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Maximal oxygen consumption in Systemic Lupus Erythematosus

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MAXIMAL OXYGEN CONSUMPTION IN SYSTEMIC LUPUS ERYTHEMATOSUS

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SUMMARY

Systemic Lupus Erythematosus (SLE) is a systemic, chronic, inflammatory, autoimmune disease associated with poor physical fitness. In fact, maximal oxygen consumption (VO_{2max}) is known to be reduced in this population. This reduction is associated with disability and can partially explain the high incidence of fatigue found in SLE patients. Although VO_{2max} has been the focus of many investigations, the mechanisms of reduced VO_{2max} in SLE are not fully understood.

In this thesis the results of four studies are presented. In the first study (presented in two chapters), we validated two submaximal exercise tests for the prediction of VO_{2max} in SLE patients. The use of these inexpensive and practical tests should facilitate the measurement of VO_{2max} in future epidemiological studies and clinical practice. In the second and third study, we investigated the mechanisms of reduced VO_{2max} using two different approaches. In the second study, we demonstrated for the first time that cardiac output was limiting VO_{2max} during whole body exercise. Furthermore, we demonstrated for the first time a significant reduction in a muscle endurance test which is not limited by cardiac output, suggesting that other limitations to exercise might be present. In study three and four, we specifically addressed oxygen metabolism at muscle level, a factor that could explain reduced muscle endurance. We were able to demonstrate that, during exercise, this variable is not affected in our cohort. In conclusion, VO_{2max} in SLE patients with low disease activity and no organ damage is limited by cardiac output and can easily and safely be measured with submaximal exercise tests.

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THESIS FORMAT

This thesis has been written following the "journal format" proposed by Thomas *et al.* (2005). In brief, the main section of the thesis consists of independent stand-alone manuscripts either submitted or to be submitted to relevant peer reviewed journals. Because of this format, there is a necessary overlap between the manuscripts.

This thesis consists of a general introduction, four physiological studies and a general discussion. The first physiological study is split into two chapters presenting the validity of predicting maximal oxygen consumption using two submaximal exercise tests in SLE patients **[chapter two and three]**. **Chapter two** includes detailed information about the methods and the results not present in **chapter three** due to the limitations imposed by the journal in which **chapter three** has been published. In **chapter four** we report the findings of a study investigating the mechanisms of reduced maximal oxygen consumption in SLE patients. The final experimental chapter **[five]** combines two different studies measuring muscle oxygen consumption**.** A general introduction **[chapter one]** provides a background and a justification for the research program. A general discussion **[chapter six]** contains a summary and a critical analysis of the main findings and highlights the strengths of the research programs. Limitations of the thesis and areas of potential future research are discussed separately.

To enhance readability, all chapters are formatted using a modified Harvard style with single reference list appearing at the end of the thesis. Abbreviations are defined at first use in each chapter and a list of abbreviations is included. Tables and figures are numbered consecutively, restarting each chapter. [Square brackets] and/or **bold type** is used when referral is required to section elsewhere within the thesis.

AKNOWLEDGEMENTS

I don't know where to start. I guess it's quite a common feeling for a Ph.D. student to be quite unsure what to write in the acknowledgments. I look back at the last four years of my life and I can think of hundreds of people that should be mentioned here. I've learned something from each one of them, so if you think that your name should be in here, you are right. Some special people do deserve to be mentioned, though.

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LIST OF ABBREVIATIONS

ACSM:	American college of sports medicine
ANOVA:	Analysis of variance
AT:	Anaerobic threshold
ATT:	Adipose tissue thickness
A-V O_2diff:	Difference between arterial and mixed venous blood oxygen content
BP:	Blood pressure
CFS:	Chalder Fatigue Scale
CO:	Cardiac output
CV:	Coefficient of variation
D_{LCO}:	Single breath carbon monoxide diffusing capacity
DMARTs:	Disease modifying anti rheumatic drugs
ECG:	Electrocardiogram
FEV_1:	Forced expiratory volume in one second
FSS:	Fatigue severity scale
FVC:	Forced vital capacity
Hb:	Haemoglobin concentration
HHB:	NIRS derived deoxyhaemoglobin concentration
HbO_2:	NIRS derived oxyhaemoglobin concentration
HR:	Heart rate
ICC:	Intra-class correlation
MAP:	Mean arterial pressure
min:	Minute(s)
MVC:	Maximal voluntary contraction
MVO_2:	Muscle oxygen consumption
MVV:	Maximal voluntary ventilation
NIRS:	Near infra-red spectroscopy
NSAIDs:	Non steroidal anti-inflammatory drugs
O_2 pulse:	Oxygen pulse (VO_2/HR)
r:	Pearson's product-moment correlation

RER:	Respiratory exchange ratio (VO_2/VCO_2)
RPE:	Rate of perceived exertion
s:	Second(s)
SBT:	Siconolfi bike test
SEE:	Standard error of the estimate
SST:	Siconolfi step test
SLE:	Systemic lupus erythematosus
SLEDAI:	Systemic lupus erythematosus disease activity index
SLICC:	Systemic lupus international collaborating clinics
SV:	Stroke volume
VCO_2:	Ventilatory equivalent of carbon dioxide production
VO_2:	Ventilatory equivalent of oxygen consumption
VO_{2max}:	Maximal oxygen consumption

CHAPTER 1

GENERAL INTRODUCTION

This chapter will present the main areas covered in this thesis by reviewing the relevant literature. Because of the multidisciplinary nature of this thesis the first part will be dedicated to provide important information about systemic lupus erythematosus (SLE) and maximal oxygen consumption (VO_{2max}) to facilitate the understanding of the subject to non expert readers in both areas. The second part of the introduction will analyse the factors that might limit VO_{2max} in SLE and the consequences of low VO_{2max} in SLE patients. In particular, this second part of the chapter will analyse in details the clinical features of SLE that could be limiting VO_{2max}.

What is SLE?

Systemic lupus erythematosus is a chronic, inflammatory, autoimmune disease with a complex multifactorial aetiology. It is a multisystem disorder characterised by remissions and exacerbations of symptoms manifested in the musculoskeletal system, skin, kidneys, heart, lungs, and central nervous system (Isenberg and Ehrenstein, 2004). Abnormalities of the immune system are prominent and include the presence of autoantibodies, some of which are characteristic of SLE such as anti-nuclear antibodies (ANA) and anti-double stranded DNA (dsDNA; Isenberg and Ehrenstein, 2004).

The diagnosis of SLE is usually established by a combination of clinical assessment and laboratory analysis and, in 1997, the American College of Rheumatology published revised criteria for the classification of SLE (Hochberg, 1997). In these criteria, eleven major clinical manifestations of the disease were identified. When 4 or more of these criteria are present, the patient can be recognised as having SLE. Clinically, SLE usually manifests itself with non-specific features such as lethargy and fatigue, as well as other features

specific to the organs involved (including arthralgia, rashes, and cardiopulmonary complications; Isenberg and Ehrenstein, 2004).

The disease is more common in women than in men (9:1 ratio), with age of onset typically being between late teens and 40s (D'Cruz *et al.*, 2007). The incidence of SLE is approximately 5 per 100,000 in the USA (Naleway *et al.*, 2005) and 4 per 100,000 in the UK (Hopkinson *et al.*, 1993), but race specific incidence rates show values as high as 31.9 per 100,000 in Afro-Caribbean's compared to the 3.4 per 100,000 in the Caucasian community in Nottingham (Hopkinson *et al.*, 1993).

The highest prevalence has been reported in Italy and Spain with values as high as 71.0 and 91. 0 per 100,000 respectively (Danchenko *et al.*, 2006), compared to an overall prevalence of 24.6 per 100,000 in the UK (Hopkinson *et al.*, 1993). These data are difficult to interpret as these differences might reflect the multifactorial nature of SLE but could also be explained by country specific health care and methodological differences between the studies.

Furthermore, Uramoto and colleagues (1999) reported that the incidence of SLE in the USA is tripled in the last 40 years, with an overall incidence of 5.56 per 100,000 between 1980 and 1992 compared with an incidence of 1.51 per 100,000 in the period from 1950-1979. The authors suggest that this increase might be due to an improved recognition of mild disease rather than a true increase of the incidence of the disease itself.

Aetiopathogenesis

The pathogenesis of SLE is not fully understood and likely to be multifactorial with both genetic and environmental factors involved. Genetic susceptibility to lupus has been linked to several different genes (Harley *et al.*, 2006), in particular genes in the human leukocyte antigen region and deficiency of the complement component of chromosome 1 (C1q). In mice models, C1q deficiency leads to impairment in the clearance of apoptotic cells and results in the accumulation of cellular debris. The acquisition by dendritic cells of the autoantigens displayed by apoptotic cells may activate T cells supporting the secretion of autoantibodies by B cells, and thus starting the autoimmune and inflammatory process (Herrmann *et al.*, 2000, Munoz *et al.*, 2005). Moreover, the high incidence of lupus in females suggests a role for estrogens in the development of SLE, with studies in mice supporting this notion (Cooper *et al.*, 1998). Indeed, it has been shown that sex hormone levels have an effect on immune function in humans (Bouman et al., 2005) and, in vitro, testosterone may suppress anti-DNA antibody production, one of the serological features of SLE, in peripheral blood mononuclear cells (Kanda *et al.*, 1997).

Environmental factors associated with the development of SLE include sunlight and exposure to a number of substances and viruses (D'Cruz et al., 2007). The critical role of sunlight is very apparent, with exposure to the sun seemingly exacerbating disease activity in most patients. One explanation may be the accumulation of apoptotic cells in the skin, which form highly immune-competent tissue (Herrmann et al., 2000).

Some studies suggest that exposure to several substances such as silica dust, aromatic amines, or tobacco (Cooper *et al.*, 1998) could increase the risk of developing SLE. In a review by Parks and Cooper (2006), higher relative risk of developing lupus has been reported in subjects exposed to silica and solvents with odd ratios between 1.5 and 3.0. The association between SLE and smoking is suggested by the increased risk of double stranded DNA seropositivity, a key serological feature of SLE, found in current smokers (Freemer *et al.*, 2006).

Epstein-Barr virus has also been identified as a possible factor in developing SLE. For instance, a high frequency of B cells infected with the Epstein-Barr virus has been reported in lupus patients (D'Cruz et al., 2007). Finally, many drug induced SLE cases have been reported in the literature, with more than 80 different drugs associated with the development of SLE. Amongst this, minocycline, statins, and anti-TNF-alpha agents have been recently implicated (Sarzi-Puttini *et al.*, 2005).

Autoantibodies

Autoantibodies are a key feature for the diagnosis of SLE and are therefore central to the concept of SLE itself. Autoantibodies can be defined as antibodies directed against one or more of the individual's own organs, tissues or proteins. In SLE, the great diversity of clinical manifestation is accompanied by the large number of autoantibodies found in this population. In fact, more than 100 autoantibodies have been detected in SLE patients (Sherer *et al.*, 2004), some of which are present in the vast majority while others are detected only in a minority of patients.

The importance of detecting autoantibodies in SLE patients is demonstrated by the study by Arbuckle *et al.* (2003). This group demonstrated that some autoantibodies (i.e. ANA) are present in patient's serum years before the diagnosis of the disease, while others appear only months before diagnosis (anti Sm). Interestingly, the steadily increase in autoantibodies ceases after treatment is initiated, with levels remaining constant thereafter (Arbuckle *et al.*, 2003).

Because of the huge number of autoantibodies detected, this paragraph will focus on autoantibodies with high diagnostic relevance as a more thorough description of this complex topic is beyond the scope of this thesis.

Antinuclear antibodies (ANA) are present in the vast majority (90-95%) of the patients (Sherer *et al.*, 2004) despite not being specific for SLE; in fact, they can be found in serum of patients with other autoimmune and rheumatic diseases (Hiepe *et al.*, 2000). Due to their frequency in this population they are included as one of the diagnostic criteria for SLE (Hochberg, 1997). Moreover, the clinical value of detecting these autoantibodies is proven by the fact that specific reactivities of ANA are associated with different clinical features and symptoms: for example antiribosomal P antibodies are associated with haematological symptoms (Hoffman *et al.*, 2004).

Anti DNA antibodies are a subgroup of ANAs that bind single or double stranded DNA with a high prevalence in SLE patients (40-80%)(Hiepe *et al.*, 2000). Anti double stranded DNA are highly specific to SLE and therefore are included as diagnostic criteria

(Hochberg, 1997). Interestingly, this class of autoantibodies is believed to contribute substantially to the development of associated disease, such as nephritis (Hiepe *et al.*, 2000).

Finally, the third autoantibodies included in the diagnostic criteria are the anti-Sm antibodies. This group of antibodies target a family of RNA binding proteins which are very important for maintaining the proper three dimensional structure of RNA. The prevalence is very variable (5-70%) and, similarly to anti DNA antibodies are characteristic of SLE patients (Hiepe *et al.*, 2000).

Clinical features and treatment

The clinical manifestations of SLE are diverse and include non-specific as well as organ/system specific features (Isenberg and Ehrenstein, 2004). Of the non specific features, fatigue is very common and disabling in this population. A paragraph dedicated to this symptom will be found in this general introduction [Consequences of low VO_{2max} in SLE-VO_{2max} fatigue and disability].

A list of organs/systems specific features can be found in table 1 in the next page. A thorough discussion of each one of them is beyond the scope of this thesis. However, in the second part of this introduction a detailed analysis of common clinical features that could potentially limit VO_{2max} in SLE will be found. In particular, cardiovascular, pulmonary, haematological and muscle involvement will be reviewed. Moreover, in the final part of the

introduction the high incidence of cardiovascular mortality in SLE patients will be discussed.

Usually, SLE patients are treated with a combination of non steroideal anti inflammatory drugs (NSAIDs), corticosteroids, and disease modifying anti-rheumatic drugs (DMARDs) such as immunosuppressant (Methotrexate and Azathioprine) and anti-malarian drugs (Hydroxychloroquine). New treatments using low dose cyclosphamide and biological agents such as Rituximab have proven to be effective especially in patients unresponsive to standard treatment (D'Cruz *et al.*, 2007).

Table 1. Clinical features in SLE patients

Organs and systems	Clinical features
Muscles and joints	Arthralgia
	Subcutaneus nodules
	Myalgia
Skin	Butterfly rash
	Photosensitivity
	Chronic discoid lesions
Cardiovascular system: Heart	Pericardial disease
	Myocardial disease
	Valvular disease
Blood vessels	Atherosclerosis
	Arterial stiffness
	Microvascular disease
Blood	Anaemia of chronic disease
	Autoimmune hemolytic anaemia
	Iron deficiency anaemia
Kidneys	Lupus nephritis
Nervous system	Cerebral involvement
	Peripheral neuropathy
	Psychological aspects
Lungs	Pleurisy
	Pleural effusion
	Interstitial fibrosis
	Pulmonary hypertension

Some of the most common clinical feature of SLE (modified from Isenberg and Ehrenstein, 2004)

Disease activity and organ damage

The complexity of SLE makes it very difficult to clinically monitor disease activity and organ damage progression. *Disease activity* refers to the potentially reversible impairment, whilst *damage* refers to the irreversible changes caused by SLE.

Disease activity relates to the transient exacerbations and remission of the disease; periods of increased disease activity, which are often referred to as "flares", are characterised by a wide range of changes in the clinical manifestations of symptoms. Increased levels of blood markers of inflammation (erythrocyte sedimentation rate and, sometimes but not always, C reactive protein) and self-reported symptoms (fatigue and pain) can be measured during disease exacerbations. Flares usually require changes in medication such as an increase in corticosteroids and/or immunosuppressant dose. During "flares" there are increases in levels of endothelial adhesion molecules (Belmont *et al.*, 1994), interpheron alpha, and markers of complement dysregulation (Merrill and Buyon, 2005).

