{-1}$ to 4 $km.h^{-1}$ – 0% grade [Δ2 WL], 4 $km.h^{-1}$ – 0% grade to 2.5% grade [Δ3 WL] and 2.5% to 5% grade [Δ4 WL]). Values are means ± SD. * Significant differences between groups ($p < 0.0001$).

3.3.5.2. Fractional utilization of VO_{2max} and respiratory exchange ratio

FU analysis reported a significant group main effect ($F = 62.61$, $p < 0.0001$), a significant time effect ($F = 274.11$, $p < 0.0001$) and a significant group x time interaction ($F = 43.26$, $p < 0.0001$). As reported in table 3.9, participants with DS relied on higher FU of the VO_{2max} than controls under resting conditions and at all walking stages ($p < 0.01$). Further, except for the transition from walking at 2.5 to a 5% treadmill grade, they increased more their FU than controls as work load increased.

Table 3.9 Resting and submaximal cardiopulmonary data of participants with DS and of non-disabled controls.

	HR (bpm)		**Abs. Ve ($L.min^{-1}$)**		**Ve_{BSA} ($L.min^{-1}.m^2$)**		**FU VO_{2max} (%)**	
	DS	Controls	DS	Controls	DS	Controls	DS	Controls
Rest	68.2±11.2	73.3±15.3	7.9±1.3	10.9±2.1*	4.9±0.8	6.1±1.1†	14.6±3.5	9.4±2.9#
Ex.1	89.9±13.2	81.7±14.5	17.6±3.2	19.8±3.1	11.1±2.0	10.8±1.9	33.1±9.9	21.5±8.0#
Ex.2	102.8±13.3	88.4±17.2#	25.0±5.0	21.7±4.1†	15.6±2.8	12.2±2.4†	54.8±12.0	25.5±8.0#
Ex.3	112.4±16.8	93.8±18.4#	29.4±5.4	24.5±4.8†	18.4±2.9	13.7±2.6†	62.2±12.6	30.2±9.0#
Ex.4	122.4±17.4	100.8±18.4#	34.3±5.9	28.0±4.0†	21.4±3.2	15.7±2.3†	69.4±13.4	36.2±10.3#

Values are means ± SD.

Abbreviations: Ex.1, submaximal exercise at 2.5 $km.h^{-1}$ 0% grade; Ex.2, submaximal exercise at 4 $km.h^{-1}$ - 0% grade; Ex.3, submaximal exercise at 4 $km.h^{-1}$ - 2.5% grade; Ex.4, submaximal exercise at 4 $km.h^{-1}$ - 5% grade; HR, heart rate; Abs. Ve, submaximal absolute minute ventilation; Ve_{BSA}, submaximal body surface area adjusted pulmonary minute ventilation; FU VO_{2max}, fractional utilization of the maximum oxygen uptake. †Participants with DS differ from controls ($p < 0.05$); #Participants with DS differ from controls ($p < 0.01$); *Participants with DS differ from controls ($p < 0.0001$).

RER analysis also reported a significant group main effect ($F = 16.60$, $p < 0.0001$), a significant time main effect ($F = 7.44$, $p < 0.0001$) and a significant group x time effect ($F = 8.5$, $p < 0.0001$). As depicted in figure 3.5, despite similarities between groups at resting conditions, participants with DS had higher RER values when walking on the treadmill at all speeds and grades ($p < 0.05$). Additionally, while remaining unchanged for controls over the entire spectrum of treadmill workloads, RER of participants with DS significantly increased from the horizontal walk at 4 $km.h^{-1}$ (0.91 ± 0.09) to the graded one at 2.5% (0.97 ± 0.07),and from there to that at 5% (1.0 ± 0.05) ($p < 0.05$).

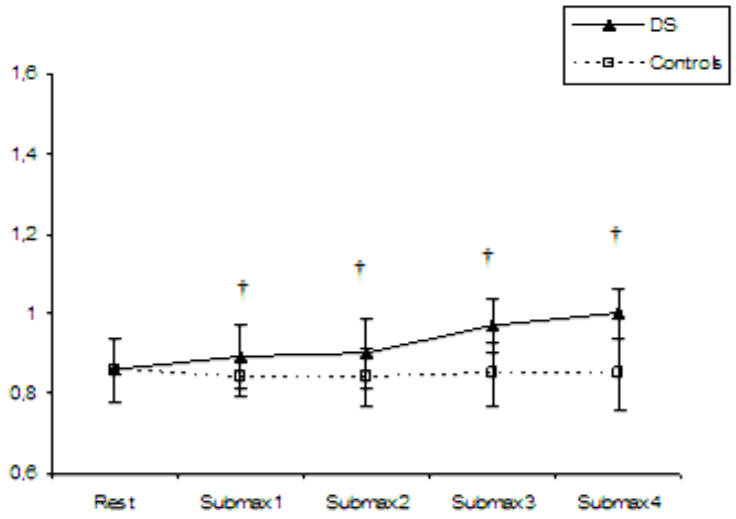

Fig. 3.5 Respiratory Exchange Ratio (RER) at rest and during four different treadmill workloads (Submax1, 2.5 km.h^{-1} - 0% grade; Submax2, 4 km.h^{-1} - 0% grade; Submax3, 4 km.h^{-1} - 2.5% grade; Submax4, 4 km.h-1 - 5% grade) in individuals with Down syndrome (DS) and non-disabled controls. Values are means ± SD. † Significant differences between groups ($p < 0.05$).

3.3.5.3. Chronotropic and ventilatory response

Heart rate analysis also reported significant main effects for group ($F = 5.76$, $p < 0.05$), time ($F = 127.25$, $p < 0.0001$) and group x time interaction ($F = 14.21$, $p < 0.0001$). While heart rate progressively increased as a function of treadmill speed for participants with DS, controls did not report significant differences from resting to walking conditions at 2.5 km.h^{-1} or from there to locomotion at 4 km.h^{-1}. In opposition, both groups increased their heart rate in response to the selected treadmill grade increments ($p < 0.05$). As shown in table 3.9, participants with DS had higher chronotropic response while walking at the same 4 km.h^{-1} treadmill work loads than controls ($p < 0.01$). Analyses conducted on Ve and Ve_{BSA} revealed consistent findings (table 3.9). A significant group x time interaction was observed for Ve ($F = 19.40$, $p < 0.0001$). The participants with DS had significantly lower Ve at rest than those without DS ($p < 0.0001$; table 2), but comparable values while walking at 2.5 km.h^{-1}. However, they increased more their Ve in response to the increment in treadmill speed from 2.5 to 4 km.h^{-1}. As a consequence, this resulted in higher levels of Ve on subsequent treadmill workloads ($p < 0.05$).

3.3.6. Discussion

Overall, our results show that adults with DS demonstrate lower walking economy than non-disabled controls while walking on a flat or graded treadmill surface at a speed faster than their preferred walking speed. As previously reported (Mendonca et al. 2009), individuals with DS exhibited similar walking economy as controls during horizontal locomotion at their preferred walking speed. We also found that, they responded with higher delta VO_2 to a given delta speed, suggesting that, under these conditions, they are less efficient walkers than controls. Interestingly, increases in treadmill grade while holding speed constant yielded similar delta VO_2 for both groups, thus suggesting that adults with DS are as efficient as controls in response to increments in walking grade. Thus, lower walking economy in individuals with DS is mainly related to their inability to adapt efficiently to increases in walking speed.

It would be reasonable to assume that the higher submaximal VO_2 of individuals with DS could be related to their reliance on increased VO_{2peak} FU to sustain a given workload. This could lead to the appearance of the VO_2 slow component that is traditionally described for exercise intensities above the ventilatory threshold (Whipp 1994). However, as reported by Baynard et al. (2004), the determination of the ventilatory threshold is difficult in individuals with intellectual disabilities, especially in those with DS. Nevertheless, minute ventilation has been shown to have the highest sensitivity for ventilatory threshold detection in these individuals (Baynard et al. 1994). Therefore, we repeated our statistical analyses while controlling for the effects of Ve_{BSA}. We found that between-group differences in walking economy persisted at all treadmill submaximal stages, with exception for both the slowest and highest workloads. Therefore, at speeds faster (4 $km.h^{-1}$) than their preferred walking speed (2.5 $km.h^{-1}$), adults with DS walk at higher energy expenditure than controls and, apparently, this is not a function of working at higher relative intensities.

Previous research conducted on the kinematics and kinetics of walking in individuals with DS has found consistent deviations from the patterns typically seen in non-disabled peers, both over-ground and on the treadmill. Specifically, their walking kinetics is characterized by the use higher levels of stiffness and angular impulse (forcing) when on a treadmill, but not stiffness during over-

ground locomotion (Ulrich et al. 2004). It has also been shown that anteroposterior and mediolateral balance during locomotion are particularly challenging to individuals with DS and may implicate a higher metabolic cost at a given walking speed (Black et al. 2007). This is also consistent with the biomechanical gait adjustments performed by these individuals while walking at faster treadmill speeds. Increased walking speed is known to result in both higher levels of lower limb stiffness and increased instability at the thigh in persons with DS, which could further contribute to their higher energy expenditure during locomotion (Buzzi and Ulrich 2006). It is possible that, in order to achieve successful motor behaviour during a treadmill walking task at faster speeds, adults with DS use different operational strategies, thus implicating an increased metabolic cost for their ambulation. This is consistent with findings showing that individuals with DS prefer the adoption of atypical walking patterns focused on stability enhancement strategies (Parker et al. 1986; Buzzi and Ulrich 2006; Black et al. 2007; Smith and Ulrich 2008; Kubo and Ulrich 2006), which allows them to obtain motor task success, but at the expense of higher energy expenditure. Conversely, at slower walking speeds, individuals with DS are known to produce a more efficient gait pattern, characterized by a decrease in step frequency and thus stiffness (Ulrich et al. 2004). Additionally, as reported by Smith et al. (2007), with sufficient practice, they become more efficient without modifying stiffness and angular impulse as much as non-disabled controls. In support of this, Mendonca et al. (2009) showed that adults with DS have similar walking economy as controls during a slow speed treadmill task, after 24 familiarization sessions. Our results agree and further extend those findings, as we obtained no between-group differences in walking economy during locomotion at our slowest treadmill speed, and four practice sessions were sufficient for proper adaptation of adults with DS to this specific motor task. However, this was not the case for faster walking speeds.

Contrasting to the findings obtained with positive variations on treadmill speed, delta grade elicited similar delta VO_2 between adults with DS and controls. To the best of our knowledge, this is the first study analysing the effects of submaximal graded exercise on individuals with DS. Thus, it is unknown if their graded walking biomechanics or efficiency are substantially deviated from what is typically reported in healthy individuals. For these, internal work (ie, due to speed changes of body

segments in respect to the body centre of mass in the environment) is known to increase as a function of treadmill grade at constant speeds. Changes in internal work result from increases in stride frequency while walking at steeper treadmill grades (Minetti et al. 1993; Minetti et al. 1994). Therefore, successful locomotion at a constant speed and increased grade is accomplished by performing more frequent walking strides. This requires higher levels of internal work that is known to be dependent on additional energy expenditure (Minetti et al. 1993). As in the present study, adults with DS responded to delta treadmill grade increments with similar delta VO_2 amplitude as controls; it is possible that, under these circumstances, they both adapt their walking kinematics (ie, stride frequency) similarly.

The greater FU among individuals with DS implies a greater potential for fatigue, especially if walking is performed at fast speeds. Increased levels of fatigue may lead to fewer episodes of spontaneous physical activity (Whitt-Glover et al. 2006), thus partially explaining the increased obesity prevalence in this population (Rubin et al. 1998). The greater relative intensity during walking of individuals with DS further corroborates the importance of designing specific exercise interventions aimed at promoting a healthy mobility in persons with DS. Accordingly, prescribing an appropriate intensity may improve the long-term adherence of persons with DS to exercise programs.

In conclusion, we found that adults with DS exhibit lower walking economy than non-disabled controls at a treadmill speed faster than their preferred walking speed. Additionally, in comparison to controls, individuals with DS show a greater change in energy expenditure with a change in walking speed. This is probably related to atypical deviations from normal gait biomechanics and results from the combined effects of ligamentous laxity, low tone, morphological characteristics and functional impairments. Decreased walking economy in individuals with DS may provide a partial explanation for the high levels of sedentary behaviour reported in this population (Fernhall and Unnithan 2002; Stanish and Draheim 2005).

3.3.7. Limitations

From a classical point of view, preferred walking speed corresponds to an important marker of physical function in which the energetic cost of locomotion is at its minimum (Corcoran and Brengelmann 1970). More recently, the energetic cost at the preferred walking speed was found to be higher than the minimal energetic cost among women with and without obesity (Browning and Kram 2005). It is unknown if this occurs similarly in individuals with DS. In the present study we did not measure the energetically optimal speed and this can be viewed as a limitation. Nevertheless, since adults with DS responded to faster treadmill speeds with poorer walking economy, our findings provide a reasonable explanation for their preference for slow walking speeds, and this is novel.

Another limitation of the present study is that neither external nor internal work was assessed across our experimental design. This would have required having to take into account variables such as the displacement of the centre of mass (COM), of body segments relative to COM, co-excitation of antagonist muscles responsible for variable amounts of positive and negative work, elastic energy storage and release, tendon stiffness, among others, which was not possible in the present study.

3.3.8. References

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3.4. Oxygen uptake kinetics during exercise in adults with Down syndrome

3.4.1. Abstract

Persons with Down syndrome (DS) have diminished submaximal and peak work capacity. This study evaluated the dynamic response of oxygen uptake at onset and recovery (VO_2 kinetics) of constant-load exercise (moderate intensity - 45% VO_{2peak}) in adults with DS. A total of 27 healthy participants (14 with DS [9 males; 5 females], 13 controls without disabilities [9 males; 4 females]), aged 18 to 50 yr, performed graded treadmill exercise to assess peak VO_2. Subjects also performed constant-load exercise tests at 45% VO_{2peak} to determine VO_2 on- and off-transient responses. Peak VO_2 was lower in participants with DS compared to controls (DS: 30.2 ± 7.1; controls: 46.1 ± 9.6 $mL.kg^{-1}.min^{-1}$, $p < 0.05$). In contrast, at 45% VO_{2peak}, the time constants for the VO_2 on- (DS: 34.6 ± 9.1; controls: 37.6 ± 9.0 s) and off-transients (DS: 36.5 ± 12.3; controls: 37.7 ± 7.0 s) were not significantly different between groups. Additionally, there were no differences between on- and off-transient time constants in participants with DS or controls. These data demonstrate that the VO_2 kinetics at onset and recovery of moderate intensity exercise is similar between adults with DS and controls. Therefore, the submaximal exercise performance of these individuals is not affected by slowed VO_2 kinetics.

3.4.2. Key words

Kinetics; oxygen uptake; Down syndrome; exercise

3.4.3. Introduction

The finite rate of adjustment of oxidative phosphorylation to sudden increases and decreases in energy demand has been termed oxygen uptake (VO_2) kinetics. Sudden changes in work output are frequently encountered in sports as well as during many activities of everyday life. Faster VO_2 kinetics are associated with less disturbance of cellular and organ homeostasis (lower degradation of phosphocreatine - PCr and glycogen stores, lower accumulation of lactate and H^+) and with positive consequences on exercise tolerance and muscular fatigue (Grassi 2006). The VO_2 response to dynamic exercise displays a biphasic pattern, with an early increase influenced predominantly by increased pulmonary blood flow and a second phase to steady-state levels additionally influenced by O_2 depleted venous blood from exercising muscle (Whipp and Ward 1990). Modeling of VO_2 and muscle O_2 uptake kinetics during exercise on-transients (Barstow and Molé 1987) and observations of equivalence between PCr kinetics and the off-transient time constants (Barstow et al. 1994; Brown 1992; McCreary et al. 1996) suggest a close association between these physiological parameters across a variety of exercise intensities (Rossiter et al. 2001).

According to previous studies, the on-transient VO_2 response to moderate exercise is well characterized by a single mono-exponential function (Whipp 1970). Conversely, above the ventilatory threshold (VT), the VO_2 kinetics becomes more complex (emergence of the VO_2 slow-component) (Whipp and Wasserman 1972) and several groups have demonstrated a greater O_2 cost per increment of workload (Whipp and Mahler 1980; Casaburi et al. 1987; Tschakovsky and Hughson 1999). The VO_2 off-transient response for moderate exercise has also been characterized by a first-order model similar to that of the on-transient, incorporating a single time constant, delay and amplitude. Interestingly, while the on- and off-transient dynamics are symmetrical in response to moderate exercise, asymmetries have been reported at intensities above the VT (Donald and Whipp 1991; Özyener et al. 2001). It has also been shown that the VO_2 slow component becomes demonstrable in recovery at higher absolute supra-VT work rates than for the on-transient (Özyener et al. 2001). There is compelling evidence that the on-off asymmetry in the VO_2 kinetics results from a combined effect of lactate accumulation (i.e. within the muscle and blood) and the requirement of different

mechanisms for its subsequent metabolism at different exercise intensities (Gladden 1996). Accordingly, any lactate cleared oxidatively during recovery may not be seen as an additional kinetic component in VO_2 if the lactate metabolism follows the same pathways utilized during moderate exercise. In contrast, any lactate that serves as a source to glyconeogenesis (in the liver and skeletal muscle) has an obligatory O_2 cost (Özyener et al. 2001).

Changes in VO_2 kinetics in populations with disease may provide insight regarding alterations in physiological regulation which may be associated with impairment in exercise performance and functional capacity (Sietsema 1992; Bauer et al. 1999). Slower O_2 onset and recovery kinetics contribute to larger O_2 deficit and O_2 debt, both of which may also be affected by a greater amplitude of VO_2 above baseline for a given exercise intensity (Demarle et al. 2001). Compared to nondisabled individuals, adults with Down syndrome (DS) show a greater VO_2 change in transition from rest to a given absolute treadmill workload (Mendonca et al. 2010). This increased amplitude in the VO_2 response to submaximal exercise can lead to early fatigue and delayed recovery from exertion (Koike et al. 1995). It has also been observed that persons with DS have a reduced peak oxygen uptake (VO_{2peak}) compared with nondisabled controls (Fernhall et al. 1996). Taken together, these data suggest that the reduced levels of work performance in DS may not simply be due to early termination of exercise, or deconditioning, but to a qualitative difference in the change of VO_2 with exercise. Despite the relevance of VO_{2peak} and walking economy as measures of aerobic function in DS, VO_2 kinetics may provide additional objective information on the ability to adapt to and recover from exercise (Kemps et al. 2007). To explore the effects of DS on VO_2 kinetics, we evaluated 14 healthy adults with DS who performed bouts of constant-load treadmill exercise at 45% VO_{2peak} (moderate intensity exercise). Furthermore, to exclude the possibility of group differences in the VO_2 kinetics due to the effects of overweight/obesity (commonly seen in DS), we included a group of controls matched with the participants with DS for body mass index (BMI).

3.4.4. Methods

3.4.4.1. Subjects

A total of 27 healthy participants (14 with DS [9 males; 5 females], 13 controls without disabilities [9 males; 4 females]), aged 18 to 50 yr, were included in the present study. Descriptive statistics are presented in table 3.10. A health screening questionnaire was completed by each participant and/or her parent or legal guardian. Exclusionary criteria included any contraindications to exercise, severe or profound mental retardation, active smoking status, congenital or atherosclerotic heart disease, metabolic disease, respiratory disorders including asthma, atlantoaxial instability, orthopaedic issues that would limit treadmill performance and heart rate altering medications. Also, all participants had normal thyroid function per family member or physician report. Subjects in both groups were either sedentary or moderately active, but none were involved in any formal exercise endurance training for at least 6 months. All subjects, and in addition, parents and/or guardians of the participants with DS, signed informed consent to participate in the study. This study was approved by the University's internal review board.

Participants with DS were recruited from a vocational center for individuals with intellectual disabilities. They all lived at home and were bussed to the center daily. Most of the vocational activities at this center involved light physical work for 5 to 6 hours, 5 days a week. Control participants were recruited from the local and university communities. Before data collection, each participant was familiarized with the laboratory setting, treadmill protocols, and use of the headgear and face mask. Familiarization sessions were continued until the subject could comfortably walk on the treadmill with the headgear and mouthpiece. All subjects were adequately familiarized within 1 or 2 sessions.

Table 3.10 Characteristics of participants with Down syndrome (DS) and of nondisabled controls.

Variables	**DS** (n=14)	**Controls** (n=13)
Age (yr)	35.3 ± 7.8	37.5 ± 8.0
Height (cm)	151.4 ± 8.7*	173.0 ± 7.0
Body mass (kg)	66.5 ± 8.5*	79.7 ± 18.9
Body mass index (kg/m^2)	29.1 ± 4.3	26.3 ± 4.8
Peak heart rate (bpm)	172.6 ± 17.5*	184.5 ± 11.3
Peak VO_2 ($mL.min^{-1}$)	2018.7 ± 571.5*	3592.3 ± 743.7
Peak VO_2 ($mL.kg^{-1}.min^{-1}$)	30.2 ± 7.1*	46.1 ± 9.6
Peak RER	1.13 ± 0.09	1.18 ± 0.07
Peak Ve ($mL.min^{-1}$)	62.4 ± 20.5*	112.3 ± 22.9
VO_{2VT} ($mL.min^{-1}$)	1341.3 ± 355.5*	2242.9 ± 470.1
VO_{2VT} ($mL.kg^{-1}.min^{-1}$)	20.1 ± 4.2*	28.6 ± 4.4

Values are mean ± SD.

Abbreviations: VO_2, oxygen uptake; RER, respiratory exchange ratio; Ve, minute ventilation; VO_{2VT}, oxygen uptake at ventilatory threshold. * Participants with DS differ from controls ($p < 0.05$)

3.4.4.2. Study design

After the familiarization period, participants were evaluated over the course of 2 visits on separate days (within a 7-day period). Testing was separated by at least 48 h and, to minimize the effects of circadian and other similarly induced variations in performance, was performed at approximately the same time of day (between 07.00 and 11.00 h). All subjects abstained from caffeine and vigorous exercise for 24 h prior to testing and were at least 3-h post-prandial upon arrival for testing. During the first visit, standing height and weight measurements were taken with participants wearing light-weight clothing and no shoes. Height was obtained using a stadiometer with measures obtained to the nearest 0.5 cm. Weight was measured on a balance-beam scale. BMI was calculated by dividing the participants' mass in kilograms by the square of their height in meters. Subsequently,

participants performed a treadmill graded exercise test (GXT) to determine their submaximal VO_2 and VO_{2peak}. This test was used to determine the treadmill workload required to elicit a given pre-selected fractional utilization (FU) of the VO_{2peak} in each individual. On the second visit, each participant performed two 6-min walking bouts at 45% VO_{2peak} and each walking bout was separated by a rest period of sufficient length of time to return VO_2 and heart rate to baseline values (20-30 min). This period of time was selected based on previous research showing that 20-30 min is sufficient for complete recovery of leg muscle blood flow and for VO_2 values return to pre-exercise conditions (Whipp and Wasserman 1972; Burnley et al. 2006).

As in previous studies, the kinetics of VO_2 and heart rate were analyzed on the average of the two curves in an attempt to establish a "characteristic" time constant and gain for the response (Puente-Maestu et al. 2000). All tests were performed on a motorized treadmill (H/P/Cosmos® Mercury Med. 4.0, Sports & Medical gmbh, Nussdorf-Traunstein, Germany). Expired gas measurements were made using a computerized on-line breath-by-breath system (Quark b^2, Cosmed® Srl-Italy), which was calibrated before each test with a known volume and with known gas concentrations. Testing was carried out in the laboratory with an environmental temperature between 21-24ºC and a relative humidity between 44-56%.

3.4.4.3. Graded exercise test

Before testing, each subject rested quietly in a seated position for 5 min. VO_2 and heart rate data were collected for an additional 5 min of seated rest. Testing began with a submaximal horizontal walk at a constant speed of 4 $km.h^{-1}$. Grade was increased 2.5% every 5 min until a 7.5% grade was reached. Grade was then increased every 2 min by 2.5% until a 12.5% grade was reached. From this point, grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every minute until exhaustion. This protocol has been shown to be a valid and reliable measure of maximal cardiorespiratory fitness in both individuals with DS and control participants without disabilities (Fernhall et al. 1990). The VO_2 data were displayed in 20-s averages. A valid VO_{2peak} was defined as the highest value obtained during the last stage of exercise with a respiratory exchange ratio (RER) over 1.0 (Fernhall and Otterstetter, 2003). To indentify the VT, breath-by-breath VO_2 and VCO_2 were

smoothed using a three-breath bin average to reduce breath-by-breath fluctuations whilst at the same time retaining the underlying response to progressive increases in exercise intensity. The VT was indentified independently by two experienced investigators using the V-slope method (Beaver et al. 1986).

3.4.4.4. Constant intensity exercise tests

To ensure that all participants exercised at the same FU of the VO_{2peak} (same relative intensity), treadmill workloads were individualized. For this purpose, the mean of the last 3 min of each 5-min walk (GXT stages at constant speed of 4 $km.h^{-1}$) was defined as the participants submaximal steady-state VO_2 (Whipp, 1971). Equations were computed using least squares linear regression on data derived from each participant's steady state VO_2 (0, 2.5, 5 and 7.5% grades). Then, the treadmill grade required to elicit 45% VO_{2peak} at a 4 $km.h^{-1}$ walking speed was calculated. This relative intensity was chosen because of its compatibility with moderate intensity exercise (i.e. below the ventilatory threshold - VT). It is acknowledged that gas exchange become more complex near the VT and are perhaps not well described by the mono-exponential equation (Whipp and Wasserman 1972). Subjects subsequently performed two 6-min treadmill walking bouts at 45% VO_{2peak}. In all tests, the exercise was preceded by a 5-min standing period and followed by another 5 min of standing recovery. All tests were separated by 20-30 min of seated rest, which was found to be adequate for gas-exchange and heart rate to return to baseline levels. To define precisely the starting point of walking, subjects stood on the edges of the treadmill and started walking after the device had reached the programmed speed and elevation. To reduce the risk of falling, there was one member of the research staff spotting the participants from behind while they stepped on the moving treadmill.

3.4.4.5. VO_2 kinetic measurements

Breath-by-breath VO_2 data were linearly interpolated to yield VO_2 values for every second during the test. The data from both exercise bouts were time-aligned and ensemble averaged to produce a single data set that was representative of the participant's underlying VO_2 response. As the initial "cardiodynamic" phase of the VO_2 response does not directly represent active muscle O_2 utilization, the first 20 s of the on-transient was omitted from the field (Özyener et al. 2001).

Therefore, to characterize the on-transient VO_2 kinetics, data were modeled from 20 s post-onset of exercise, thereby excluding phase 1, until end exercise, by non-linear least squares regression to mono-exponential model incorporating a time delay. The on-transient exponential model was of the form:

$$VO_2\,(t) = VO_{2(b)} + A_{on}\,(1 - e^{-(t - Td)/\tau}) \quad (1)$$

where t is time, $VO_{2(b)}$ is baseline VO_2, A_{on} is the amplitude of VO_2 from exercise to recovery, Td is the time delay and τ is the time constant. Although the duration of phase 1 is likely to be less in recovery, as blood flow is higher at the off- than the on-transient, little is known about this duration and therefore omission of the first 20 s of the off-transient was thought to be more than sufficient to obviate any distorting influence of subsequent kinetics. Accordingly, to characterize the off-transient VO_2 kinetics, the following mono-exponential model was used from 20 s post-offset of exercise (Özyener et al. 2001):

$$VO_2\,(t) = VO_{2(m)} - A_{off}\,(1 - e^{-(t - Td)/\tau}) \quad (2)$$

where $VO_{2(m)}$ is moderate intensity (45% VO_{2peak}) exercise VO_2. Other parameters have been previously described in Eq. 1. Occasional errant breaths (i.e. due to coughing, swallowing or talking) were deleted from the data set when VO_2 exceeded three standard deviations of the mean, defined as the average of two following and two preceding sampling intervals (Lamarra et al. 1987). In total, about 1% of the breaths had to be deleted. To provide an indication of the overall rate of VO_2 adaptation, the mean response time (MRT; the sum of the time constant and time delay values) was additionally calculated. The equations of Lamarra et al. (1987) were used to determine the 95% confidence interval for τ_{on} and τ_{off} in all participants. Finally, the increment in VO_2 between the 3rd and 6th min of the transition ($\Delta VO_{2,\,6\text{-}3}$) was also determined to confirm the attainment of steady-state by participants with DS and controls.

3.4.4.6. Heart rate kinetic measurements

Beat-by-beat heart rate data were obtained by means of a Polar RS 800 G3 heart rate monitor (Polar R-R Recorder, Polar Electro, Kempele, Finland). As in previous studies, the data from the two

repetitions performed at 45% VO_{2peak} were temporally aligned to a time at the start of exercise and superimposed to yield a single second-by-second averaged record of the tests for each subject (Regensteiner et al. 1998). Two exponential curves were fit to the data: 1) from the onset of exercise to the end of the sixth minute exercise; 2) from the offset of exercise to the end of recovery.

3.4.4.7. Statistical analysis

Descriptive statistics were calculated for all variables. Before comparing both groups, data were tested for normality and homocedasticity with the Kolmogorov-Smirnov and Levene's tests, respectively. Potential group differences were evaluated using analyses of variance (ANOVAs). Paired *t*-tests were used to determine the symmetry between VO_2 on- and off-transient responses within groups. Attainment of steady-state VO_2 during exercise was confirmed by exploring if $\Delta VO_{2,\ 6\text{-}3}$, in each group, differed significantly from zero. All data are reported as mean ± SD. Statistical significance was set at $p < 0.05$. All data analysis was carried out using Statistical Package for the Social Sciences (SPSS, v 16.0, SPSS, Inc., Chicago, IL).

3.4.5. Results

Descriptive and peak exercise data are presented in table 3.10. No differences were observed between groups for age. Participants with DS were shorter and had lower body mass than controls ($p < 0.05$). Furthermore, subjects with DS attained lower peak exercise values for VO_2, heart rate and minute ventilation ($p < 0.05$). Conversely, peak respiratory exchange ratio values were similar between groups. Even though both groups showed different VO_2 values at the VT (table 3.10), no differences between them were found after expressing it as a percentage of VO_{2peak} (DS: 67.5 ± 7.8; controls: 63.3 ± 9.1%).

Table 3.11 Pulmonary VO_2 kinetics of participants with Down syndrome (DS) and nondisabled controls at 45% VO_{2peak}.

Variable	DS (n=14)	Controls (n=13)
On-transient VO_2 kinetics		
$VO_{2(b)}$ ($mL.min^{-1}$)	228.3 ± 62.6*	291.1± 52.7
$VO_{2(m)}$ ($mL.min^{-1}$)	911.1 ± 259.3*	1764.6 ± 493.8
VO_2 amplitude ($mL.min^{-1}$)	682.8 ± 240.3*	1473.6 ± 465.8
τ (s)	34.6 ± 9.1	37.6 ± 9.0
Td (s)	22.8 ± 3.3	23.0 ± 3.0
MRT (s)	57.4 ± 10.8	60.6 ± 11.5
$\Delta VO_{2,\ 6\text{-}3}$ ($mL.min^{-1}$)	8.0 ± 59.8	10.7 ± 35.5
Off-transient VO_2 kinetics		
$VO_{2(b)}$ ($mL.min^{-1}$)	237.7 ± 61.8*	327.9 ± 63.8
$VO_{2(m)}$ ($mL.min^{-1}$)	919.2 ± 267.9*	1758.4 ± 510.4
VO_2 amplitude ($mL.min^{-1}$)	673.4 ± 234.4*	1436.7 ± 451.5
τ (s)	36.5 ± 12.3	37.7 ± 7.0
Td (s)	24.3 ± 4.3	23.8 ± 4.6
MRT (s)	60.7 ± 14.9	61.5 ± 9.1

Values are mean ± SD.

Abbreviations: $VO_{2(b)}$, oxygen uptake at baseline; $VO_{2(m)}$, oxygen uptake during moderate intensity exercise; τ, oxygen uptake time constant; Td, time delay; MRT, mean response time; $\Delta VO_{2,\ 6\text{-}3}$, change in oxygen uptake between the 3rd and 6th min of exercise. * Participants with DS differ from controls ($p < 0.05$).