This wide range of clinical manifestations of SLE illustrates the difficulties in monitoring disease activity. To address this problem, standardised indices for disease activity have been developed, and these can be broadly divided into two categories: individual organ/system scales and global score systems (Griffiths *et al.*, 2005). An example of the former is the BILAG (British Isles Lupus Assessment Group), while examples of the latter include the SLEDAI (Systemic Lupus Erythematosus Disease Activity Index), the ECLAM (European Community Lupus Activity Measures) and the SLAM (Systemic Lupus Activity Measures). All these indices are reliable and sensitive to changes in disease activity (Haq

and Isenberg, 2002, Lam and Petri, 2005). Recently the BILAG has been demonstrated as more suitable than the SLEDAI to detect changes in disease activity requiring adjustments in therapy (Yee *et al.*, 2007). These scales assess disease activity by monitoring different organs and systems potentially affected by SLE, as well as blood test results, to produce a score reflecting overall disease activity.

Implementation of these instruments has led to the identification of three different patterns of disease activity: Relapsing-remitting, chronic active, and long quiescent (Barr *et al.*, 1999). *Relapsing-remitting* is characterised by period of high disease activity interspersed by a period of disease inactivity. *Chronic active* is defined as disease activity being persistently active for at least a year. *Long quiescent* refers to disease activity being persistently inactive for at least one year. The authors report that the most common patterns identified, when using a modified SLEDAI, are chronic active (40%) and relapsing-remitting (35%).

With improved survival of the patients due to early diagnosis and improved treatment, the cumulative organ damage due to SLE has received more attention in the literature. Organ damage appears to be linked to the presence of the autoantibodies forming immune complexes in the tissues. For example, in glomerulonephritis, autoantibodies to double stranded DNA appear to lead to complex formation in situ and a similar mechanism could account for other SLE related manifestation such as vasculitis (Kotzin, 1996). The importance of monitoring damage over time is demonstrated by the fact that this variable has a high mortality predictive value. Rahman *et al.* (2001) found that damage within 1 year from diagnosis was able to predict mortality and a similar result was published by

Nived *et al.*(2002). In fact, this group demonstrated that damage scores registered 5 years after diagnosis could predict mid term (7 years) survival in SLE patients.

In order to assess progression of damage in SLE, the SLICC (Systemic Lupus International Cooperative Clinics) damage index has been created (Gladman *et al.*, 1996a). The SLICC damage index is both a reliable and valid measure (Gladman *et al.*, 1997) and the scoring is based on the number of permanently damaged organs that affect the patient (Gladman *et al.*, 1996a, Gladman *et al.*, 1997). Damage is not necessarily related to disease activity; in fact, Gilboe *et al.* (2001) found an increase in damage over time in spite of stable health status. The authors suggest that this could be due to the metric properties of the scales or side effects of treatment as the index is not able to differentiate between damage due to disease, damage due to co-morbidities and damage due to medications. This study suggests that in spite of the absence of a correlation between damage and disease activity in cross-sectional studies (Gladman *et al.*, 1996b), the latter is nonetheless the best predictor of the former over time. The same finding has been replicated in other studies (Stoll *et al.*, 2004, Becker-Merok and Nossent, 2006), providing further evidence to support the need to constantly monitor disease activity.

Maximum oxygen uptake

What is it?

Maximum oxygen uptake can be defined as the highest rate at which oxygen can be utilised by the human body during strenuous exercise (Bassett and Howley, 2000). Wagner in 1996 pointed out that, due to the different values of VO_{2max} found in different environmental

conditions (i.e. different oxygen concentration such as high versus low altitude conditions) within the same individual, this definition of VO_{2max} is referred to any given circumstances determining physiological end environmental conditions (Wagner, 1996). The definition of this classical exercise physiology variable was developed from the seminal works of Hill and colleagues in the 1920s (Hill and Lupton, 1923).[1]

Oxygen consumption increases linearly with muscle work output and is considered to be maximal when a plateau in oxygen uptake is demonstrated despite an increase in exercise intensity. The operational definition of plateau is matter of debate with the most commonly used definition developed for multi-day discontinuous protocols (change in $VO_2 \leq 150$ ml min^{-1}; Taylor *et al.*, 1955) often challenged by more stringent definitions (for example, change in $VO_2 \leq 80$ ml min^{-1}; Astrand 1960). Recently, an even more strict definition of plateau (change in $VO_2 \leq 50$ ml min^{-1}; Astorino *et al.*, 2005) has been proposed and applied.

The existence of the plateau phenomenon itself has been questioned as some populations (sedentary and elderly healthy subjects) do not commonly demonstrate this phenomenon (Howley *et al.*, 1995). For this reason, alternative criteria such as respiratory exchange ratio (RER ; the ratio between oxygen consumption and carbon dioxide production), blood lactate concentration, and age adjusted estimate of some percentage of maximal heart rate (HR) have been used as secondary criteria.

[1] It has to be noted that in recent years the concept of VO_{2max} itself has been criticised by some authors. The debate concerning this topic is very interesting and important but goes beyond the scope of this thesis. The interested reader can analyse thoroughly this topic by consulting the article Bassett & Howley (2000).

The American College of Sports Medicine (ACSM, 2000) proposed a combination of different secondary criteria in order to establish if oxygen consumption really reached a maximal value during exercise. The ACSM criteria include an RER > 1.15, blood lactate concentration > 8mMol L^{-1}, rate of perceived exertion ≥ 17 on 6-20 Borg scale, and achievement of a plateau in HR (ACSM, 2000). These criteria have been subject to some criticism themselves as other cut off values also have been used, but it has been suggested that the use of predetermined objective criteria such as RER and blood lactate concentration increases the probability that a true VO_{2max} has been achieved (Duncan *et al.*, 1997).

Factors affecting VO_{2max}

The main factors affecting VO_{2max} in humans are age, physical activity, genetic background, gender, and health status. Studies from the middle of the twentieth century, like the classic Astrand study in 1960, were the first to show a decline in VO_{2max} with age. This finding has been confirmed by both cross sectional (Weiss *et al.*, 2006) and longitudinal investigations (McGuire *et al.*, 2001, Stathokostas *et al.*, 2004). The mechanism for this age related decline in VO_{2max} is believed to be multifactorial, although primarily due to a concomitant decrease in cardiac function and peripheral function, as reflected by arterial-venous oxygen difference (McGuire *et al.*, 2001, Weiss *et al.*, 2006). Indeed other factors such as loss of muscle mass due to alterations in endocrine function, loss of neuromuscular function, and change in protein metabolism (Thomas, 2007) contribute to the overall loss of fitness observed in healthy elderly humans.

Gender differences in oxygen consumption are evident from childhood throughout adulthood and are believed to be due to dimensional dissimilarities in variables such as cardiac volume, haemoglobin concentration, and muscle mass (Jones, 1997). Indeed gender differences affect the aging process itself as men demonstrate a greater decline in VO_{2max} than women (Weiss *et al.*, 2006).

In recent years progresses in understanding of the human genome have highlighted the presence of a range of genes contributing to physical performance. A range of genes linked to better response to exercise training and associated with better exercise haemodynamics have been identified (Rankinen *et al.*, 2006). This area of research is still in its infancy and the relationship between genotypes and phenotypes is still not clear. It is important to highlight that genetic differences in physical performance are not related to ethnic background. In fact, when elite athletes from different ethnic background (African vs Caucasian) were compared, no differences in VO_{2max} were found (Lucia *et al.*, 2006) thus demonstrating that other factors (for example exercise efficiency) are responsible for the better results obtained in international competition by eastern Africa athletes.

Health status is a better recognised determinant of VO_{2max} as demonstrated by a plethora of studies in patients affected by a wide range of diseases (Marrades *et al.*, 1996, Gosker *et al.*, 2000, Jones and Killian, 2000, Keyser *et al.*, 2003). In chronic diseases, exercise is limited both directly and indirectly by changes due to the illness. Direct limitations to exercise refer to changes in the organ affected by the pathology; for example, changes in cardiac function in patients with chronic heart disease (Sullivan and Cobb, 1992). Indirect limitations to exercise refer to changes in the body caused by the pathology but not in the

organs primarily affected. An example of this is the changes in muscle mass and structure observed in patients with chronic obstructive pulmonary disease (Gosker *et al.*, 2000).

It can be added that in diseased populations physical inactivity is often regarded as the cause of the decline in VO_{2max}. Indeed, in healthy subjects, physical activity is a major determinant of VO_{2max} as demonstrated by cross sectional investigations (Wagner *et al.*, 1992) and classic studies on physical training and detraining (Saltin *et al.*, 1968). The same is true in patients affected by chronic diseases; however, in these populations physical activity could be contributing, and probably not causing, the overall decline in VO_{2max}. In fact, comparing VO_{2max} between patients and sedentary healthy subjects (Tench *et al.*, 2002, Keyser *et al.*, 2003) suggests that disease related mechanism could be more important. Certainly, one of the problems with this approach is that healthy controls are often self reported as sedentary and actual physical activity is not measured, thus limiting the validity of this conclusion. Studies directly measuring physical activity and VO_{2max} are needed to address this problem.

Maximum oxygen uptake in SLE

Maximal oxygen consumption in SLE patients has been measured in different studies in the last twenty years (Robb-Nicholson *et al.*, 1989, Sakauchi *et al.*, 1995, Forte *et al.*, 1999, Ramsey-Goldman *et al.*, 2000, Tench *et al.*, 2002, Keyser *et al.*, 2003, Carvalho *et al.*, 2005, Clarke-Jenssen *et al.*, 2005, Bostrom *et al.*, 2008). Exercise modality and aim of the studies have been different among these investigations and the following section will analyse in details each publication individually.

Robb-Nicholson and colleagues in 1989 were the first to measure VO_{2max} in their training study. At baseline they found an average VO_{2max} of 18.8 ml $kg^{-1}min^{-1}$ using an incremental protocol on a cycle ergometer in a group of SLE patients with low disease activity. After 8 weeks of moderate intensity aerobic training they found a non significant 18.6% improvement in VO_{2max}. The absence of a healthy subjects control group limited the possibility of interpreting the baseline data. However, they reported high values of peak HR and RER as secondary criteria for maximal effort demonstrating that a true maximum was achieved by the subjects.

In 1995, Sakauchi *et al.* tried to increase the understanding of exercise limitation in SLE patients by analysing cardiac response during a cycle-ergometer test. Similarly to Robb-Nicholson *et al.* (1989), Sakauchi and colleagues found an average VO_{2max} of 18.4 ml $kg^{-1}min^{-1}$ but did not report any maximal value for RER or HR. This study claimed reduced muscle uptake using the oxygen pulse method but unfortunately that finding is not supported by their data. Oxygen pulse (O_2 pulse) is calculated as the ratio between oxygen consumption and HR. The Fick equation states that:

(Equation 1) $$VO_2 = HR \times SV \times (A\text{-}V\ O_2diff)$$

where SV is stroke volume (the amount of blood pumped every beat) and A-V O_2diff is the difference between arterial and mixed venous blood oxygen content. Therefore O_2 pulse can be calculated as:

(Equation 2) O_2 pulse = VO_2 / HR = SV x (A-V O_2diff)

From Equation 2 it follows that a reduction in O_2 pulse can be caused by a reduction in SV and/or a reduction in A-V O_2diff; the authors' assumption that SV should be unchanged because they selected a group with no cardiopulmonary complications is not valid as many studies demonstrate a very high incidence of subclinical heart disease in SLE (Doherty and Siegel, 1985).

The investigation by Forte *et al.* (1999) was the first to measure VO_{2max} in SLE patients and a group of self reported sedentary healthy controls. They reported an average result of a cycle-ergometer of 17.4 ml $kg^{-1}min^{-1}$ for the SLE patients, and suggested that this reduction in VO_{2max} is due to peripheral factors because the difference in maximal HR was only 12% compared to the 50% reduction in VO_{2max}. Moreover they used an indirect measure similar to Sakauchi and colleagues (1995) to reinforce their hypothesis. In fact, they reported that cardiovascular adaptations to exercise were adequate. These arguments cannot be accepted, similarly to Sakauchi's hypothesis, because in this study no measure of SV and haemoglobin were performed and therefore no estimation of oxygen delivery can be determined from HR alone.

More information about exercise limitations were obtained by the studies by Ramsey-Goldman *et al.* (2000) and Tench *et al.* (2002) by adding a measure of muscle strength. Ramsey-Goldman *et al.* (2000) was the first study to use exercise on a treadmill to measure VO_{2max} but, unfortunately, the absence of a control group and no criteria for maximal exercise in this study makes it hard to draw any definite conclusions. Moreover, this study

was designed only as a small pilot investigation (n = 5) on the effect of different exercise regimes in SLE and they reported no significant increase in VO_{2max} from the value measured at baseline (30.87 ml $kg^{-1}min^{-1}$). The high VO_{2max} reported seem to indicate that this group was less representative of the SLE population compared to previous studies.

By contrast, the study by Tench *et al.* (2002) adds a significant amount of knowledge to the previous literature. The authors reported their results as VO_{2peak} rather than VO_{2max} because approximately 30% of the participants failed to meet the criteria for maximal exercise. In this study there was a significant decrease in VO_{2peak} (23.2 ml $kg^{-1}min^{-1}$) during treadmill exercise, muscle strength, and static lung volumes in the SLE group compared to healthy sedentary controls. The authors tentatively suggested that this reduction in exercise capacity could be partly explained by impaired lung function, but this explanation is in contrast with data demonstrating no arterial desaturation at peak exercise in SLE patients (Forte *et al.*, 1999). Interestingly, the authors reported no significant differences in perceived exertion at submaximal level during the treadmill exercise test. The implication of this finding will be discussed in another paragraph of this introduction [Consequences of low VO_{2nax} in SLE].

Data from Tench *et al.* 2002 were used as baseline for a 12 weeks training study by the same group (Tench *et al.*, 2003). They reported no significant training effect on VO_{2peak} and argued that this could be due to the intensity chosen for their mixed supervised-unsupervised exercise intervention (60% of VO_{2peak}). According to ACSM guidelines (ACSM, 2000) for exercise prescription this intensity is appropriate in healthy subjects thus raising the question if SLE patients might require more intense exercise to show changes in

VO_{2peak}. Alternatively, lack of supervision in some part of the exercise training might explain their results.

Keyser *et al.* (2003) confirmed previous findings on exercise limitation in SLE patients reporting a VO_{2peak} of 19.2 ml $kg^{-1}min^{-1}$ during treadmill exercise. They suggested that microangiopathy, leading to diminished oxygen delivery and utilisation at muscle level, were the most likely mechanisms causing this reduction in VO_{2peak}. The authors did not support this hypothesis with any data and therefore their suggestion remains purely speculative. The studies described above (Tench *et al.*, 2002, Tench *et al.*, 2003, Keyser *et al.*, 2003) presented some interesting analysis of the relationship between exercise capacity, fatigue and disability that will be discussed separately in this thesis [Consequences of low VO_{2nax} in SLE].

Two small pilot studies (Carvalho *et al.*, 2005, Clarke-Jenssen *et al.*, 2005) on the effect of exercise in SLE patients have been recently published, confirming the safety of exercise programs in SLE and showing some interesting significant changes in VO_{2max}. Clarke-Jenssen *et al.* (2005) reported that patients significantly increased VO_{2max} from 25.1 ml $kg^{-1}min^{-1}$ to 28.1 ml $kg^{-1}min^{-1}$ after 12 weeks of treadmill exercise at 70% of maximum HR and this increase was still significant at the follow up, 15 weeks after the end of the training.