All VO_2 kinetic parameters obtained during the on- and off-transients for both groups are presented in table 3.11. A representative example of VO_2 on- and off- transient responses from one participant with DS and another control, at 45% VO_{2peak}, is illustrated in figure 3.6. Resting VO_2 was lower in subjects with DS compared to controls ($p < 0.05$). The time constants for the VO_2 on- and off-transients (τ_{on} and τ_{off}) were not significantly different between groups. Using the equation of Lamarra et al. (1987), the 95% confidence interval for the estimation of τ_{on} and τ_{off} for participants with DS were 3.0 ± 0.6 s and 1.9 ± 0.5 s, respectively. For the controls, the 95% confidence interval of τ_{on} and τ_{off} were 2.7 ± 0.6 and 1.7 ± 0.4 s, respectively. In proportion to the mean τ_{on} and τ_{off} of both groups, this corresponded to 8.7 and 5.2% for participants with DS and 7.2 and 4.5 % for controls. Accordingly, the magnitude of the effects of noise on kinetic parameter estimations was similar between groups. As shown in table 2, the change in VO_2 from rest to 45%VO_{2peak} (VO_2 amplitude)

was smaller in participants with DS than in controls ($p < 0.05$). Given the differences between participants with DS and controls in peak exercise capacity, we repeated this analysis while expressing the VO_2 amplitude in each group as a percentage of VO_{2peak}. This analysis revealed that the participants with DS responded to exercise with lower relative gain in VO_2 compared to controls (DS: 33.9 ± 6.6; controls: 40.4 ± 6.0 %, $p < 0.05$). Similarly, subjects with DS also presented a smaller change in normalized VO_2 than controls in transition from exercise to recovery (DS: 33.6 ± 6.5; controls: 39.4 ± 5.9 %, $p < 0.05$). In contrast, the values of Td and MRT (on and off) were not different between groups (table 3.11). Furthermore, there were no differences between groups for the respiratory exchange ratio during the 6th min of exercise (DS: 0.91 ± 0.08; controls: 0.91 ± 0.05). In addition, during submaximal testing, all participants exercised below their VT and therefore within the moderate intensity domain. Since the $\Delta VO_{2,\ 6\text{-}3}$ was of comparable magnitude in both groups and did not differ from zero in either one, this further supports adequacy of the selected protocol in eliciting steady-state VO_2 in participants with DS and controls. Table 2 also characterizes the symmetry of on- and off-VO_2 kinetics, showing that τ_{on} was not significantly different from τ_{off} in participants with DS or controls. As shown in table 3.12, heart rate was similar between groups before and after exercise. During the last minute of steady-state, both groups exercised at similar FU of their peak heart rates (DS: 61.4 ± 6.5; controls: 63.9 ± 7.1%). However, while the heart rate on-kinetics was not different between groups, participants with DS showed slower heart rate off-kinetics than the controls during recovery from exercise ($p < 0.05$).

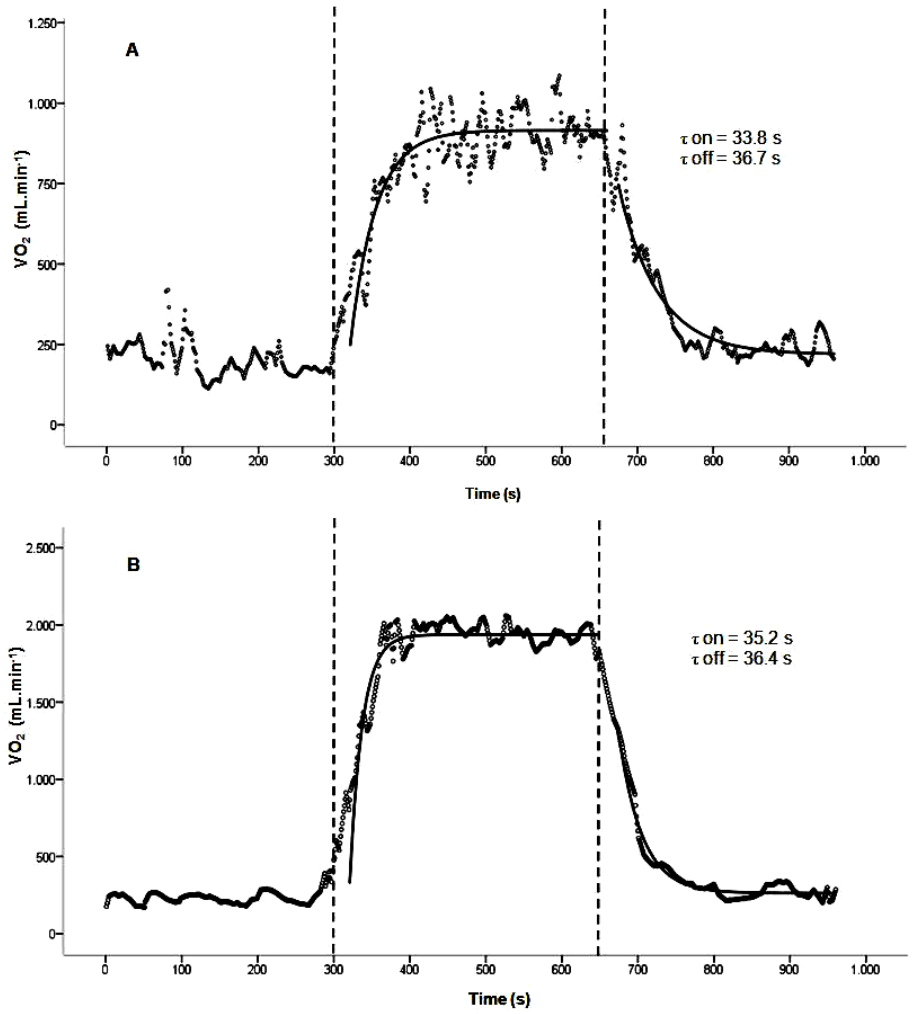

Fig. 3.6 Interpolated VO_2 response to steady-state treadmill exercise at 45% VO_{2peak} in two representative subjects (A, participant with Down syndrome; B, control participant). The curved solid line is the computer-derived representation of the best fit of the mono-exponential model to the VO_2 response.

Abbreviations: VO_2, oxygen uptake; VO_{2peak}, peak oxygen uptake; τ, oxygen uptake time constant.

Table 3.12 Heart rate responses of participants with Down syndrome (DS) and of nondisabled controls during exercise at 45% VO_{2peak}.

Variable	DS (n=14)	Controls (n=13)
Resting HR (bpm)	71.8 ± 11.0	68.0 ± 11.5
Exercise HR (bpm)	105.6 ± 13.3*	117.8 ± 13.0
Post-exercise HR (bpm)	74.6 ± 10.7	74.5 ± 10.9
τ on (s)	21.9 ± 9.2	21.7 ± 10.1
τ off (s)	58.5 ± 33.8*	27.0 ± 17.3

Values are mean ± SD.

Abbreviations: HR, heart rate; τ, heart rate time constant. * Participants with DS differ from controls ($p < 0.05$).

3.4.6. Discussion

The main finding of the present study is that the VO_2 kinetics at onset and recovery of constant-load treadmill exercise (45% VO_{2peak}) is comparable between adults with DS and controls (τ of ~35 s). We had previously observed an increased VO_2 change in persons with DS while responding to submaximal treadmill exercise at a given absolute workload (Mendonca et al. 2010). Since adults with DS show lower VO_{2peak} and poorer walking economy than nondisabled controls (Fernhall et al. 1996; Mendonca et al. 2010), in the present study, we used treadmill exercise of constant relative intensity to explore their VO_2 kinetic responses. Our results suggest that the submaximal exercise performance of these individuals is not affected by slowed VO_2 kinetics.

During exercise at 45% VO_{2peak}, the steady-state increase in VO_2 was smaller in individuals with DS than in controls owing to their lower functional capacity, resulting in a lower absolute workload at this relative intensity. Lower VO_{2peak} values are generally associated with lower peak cardiac outputs (di Prampero and Ferretti 1990) and have been extensively reported in this population (Fernhall et al. 1990; Fernhall et al. 1996; Guerra et al. 2003). The present study indicates that, despite having a reduced VO_{2peak}, adults with DS show normal VO_2 kinetics at onset of moderate intensity exercise, and this is novel. Importantly, our results are in agreement with those of Whipp et al. (2002), showing that, in healthy humans, τ is of ~35 s. While it is generally accepted that the main limiting factor for VO_{2peak} resides in the capacity by the cardiovascular system to deliver O_2 to muscle fibers

(di Prampero and Ferretti 1990), the VO_2 kinetics is mainly limited by factors located within muscle fibers (skeletal muscle oxidative metabolism) (Hughson and Kowalchuk 1995; Williamson et al. 1996). Specifically, the kinetics of muscle and pulmonary VO_2 are thought to be primarily determined by intramuscular processes (Grassi et al. 2000). Nevertheless, the kinetics of VO_2 can be slowed by decreasing arterial O_2 content and/or delivery (Engelen et al. 1996). On the contrary, there is no compelling evidence that increased muscle O_2 delivery can increase VO_2 kinetics in healthy humans. It has been postulated that faster VO_2 kinetics is mostly caused by an increase of the parallel activation of ATP usage and ATP supply pathways and, therefore, of metabolic stability. The oscillation of calcium ions in the cytosol and their uptake into the mitochondria play an important role in the parallel activation of ATP hydrolysis (myosin ATPase) and of ATP synthesis (pyruvate dehydrogenase - PDH, NAD^+-isocitrate dehydrogenase and oxoglutarate dehydrogenase) (Denton et al. 1980). It is well documented that oxidative muscle fibers are characterized, during rest-to-work transitions, by higher metabolic stability (shorter half transition time for VO_2 during on- and off-transients), compared to glycolytic muscle fibers (Hochachka and McClelland 1997).

Mitochondrial content, levels of critical mitochondrial proteins such as cytochrome *c* and the electron transport chain proteins and respiration rate are grossly normal in the mouse model of DS (TS16). TS16 also shows normal number of mitochondria and a preserved capacity for maximal calcium uptake (Bambrick and Fiskum 2008). Consequently, our results of comparable VO_2 kinetics between adults with DS and controls are supported by previous findings. In contrast, selective defects were demonstrated in complex I mitochondrial subunit and PDH of TS16 (Bambrick and Fiskum 2008; Prince et al. 1994). It was speculated that PDH damage might be associated with an increased generation of free radicals in DS which would lead to a defective complex I (Bambrick and Fiskum 2008). Rossiter et al. (2003) showed that PDH activation by dichloroacetate (DCA) does not determine, in humans, faster pulmonary VO_2 kinetics. These authors, however, observed after DCA, for the same absolute workload, lower amplitude of VO_2 and less blood lactate accumulation. Interestingly, Agiovlasitis et al. (2009) showed greater O_2 cost of walking in adults with DS compared

to nondisabled controls. Taken together, results suggest that, while the selective PDH defect in DS may not disturb their VO_2 kinetics, it might contribute to their reduced walking economy.

We found symmetrical on- and off-transient dynamics in both groups (τ_{on} were not different from τ_{off}) and this is in accordance with the findings of Donald and Whipp (1991) for exercise intensities below the VT, in which VO_2 is in a steady-state by ~ 3 min. Since, in each group, the VT corresponded to ~ 65% VO_{2peak} and the walking bouts were performed at 45% VO_{2peak}, we are confident that all participants exercised in the moderate intensity domain. This is further supported by $\Delta VO_{2,\ 6\text{-}3}$ which was of similar magnitude between groups and not significantly different from zero in either one (Wasserman 1990). Symmetry between VO_2 on- and off-transients is to be expected if in recovery the depleted O_2 and local creatine phosphate stores are replenished to the same pre-exercise levels. The fast component of the VO_2 off-transient response is a reflection of the rate of readjustment of oxidative phosphorylation during recovery and is not affected by anaerobic metabolism at intensities within the moderate and heavy exercise domains (Cerretelli and di Prampero 1987; Özyener et al. 2001). Therefore, the similarity of on- and off-transient time constants of VO_2 [symmetrical fast component (Barstow et al. 1996; Engelen et al. 1996)] in both groups suggests that muscle O_2 utilization during recovery in adults with DS is not different that of nondisabled controls at exercise intensities below the VT.

In terms of chronotropic response, despite the similarities between groups at submaximal exercise onset, participants with DS showed slower heart rate off-kinetics than controls. We have previously shown that adults with DS have reduced heart rate recovery from maximal exercise (at 1 and 2 min of recovery) and that this is independent of their chronotropic incompetence (Mendonca and Pereira 2010). Therefore, the present findings of delayed heart rate recovery from submaximal exercise in adults with DS not only corroborate, but also extend those of previous studies. Taken together, results suggest an attenuated vagal tone reactivation during recovery in persons with DS that is independent of exercise intensity (Androne et al. 2003). Interestingly, even though participants with DS presented slower heart rate off-kinetics, their VO_2 off-transient was similar to that of controls. This suggests no relationship between heart rate and other exercise variables during recovery, and it further

confirms the work of others (Pavia et al. 1999). Subjects with DS have lower blood pressure than healthy controls at all ages and the reported sex difference in the general population is not seen in these individuals (Richards and Enver 1979). Furthermore, Eberhard et al. (1991) also showed that blood pressure does not rise regularly with exercise intensity in adolescents with DS. Therefore, it is possible that their attenuated cardiodeceleration after exercise results from a compensatory mechanism that further prevents the onset of post-exercise hypotension.

In conclusion, we found that despite having a reduced peak functional capacity, adults with DS exhibit normal rate of oxygen uptake (VO_2) during onset and recovery of exercise at 45% VO_{2peak}. These findings indicate an adequate dynamics of O_2 delivery and utilization in DS during the early response to submaximal exercise. Additionally, they corroborate the hypothesis of a different localization for the main limiting factors for VO_2 kinetics and VO_{2peak}.

3.4.6.1. Clinical implications

Persons with DS have reduced VO_{2peak}, poor exercise tolerance and chronotropic incompetence (Guerra et al. 2003). Nevertheless, post-mortem examination of adults with DS usually exhibits a complete absence of atheroma and this has been associated with a "genetic triple dose effect" (Murdoch et al. 1977). Prolonged VO_2 kinetics during low-intensity exercise is related to poor prognosis in patients with reduced cardiac function (Schalcher et al. 2003). Time constants, often exceeding 70 s, have been observed in those suffering from cardiopulmonary insufficiency (Sietsema 1992). We did not find altered VO_2 kinetics in adults with DS. Thus, this is an important marker of differentiation between their physiological profile and that of patients with heart disease. Our findings suggest that although VO_{2peak} values are low in persons with DS, they do not exhibit a profile otherwise compatible with increased risk of disease, and this is consistent with prior past-mortem findings.

3.4.6.2. Limitations

There are 4 main limitations to this study. First, DS is a genetic disorder with diverse physiological consequences. However, physical work capacity and heart rate responses to peak exercise are remarkably consistent in the literature, and our present data are similar to that previously reported. Second, peak treadmill exercise is effort dependent; thus it is possible that participants with DS may have produced lower effort than the control subjects. We used validated protocols and accepted criteria for peak effort during treadmill testing. Additionally, the lack of group differences in the respiratory exchange ratio at peak exercise intensities further corroborates the assumption of comparable effort between subjects with DS and controls. Therefore, we do not believe that our data were substantially influenced by lack of effort in participants from either group. Third, we only included two repetitions in our square-wave constant load exercise protocol. It has been documented that to determine phase 2 time constant accurately, several transitions need to be ensemble averaged; the number of repetitions depends on the noise, steady state amplitude, and the underlying time constant. As a double square-wave was conducted and this may limit the accurate determination of the phase 2 time constant, we also calculated the MRT. Kidling et al. (2005) demonstrated no significant differences in test-retest MRT values derived from a treadmill running protocol on two separate days. Evidence also exists to confirm that the MRT can be used as more reliable oxygen uptake kinetic modeling tool than peak cross-correlation time for a pseudorandom binary sequence exercise test (Edwards et al. 2005). According to these authors, a valid MRT may even be derived from a single test measurement of oxygen uptake kinetics. Finally, considering that the participants with DS showed smaller relative VO_2 amplitude (on and off) than controls while exercising at 45% VO_{2peak}, it is possible that these individuals show a decreased slope of VO_2 as a function of work rate (WR) or, alternatively, a loss of linearity in the VO_2 response to exercise at work rates within the moderate intensity domain. Unfortunately, because several variables make it difficult to estimate the subjects' actual power output during treadmill exercise, our experimental design does not allow further insight into this issue. Specifically, a predictable adjustment for body mass is not possible for treadmill exercise because of complex mechanical factors such as varying center of gravity as the angle of the treadmill is changed and the variable length of stride as the speed and/or grade are altered (Wasserman

et al. 2005). Therefore, future studies should consider the use of the cycle ergometer to determine the slope and linearity of the VO_2/WR relationship in persons with DS.

3.4.7. References

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3.5. Effects of combined aerobic and resistance exercise training in adults with and without Down syndrome

3.5.1. Abstract

Objective: To determine whether adults with Down syndrome (DS) could improve their submaximal and peak exercise capacity as adults without disabilities, after 12 wks of combined (aerobic and resistance) exercise training.

Design: Prospective study comparing the effects of a 12-wk combined exercise program on submaximal and peak exercise capacity of adults with and without DS.

Participants: Thirteen participants with DS (mean age ± standard deviation, 36.5 ± 5.5 yr) and 12 participants without disabilities (38.7 ± 8.3 yr).

Intervention: Combined exercise training for 12 wks. Endurance training was performed 3 days/wk for 30 min at 65-85% VO_{2peak}. Resistance training was prescribed for 2 days/wk and consisted of 2 rotations in a circuit of 9 exercises at 12-repetition-maximum.

Main outcome measure: Submaximal and maximal graded exercise tests with metabolic and heart rate measurements. Walking economy and VO_{2peak} were considered the main outcomes.

Results: Participants with DS and those without disabilities showed similar values for body mass index and relative fat mass at pre- and post-training periods. Walking economy and VO_{2peak} were overall lower in participants with DS compared to participants without disabilities ($p < 0.05$). Training improved walking economy and VO_{2peak} in participants with DS and in those without disabilities ($p < 0.05$); additionally, the magnitude of changes induced by training was similar between groups.

Conclusions: Overall, a 12-wk combined exercise regimen induced gains of similar magnitude between adults with and without DS for submaximal and peak exercise capacity.

3.5.2. Key words

Exercise; training; Down syndrome; rehabilitation

3.5.3. Introduction

Down syndrome (DS) is the most commonly inherited form of developmental disability in North America (Barnhart and Connolly 2007). It is estimated that 1 out of every 700-1000 newborns in the United States will be diagnosed with DS, and there are currently 350.000 persons with DS living in the United States (Barnhart and Connolly 2007). In more than 90% of cases, DS results from an excess of genetic material from chromosome 21 (i.e. trisomy 21). Numerous studies have shown that persons with DS have diminished work capacity; concomitant with reduced peak oxygen uptake (VO_{2peak}) (Fernhall et al. 1996; Fernhall and Pitetti 2001) and low levels of muscle strength (Guerra et al. 2000; Pitetti and Boneh 1995; Cowley et al. 2010). More recently, it was also demonstrated that adults with DS exhibit reduced walking economy and thus, an impaired response to submaximal exercise (Agiovlasitis et al. 2009; Mendonca et al. 2010). Taken together, such findings suggest that persons with DS might be particularly susceptible to loss of basic function because of poor physical fitness (Pitetti and Boneh 1995; Cowley et al. 2010; Carmeli et al. 2004; Carmeli et al. 2002a; Carmeli et al. 2002b).

To the best of our knowledge, few prospective studies have examined the effects of structured exercise training in persons with DS and their findings are somewhat contradictory. Millar et al. (1993) observed increases in work capacity but no changes in the VO_{2peak} of 14 adolescents with DS after 10 wks of aerobic training. These findings were subsequently confirmed by Varela et al. (2001) after a 16-wk rowing program. Accordingly, this led the authors to speculate that the capacity for improving aerobic functioning in DS might be limited. Nevertheless, both studies reported gains in exercise endurance after training; however, they were unable to provide a definite explanation for these findings. More recently, Tsimaras et al. (2003) were the first to document a significant increase in the VO_{2peak} of adults with DS after an aerobic training regimen consisting of 3 sessions/wk for 12 wks. Similar findings were also reported after 12 wks of a combined exercise program in older adults with DS (Rimmer et al. 2004) and after 28 wks of multi-ergometer aerobic conditioning (Mendonca and Pereira 2009). Consequently, given these latest reports, there is evidence that adults with DS respond positively to structured exercise training, particularly under well controlled conditions and

this is further supported by the meta-analytic findings of Dodd and Shields (2005). As importantly, only 4 trials have investigated whether persons with DS have the capacity to improve their levels of muscle strength in response to progressive resistance training. Three of these trials included progressive resistance training (Davis 1987; Weber and French 1988; Shields et al. 2008) and 1 used a combined exercise program (Rimmer et al. 2004). Overall, findings indicate that programs prescribed for a frequency of 3 days/wk improve the upper- and lower-limb muscle strength of persons with DS (Rimmer et al. 2004; Davis 1987; Weber and French 1988). In contrast, training regimens of lower frequency (2 days/wk) are apparently associated with gains in upper-limb muscle endurance (Shields et al. 2008). Unfortunately, none of the above training studies compared the relative gains in physical fitness of people with DS with those of a nondisabled group of similar characteristics. Considering the differential training responses of the previous studies in persons with DS it would be important to explore if adults with DS exhibit a different pattern of response to training compared to adults without disabilities. Furthermore, it is not known if exercise training is an effective intervention for improving walking economy of adults with DS. It has been shown that a combination of resistance training and endurance training enhances exercise performance and economy in healthy adults (Millet et al. 2002); however this has never been explored in persons with DS. Consequently, the main purpose of this study was to determine whether 12 wks of combined exercise training, using both aerobic and resistance exercise, produces differential responses in exercise economy and peak exercise capacity in adults with and without DS.

3.5.4. Methods

A total of 25 healthy participants (13 with DS [10 men; 3 women], 12 without disabilities [9 men; 3 women]), aged 27-50 yr, were included in the present study. A health screening questionnaire was completed by each participant and/or a parent or legal guardian. As in previous studies, exclusionary criteria included any contraindications to exercise, severe or profound intellectual disability, active smoking status, congenital or atherosclerotic heart disease, metabolic disease, respiratory disorders including asthma, atlantoaxial instability, orthopaedic issues that would limit treadmill performance and heart rate altering medications (Cowley et al. 2010). Also, all participants

had normal thyroid function per family member or physician report. Participants in both groups were either sedentary or moderately active, but none were involved in any formal exercise endurance training for at least 6 months. All participants, and in addition, parents and/or guardians of the participants with DS, signed informed consent to participate in the study. Informed consent was attained after listening to an explanation of the nature of study participation and initial eligibility screening. Additionally, before signing the informed consent, participants were given the opportunity to visit the facilities where testing and exercise sessions would be conducted. This study was approved by the University's Institutional Review Board.

Participants with DS were recruited from a vocational center for adults with intellectual disabilities. They all lived at home and were bussed to the center daily. Most of the vocational activities at this center involved light physical work for 5 to 6 h, 5 days/wk. Participants without disabilities were recruited from the local and university communities via word of mouth, flyers posted on boards, and throughout community organizations. For inclusion, participants without disabilities had to fulfil the following criteria: (1) healthy medical status, (2) non-smoking condition, (3) sex match with the participants with DS, (4) age match with the participants with DS and (5) agreement with the study procedures confirmed by signature of the written informed consent. Before data collection, each participant was familiarized with the laboratory setting, treadmill protocols, and use of the headgear and face mask. Familiarization sessions were continued until each participant could comfortably walk on the treadmill with the headgear and mouthpiece without hand-rail support. As in previous studies (Rimmer et al. 2004), participants were also given the opportunity to practice using strength-testing equipment. Approximately 30 min were spent demonstrating the correct use and technique of the 9 different exercises. Each participant performed 2 sets of 8 to 15 repetitions in each exercise. The familiarization sessions were helpful in teaching the participants the correct procedures and identifying close approximations of their 12-repetition-maximum (12-RM). All participants were adequately familiarized within 1 or 2 sessions.

3.5.4.1 Study design

Measurements were taken at baseline (Pre) and following a 12-wk combined exercise intervention (Post). During the exercise intervention, participants were instructed not to participate in any other form of exercise training. At baseline, participants completed a whole-body composition assessment following a 12-h overnight fast. Immediately following the body composition assessment, participants underwent resting, submaximal and maximal exercise testing. Testing was carried out in the laboratory with an environmental temperature between 21-24 ºC and a relative humidity between 44-56%. During a second visit (48 h after exercise testing), participants underwent a 12-RM multi-exercise testing. All within-participant sessions were conducted at the same time of the day (between 07.00 and 11.00 h) to reduce possible diurnal variation. Participants were asked to refrain from exercise 24 h before testing and caffeine ingestion on testing days. Post-measures were conducted ~ 48 h after the last exercise session.

3.5.4.2. Body composition

Body mass was measured using a calibrated digital scale, and height was measured using a stadiometer (standing digital scale/height rod attached; Seca, 770, GmbH Hambugh Germany). Body mass index (BMI) was calculated by dividing the participants' mass in kilograms by the square of their height in meters. The Dubois and Dubois formula was used for body surface area (BSA) calculations (Dubois and Dubois 1916). Bioelectrical impedance spectroscopy was additionally performed, using a multifrequency bioimpedance meter (SEAC, SFB3, Brisbane Australia), for the measurement of whole-body composition fat mass, fat-free mass, and intracellular and extracellular body water volumes. The participants were asked to empty their bladders before each measurement. For these experiments, a non-conducting bed was used. All metal items were removed from the participants to ensure the accuracy of the measurement. The participants lay in the supine position with their arms and legs abducted at an angle of 45º. Measurements were carried out after a latency period of 10 min to stabilize the organic water compartments prior to assessments, with each participant lying still. After the skin was cleaned with alcohol, 4 Ag/AgCl electrodes were placed in the dorsal surfaces of the right hand and right foot. Two electrodes were placed on the right wrist with one proximal to the

third metacarpophalangeal joint (positive) and the other next to the ulnar head (negative). Two electrodes were also placed on the right ankle with one just proximal to the third metatarsophalangeal joint (positive) and the other between the medial and lateral malleoli (negative). Currents were introduced from the positive leads and travelled throughout the body to the negative leads. Each set of measurements were taken at 496 frequencies between 4 kHz and 1012 kHz. The percentage of body fat was then calculated using the resistance at 50 kHz. This single frequency was used to derive fat-free mass by using the Segal et al. (1988) equation. According to Sardinha et al. (1998), the accuracy of bioelectrical impedance spectroscopy, cross-validated with dual-x-ray-absorptiometry, increases using the resistance at 50 kHz for percent body fat calculations (in comparison to the standardized use of the manufacturer's equation).

3.5.4.3. Resting and submaximal exercise protocol

As in previous studies, the participants' resting VO_2 was obtained during a 5-min standing period, following a quiet rest of 10 min in the seated position (Mendonca et al. 2010). Cardiorespiratory data were collected while exercising on a motorised treadmill (Jaeger, Laufergotest, Hoechberg Germany) and expired gas measurements were made using a portable mixing chamber system (Cortex, Metamax I; Biophysik GmbH, Leipzig Germany), which was calibrated before each test with a known volume and with known gas concentrations. The protocol involved 5 min of continuous horizontal walking at a constant speed of 2.5 $km.h^{-1}$. Subsequently, speed was increased to 4 $km.h^{-1}$ and held constant at 3 different treadmill grades (0, 2.5 and 5%), for 5 min each. Heart rate data were obtained by means of a Polar R-R recorder (Polar, RS 800 R-R recorder, Kempele Finland). The submaximal treadmill speeds were selected on the basis of the results reported in previous studies (Smith and Ulrich 2008; Mendonca et al. 2009). Accordingly, adults with DS prefer walking velocities of approximately 2.5 $km.h^{-1}$ (Smith and Ulrich 2008) and present similar walking economy as adults without disabilities at this specific treadmill workload (Mendonca et al. 2009). Additionally, this protocol has been validated for both adults with and without DS (Fernhall et al. 1990; Mendonca et al. 2008). The VO_2 data were displayed as 30-s averages. The mean of the last 3 min of the 5-min resting period was defined as the participants' resting steady-state VO_2. For the submaximal exercise test, the

mean of the last 3 min of each 5-min walk was also defined as the participants' submaximal steady-state VO_2 (Whipp 1971). Additionally, the submaximal relative work intensities were determined as percentages of VO_{2peak} (fractional utilization - FU).

3.5.4.4. Maximal protocol

VO_{2peak} was determined by means of a continuous graded exercise test to volitional exhaustion commencing immediately after the last submaximal walk. For this purpose, treadmill grade was increased every 2 min by 2.5% until a 12.5% grade was reached. From this point, grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every minute until exhaustion. The test was terminated when the subject reached exhaustion and grasped the hand rails of the treadmill. This protocol has been shown to be both a valid and reliable measure of peak cardiorespiratory fitness in both adults with and without DS (Fernhall et al. 1990). The VO_2 data were displayed in 20-s averages. A valid VO_{2peak} was defined as the highest value obtained during the last stage of exercise with a respiratory exchange ratio (RER) over 1.0 (Fernhall and Otterstetter 2003).

3.5.4.5. 12-RM protocol

Given that, in this study, the exercise program was designed for 12-RM and that there is no information available on the equivalence between RM percentages and number of repetitions for adults with DS, muscle strength was determined using the 12-RM method directly. The 12-RM was measured for 5 exercises using variable resistance machines (Technogym selection line, Gabetolla Itally) as follows: (1) leg press, (2) chest press, (3) vertical traction, (4) lower back and (5) leg extension. Prior to the 12-RM determination, all participants warmed-up for 15 min on a treadmill at 50% of their heart rate reserve (Spring et al. 1991). Subsequently, each participant was asked to perform 15 repetitions using a relatively light resistance, followed by 30 s of recovery. The resistance was then increased and each participant performed a maximum of 5 sets of 12 repetitions until the 12-RM was attained. The recovery period between sets was of exactly 2 min and increments of 2.5-5 kg were used whenever each participant approached fatigue. 12-RM was defined as the maximal load lifted in a full range of motion for a total of 12 repetitions. For most participants, 12-RM was determined within 3-4 attempts.

3.5.4.6. Exercise training program

Participants exercised 3 days/wk and each session was conducted with a maximum of 5 participants. The exercise sessions were supervised by an exercise physiologist and 1 assistant. The intervention consisted of 2 days of combined exercise training (Monday and Friday) separated by 1 day of endurance training (Wednesday). The endurance training consisted of 30 min of treadmill walking or running at target heart rates compatible to 65-85% VO_{2peak}. Endurance exercise was preceded by a 5-min warm-up and followed by another 5 min of recovery. Warm-up and recovery consisted of treadmill walking at light intensity. During the first 3 wk, participants exercised for 30 min at 65% VO_{2peak} and then, emphasis was placed on reaching and maintaining an exercise intensity of approximately 85% VO_{2peak} for 30 min. Heart rate watch monitors (Vantage Night Vision, Polar heart rate monitor, Kempele Finland) were programmed for each participant (upper and lower training heart rate) to assure they were exercising in the appropriate target heart rate zone. Participants were given the opportunity to select between walking and running gaits as long as they exercised continuously within the prescribed heart rate zone. While the participants with DS preferred to exercise using graded treadmill walking, those without disabilities generally choose running at 0 % grade. Each staff member was responsible for 1 to 3 participants to encourage them to sustain a treadmill workload compatible with a heart rate closer to the upper limit of the training zone.

The resistance training consisted of 2 rotations in a circuit of 9 exercises with less that 30 s of rest between them. Participants trained on the same equipment used for the 12-RM assessments and each session included the following dynamic exercises: leg press, chest press, vertical traction, shoulder press, lower back, leg extension, biceps curl and triceps pushdown. The exercise stations were prescribed for 12-RM and organized in alternate agonist/antagonist interplay to avoid the early onset of local/regional fatigue. Additionally, the participants performed 1 set of 15 repetitions of abdominal curls in each rotation. When participants were able to complete 14 repetitions for 2 consecutive sessions with the proper lifting technique (i.e. proper biomechanical motion; avoidance of the Valsalva maneuver, which involves holding the breath), the load was increased by 10% of their 12-RM.

3.5.4.7. Statistical analysis

Descriptive statistics were calculated for all variables. Before comparing both groups, data were tested for normality and homocedasticity with the Kolmogorov-Smirnov and Levene's tests, respectively. A repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of the training program on the participants' body composition, submaximal and peak exercise capacity and muscle strength. When a significant effect was detected at a significance level of $p < .05$, *t*-tests were used for *post hoc* comparisons. Adjustment for multiple comparisons was made with Bonferroni's correction. Because previous studies provided no definite explanation for the improvements seen in work capacity of adults with DS after training, we repeated the ANOVAs conducted on graded exercise test time using the training-induced changes in walking economy and peak VO_2 as covariates. This analysis was performed separately in participants with and without DS. All data are reported as mean ± SD unless otherwise specified. Statistical significance was set at $p < .05$. All data analysis was carried out using Statistical Package for the Social Sciences (Version 17.0, SPSS Inc, Chicago Illinois USA).

Table 3.13 Body composition and resting cardiorespiratory data of participants with and without Down syndrome at pre- and post-training conditions.

Variables	Down syndrome (n=13)		Participants without disabilities (n=12)	
	Pre-training	Post-training	Pre-training	Post-training
Body composition				
Body mass (kg)	68.6 ± 9.2	67.7 ± 9.2*	81.2 ± 17.1	81.4 ± 16.3
Body surface area (m^2)	1.7 ± 0.1	1.7 ± 0.1*	1.9 ± 0.2	1.9 ± 0.2
Body mass index (kg/m^2)	29.3 ± 3.7	28.9 ± 3.6	26.6 ± 4.5	26.6 ± 4.2
Fat mass (kg)	19.5 ± 4.0	19.1 ± 4.1	21.7 ± 8.5	21.8 ± 7.7
Fat-free mass (kg)	48.1 ± 6.9	47.8 ± 7.0*	59.4 ± 9.2	59.5 ± 9.2
Relative fat mass (%)	28.4 ± 4.3	28.4 ± 4.4	25.6 ± 5.3	26.2 ± 4.5
Resting cardiorespiratory data				
VO_2 ($mL.^{-1}kg.min^{-1}$)	4.2 ± 0.8	3.9 ± 0.5	4.0 ± 0.9	3.9 ± 0.8
BSA Ve ($L.min^{-1}.m^2$)	4.0 ± 0.6	4.0 ± 0.7	4.5 ± 0.5	4.5 ± 0.9
RER	0.88 ± 0.09	0.80 ± 0.06	0.85 ± 0.06	0.85 ± 0.09
HR (bpm)	69.6 ± 12.2	67.8 ± 12.3	70.6 ± 16.0	66.1 ± 9.0

Values are mean ± SD.

Abbreviations: VO_2, resting oxygen uptake; BSA Ve, body surface area adjusted minute ventilation; RER, respiratory exchange ratio; HR_{rest}, rest heart rate. * Between-group differences at pre- and post-training periods ($p < 0.05$).

3.5.5. Results

No differences were observed between groups for age (DS: 36.5 ± 5.5; participants without disabilities: 38.7 ± 8.3 yr). Participants with DS were shorter (DS: 152.9 ± 7.9; participants without disabilities: 174.3 ± 6.1 cm) and presented lower body mass than participants without disabilities at pre- and post-training periods ($p < 0.05$) (Table 3.13). BSA was also lower for participants with DS ($p < 0.05$). In contrast, both groups showed similar values for BMI, absolute fat mass and relative fat mass at both periods. Nevertheless, participants with DS had lower fat-free mass than participants without disabilities before and after training ($p < 0.05$). As depicted in table 3.13, the exercise program had no significant effect on body composition. Of relevance, there were no withdrawals from the study and no adverse events for the intervention were noted.

3.5.5.1. Submaximal exercise

There were no significant differences between groups at rest before or after training (Table 3.13). However, participants with DS exhibited higher overall VO_2 (lower exercise economy) than participants without disabilities during submaximal exercise (group main effect: $F = 23.7$, $p < 0.05$) (Fig. 3.7A and Fig. 3.7B). As shown in table 3.14, there was an overall decrease in submaximal VO_2 in both groups after training (time main effect: $F = 30.5$, $p < 0.05$). However, while the improvements of participants with DS were extensive to all submaximal workloads performed at 4 $km.h^{-1}$, those of participants without disabilities only reached significance at 0 and 2.5% treadmill grades (time by group interaction: $F = 5.7$, $p < 0.05$). Training had no effects on submaximal VO_2 of either group at a walking speed of 2.5 $km.h^{-1}$.

As can be seen in table 3.14, participants with DS relied on higher FU of the VO_{2peak} than participants without disabilities at all submaximal workloads both during the pre- and post-training periods (group main effect: $F = 50.5$, $p < 0.05$). Nevertheless, after training, both groups showed lower FU of their VO_{2peak} at each treadmill workload (time main effect: $F = 35.7$, $p < 0.05$). However, compared to participants without disabilities, those with DS exhibited a greater reduction in their FU of VO_{2peak} during the horizontal treadmill walk at 4 $km.h^{-1}$ (time by group interaction: $F = 6.3$, $p < 0.05$).

Pre-training, participants with DS had higher BSA adjusted minute ventilation than those without disabilities at all submaximal workloads ($p < 0.05$) except for the 2.5 $km.h^{-1}$ treadmill walking speed (Table 3.14). However, while training induced no significant changes in the nondisabled group, participants with DS reduced their BSA adjusted minute ventilation during horizontal and graded (2.5 and 5%) treadmill walking at 4 $km.h^{-1}$ ($p < 0.05$). Furthermore, the differences between groups seen at baseline were no longer significant after training.

Compared to participants without disabilities, those with DS showed higher RER values during exercise at 4 $km.h^{-1}$ (horizontal and graded) ($p < 0.05$) (Fig. 3.7C). With exception for the 2.5 $km.h^{-1}$ treadmill walking speed, training resulted in significant RER reductions at all submaximal

workloads; however this only occurred in participants with DS (time by group interaction: $F = 8.1$, $p < 0.05$) (Table 2). Due to these improvements, the pre-training group differences seen during exercise at 4 km.h^{-1} were no longer significant after training (Fig. 3.7D).

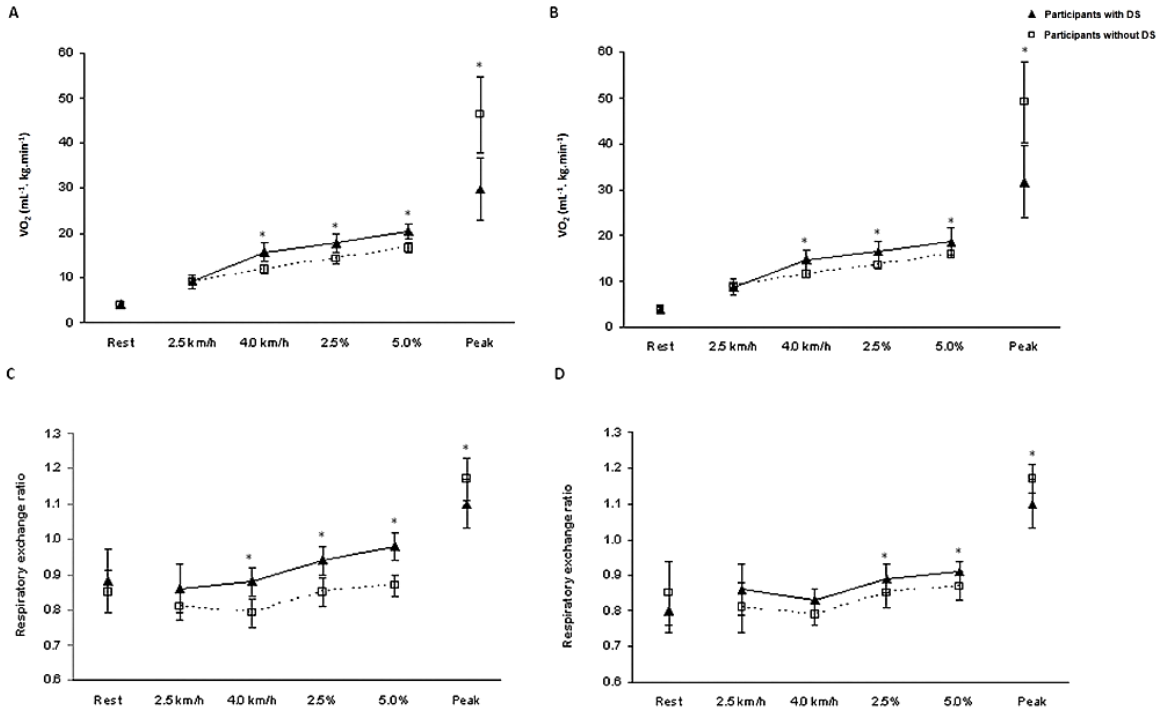

Fig. 3.7 Oxygen uptake (VO_2) and respiratory exchange ratio (RER) of participants with and without Down syndrome at rest, during four different submaximal treadmill workloads (2.5 km.h^{-1} – 0% grade; 4 km.h^{-1} – 0% grade; 4 km.h^{-1} – 2.5% grade; 4 km.h^{-1} – 5% grade) and at peak exercise. (A) Pre-training VO_2; (B) Post-training VO_2; (C) Pre-training RER; (D) Post-training RER. * Between-group differences ($p < 0.05$).

Participants with DS exercised at higher heart rate than controls while walking at a 5% treadmill grade (Table 3.14) ($p < 0.05$). Conversely, the magnitude of increase in heart rate across submaximal workloads was similar between groups before training. After training, the nondisabled participants exhibited a more attenuated chronotropic response to submaximal exercise than participants with DS. This was manifested by a lower increase in heart rate during the transition between walking speeds (2.5 to 4 km.h^{-1}) and grades (0 to 2.5%) ($p < 0.05$).

Table 3.14 Submaximal cardiorespiratory data of participants with and without Down syndrome at pre- and post-training conditions.

Variables	Down syndrome (n=13)		Participants without disabilities (n=12)	
	Pre-training	Post-training	Pre-training	Post-training
VO_2 (mL. $^{-1}$kg.min^{-1})				
2.5 km.h^{-1}	9.3 ± 1.5	8.8 ± 1.8	9.2 ± 0.7	8.9 ± 0.6
4.0 km.h^{-1}	15.6 ± 2.1	14.6 ± 2.2*†	12.0 ± 0.9	11.5 ± 0.8*
2.5%	17.9 ± 2.1	16.6 ± 2.2*†	14.2 ± 1.0	13.6 ± 0.8*
5%	20.4 ± 1.7	18.7 ± 2.8*†	16.7 ± 1.1	16.3 ± 0.8
FU VO_{2peak} (%)				
2.5 km.h^{-1}	32.6 ± 9.1	29.4 ± 10.3†	20.6 ± 4.0	18.5 ± 3.2*
4.0 km.h^{-1}	54.7 ± 12.7	48.1 ± 10.5*†	26.8 ± 4.9	24.0 ± 3.9*
2.5%	62.9 ± 14.8	55.2 ± 11.9*†	31.8 ± 6.1	28.5 ± 4.7*
5%	71.4 ± 15.6	61.1 ± 10.6*†	37.4 ± 7.3	34.1 ± 5.9*
BSA Ve (L.min^{-1}.m^2)				
2.5 km.h^{-1}	10.0 ± 1.6	9.6 ± 1.2	9.3 ± 1.1	9.1 ± 1.0
4.0 km.h^{-1}	14.5 ± 2.8	12.9 ± 2.3*‡	11.3 ± 1.2	10.9 ± 1.2
2.5%	17.2 ± 3.1	15.5 ± 2.6*‡	13.8 ± 1.3	13.2 ± 1.5
5%	20.5 ± 3.2	17.7 ± 3.2‡	16.4 ± 1.6	15.9 ± 1.8
RER				
2.5 km.h^{-1}	0.86 ± 0.07	0.86 ± 0.07	0.81 ± 0.04	0.81 ± 0.07
4.0 km.h^{-1}	0.88 ± 0.04	0.83 ± 0.03*‡	0.79 ± 0.04	0.79 ± 0.04
2.5%	0.94 ± 0.04	0.89 ± 0.04*†	0.85 ± 0.04	0.85 ± 0.03
5%	0.98 ± 0.04	0.91 ± 0.03*†	0.87 ± 0.03	0.87 ± 0.03
HR (bpm)				
2.5 km.h^{-1}	88.2 ± 13.2	81.6 ± 10.2	77.8 ± 11.3	77.5 ± 9.2
4.0 km.h^{-1}	100.5 ± 12.4	96.5 ± 11.2	88.1 ± 15.8	84.1 ± 11.2
2.5%	109.8 ± 13.6	106.5 ± 10.7§	95.0 ±17.6	90.9 ± 10.6
5%	121.2 ± 13.8	116.9 ± 10.5†	103.0 ± 18.6	97.9 ± 11.2

Values are mean ± SD.

Abbreviations: VO_2, oxygen uptake; FU VO_{2peak}, fractional utilization of peak oxygen uptake; BSA Ve, body surface area adjusted minute ventilation; RER, respiratory exchange ratio; HR, heart rate. * Within-group differences after training ($p < 0.05$); † between-group differences at pre- and post-training ($p < 0.05$); ‡ between-group differences at pre-training ($p < 0.05$); § between-group differences at post-training ($p < 0.05$).

3.5.5.2. Peak exercise

Table 3.15 shows the peak cardiorespiratory data of participants with and without DS at pre- and post-training. Compared to participants without disabilities, those with DS exhibited lower peak values for VO_2, BSA adjusted minute ventilation, RER, heart rate and graded exercise test time at both time-points. Yet, training resulted in significant improvements in the peak values of VO_2, BSA adjusted minute ventilation and graded exercise test time in both participants with and without DS ($p < 0.05$). Importantly, there were no differences in the magnitude of these changes between groups (Fig. 3.8). Peak heart rate or peak RER did not change with training (Table 3.15).

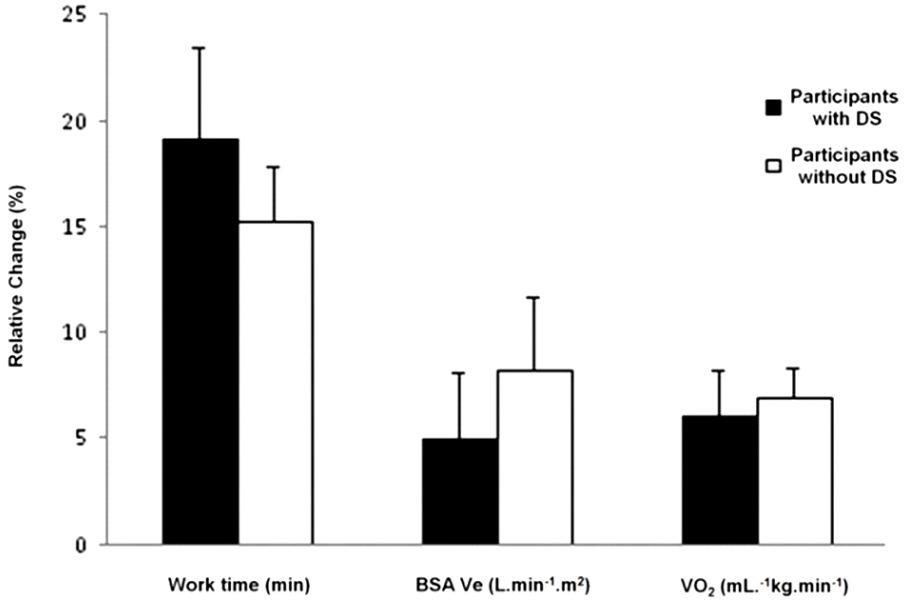

Fig. 3.8 Relative change in work time, body surface area adjusted minute ventilation (BSA Ve) and peak oxygen uptake (VO_{2peak}) in participants with and without Down syndrome (DS) after training.

We also repeated the ANOVAs conducted on graded exercise test time to explore possible relationships between increased work capacity and improvements in physiological function resulting from training. We found that, after controlling for the training-induced improvements in submaximal VO_2 and VO_{2peak}, there were no significant differences between pre- and post-training graded exercise test time in either group (DS: $F = 2.2$; participants without disabilities: $F = 3.8$, $p > 0.05$).

Additionally, for participants with DS, similar findings were obtained after controlling exclusively for gains in walking economy ($F = 2.4$; $p > 0.05$)

3.5.5.3. Muscle Strength

Participants with DS showed lower muscle strength than participants without disabilities in all dynamic exercises both pre-and post-training (group main effect: $F = 15.3$, $p < 0.05$). As can be seen in table 3.15, training was effective in eliciting generalized improvements in the 12-RM of both groups ($p < 0.05$). The magnitude of these improvements was similar between participants with and without DS.

Table 3.15 Peak exercise and muscle strength data of participants with and without Down syndrome at pre- and post-training periods.

Variables	**Down syndrome (n=13)**		**Participants without disabilities (n=12)**	
	Pre-training	Post-training	Pre-training	Post-training
Peak exercise				
VO_2 (mL. $^{-1}$kg.min^{-1})	29.8 ± 7.1	31.7 ± 7.9*†	46.3 ± 9.6	49.1 ± 8.5*
BSA Ve (L.min^{-1}.m^2)	37.8 ± 11.1	39.9 ± 12.2*†	61.0 ± 10.7	65.4 ± 10.0*
RER	1.10 ± 0.07	1.10 ± 0.07†	1.17 ± 0.06	1.17 ± 0.04
HR (bpm)	166.8 ± 19.9	167.6 ± 19.1†	183.0 ± 10.9	182.4 ± 9.5
GXT time (min)	4.8 ± 0.6	5.4 ± 0.8*†	6.3 ± 0.9	6.9 ± 0.8*
Muscle strength				
Leg press (kg)	94.1 ± 47.9	110.2 ± 52.6*†	149.6 ± 56.6	171.3 ± 56.5*
Vertical traction (kg)	31.5 ± 13.1	39.2 ± 14.1*†	51.5 ± 12.2	59.4 ± 15.3*
Chest press (kg)	27.5 ± 9.5	35.3 ± 12.2*†	44.4 ± 15.5	51.3 ± 21.0*
Lower back (kg)	31.3 ± 7.3	35.6 ± 7.4*†	44.8 ± 18.4	51.9 ± 19.3*
Leg extension (kg)	22.2 ± 7.0	30.1 ± 10.3*†	43.5 ± 17.8	52.7 ± 17.6*

Values are mean ± SD.

Abbreviations: VO_2, oxygen uptake; BSA Ve, body surface are adjusted minute ventilation; RER, respiratory exchange ratio; HR, heart rate; GXT, graded exercise test. * Within-group differences after training ($p < 0.05$); † Between-group differences at pre- and post-training ($p < 0.05$).

3.5.6. Discussion

Overall, our results show that adults with DS improve their physical fitness in similar magnitude as adults without disabilities after 12 wks of combined exercise. Additionally, to the best of our knowledge, this is the first study to report significant improvements in walking economy after an exercise intervention in persons with DS (6.6 - 10.4%). Given their increased O_2 cost of locomotion (Agiovlasitis et al. 2009; Mendonca et al. 2010); these improvements may have a large impact in their ability to perform daily activities. Noteworthy, we also found that the gains in work capacity resulting from exercise training were dependent on those seen in walking economy and VO_{2peak}. Importantly, all of these improvements were similar between participants with and without DS.

3.5.6.1. Maximal exercise

Our results agree with previous reports showing that, compared to nondisabled participants; adults with DS exhibit diminished work performance, reduced VO_{2peak}, and chronotropic incompetence (Fernhall et al. 1996; Fernhall and Pitetti 2001). Despite the ubiquitous nature of these findings, only five previous training studies were conducted on adolescents and adults with DS (Millar et al. 1993; Varela et al. 2001; Tsimaras et al. 2003; Rimmer et al. 2004; Mendonca and Pereira 2009). In the present study we found that a 12-wk combined exercise program elicited significant improvements in the VO_{2peak} of adults with DS. Of relevance, there were no differences between their pre- and post-training peak values of RER or heart rate. This suggests that participants produced valid peak efforts at both time points. Moreover, since there were no significant changes in body composition or body mass after training, the gains in peak aerobic capacity did not depend on reductions of participants' body mass or increases in fat-free mass. Our results agree with those of Rimmer et al. (2004) who also found significant increases in VO_{2peak} after 12 wks of combined exercise training. Therefore, a combined exercise prescription constitutes an effective stimulus for increasing peak aerobic capacity of adults with DS. Importantly, participants with DS showed similar magnitude of improvement as participants without disabilities for peak values of VO_2 (DS: 6%; participants without disabilities: 6.9%), minute ventilation (DS: 5.0%; participants without disabilities: 8.2%) and graded exercise test time (DS: 19.1%; participants without disabilities: 15.2%). Our

findings suggest that adults with DS respond to combined exercise training as participants without disabilities, thus supporting that the conventional exercise prescription guidelines, as those recommended by the American College of Sports Medicine (ACSM 2009), are well suited for this population. We found that the increases in VO_{2peak} were paired by gains peak minute ventilation, both in participants with and without DS. This agrees with the findings of Boutellier (1988) and Johnson et al. (1996), thus indicating that the improvements in VO_{2peak} were associated with higher ventilatory ability after training. As previously reported, training enhances the ability to sustain high levels of minute ventilation due to a coupling between improved ventilatory muscle endurance and increases in inspiratory muscle capacity to generate force and sustain a given level of inspiratory pressure (Clanton et al. 1987).

3.5.6.2. Submaximal exercise

As in previous studies, participants with DS showed poorer walking economy than participants without disabilities at all treadmill speeds other than their preferred walking speed (2.5 $km.h^{-1}$) (Mendonca et al. 2010). Nevertheless, we found that combined exercise training was an effective intervention for improving walking economy of adults with DS and this is novel. Given that the participants without disabilities also showed lower submaximal VO_2 during steady state exercise after training, the improvements in walking economy were not exclusive to adults with DS. However, according to our results, the mechanisms underlying these findings may be different between groups. While in participants with DS these improvements were paralleled by a reduction in submaximal minute ventilation, this was not the case for participants without disabilities. Therefore, persons with DS exhibit greater walking economy after training and this is apparently associated with reductions in the ventilatory demands during submaximal exercise. This agrees with previous studies showing that lower minute ventilation decreases the O_2 cost of locomotion (Thomas et al. 1999). Since, in these participants, training also contributed to a generalized reduction in RER values during submaximal workloads, it is possible that the reduction in minute ventilation may have resulted from lower levels of CO_2 being produced during exercise. Our results corroborate previous findings showing a considerable reduction of submaximal RER values in persons with DS after exercise training

(Mendonca and Pereira 2009). Conversely, in participants without disabilities, these improvements were not paired by such findings. Rossiter et al. (2003) showed that activation of pyruvate dehydrogenase by dichloroacetate determined lower O_2 cost during exercise. This is to due to a more efficient coupling between the amounts of ATP being synthesized per mole of O_2 being used within the muscle (Green et al. 2000). Therefore, improvements in walking economy, as those seen in adults without disabilities after training, may well be related to increases in the functionality of specific skeletal muscle mitochondrial enzymes. Importantly, this does not necessarily implicate changes in the nature of the substrate being oxidized for ATP synthesis.

In contrast to the participants without disabilities, those with DS showed a training effect on steady-state VO_2 while walking at a 5% treadmill grade. This may have been a function of how they trained as the participants with DS included in this study preferred to exercise (within their training zones) using graded treadmill walking. Conversely, the participants without disabilities generally choose running at 0% treadmill grade. Considering that the metabolic adaptations which occur within the skeletal muscle are specific to the training stimulus (Henriksson 1976), the lack of improvement in adults without disabilities at a steeper treadmill grade may be related to the form of exercise selected by these participants during the training sessions. Nevertheless, both groups exercised at lower FU of their VO_{2peak} after training. While these training-induced reductions in relative intensity resulted from a combination of improved walking economy and increased VO_{2peak} during most submaximal workloads, this was not the case for the 2.5 $km.h^{-1}$ treadmill walking speed in either group or during the 5% graded walk for participants without disabilities. Under these conditions, the decrease in FU was simply due to the gains seen in the participants' VO_{2peak} after training.

3.5.5.3. Relationships between work capacity and physiological adaptations

Even though this is the first evidence of improvements in walking economy of adults with DS, all previous studies in persons with DS had observed significant gains in work capacity after training (Millar et al. 1993; Varela et al. 2001; Tsimaras et al. 2003; Rimmer et al. 2004; Mendonca and Pereira 2009). Interestingly, as reported by Millar et al. (1993) and Varela et al. (2001), this occurred without concomitant increases in VO_{2peak}. As reviewed by Saunders et al. (2004) and McCann and

Higginson (2008), several studies support that improvements in exercise economy can lead to gains in peak work capacity, even without significant changes in VO_{2peak}. Therefore, we hypothesized that the changes seen in work capacity of persons with DS after training might result from a combination between improvements in exercise economy and VO_{2peak}. After repeating our statistical analyses on graded exercise test time, while controlling for the training-induced changes in walking economy and VO_{2peak}, we found that the differences in work capacity between pre- and post-training periods were no longer significant in either group. This indicates that the enhancements in work capacity, as those obtained in both groups after training, were largely dependent on a combination between improvements in walking economy and in VO_{2peak}. Consequently, although not measured in their studies, the gains in work capacity reported by Millar et al. (1993) and Varela et al. (2001) were most likely due to improved exercise economy after training.

3.5.5.4. Muscle Strength

We found that both groups showed generalized improvements in muscle strength after training. Noteworthy, as for peak endurance capacity, the magnitude of gains in muscle strength were not different between participants with and without DS (DS: 15.3% - 35.9%; participants without disabilities: 15.8% - 30.5%). According to recent findings, knee extensor strength predicts time performance of daily tasks in adults with DS (Cowley et al. 2010). Participants with DS included in the present study improved their leg extension 12-RM by ~ 35%. Therefore, it may be speculated that training represented a positive stimulus to enhance the ability of these persons to perform common daily tasks of daily living. Additionally, increased leg-extensor strength leads to a reduction in the relative load placed on the muscle during submaximal exercise (Osteras et al. 2001). Because persons with DS are documented to adjust their gait pattern to favour greater gait stability (Smith and Ulrich 2008; Black et al. 2007; Buzzi and Ulrich 2006; Kubo and Ulrich 2006; Parker et al. 1986), the reduced relative load could have improved their stability during walking, thereby leading to a reduced demand for O_2 when walking.

3.5.5.5. Study limitations

There are several limitations to this study. First, DS is a genetic disorder with diverse physiological consequences. However, physical work capacity and heart rate responses to peak exercise are remarkably consistent in the literature, and our present data are similar to that previously reported. Second, peak treadmill exercise is effort dependent; thus it is possible that participants with DS may have produced lower effort than the participants without disabilities. We used validated protocols and accepted criteria for peak effort during treadmill testing. Therefore, we do not believe that our data were substantially influenced by lack of effort in participants from either group. Third, since 12-RM represents a complex combination of muscle endurance and muscle strength we are unable to determine the exact origin of the improvements induced by training in either group. Fourth, our experimental design did not include non-exercising control groups; therefore, it is not known to what extent these findings are due to the effects of the combined exercise program rather than to series effects. Finally, the lack of blinded assessors to collect data at pre- and post-training periods may also correspond to a limitation the present study.

3.5.6. Conclusions

We found that adults with DS showed similar gains in peak exercise capacity and muscle strength, as participants without disabilities, after 12 wks of combined exercise training. Furthermore, these data provide preliminary evidence that combined exercise training may represent an effective intervention strategy to improve their reduced walking economy. As importantly, the present findings suggest that enhanced work capacity in adults with DS following training depends on a combination between improved exercise economy and higher VO_{2peak} and this is similar to that observed in participants without disabilities.

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4. Cardiac autonomic function:

Spectral methods of heart rate variability.

Cardiac autonomic function of adults with Down syndrome.

4.1. Spectral methods of heart rate variability analysis during dynamic exercise

4.1.1. Abstract

Objectives: To apply both autoregressive (AR) and fast Fourier transform (FFT) spectral analysis at rest, during two different dynamic exercise intensities and in recovery from maximal exercise and to compare raw and normalized powers obtained with both methods.

Methods: Sixteen participants (age 22.3 ± 4.3 yr) performed resting, submaximal and maximal protocols. The submaximal protocol consisted of two 5-min walks at 4 $km.h^{-1}$ at treadmill grades of 0 and 7.5%. Beat-to-beat R-R series were recorded. FFT and AR analyses were preformed on the same R-R series.

Results: Compared to AR, FFT provided higher total power (TP) and raw high frequency (HF) power at rest and exercise. Furthermore, FFT LF/HF ratio was lower than with the AR, except under resting conditions. Both methods showed reductions in TP, raw HF and LF powers during exercise and recovery. Only the AR revealed a significant reduction for normalized HF power and increase for normalized LF power in transition from rest to exercise conditions.

Interpretation: AR and FFT methods are not inter-changeable at rest or during dynamic exercise conditions. The AR method is more sensitive to the effects of exercise on the normalized power spectra of HRV than FFT. Finally, as both approaches are equally insensitive to the increase of exercise relative intensity, there is no practical advantage of performing HRV spectral analyses by the AR or FFT at higher workloads.

4.1.2. Key words:

Heart rate variability; spectral analysis; dynamic exercise; cardiac autonomic control

4.1.3. Introduction

Analysis of heart rate variability (HRV) is a valuable non-invasive marker of autonomic nervous system modulation of the SA node and is regularly used to study underlying physiological processes involved in cardiovascular control, both at rest and during exercise (Malik 1998). The HRV is frequently analyzed in both the time and frequency domains (Task Force 1996) and is most often evaluated at rest. During exercise, an increase in heart rate results from vagal withdrawal at low exercise intensities and from both vagal withdrawal and sympathetic activation at moderate and higher exercise intensities (Orizio et al. 1998; Robinson et al. 1966). Heart rate recovery following the cessation of exercise is mainly due to vagal re-activation immediately after exercise termination and further reductions are mediated by both vagal and sympathetic influences (Imai et al. 1994; Perini et al. 1989). Studies using conventional spectral analysis of HRV have shown that raw total, low frequency (LF) and high frequency (HF) powers decrease with exercise (Arai et al. 1989; Perini et al. 1990), but results for LF and HF normalized to total power have been inconsistent (Bernardi et al. 1990; Casadei et al. 1996; Perini et al. 1990; Rimoldi et al. 1992; Tulppo et al. 2001; Warren et al. 1997). During recovery, the reduced HRV gradually returns to pre-exercise levels within several minutes or hours depending on exercise intensity (Bernardi et al. 1990; Perini et al. 1990; Terziotti et al. 2001).

Frequency domain analyses of the HRV signal are usually made using either fast Fourier transform (FFT) or autoregressive (AR) models (Badilini et al. 1998). There is general agreement that AR and FFT spectral analyses are not inter-changeable (Badilini et al. 1998; Pichon et al. 2006). Nevertheless, dynamic trends provided by the two approaches are generally equivalent (Badilini et al. 1998), but to date, there are no reports on whether AR and FFT data processing of HRV during submaximal dynamic exercise provide similar results. Indeed, literature still presents conflicting results (Martinmäki and Rusko 2008; Leicht et al. 2008; Perini et al. 1998; Winsley et al. 2003) and a critical dilemma is whether both methods are equally sensitive to the effects of exercise on autonomic function. Thus, it is important to determine whether one methodology is more suitable than the other for HRV assessments during dynamic exercise. The purpose of this study was to apply both AR and

FFT spectral analysis at rest, during two different exercise intensities and during recovery from maximal exercise and to compare raw and normalized LF and HF powers obtained with the two methods.

4.1.4. Methods

4.1.4.1. Participants

A total of 16 participants, 9 male and 7 female physical education students volunteered to take part in the study (22.3 ± 4.3 yr). Participants' characteristics are presented in table 4.1. All participants were experienced treadmill walkers and runners and they all were similarly active, accumulating nine-hours of physical activity per week as part of their academic work. Medical histories were obtained through direct interviews and exclusion criteria were as follows: 1) history of thyroid or cardiovascular disease, 2) history of diabetes or other metabolic disease that might affect outcome measures, 3) heart rate altering medications, 4) smoking, 5) pulmonary or respiratory disorders, including asthma and 6) orthopaedic injury preventing successful completion of the exercise protocol. After thorough explanation of the study protocol to participants, and after having been shown the equipment, written informed consent was attained. All procedures in this study complied with the Declaration of Helsinki and were approved by the Ethics Committee from the Human Kinetics University at Lisbon-Portugal.

Table 4.1 Descriptive characteristics of the participants.

Variable	**Participants (n = 16)**
Age (yr)	22.3 ± 4.3
Body mass (kg)	65.6 ± 10.3
Height (cm)	171.4 ± 6.7
BMI (kg/m^2)	22.3 ± 2.5
VO_{2max} ($mL.min^{-1}$)	2892.1 ± 695.8
VO_{2max} ($mL.kg^{-1}.min^{-1}$)	43.9 ± 8.6
HR_{max} (bpm)	191.7 ± 10.1

Values are mean ± SD.