Similarly, Carvalho *et al.* (2005) showed a significant increase in VO_{2max} from 22.6 ml $kg^{-1}min^{-1}$ to 24.3 ml $kg^{-1}min^{-1}$ after 12 weeks of treadmill exercise at the anaerobic threshold (approximately 65% VO_{2max} in this study). Both studies used only slightly higher exercise intensity than the one used by Tench *et al.* (2003) but they supervised exercise, thus

suggesting that the lack of increase in Tench *et al* (2003) could be due to poor compliance to exercise, especially in the self reported home exercise sessions.

Finally, a very recent study by Bostrom *et al.* (2008) reported a VO_{2max} of 21.2 ml $kg^{-1}min^{-1}$ and analysed the relationship between aerobic capacity, physical function and disease activity. Their findings on this relationship will be discussed in another paragraph of this thesis [Consequences of low VO_{2nax} in SLE].

There is general agreement across research studies that there is a reduction in exercise capacity in subjects suffering with SLE compared to healthy controls (Robb-Nicholson *et al.*, 1989, Daltroy *et al.*, 1995, Sakauchi *et al.*, 1995, Forte *et al.*, 1999, Ramsey-Goldman *et al.*, 2000, Tench *et al.*, 2002, Keyser *et al.*, 2003, Carvalho *et al.*, 2005, Clarke-Jenssen *et al.*, 2005, Bostrom *et al.*, 2008). Most of the studies directly measuring VO_{2max} report values between 17.4 and 25.1 ml $kg^{-1}min^{-1}$.

An interesting implication that can cautiously be drawn from all the training studies previously described is that the reduction in VO_{2max} can be considered a direct consequence of the disease process and not a result of physical inactivity. Indeed, the fact that no studies have directly measured physical activity and the short term of these interventions limit the validity of this suggestion. Taking these very important limitations into account, subjects at the end of these studies are less sedentary than the rest of the SLE population, however they still present low levels of oxygen consumption suggesting that factors other than physical inactivity must contribute to the reduction in VO_{2max}.

Taken together, very low results of incremental tests do not seem to be due to poor effort during the incremental tests because RER and percent of maximal HR, when reported, show values that can be considered associated with maximal effort. In fact, an average RER of approximately 1.15 and a average maximal HR above 90% of the age predicted maximal value have been consistently presented (Robb Nicholson *et al.* 1989, Tench *et al.* 2002, Keyser *et al.* 2003, Carvalho *et al.*2005). These data, therefore, clearly demonstrate a severe impairment of the oxygen transport system.

An interesting question is how much these studies can be considered representative of the totality of the SLE population. The heterogeneity of SLE suggests that external validity of these results could be limited. In fact, participants in these investigations are drawn from a selected group of moderately young patients with a calculated mean age of approximately 37 years, without organ involvement and low disease activity. As this cohort does not necessarily represent the majority of SLE patients it is possible to speculate that the real average VO_{2max} for the totality of SLE patients could be lower then indicated in these investigations.

Factors limiting VO_{2max} in healthy humans and SLE patients

Each one of the steps of the oxygen pathway from ambient air to mitochondria could potentially limit VO_{2max}. It is still not clear whether a single limiting factor or multiple limiting factors exist. The mechanisms through which each step can lead to a decrease in VO_{2max} will be individually discussed below, focusing on the specific mechanisms which may limit oxygen consumption in SLE.

Recalling the Fick principle (Equation 1), we can now state that for maximal exercise:

(Equation 3) $$VO_{2max} = HR_{max} \times SV_{max} \times (A\text{-}V\ O_2diff_{max})$$

In this thesis we will use Equation 3 as a framework to describe the physiology of the oxygen transport system and to discuss the possible VO_2 limitations in SLE by analysing separately the factors limiting VO_{2max}, which is the variables affecting the right hand side of Equation 3: $HR_{max} \times SV_{max} \times (A\text{-}V\ O_2diff_{max})$.

Factors limiting VO_{2max} in healthy humans

There is an overall agreement that VO_{2max}, in healthy individuals performing whole body exercise at sea level, is mainly limited by convective delivery of oxygen to the exercising muscles (di Prampero and Ferretti, 1990, Bassett and Howley, 2000, Bergh *et al.*, 2000, Saltin and Calbet, 2006, Brink-Elfegoun *et al.*, 2007, Levine, 2008, Noakes *et al.*, 2008), with some additional limitation provided by oxygen diffusion from capillary to mitochondria (Roca *et al.*, 1989).

Di Prampero and Ferretti in 1990 created a mathematical model to investigate the oxygen pathway from the lungs to the mitochondria in order to determine the factors limiting oxygen consumption in healthy non athletic humans at sea level. Given the limitation of being purely theoretical, the model shows that oxygen consumption is not limited by a single factor. The role of pulmonary ventilation and diffusion seem to be minor (changing their values leads to negligible modification in VO_2), while convective blood oxygen

transport is found to be responsible of 70% of overall limits of VO_{2max} with the remaining 30% shared between capillary oxygen diffusion and mitochondrial utilisation.

Ekblom (2006) proposed that the vast majority of experimental evidence identifies cardiac function as limiting VO_{2max}, while scientists arguing peripheral limitation rely instead on theoretical models. Experimental evidence has demonstrated that the absolute value of VO_{2max} is dependent on the muscle mass activated during exercise, but the limiting factor is the heart's pumping ability and not peripheral extraction (Brink-Elfegoun *et al.*, 2007). In fact, this study found a VO_{2max} of 3.32 L min^{-1} for maximal arm exercise, 4.46 L min^{-1} for maximal leg exercise, and 4.70 L min^{-1} for maximal arm and leg exercise combined. These data show very clearly that convective delivery of oxygen and not peripheral factors limit VO_{2max}: If peripheral factors were limiting VO_{2max} the total value during arm and leg exercise should be equal to the sum of leg VO_{2max} and arm VO_{2max} individually. The value obtained by doing this (7.78 L min^{-1}) is 40% higher than the actual measurement (4.70 L min^{-1}), thus proving the cardiac limitation here.

The mechanism behind this finding can be found in data published by Secher *et al.* (1977) where the authors demonstrated that adding arm exercise while maintaining the leg power output caused a reduction in leg blood flow. This finding demonstrates that cardiac output (CO) was unable to maintain blood flow when another metabolically active area was added. Indeed, additional data from controlled experiments manipulating CO, for example by reducing blood volume, show a reduction in VO_{2max} when the former variable is diminished (Krip *et al.*, 1997).

Theoretical models from Wagner and colleagues, supported by some experimental evidence (Roca *et al.*, 1989), show that peripheral limitations, such as muscle oxygen conductance, are also important (Wagner, 1996). Certainly, these authors do not dispute that part of the limitation comes from convective oxygen delivery to the muscles, but they highlight that VO_{2max} is a balance between delivery and subsequent diffusive oxygen movement to the mitochondria (Roca *et al.*, 1989). This idea is in agreement with the previously mentioned mathematical model by di Prampero and Ferretti (1990).

Possible mechanism of reduced VO_{2max} in SLE

No studies have specifically investigated the factors limiting VO_{2max} in SLE patients and therefore the following paragraphs will be dedicated to the analysis of the possible mechanisms causing this limitation. In the following pages published studies will be presented in order to understand potential limitation in oxygen consumption. As previously mentioned this part of the introduction will be divided according to the Fick equation to facilitate understanding.

Cardiac function

Cardiac output is the amount of blood ejected in one minute by either the left or right ventricle. It is a very important functional parameter of the cardiac muscle: It is determined by the product of HR and SV, that is the amount of blood ejected in one beat. In humans stroke volume is determined by left ventricular contractility and by other important parameters as shown by Equation 4:

(Equation 4) $SV = [LVEDV \times LVEF \times (1\text{-}MRF)]$

where LVEDV is the left ventricular end diastolic volume, LVEF is the left ventricular ejection fraction, and MRF is the fraction of blood that is not ejected in the aorta but is "ejected back" in the atrium due to a malfunctioning mitral valve. The latter parameter is normally zero in healthy humans but might change in diseased populations reducing SV. From the Fick equation (Equation 3), it is clear that a reduction in CO_{max} will determine a reduction in VO_{2max} and therefore any changes in the variables in the right hand of the SV equation and/or in HR_{max} will lead to this result.

While previous studies reported no evidence of reduction in HR_{max} during exercise in SLE (Tench *et al.*, 2002, Keyser et al., 2003), no data on SV have been published so far. Despite this, some investigations on factors affecting Equation 4 at rest allow us to speculate that this variable could limit CO_{max} and VO_{2max} in SLE.

In the study of cardiac function, a reduction in LVEF is referred to as *systolic dysfunction*, while an increase in left ventricular diastolic pressure, which in turn is affecting LVEDV, is referred to as *diastolic dysfunction*. Changes in these variables will reduce SV and, in fact, this has been demonstrated in patients with chronic heart failure (Sullivan and Cobb, 1992, Farr *et al.*, 2008).

Left ventricular dysfunctions, both systolic and diastolic, have been demonstrated, not always consistently, in SLE patients (Sasson *et al.*, 1992, Astorri *et al.*, 1997, Kalke *et al.*, 1998, Pieretti *et al.*, 2007). The results of studies on diastolic dysfunction in SLE seem to

show an impaired relaxation of the left ventricle at rest, with some data suggesting higher incidence in patients with active disease. Sasson *et al.* in 1992 and Kalke *et al.* (1998) found impairment in left ventricular relaxation in a cohort of SLE patients with no evidence of cardiac disease. Interestingly, in both studies patients with active disease had more frequent and more pronounced left ventricular impairment. Sasson and colleagues reported that this impairment persisted at the 7 month follow up, with only a trend towards improvement in association with a decrease in disease activity. This finding was not confirmed by the study by Astorri *et al.* (1997). This group in fact found diastolic impairment at rest but no relationship with disease activity. The authors suggest that this could be due to the small number of patients with active disease in their cohort.

More controversy exists on the existence and extent of systolic dysfunction in SLE patients. While Bahl *et al.* (1992) found no difference in systolic function at rest, Paran and colleagues (2007) propose that a lower fractional shortening might be indicative of systolic dysfunction. Unfortunately neither of these studies included a control group therefore it is hard to draw definitive conclusions from these results. Pieretti *et al.* (2007) found no difference in systolic function but interestingly they demonstrated a higher prevalence of left ventricular hypertrophy in SLE compared to controls. The authors suggested that left ventricular hypertrophy is associated with the presence of SLE (*per se)*, especially in the contest of higher blood pressure and vascular stiffness. Finally, a very recent study patients using cardiovascular magnetic resonance and involving a small number of patients suggested that ejection fraction is preserved in SLE (Keenan *et al.*, 2008).

The mechanisms causing systolic and diastolic dysfunction in SLE are not fully understood but pathologies such as myocarditis, pericarditis, and mitral regurgitation (Schlant and Roberts, 2001) could lead to these dysfunctions. Myocarditis can reduce systolic function by reducing cardiac contractility (Tschope *et al.*, 2004), pericarditis by increasing mechanical constraints on the heart (Rowell, 1993) and mitral regurgitation by affecting SV, as shown in Equation 4.

Cardiovascular manifestations of SLE are common, and were brought to the attention of the medical community when Libman and Sacks in 1924 described cases of "nonbacterial verrucous endocarditis" (cited in Doherty and Siegel, 1985). Since then, many studies have investigated the nature and prevalence of cardiac involvement in patients with SLE. It is believed that the prevalence is more than 50% (Moder *et al.*, 1999), depending on what manifestations are included and whether symptomatic or asymptomatic cardiac diseases are included in the analysis. Cardiac abnormalities can involve many anatomical structures of the heart, including the pericardium, myocardium, valves, coronary arteries, and conduction system (Manzi *et al.*, 2004, Roman and Salmon, 2007).

Myocarditis has a prevalence of 9% in SLE (Wijetunga and Rockson, 2002) but asymptomatic myocarditis might happen in a higher proportion of the patients. In fact, post mortem studies show a higher prevalence of subclinical myocardial disorders with an average prevalence ranging between 40 and 57% (Doherty and Siegel, 1985). Some studies indicate that myocarditis in SLE seems to be mediated by immune-complex (Wijetunga and Rockson, 2002) and circulating autoantibodies (Nihoyannopoulos *et al.*, 1990), but indeed other contributing factors such as hypertension, coronary artery disease, and medication might be directly or indirectly related (Moder *et al.*, 1999).

Pericarditis, the acute or chronic inflammation of the pericardium, has been found to be the most common cardiac manifestations of SLE with prevalence between 11 and 54% (Moder *et al.*, 1999, Doria *et al.*, 2005). It is believed that symptomatic pericarditis occurs in approximately a quarter of SLE patients, and more than a half might have asymptomatic pericarditis during the course of their life (Doria *et al.*, 2005). This is supported by the high percentage of evidence of pericarditis found in combined autopsy studies (62%; Doherty and Siegel, 1985). Complications of pericarditis appear to be rare in SLE patients, with only a small percentage of cases demonstrating pericardial tamponade (Doherty and Siegel, 1985) and constrictive pericarditis (Doria *et al.*, 2005).

Other cardiovascular manifestations of SLE include valvular disease and conduction tissue abnormalities. Libman-Sacks verrucous vegetations are usually clinically silent but have been found in 47% of patients in autopsy studies (Doherty and Siegel, 1985) and can affect all valves but are most frequently found on the left sided valves (Schlant and Roberts, 2001). Moreover, mitral regurgitation and valve thickening have been described (Moder *et al.*, 1999), with the former potentially affecting Equation 4. Conduction tissue abnormalities are less common in SLE and include atrio-ventricular and bundle branch blocks (Doria *et al.*, 2005).

It should also be noted in this section that both atheritis and atherosclerosis can affect the coronary arteries of SLE patients, but these co-morbidities will be reviewed in details in another paragraph of this introduction [Consequences of low VO_{2nax} in SLE], which is

dedicated to this very important topic and its relevance to the risk of developing myocardial infarction and coronary heart disease in SLE patients.

Arterial oxygen content

A decrease in arterial oxygen content could limit VO_{2max} in SLE by reducing the availability of oxygen to be extracted by the exercising muscles. This decrease in arterial oxygen content can result from two different factors: Impairment in respiratory function and/or decreased oxygen carrying capacity of the blood. Systemic oxygen delivery is in fact the product of CO and the arterial oxygen content. The latter factor is determined by the concentration of haemoglobin, its saturation and binding ability (Hebert *et al.*, 2004).

Exercise limitation due to respiratory system impairment mainly manifests itself in lower oxygen saturation at maximal exercise and it is believed to affect VO_{2max} only when arterial saturation falls below 95% (Dempsey and Wagner, 1999). Mayers (2005) suggested an alternative approach to assess pulmonary limitation by analysing breathing reserve at maximal exercise: Breathing reserve is the difference between maximal minute ventilation during exercise and the maximal voluntary ventilation (MVV). Breathing reserve suggests pulmonary limitation to maximal exercise when the value is lower than 11 L min^{-1} or 20% MVV.

Respiratory function

Ambient air is moved in and out of the respiratory system by the action of the respiratory muscles and the elastic properties of the chest. When inspired air reaches the respiratory

bronchioles, the convective movement is slowed down by the increase in total surface area and the process of diffusion moves the oxygen molecules from areas of higher partial pressure to areas of lower partial pressure. Oxygen partial pressure in the alveoli is always higher than in venous blood, and therefore oxygen molecules diffuse into the blood stream. The rate of diffusion is mainly determined by the pressure gradient between alveoli and blood, but the relation between alveolar ventilation and alveolar capillary perfusion as well as the diffusive properties of the alveolar capillary membrane are important.

In healthy subjects, a reduction in pulmonary function is less likely to reduce maximal oxygen consumption than reductions in other steps of the oxygen pathway (di Prampero and Ferretti, 1990). Nonetheless, functional impairment of the respiratory system could manifest itself in various clinical populations as feeling of dyspnea during exertion.

From a clinical perspective the most common feature affecting the respiratory system in SLE is pain due to pleurisy, the inflammation of the pleura. Pleuritic pain –pleuritis- is present in 40–60% of patients; pleural effusions are found in 20–30% (Isenberg, 2004), with a higher percentage of pathologic pleural findings in autopsy studies (50-83%; Paran *et al.*, 2004). Other complications such as pneumonitis, interstitial lung disease and "shrinking lung" syndrome are less common in SLE patients (Kao and Manzi, 2002).

Pulmonary hypertension (PH) found in SLE patients could be of particular interest for the pathogenesis of exercise limitation. PH is a well described, not very common, consequence of SLE with prevalence between 5 and 14% (Kao and Manzi, 2002), with a possible higher prevalence masked by mild unrecognised forms of the co-morbidity (Paran *et al.*, 2004).

The aetiology of PH in SLE is still not understood (Fagan and Badesch, 2002), but the pathogenesis and clinical features are similar to idiopathic pulmonary artery hypertension (Chung *et al.*, 2006). These similarities are of great interest because it has been demonstrated that subjects suffering with idiopathic pulmonary arterial hypertension have severe reduction in VO_{2max}, even in mild cases of the disease (Sun *et al.*, 2001), and a reduced left ventricular stroke volume response to exercise (Nootens *et al.*, 1995, Holverda *et al.*, 2006).

Interstitial lung disease is another rare complication of SLE that could potentially affect respiratory physiology. The estimated prevalence of symptomatic interstitial lung disease is 3%, with higher prevalence (13%) found in autopsy studies (Pego-Reigosa *et al.*, 2009). From a functional point of view it manifests itself as a restrictive reduction of lung volume and a reduction in lung diffusing capacity. Both these changes can potentially affect the ability of the patients to perform exercise.

To our knowledge, only one study investigated pulmonary and systemic haemodynamic response to exercise in SLE. Winslow *et al.* (1993) found an increased mean pulmonary arterial pressure during submaximal and maximal exercise, accompanied by a difference in systemic diastolic pressure at maximal exercise. Unfortunately, this study did not measure stroke volume to better understand cardiac function but it is possible to speculate based on results in patients with idiopathic pulmonary arterial hypertension that this variable could be causing the reduction in exercise duration. The authors proposed that the most likely mechanism for increased pulmonary pressure was an increased pulmonary vascular resistance.

From a functional point of view, patients with SLE often demonstrate reduced forced vital capacity and reduced forced expired volume in 1 second (Worth *et al.*, 1988, Tench *et al.*, 2002). Tench and colleagues (2002) suggested that these reductions could potentially contribute to exercise limitation and this principle is supported by studies in chronic obstructive pulmonary disease patients. In this population it was traditionally believed that limitation to exercise was the result of a combination of reduced ventilatory capacity and increased ventilatory demand (Butcher and Jones, 2006). Although this idea has recently been challenged with the proposal that other factors such as cardiac and muscular deficiencies play an important role (Gosker *et al.*, 2000), Marin *et al.* (2001) showed that parameters like inspiratory capacity correlate with VO_{2max} and the sensation of dyspnea.

The most common functional impairment in SLE patients is a reduction in lung diffusion capacity (Paran *et al.*, 2004). A study on 110 Japanese SLE patients found that 52% had a reduction in single breath diffusion capacity and this was seen even in patients without restrictive pattern or pulmonary fibrosis (Nakano *et al.*, 2002). Isenberg (2004) suggested that diffusion capacity is reduced when there is a reduction in number of alveolar capillaries, a mismatch between ventilation/perfusion and a reduction in haemoglobin concentration. The possible role of diffusion limitation as a factor contributing to the development of exercise intolerance in SLE patients is suggested by studies on chronic heart failure patients, where a correlation between reduced diffusion and reduce VO_{2max} has been found (Agostoni *et al.*, 2002). Indeed, Ville *et al.* (1998) showed that after transplant, cardiac patients with lower diffusion capacity have a lower exercise capacity than patients with normal diffusion capacity. The possibility that diffusion capacity is the main factor

limiting exercise is questioned though by the lack of hypoxemia (a sign of ventilatory limitation) in the low diffusion capacity group in Ville *et al.* (1998).

Oxygen carrying capacity and the effects of anaemia

The amount of oxygen carried in solution in the blood is regulated by Henry's law, and it is determined by oxygen partial pressure and its solubility coefficient. Due to the low solubility of oxygen, only a very small amount is dissolved in plasma, with most of the molecules forming a reversible chemical bond with haemoglobin, an iron containing protein found in red blood cells with a very high affinity for oxygen. The oxygen saturation of haemoglobin is dependent on oxygen partial pressure, and the relationship between the two is not linear but rather sigmoid, S-shaped: At low oxygen concentration (muscles) haemoglobin unloading is favoured, while at high oxygen concentration (alveoli) uploading is favoured.

Exercise limitation is a well recognised consequence of acute and chronic anaemia with a decrease in performance directly proportional to a decrease of arterial haemoglobin content (Sproule *et al.*, 1960, Woodson et al., 1978). This has been demonstrated in both healthy subjects with experimentally induced anaemia and patients with chronic diseases (Woodson *et al.*, 1978, Marrades *et al.*, 1996).

Lupus patients demonstrate different forms of anaemia: Anaemia of chronic disease, autoimmune haemolytic anaemia, and iron deficiency anaemia (Voulgarelis *et al.*, 2000). Voulgarelis *et al.* (2000) showed that anaemia of chronic disease, a mild normocytic-

hypochromic anaemia linked to chronic inflammation (Means and Krantz, 1992), is the most common form of anaemia in SLE, and is associated with higher scores in disease activity measures.

The main mechanism causing anaemia in SLE does not appear to be autoimmune haemolysis, whereas reduced EPO secretion and T cell mediated inhibition of haemopoiesis have been proposed as more likely mechanism for anaemia (Schett *et al.*, 2001, Giannouli *et al.*, 2006). Nevertheless, autoantibodies to human EPO have been found in anaemic SLE patients (Voulgarelis *et al.*, 2000), therefore anaemia in this population is likely to be caused by multiple mechanisms operating independently or simultaneously.

From a clinical perspective, SLE patients demonstrate only mild anaemia with haemoglobin values ranging between 9 and 11 g/dl (Voulgarelis *et al.*, 2000) but such values are associated with a significant reduction in exercise capacity. In fact, Woodson *et al.* (1978) experimentally induced a reduction in haemoglobin from 15.3 to 10.0 g/dl in a group of healthy subjects without changes in total blood volume. The induction of anaemia caused a significant reduction in VO_{2max} from 43 to 36 ml $kg^{-1}min^{-1}$ acutely, and a further reduction to 30 ml $kg^{-1}min^{-1}$ after two weeks of sustained low haemoglobin concentration.

Mixed venous blood oxygen content

An increase in mixed venous blood oxygen content for the same level of arterial oxygen is associated with a lower utilisation by exercising muscles. Recalling the Fick equation (Equation 3), the increase in venous blood content will in turn diminish VO_{2max} by reducing

A-V O_2diff_{max}. Decreased consumption of oxygen in the muscle could be due to impairment in blood distribution and/or decreased oxygen utilisation at muscle level.

Cardiac output is distributed to the different areas of the body according to the metabolic needs of each area by controlling the contraction of the smooth muscles in the arteries. Impairment in blood distribution, i.e. lack of appropriate vasodilation in arteries serving muscle districts or impaired vasoconstriction of non active areas, could limit VO_{2max} by reducing oxygen availability. Moreover, changes in the microcirculation in the muscles, such as decreased capillary density or capillary wall thickening, could increase the distance between blood and muscle cells, thus reducing their function.
Another possible source of impairment could be found at the muscle cell level where mitochondrial dysfunction could lead to a diminished oxygen utilisation resulting in a reduced A-V O_2diff_{max}.

Blood flow

During exercise blood is redistributed to the appropriate areas by vasodilation of peripheral arteries connected to exercising muscles and vasoconstriction of other arteries. When oxygen reaches the capillary bed it diffuses from high to low partial pressure areas, i.e. from the blood stream into the interstitial space and from there into the muscle cells where, facilitated by the presence of myoglobin, it reaches the mitochondria.

Reduced blood flow to active muscles could be caused by changes in all parts of the vascular tree from arteries to capillaries. Poor muscle perfusion was demonstrated both at

rest and during plantar flexor exercise in a group of SLE patients using radionuclide methods (Hsu *et al.*, 2004, Lin *et al.*, 2004). The authors suggested that a diminution in blood flow to the limbs could be caused by alterations in peripheral arterial function, an impairment of the microcirculation or, most likely, by a combination of these factors.

Peripheral arterial disease (PAD) has been described in SLE patients, and it is believed to be caused by accelerated atherosclerosis due to endothelial injury caused by chronic inflammation (McDonald *et al.*, 1992, Henke *et al.*, 2003). Exercise intolerance is a hallmark of PAD, where the pathological restriction of blood flow to the peripheral arteries can lead to muscle pain, claudication, and gangrene (Beard, 2000). Interestingly, even in asymptomatic PAD patients there is a reduction in physical function independent of other co-morbidities (McDermott *et al.*, 2000).

Endothelial dysfunction is believed to be the starting point responsible for arterial disease in SLE patients by facilitating the formation of atherosclerotic plaques. A brief review of the development of atherosclerosis in SLE patients can be found in another paragraph of this thesis [Consequences of low VO_{2max}]. Impairment in endothelium dependent vasodilation following experimental ischemia has been largely demonstrated in SLE (Piper *et al.*, 2001, Lima *et al.*, 2002, El-Magadmi *et al.*, 2004, Piper *et al.*, 2007). Hyperaemia following arterial occlusion is believed to be dependent on the activity of the endothelial cells, mainly due to their release of nitric oxide (NO; Celermajer, 1997). The role of traditional risk factors in developing endothelial dysfunction in SLE is not fully understood. Piper *et al.* (2001) found a correlation between cholesterol levels and flow mediated dilation, while result from El-Magadami *et al.* (2004) showed that the impairment persisted

even after controlling for traditional risk factors (older age, high blood pressure, high cholesterol, diabetes etc.). This study suggested that some SLE specific factors might be involved. Of all the possible candidates, inflammation is believed to be the main factors causing endothelial dysfunction. In fact it has been demonstrated that experimentally induced acute inflammation impairs endothelial function mainly by reducing NO availability (Hingorani *et al.*, 2000, Clapp *et al.*, 2004).

An interesting study by Wright *et al.* (2006) showed that reduced flow mediated dilation in SLE patients might be caused by microvascular impairment as well as peripheral arteries endothelial dysfunction. This is very relevant as it is currently believed that endothelial dysfunction and autoantibodies are mainly involved with the genesis of vasculitis in the small vessels as much as they are in larger arteries (Guillevin and Dorner, 2007).

General agreement can be found on the frequency of microvascular disease in SLE but the extent and the nature of the changes are sometimes controversial. Functional capillary density, the number of capillaries with moving blood cells per mm^2, was found to be reduced in the skin of SLE patients (Bongard *et al.*, 1997). By contrast, Dancour and colleagues (2006) found no difference in functional capillary density using nailfold videocapillaroscopy. The most likely explanation of these contradicting results is the lower disease activity in the group studied by Dancour and colleagues (2006). Finol *et al.* (1990) and Pallis *et al.* (1994) found a thickening of the basement membrane in muscle biopsies. This finding was not confirmed by Dancour and colleagues (2006), but the authors suggest that vascular parameter could be altered during periods of exacerbation.

Reduction in capillary density and an increase in membrane thickness will reduce the amount of oxygen available to the muscles and affect VO_{2max} (Wagner, 2000), but studies on acute inflammation suggest that other factors could contribute to reduced blood flow. Animal models of acute inflammation show a patchy maldistribution within the same muscle, with some areas over-perfused and other areas under-perfused (Ellis *et al.*, 2002). Moreover, in similar animal models, problems with coagulation and formation of microthrombi in small vessel have been shown, with further decreased flow by obstructing otherwise perfused intact capillaries (Levi *et al.*, 2003).

Mitochondrial function

Mitochondria are specialised organelles within the cell where oxygen is used to oxidise high energy substrates, such as glycogen and fatty acid, to produce adenosine tri-phosphate (ATP) in order to allow muscles to contract and maintain external work. Adenosine tri-phosphate is in fact used by the sliding myofilaments within muscle cells to detach from each other at the end of a contraction. The biochemical reactions within the mitochondria are commonly referred to as aerobic metabolism and can be broadly divided into two main processes: Krebs cycle and the electron transfer chain.

Oxygen utilisation in the mitochondria at the muscle cell level has been identified as a limiting factor in healthy humans (Roca *et al.*, 1989). To the best of our knowledge no studies have used muscle mitochondria to investigate oxidative phosphorylation and its potential role in limiting energy production in SLE patients. On the other hand, different studies suggest that mitochondrial dysfunction could be present in this population.

An investigation by Perl *et al.* (2004) found evidence of mitochondrial membrane disruptions in lymphocytes taken from a group of SLE patients. These changes will disrupt oxidative phosphorylation and are believed to be a starting point for cell apoptosis. Moreover, mitochondria in lymphocyte cells have been previously used to differentiate between patients with muscle mitochondrial disorders and healthy controls (Marriage *et al.*, 2003), and are more easily available than muscle mitochondria because of the less invasive nature of the procedure. Therefore, it is reasonable to speculate that muscle mitochondria might demonstrate the same mitochondrial membrane disruptions of lymphocyte mitochondria.

This suggestion is supported by other studies in SLE patients and studies on different clinical populations. The presence of anti mitochondria antibodies (AMA) has been demonstrated in SLE patients, especially subjects with liver complications (Li *et al.*, 2006). More generally, high prevalence of AMA has been found in patients with connective tissue disease, with approximately 40% of the lupus cohort found to be AMA positive (Mouritsen *et al.*, 1986).

Disease related direct mechanisms suggesting mitochondrial dysfunction, such as AMA, can be accompanied by the damage due to indirect factors such as drug treatment. In fact, Mitsui and colleagues (2002) found that long term administration of prednisolone caused mitochondrial dysfunction in a group of patients with mixed chronic diseases compared with healthy controls. Mitochondrial dysfunction was measured as difference in lactate production following constant load exercise and with the use of muscle biopsies.

Interestingly, this investigation found a correlation between corticosteroids total dose and post exercise lactate production. To confirm the importance of steroid administration and overcome some of the limitations of the cross sectional comparison, 25 subjects were then tested before the start and throughout prednisolone therapy. The post exercise lactate production was significantly elevated in all the patients, demonstrating a disruption of oxidative phosphorylation (Mitsui *et al.*, 2002).