Abbreviations: BMI, body mass index; VO_{2max}, maximum oxygen uptake; HR_{max}, maximum heart rate.

4.1.4.2. Measurements

All subjects were tested in a postprandial state, approximately 2-4 hours after their last meal. Participants refrained from exercise 24 hours before testing and caffeine ingestion on testing days. The days of testing consisted of: (1) a standardized body composition assessment, (2) a resting protocol, (3) a continuous submaximal steady-state exercise protocol, and (4) a maximal graded exercise protocol. Testing was carried out in the laboratory with an environmental temperature between 21-24ºC and a relative humidity between 44-56%. In an attempt to control for possible circadian variations in HRV, the measurements were performed between 07.00 and 11.00 h at approximately the same time of day for all individuals.

Body mass was measured at both visits using a calibrated digital scale, and height was measured using a stadiometer (Secca 770, Hamburg, Germany - standing digital scale/height rod attached). Body mass index (BMI) was calculated by dividing the participants' mass in kilograms by the square of their height in meters. Expired gas measurements were made using a computerized on-line breath-by-breath system (Quark b^2, Cosmed® Srl-Italy), which was calibrated before each test with a known volume and with known gas concentrations. HRV data were obtained by means of a Polar RS 800 G3 heart rate monitor (Polar R-R Recorder, Polar Electro, Kempele, Finland).

4.1.4.3. Testing protocols

Each test was conducted after a 15-min resting period. As previously reported by Perini et al. (2000), under free breathing conditions, there are no substantial differences between the power spectra obtained with the subjects in the supine and sitting position. In conformity and to allow comparisons with previous studies (Arai et al. 1989; Kaikkonen et al. 2007; Leicht et al 2008; Martinmäki and Rusko 2008; Perini et al. 1990; Rimoldi et al. 1992; Tulppo et al. 2001; Tulppo et al. 1998), resting HRV and VO_2 were obtained during the last 3 min of the resting period in sitting position. Subsequently, participants' HRV and VO_2 data were collected while exercising on a motorised treadmill (h/p/cosmos® mercury med 4.0). The protocol involved continuous walking at a constant speed of 4 $km.h^{-1}$ at two different treadmill grades (0 and 7.5%), for 5 min each. According to Sandercock and Brodie, there is some evidence to suggest that the expected augmentation and

reduction of normalized LF and HF powers only occurs at low-to-moderate relative intensities (Sandercock and Brodie 2006). Furthermore, it is also known that 50% of tachograms recorded at intensities above the anaerobic threshold can not be analysed due to low signal-to-noise ratio (Gregoire et al. 1996). Therefore, in the present study, submaximal treadmill workloads were selected on the basis of previous research conducted in our laboratory with a group of similar characteristics (Mendonca et al. 2008). As reported, both these absolute treadmill workloads are compatible with reliable cardiopulmonary data collection within a seven day period test-retest experimental design; and effective in eliciting different VO_2 fractional utilization (FU) while remaining below 60% of the VO_{2max}.

HRV and on-line VO_2 uptake measurements were obtained during the submaximal protocol. The VO_2 data were displayed as 30-s averages. The last 3 min of each 5-min walk were used for subsequent HRV and cardiorespiratory data analysis (Whipp 1971). Additionally, the submaximal relative work intensities were determined as percentages of VO_{2max} (fractional utilization).

4.1.4.4. Maximal protocol

VO_{2max} was determined by means of a continuous incremental test to volitional exhaustion commencing immediately after the second submaximal walk. For this purpose, treadmill grade was increased from 7.5 to 10% while maintaining a speed of 4 $km.h^{-1}$ for three minutes and then to 12.5% for a further minute. From this point, grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every minute until exhaustion. The test was terminated when the subject reached exhaustion and grasped the hand rails of the treadmill. The VO_2 data were displayed in 20-s averages. Data were then examined to determine if VO_{2max} had been attained according to the following criteria (McArdle et al. 2001): 1) attainment of the age-predicted maximum heart rate, 2) respiratory exchange ratio $\geq$ 1.15, and 3) a plateau or decrease in VO_2. If one of the first two and the 3rd criteria were not achieved, the subject was required to repeat both the submaximal and maximal protocols after a recovery period ranging from 2-7 days. Only one participant required a second test. Recovery from maximal exercise consisted of a 5-min treadmill horizontal walk at 4 $km.h^{-1}$ with the last 2 min being used for HRV and cardiorespiratory data analysis.

4.1.4.5. Measurement and analysis of HRV

The R-R intervals were recorded (Polar R-R Recorder, Polar Electro, Kempele, Finland) at a frequency of 1000 Hz, providing an accuracy of 1 ms for each R-R interval. Recorded R-R intervals were first transferred to the Polar Precision Performance Software (Kempele, Finland) and visually inspected for undesirable premature beats and noise. An R-R interval was interpreted as premature if it deviated from the previous quantified interval by >30%. No premature beats were observed in the complete set of R-R intervals obtained from each individual; therefore, there was no need for interpolation due to ectopy. The R-R intervals of the last 3 min at rest and each submaximal exercise stage were then chosen for analysis. The same procedure was used for the last 2 min of post-exercise recovery period. AR and FFT calculations were then performed with HRV Analysis Software 1.1 for Windows (The Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) (Niskanen et al. 2004).

4.1.4.6. Frequency Domain

Prior to HRV analysis in the frequency domain, R-R data were detrended (Tarvainen et al. 2001), and resampled at 2 Hz. The FFT spectrum was then calculated using a Welch periodogram method. In this method, the R-R data were first divided into overlapping segments of 128 R-R intervals. Each segment was then windowed using a Hanning window (Kesselbrener and Akselrod 1996), and FFT spectrum was calculated for each windowed segment with subsequent spectra averaging. The AR spectrum was calculated fitting a 16^{th}-order model (Boardman et al. 2002) to the R-R data. The AR model parameters were solved using a forward-backward least squares method, and finally, the AR spectrum was obtained from the estimated AR parameters. The frequency-domain variables included the total power (TP) spectrum (0 to 1.0 Hz) and the power spectra integrated over the very low frequency (VLF, 0 to 0.04 Hz), low frequency (LF, 0.04 to 0.15 Hz), and high frequency (HF, 0.15 to 1.0 Hz) bands. As in previous studies, the higher frequency limit of 1.0 Hz was chosen to include the respiratory frequency during exercise and post-exercise recovery (Kaikkonen et al. 2007; Martinmäki and Rusko 2008).

It is widely accepted that the HF power reflects vagal modulation of heart rate and that both the LF power and the LF/HF ratio reflect a complex interplay between sympathetic and parasympathetic modulation. The physiological meaning of the VLF power assessed from short-term recordings is less defined and its interpretation is not recommended when analysing power spectra density results (Task Force 1996). Data were expressed as raw and normalized values. The LF/HF ratio (which is independent of normalization) was then calculated. Finally, for the AR modelling, the centered frequency value of each component was also calculated.

4.1.4.7. Statistical analysis

Standard descriptive statistics were used to summarize the data. Resting, submaximal, maximal and post-exercise recovery cardiorespiratory data were studied using repeated measures analysis of variance to test for treadmill workload effects. Raw and normalized powers obtained with FFT and AR approaches were compared with the paired Wilcoxon test to determine possible differences between them. Furthermore, the Bonferroni correction for multiple comparisons was used to describe the overall changes in spectral powers, obtained by both methods, following the transition from rest to exercise and post-exercise recovery. We also examined the Pearson correlation coefficients between delta heart rate and delta normalized LF and HF powers in response to the selected workloads (Δ1 WL, from rest to submaximal exercise 1; Δ2 WL, from submaximal exercise 1 to submaximal exercise 2). All statistical calculations were computed using SPSS version 16.0 and a significance level of 0.05 was used.

4.1.5. Results

4.1.5.1. Morphological and cardiopulmonary data

As shown in table 4.2, the increase in treadmill grade from 0% to 7.5%, while maintaining a constant speed of 4 $km.h^{-1}$, was effective in eliciting a significant increase in VO_2 from 10.1 ± 1.2 to 17.2 ± 1.3 $mL.kg^{-1}.min^{-1}$ ($p < 0.05$). Since the participants attained a mean VO_{2max} of 43.9 ± 8.6 $mL.kg^{-1}.min^{-1}$, these treadmill grades corresponded to 23.5 ± 4.0% (~ 20%) and 40.2 ± 7.0% (~ 40%) of VO_{2max}, respectively ($p < 0.0001$). While the respiratory rate (RR) increased in a continuous fashion

from rest to exercise and post-exercise recovery ($p < 0.05$), tidal volume (TV) was not different between treadmill workloads.

Table 4.2 Respiratory variables at submaximal walking grades.

Variable	Rest	0%	7.5%	Recovery
VO_2 ($mL.kg^{-1}.min^{-1}$)	3.5 ± 0.7	10.1 ± 1.2*	17.2 ± 1.3 #	12.2 ± 2.8 †
TV (L)	0.6 ± 0.2	1.1 ± 0.6 *	1.4 ± 0.4 *	1.6 ± 0.5 #
RR (cpm)	15.0 ± 2.8	19.0 ± 5.4 *	22.4 ± 6.3 #	27.9 ± 4.6 †

Values are mean ± SD.

Abbreviations: VO_2, oxygen uptake; TV, tidal volume; RR, respiratory rate. * $p < 0.05$ with respect to rest; # $p < 0.05$ with respect to rest and 0%; † $p < 0.05$ with respect to rest, 0% and 7.5%.

Figure 4.1 shows the between-day heart rate dynamics from rest to exercise at different intensities, followed by the 5-min active recovery period. Heart rate increased significantly as a function of treadmill workload from resting conditions to submaximal and maximal exercise ($p < 0.0001$). A significant decrease in the successive heart rate values was only observed for the first 3 min of active recovery after maximal exercise. Thereafter, during the 4^{th} and 5^{th} min of active recovery, heart rate stabilized ($p > 0.05$).

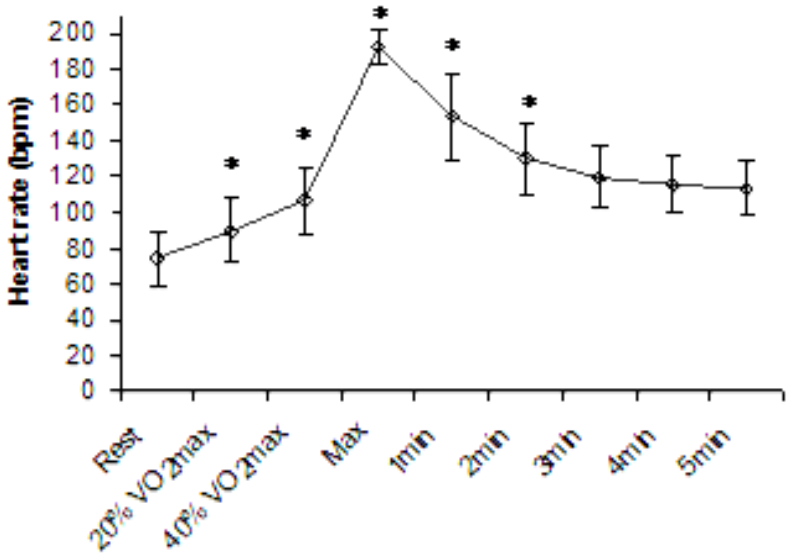

Fig. 4.1 Heart rate response in transition from resting to exercise and active recovery at a constant speed of 4 $km.h^{-1}$. Values are mean ± standard deviation. *p < 0.05 for comparisons of heart rate between adjacent data points.

4.1.5.2. Power spectra of Autoregressive versus Fast Fourier Transform

Mean values of comparisons between AR and FFT over visits are shown in table 4.3. At rest, both TP and HF raw power derived from the FFT calculations were significantly higher than those provided by the AR method ($p < 0.05$). No between-method differences were obtained for LF raw power ($p > 0.05$). At both relative intensities, FFT TP was also higher than that of AR. Additionally, when compared to AR, FFT provided higher HF raw power at ~ 20 (284.3 ± 154.6 vs 156.9 ± 99.5 ms^2, $p < 0.05$) and ~ 40% VO_{2max} (109.1 ± 66.1 vs 51.5 ± 33.7 ms^2, $p < 0.05$). Similarly, normalized HF power derived from FFT was higher than that of AR at ~ 20 (29.0 ± 3.8 vs 15.5 ± 2.2 nu, $p < 0.05$) and ~ 40% VO_{2max} (31.6 ± 4.5 vs 17.4 ± 3.5 nu, $p < 0.05$). On the contrary, LF normalized power and the LF/HF ratio derived from AR were higher than those obtained with FFT during exercise ($p < 0.05$). Finally, while LF powers did not differ between approaches during recovery, FFT derived raw HF power was higher than that obtained by the AR approach (12.8 ± 4.1 vs 7.0 ± 2.7 ms^2, $p < 0.05$), with the opposite being reported for the LF/HF ratio.

Table 4.3 Power spectra of heart rate variability derived from autoregressive and fast Fourier transform methods.

Variable	AR	FFT
	Rest	
TP (ms^2)	2731.9 ± 1186.6	3320.8 ± 1513.1*
VLF (ms^2)	328.7 ± 108.9	744.6 ± 343.4*
LF (ms^2)	1278.9 ± 649.1	1186.3 ± 475.2
HF (ms^2)	1124.2 ± 566.6	1389.9 ± 721.4*
LF (nu)	61.4 ± 5.1	57.1 ± 5.2
HF (nu)	38.1 ± 5.0	42.9 ± 5.2
LF/HF	2.4 ± 0.5	1.9 ± 0.4
	20% VO_{2max}	
TP (ms^2)	823.1 ± 244.8	1000.3 ± 294.1*
VLF (ms^2)	123.1 ± 39.0	203.5 ± 55.1
LF (ms^2)	543.1 ± 155.0	512.6 ± 122.7
HF (ms^2)	156.9 ± 99.5	284.3 ± 154.6*
LF (nu)	84.5 ± 2.2	71.0 ± 3.8*
HF (nu)	15.5 ± 2.2	29.0 ± 3.8*
LF/HF	7.8 ± 1.5	3.6 ± 0.8*
	40% VO_{2max}	
TP (ms^2)	260.1 ± 99.4	332.6 ± 113.2*
VLF (ms^2)	40.2 ± 22.2	54.6 ± 13.2*
LF (ms^2)	168.4 ± 47.9	168.8 ± 43.8
HF (ms^2)	51.5 ± 33.7	109.1 ± 66.1*
LF (nu)	82.6 ± 3.5	68.4 ± 4.5*
HF (nu)	17.4 ± 3.5	31.6 ± 4.5*
LF/HF	8.6 ± 1.5	3.2 ± 0.6*
	Recovery	
TP (ms^2)	46.9 ± 17.9	48.5 ± 14.2
VLF (ms^2)	12.1 ± 4.2	16.5 ± 4.8
LF (ms^2)	25.7 ± 15.7	19.1 ± 7.1
HF (ms^2)	7.0 ± 2.7	12.8 ± 4.1*
LF (nu)	61.5 ± 7.6	56.8 ± 5.2
HF (nu)	32.9 ± 7.2	43.2 ± 5.2
LF/HF	6.9 ± 2.8	2.7 ± 1.2*

Values are mean ± SEM.

Abbreviations: LF, low frequency; HF, high frequency; TP, total power; VLF, very low frequency. *$p < 0.05$.

4.1.5.3. Comparisons of power spectra between rest, exercise and recovery

As shown in figure 4.2, the comparisons of TP and raw LF power between rest, exercise and recovery provided homogenous results with both approaches. Specifically, TP and raw LF power decreased in a continuous fashion from rest to exercise and recovery. Raw HF powers did decrease with both methods, but significant differences between recovery and exercise at ~ 40% VO_{2max} were only attained with the FFT ($p < 0.05$). While HF power centered frequency increased progressively from rest (0.25 Hz ± 0.01) to ~ 20% VO_{2max} (0.31 Hz ± 0.02), ~ 40% VO_{2max} (0.36 Hz ± 0.03) and recovery (0.48 Hz ± 0.04), LF centered frequency was similar over conditions (~ 0.1 Hz, $p > 0.05$).

Despite showing a tendency, while the AR calculated normalized LF power and LF/HF ratio both increased significantly from rest to exercise ($p < 0.05$), FFT did not reach significance. Findings for normalized HF power followed those of normalized LF powers, but in the opposite direction. Thus, whereas AR attained a significant decrease for normalized HF power in transition from rest to exercise, FFT only presented such tendency. Nevertheless, both methods were equally insensitive to the effects of an increase in exercise relative intensity. Moreover, the above mentioned differences between approaches for raw HF power during recovery were dissipated after normalization procedures. Finally, the increase in heart rate from rest to exercise at ~ 20% VO_{2max} (17.9 ± 4.9 bpm, $p < 0.05$) was significantly correlated with changes obtained in normalized powers with the AR (LFnu, $r = 0.56$, $p < 0.05$; HFnu, $r = -0.55$, $p < 0.05$) and FFT (LFnu, $r = 0.54$, $p < 0.05$; HFnu, $r = -0.54$, $p < 0.05$).

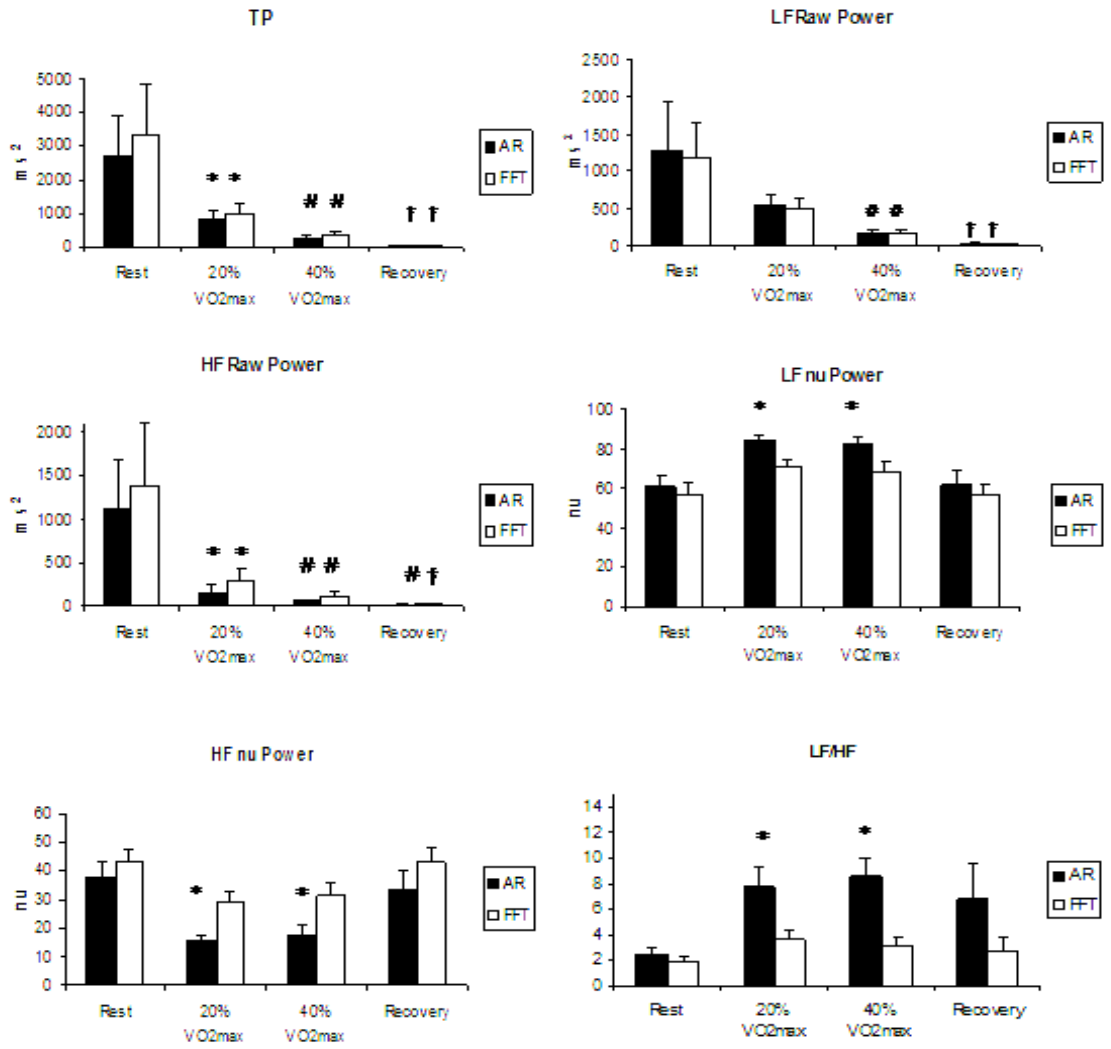

Fig. 4.2 Absolute and normalized spectral powers at rest, exercise and active recovery. In each panel, vertical bars and vertical lines indicate means and SEM of the power considered. Spectral parameters are given independently for autoregressive (AR) and fast Fourier transform (FFT) methods. TP, total power; HF, high frequency; LF, low frequency. * $p < 0.05$ with respect to rest; # $p < 0.05$ with respect to rest and 20% VO_{2max};† $p < 0.05$ with respect to rest, 20% VO_{2max} and 40% VO_{2max}.

4.1.6. Discussion

To our knowledge this is the first study in which FFT and AR methods were applied to the same population over a spectrum that included resting, exercise and post-exercise recovery conditions. The major findings can be summarized as follows:

1. Significant differences between the AR and FFT methods suggest the data generated from these methods are not inter-changeable.
2. Despite the existence of a general common trend between spectral approaches for changes in HRV from rest to exercise and active recovery, the AR method is more sensitive than FFT to the effects of dynamic exercise on the normalized power spectra of HRV.
3. Both approaches are equally insensitive to the increase of exercise relative intensity, thus suggesting that there is no practical advantage of performing HRV spectral analyses by the AR or FFT at higher workloads.

The analysis of heart rate variability provides a useful method for studying autonomic function of the cardiovascular system during different physiological and pathophysiological conditions. The development of increasingly sophisticated means of analysing HRV has led to better identification of the role played by the autonomic nervous system under different conditions. Many studies have examined the influence of acute exercise upon raw HF and LF powers of HRV spectrum and there is general agreement that, independently of the HRV spectral approach, they both decrease as a function of exercise intensity (Arai et al. 1989; Breuer et al. 1993; Casadei et al. 1995; Dixon et al. 1992; Furlan et al. 1990; Leicht et al. 2008; Martinmäki and Rusko 2008; Perini et al. 1990; Pichon et al. 2004; Rimoldi et al. 1992; Tulppo et al. 1998). On the other hand, the patterns of change reported for the normalized LF and HF powers or the LF/HF ratio, have been more inconsistent. Inconsistency in findings probably reflect, at least in part, differences in methodology (Casadei et al. 1995), including differences in exercise intensity and duration and completeness of the steady state, the number of heart beats analysed and, additionally, the selected spectral approach for HRV data analysis (Leicht et al. 2008; Martnimäki and Rusko 2008; Perini et al. 1998; Rimoldi et al. 1992; Tulppo et al. 2001; Winley et al. 2003). Although many spectral methods have been applied, the most popular two are the AR and

FFT-based approaches. As shown in table 4.3, these two methods give numerically different results at rest, during exercise and at post-exercise recovery and this agrees with previous findings for supine and sitting rest (Badilini et al. 1998; Pichon et al. 2004), passive tilt test (Badilini et al. 1998), active orthostatism (Badilini et al. 1998; Pichon et al. 2004), specific circadian periods and pharmacological autonomic modulation (Badilini et al. 2000). Consequently, the present study corroborates that FFT and AR analyses are not interchangeable at rest and further extend these findings to exercise and active recovery conditions.

According to our results, FFT provided higher total power than AR at rest and during exercise at both relative intensities. These findings are similar to those of Pichon et al. who also reported more total power with FFT than with AR in sitting, but not in standing position (Pichon et al. 2004). Differences between methods could be due to the Hanning windowing preprocessing before the FFT, which influences, indeed, the overall variability of the signal (Pichon et al. 2004). Other authors reported different results from the ones of the present study and those of Pichon et al. (2004) at rest in sitting position (Badilini et al. 2000; Badilini et al. 1998; Chemla et al. 2005). Specifically, Badilini et al. (1998) found no differences between FFT and AR for total power obtained from a group of healthy young participants performing supine rest and passive tilt test under metronomic breathing (0.25 Hz). On a different experimental design which included 24-h HRV assessments performed on patients with mild hypertension, Badilini et al. concluded that total power obtained by AR was significantly higher than that of FFT, irrespectively of the circadian period of interest (Badilini et al. 2000). The authors interpreted these differences as being related to the uncontrolled nature of the experiment in which, a permanently changing environment might had contributed for strong heart rate trends (physical activity, sleep states). In line with Badilini et al. (2000), Chemla et al. (2005) also reported higher total power with AR than FFT during a 10-min resting period in diabetic patients. Unfortunately, adding to the fact that Chemla et al. (2005) included a population subset with high prevalence of autonomic dysfunction (Ewing et al. 1978), authors did not specify the nature of the selected resting period (i.e. supine vs sitting or free vs controlled breathing), thus limiting further comparisons. Interestingly, irrespectively of discrepancies between studies for total power derived from both approaches under

different physiological conditions, there is general agreement that raw HF power is higher with FFT than with AR (Badilini et al. 2000; Badilini et al. 1998; Fagard et al. 1998; Pichon et al. 2004). Therefore, FFT derives more raw HF power than AR during exercise at different relative intensities and this is in agreement to that obtained from short-term HRV recordings performed at rest in supine, sitting and standing position (Badilini et al. 1998; Fagard et al. 1998; Pichon et al. 2004) and from long-term HRV recordings performed over day and night with or without pharmacological sympathetic blockade (Badilini et al. 2000). Furthermore, similarly to resting conditions, short-term HRV recordings during exercise, particularly at ~ 40% VO_{2max}, result in higher VLF spectral power with FFT compared to AR and this further contributes to the increased total power reported by the former. As the VLF assessed from short-term recordings is a dubious measure (Task Force 1996) that apparently adds significance to an increase in total power from FFT over AR, these differences between methods may not be physiologically relevant.

Generally, whatever the analysis, the decrease in total power and raw HF power in transition from rest to exercise at ~ 20% VO_{2max}, ~ 40% VO_{2max} and active recovery were significant. Furthermore, both methods confirmed that exercise raw LF power was not significantly reduced from resting conditions during exercise at ~ 20% VO_{2max}. On the other hand, only the AR calculations provided significant increases for normalized LF power and significant decreases for normalized HF power during exercise over resting conditions. Similarly, opposing to FFT, AR derived LF/HF ratio also increased from rest to exercise. There was no further change in normalized LF and HF powers or LF/HF ratio with increasing exercise relative intensity and this was transversal to both methods. As it is usually reported by the use AR analysis during dynamic exercise, both the HRV normalized spectral components and the LF/HF ratio present a biphasic pattern of behaviour in response to increases in relative intensity (Sandercock and Brodie 2006). In general, while these parameters change in the expected directions at low exercise intensities, the opposite has been reported for higher relative intensities. This appears to be related the non-neural genesis of some HF oscillations at higher exercise intensities that further constitute a confounding factor, presently quantified at ~ 32% of this component (Casadei et al. 1996). In the present study, as there were no additional modifications of

normalized power spectrum or LF/HF ratio despite the significant increase in relative intensity, it seems reasonable to assume that HRV spectral analysis should be restricted to lower exercise intensities for purposes of meaningful physiologic interpretations. In support of this, we only obtained significant correlations between delta heart rate and delta normalized HF and LF powers from rest to exercise at ~ 20% VO_{2max}. Curiously, HF centered frequency increased linearly with metabolic demand, reflecting the increase in respiratory rate. This corroborates previous findings (Bernardi et al. 1990; Casadei et al. 1996; Perini et al. 1998) and is probably related to a direct mechanical effect of enhanced respiratory activity on the heart, occurring not only during low and moderate relative intensities, but also in recovery from maximal exercise. Overall, these results indicate that, both raw and normalized AR powers are sensitive to a reduction in vagal modulation induced by exercise (Arai et al. 1989; Perini et al. 2000; Perini et al. 1990; Rimoldi et al. 1992; Tulppo et al. 2001; Tulppo et al. 1998). Additionally, the increase reported by normalized LF power and the LF/HF ratio suggest sympathetic activation with a well defined shift towards sympathetic dominance during low and moderate intensity dynamic exercise.

The vast majority of studies on autonomic function during exercise have been conducted using cycle ergometry, but findings are contradictory (Perini et al. 2000; Perini et al. 1990; Tulppo et al. 1998; Winsley et al. 2003; Yamamoto et al. 1991). Some report that the AR normalized LF component decreases linearly and the normalized HF power shows a tendency to increase above resting values at exercise intensities beyond 30% VO_{2max} (Perini et al. 2000; Perini et al. 1990). On the contrary, for approximately the same exercise intensities, others have demonstrated an increase in the AR normalized LF component above resting values that was accompanied by a decrease in the normalized HF power (Tulppo et al. 1998; Winsley et al. 2003; Yamamoto et al. 1991). Findings from treadmill exercise have been more consistent. In general, LF/HF ratio increases with treadmill exercise and HF decreases (Rimoldi et al. 1992; Tulppo et al. 2001). Our results agree with those of previous studies conducted on the assessments of spectral HRV during treadmill exercise at comparable workloads by the AR approach (Rimoldi et al. 1992; Tulppo et al. 2001). Moreover, present findings also corroborate those of two recent reports using FFT approach for HRV spectral analysis during low to

moderate intensity dynamic exercise (Leicht et al. 2008; Martinmäki and Rusko 2008). Specifically, the authors reported non significant changes in FFT normalized LF and HF powers in response to steady state exercise on the cycle ergometer at ~ 29% VO_{2max} (Martinmäki and Rusko 2008) and at ~ 25 and ~ 40% VO_{2max} on the treadmill (Leicht et al. 2008) ($p > 0.05$). Nevertheless, in accordance with our results, there was a clear tendency towards the increase of normalized LF power paired by a decrease in normalized HF power at the selected relative intensities.

We interpret these findings as related to an overestimation of the HF components with FFT analysis. Of several explanations that could be proposed to explain this overestimation, a relevant one is related to the known wide-band noise that is isolated and suppressed with AR, but constitutes a part of total power spectrum with FFT analysis (Fagard et al. 1998; Malliani et al. 1991). A second explanation for the differences between-methods might be a consequence of tail effect (Badilini et al. 1998). In fact, with FFT, the calculation of a component between one frequency band includes the power corresponding to the tail of the neighbouring component, whereas with AR analysis, spectral power corresponds only to a specific oscillatory pattern representing one HRV single component. Finally, in line with the available literature, the systematic overestimation of the HF by the FFT component in our study could be explained by the possibility that the AR extracts only the power corresponding to respiratory sinus arrhythmia and not the associated noise contained within a predefined frequency range (Fagard et al. 1998; Pichon et al. 2004).

In conclusion, AR and FFT spectral analyses provide different quantitative results at rest, during exercise and at post-exercise recovery period. Specifically, AR methodology is apparently more sensitive to the effects of dynamic exercise on autonomic function when quantified by changes in HRV normalized power. Differences between the two methodologies in normalized units may depend on the intrinsic effects of dynamic exercise itself and may be possibly related to the mode of spectrum integration specific of each approach. Therefore, normalization procedures that do not account for VLF powers are capable of showing significant increases of LF and decreases of HF in transition from rest to whole-body dynamic exercise with AR, but not with FFT. Finally, as both approaches are

equally insensitive to the increase of exercise relative intensity, there is no practical advantage of performing HRV spectral analyses by the AR or FFT at higher workloads.

4.1.7. References

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4.2. Cardiac autonomic function during submaximal treadmill exercise in adults with Down syndrome

4.2.1. Abstract

This study determined whether the cardiac autonomic function of adults with Down syndrome (DS) differs from that of nondisabled persons during submaximal dynamic exercise. Thirteen participants with DS and 12 nondisabled individuals performed maximal and submaximal treadmill tests with metabolic and heart rate (HR) measurements. Spectral analysis of HR variability was performed on the last 256 consecutive R-R intervals obtained under the following conditions: (1) rest, (2) submaximal treadmill exercise (at constant relative intensity below the ventilatory threshold) and (3) recovery. Participants with DS presented lower chronotropic response than those without DS to peak and submaximal exercise ($p < 0.05$). Nevertheless, the fractional utilization of peak HR during treadmill walking was similar between groups (~60% peak HR). Even though there were no between-group differences at rest or during recovery, the participants with DS showed a higher LF/HF ratio during exercise ($p < 0.05$). Similarly, the LF power of participants with DS during exercise was greater than that of nondisabled participants ($p < 0.05$). In contrast, both groups exhibited similar HF power at each physiological condition. In conclusion, these findings suggest that adults with DS demonstrate appropriate vagal withdrawal, but heightened sympathetic modulation of HR variability at ~ 60% of their peak HR. Despite this, the absolute change in HR from rest to exercise was attenuated in these individuals compared to persons without disabilities. This indicates that DS may be associated with poor cardiac responsiveness to changes in autonomic modulation during exercise at intensities below the ventilatory threshold.