More support for the hypothesis that mitochondrial function could be affected by SLE is provided by studies investigating the effect of acute inflammation in septic patients. The reason for this is that, theoretically, chronic long term low grade inflammation could cause mitochondrial changes similar to the ones caused by acute inflammation.
Both patients with acute septic shock and patients in a later phase of sepsis demonstrate mitochondrial dysfunction, and this appears to be caused by a lower mitochondrial density rather then inhibition of specific enzymes (Brealey *et al.*, 2002, Fredriksson *et al.*, 2006). The pathogenesis of mitochondrial dysfunction in response to acute and chronic stress, such as inflammation, is not fully understood; however mitochondria appear to play a crucial role in response to different stressors and are currently receiving great attention from researchers (Manoli *et al.*, 2007).

Consequences of low VO_{2max} in SLE

VO_{2max} fatigue and disability

The relationship between VO_{2max} and fatigue has been the focus of attention for many investigations on physical fitness in SLE patients. The rationale behind this hypothesis is

clearly explained by Keyser *et al.* (2003): These authors demonstrated that oxygen required for activity of daily living in SLE patients is often close to peak VO_2, and even lighter activities are above the ventilatory threshold, the level at which exercise is beginning to be perceived as fatiguing. Perceived exertion during exercise is highly correlated with the percentage of percent of VO_{2max} (Robertson *et al.*, 2004) needed to perform physical tasks. Therefore, SLE patients perform activity of daily living work at a higher percentage of their maximum than healthy subjects and perceive these tasks as more physically demanding. For that reason it is not surprising that Keyser *et al.* (2003) found a correlation between VO_{2peak} and fatigue.

The correlation found by Keyser *et al.* (2003) was only moderate though, suggesting that other factors might be involved with fatigue. This idea is supported by Tench *et al.* (2002) who found that in their regression model the best predictor of fatigue measured by the fatigue severity scale (FSS) were depression and quality of sleep, with no significant changes in the prediction when VO_{2peak} was included as a factor. In the same study, VO_{2peak} explained only 3% variability in the regression model when visual analog scales for fatigue were used.

It has to be highlighted that the measures of fatigue used in these studies, and in many other studies in SLE, are designed to assess fatigue as a whole, thus including factors such as mental fatigue. Consequently, these results can be explained by the fact that fatigue is a multifactorial construct and both physiological and psychological elements are involved in determining the severity of this symptom. A better operational definition might be needed to determine the relationship between physical fitness and fatigue. Perceived exertion

during physical tasks could be a valid measure of fatigue when exercise is considered. To the best of our knowledge only one study reported RPE during submaximal exercise (Tench *et al.*, 2002) and found no difference between SLE patients and controls. Indeed, this was not the primary aim of this study and thus further research is needed to draw a definitive conclusion.

The relationship between VO_{2max} and disability seems to be more significant with the former variable explaining a substantial amount variability of the latter variable. Tench *et al.* (2002) found that VO_{2peak} was the best predictor of disability as determined by physical function using the SF36 questionnaire and exercise duration. In their regression models, physical function from SF36 was used as a more subjective assessment of disability, while exercise duration was chosen as a more objective measure: In the first model VO_{2peak} on its own explained 35% of the variance, while in the second model VO_{2peak} explained 57% of variance. Similarly, Bostrom *et al.* (2008) recently demonstrated that VO_{2max} correlated with self reported physical function.

On the contrary, Houghton *et al.* (2008) found no correlation between VO_2 and disability in her group of patients with pediatric lupus. The difference between the studies could be explained by the different populations recruited in these studies, especially given the young age of the participants in Houghton *et al.* (2008).

VO_{2max} and cardiovascular risk

Since the bimodal mortality pattern was described (Urowitz *et al.*, 1976), great attention has been given in the literature to the increased cardiovascular risk found in SLE patients

(Manzi *et al.*, 1997, Ward, 1999, Svenungsson *et al.*, 2001, Bruce *et al.*, 2003). Urowitz and colleagues (1976) showed that active SLE and high incidence of infection was the main cause of death in patients who die early in the course of the disease. In contrast, those who die late in the course of the disease have low disease activity and an increased incidence of myocardial infarction.

Results from many different epidemiological studies agree that SLE patients are at higher risk of cardiovascular diseases compared to the general population. Most of the studies report a 6-20% increase in prevalence of cardiovascular events in women with SLE (Ward, 1999). This risk is more evident in the younger females, where the risk of myocardial infarction in the age group 35-44 has been found to be 50 times higher (Manzi *et al.*, 1997).

It is of great interest that traditional risk factors do not fully explain the relative odds in the SLE population (Esdaile *et al.*, 2001) and therefore some Lupus specific factors must contribute to this increased risk. After correcting for traditional risk factors, Esdaile *et al.* (2001) found that SLE patients had 8 times higher risk of cardiovascular events than the control population. Traditional risk factors associated with cardiovascular events in SLE include hypertension, presence of diabetes (Bruce *et al.*, 2003) and dyslipidemia (Svenungsson *et al.*, 2001) while among SLE specific risk factors older age at time of diagnosis (Manzi, 1997) and presence of lupus antiphospholipid antibodies have also been proposed (Bessant *et al.*, 2006).

It is generally believed that the mechanism for the increased risk of cardiovascular disease in SLE is likely to be multifactorial with most research focusing on accelerated atherosclerosis in the coronary arteries as the leading cause. In fact, Asanuma *et al.* (2003) demonstrated an increased prevalence of atherosclerotic plaques in coronary arteries with chronic inflammation regarded as the main pathogenic pathway.

Accelerated atherosclerosis is a well recognised feature of SLE with a number of studies demonstrating an increased prevalence of atherosclerotic plaques in various arteries (including coronary, carotid etc.) in this population (Asanuma *et al.*, 2003, Roman *et al.*, 2003, Mercado and Avendano, 2005, Ahmad *et al.*, 2007, Westerweel *et al.*, 2007). These studies have found that, similarly to cardiovascular risk, traditional risk factors for the development of atherosclerotic plaques do not fully explain this high prevalence (Esdaile *et al.*, 2001). Ahmad *et al.* (2007) showed that some SLE specific factors such as SLE phenotype, treatment with azathioprine, and presence of autoantibodies are better predictors than traditional risk factors of cardiovascular events. Not surprisingly, patients with longer disease duration and higher damage score tend to have more plaques. (Ahmad *et al.*, 2007; Roman *et al.*, 2003).

To the best of our knowledge, the relative contribution of reduced aerobic fitness to increased cardiovascular risk has not previously been investigated, and is likely to be significant in SLE. It has, in fact, been widely demonstrated that in the healthy population a lower VO_{2max} is associated with higher risk of cardiovascular disease (Carnethon *et al.*, 2005, Gulati *et al.*, 2005, Kallinen *et al.*, 2006). For example, after adjusting for traditional Framingham cardiovascular risk factors, Gulati *et al.* (2005) observed a relative risk of all-

cause mortality in women of low fitness (< 17.5 ml kg^{-1} min^{-1}) 3.1 times higher, compared with women of moderate-high fitness (> 27 ml kg^{-1} min^{-1}).

Moreover, in patients with chronic heart failure, a higher VO_{2max} is associated with a more favourable prognosis at two years follow up (Francis *et al.*, 2000). The most likely reason for this lack of research in SLE is the problems associated with direct measurements of VO_{2max} during incremental exercise. These tests, in fact, require expensive equipment, well trained personnel, and maximal effort from the subjects.

Importantly, low VO_{2max} is a risk factor that can be modified with exercise training. This means that, if the relationship demonstrated in the healthy population in valid in SLE patients as well, exercise studies in the future could focus on the analysis of cardiovascular risk reduction associated with improvement in VO_{2max}. Indeed, a measure of VO_{2max} that can be easily performed would facilitate this important area of research.

AIM OF THE THESIS

The main aim of this thesis was to explore the mechanisms limiting VO_{2max} in a group of SLE patients with low disease activity and no organ damage. In order to do that, we measured all the steps of the oxygen transport chain to demonstrate which ones are primarily affected by SLE. Because of the systemic nature of the disease it is not possible to formulate specific hypotheses. Therefore, our studies can be considered exploratory.

From a clinical point of view, it is desirable to have a method of assessing physical fitness without the risks and costs associated with maximal exercise. Therefore, the secondary aim of this thesis was two validate two submaximal exercise tests to predict VO_{2max} in SLE patients. If proved valid and reliable, these tests may also be used in large epidemiological studies and in clinical practice. Such studies are necessary to determine whether poor VO_{2max} is associated with the high cardiovascular morbidity and mortality found in SLE patients.

CHAPTER 2

VALIDITY AND RELIABILITY OF THE SICONOLFI CYCLE ERGOMETER TEST FOR ASSESSMENT OF PHYSICAL FITNESS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune, inflammatory disease of unknown aetiology (Uramoto *et al.*, 1999). It is a multisystem disease characterised by the presence of multiple autoantibodies. It affects mainly the heart, skin, joints, kidneys, lungs, nervous system, serous membranes, and in some cases other systems (Isenberg and Ehrenstein, 2004). The disease has a high impact on quality of life of the patients as a result of psychological and physical distress (Doria *et al.*, 2004). One of the most common complaints of the patients is fatigue, being the most disabling symptom for 53% of the patients in a study by Krupp and colleagues (1990)

Interestingly, physical fitness as measured by maximal oxygen uptake (VO_{2max}) is an important predictor of physical disability in SLE patients (Tench *et al.*, 2002) and correlates moderately with the symptom of fatigue (Tench *et al.*, 2002). These results are not surprising as the average VO_{2max} measured in SLE patients is of the same magnitude as the energy requirement of many activities of daily living (10 to 20 ml kg^{-1} min^{-1}; Gunn *et al.*, 2005). In fact, in this population VO_{2max} has been found to range between 17.4 ml kg^{-1} min^{-1} and 23.1 ml kg^{-1} min^{-1} (Forte *et al.*, 1999, Tench *et al.*, 2002, Keyser *et al.*, 2003).

Furthermore, reduced VO_{2max} might contribute to the higher risk of dying from coronary heart disease (CHD) and stroke found in this population (Manzi *et al.*, 1997). It has, in fact, been widely demonstrated that in the healthy population a lower VO_{2max} is associated with higher risk of cardiovascular disease (Gulati *et al.*, 2003). It is noteworthy that patients suffering with SLE have a higher risk of CHD even after correcting for traditional risk

factors. Therefore the relative contribution of poor physical fitness to increased cardiac risk in this population is unknown and likely to be substantial (Gulati *et al.*, 2003).

The main reason for this lack of understanding of the contribution of physical fitness towards higher CHD risk is that direct measurements of VO_{2max} during incremental exercise tests require expensive equipment, well trained personnel and maximal effort from the subject. These make the measure very unpractical in the routine clinical assessment and even for research purposes, due to the increased risk of a cardiac event during maximal exercise (Albert *et al.*, 2000).

A more practical alternative to maximal exercise tests is the use of prediction equations based on a) the relationship between heart rate (HR), VO_2 and workload during steady-state submaximal exercise, and b) age-predicted maximum HR (Noonan and Dean, 2000).
To our knowledge only one such submaximal exercise test has been validated in patients with rheumatic diseases (Minor and Johnson, 1996). This test consists of walking at a self-selected pace for two bouts of 4 min at 0% and 5% incline. However, only 5 of the 30 patients involved in that study suffered from SLE. Therefore, the validity of this predictive test remains uncertain in this specific population. Moreover, some patients might not be able to perform the test because musculoskeletal manifestations are very common in SLE. In fact, 88% of patients has evidence of arthritis (pain, tenderness, effusion, soft-tissue swelling) at the time of diagnosis with involvement of the knee in 76% of the patients (Rothfield *et al.*, 2006).

Therefore, the aim of this study was to assess the validity and reliability of the Siconolfi bike test (SBT), a modification of the Astrand Rhyming test, originally developed in healthy subjects (Siconolfi *et al.*, 1982) to predict VO_{2max} in SLE patients.

Methods

Subjects

Thirty adult SLE patients willing to take part in the study and able to give written informed consent were recruited for this criterion validity study. All the subjects had to fulfil the American College of Rheumatology 1997 revised criteria for SLE (Hochberg, 1997). Patients were excluded if they had severe cardio-pulmonary pathology, history of ischemic heart disease, severe myositis, active nephritis, active neurological disease, or pregnancy. Clearance for participation in the study was given by consultant rheumatologists from the rheumatology departments in Ysbyty Gwynedd, Ysbyty Glan Clwyd, and Maelor Wrexham Hospital. The study was approved by the North West Wales Local Research Ethics Committee.

Procedures and risk assessment

All recruited subjects attended two testing sessions in the Physiology Lab of the School of Sport, Health and Exercise Sciences, Bangor University. These two sessions were separated by at least one and not more than two weeks, and were scheduled at the same time of the day. Subjects were instructed to avoid any strenuous activity for 24 hours prior to testing and to avoid a heavy meal, caffeine, or nicotine within 3 hours from testing. During visit 1, patients performed the SBT as described below. Before the exercise test, participants were screened for risk factors of coronary heart disease (ACSM, 2000) as maximal exercise increases the risk of a cardiac event (Albert *et al.*, 2000). This screening was performed by a research assistant trained in the relevant procedures. For this purpose resting blood pressure (BP) and total serum cholesterol were measured. Blood pressure was measured by

auscultation of the brachial artery and total serum cholesterol was measured from a 30 μL sample of arterialised blood taken from the fingertip using an enzymatic technique (Reflotron. Boehringer Manneheim, Germany). The risk factors, the risk category and the action taken are shown in Table 1.

During visit 2, patients' lung function was assessed and the maximal exercise test was performed as described below.

Siconolfi bike test (SBT)

After this evaluation, subjects performed the SBT as previously validated in a cohort of healthy subjects (Siconolfi *et al.*, 1982).The protocol started with an initial exercise load of 0W for women and men older than 35 years. No men younger than 35 years were involved in the study therefore the different protocol required for this age group was not performed. After the initial stage the work rate was incremented by 25 W every 2 minutes until subjects achieved the target heart rate of 70% of the predicted maximal heart rate as calculated from the equation 220-age. Throughout the test heart rate was monitored continuously by telemetry (Model S810, Polar, Finland) and recorded at the end of each stage.

Maximal exercise test

The incremental exercise test was performed on a cycle ergometer (874E, Monark, Sweden) keeping a constant pedalling rate of 50 rpm. The protocol used in this study is the same as the one used by Siconolfi *et al.* (1982). This protocol consisted of two minutes cycling against no resistance followed by increments of 25 W every 2 min until volitional exhaustion. Throughout the test, expired gases and flow were analysed breath-by-breath using an automated system (600Ergo Test, ZAN Messgeräte, Germany) from which VO_2 and carbon dioxide production (VCO_2) were calculated and averaged every 30 seconds. This device was calibrated prior to each test using a 3-litre syringe across a wide range of flow rates, and using a gas of known concentrations of oxygen (12%) and carbon dioxide (5%) according to the manufacturer instruction manual.

During the incremental bike test, heart rate was monitored continuously by telemetry (Model S810, Polar, Finland) and recorded at the end of each stage. Rate of perceived exertion was assessed in the last 10 seconds of each incremental stage using the Borg 6-20 scale using a memory anchoring procedure as suggested by the author of the scale (Borg, 1982). Finally, lactate concentration in arterialised blood (fingertip) was measured five minutes after the end of the maximal test using an enzymatic technique (Lactate Pro, Arkray, Japan).

For this study measured VO_2 was considered to be maximal when the criteria used in the original validation study by Siconolfi *et al.* (1982) were met. These were 1) a plateau defined as a difference less than 0.25 L min^{-1} between the two final VO_2 measurements

taken every 30s, and 2) a respiratory quotient (RER = VCO_2/VO_2) for the last measurement equal or greater than 1.0.

Other measures

In the first testing session, subjects' height and weight were measured using standard equipment and procedures and body mass index (BMI) calculated as weight in kg height^{-1} in m^2. Haemoglobin concentration was also measured (B-hemoglobin, Hemocue AB, Sweden) from arterialised blood taken from the finger tip.

On the second testing session, subjects performed two pulmonary function tests before the incremental maximal test. Pulmonary function tests were performed using a Keystone3 spirometer (Ferraris Group Plc, U.K.) according to the procedures recommended by the American Thoracic Society (ATS, 1994). This device was calibrated prior to each test using a 3-litre syringe according to the manufacturer instruction manual. Pulmonary function tests included Forced Vital Capacity (FVC, the maximal volume of air exhaled with maximally forced effort from maximal inspiration) and one second Forced Expiratory Volume (FEV_1, the volume of air exhaled during the first second of FVC). Every subject performed three acceptable FVC manoeuvres and the best performance was recorded.

VO_{2max} estimation

To estimate VO_{2max} from the submaximal test the calculation validated by Siconlfi *et al.* (1982) were used. The steady-state absolute VO_2 at the final stage of the SBT was calculated from the workload according to the ACSM equation for estimating VO_2 during cycle ergometry:

Stage VO_2 (L min^{-1}) = [(load in Kgm min^{-1} x 2 ml O_2 kgm^{-1}) + (3.5 ml O_2 kg^{-1} min^{-1} x body weight in kg] / 1000

Then, each subject's uncorrected VO_{2max} was estimated using the mathematical version of the original Astrand-Ryhming nomogram (Astrand, 1960) developed by Steven Siconolfi (personal communication):

Uncorrected VO_{2max} (L min^{-}) = Stage VO_2 / $\%VO_{2max}$

where $\%VO_{2max}$ is calculated as:

% VO_{2max} male = (0.769 x Stage HR) – 48.5

% VO_{2max} female = (0.667 x Stage HR) – 42

Alternatively, the uncorrected VO_{2max} can be predicted using the original graphic version of the Astrand-Ryhming nomogram (Astrand, 1960).

Finally, the uncorrected VO_{2max} calculated from the above equations was entered in the following equations developed by Siconolfi *et al.* (1982) to predict VO_{2max} according to age and sex:

VO_{2max} male (L min^{-1}) = (0.348 x uncorrected VO_{2max}) – (0.035 x age in years) + 3.011

VO_{2max} female (L min^{-1}) = (0.302 x uncorrected VO_{2max}) – (0.019 x age in years) + 1.593

Clinical measures

Global disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Bombardier *et al.*, 1992). The SLEDAI is a valid measure of SLE activity (Ward *et al.*, 2000) and it has been found to be responsive to changes in SLE disease activity (Chang *et al.*, 2002). The SLEDAI generates a total score between 0 and 105 with high scores indicating higher disease activity. Organ damage was assessed by the Systemic Lupus International Collaborating Clinics (SLICC) damage index (Gladman *et*

al., 1997). The SLICC produces a score between 0 and 46 with high scores indicating more severe damage. All these measures were completed by each patient's consultant rheumatologist within a maximum of three weeks from exercise testing. Fatigue was self-assessed by the patients using two questionnaires, the Chalder Fatigue Scale (CFS) (CFS; Chalder *et al.*, 1993)(Chalder 1993) and the Fatigue Severity Scale (FSS; Krupp *et al.*, 1990). The CFS is a 14-item questionnaire, each item being scored from 0 to 3 on a Likert scale, generating a total score between 0 and 42 and two sub-scores, a mental fatigue score and a physical fatigue score. High scores represent more severe fatigue. The FSS is a validated nine-item questionnaire, each item being scored from 1 to 7 on a Likert scale and an overall score computed in the range of 1 to 7 with high scores representing more severe fatigue. Both questionnaires have been previously used in studies with SLE patients (Tench *et al.*, 2002).

Data comparison and statistical analysis

To asses the validity of SBT, the highest recorded VO_2 during the maximal incremental test was used as our criterion measure for the comparison with the VO_{2max} predicted by the SBT. To asses reliability of the SBT we compared results of the SBT during the first visit with the results obtained during the appropriate submaximal stage of the maximal incremental test in visit two. The heart rate at the end of same stage reached in test 1 was used to estimate VO_{2max} for the second visit.

Data are reported as mean (standard deviation) for normally distributed variables; otherwise, data are reported as median [interquartile range]. The criterion validity of VO_{2max} predicted from the SBT was assessed using the 95% limits of agreement by Bland and Altman (Bland and Altman, 1986), paired *t* test, Pearson's product-moment correlation

coefficient and intraclass correlation coefficient (ICC) against VO_{2max} measured during the cycling test. Standard error of the estimate (SEE) was also calculated. Reliability of predicted VO_{2max} was assessed using 95% LOA, Pearson's product-moment correlation coefficient and ICC between the first and second visit SBT. Coefficient of variation (CV) was also calculated. For both validity and reliability a two way mixed effects, single measure model of ICC was used. Significance level was set at 0.05 for all analyses.

Results

Patients characteristics

Thirty patients (26 female, 4 males) were recruited and performed all exercise tests. One female patient was excluded after failing to meet the criteria for the attainment of VO_{2max} during the cycling test leaving 29 patients for analysis. The anthropometric, demographic and descriptive characteristics of these subjects are presented in Table 2.

Patients in this study were not anaemic, only slightly overweight with normal blood pressure and blood cholesterol. Two subjects were currently smokers and five were ex smokers at the time of the test. According to our screening all the subjects belonged to the low risk category as previously defined. Clinical characteristics of the SLE patients are presented in table 3.

This group of patients had long standing disease, low disease activity and very little damage but disabling fatigue. None of the subjects were on beta-blockers at the time of exercise testing, 12 patients were receiving oral prednisolone with daily dosages ranging between 2 and 10 mg, 7 were receiving hydroxychloroquine with daily dosage ranging between 200 and 400mg, 5 were receiving non steroidal anti-inflammatory drugs, 2 were receiving immunosuppressants and 2 were receiving antidepressants. None of these drugs are known to affect VO_{2max} directly.

Exercise tests

The results of the measured VO_{2max} test and the results of the prediction based on the submaximal bike test are presented in table 4. No adverse event occurred during the study, and patients tolerated well both maximal and submaximal exercise testing.

Validity

The scatterplot and Bland-Altman plot of VO_{2max} estimated by the first visit SBT (1.53 ± 0.39 L min^{-1}) against the direct measurement of VO_{2max} during the cycling test (1.57 ± 0.39 L min^{-1}) are displayed in Figure 1 and 2 respectively. The residuals showed no evidence of significant heteroscedasticity. There was a moderately strong correlation ($r = 0.71$, $p < 0.001$), moderately high ICC (0.71, $p < 0.001$) between predicted and measured VO_{2max}, and a non significant negative bias (-0.04 L min^{-1}, $p = 0.504$). The 95% LOA were ± 0.60 L min^{-1}, and SEE was 0.29 L min^{-1}.

The scatterplot and Bland-Altman plot of VO_{2max} estimated by the second visit SBT (1.51 ± 0.44 L min^{-1}) against the direct measurement of VO_{2max} during the cycling test are displayed in Figure 3 and 4 respectively. The residuals showed no evidence of significant heteroscedasticity. There was a moderately strong correlation ($r = 0.73$, $p < 0.001$), moderately high ICC (0.72, $p < 0.001$) between predicted and measured VO_{2max}, and non significant negative bias (-0.06 L min^{-1}, $p = 0.316$). The 95% LOA were ± 0.61 L min^{-1}, and SEE was 0.28 L min^{-1}.

Reliability

The scatterplot and Bland-Altman plot of VO_{2max} estimated by the first and the second visit SBT are displayed in Figure 5 and 6. The residuals showed no evidence of significant

heteroscedasticity. There was a very strong correlation ($r = 0.97$, $p < 0.001$), very high ICC (0.97 $p < 0.001$) between VO_{2max} estimated by the first and second visit SST, and a non significant positive bias (0.02 L min^{-1}, $p = 0.275$). The 95% LOA were ± 0.10 L min^{-1}, and CV was 3.5%.

Discussion

The results of this study demonstrate that the SBT is well tolerated, valid and reliable in SLE patients with well-controlled disease. There was a non significant negative bias between VO_{2max} directly measured during the cycling test both in visit 1 and visit 2 (-0.04 and -0.06 L min^{-1} respectively). This bias is of the same magnitude but opposite direction of the bias found by Siconolfi *et al* (1982) in healthy subjects. In fact, Siconolfi and colleagues found an overestimation of 0.02 L min^{-1}using the SBT for the overall group in their original validation study. Interestingly, the estimation in the female only group in Siconolfi's study (-0.04 L min^{-1}) is in the same direction of the estimation in the present study where most of the subjects (25/29) were females. This might be simply due to chance but there might be a minor underestimation in the gender specific equation created for women in Siconolfi's study.

The validity of the SBT in both visit 1 and 2 is also supported by the significant Pearson's correlation between measured and predicted VO_{2max} ($r = 0.71$ and 0.73 respectively), an ICC > 0.70 and a moderate SEE (0.29 and 0.28 L min^{-1} respectively). Again, these values compare favourably with the results of the original validation study by Siconolfi and colleagues where a SEE of 0.25 L min^{-1} and a correlation of 0.94 were found. The higher correlation in Siconolfi's study is probably due to the larger spread of data due to the bigger number of men involved in that study.

The results from this investigation compare well with Minor and Johnson (1996) who validated a submaximal walking test in patients with a variety of rheumatic diseases,

including SLE. Minor and Johnson found a similar correlation (r = 0.77) and a slightly higher VO_{2max} overestimation (3.1 ml kg^{-1} min^{-1}) to the one obtained in this study in both visit 1 and visit 2 (0.6 and 1.7 ml kg^{-1} min^{-1}respectively). The attractiveness of SBT compared to the treadmill test by Minor and Johnson is that cycling is a non weight bearing exercise and therefore better tolerated in a population which such an high incidence of arthritis or arthritis like symptoms. Furthermore, a cycle ergometer might be more readily available in most physiotherapy departments, thus increasing the applicability of the test.

The estimation error found in our study (3.8% underestimation) is similar to the prediction error of studies validating other submaximal cycle ergometer exercise tests (Teraslinna *et al.*, 1966, Jette, 1979, Macsween, 2001). These investigations found conflicting results using the Astrand Rhyming cycle ergometer test, of which SBT is a modification. In healthy subjects Teraslinna *et al.* (1966) and Macsween (2001) found an overestimation of 7.1% and 6.5% respectively compared to results of a maximal cycle ergometer test. By contrast, Jette (1979) found a 14.4% underestimation using the same test. Pearson's correlation between predicted and measured VO_{2max} in these studies ranged between 0.47 and 0.92. Even bigger overestimation was found by Greiwe *et al.* (1995) when validating the ACSM submaximal cycle ergometer test (percent error 25.3% and correlation r = 0.86).

Overall, the SBT developed in healthy subjects cross-validate well in SLE patients and is well tolerated. However, participants in our study represent a subgroup of SLE patients with well-controlled disease. Therefore, our results can not be readily generalised to the full SLE population. Nevertheless, health professionals should be aware of the relatively high margin of error of the SBT when predicting VO_{2max} in individual patients as revealed by the

wide 95% limits of agreements (Figure 2 and 4). Therefore, maximal exercise tests can not be substituted when a precise individual assessment of physical fitness is required. This is true not only for submaximal tests in SLE patients but also for the healthy population.

The very strong test-retest Pearson's correlation, very high ICC, low CV, non-significant bias, and relatively narrow limits of agreement (Figure 6) demonstrate that the SBT is highly reliable in SLE patients. Again, these results compare well with reliability of the test validated by Minor and Johnson (1996). Similarly, these results are in agreement with the reliability result of other submaximal cycle ergometer test in healthy people (Greiwe *et al.*, 1995, Hartung *et al.*, 1995). Overall these results highlight the fact that, like in most submaximal tests in healthy, a familiarization session for the SBT does not seem to be necessary as results obtained during visit one are not statistically different form results obtained in visit two ($p = 0.134$).

It is clear from these results that physical fitness is poor even in non-anaemic, non-obese SLE patients with well-controlled disease and preserved lung function such as the ones included in our study. If we consider that moderate activities of daily living require up to 21 ml kg^{-1} min^{-1} (Gunn *et al.*, 2005), it is no surprise that low VO_{2max} contributes to physical disability and fatigue in SLE as patients are forced to use a high percentage of their functional reserve (Tench *et al.*, 2002, Keyser *et al.*, 2003).

Furthermore, we calculated that only 26% of our cohort had a VO_{2max} higher than 85% of the normal predicted value (Gulati *et al.*, 2005). This finding is clinically relevant given that healthy women with an exercise capacity less than 85% of their predicted value have

twice as high risk of death from all causes (2.44 for cardiac disease) than women with a normal VO_{2max} (Gulati *et al.*, 2005). Unfortunately, available epidemiological studies on CHD in SLE patients have focused on other traditional risk factors (e.g., high cholesterol) and inflammation (Manzi, 1997, Svenungsson *et al.*, 2001), and did not include a measure of VO_{2max}. Poor physical fitness is a highly modifiable risk factor for CHD, and we hope that the validation of a simple, inexpensive, submaximal exercise test to predict VO_{2max} in SLE patients will facilitate future research in this important area.

In conclusion, the SBT is reasonably valid, highly reliable and safe in SLE patients with well-controlled disease. The use of a cycle ergometer increases the feasibility of the test in SLE patients with musculoskeletal manifestations compared to the test published by Minor and Johnson (1996). Indeed, a cycle ergometer might not be available in a clinical setting and therefore validation of other tests using equipment more accessible in a busy clinic might be needed.

Tables and figures

Table 1. Risk factors for coronary heart disease (ACSM, 2000), along with inclusion/exclusion criteria.

	Risk Factor
1	Age above 45 for men and above 55 for women;
2	Family history: myocardial infarction or sudden death before 55 years of age in father or other male first-degree relative, or before 65 years of age in mother or other female first degree relative;
3	Current cigarette smoking
4	Hypertension: Uncontrolled blood pressure ≥140/90 mmHg
5	Hypercholesterolemia: total serum cholesterol ≥200 mg dL^{-1} (5.2 mmol L^{-1}) or HDL≤ 35 mg dL^{-1} (0.9 mmol L^{-1}).
6	Diabetes mellitus
7	Sedentary lifestyle: defined as less than 30 minutes of moderate physical activity two times a week.
8	Body weight: percentage of body fat ≥ 39% for women aged 20-39 years, ≥ 41% for women aged 40-59 years and ≥ 43% for women aged 60-79 years. Percentage of body fat ≥ 26% for men aged 20-39 years, ≥ 29% for men aged 40-59 years and ≥ 31% for men aged 60-79 years.
	Risk Category and Action
High risk	One or more symptoms of cardiopulmonary disease or with known cardiovascular or pulmonary disease. Action taken: not admitted to the study
Moderate risk	Three or more of the above listed risk factors. Action taken: admitted to the study. Three lead ECG will be used during the maximal test
Low risk	Less than three of the above listed risk factors. Action taken: admitted to the study with no further actions.