4.2.2. Key Words

Heart rate variability; cardiac autonomic function; exercise; Down syndrome

4.2.3 Introduction

Individuals with Down syndrome (DS), without concomitant congenital heart disease, exhibit reduced heart rate (HR) responses to exercise, or chronotropic incompetence (Guerra et al. 2003). Chronotropic incompetence is manifested by an inability to achieve 85% of the age-predicted maximal HR and delayed achievement of maximal HR (Gentlesk et al. 2004). Reduced HR response to aerobic exercise has been identified as the primary contributor to the low physical work capacity and cardiorespiratory fitness in this population (Fernhall et al, 1996; Fernhall et al, 2001; Fernhall and Pitetti 2001). Chronotropic incompetence is also clinically relevant and it has been related to early mortality and morbidity both in patients with and without established coronary heart disease (Lauer et al. 1996; Lauer et al, 1999). Nonetheless, it is unknown whether chronotropic incompetence is equally predictive of early mortality and morbidity in adults with DS.

There is compelling evidence that the chronotropic incompetence experienced by persons with DS, in response to classical adrenergic stressors, may be associated with alterations in cardiac autonomic control (Fernhall and Otterstetter 2003; Fernhall et al. 2005; Figueroa et al. 2005; Iellamo et al. 2005). Fernhall and Otterstetter (2003) first reported reduced blood pressure and HR responses to cold pressure testing and static handgrip in healthy adults with DS. Subsequently, Figueroa et al. (2005) showed that the attenuated hemodynamic responses to handgrip exercise, in these individuals, results from blunted vagal withdrawal. Hypotension and attenuated HR response to other adrenergic perturbations (i.e., orthostatic challenge and passive head-up tilt) have also been noted in individuals with DS (Fernhall et al. 2005). Accordingly, Iellamo et al. (2005) further described that people with DS exhibit reduced HR response to orthostatic stress, and that this is associated with blunted sympathetic activation and vagal withdrawal and with lesser reductions in baroreflex sensitivity (BRS) during active orthostatism. Hence, these atypical responses to common adrenergic stressors support the hypothesis of autonomic dysfunction in persons with DS.

Even though individuals with DS demonstrate marked attenuation of HR during peak dynamic exercise compared with persons without disabilities (Fernhall et al. 1996; Guerra et al. 2003), less is known about their cardiac autonomic modulation during submaximal dynamic exercise at intensities

below the ventilatory threshold (VT). During higher exercise intensities (above the VT), nondisabled individuals increase their HR by concomitant vagal withdrawal and sympathetic activation (Orizio et al. 1998). Recently, Fernhall et al. (2009) demonstrated that, in comparison with adults without disabilities, those with DS show reduced catecholamine responsiveness to peak dynamic exercise. In conformity, individuals with DS present reduced sympatho-adrenergic drive during peak dynamic exercise and this is, apparently, responsible for their chronotropic incompetence and low levels of cardiorespiratory fitness (Fernhall et al. 2009). In contrast to that seen at peak exercise intensities, the positive chronotropic response of nondisabled individuals to submaximal dynamic exercise (below the VT) depends primarily on vagal withdrawal (Orizio et al. 1998). To date, the only study that examined cardiac autonomic modulation in adults with DS observed similar vagal activity during submaximal dynamic exercise between adolescents with intellectual disabilities (ID) with and without DS (Baynard et al. 2004). Unfortunately, since the authors did not include a group of nondisabled individuals nor the participants exercised at the same submaximal intensity, these results are difficult to interpret. Considering the clinical significance of reduced work capacity, chronotropic incompetence and autonomic dysfunction, it is important to determine whether the cardiac autonomic response of adults with DS differs from that of nondisabled participants in response to exercise, at a given submaximal intensity, performed below the VT. Therefore, the main purpose of this study was to evaluate the cardiac autonomic response, by means of heart rate variability (HRV), in response to submaximal exercise intensity (below the VT) in individuals with DS and compare their responses with nondisabled participants of similar age and body mass index (BMI). We hypothesized that the changes in HR from rest to submaximal exercise would be attenuated in participants with DS due to blunted vagal withdrawal.

4.2.4. Methods

4.2.4.1. Participants

A total of 25 healthy participants (13 with DS [9 males; 4 females], 12 controls without disabilities [8 males; 4 females]), aged 27 to 48 years, were included in the present study. Those with DS had mild-to-moderate ID, as reported by their parents or direct caregivers. Descriptive statistics are

presented in table 4.4. All participants with DS had medical approval for physical activity participation from their personal physicians. Active smokers and those with congenital heart disease, ambulatory, musculoskeletal, visual, or auditory problems were not included in the study. All participants, and in addition, parents and/or guardians of the participants with DS, signed informed consent to participate in the study. Participants in both groups were either sedentary or lightly active (light intensity walking bouts of at least 30 min 1-2 days/week), but none were involved in any extensive exercise endurance or resistance training for at least six months.

The participants without disabilities were selected based on the following: (1) healthy medical status, (2) non-smoking condition, (3) gender match with the participants with DS, (4) age match with the participants with DS, (5) absence of involvement in any formal exercise endurance or resistance training for at least six months, and (6) agreement with the testing procedures confirmed by signature of the informed written consent. None of the participants included in this study took any medication. This study was approved by the University's internal review board.

The participants with DS were recruited from a vocational center for individuals with ID. They all lived at home and were bussed to the center daily. Most of the vocational activities at this center involved light physical work for 5 to 6 hours, 5 days/week (gardening, carpentry, cooking, plumbing, ironing, tailoring and hand sewing, typing on a computer, house cleaning, bakery and printing). Participants without disabilities were recruited from the local and university communities.

Table 4.4 Characteristics of participants with Down syndrome (DS) and of nondisabled controls.

Variables	DS (n=13)	Controls (n=12)
Physical characteristics		
Age (years)	34.9 ± 1.1	34.8 ± 2.0
Height (cm)	153.8 ± 2.2*	172.0 ± 1.9
Body mass (kg)	67.8 ± 2.6	76.4 ± 4.6
BMI (kg/m^2)	28.6 ± 1.0	25.7 ± 1.2
Physiological characteristics		
HR_{peak} (bpm)	171.5 ± 4.9*	187.3 ± 3.1
VO_{2peak} ($mL.kg^{-1}.min^{-1}$)	30.4 ± 2.1*	46.7 ± 2.7
RER_{peak}	1.13 ± 0.02	1.17 ± 0.01
Ve_{peak} ($L.min^{-1}$)	64.9 ± 5.7*	113.5 ± 5.8
VO_{2VT} ($mL.kg^{-1}.min^{-1}$)	20.2 ± 1.3*	28.4 ± 1.2

Values are mean ± SEM.

Abbreviations: BMI, body mass index; HR_{peak}, peak heart rate; VO_{2peak}, peak oxygen uptake; RER_{peak}, peak respiratory exchange ratio; Ve_{peak}, peak minute ventilation; VO_{2VT}, oxygen uptake at ventilatory threshold. * $p < 0.05$ between participants with and without DS.

4.2.4.2. Familiarization

Before data collection, each participant was familiarized with the laboratory setting, treadmill protocols, and use of the headgear and face mask. As in previous studies (Baynard et al. 2004), familiarization sessions were continued until the participant could comfortably walk on the treadmill with the headgear and mouthpiece. All participants were adequately familiarized within 1 or 2 sessions.

4.2.4.3. Study design

After the familiarization period, participants were evaluated over the course of two visits on separate days. All participants abstained from caffeine and vigorous exercise for 24-hours prior to testing and were at least 3 hrs post-prandial upon arrival for testing. During the first visit, standing height and weight measurements were taken with participants wearing light-weight clothing and no shoes. Height was obtained using a stadiometer with measures obtained to the nearest 0.5 cm. Weight was measured on a balance-beam scale. BMI was calculated by dividing the participants' mass in kilograms by the square of their height in meters. Subsequently, participants performed a treadmill

graded exercise test (GXT) to determine their submaximal oxygen uptake (VO_2) and VO_{2peak}. This test was used for two specific purposes: (1) to confirm chronotropic incompetence in participants with DS and to explore whether those without disabilities had a normal HR response to exercise; (2) to determine the treadmill workload required to elicit a given pre-selected fractional utilization (FU) of the VO_{2peak} in each participant. On the second visit, each participant performed one walking bout at 45% VO_{2peak}. Expired gas measurements were made using a computerized on-line breath-by-breath system (Quark b^2, Cosmed® Srl-Italy), which was calibrated before each test with a known volume and with known gas concentrations. R-R interval data were obtained by means of a Polar RS 800 G3 HR monitor (Polar R-R Recorder, Polar Electro, Kempele, Finland). Testing was carried out in the laboratory with an environmental temperature between 21-24 ºC and a relative humidity between 44-56%. In an attempt to control for possible circadian variations in HRV, the measurements were performed between 7.00 and 11.00 h at approximately the same time of day for all individuals. Visits were a minimum of 2 days apart and a maximum of 7 days apart.

4.2.4.4. Graded exercise testing

As in Baynard et al. (2004), participants rested quietly in a seated position for 5 min. R-R interval and VO_2 data were collected for an additional 5 min of seated rest. Testing began with a submaximal horizontal walk on a motorized treadmill at a constant speed of 4 $km.h^{-1}$ (h/p/cosmos® mercury med 4.0). Grade was increased 2.5% every 5 min until a 7.5% grade was reached. Grade was then increased every 2 min by 2.5% until a 12.5% grade was reached. From this point, grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every min until exhaustion. This protocol has been shown to be a valid and reliable measure of maximal cardiorespiratory fitness in both individuals with and without DS (Fernhall et al. 1990). The VO_2 data were displayed in 20-s averages. A valid VO_{2peak} was defined as the highest value obtained during the last stage of exercise with a respiratory exchange ratio (RER) over 1.0 (Fernhall and Otterstetter 2003; Fernhall and Pitetti 2001). To indentify the VT, breath-by-breath VO_2 and VCO_2 were smoothed using a three-breath bin average to reduce breath-by-breath fluctuations while at the same time retaining the underlying response to progressive

increases in exercise intensity. The VT was indentified independently by two experienced investigators using the V-slope method (Beaver et al. 1986).

4.2.4.3. Constant load submaximal exercise testing

Each participant performed treadmill walking with metabolic and R-R interval measurements, at 45% VO_{2peak}. Testing began with a 5-min standing period (baseline measurements) and terminated with passive recovery in the standing position, for an additional 5 min. To ensure that all participants exercised at the same FU of the VO_{2peak} (same relative intensity), treadmill workloads were individualized. For this purpose, the mean of the last 3 min of each 5-min walk (GXT stages at constant speed of 4 $km.h^{-1}$) was defined as the participants submaximal steady-state VO_2 (Whipp 1971). Equations were computed using least squares linear regression on data derived from each participant's steady state VO_2 (0, 2.5, 5 and 7.5% grades). Then, the treadmill grade required to elicit 45% VO_{2peak} at a 4 $km.h^{-1}$ walking speed was calculated. This relative intensity was chosen because of its compatibility with moderate intensity exercise (i.e. below the VT). It is acknowledged that the increase in HR at exercise intensities below the VT depends primarily on vagal withdrawal (Orizio et al. 1998). In contrast, it has been shown that changes in catecholamines are largely responsible for increases in HR at exercise intensities above the VT (Kjaer 1998).

4.2.4.5. R-R interval signal acquisition

The R-R intervals were recorded at a frequency of 1000 Hz, providing an accuracy of 1 ms for each R-R interval. Recorded R-R intervals were first transferred to the Polar Precision Performance Software (Kempele, Finland) and visually inspected for undesirable premature beats and noise. An R-R interval was interpreted as premature if it deviated from the previous quantified interval by > 30%. No premature beats were observed in the complete set of R-R intervals obtained from each individual; therefore, there was no need for interpolation due to ectopy. HR and power spectral analyses were performed on the last 256 consecutive R-R intervals obtained under the following conditions: (1) standing rest, (2) constant load submaximal treadmill exercise and (3) standing post-exercise recovery. All analyses were carried out using Kubios HRV Analysis Software 2.0 for Windows (The Biomedical

Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). The time series was detrended (Tarvainen et al. 2001) and re-sampled at 4 Hz.

4.2.4.6. Heart rate variability

Power spectral analysis was performed following data detrending. Spectral decomposition of HRV was conducted using a parametric; autoregressive modeling (AR) based spectrum estimates. The AR spectrum was calculated fitting a 16^{th}-order model (Boardman et al. 2002) to the R-R data. The AR model parameters were solved using a forward-backward least squares method, and finally, the spectrum was obtained from the estimated AR parameters. The power was calculated by measuring the area under the peak of the power spectra density curve and corresponding bandwidths interpreted as follows: the high frequency (HF) component or region (0.15 - 1.0 Hz) is considered to be of vagal origin, and, therefore was used in this study as indicative of vagal modulation of the heart (Berntson et al. 1997). The low frequency (LF) component (0.04 - 0.15 Hz) is regarded as both sympathetic and parasympathetic in origin (Berntson et al. 1997). The ratio of LF/HF was then calculated and used as an index of sympathovagal balance (Pagani et al. 1986; Malliani et al. 1994; Montano et al. 1994). All data acquisition and post-acquisition analyses were carried out in accordance with standards put forth by the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology (Task Force 1996).

4.2.4.7. Statistical Analysis

Descriptive statistics were calculated for all variables. Before comparing both groups, data were tested for normality and homocedasticity with the Kolmogorov-Smirnov and Levene's tests, respectively. Subsequently, independent *t* tests were performed to determine group differences among descriptive characteristics (age, height, body mass, BMI, peak cardiopulmonary measures and VT). A 2-way analysis of variance [group (participants with DS vs participants without DS) by condition (rest, submaximal exercise, post-exercise recovery)], with repeated measures, was conducted on all dependent variables to compare HRV differences between participants with DS and those without disabilities. When the interaction was significant, between-group differences at each level were evaluated with independent *t*-tests, whereas the responses over the different physiological conditions

were examined with paired *t*-tests within each group. LF and HF power were transformed to their natural logarithm (ln) for statistical analysis because of their skewed distribution. All data are reported as mean ± SEM. Statistical significance was set at $p < 0.05$. All data analysis was carried out using Statistical Package for the Social Sciences (SPSS, v 16.0, SPSS, Inc., Chicago, IL).

4.2.5. Results

Descriptive and peak exercise data are presented in table 4.4. No between-group differences were presented for age, body mass or BMI; however the participants with DS were significantly shorter than those without disabilities ($p < 0.05$). The participants with DS also had lower peak exercise values for HR, VO_2 and minute ventilation ($p < 0.05$). Conversely, no between-group differences were found for peak RER. Even though both groups showed different VO_2 values at the VT (table 4.4), no differences between them were found after expressing it as a percentage of the VO_{2peak} (DS: 66.7 ± 2.1; non-DS: 61.6 ± 2.0%).

During submaximal exercise, both groups walked at the same relative intensity (DS: 45.3 ± 0.4; non-DS: 45.4 ± 0.4% VO_{2peak}) and at comparable RER values (DS: 0.89 ± 0.01; non-DS: 0.90 ± 0.01). The HR response to exercise at 45% VO_{2peak} was significantly reduced in participants with DS compared to those without DS (significant interaction: $F = 20.6$, $p < 0.05$). As depicted in figure 4.3, follow-up analyses demonstrated that HR increased in both groups from rest to exercise ($p < 0.05$) and then decreased during recovery ($p < 0.05$) while remaining elevated above baseline ($p < 0.05$). There were no between-group differences in HR at rest or during recovery. During exercise, however, the participants with DS exhibited lower HR than those without DS ($p < 0.05$). Nevertheless, the FU of peak HR during treadmill walking was similar between participants with and without DS (DS: 62.1 ± 1.9; non-DS: 64.2 ± 1.8% HR_{peak}).

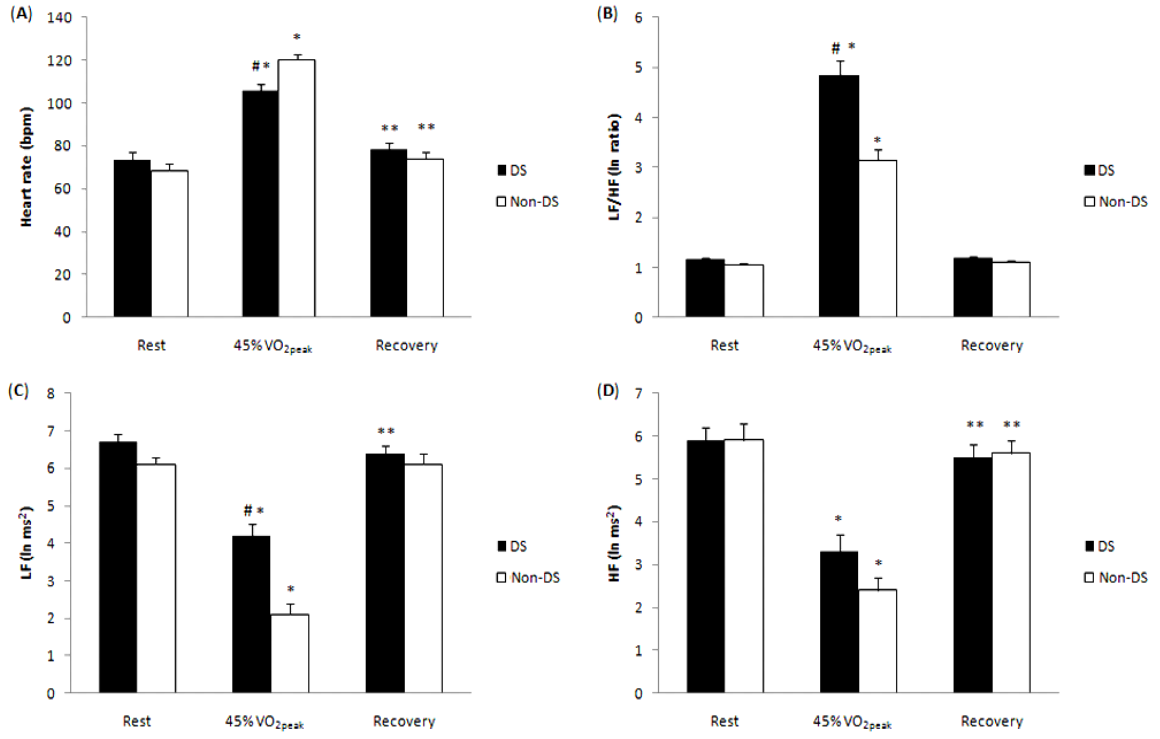

Fig. 4.3 Spectral components of heart rate variability at rest, during submaximal exercise at 45% VO_{2peak} and recovery in participants with Down syndrome (DS) and individuals without Down syndrome (non-DS). (A) heart rate; (B) low frequency (LF) to high frequency (HF) power ratio; (C) raw low frequency power; (D) raw high frequency power. LF, HF and LF/HF ratio are the natural logarithm (ln). Values are mean ± SEM. * $p < 0.05$ between rest and exercise for participants with and without DS; # $p < 0.05$ between participants with and without DS; ** $p < 0.05$ between exercise and recovery for participants with and without DS.

As show in figure 4.3, the responses to exercise of the raw LF and LF/HF HRV were significantly different between participants with and without DS (significant interactions: $F = 8.9$ and $F = 16.7$, respectively; $p < 0.05$). Specifically, the LF power decreased ($p < 0.05$) whereas the LF/HF ratio increased ($p < 0.05$) in both groups from rest to exercise. However, during exercise, both the LF power and the LF/HF ratio were higher in individuals with DS than in those without DS ($p < 0.05$) (Fig. 1). Figure 4.3 also shows that while the LF power recovered towards baseline at post-exercise period in participants without DS, this did not occur in those with DS ($p < 0.05$). Nevertheless, there were no between-group differences in LF power during recovery. As importantly, both groups showed a significant reduction in the LF/HF ratio after exercise ($p < 0.05$). However, in contrast to LF power, the LF/HF ratio showed complete recovery to pre-exercise values in participants with and without DS ($p > 0.05$). HF power did not differ between groups and showed similar response to exercise and recovery in individuals with and without DS (main effect for condition: $F = 108.0$, $p < 0.05$).

Accordingly, both groups showed a significant decrease in HF power from rest to exercise ($p < 0.05$) and this was followed by incomplete recovery of this spectral component to resting values at post-exercise period ($p < 0.05$) (Fig. 4.3). Finally, figure 4.4 demonstrates that the respiratory rate of participants with DS was similar to that of controls at each physiological condition. Because the respiratory rate is known to affect the spectral analysis of HRV (Brown et al. 1993), this further corroborates that between-group comparisons were conducted under comparable conditions at rest, during exercise and recovery.

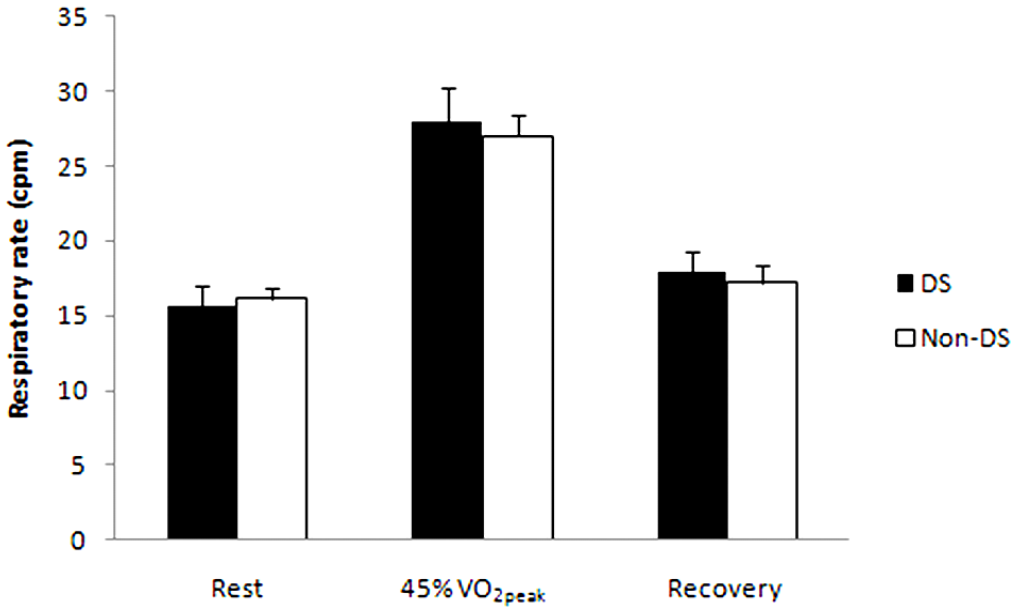

Fig. 4.4 Respiratory rate at rest, during submaximal exercise at 45% VO_{2peak} and recovery in participants with Down syndrome (DS) and individuals without Down syndrome (non-DS).

4.2.6. Discussion

This study explored differences in cardiac autonomic function between adults with and without DS in response to dynamic exercise, performed at the same relative intensity (below the VT). We found that, compared to persons without disabilities, adults with DS showed appropriate vagal withdrawal (HF power) in transition from rest to submaximal exercise. However, under these conditions, participants with DS demonstrated an exacerbated shift in their sympathovagal balance towards greater sympathetic dominance (LF/HF ratio). Despite this, their absolute change in HR from rest to exercise was lower than that of participants without DS. Therefore, contrary to that

hypothesized, our results indicate that reduced chronotropic response in persons with DS is not accompanied by blunted vagal withdrawal during submaximal dynamic exercise. Alternatively, the present findings suggest that this may originate from poor end-organ (cardiac) responsiveness to a given level of change in autonomic efferent activity.

At rest, the lack of between-group differences in HR or in any of the spectral components of HRV indicates similar resting vagal and sympathetic modulation in adults with and without DS. These findings support those of previous studies (Figueroa et al. 2005; Goulopoulou et al. 2006; Iellamo et al. 2005; Agiovlasitis et al. 2010). To the best of our knowledge, only one study found greater resting vagal modulation in persons with DS (Baynard et al. 2004). However, this study compared cardiac autonomic modulation as measured by HRV between adolescents with DS and those with ID without DS. Because there was no comparison group without disabilities, our current findings may not be directly comparable to those of Baynard et al. (2004). In addition, the fact that adolescents with DS had high resting vagal activity does not necessarily imply that adults with DS would also demonstrate such findings. Previous studies have shown that the autonomic nervous system progressively develops from infancy to adulthood and that vagal modulation decreases during adulthood in nondisabled individuals (Migliaro et al. 2001). However, the development with growth of the autonomic nervous system in persons with DS is unknown.

There is general agreement that, in response to submaximal exercise below the VT, neural activation of central command evokes a decrease in vagal outflow, which in turn mediates the increase in HR during light to moderate exercise intensities (Raven et al. 2006). Considering that the reduction of vagal modulation to the SA node is indicated by the HF power of HRV (Arai et al. 1989; Iellamo et al. 1999; Taylor et al. 1995; Tulppo et al. 1998) and that both groups presented similar decrease in this spectral component from rest to exercise, our data supports similar vagal withdrawal between participants with and without DS. In contrast, sympathetic activity at the SA node increases with exercise intensity, and this is generally observed by a rise in the LF/HF ratio (Yamamoto et al. 1991). Yamamoto et al. (1991) found LF/HF ratios between 3.6 and 7.0 for exercise intensities within 30 to 100% of VT in nondisabled individuals using a general spectral analysis technique. We also observed

a significant increase in the LF/HF ratio from rest to exercise in both participants with and without DS. Additionally, as expected for exercise performed below the VT, both groups increased their LF/HF ratios within the limits of variation previously reported in the literature (Yamamoto et al. 1991) (Fig. 4.3). However, because the increase in the LF/HF ratio from rest to exercise was greater in participants with DS, our results suggest that these individuals may exhibit heightened sympathetic modulation of the SA node at a given relative intensity of exercise. The higher LF power in participants with DS during submaximal exercise may further corroborate this assumption. Taken together, our findings indicate that, for achieving similar FU of peak HR during dynamic exercise, persons with DS adjusted their cardiac autonomic function differently from participants without disabilities. Specifically, while both groups responded to exercise with similar levels of vagal withdrawal, participants with DS required greater sympathetic activation to attain ~ 60% of peak HR. From a different point of view, it is also interesting to note that, despite showing a greater shift in their sympathovagal balance towards sympathetic dominance and similar vagal withdrawal as nondisabled participants, adults with DS responded to exercise with an attenuated increase in HR (absolute change from rest to exercise). This may indicate that DS is associated with intact and even heightened cardiac autonomic modulation during submaximal exercise; however, there may be poor cardiac responsiveness to a given level of autonomic activity change in these individuals. Nevertheless, the validity of this argument should be tested empirically and pharmacological testing (i.e. vagal blockade with atropine) together with HRV and HR measurements would be valuable to help clarifying this issue.

It should be noted that, while the most likely candidate explanation for attenuated chronotropic response of persons with DS to exercise above the VT is poor ability to produce catecholamines (Bricout et al. 2008; Fernhall et al. 2009); the mechanism responsible for their reduced HR increase at intensities below the VT remains largely unknown. Past findings on autonomic control of HR during treadmill exercise have reported no differences in submaximal HR or spectral HRV, between adolescents with DS and those with other ID (Baynard et al. 2004). Although that study cannot be directly compared with the present study, it is of note that there was no attempt to control exercise relative intensity, which may probably have influenced the results. Additionally, as already mentioned,

part of HRV is determined by respiratory sinus arrhythmia and it has been shown that any change in respiratory rate influences the distribution of total power within each spectral component (Brown et al. 1993). Baynard et al. (2004) did not report the respiratory rate of either group during submaximal exercise and this further limits interpretations of their data. We found that both groups of participants had similar values for the respiratory rate at each physiological condition; therefore, it is not likely that our data were substantially influenced by this confounding factor.

Even though our data suggests that adults with DS exhibit poor cardiac responsiveness to a given change in cardiac autonomic modulation during submaximal dynamic exercise, the causes for such findings are difficult to discern from this study. Interestingly, most of the existent research suggests a relationship between reduced ability of persons with DS to suppress vagal activity and blunted HR responses to autonomic provocative maneuvers such as handgrip exercise, active orthostatism and tilt testing (Figueroa et al. 2005; Iellamo et al. 2005; Agiovlasitis et al. 2010). Although dynamic exercise and most of the other autonomic provocative maneuvers cause similar changes in cardiac sympathetic and vagal outflow, there are obvious differences in cardiovascular regulation between them (Iellamo 2001; Rössler et al. 1999; Robinson et al. 1988; Tulppo et al. 2001). Furthermore, to the best of our knowledge, this is the first study to explore differences in spectral HRV between persons with and without DS at similar FU of VO_{2peak} and peak HR. This is relevant because the relative contribution of central command and muscle metaboreflex, affecting the cardiac sympathovagal balance, has been shown to change as a function of exercise intensity (Iellamo et al. 1997; Iellamo et al. 1998). Muscle hypotonia, which is common in these individuals (American Academy of Pediatrics 2001), may be one candidate explanation for reduced cardiac responsiveness to neural autonomic changes during dynamic submaximal in DS. Accordingly, hypotonia may also affect myocardiocyteal muscle cells potentially lowering their response to a given level of vagal deactivation and sympathetic activation and resulting in lower absolute change in HR during dynamic exercise; however, there are presently no data to support this hypothesis.

During recovery from submaximal exercise, when the sympathetic nervous system has not been significantly stimulated, reduction in HR is governed mainly by vagal reactivation (Pierpont and

Voth 2004). Vagal reactivation is known to occur rapidly within the first minute of recovery and then more slowly until the fourth min of exercise cessation. Subsequently, vagal activity remains fairly constant until 10 min post-exercise (Kannankeril et al. 2004). We found that both participants with and without DS showed incomplete recovery of HF power after 5 min of exercise cessation and this agrees with previous studies in healthy and nondisabled individuals (Martinmäki and Rusko 2008). As with vagal reactivation, we found that HR did not recover towards baseline in either group of participants after exercise. Nevertheless, the between-group differences in HR, observed during exercise, were no longer evident at 5 min of recovery. Finally, we found that while the participants without DS demonstrated complete recovery of LF power to resting values, this did not occur in those with DS. Yet, in contrast to that seen during exercise, the LF power was no longer different between groups at recovery. Published literature on autonomic mediation of LF HRV is controversial (Berntson et al. 1997) and for this reason it is preferable to draw physiological interpretations based on the information derived from the LF/HF ratio (Lombardi 2002). In parallel to that seen at resting conditions, both groups of participants showed similar values for the LF/HF ratio during recovery from exercise. Moreover, there were no differences in the sympathovagal balance between resting and recovery conditions for participants with DS and those without DS. This indicates that the between-group differences in cardiac autonomic function were exclusively manifested during submaximal exercise and then reverted back to baseline at 5 min of passive recovery.

In conclusion, we investigated cardiac autonomic function in response to submaximal dynamic exercise in adults with DS and observed appropriate vagal withdrawal, but heightened sympathetic modulation of HRV at ~ 60% of FU their peak HR. Despite this, the absolute change in HR from rest to exercise was attenuated in these individuals compared to persons without disabilities. These findings suggest that DS may be associated with poor cardiac responsiveness to changes in autonomic modulation during exercise at below VT intensities. Consequently, our findings provide novel information that may help to explain chronotropic incompetence typically reported in people with DS without congenital heart disease.

There are three main limitations to this study. First, the use of indirect methods may have introduced some error in the assessment of autonomic function. However, these methods are considered valid (Task Force 1996). Importantly, any measurement error should apply equally to both groups without systematically affecting between-group comparisons. Second, DS is a genetic disorder with diverse physiological consequences. However, physical work capacity and HR responses to peak exercise are remarkably consistent in the literature, and our present data are similar to that previously reported. Third, peak treadmill exercise is effort dependent; thus it is possible that participants with DS may have produced lower effort than the control subjects. We used validated protocols and accepted criteria for peak effort during treadmill testing. Additionally, the lack of group differences in the RER at peak exercise intensities further corroborates the assumption of comparable effort between subjects with DS and controls. Therefore, we do not believe that our data were substantially influenced by lack of effort in participants from either group.