Table 2. Characteristics of the SLE patients (25 females and 4 males) included in the analysis

Characteristic	Mean	(SD)
Age (yrs)	48	(14)
Height (cm)	164	(9)
Body mass (kg)	71.5	(13.7)
BMI (kg m^{-2})	26.5	(4.5)
Cholesterol (mMol L^{-1})	4.56	(0.93)
Systolic Resting BP (mmHg)	123	(12)
Diastolic Resting BP (mmHg)	80.4	(8.0)
Haemoglobin (g dL^{-1})	13.1	(1.3)
FVC (L)	3.17	(0.81)
FVC (% of predicted value)	92	(22)
FEV_1 (L)	2.49	(0.72)
FEV_1/FVC (%)	79.0	(10)

BMI = Body Mass Index; BP = Blood Pressure; FVC = Forced Vital Capacity; FEV_1 = forced expired volume in 1 second.

Table 3. Characteristics of the SLE patients (25 females and 4 males) included in the analysis

Characteristic	Mean or Median	(SD) or [interquartile range]
Disease Duration (yrs)	10	(9)
SLEDAI (0-105)	0.0	[0.8]
SLICC damage score (0-46)	0.0	[0.0]
Fatigue Severity Scale (1-7)	4.9	[1.2]
CFS Total Score (0-42)	17	[10.5]
CFS Physical Fatigue	10	[6.5]
CFS Mental Fatigue	7	[4.5]

SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; SLICC = Systemic Lupus International Collaborating Clinics; CFS = Chalder Fatigue Scale.

Table 4. Characteristics of the SLE patients (25 females and 4 males) included in the analysis

Characteristic	Mean	(SD)
Absolute VO_{2max} (L min^{-1})		
Measured	1.57	(0.39)
Visit 1 Predicted	1.53	(0.39)
Visit 2 Predicted	1.51	(0.44)
Relative VO_{2max} (mL kg^{-1} min^{-1})		
Measured	22.4	(6.0)
Visit 1 Predicted	21.8	(5.1)
Visit 2 Predicted	20.7	(6.4)
Max Heart rate (beats min^{-1})	162	(17)
% of the Predicted max HR achieved during VO_{2max} test	94.4	(7.1)
Max RER	1.19	(0.08)
Max RPE	18.0	(1.49)
Post exercise Lactate (mMol L^{-1})	6.55	(1.88)

VO_{2max} = maximal oxygen uptake; RER = Respiratory Exchange Ratio; RPE = Rate of Perceived Exertion

FIGURE 1: Scatterplot of VO_{2max} predicted by the first visit SBT against the direct measurement of VO_{2max} during the cycling test. The diagonal line represents the identity line (slope = 1; intercept = 0).

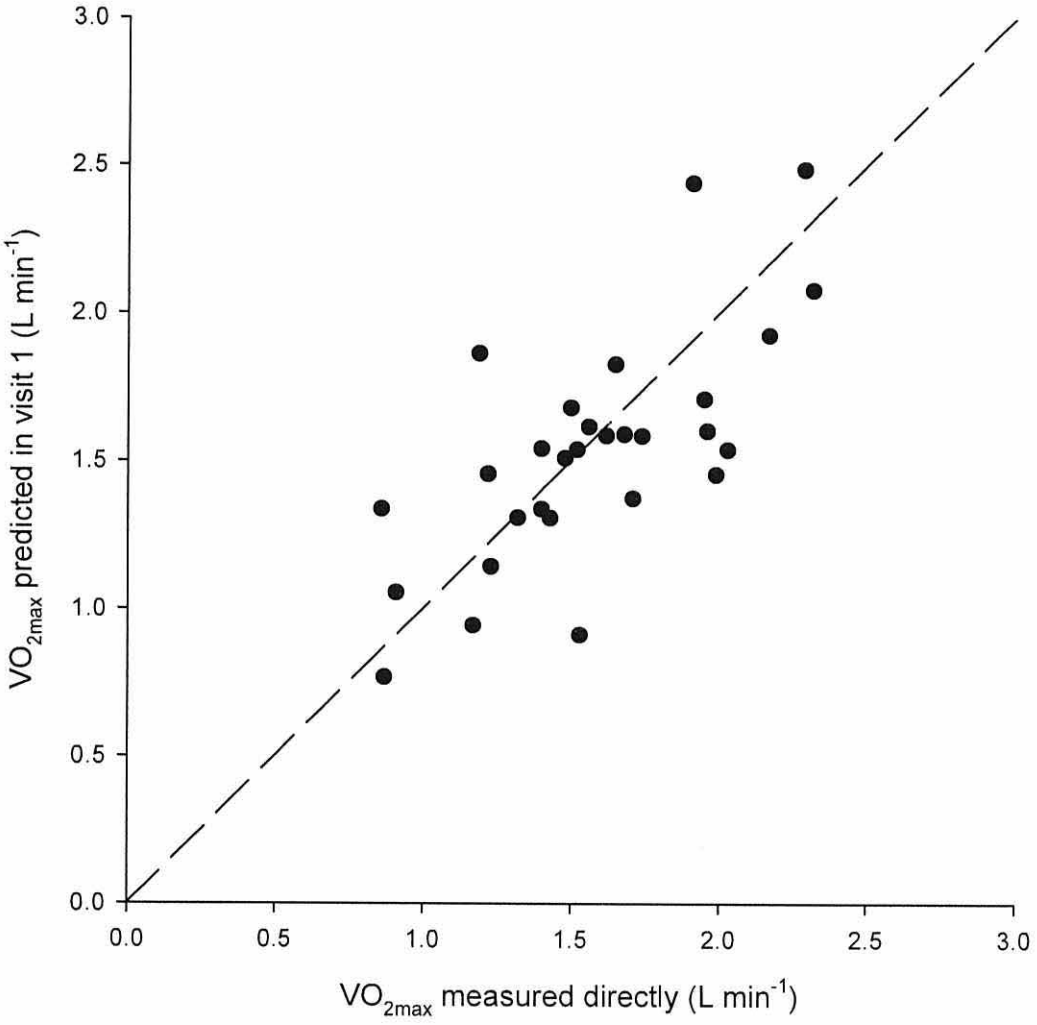


FIGURE 2: Bland-Altman plot of VO_{2max} predicted by the first visit SBT against the direct measurement of VO_{2max} during the cycling test. The solid bold line within the graph represents the bias, the dashed lines represent the upper and lower 95% limits of agreement.

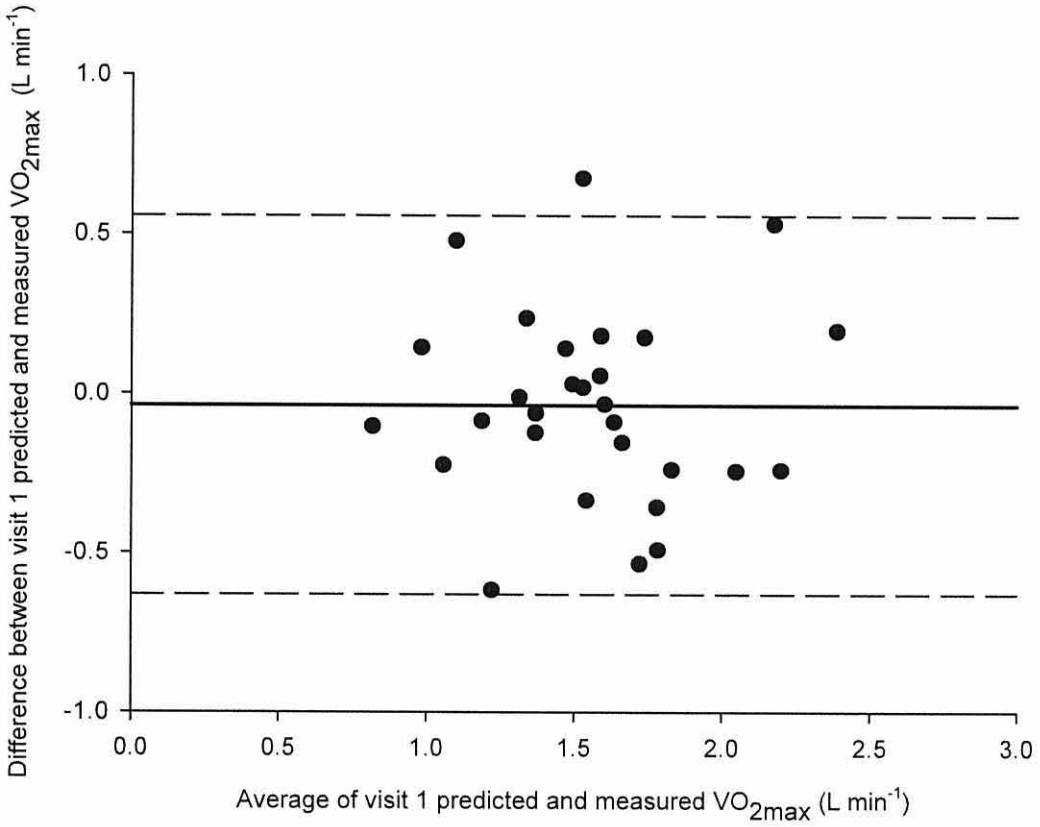


FIGURE 3: Scatterplot of VO_{2max} predicted by the second visit SBT against the direct measurement of VO_{2max} during the cycling test. The diagonal line represents the identity line (slope = 1; intercept = 0).

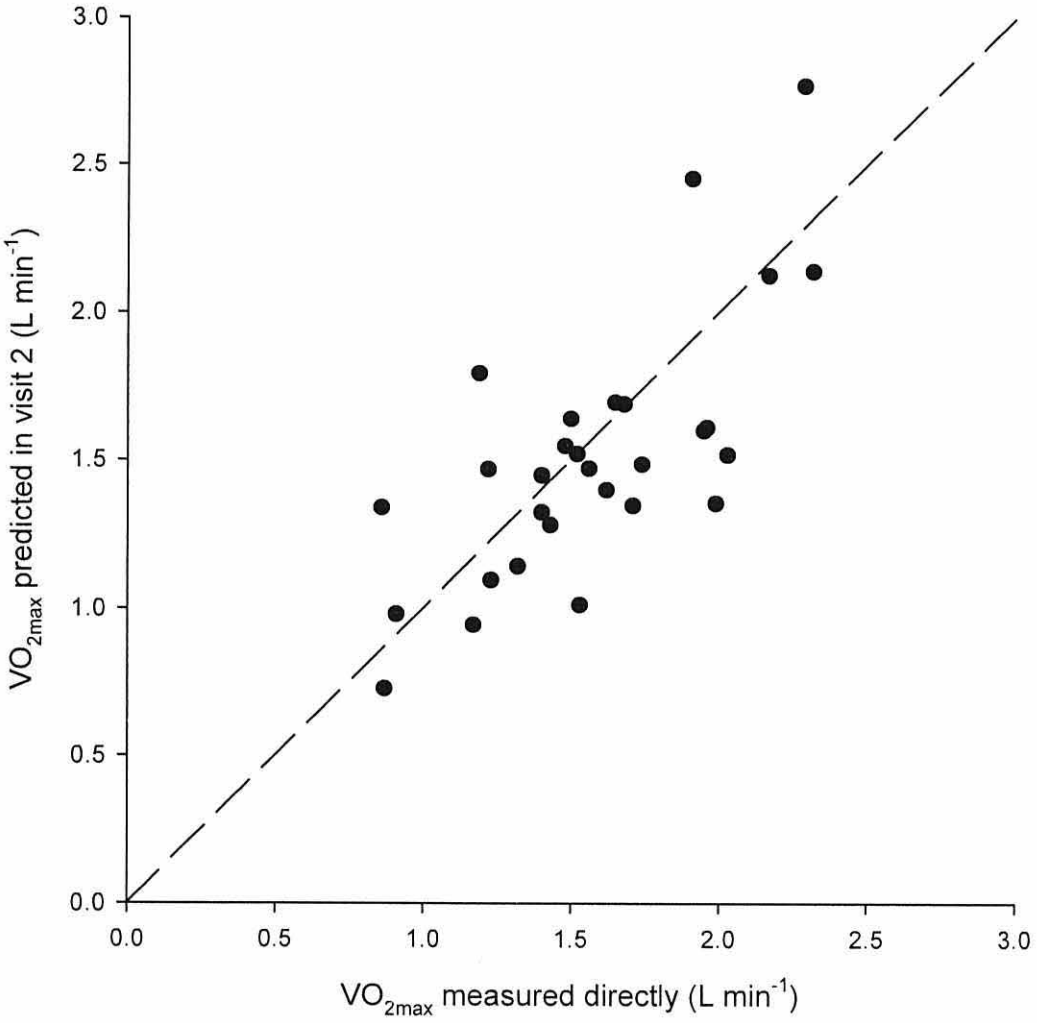


FIGURE 4: Bland-Altman plot of VO_{2max} predicted by the second visit SBT against the direct measurement of VO_{2max} during the cycling test. The solid bold line within the graph represents the bias, the dashed lines represent the upper and lower 95% limits of agreement.

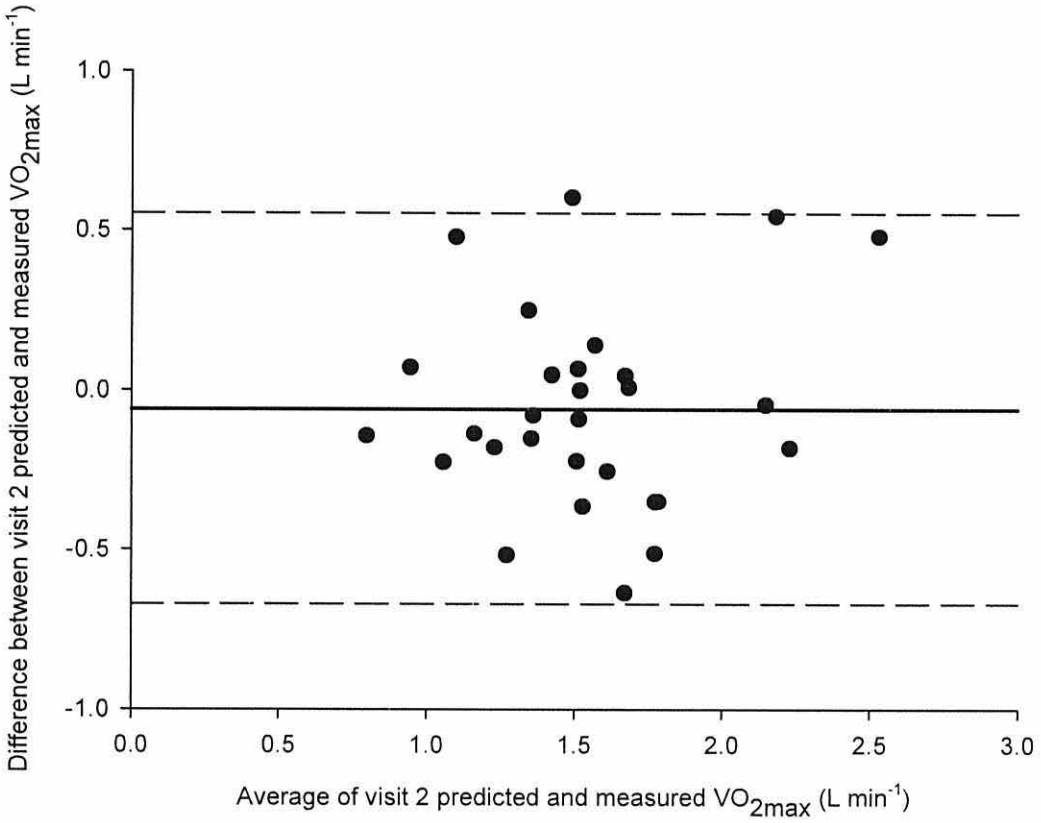


FIGURA 5: Scatterplot of VO_{2max} predicted by the first and the second visit SBT. The diagonal line represents the identity line (slope = 1; intercept = 0).