4.2.7. References

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4.3. Fractal scaling properties of heart rate dynamics in persons with Down syndrome

4.3.1. Abstract

It has been shown that the fractal scaling properties of heart rate dynamics, in healthy aging, differ from that seen in heart disease and this favors the use of fluctuation measures as diagnostic tools. The purpose of this study was to evaluate the fractal heart rate dynamics in adults with Down syndrome (DS) under different physiological conditions (rest, exercise and post-exercise recovery) and compare their responses with those of nondisabled individuals. Fourteen participants (10 males and 4 females) with DS and 13 nondisabled (9 males and 4 females) controls performed maximal and submaximal treadmill tests with metabolic and heart rate measurements. Detrended fluctuation analysis was performed on the last 256 consecutive R-R intervals obtained under the following conditions: (1) standing rest, (2) submaximal treadmill exercise and (3) standing post-exercise recovery. Participants with DS presented lower chronotropic response than controls to peak and submaximal exercise ($p < 0.05$). The short-term scaling exponent (α_1) was greater in the DS group at rest (1.29 ± 0.06 vs 1.07 ± 0.08), during exercise (1.24 ± 0.09 vs 0.99 ± 0.08) and recovery (1.31 ± 0.06 vs 1.21 ± 0.06) ($p < 0.05$). Furthermore, the fractal scaling distance score ($|1 - \alpha_1|$) of participants with DS was also greater than that of controls under each physiological condition ($p < 0.05$). This confirmed their greater fractal distance from the healthy value of 1.0. In conclusion, adults with DS show a breakdown of scale-invariant organization in heart rate dynamics towards Brownian noise and this is similar to that described in healthy aging.

4.3.2. Key words

Down syndrome; detrended fluctuation analysis; heart rate; cardiac autonomic function

4.3.3. Introduction

Several studies have shown that the normal beat-to-beat fluctuations of the healthy sinus rhythm heartbeat are neither strictly regular nor completely random, but demonstrate an underlying fractal-like structure, characterized by the presence of similar detrended behaviors operating over multiple scales in time (long-range correlations) (Peng et al. 1993; Yamamoto et al. 1995). The application of fractal analysis (through dynamic fluctuation analysis - DFA) has provided new approaches to assessing cardiac risk and forecasting sudden cardiac death, as well as to monitoring the aging process (Iyengar et al. 1996; Mäkikallio et al. 1999; Huikuri et al. 2000; Mäkikallio et al. 2001; Goldberger et al. 2002). Accordingly, the short-term scaling exponent (α_1) of the elderly differs from that of young adults. Further, the alterations of scaling behavior associated with physiologic aging exhibit different patterns compared with the changes associated with heart failure, and this suggests that DFA may be of practical diagnostic and prognostic use (Lipsitz and Goldberger 1992; Iyengar et al. 1996; Goldberger et al. 2002). While the loss of fractal heart rate dynamics with healthy aging is typically associated with excessive order (Brownian noise, $\alpha_1 \sim 1.5$) (Lipsitz and Goldberger 1992; Iyengar et al. 1996; Goldberger et al. 2002), it resembles uncorrelated randomness in heart disease (white noise, $\alpha_1 \sim 0.5$) (Mäkikallio et al. 1999). Furthermore, interpretations based on DFA are not limited by several methodological issues known to affect more conventional measures of heart rate variability such as spectral analysis. In contrast to DFA, these measures are highly sensitive to nonstationarities in time series data and therefore cannot identify the underlying structure of physiological fluctuations if there are trends due to external environmental influences (Peng et al. 1995).

Down's syndrome (DS) is a chromosomal disorder affecting 1 per 650 to 1000 live births (Stoll et al. 1998; Frid et al. 1999) and it is the most common inherited cause of intellectual disability in North America (Barnhart and Connolly 2007). Individuals with DS have reduced aerobic capacity (VO_{2peak}) and chronotropic incompetence compared to nondisabled controls of similar age (Fernhall et al. 1996; Guerra et al. 2003). There is compelling evidence that the chronotropic incompetence experienced by persons with DS may be associated with alterations in cardiac autonomic control

(Fernhall and Otterstetter 2003; Figueroa et al. 2005; Fernhall et al. 2009). In accordance, adults with DS also show blunted vagal withdrawal in response to submaximal exercise (Figueroa et al. 2005), and this is similar to the effects of advanced aging on cardiac autonomic control (Levy et al. 1998). Short-term resting heart rate variability (HRV) (Tulppo et al. 1998) and 24-hour HRV (Ramaekers et al. 1998) both decrease with age and there is less withdrawal of cardiac vagal modulation during submaximal and maximal exercise in the healthy elderly (Levy et al. 1998). Evidence indicates that persons with DS age prematurely (Roth et al. 1996). Biological age in DS has been estimated to be nearly twofold to that of chronological age and this senescence is global as it occurs at multiple organ levels (Nakamura and Tanaka 1998). Not only do individuals with DS show degenerative changes in physical appearance, such as skin and hair, earlier than intellectually disabled without DS (LeMay and Alvarez 1990), they also show the neuropathologic features of Alzheimer's disease earlier than the general population. *Post mortem* studies show that at 40 years of age, virtually all persons with DS have senile plaques, neurofibrillary tangles, and granulovacuolar degeneration of nerve cells (Mann et al. 1984). Individuals with DS also have a shorter life expectancy than the general population (Glasson et al. 2002). Thus, it is possible that accelerated neural aging in DS may contribute to declines in cardiac autonomic modulation as is seen in normal aging.

The purpose of this study was to evaluate the fractal scaling properties of heart rate in adults with DS under different physiological conditions (rest, exercise and post-exercise recovery) and compare their responses with those of nondisabled individuals of similar age, gender and body mass index. We hypothesized that adults with DS would show a degradation of fractal heart rate dynamics compared controls without disabilities.

4.3.4. Methods

4.3.4.1. Participants

A total of 27 healthy participants (14 with DS [10 males and 4 females], 13 controls without disabilities [9 males and 4 females]), aged 18 to 50 yr, were included in the present study. Descriptive statistics are presented in table 4.5. All participants with DS had medical approval for physical activity

participation from their personal physicians. Those with congenital heart disease, endocrine disorders, ambulatory, musculoskeletal, visual, or auditory problems were not included in the study. All subjects, and in addition, parents and/or guardians of the participants with DS, signed informed consent to participate in the study. Subjects in both groups were either sedentary or lightly active (light intensity walking bouts of at least 30 min 1-2 days/wk), but none were involved in any extensive exercise endurance or resistance training for at least six months.

Table 4.5 Characteristics of participants with Down syndrome (DS) and of nondisabled controls.

Variables	**DS (n=14)**	**Controls (n=13)**
Age (years)	35.1 ± 7.8 (18-50)	36.0 ± 7.7 (20-49)
Height (cm)*	152.8 ± 8.9 (137-169)	172.0 ± 6.4 (160-180)
Body mass (kg)	67.1 ± 9.3 (48.8-81.5)	76.6 ± 15.3 (60.4-113.2)
BMI (kg/m^2)	28.8 ± 3.9 (23.2-37.0)	25.8 ± 4.1 (20.1-35.3)
HR_{peak} (bpm)*	171.1 ± 17.9 (132-190)	186.5 ± 10.6 (170-203)
VO_{2peak} ($mL.kg^{-1}.min^{-1}$)*	30.5 ± 6.7 (19.8-40.5)	46.5 ± 9.1 (31.1-52.0)
RER_{peak}	1.14 ± 0.1 (1.05-1.40)	1.17 ± 0.1 (1.07-1.24)
Ve_{peak} ($L.min^{-1}$)*	62.9 ± 20.8 (33.2-96.4)	113.5 ± 19.2 (76.8-139.8)

Values are mean ± SD and range.

Abbreviations: BMI, body mass index; HR_{peak}, peak heart rate; VO_{2peak}, peak oxygen uptake; RER_{peak}, peak respiratory exchange ratio and Ve_{peak}, peak minute ventilation. * Participants with DS differ from controls ($p < 0.05$).

The control group was selected based on the following: (1) healthy medical status, (2) non-smoking condition, (3) gender match with the DS participants, (4) age match with the DS participants, (5) absence of involvement in any formal exercise endurance or resistance training for at least six months, (6) previous familiarization with treadmill walking, and (7) agreement with the testing procedures confirmed by signature of the informed written consent. None of the participants took any medication. This study was approved by the University's internal review board.

Before data collection, each participant was familiarized with the laboratory setting, treadmill protocols, and use of the headgear and face mask. Familiarization sessions were continued until the

subject could comfortably walk on the treadmill with the headgear and mouthpiece. All subjects were adequately familiarized within 1 or 2 sessions.

4.3.4.2. Study Design

Following the familiarization sessions (within a 7-day period), participants were evaluated over the course of 2 visits on separate days (within a 7-day period). Testing was separated by at least 48 h and, to minimize the effects of circadian and other similarly induced variations in performance, was performed at approximately the same time of day (between 07.00 and 11.00 h). All subjects abstained from caffeine and vigorous exercise for 24 h prior to testing and were at least 3-h post-prandial upon arrival for testing. During the first visit, standing height and weight measurements were taken with participants wearing light-weight clothing and no shoes. Body mass index (BMI) was calculated by dividing the participants' mass in kilograms by the square of their height in meters. Subsequently, participants performed a treadmill graded exercise test (GXT) to determine their submaximal VO_2 and VO_{2peak}. This test was used for two specific purposes: (1) to confirm chronotropic incompetence in subjects with DS and whether subjects in the control group had a normal heart rate response to exercise, defined as the ability to achieve 85% of age-predicted maximal heart rate (Lauer et al. 1996); and (2) to determine the treadmill workload required to elicit a given pre-selected fractional utilization (FU) of the VO_{2peak} in each individual. On the second visit, each participant performed one walking bout at 50% VO_{2peak}. All tests were performed on a motorized treadmill (Jaeger® Laufergotest, Germany). Expired gas measurements were made using a portable mixing chamber system (MetaMax® I, Cortex, Leipzig, Germany), which was calibrated before each test with a known volume and with known gas concentrations. Heart rate data were obtained by means of a Polar RS 800 G3 heart rate monitor (Polar R-R Recorder, Polar Electro, Kempele, Finland). Testing was carried out in the laboratory with an environmental temperature between 21and 24 ºC and a relative humidity between 44 and 56%.

4.3.4.3. Graded exercise test

Before testing, each subject rested quietly in a seated position for 5 min. VO_2 and heart rate data were collected for an additional 5 min of seated rest. Testing began with a submaximal horizontal

walk at a constant speed of 4 $km.h^{-1}$. Grade was increased 2.5% every 5 min until a 7.5% grade was reached. Grade was then increased every 2 min by 2.5% until a 12.5% grade was reached. From this point, the grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every minute until exhaustion. This protocol has been shown to be a valid and reliable measure of maximal cardiorespiratory fitness in both individuals with DS and control participants without disabilities (Fernhall et al. 1990). The VO_2 data were displayed in 20-s averages. A valid VO_{2peak} was defined as the highest value obtained during the last stage of exercise with a respiratory exchange ratio (RER) over 1.0 (Fernhall and Otterstetter 2003).

4.4.4.4. Constant intensity exercise tests

To ensure that all participants exercised at the same FU of the VO_{2peak} (same relative intensity), treadmill workloads were individualized. For this purpose, the mean of the last 3 min of each 5-min walk (GXT stages at constant speed of 4 $km.h^{-1}$) was defined as the participants submaximal steady-state VO_2. Equations were computed using least squares linear regression on data derived from each participant's steady state VO_2 (0, 2.5, 5 and 7.5% grades). Then, the treadmill grade required to elicit 50% VO_{2peak} at a 4 $km.h^{-1}$ walking speed was calculated. Subjects subsequently performed a 6-min treadmill walking bout at 50% VO_{2peak}. Exercise was preceded by a 5-min standing period and followed by another 5 min of standing recovery.

4.3.4.5. R-R interval signal acquisition

The R-R intervals were recorded (Polar R-R Recorder, Polar Electro, Kempele, Finland) at a frequency of 1000 Hz, providing an accuracy of 1 ms for each R-R interval. Recorded R-R intervals were first transferred to the Polar Precision Performance Software (Kempele, Finland) and visually inspected for undesirable premature beats and noise. No premature beats were observed in the complete set of R-R intervals obtained from each individual; therefore, there was no need for interpolation due to ectopy. Heart rate and fractal scaling analyses were performed on the last 256 consecutive R-R intervals obtained under the following conditions: (1) standing rest, (2) constant load submaximal treadmill exercise and (3) standing post-exercise recovery.

4.3.4.6. Detrended Fluctuation Analysis

The fractal nature of heart rate time-series allows measurement of self-similarity correlations using DFA. In brief, DFA is a modified root mean square analysis of a random walk and has previously been described in detail (Peng et al. 1995). In this analysis, the time-series is integrated and divided into boxes of equal length. A least squares line is fit to the data in each box and then detrended by subtracting the trends in each box (Goldberger et al. 2000). This calculation was repeated over all box sizes to describe the relationship between the average fluctuation and the box size. In this context, the scaling exponent (α) represents the slope of the line, which relates (log) fluctuation to (log) window size (Goldberger et al. 2000). The values of α reflect the self-similarity of a time-series. Based on previous research (Peng et al. 1995) we utilized the short-term (4 to 16 beats) scaling exponent (α_1) to analyze our heart rate time-series data. An α_1 value of 0.5 reflects white noise (i.e. no correlations), whereas values < 0.5 imply that the data are anticorrelated. A value of 1.5 reflects Brownian noise (i.e. random walk). An α_1 value near 1.0 reflects pink noise (i.e. fractal like behaviour) and is associated with healthy heart rate dynamics (Heffernan et al. 2008). Analyses were carried out using Kubios HRV Analysis Software 2.0 for Windows (The Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland).

4.3.4.7. Statistical Analysis

Descriptive statistics were calculated for all variables. Before comparing both groups, data were tested for normality and homocedasticity with the Kolmogorov-Smirnov and Levene's tests, respectively. Subsequently, independent *t* tests were performed to determine group differences among the descriptive characteristics (ie, age, height, body mass, BMI and peak cardiopulmonary measures). A 2-way analysis of variance (ANOVA), with repeated measures [group (DS vs controls) by time (standing rest, submaximal exercise and post-exercise standing recovery)], was conducted on all dependent variables to compare differences between participants with DS and controls. When a significant main effect was detected at a significance level of $p < 0.05$, *t*-tests were used for post hoc comparisons. Adjustment for multiple comparisons was made with Bonferroni's correction. Because heart rate during exercise differed between groups, the ANOVA was repeated by using exercise heart

rate as a covariate. All data are reported as mean ± SEM unless otherwise specified. Statistical significance was set at $p < 0.05$. All data analysis was carried out using Statistical Package for the Social Sciences (SPSS, v 16.0, SPSS, Inc., Chicago, IL).

4.3.5. Results

Subjects' characteristics and peak exercise data are shown in table 4.5. No differences were observed between participants with DS and controls for age. Both groups were of similar body mass and BMI, but the participants with DS were shorter than controls. The control group exhibited significantly higher peak values for heart rate, VO_2 and minute ventilation compared with the subjects with DS. In opposition, both groups attained similar RER values at peak exercise.

At rest, there were no differences in heart rate between the participants with DS and the controls (DS: 72.3 ± 2.8; controls: 68.2 ± 2.9 bpm). Furthermore, both groups exercised at the same relative intensity (DS: 48.3 ± 1.1; controls: 49.1 ± 1.1% VO_{2peak}) and at comparable RER values (DS: 0.91 ± 0.02; controls: 0.90 ± 0.01). There was a statistically significant group by time interaction for heart rate ($F = 19.9$; $p < 0.05$), indicating that the subjects with DS exhibited a smaller change in heart rate while responding to exercise at 50% VO_{2peak}. Heart rate increased in both groups (DS: 33.2 ± 2.2, controls: 51.5 ± 3.5 bpm), but the change during exercise was significantly lower in participants with DS ($p < 0.05$). Post-exercise recovery elicited a significant decrease of heart rate in control subjects (45.9 ± 3.2 bpm) compared to individuals with DS (28.4 ± 1.9 bpm) ($p < 0.05$). This was sustained even after controlling for group differences in heart rate during exercise. As can be seen in table 4.6, the respiratory rate of participants with DS was similar to that of controls at each physiological condition. Furthermore, the respiratory rate increased similarly in both groups during exercise and then returned to baseline during recovery. Tidal volume and minute ventilation were also similar between participants with DS and controls at rest and after exercise. However, participants with DS exhibited a smaller change in tidal volume (time by group interaction: $F = 20.9$; $p < 0.05$) and minute ventilation (time by group interaction: $F = 32.6$; $p < 0.05$) from rest to exercise and this resulted in differences between groups while walking at 50% VO_{2peak} ($p < 0.05$) (Table 4.6). As importantly, even

though both groups showed a significant decrease in tidal volume and minute ventilation after exercise, full recovery towards baseline was only seen in the participants with DS.

Table 4.6 Submaximal pulmonary data of participants with Down syndrome (DS) and of nondisabled controls.

Group	RR (cpm)	TV (mL)	Ve (L.min^{-1})
		Rest	
DS	16.7 ± 1.2	526.8 ± 37.1	8.7 ± 0.7
Controls	16.3 ± 0.6	535.9 ± 31.5	8.7 ± 0.5
		Treadmill exercise at 50% VO_{2peak}	
DS	27.0 ± 2.1 #	918.7 ± 75.1 * #	24.3 ± 1.0 * #
Controls	28.1 ± 1.3 #	1567.8 ± 130.4 #	41.3 ± 2.8 #
		Recovery	
DS	18.2 ± 1.4	546.9 ± 47.4	9.8 ± 0.9
Controls	17.5 ± 1.0	721.8 ± 73.3 §	12.0 ± 0.8 §

Values are means ± SEM.

Abbreviations: RR, respiratory rate; TV, tidal volume and Ve, minute ventilation. * Participants with DS differ from controls ($p < 0.05$); # significantly different from resting and recovery conditions ($p < 0.05$); § significantly different from resting conditions ($p < 0.05$).

Overall, as depicted in figure 4.5A, the participants with DS showed higher α_1 values than the controls under each physiological condition (main effect for group: $F = 6.0$, $p < 0.05$). There was a main effect for time in α_1 ($F = 3.5$, $p < 0.05$). As we did not find any interaction effect, results show that α_1 responded in a similar manner in both groups (Fig. 4.5A). Specifically, there were no differences in α_1 values between rest and exercise. Conversely, post-exercise recovery resulted in higher values of α_1 compared to resting and exercise conditions ($p < 0.05$). However, as mentioned previously (Heffernan et al. 2008), the bidirectional nature of fractal scaling properties may mask important data information. For this reason we calculated distance scores from the optimal value of α_1 (i.e. $|1 - \alpha_1|$) (Millar et al. 2009). This score represented a distance from 1.0, in participants with DS and controls, under each condition. A significant group main effect was detected as the participants with DS showed higher $|1 - \alpha_1|$ values than the controls at rest, during exercise and recovery ($F = 4.3$, $p < 0.05$). In contrast with α_1, we found no main effect for time in $|1 - \alpha_1|$ (Fig. 4.5B).

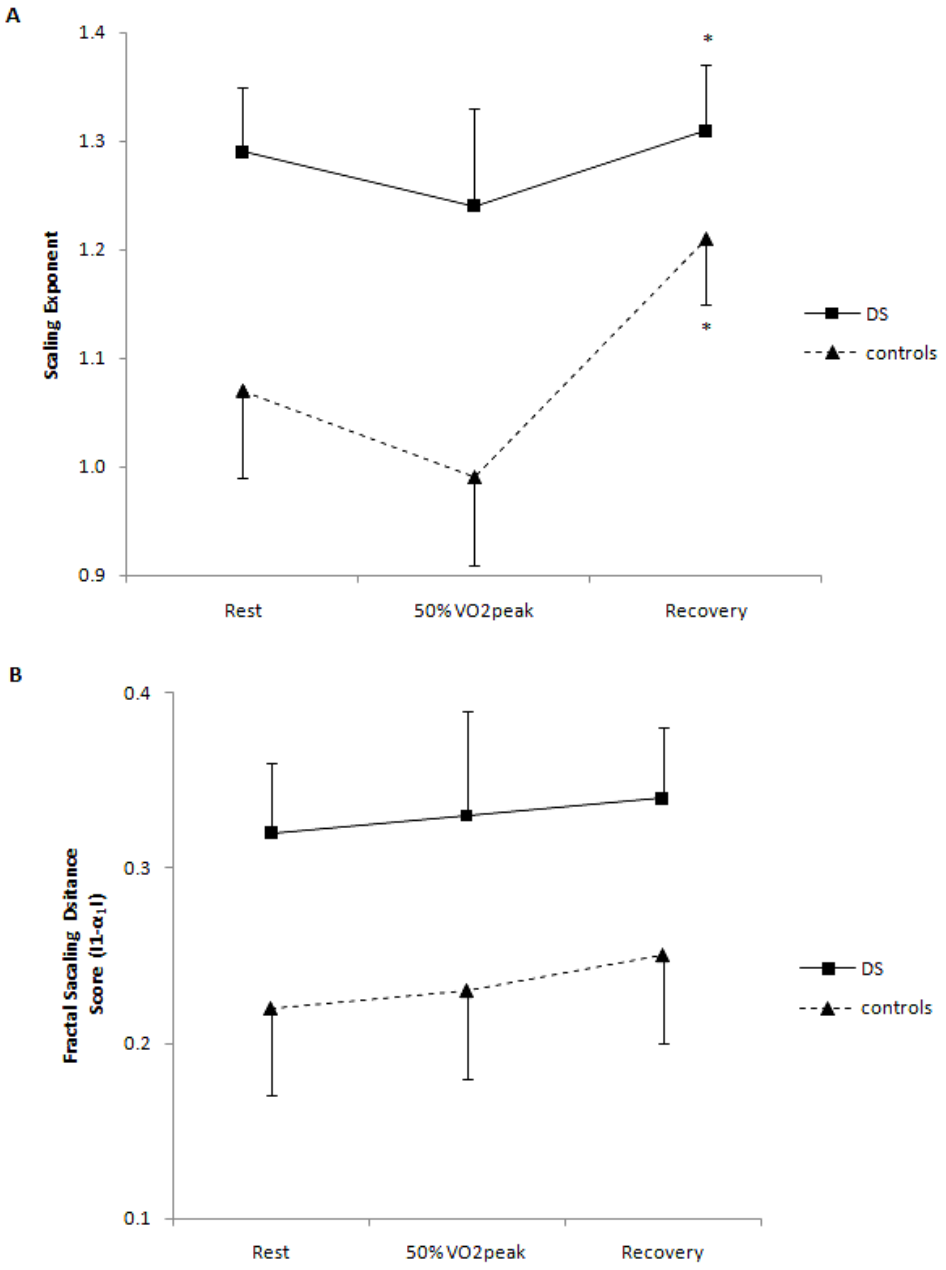

Fig. 4.5 Short-term scaling exponent (α_1) and B: distance score of the short-term scaling exponent ($|1 - \alpha_1|$) of participants with Down syndrome (DS) and controls during standing rest, treadmill exercise at 50% VO_{2peak} and standing recovery. y-axes are unitless. Data are presented as mean ± SEM. * significantly different from resting and exercise conditions ($p < 0.05$)

Considering that the participants included in this study spanned a wide range of ages (18 to 50 yr), we repeated the ANOVA on α_1 while limiting this analysis to participants between 30 and 46 yr of age (11 participants with DS: 36.0 ± 1.3; 9 control participants: 35.1 ± 1.8 yr). Overall, findings were

similar to those previously obtained. Specifically, the α_1 values were higher in participants with DS compared to controls at each physiological condition (main effect for group: $F = 13.7$; $p < 0.05$).

4.3.6. Discussion

This study provides new information about DS-related alterations in cardiovascular dynamics. Our main finding is that there is a breakdown in the fractal scaling properties of interbeat interval fluctuations in DS under different physiological conditions (rest, exercise and recovery). Importantly, while the interbeat interval fluctuations in adults with DS approached a random walk process (Brownian noise), that of nondisabled controls remained closer to a fractal-like behavior ($1/f$ noise). This apparent loss of fractal organization in heartbeat dynamics may reflect the degradation and decoupling of integrated physiological systems in DS.

Healthy autonomic function is characterized by a complex interaction of multiple control mechanisms that enable an individual to adapt to the exigencies and unpredictable changes of everyday life. It is likely that the complex dynamics of the healthy heartbeat arise from numerous coupled control systems and feedback loops that regulate the cardiac cycle on different time scales (Lipsitz 1995). Under normal conditions a short-term scaling exponent value of 1.0, reflecting fractal-like behavior, is associated with healthy heart rate dynamics (Goldberger et al. 2000; Heffernan et al. 2008). Hautala et al. (2003) showed that, α_1 responds with bidirectional pattern of change during the course of dynamic exercise. The diversion point of α_1 from increasing values at low exercise intensity (< 40% VO_{2peak}) to decreasing values at higher intensity levels (> 40% VO_{2peak}) represent the intensity level where increased sympathetic activation start to dominate after vagal withdrawal (Hautala et al. 2003). Accordingly, exercise has been viewed as an effective means for assessing cardiac autonomic function under physiological conditions and thus, without the influence of more invasive approaches such as pharmacological manipulation (Orizio et al. 1998). In addition, the recovery of cardiac autonomic function towards pre-exercise levels reflects a complex interplay between vagal reactivation and sympathetic deactivation and is associated with the individual health status (Cole et al. 1999; Shetler et al. 2001). Consequently, DFA derived from post-exercise recovery may also be useful for discriminating different autonomic profiles in specific populations. We found that both

groups showed a similar pattern of α_1 response to exercise and recovery. Accordingly, while there were no differences between resting and exercise conditions, α_1 increased in participants with DS and controls during recovery. At a relative intensity of 50% VO_{2peak}, such as the one selected for the present study, α_1 is expected to reflect some degree of sympathetic activation (Hautala et al. 2003). Given that sympathetic activation leads to a decrease in α_1, this may justify the observation of comparable values between resting and exercise conditions. Conversely, after 50 s of recovery from moderate exercise, the plasma norepinephrine clearance rate is high (Perini et al. 1989) and this unmasks the vagal reactivation (Borresen and Lambert 2008), possibly leading to an increase in α_1 values.

It has been suggested that subjects with DS not suffering from concomitant congenital heart disease may exhibit a dysfunction in autonomic cardiac regulation that is mainly manifested by a reduced heart rate response to excitatory stimuli (Fernhall et al. 1996; Fernhall et al. 2001; Fernhall and Otterstetter 2003; Guerra et al. 2003). In the present study participants with DS also showed an attenuated increase in heart rate while responding to peak and submaximal exercise, therefore our results agree with previous findings. According to the results of Figueroa et al. (2005), there is an association between chronotropic incompetence and impaired vagal withdrawal in DS. In their study, the authors further speculated that the impairment of vagal withdrawal, in this population, might result from disturbed baroreflex sensitivity (BRS) during exercise. Reduced BRS in DS was later confirmed with the use of several provocative maneuvers such as isometric handgrip exercise (Heffernan et al. 2005), active orthostatism (Iellamo et al. 2005) and passive upright tilt (Agiovlasitis et al. 2010). It has also been reported that HRV (i.e. SDNN), during supine rest, is decreased in DS (Goulopoulou et al. 2006). This indicates that the resting cardiac autonomic function of persons with DS is characterized by lower vagal activity than that of healthy controls. In a different study, it was recently found that adults with DS exhibit reduced heart rate recovery after peak exercise cessation. Thus, these individuals may also show a slower recovery of vagal tone after physical exertion (Mendonca and Pereira 2010).

Interestingly, even though this has not been previously investigated, the autonomic profile of adults with DS shares multiple common features with that of healthy elders. In support of this, aging is known to be associated with a marked decrease in chronotropic response to sympathetic stimulation due to biochemical changes in β receptor-coupling and post-synaptic signaling (Novak and Lipsitz 2004). It has also been found that resting HRV decreases with age (Tulppo et al. 1998) and that there is less withdrawal of vagal tone during submaximal and maximal exercise in the healthy elderly (Levy et al. 1998). Normal human aging is also associated with impairment in BRS which is evident from the blunted cardioacceleration to stimuli that decrease blood pressure, such as active orthostatism or passive upright tilt (Lipsitz 1989; Lipsitz et al. 1990). Furthermore, it has been demonstrated that older adults show a slower recovery of heart rate after peak exercise compared to younger individuals (Antelmi et al. 2008). Persons with DS present many clinical features associated with global senescence in early adulthood (Nakamura and Tanaka 1998), for example premature graying of hair, hair loss, increased lipofuscin, increased neplasms and leukemia, increased autoimmunity, amyloidosis, degenerative vascular disease, and cataracts (Brown 1987). There are several lines of evidence suggesting that premature aging in DS may be a consequence of increased oxidative stress (de Hann et al. 1997; Jovanovic et al. 1998). This is supported by studies showing that DS neuron degeneration in vitro is completely averted by treatment with free radical scavengers, such as antioxidant vitamins (vitamin E and C), or catalase (Busciglio and Yankner 1995).

It has been previously shown that the fractal scaling properties of heart rate dynamics, in healthy aging, differ from that seen in heart disease and this favors the use of fluctuation measures as diagnostic tools to distinguish normal aging from occult disease. Specifically, the age-related loss of fractal scaling is in the opposite direction of that typically seen in heart disease (Brownian vs white noise) (Lipsitz and Goldberger 1992; Iyengar et al. 1996; Goldberger et al. 2002). Compared to controls, participants with DS showed greater α_1 values under resting, exercise and recovery conditions. Interestingly, while α_1 values of the control group remained close to the beneficial value of 1.0 (fractal behavior), participants with DS approached Brownian noise (~ 1.5) under each physiological condition. To explore the clinical significance of this apparent fractal collapse in the

beat-to-beat fluctuations of persons with DS, we additionally calculated the fractal scaling distance score in each group. This analysis confirmed that, compared to controls, participants with DS showed a greater fractal scaling distance from the healthy value of 1.0. Taken together, our results suggest that adults with DS show premature breakdown of fractal scaling properties of heart rate dynamics towards Brownian noise, and that this may be clinically relevant. Because vagal blockade with atropine appears to increase the values of α_1 (i.e. reduce the fractal scaling) of resting (Yamamoto et al. 1995) and exercise (Hautala et al. 2003) R-R intervals in healthy humans, the fractal nature of heart rate dynamics may be in part mediated by vagal neural activity. Therefore, the observed DS-related alterations in fractal scaling of interbeat interval dynamics may be partially due to degradation of autonomic nervous system influences.

In conclusion, we found that adults with DS show a breakdown of scale-invariant organization in heart rate dynamics towards Brownian noise under different physiological conditions. Interestingly, this is similar to that described in healthy aging. Therefore, it is reasonable to speculate that persons with DS may show premature aging of cardiac autonomic function.

4.3.6.1. Clinical implications

Reduced α_1 values predict mortality in patients with depressed left ventricular function after acute myocardial infarction and in those with heart failure (Mäkikallio et al. 1999; Huikuri et al. 2000; Mäkikallio et al. 2001). In opposition, increased α_1 values may reflect the degradation of integrated physiological regulatory systems with aging (Lipsitz and Goldberger 1992; Iyengar et al. 1996; Goldberger et al. 2002). *Post mortem* examination of adults with DS usually exhibits a complete absence of atheroma (Murdoch et al. 1977) and some have suggested that a "genetic triple-dose effect" is responsible for the apparent protection (Ylä-Herttuala et al. 1989). Our findings suggest that, despite persons with DS show loss of scale invariance and emergence of a dominant time scale in cardiac interbeat intervals, they do not exhibit a profile otherwise compatible with increased risk of disease, and this is consistent with prior *post mortem* findings. Nevertheless, a progressive impairment in these mechanisms may result in a loss of dynamic range in physiologic function and, possibly, in a reduced capacity to adapt to stress (Lipsitz and Goldberger 1992).

4.3.6.2. Limitations

There are 4 main limitations to this study. First, we did not include a group of older adults in the present study. This would have allowed direct comparisons between participants with DS and nondisabled controls of different age groups. Nevertheless, the physiological alterations in fractal dynamics with aging are well described in the literature which further supports our conclusions. Second, we did not control for the effects of menstrual cycle on heart rate dynamics in this study. As previously reported, spectral analysis of heart rate variability is sensitive to the influence of estrogen in the follicular phase of the menstrual cycle (Sato et al. 1995). In contrast, the fractal properties of heart rate dynamics do not show significant differences among the 3 phases of the menstrual cycle (menstruation, follicular phase and luteal phase) (Princi et al. 2005). For this reason, although this possibility cannot be completely excluded, it is not likely that the differences seen in α_1 between groups resulted from the effects of varying levels of serum sex hormones on cardiac autonomic function. Third, DS is a genetic disorder with diverse physiological consequences. However, physical work capacity and heart rate responses to peak exercise are remarkably consistent in the literature, and our present data are similar to that previously reported. Fourth, peak treadmill exercise is effort dependent; thus it is possible that participants with DS may have produced lower effort than the control subjects. We used validated protocols and accepted criteria for peak effort during treadmill testing. Additionally, the lack of group differences in the RER at peak exercise intensities further corroborates the assumption of comparable effort between subjects with DS and controls. Therefore, we do not believe that our data were substantially influenced by lack of effort in participants from either group.

4.3.7. References

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4.4. Heart Rate Recovery After Exercise in Adults with Down Syndrome

4.4.1. Abstract

The main purpose of this study was to evaluate heart rate recovery (HRR) in individuals with Down syndrome (DS) after peak dynamic exercise and compare their response with that of nondisabled subjects of similar age, gender and body mass index (BMI). 18 participants (14 males; 4 females) with DS (33.6 ± 7.6 yr) and 18 nondisabled (14 males; 4 females) controls (33.8 ± 8.5 yr) performed peak treadmill tests with metabolic and heart rate measurements. Compared to controls, individuals with DS presented lower peak values for heart rate, oxygen uptake and minute ventilation ($p < 0.05$). In opposition, both groups attained similar respiratory exchange ratio values at peak exercise. Even after controlling for the effects of reduced peak heart rate and BMI, participants with DS showed slower HRR than controls both at 1 minute (DS: 25.3 ± 7.2; controls: 34.1 ± 12.1 bpm) and 2 minutes (DS: 36.3 ± 5.8; controls: 53.6 ± 14.1 bpm) of recovery ($p < 0.05$). Therefore, adults with DS have reduced HRR (at 1 and 2 minutes of recovery) compared with nondisabled controls and this is independent of their lower chronotropic response to peak exercise. Additionally, despite showing attenuated HRR from peak exercise, adults with DS do not present increased cardiovascular risk by general diagnostic criteria (HRR > 12 bpm and 22 bpm, respectively).

4.4.2. Key Words

Down syndrome; heart rate recovery; exercise treadmill testing

4.4.3. Introduction

Individuals with Down syndrome (DS) have reduced aerobic capacity (VO_{2peak}) and chronotropic incompetence (Guerra et al. 2003). Reduced heart rate response to exercise has been identified as the primary contributor to the low physical work capacity and cardiorespiratory fitness in this population (Fernhall et al. 2001). There is compelling evidence that the chronotropic incompetence experienced by persons with DS, in response to exercise, may be associated with alterations in cardiac autonomic control (Fernhall and Otterstetter 2003; Figueroa et al. 2005; Fernhall et al. 2009). Although attention has been given to the clinical implications of changes in heart rate during exercise (Lauer et al. 1996), the prognostic value of the rate of decline in heart rate after the cessation of exercise is of considerable relevance (Cole et al. 1999; Shetler et al. 2001). Delayed heart rate recovery (HRR) has been found to be associated with several negative health outcomes and is an independent predictor of subsequent mortality among adults undergoing exercise testing for screening purposes (Cole et al. 1999). Because previous studies have demonstrated delayed HRR in adults with DS performing isometric exercise (Figueroa et al. 2005), we hypothesized that their HRR, after peak dynamic exercise, would also be reduced. Consequently, the purpose of this study was to evaluate HRR in individuals with DS after peak dynamic exercise and compare their response with that of nondisabled subjects of similar age, gender and body mass index (BMI).

4.4.4. Methods

A total of 36 healthy participants (18 with DS [14 males; 4 females], 18 controls without disabilities [14 males; 4 females]), aged 18 to 50 yr, were included in the present study. Descriptive statistics are presented in Table 4.7. A health screening questionnaire was completed by each participant and/or her parent or legal guardian. Exclusionary criteria included any contraindications to exercise, severe or profound mental retardation, active smoking status, congenital or atherosclerotic heart disease, metabolic disease, respiratory disorders including asthma, atlantoaxial instability, orthopaedic issues that would limit treadmill performance and heart rate altering medications. Also, all participants had normal thyroid function per family member or physician report. Subjects in both groups were either sedentary or moderately active, but none were involved in any formal exercise

endurance training for at least 6 months. All subjects, and in addition, parents and/or guardians of the participants with DS, signed informed consent to participate in the study. Participants with DS were recruited from a vocational center for individuals with intellectual disabilities. They all lived at home and were bussed to the center daily. Most of the vocational activities at this center site involved light physical work for 5 to 6 hours, 5 days a week. Control participants were recruited from the local and university communities. This study was approved by the University's internal review board. Before data collection, each participant was familiarized with the laboratory setting, treadmill protocols, and use of the headgear and face mask. Familiarization sessions were continued until the subject could comfortably walk on the treadmill with the headgear and mouthpiece. All subjects were adequately familiarized within 1 or 2 sessions.

Table 4.7 Characteristics of participants with Down syndrome (DS) and of nondisabled controls.

Variable	**DS (n=18)**	**Controls (n=18)**
Age (yr)	34 ± 8	34 ± 8
Height (cm)	153.9 ± 8.7†	174.3 ± 6.7
Body mass (kg)	67.2 ± 9.1*	78.6 ± 16.1
Body mass index (kg/m^2)	28.5 ± 4.3	25.7 ± 4.5
Resting heart rate (bpm)	68 ± 11	69 ± 11
Resting oxygen uptake ($mL.kg^{-1}.min^{-1}$)	4.1 ± 0.7	3.8 ± 0.9
Resting minute ventilation ($L.min^{-1}$)	7.8 ± 1.3#	9.2 ± 1.1
Resting respiratory exchange ratio	0.86 ± 0.07	0.85 ± 0.06

Values are mean ± SD.

† Participants with DS differ from controls ($p < 0.0001$); # participants with DS differ from controls ($p < 0.01$); * participants with DS differ from controls ($p < 0.05$).

All subjects were tested in a postprandial state, approximately 2-4 hours after their last meal. Participants refrained from vigorous exercise 24 hours before testing. Subjects were also asked to refrain from caffeine ingestion on the testing day. Testing consisted of: (1) a standardized anthropometric assessment and (2) a peak graded exercise protocol. Testing was carried out in the

laboratory with an environmental temperature between 21-24ºC and a relative humidity between 44-56%. In an attempt to control for possible circadian variations, the measurements were performed between 07.00 and 11.00 hours at approximately the same time of day for all individuals. Body mass was measured using a calibrated digital scale, and height was measured using a stadiometer (Secca 770, Hamburg, Germany - standing digital scale/height rod attached). BMI was calculated by dividing the participants' mass in kilograms by the square of their height in meters. Expired gas measurements were made using a respiratory gas analysis system (Quark b^2, Cosmed® Srl-Italy), which was calibrated before each test with a known volume and with known gas concentrations. HR data were obtained by means of a Polar RS 800 G3 heart rate monitor (Polar R-R Recorder, Polar Electro, Kempele, Finland).

The participants resting cardiopulmonary data were obtained during a 5-minute seating period, following a quiet rest of 10 minutes in the same position. Subsequently, participants' cardiorespiratory data were collected while exercising on a motorised treadmill (h/p/cosmos® mercury med 4.0). Testing began with a submaximal horizontal walk on a treadmill at a constant speed of 4 $km.h^{-1}$. Grade was increased 2.5% every 5 minutes until a 7.5% grade was reached. Grade was then increased every 2 minutes by 2.5% until a 12.5% grade was reached. From this point, grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every minute until exhaustion. This protocol has been shown to be a valid and reliable measure of maximal cardiorespiratory fitness in both individuals with DS and control participants without disabilities (Fernhall et al. 1990). The VO_2 data were displayed in 20 second averages. A valid VO_{2peak} was defined as the highest value obtained during the last stage of exercise with a respiratory exchange ratio (RER) over 1.0 (Fernhall and Otterstetter 2003). Recovery from peak exercise consisted of a 3-minute treadmill walk at a speed of 2.4 $km.h^{-1}$ and a grade of 2.5% (Cole et al. 1999). HRR was defined as the reduction in heart rate from the rate at peak exercise to the rate at 1 and 2 minutes after the cessation of exercise (Cole et al. 1999; Shetler et al. 2001).

Descriptive statistics were calculated for all variables. Before comparing both groups, data were tested for normality and homocedasticity with the Kolmogorov-Smirnov and Levene's tests, respectively. Potential group differences were evaluated using analyses of variance (ANOVAs).

Analyses of covariance (ANCOVAs) were conducted to compare HRR between groups, controlling for peak heart rate and BMI. All data are reported as mean ± SD. Statistical significance was set at $p < 0.05$. All data analysis was carried out using Statistical Package for the Social Sciences (SPSS, v 16.0, SPSS, Inc., Chicago, IL).

4.4.5. Results

Descriptive and resting data are presented in Table 4.7. No differences were observed between groups for age. Participants with DS were shorter and presented lower body mass than controls. Additionally, they had lower minute ventilation at resting conditions. The comparisons between individuals with DS and control subjects at peak exercise intensities are shown in Table 4.8. Subjects with DS achieved lower peak values for heart rate, VO_2 and minute ventilation. In opposition, both groups attained similar respiratory exchange ratio values at peak exercise. Post-exercise recovery resulted in significantly different HRR, between individuals with DS and controls, even after controlling for the effects of peak heart rate and BMI. Participants with DS showed lower HRR than controls at both minutes of recovery (Table 4.8).

Table 4.8 Physiological responses of participants with Down syndrome (DS) and of nondisabled controls at peak exercise and during recovery.

Variable	DS (n=18)	Controls (n=18)
Peak heart rate (bpm)	162 ± 14†	188 ± 10
Peak oxygen uptake ($mL.kg^{-1}.min^{-1}$)	29.1 ± 6.3†	45.1 ± 9.5
Peak minute ventilation ($L.min^{-1}$)	61.0 ± 17.6†	122.9 ± 33.9
Peak respiratory exchange ratio	1.19 ± 0.17	1.25 ± 0.12
Heart rate recovery at 1 minute post-exercise	25 ± 7*	34 ± 12
Heart rate recovery at 2 minutes post-exercise	36 ± 6#	54 ± 14

Values are mean ± SD.

† Participants with DS differ from controls ($p < 0.0001$); # participants with DS differ from controls ($p < 0.01$); * participants with DS differ from controls ($p < 0.05$).

4.4.6. Discussion

Overall, the present study demonstrates that adults with DS have reduced HRR after peak exercise cessation (at 1 and 2 minutes of recovery) compared with their nondisabled counterparts, and

this is novel. Our findings also suggest that the attenuated post-exercise HRR in individuals with DS is independent of their lower chronotropic response to peak intensities.

As in previous studies, adults with DS showed attenuated heart rate responses to peak exercise concomitant with lower VO_{2peak} values (Guerra et al. 2003; Fernhall et al. 2001). Heart rate increase from rest to peak exercise depends on vagal withdrawal and increased sympathetic activity (Orizio et al. 1998). Since individuals with DS demonstrate blunted vagal withdrawal (Figueroa et al. 2005) and attenuated catecholamine responsiveness during exercise (Fernhall et al. 2009), their chronotropic incompetence probably results from a combination of both.

To our knowledge, this is the first study to compare the reduction of heart rate from peak exercise to recovery between adults with DS and nondisabled controls. Our data are in agreement with that of Antelmi et al. (2008) that used peak intensities to explore post-exercise HRR in healthy individuals. HRR after exercise predicts cardiovascular mortality and is related with arrhythmia and sudden cardiac death (Chaitman 2003; Cole et al. 1999). Slow HRR after exercise is associated with impaired fibrinolysis, increased inflammation, and increased carotid atherosclerosis (Jae et al. 2008a; Jae et al. 2007; Jae et al. 2008b). Abnormal HRR from peak exercise corresponds to a reduction in heart rate $\leq$ 12 bpm in 1 minute of active cool down (Cole et al. 1999). A value of HRR $\leq$ 22 bpm in 2 minutes of recovery has also been validated as a useful prognostic treadmill measurement (Shetler et al. 2001). We found that, even though adults with DS have slower cardiodeceleration than healthy controls, their HRR is not within pathological range at either 1 or 2 minutes of recovery. Therefore, despite showing attenuated HRR from peak exercise, adults with DS do not present increased cardiovascular risk by general diagnostic criteria. Our findings are in agreement with previous studies showing that post-mortem examination of adults with DS reveals a complete absence of atheroma (Murdoch et al. 1977). Some have suggested that a "genetic triple-dose effect" is responsible for the apparent protection because the genes for cysthationine beta synthase and superoxide dismutase are located on chromosome 21 (Ylä-Herttuala et al. 1989; Meade et al. 1993). The observations of Hopkins et al. (Hopkins et al. 2000) further indicate that adults with DS show lower levels of plasminogen activator inhibitor type-1 (PAI-1) which protects them against macroangiopathy and

complications resulting from plaque instability (unstable angina and myocardial infarction). Interestingly, this is just the opposite of that occurring in obese subjects who exhibit insulin resistance with hyperinsulinemia and hypertriglyceridemia (McGil et al .1994). Therefore, while showing some of the most common features of metabolic syndrome (ie, insulin resistance, overweight, hypertriglyceridemia and reduced levels of high density lipoprotein), adults with DS do not present increased levels of PAI-1 or a pathological HRR from maximal exercise, and this is in conformity with their decreased prevalence of atherosclerotic lesions throughout adulthood (Fonseca et al. 2005; Rubin et al. 1998; Pueschel et al. 1992).

Even though it did not attain statistical significance, the difference in BMI between participants with DS and controls was still considerable. Previous studies have shown that autonomic function is impaired in obese subjects (Grassi et al. 1998). Specifically, a linear association between BMI and HRR has been previously reported in individuals with metabolic syndrome (Kiziblash et al. 2006). To evaluate the influence of overweight/obesity in this study, we statistically controlled for this variable and found that this did not alter our results. These results are consistent with previous findings on adults with DS, which showed that obesity did not influence their heart rate and blood pressure responses to adrenergic stressors (Fernhall and Otterstetter 2003). Therefore, our findings suggest that obesity has little influence on HRR in persons with DS.

HRR is mediated by both branches of the autonomic nervous system. The initial decrease in heart rate is mediated via prompt parasympathetic reactivation, with latter reductions due to continued parasympathetic reactivation and sympathetic withdrawal (Pierpont and Voth 2004). After high levels of exercise, the sympathetic drive may continue well into the first minute of recovery, masking the reactivation of parasympathetic system and contributing to a slower non-exponential decrease in heart rate. On the other hand, during the second minute of recovery, the plasma norepinephrine clearance rate is higher and this unmasks the parasympathetic reactivation (Borresen and Lambert 2008). Individuals with DS show less change in catecholamine concentrations after peak exercise than nondisabled controls (Fernhall et al. 2009). For this reason, it is not likely that their reduced HRR after peak exercise results from a persistently increased sympathetic activity during recovery. According to

Androne et al. (2003), as cardiodeceleration after exercise is improved by the inhibitory effects of pyridostigmine on acetylcholinesterase, HRR is an index of vagal tone. In this context, our findings suggest that adults with DS may show attenuated recovery of vagal tone (ie, decreased acetylcholine released from the vagus and/or reduced M2 muscarinic receptor sensitivity), compared to healthy controls, after cessation of peak exercise. This agrees with previous studies in which adults with DS were found to exhibit blunted HRR and vagal reactivation (R-R interval high frequency spectral power) after handgrip exercise at 30% of the maximal voluntary contraction (Figueroa et al. 2005). Taken together, adults with the DS present reduced HRR after exercise and this may be independent of exercise intensity or modality. Although blood pressure was not measured in the present study, a marked fall in its values immediately after exercise elicits slower HRR (Cole et al. 1999). Subjects with DS have lower blood pressure than healthy controls at all ages and the reported sex difference in the general population is not seen in these individuals (Richards and Enver 1979). Furthermore, Eberhard et al. (1989) also showed that blood pressure does not rise regularly with exercise intensity in adolescents with DS. Therefore, it is possible that the attenuated cardiodeceleration after exercise results from a compensatory mechanism that further prevents the onset of post-exercise hypotension in persons with DS.

4.4.6.1. Limitations

There are three main limitations to this study. First, we did not measure the blood pressure responses of participants with DS or controls during exercise or recovery. Thus, our inferences on the interactions between blood pressure and heart rate regulation after peak exercise are only based on information from prior studies regarding control of these responses during dynamic exercise. For this reason, we can only speculate on possible mechanisms for the observed differences between persons with DS and controls. Second, DS is a genetic disorder with diverse physiological consequences. However, physical work capacity and heart rate responses to peak exercise are remarkably consistent in the literature, and our present data are similar to that previously reported. Third, peak treadmill exercise is effort dependent; thus it is possible that participants with DS may have produced lower effort than the control subjects. We used validated protocols and accepted criteria for peak effort

during treadmill testing. Additionally, the lack of group differences in the respiratory exchange ratio at peak exercise intensities further corroborates the assumption of comparable effort between subjects with DS and controls. Therefore, we do not believe that our data were substantially influenced by lack of effort in participants from either group.

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4.5. Heart rate recovery and spectral heart rate variability following combined aerobic and resistance training in adults with and without Down syndrome

4.5.1. Abstract

Objective: To determine whether adults with Down syndrome (DS) could improve their cardiac autonomic function as adults without disabilities, after 12 weeks of combined exercise training.

Design: Prospective study comparing the effects of a 12-week combined exercise program on the cardiac autonomic function of adults with and without DS.

Participants: Thirteen participants with DS (mean age ± SEM, 36.5 ± 1.5 yr) and 12 participants without disabilities (38.7 ± 2.4 yr).

Intervention: Combined exercise training for 12 weeks. Aerobic training was performed 3 days/week for 30 min at 65-85% VO_{2peak}. Resistance training was prescribed for 2 days/week and consisted of 2 rotations in a circuit of 9 exercises at 12-repetition-maximum.

Main outcome measure: Heart rate recovery (HRR) from peak exercise (1 and 2 min post-exercise) and spectral heart rate variability during supine rest.

Results: Participants with DS and those without disabilities showed similar increase in VO_{2peak} after training. Training improved HRR at 1 min post-exercise in participants with DS, but not in those without DS ($p < 0.05$). Both groups of participants exhibited a similar magnitude of increase in normalized high frequency power and of decrease in normalized low frequency power at post-training ($p < 0.05$).

Conclusions: A 12-week program of combined aerobic and resistance training enhanced the HRR from peak exercise in adults with DS, but not in those without DS. In contrast, the exercise intervention elicited gains of similar magnitude between persons with and without DS for cardiac autonomic modulation.

4.5.2. Key words

Exercise; training; Down syndrome; heart rate recovery; autonomic function

4.5.3. Introduction

Both time and frequency domain analyses of heart rate variability (HRV), which represent the changes in beat-to-beat intervals occurring between consecutive heartbeats, constitute adequate and noninvasive methods to assess the autonomic influence on the heart (Task Force 1996). Specifically, high frequency power and especially the normalized high frequency ratio (i.e., high frequency power/[total power – very low frequency power]) have been recognized to give insight into vagal activity (Berntson et al. 1997). Heart rate recovery (HRR) after peak exercise has also been advanced as an index of vagal activity (Pierpont and Voth 2004). These measures are particularly important in the clinical context because vagal activity has been shown to exert a cardioprotective effect through enhanced cardiac electrical stability (Billman 2002). This is further supported by previous research showing that low HRV and reduced HRR, both representing poor vagal activity to the SA node, are prognostic markers of sudden death and all-cause mortality (Molgaard et al. 1991; Jouven et al. 2005; Cole et al. 1999).

Past research has shown that vagal modulation of heart rate is influenced by several physiological factors such as aging (Tulppo et al. 1998), obesity (Christou et al. 2004), aerobic training (Melanson and Freedson 2001; Al-Ani et al. 1996; Levy et al. 1998) and physical fitness (Tulppo et al. 1998). Although it is widely accepted that cardiac vagal modulation is negatively affected by advanced age and increased body fatness, there still remains some uncertainty about the beneficial effects of exercise training on vagal-related HRV indexes and HRR. Longitudinally, increases in measures of HRV have been demonstrated following intense (≥ 85% peak oxygen consumption – VO_{2peak}) or prolonged (~ 30 weeks) moderate intensity aerobic training (Melanson and Freedson 2001; Al-Ani et al. 1996; Levy et al. 1998; De Meersman 1992). Similarly, faster HRR has also been reported after 4 weeks of aerobic training in healthy adults and after 2 weeks of cycle-ergometry in cardiac patients (Sugawara et al. 2001; Legramante et al. 2007). Nevertheless, others have failed to show any improvements in vagal-related indexes or HRV measures after exercise training (Botcher and Stein 1995; Davy et al. 1997; Loimaala et al. 2000).

Persons with Down syndrome (DS) are at high risk for cardiovascular morbidity and mortality (Esbensen et al. 2007), and have low levels of cardiovascular fitness (Fernhall et al. 1996). There is compelling evidence that these individuals may exhibit a dysfunction in autonomic cardiac regulation, which would manifest mainly with a reduced heart rate response to acute sympatho-stimulatory tasks (Agiovlasitis et al. 2010; Fernhall et al 2009; Fernhall et al. 2005; Figueroa et al. 2005; Iellamo et al. 2005; Fernhall and Otterstetter 2003). More precisely, the attenuated chronotropic response in adults with DS is associated with less vagal withdrawal than in controls without disabilities during isometric handgrip (Figueroa et al. 2005); following active standing (Iellamo et al. 2005) and upright tilt (Agiovlasitis et al. 2010). As importantly, it has been previously shown that adults with DS have reduced HRR after peak exercise cessation and that this is independent of their lower chronotropic response to peak intensities (Mendonca and Pereira 2010). Taken together, these findings support the contention of autonomic dysfunction in DS which is associated with poor vagal withdrawal in response to acute adrenergic stressors and blunted vagal reactivation during recovery from excitatory stimuli. Whether systematic exercise training can improve the autonomic profile of persons with DS is not known. Considering that there may be a relationship between improved autonomic function in the exercise-trained state and decreased overall mortality (Sandvik et al. 1993) or sudden death observed during and after exertion (Albert et al. 2000), it is important to explore if adults with DS show a similar pattern of response to training as adults without disabilities. Consequently, the primary purpose of this study was to determine whether 12 weeks of combined exercise training, using both aerobic and resistance exercise, results in differential responses in resting cardiac autonomic function and HRR after peak exercise in adults with and without DS.

4.5.4. Methods

A total of 25 healthy participants (13 with DS [10 men; 3 women], 12 without disabilities [9 men; 3 women]), aged 27-50 yr, were included in the present study. A health screening questionnaire was completed by each participant and/or a parent or legal guardian. As in previous studies, exclusionary criteria included any contraindications to exercise, severe or profound intellectual disability, active smoking status, congenital or atherosclerotic heart disease, metabolic disease,

respiratory disorders including asthma, atlantoaxial instability, orthopaedic issues that would limit treadmill performance (Cowley et al. 2010). Additionally, participants were not taking any kind of medication and they were all normotensive (systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg). Participants also had normal thyroid function per family member or physician report. Participants in both groups were either sedentary or lightly active (light intensity walking bouts of at least 30 min 1-2 days/week), but none were involved in any formal exercise endurance or resistance training for at least 6 months. All participants, and in addition, parents and/or guardians of the participants with DS, signed informed consent to participate in the study. Informed consent was attained after listening to an explanation of the nature of study participation and initial eligibility screening. Additionally, before signing the informed consent, participants were given the opportunity to visit the facilities where testing and exercise sessions would be conducted. This study was approved by the University's Institutional Review Board.

Participants with DS were recruited from a vocational center for adults with intellectual disabilities. They all lived at home and were bussed to the center daily. Most of the vocational activities at this center involved light physical work for 5 to 6 hours, 5 days/week. Participants without disabilities were recruited from the local and university communities via word of mouth, flyers posted on boards, and throughout community organizations. For inclusion, participants without disabilities had to fulfil the following criteria: (1) healthy medical status, (2) non-smoking condition, (3) normal blood pressure, (4) not taking any kind of medication, (5) absence of involvement in any formal exercise endurance or resistance training for at least 6 months, (6) sex match with the participants with DS, (7) age match with the participants with DS and (8) agreement with the study procedures confirmed by signature of the written informed consent. Before data collection, each participant was familiarized with the laboratory setting, treadmill protocols, and use of the headgear and face mask. Familiarization sessions were continued until each participant could comfortably walk on the treadmill with the headgear and mouthpiece without hand-rail support. Participants were also given the opportunity to practice using strength-testing equipment (Technogym selection line; Gambetolla, Italy). Approximately 30 min were spent demonstrating the correct use and technique of the 9 different

exercises. Each participant performed 2 sets of 8 to 15 repetitions in each exercise. The familiarization sessions were helpful in teaching the participants the correct procedures and identifying close approximations of their 12-repetition-maximum (12-RM). All participants were adequately familiarized within 1 or 2 sessions.

4.5.4.1. Study design

After the familiarization period, participants were evaluated over the course of two visits on separate days. Measurements were taken at baseline (pre) and following a 12-week combined exercise intervention (post). All testing sessions occurred following a 12-hour overnight fast. Additionally, participants were asked to refrain from exercise 24 hours before testing and caffeine ingestion on testing days. Testing was carried out in the laboratory with an environmental temperature between 21-24 ºC and a relative humidity between 44-56%. During the exercise intervention, participants were instructed not to participate in any other form of exercise training. In the first visit, standing height and body mass measurements were taken with participants wearing light-weight clothing and no shoes. Height was obtained using a stadiometer with measures obtained to the nearest 0.5 cm. Body mass was measured on a standing digital scale (Secca 770; Hamburg, Germany). Body mass index (BMI) was calculated by dividing the participants' mass in kilograms by the square of their height in meters. Subsequently, participants performed a treadmill graded exercise test to determine their VO_{2peak} and HRR. During a second visit (48 hours after the first visit), each participant rested in a supine position on a bed in a quiet, semi-dark environment for R-R interval data collection. All within-participant sessions were conducted at the same time of the day (between 07.00 and 11.00 h) to reduce possible diurnal variation. Post-measures were conducted ~ 48 hours after the last exercise session. This report only presents HRV and HRR data. Other outcomes (i.e., cardiorespiratory and metabolic data) are presented in more detail elsewhere (Mendonca et al. 2011).

4.5.4.2. Graded exercise testing

The participants' resting VO_2 was obtained during a 5-min standing period, following a quiet rest of 10 min in the seated position. Cardiorespiratory data were collected while exercising on a motorised treadmill (Jaeger Laufergotest; Hoechberg, Germany) and expired gas measurements were

made using a portable mixing chamber system (Cortex Metamax I; Leipzig, Germany), which was calibrated before each test with a known volume and with known gas concentrations. Heart rate data were obtained by means of a Polar RS 800 G3 heart rate monitor (Polar R-R recorder; Kempele, Finland). Testing began with a submaximal horizontal walk at a constant speed of 4 $km.h^{-1}$. Grade was increased 2.5% every 5 min until a 7.5% grade was reached. Grade was then increased every 2 min by 2.5% until a 12.5% grade was reached. From this point, grade was held constant whereas speed was increased by 1.6 $km.h^{-1}$ every minute until exhaustion. This protocol has been shown to be a valid and reliable measure of maximal cardiorespiratory fitness in both individuals with DS and control participants without disabilities (Fernhall et al. 1990). The VO_2 data were displayed in 20-s averages. A valid VO_{2peak} was defined as the highest value obtained during the last stage of exercise with a respiratory exchange ratio over 1.0 (Fernhall and Otterstetter 2003). Recovery from peak exercise consisted of a 3-min treadmill walk at a speed of 2.4 $km.h^{-1}$ and a grade of 2.5% (Cole et al. 1999). HRR was defined as the reduction in heart rate from the rate at peak exercise to the rate at 1 and 2 min after the cessation of exercise (Cole et al. 1999; Shetler et al. 2001).

4.5.4.3. R-R interval signal acquisition

After 15 min of rest, the participants were asked remain quietly supine for another 10 min without speaking or making any movements (Melanson and Freedson 2001). Because it has been shown that there is no need to control breathing rate to interpret the power spectra of HRV, participants were asked to breathe spontaneously (Bloomfield et al. 2001). The R-R intervals were recorded during the last 10 min of supine rest at a frequency of 1000 Hz, providing an accuracy of 1 ms for each R-R interval. Recorded R-R intervals were first transferred to the Polar Precision Performance Software (Version 5.40.171; Kempele, Finland) and visually inspected for undesirable premature beats and noise. An R-R interval was interpreted as premature if it deviated from the previous quantified interval by > 30%. No premature beats were observed in the complete set of R-R intervals obtained from each individual; therefore, there was no need for interpolation due to ectopy. Heart rate and power spectral analyses were performed on the last 256 consecutive R-R intervals obtained during supine rest. All analyses were carried out using Kubios HRV Analysis Software for

Windows (Version 2.0; Kuopio, Finland). The time series was detrended and re-sampled at 4 Hz (Tarvainen et al. 2001).

4.5.4.4. Spectral HRV analysis

Power spectral analysis was performed following data detrending. Spectral decomposition of HRV was conducted using a parametric; autoregressive modeling (AR) based spectrum estimates. The AR spectrum was calculated fitting a 16^{th}-order model to the R-R data (Boardman et al. 2002). The AR model parameters were solved using a forward-backward least squares method, and finally, the spectrum was obtained from the estimated AR parameters. The frequency-domain variables included the total power (TP) spectrum (0 to 0.4 Hz) and the power spectra integrated over the very low frequency (VLF, 0 to 0.04 Hz), low frequency (LF, 0.04 to 0.15 Hz), and high frequency (HF, 0.15 to 0.4 Hz) bands (Task Force 1996). It is widely accepted that the HF power reflects vagal modulation of heart rate and that both the LF power and the LF/HF ratio reflect a complex interplay between sympathetic and parasympathetic modulation (Berntson et al. 1997). The physiological meaning of the VLF power assessed from short-term recordings is less defined and its interpretation is not recommended when analysing power spectra density results (Task Force 1996). Data were expressed as raw and normalized values. The LF/HF ratio (which is independent of normalization) was then calculated. All data acquisition and post-acquisition analyses were carried out in accordance with standards put forth by the Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology (Task Force 1996).

4.5.4.5. Exercise training program

Participants exercised 3 days/week and each session was conducted with a maximum of 5 participants. The exercise sessions were supervised by an exercise physiologist and 1 assistant. The intervention consisted of 2 days of combined exercise training (Monday and Friday) separated by 1 day of endurance training (Wednesday). The endurance training consisted of 30 min of treadmill walking or running at target heart rates compatible to 65-85% VO_{2peak}. Endurance exercise was preceded by a 5-min warm-up and followed by another 5 min of recovery. Warm-up and recovery consisted of treadmill walking at light intensity. During the first 3 weeks, participants exercised for 30

min at 65% VO_{2peak} and then, emphasis was placed on reaching and maintaining an exercise intensity of approximately 85% VO_{2peak} for 30 min. Heart rate watch monitors (Polar Vantage Night Vision; Kempele, Finland) were programmed for each participant (upper and lower training heart rate) to assure they were exercising in the appropriate target heart rate zone. Participants were given the opportunity to select between walking and running gaits as long as they exercised continuously within the prescribed heart rate zone. Each staff member was responsible for 1 to 3 participants to encourage them to sustain a treadmill workload compatible with a heart rate closer to the upper limit of the training zone.

The resistance training consisted of 2 rotations in a circuit of 9 exercises with less that 30 s of rest between them. Participants trained on the same equipment used for the 12-RM assessments and each session included the following dynamic exercises: leg press, chest press, vertical traction, shoulder press, lower back, leg extension, biceps curl and triceps pushdown. The exercise stations were prescribed for 12-RM and organized in alternate agonist/antagonist interplay to avoid the early onset of local/regional fatigue. Additionally, the participants performed 1 set of 15 repetitions of abdominal curls in each rotation. When participants were able to complete 14 repetitions for 2 consecutive sessions with the proper lifting technique (i.e. proper biomechanical motion; avoidance of the Valsalva maneuver, which involves holding the breath), the load was increased by 10% of their 12-RM.

4.5.4.6. Statistical analysis

Descriptive statistics were calculated for all variables. Before comparing both groups, data were tested for normality and homocedasticity with the Kolmogorov-Smirnov and Levene's tests, respectively. A repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of the training program on the participants' BMI, HRV and HRR. When a significant effect was detected at a significance level of $p < 0.05$, *t*-tests were used for *post hoc* comparisons. Adjustment for multiple comparisons was made with Bonferroni's correction. Since there were group differences in peak heart rate at both time points and this may affect HRR, we included pre- and post-training peak heart rate as covariates in the ANOVAs for HRR (at 1 and 2 min after exercise cessation). LF and HF power were

transformed to their natural logarithm (ln) for statistical analysis because of their skewed distribution. All data are reported as mean ± SEM unless otherwise specified. Statistical significance was set at $p < 0.05$. All data analysis was carried out using Statistical Package for the Social Sciences (SPSS Version 17.0; Chicago Illinois, USA).

4.5.5. Results

No differences were observed between groups for age (DS: 36.5 ± 1.5; non-DS: 38.7 ± 2.4 yr). Participants with DS were shorter (DS: 152.9 ± 2.2; non-DS: 174.3 ± 1.8 cm) and presented lower body mass than participants without disabilities at pre- (DS: 68.6 ± 2.6; non-DS: 81.2 ± 4.9 kg) and post-training periods (DS: 67.7 ± 2.5; non-DS: 81.4 ± 4.7 kg) ($p < 0.05$). In contrast, both groups showed similar values for BMI at pre- (DS: 29.3 ± 1.0; non-DS: 26.6 ± 1.3 kg/m^2) and post-training period (DS: 28.9 ± 1.0; non-DS: 26.6 ± 1.2 kg/m^2). Additionally, the training program had no significant effect on improving body mass or BMI in participants with or without DS.

Exercise training induced significant gains in the relative VO_{2peak} of both groups (DS: 6; non-DS: 6.9%); however, there were no differences in the magnitude of these changes between participants with and without DS. Importantly, participants with DS showed lower chronotropic response to peak exercise intensities than those without DS before (DS: 166.8 ± 5.6; non-DS: 183.0 ± 3.2 bpm) and after training (DS: 167.6 ± 5.4; non-DS: 182.4 ± 3.2 bpm) ($p < 0.05$). Nevertheless, peak heart rate was similar within-groups both at pre- and post-training (Mendonca et al. 2011).

4.5.5.1. Heart rate recovery

As shown in figure 4.6A, the effects of exercise training on HRR at 1 min post-exercise were different between participants with and without DS (time-by-group interaction: $F = 5.3$, $p < 0.05$). Specifically, while participants with DS exhibited a significant improvement in HRR at 1 min post-exercise ($p < 0.05$), this was not seen in those without DS. Nevertheless, there were no significant differences between the HRR at 1 min post-exercise of participants with and without DS before or after training. In contrast, HRR at 2 min of recovery was not significantly different after training in

either group. However, as depicted in figure 4.6B, HRR at 2 min post-exercise was significantly lower in participants with DS compared to those without DS both during pre- and post-training periods (group main effect: $F = 4.4$, $p < 0.05$).

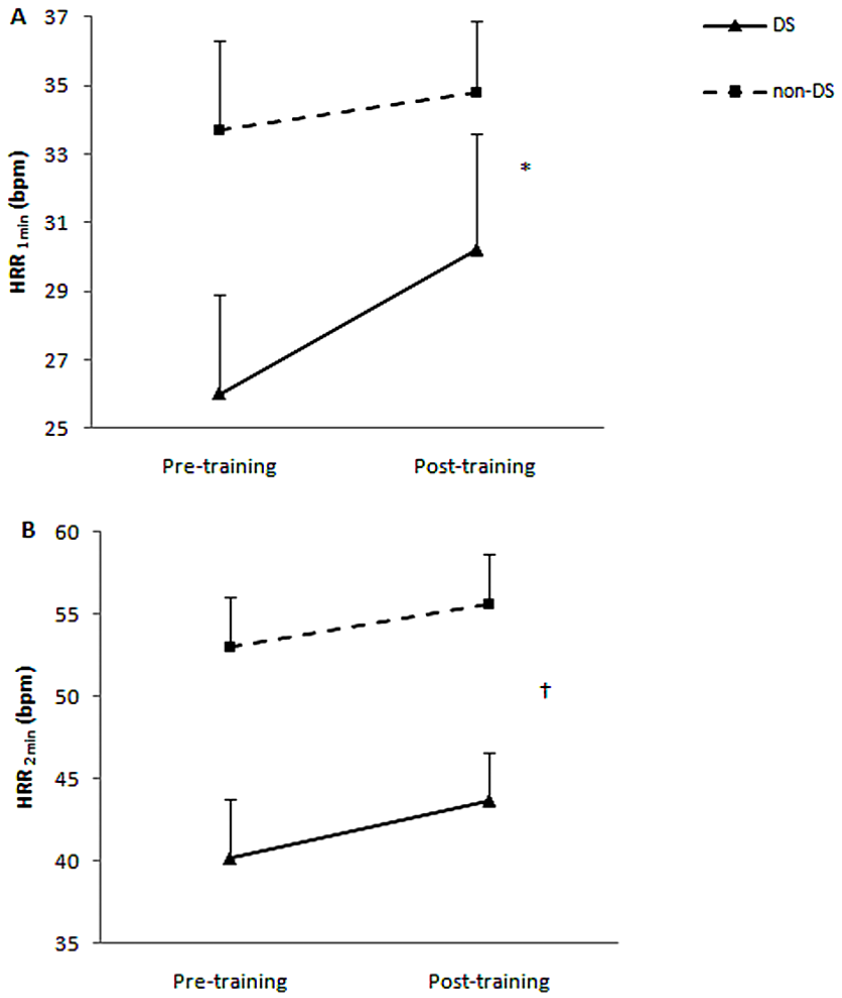

Fig. 4.6 Heart rate recovery after peak exercise cessation in participants with and without Down syndrome (DS) before and after training. (A) Heart rate recovery after 1 min of peak exercise (HRR_{1min}); (B) Heart rate recovery after 2 min of peak exercise ($HRR_{2\ min}$). * Time-by-group interaction ($p < 0.05$); † group main effect ($p < 0.05$).

Table 4.9 Raw power spectra of heart rate variability of participants with and without Down syndrome (DS) at pre- and post-training conditions.

	DS (n =13)		Non-DS (n = 12)	
Variable	**Pre-training**	**Post-training**	**Pre-training**	**Post-training**
HR (bpm)	62.7 ± 3.4	63.4 ± 1.9	62.6 ± 2.5	61.9 ± 1.7
HF (ln ms^2)	6.1 ± 0.3	6.5 ± 0.3	5.5 ± 0.3	5.7 ± 0.4
LF (ln ms^2)	6.6 ± 0.2	6.7 ± 0.2	6.4 ± 0.3	6.3 ± 0.2
TP (ln ms^2)	7.3 ± 0.2	7.5 ± 0.2	6.9 ± 0.3	6.9 ± 0.3
LF/HF (ln ratio)	1.08 ± 0.03	1.04 ± 0.03	1.17 ± 0.04	1.15 ± 0.08

Values are mean ± SEM.

Abbreviation: HR, heart rate; HF, high frequency power; LF, low frequency power; TP, total power; LF/HF, low to high frequency power ratio. HF, LF, TP and the LF/HF ratio are the natural logarithm (ln).

4.5.5.2. Spectral heart rate variability

Table 4.9 shows the mean values of heart rate of participants with and without DS at pre- and post-training during supine rest. As can be seen, participants with DS exhibited similar values of heart rate as those without DS at each time point; and training was not effective in reducing the resting heart rate of either group of participants. Additionally, table 4.9 also demonstrates the raw components of spectral HRV, TP and the LF/HF ratio of participants with and without DS, before and after training. As for heart rate, none of these variables was different between groups at baseline or after training. Furthermore, training had no effect on changing the raw power of HF, LF, TP or the LF/HF ratio in either group of participants. Despite this, there was an overall increase in the normalized HF power in participants with and without DS after training (time main effect: $F = 4.7$, $p < 0.05$) (Fig. 4.7A). In contrast, the normalized LF power displayed an overall decrease in both groups at post-training (time main effect: $F = 4.7$, $p < 0.05$) (Fig. 4.7B).

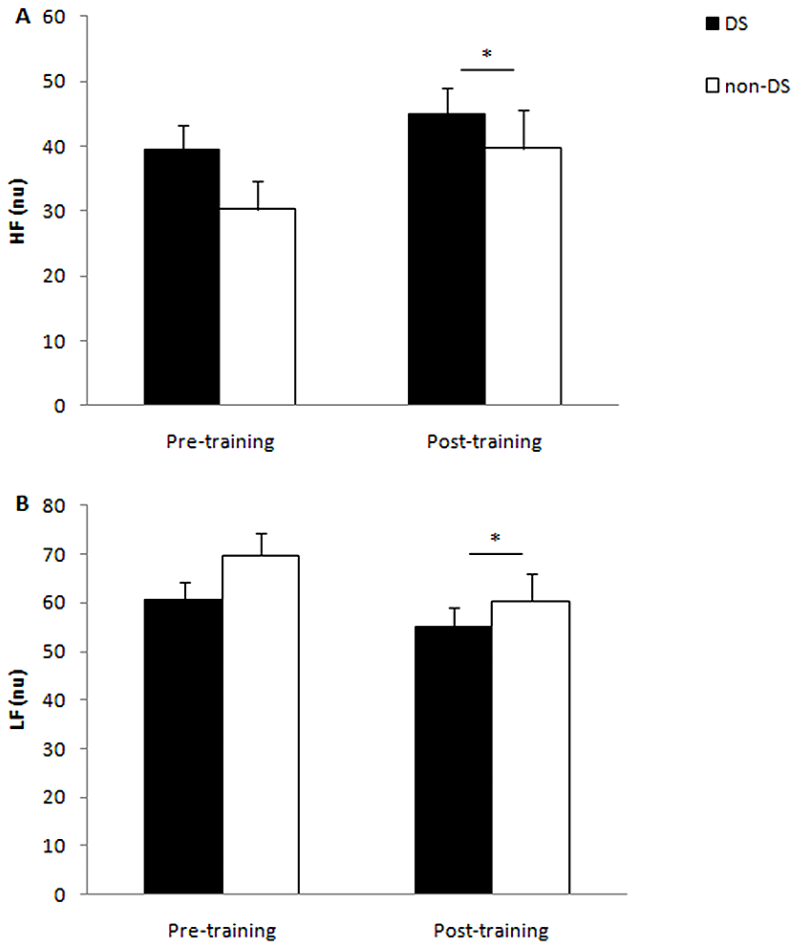

Fig. 4.7 Normalized (nu) spectral components of heart rate variability of participants with and without Down syndrome (DS) during supine rest at pre- and post-training periods. (A) High frequency (HF) power; (B) Low frequency (LF) power. * Time main effect ($p < 0.05$).

4.5.6. Discussion

This is the first study to prospectively compare the effects of combined aerobic and resistance training, on HRR after peak exercise cessation and on spectral HRV during supine rest, between persons with DS and without DS. Current findings indicate that 12 weeks of combined exercise training were sufficient to increase HRR at 1 min after peak exercise in adults with DS (16.2%), but not in those without DS. In contrast, both groups of participants showed similar improvements in the normalized power spectra of HRV after combined exercise training. Importantly, while these favorable changes in vagal-related indexes occurred in the context of improved physical fitness at post-training

period (enhanced VO_{2peak} in participants with and without DS); they were not accompanied by significant reductions in the BMI of either group after training (Mendonca et al. 2011).

4.5.6.1. Heart rate recovery

The initial decrease in heart rate after peak exercise is mediated via prompt parasympathetic reactivation, with later reductions due to continued parasympathetic reactivation and sympathetic withdrawal (Pierpont and Voth 2004). Previous research has shown that adults with DS exhibit slower HRR at 1 and 2 min of peak exercise cessation compared to persons without disabilities (Mendonca and Pereira 2010). This has also been shown to occur during recovery from moderate exercise at 45% VO_{2peak} (Mendonca et al. 2010). In the present study, reduced HRR in adults with DS was only found at 2 min of recovery from peak exercise and this was transversal to pre- and post-training periods (Fig. 4.6B). Consequently, even though our results support the hypothesis of reduced HRR in DS after peak exercise intensities, they are only in partial agreement with previous observations. Because HRR was measured using the same treadmill protocol as in that previous report (Mendonca and Pereira 2010), we speculate that the reduced number of participants included in our study is the most plausible candidate for these discrepant findings. Irrespectively of this, delayed HRR at 2 min of recovery from peak exercise has also been associated with several negative health outcomes and is an independent predictor of subsequent mortality among adults undergoing exercise testing for screening purposes (Shetler et al. 2001). However, given that an abnormal HRR at 2 min of peak exercise cessation corresponds to a reduction in heart rate < 22 bpm (Shetler et al. 2001), it is not likely that these findings represent prognostic relevance for the overall health status of adults with DS. Interestingly, slight reductions in HRR, especially at 2 min of recovery, have been documented as a marker of obstructive sleep apnea (Maeder et al. 2009). Considering that obstructive sleep apnea is highly prevalent in DS (94%) (Trois et al. 2009), our findings of reduced HRR may be associated with the effects of disordered sleep on the cardiac autonomic function of these individuals. Alternatively, as persons with DS have been shown to age prematurely (Nakamura and Tanaka 1998) and as post-exercise cardiodeceleration is considerably slower in older than in younger adults (Cole et al. 1999), it is possible that delayed HRR represents a progeroid feature inherent to DS.

We found that, while exercise training improved HRR at 1 min after peak exercise in participants with DS (16.2%), this did not occur in participants without DS; nevertheless, both groups attained similar gains in physical fitness after training (Mendonca et al. 2011). Previous research has shown that HRR is positively correlated with the training load, but not correlated with VO_{2peak} (Buchheit and Gindre 2006). For this reason, the lack of a training effect on the HRR of persons without DS, despite a significant increase in their VO_{2peak}, is well supported by past findings. These results indicate that the overall training load, associated with the combined training regimen, was sufficient to increase vagal tone (i.e., increased acetylcholine release from the vagus and increased receptor number /sensitivity) (Androne et al. 2003) in persons with DS, but not in those without DS. Even though both aerobic and resistance exercise training have been shown to be effective in increasing post-exercise HRR in healthy individuals (Sugawara et al. 2001; Hagberg et al. 1980; Heffernan et al. 2007; Heffernan et al. 2009), the exact training load required to elicit such response is presently unknown. Nonetheless, it is interesting to note that most previous studies on aerobic training used more frequent training sessions (~ 4 and 6 sessions/week) and of greater duration (~ 1 hour) compared to our exercise prescription (3 session/week; 30 min of treadmill exercise) (Sugawara et al. 2001; Hagberg et al. 1980). Similarly, investigations on resistance training have included prescriptions of greater duration (~ 60 min/session), frequency (3 sessions/week) and number of sets (3 sets/exercise) in comparison to that of the present study (~ 20 min/session; 2 sessions/week and 2 sets/exercise) (Heffernan et al. 2007; Heffernan et al. 2009). Thus, for adults without disabilities, the selected training regimen was insufficient to reach a threshold for improvement in HRR after 12 weeks of participation; however, this was not the case for participants with DS. These discrepant findings between groups are most likely dependent on higher levels of sedentary behavior among participants with DS, compared to those without DS, at baseline. Even though we only included sedentary participants for matching purposes, it may be very difficult to find such high levels of sedentary behavior, as those typically described in DS (Stanish and Draheim 2005), among middle-aged nondisabled adults.

In contrast to the improvements seen in HRR at 1 min post-exercise, participants with DS showed no effects of training on HRR at 2 min of peak exercise cessation. While the primary mechanism for cardiodeceleration at 1 min post-exercise is vagal tone reactivation (Androne et al. 2003), at 2 min of recovery the withdrawal of sympathetic activity assumes considerable relevance. This is supported by previous research showing that the plasma norepinephrine clearance rate is considerably higher at 2 min of recovery from peak exercise (Borresen and Lambert 2008). Consequently, our findings suggest that even though persons with DS exhibited enhanced vagal tone reactivation during recovery from peak exercise intensities after 12 weeks of combined exercise training, this was not be accompanied by faster sympathetic withdrawal after exertion. Nevertheless, because greater HRR after exercise is protective against the incidence of arrhythmia and sudden cardiac death (Cole et al. 1999) and training increased HRR at 1 min post-exercise in participants with DS; our results suggest that a combined exercise intervention may have favorable cardiovascular implications for these individuals.

4.5.6.2 .Spectral heart rate variability

Numerous studies acknowledge improvements in spectral measures of HRV after aerobic exercise training in adults without disabilities (Melanson and Freedson 2001; Al-Ani et al. 1996; Levy et al. 1998). Others have found no change in either HF or LF power after aerobic training (Boutcher and Stein 1995; Loimaala et al. 2000). Interestingly, it has been shown that the relationship between the exercise training stimulus and the responses in reflex control of heart rate do not follow a simple, linear dose-response relationship and it displays a bell-shaped relation with a maximal response at moderate amounts of training (Iwasaki et al. 2003). Specifically, it appears that moderate amounts of exercise training for 12 weeks, as those prescribed in the present study, are sufficient to achieve the majority of this response. In contrast, more prolonged and intense aerobic training results in dissociation between increased vagal tone (i.e., resting braycardia) and HRV parameters (HF and LF power) (Iwasaki et al. 2003). In agreement with previous studies, we found that 12 weeks of exercise training were effective in eliciting improvements in the cardiac autonomic profile of participants with and without DS during supine rest. Importantly, the magnitude of increase in normalized HF power

and of decrease in normalized LF power was similar between groups. Because TP did not change in either group after training, our results suggest that there was a favorable shift in the redistribution of each spectral component within the overall HRV power. Such improvements may be of clinical relevance because normalization allows a more precise assessment of the relative distribution of oscillatory components; in contrast this is not possible when analyzing the raw measures of spectral power (Montano et al. 1994). Physiologically, these findings indicate that the exercise program was effective in eliciting greater vagal modulation and lower sympathetic activity to the SA-node of both participants with and without DS (Task Force 1996). Considering that improved HRV is associated with lower risk of sudden cardiac death, even in asymptomatic individuals (Molgaard et al. 1991), our results suggest that 12 weeks of combined exercise training may represent a useful strategy for enhancing myocardial electrical stability of persons with and without DS at rest. Because there is compelling evidence supporting the notion of autonomic dysfunction in DS, this may be of greater relevance for the overall health status of these individuals (Agiovlasitis et al. 2010; Fernhall et al. 2009; Fernhall et al. 2005; Figueroa et al. 2005; Iellamo et al. 2005; Fernhall and Otterstetter 2003).

In the current study we did not observe any significant decrease in the resting heart rate of participants with DS or those without DS after exercise training. Even though several previous studies have reported the co-occurrence of decreased resting heart rate and enhanced HRV subsequent to exercise training (Al-Ani et al. 1996; Levy et al. 1998), others have failed to demonstrate such relationship (Melanson and Freedson 2001; Bonaduce et al. 1998). Decreases in resting heart rate after training have been attributed to a decrease in intrinsic rate, augmented blood volume, enhanced left ventricular ejection fraction, an increase in vagal efferent activity, and a decrease in sympathetic efferent activity. There is considerable evidence that decreases in intrinsic rate occur over a period of years rather than weeks or months of exercise training (Lewis et al. 1980). Additionally, moderate exercise training programs up to 10 weeks have been shown not to produce significant structural cardiac hypertrophy (McDonald et al. 1993). Accordingly, as neither a decrease in intrinsic heart rate nor an increase in ventricular intracavitary volume were expected to occur after 12 weeks of combined exercise training, resting heart rate was not likely to be affected by any of these two factors. As for the

dissociation between the responses of HRV and heart rate to training, it is interesting to note that while resting heart rate is dependent on vagal tone, HF power represents the modulation in heart rate (and presumably the changes in cardiac vagal efferent activity) that occur synchronously with respiration. The relationship between the magnitude of vagal mediated modulations in heart rate and vagal tone in not known at this time. Therefore, our findings corroborate those of previous studies which indicate that the utility of spectral HRV in determining the mechanisms of bradycardia induced by several weeks of training may be limited (Melanson and Freedson 2001).

4.5.6.3. Study limitations

Although posture, respiratory frequency and tidal volume may all affect HRV, it is unlikely that these factors influenced the findings of the current study. When measured in the supine position, virtually all of HRV is mediated solely by the parasympathetic nervous system (Pomeranz et al. 1985). It could be argued that measurements of HRV obtained in this position would create a ceiling effect, and thus make it difficult to detect changes in HRV due to the exercise intervention. The decision to measure in the supine position was based on the fact that several previous studies had shown improvements in resting supine HRV in previously sedentary adults (Melanson and Freedson 2001; Levy et al. 1998; Iwasaki et al. 2003), and resting supine HRV has been frequently shown to be greater in endurance-trained compared to sedentary individuals (De Meersman 1993). Thus, we were reasonably confident that measurements obtained in the supine position would be sensitive to the intervention. However, we do not know if they may be extended to conditions other than supine rest. It is important to note that we did note control respiratory frequency in this study. Bloomfield et al. (2001) showed that HF power during spontaneous and metronome-guided breathing differs at most by very small amounts, indicating that there is no need to control respiratory rate to interpret the spectral power of HRV. Furthermore, has previously shown, exercise training has no effect on respiratory frequency or tidal volume at rest (Iwasaki et al. 2003; Plowman and Smith 1997). For all these reasons, we do not believe that our data was substantially influenced by spontaneous breathing in both groups at pre- and post-training periods. Importantly, our experimental design did not include non-exercising control groups; therefore, it is not known to what extent these findings are due to the effects

of the combined exercise program rather than to series effects. Finally, the lack of blinded assessors to collect data at pre- and post-training periods may also correspond to a limitation the present study.

4.5.7. Conclusions

In conclusion, 12 weeks of combined aerobic and resistance exercise were effective in improving HRR at 1 min of peak exercise cessation in adults with DS, but not in those without DS. In contrast, both groups of participants showed gains of similar magnitude in the normalized power spectra of HRV after training. More precisely, the exercise training intervention enhanced the normalized power of HF and decreased the normalized power of LF of participants with and without DS at rest in the supine position. As previously shown, HRV and HRR both yield information concerning distinct and independent aspects of cardiac autonomic regulation (Dewland et al. 2007). According to Buccheit et al. (2007), although HRV more aptly reflects phasic fluctuations in vagal efferent activity (parasympathetic modulation); HRR is an index of mean cholinergic signaling at the level of the SA node (vagal tone). In this context, our findings suggest that, while adults with DS responded to the combined exercise regimen with improved vagal tone and heightened vagal efferent activity to the SA node, those without DS only demonstrated greater vagal efferent activity after training.

4.5.8. References

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5. Epilogue

5.1. Main findings

In the preceding chapters we reported several experimental results on the physiological function of adults with DS during dynamic exercise. While the main focus of chapter 3 was on the comparisons between adults with and without DS for cardiorespiratory and metabolic data, chapter 4 addressed variables related to autonomic function.

The main findings of the present thesis can be summarized as follows:

1. The treadmill protocol developed by Fernhall et al. (1990) provides reliable data for both submaximal and peak exercise assessments. Therefore, this protocol allows accurate quantification of submaximal and peak exercise capacity in persons with and without DS.
2. Even though adults with DS show similar walking economy as nondisabled controls at their preferred walking speed, their locomotion is characterized by lower cardiorespiratory efficiency.
3. Adults with DS show a greater change in energy expenditure to positive variations in walking speed. Therefore, these individuals exhibit lower walking economy at a speed faster than their preferred walking speed. In contrast, persons with DS produce similar VO_2 increase as those without DS to treadmill grade variations.
4. The kinetic VO_2 response of adults with DS is similar to that of persons without DS. Consequently, the functional capacity of individuals with DS is not limited by a delay in the VO_2 response to a given exercise intensity.
5. A combined aerobic and resistance training regimen is effective for improving the peak exercise capacity of persons with DS. Furthermore, this training intervention improves the disturbed walking economy of these individuals. As importantly, adults with DS respond to exercise training with gains of similar magnitude as those without DS.
6. For sensitivity purposes, the autoregressive approach is more suited than the fast Fourier transform for analyzing the effects of dynamic exercise on the normalized power spectra.
7. Despite showing appropriate vagal withdrawal, adults with DS exhibit heightened shift in the sympathovagal balance from rest to moderate exercise. Nevertheless, their

chronotropic response to exercise is attenuated and this may result from poor cardiac responsiveness to a given level of change in autonomic efferent activity.

8. There is a breakdown in the fractal scaling properties of heart rate towards Brownian noise in adults with DS. Importantly, this is extensive to resting, exercise and recovery conditions. These results may indicate a novel progeroid feature associated with DS.
9. DS results in reduced cardiodeceleration during recovery from peak exercise intensities and this is independent of chronotropic incompetence. Nevertheless, because the threshold for increased cardiovascular risk is not attained, these findings may simply represent a compensatory mechanism that prevents post-exercise hypotension.
10. Combined aerobic and resistance training improves cardiac vagal modulation in persons with DS and this is similar to that seen in healthy controls. In contrast, while exercise training enhances vagal tone reactivation (heart rate recovery) in adults with DS after peak exercise cessation; this is not the case for those without DS.

5.2. Clinical implications

There are several clinical implications to the findings inherent to the present thesis. First of all, the lower submaximal O_2 pulse suggests poor cardiac function in adults with DS during exercise. In fact, the O_2 pulse is surrogate marker of stroke volume (Tarazi and Levy 1982); therefore, these findings may indicate compromised stroke work and poor cardiac function in the context of dynamic exercise. Secondly, the lower walking economy in persons with DS further aggravates their limited exercise performance. We provided evidence that DS is associated to both reduced VO_{2peak} and decreased exercise economy. This implies an excessive usage of an already limited VO_2 reserve in these individuals. Interestingly, heightened energy expenditure is only seen at speeds faster than their preferred walking speed. For this reason, we believe that this may be a consequence of muscle hypotonicity and ligamentous laxity, which together may possibly disturb locomotion in DS at faster walking speeds. On the other hand, we found appropriate VO_2 kinetics in response to moderate exercise in adults with DS. Considering the close relationship between pulmonary and muscle VO_2 kinetics (Grassi 2000), this indicates a proper level of peripheral O_2 usage. Nevertheless, taken

together, these findings support the notion of disturbed submaximal exercise capacity in this population. Interestingly, because training improved both peak and submaximal exercise capacity in persons with DS, these findings corroborate the effectiveness of combined exercise regimens (aerobic and resistance training) to improve physiological function in these individuals.

Our findings also provide novel information on the autonomic function of persons with DS. As in most previous studies, we found that DS implicates chronotropic incompetence at peak exercise intensities. This is important because blunted heart rate responses to dynamic exercise have been shown to be of prognostic relevance for subsequent cardiovascular morbidity and/or mortality in several pathological conditions (Lauer et al. 1996). Unfortunately, it is not known if such relationship is also valid for individuals with DS. In an attempt to explore an etiological basis for chronotropic incompetence in DS, we investigated whether the spectral HRV responses to submaximal exercise differed between these individuals and nondisabled controls. Our findings indicate that chronotropic incompetence is also extensive to submaximal workloads. Furthermore, they suggest the presence of poor cardiac responsiveness to changes in the efferent autonomic modulation of the SA node. Therefore, in one way, this corroborates the notion of autonomic dysfunction in DS; however, it also provides evidence of a possible end-organ limitation in responding to a given level of sympathetic or vagal activity. In support of the hypothesis of disturbed autonomic function, we additionally found that adults with DS show a collapse in their fractal heart rate dynamics toward excessive order. Curiously, in contrast to that reported in patients with depressed ventricular function, loss of scale invariance towards Brownian noise has been shown consistently in the elderly (Lipsitz and Goldberger 1992; Iyengar et al. 1996; Goldberger et al. 2002). Consequently, this suggests that DS is associated with premature aging of cardiac autonomic function, and it may well provide a partial explanation for chronotropic incompetence in these individuals. Premature aging could also be a plausible candidate for reduced heart rate recovery post-maximal exercise. As previously shown, cardiodeceleration after peak exercise cessation is also attenuated in the elderly (Cole et al. 1999). Interestingly, while the cardiac autonomic physiology in DS clearly differs from that of healthy controls, most of these differences do not reach the threshold of clinical significance (they lag behind the cut-off values known to be associated with subsequent cardiovascular mortality). This is interesting because there is

compelling evidence that the presence of additional genetic material may indeed protect these individuals against atheroma (Murdoch et al. 1977). For this reason, we speculate that most these autonomic singularities may simply represent progeroid features of DS not necessarily implicating an increased risk of cardiovascular morbidity or mortality.

5.3. Future research

Some questions raised during the previous chapters might constitute interesting topics for further research in DS.

One important finding described in chapter 3 is that DS is associated with reduced walking economy. Even though this has not been previously explored, poor exercise economy may well predispose these individuals to fewer episodes of spontaneous daily physical activity. Ultimately, this would provide a partial explanation for the greater levels of sedentarism and obesity in DS (Stanish and Draheim 2005; Rubin et al. 1998). As importantly, it is plausible to assume that reduced exercise economy may also affect the performance of persons with DS during functional tasks of daily living. As mentioned in chapter 2, Cowley et al. (2010) recently demonstrated a positive relatioship between low VO_{2peak}/low levels of knee extensor strength and functional ability in this population. Considering that the vast majority of functional tasks are of submaximal intensity, it is also likely that reduced exercise economy may negatively impact the performance of persons with DS during activities of daily living. For all these reasons, future research on the relationships between reduced exercise economy, sedentarism, obesity and functional ability should prove invaluable in the field of DS.

In chapter 3 we also demonstrated that a combined aerobic and resistance exercise training regimen corresponds to an effective intervention for improving both peak and submaximal physiological function of adults with DS. Despite the importance of these findings, the field of exercise training in DS remains merely embrionary. There is an urgent need for subsequent research on specific interventions designed to improve both submaximal and peak exercise capacity in DS. It is our belief that "newer" training approaches may be useful for enhancing physiological function in DS. Kaatsu, an alternative method of exercise training with restricted venous blood flow, has proven useful in eliciting gains in muscle hypertrophy and strength in middle-aged individuals (Takarada et al.

2000). Similar findings have also been obtained after only 3 weeks of participation in a Kaatsu-walk training regimen at ~ 20% VO_{2peak} (Abe et al. 2006). Therefore, Kaatsu training (aerobic or resistance training) may be of interest for a population such as DS.

Chapter 4 integrates several studies on cardiac autonomic function of persons with DS. We found that these individuals exhibit an atypical pattern of autonomic response to submaximal exercise. Additionally, our findings indicate that chronotropic incompetence is not limited to peak exercise, as it is also present during submaximal exercise intensities. Further research on determining the chronotropic response to vagal blockade with atropine would be helpful in further clarifying this issue. Additionally, reduced heart rate increase during exercise may also be due to blunted metaboreflex activation during exercise. This is important because the metaboreflex, via type IV afferents, has been shown to greatly contribute to the increase in cardiac output during both static and dynamic exercise (Rowel and O'Leary 1990). Considering that type IV afferents convey essentially nociceptive information to the medulla, and that persons with DS do express pain and discomfort more slowly than the general population (Hennequin et al. 2000), it is plausible that chronotropic incompetence may associate with poor metaboreflex activation during exercise in this population. Finally, the hypothesis of premature aging in the cardiac autonomic function of persons with DS should be further explored. Previous studies have shown that the complexity of force output in adults with DS is similar to that seen in the elderly without DS (Heffernan et al. 2009). This may well be due to senescence of the neural networks that underlie appropriate motor control during voluntary muscle contractions. Because autonomic control is also dependent on the integrity of several neural circuits (central and peripheral autonomic control) it is plausible to speculate that the autonomic function of young adults with DS would be comparable to that of older adults without DS.

5.4. General conclusion

The present thesis provides evidence that adults with DS demonstrate reduced submaximal and peak exercise capacity. Importantly, their peak exercise capacity is not limited by delayed VO_2 kinetics. Our findings also suggest poor cardiac responsiveness to autonomic efferent modulation

under submaximal exercise conditions and a fractal collapse in their R-R interval variability suggestive of premature aging. The contention of disturbed autonomic function is further supported by our results of reduced heart rate recovery from peak exercise intensities in DS. Finally, despite all these relevant deviations from regular physiological function in response to acute exercise, adults with DS respond positively to chronic exercise training. Specifically, we found that they improve their exercise capacity and general autonomic function in similar magnitude as controls without disabilities.

5.5. References

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