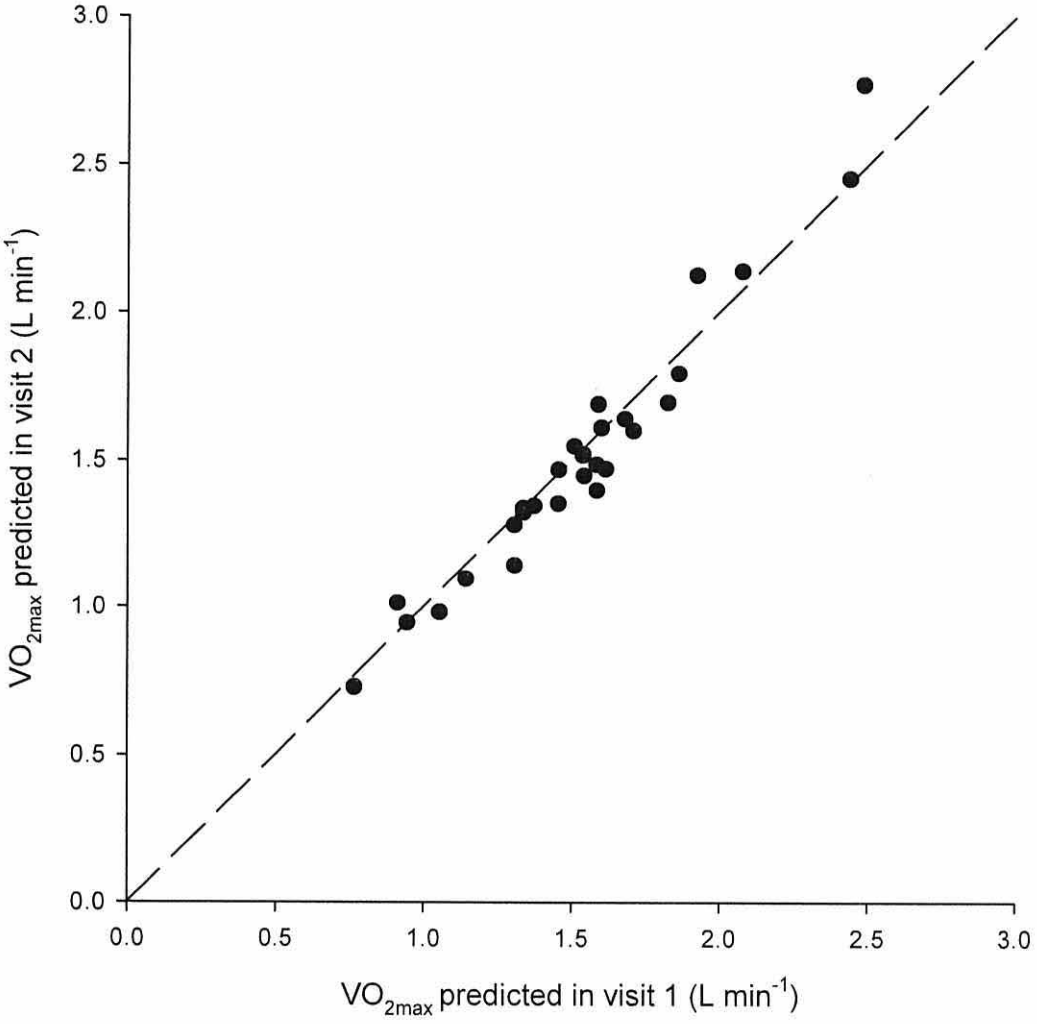
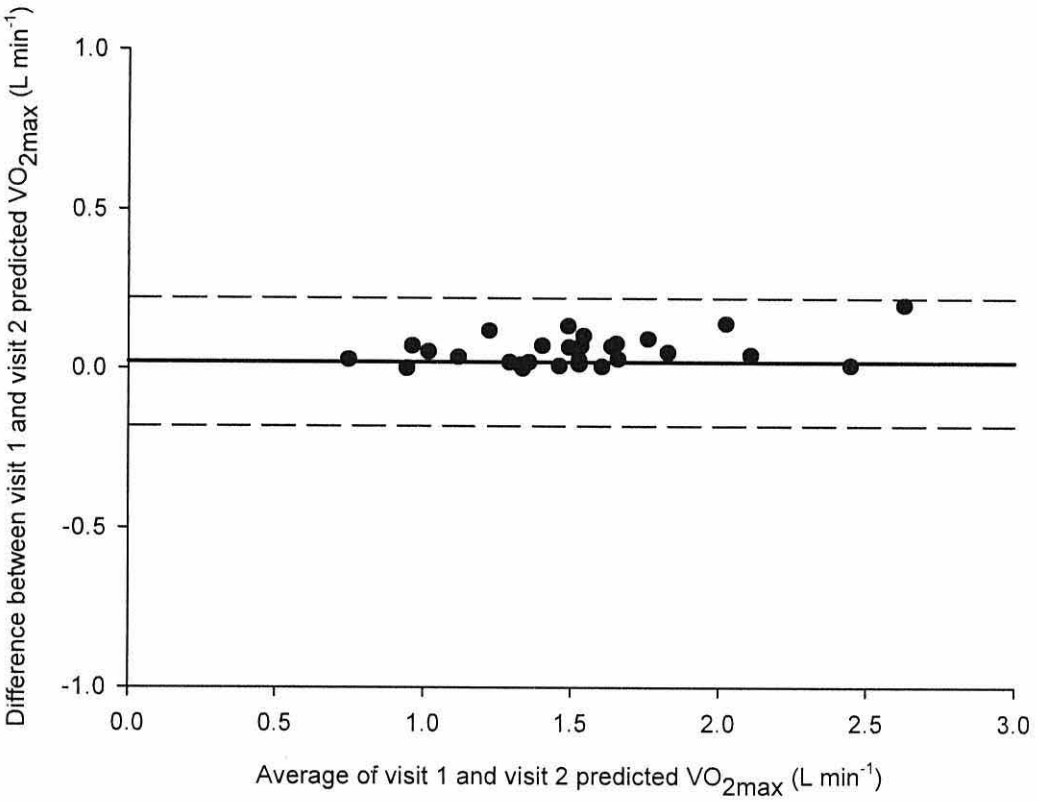


FIGURE 6: Bland-Altman plot of VO_{2max} predicted by the first and the second visit SBT. The solid bold line within the graph represents the bias, the dashed lines represent the upper and lower 95% limits of agreement.



CHAPTER 3

VALIDITY AND RELIABILITY OF THE SICONOLFI STEP TEST FOR ASSESSMENT OF PHYSICAL FITNESS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS[2]

MARCORA, S. M., CASANOVA, F., FORTES, M. B. & MADDISON, P. J. (2007) Validity and reliability of the Siconolfi Step Test for assessment of physical fitness in patients with systemic lupus erythematosus. *Arthritis Rheum,* 57, 1007-11

Introduction

Patients with systemic lupus erythematosus (SLE) have poor physical fitness as measured by maximal oxygen uptake (VO_{2max}) which ranges between 17.4 mL/kg/min and 23.1 mL/kg/min (Forte *et al.*, 1999, Tench *et al.*, 2002, Keyser *et al.*, 2003). Reduced VO_{2max} is an important predictor of physical disability in SLE patients (Tench *et al.*, 2002) and correlates significantly with the symptom of fatigue (Keyser *et al.*, 2003). Therefore, routine assessment of physical fitness in clinical practice might be useful to identify and monitor SLE patients who would benefit from aerobic exercise training programs to improve exercise tolerance and fatigue (Tench *et al.*, 2003).

A simple measure of VO_{2max} would also be useful for epidemiological studies on coronary heart disease (CHD) in SLE patients as the contribution of poor physical fitness to increased cardiac risk in this population is currently unknown and is likely to be substantial (Gulati *et al.*, 2005). Unfortunately, direct measurements of VO_{2max} during incremental exercise tests require expensive equipment, well trained personnel, and maximal effort from the subject.

A more practical alternative to maximal exercise tests is the use of prediction equations based on a) the relationship between heart rate (HR), VO_2 and workload during steady-state submaximal exercise, and b) age-predicted maximum HR (Noonan and Dean, 2000). To our knowledge only one such submaximal exercise test has been validated in patients with rheumatic diseases (Minor and Johnson, 1996). This consists of walking at a self-selected pace for two bouts of 4 min at 0% and 5% incline. However, only five of the 30 patients

involved in that study suffered from SLE. Therefore, the validity of this predictive test remains uncertain in this specific population. Moreover, it requires a motorised treadmill which might limit the applicability of this submaximal walking test in clinical practice and epidemiological studies.

Therefore, the aim of this study was to assess the validity and reliability of the Siconolfi step test (SST) originally developed in healthy subjects (Siconolfi *et al.*, 1985) to predict VO_{2max} in SLE patients. This test is submaximal, very short (3 to 9 min of exercise), and does not require expensive and bulky equipment, or highly trained personnel. However, predictive exercise tests tend to be population-specific and cross-validation in a group of SLE patients is required before the SST can be used in this population.

Methods

Subjects

Thirty adult SLE patients willing to take part in the study and able to give written informed consent were recruited for this criterion validity study. All the subjects had to fulfil the American College of Rheumatology 1997 revised criteria for SLE. Patients were excluded if they had severe cardio-pulmonary pathology, history of ischemic heart disease, severe myositis, active nephritis, active neurological disease, or pregnancy. Clearance for participation in the study was given by consultant rheumatologists from the rheumatology departments in Ysbyty Gwynedd, Ysbyty Glan Clwyd, and Maelor Wrexham Hospital. The study was approved by the North West Wales Local Research Ethics Committee.

Exercise test and procedures

All participants attended two testing sessions in the Physiology Lab of the School of Sport, Health and Exercise Sciences, University of Wales-Bangor. These two visits were separated by one to two weeks, and were scheduled at the same time of the day. Subjects were instructed to avoid any strenuous activity for 24 hours prior to testing and to avoid a heavy meal, caffeine, or nicotine within 3 hours of testing. In the first visit, subjects' height and weight were measured using standard equipment and procedures and body mass index (BMI) calculated as weight in kg/height in m^2. Haemoglobin concentration was also measured (B-hemoglobin, Hemocue AB, Sweden).

After this evaluation, subjects performed the SST described in detail elsewhere (Siconolfi *et al.*, 1985). Briefly, subjects were required to step for three minutes up and down a

portable 10 inch bench at a rate of 17 steps per minute which was kept constant with the help of a metronome. Heart rate was monitored continuously during the test by telemetry (Model S810, Polar, Finland) and recorded at the end of the stage. If target HR (65% of the predicted [220-age] maximum HR) was reached, the protocol was terminated. Otherwise, a second (26 steps min^{-1}) and, eventually, a third (34 steps min^{-1}) stage were completed with a minute rest between each stage. The steady-state absolute VO_2 at each stage was calculated according to the following equations (Siconolfi *et al.*, 1985):

Stage 1 VO_2 (L min^{-1}) = (16.287 x body weight in kg)/1000

Stage 2 VO_2 (L min^{-1}) = (24.910 x body weight in kg)/1000

Stage 3 VO_2 (L min^{-1}) = (33.533 x body weight in kg)/1000

Then, each subject's uncorrected VO_{2max} was estimated using the mathematical version of the original Astrand-Ryhming nomogram (Astrand, 1960) developed by Steven Siconolfi (personal communication):

Uncorrected VO_{2max} (L min^{-1}) = Stage VO_2 / $\%VO_{2max}$

where $\%VO_{2max}$ is calculated as:

$\%VO_{2max}$ male = (0.769 x Stage HR) – 48.5

$\%VO_{2max}$ female = (0.667 x Stage HR) – 42

Alternatively, the uncorrected VO_{2max} can be predicted using the original graphic version of the Astrand-Ryhming nomogram (Astrand, 1960).

Finally, the uncorrected VO_{2max} calculated from the above equations was entered in the following equations developed by Siconolfi *et al.* (1985) to predict VO_{2max} according to age and sex:

VO_{2max} male (L min^{-1}) = (0.348 x uncorrected VO_{2max}) – (0.035 x age in years) + 3.011

VO_{2max} female (L min^{-1}) = (0.302 x uncorrected VO_{2max}) – (0.019 x age in years) + 1.593

At end of this first testing session, subjects were familiarised with the maximal exercise test to be performed in the second visit.

On the second testing session, subjects repeated the SST and, after a minimum of 30 min rest, performed an incremental exercise test on a cycle ergometer (874E, Monark, Sweden) keeping a constant pedalling rate of 50 rpm. This protocol consisted of two minutes with no resistance followed by increments of 25 W every 2 min until volitional exhaustion. Throughout the test, expired gases and flow were analysed breath-by-breath using an automated system (600Ergo Test, ZAN Messgeräte, Germany) from which VO_2 and carbon dioxide production (VCO_2) were calculated and averaged every 30 seconds. The highest VO_2 recorded during the test (our criterion measure) was considered to be maximal when the criteria used in the original validation study by Siconolfi *et al.* (1985) were met. These were 1) a plateau defined as a difference less than 0.25 L/min between the two final VO_2 measurements taken every 30s, and 2) a respiratory quotient (VCO_2/VO_2) for the last measurement equal or greater than 1.0.

When describing patient characteristics, VO_{2max} values are reported relative to body mass (ml kg^{-1} min^{-1}) as this is the measure of physical fitness commonly used for comparative purposes. For validity and reliability analyses, however, we used absolute VO_{2max} values (L min^{-1}) as these are the values directly measured by the automated breath-by-breath analysis system and predicted by Siconolfi's equations.

Clinical Measures

Disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and damage by the Systemic Lupus International Collaborating Clinics (SLICC) damage index. These measures were completed by each patient's consultant rheumatologist within a maximum of three weeks from exercise testing. Fatigue was self-assessed by the patients with the Fatigue Severity Scale previously validated in SLE (Krupp et al., 1989).

Statistical analysis

Data are reported as mean (SD) for normally distributed variables; otherwise, data are reported as median [interquartile range]. The criterion validity of VO_{2max} predicted from the SST was assessed using the 95% limits of agreement (LOA) by Bland and Altman (Bland and Altman, 1986), paired *t* test and intraclass correlation coefficient (ICC) against VO_{2max} measured during the cycling test. Standard error of the estimate (SEE) was also calculated. Reliability of predicted VO_{2max} was assessed using 95% LOA, paired *t* test and ICC between the first and second visit SST. Coefficient of variation (CV) was also calculated. For validity and reliability, two-way, mixed effects, single measure models of ICC were used. For validity, methods of VO_{2max} assessment (measured during the cycling test vs. predicted from the SST) are a fixed effect while the participants are a random effect. For reliability, the first and second visit SST are a fixed effect while the participants are a random effect. Significance level was set at 0.05 for all analyses.

Results

Patients characteristics

Thirty patients (26 female, 4 males) were recruited and performed all exercise tests. One female patient was excluded after failing to meet the criteria for the attainment of VO_{2max}, during the cycling test leaving 29 patients for analysis. The characteristics of these subjects are presented in Table 1. This group of patients had low disease activity (SLEDAI scores ranging from 0 to 4 with 75% of patients scoring 0) and very little damage (SLICC scores ranging from 0 to 1 with 88% of patients scoring 0), and were not anaemic and only slightly overweight. Yet, patients had poor physical fitness and disabling fatigue. As expected, male patients had higher measured (24.5 ± 3.1 ml kg^{-1} min^{-1}) and predicted (26.9 ± 6.4 ml kg^{-1} min^{-1}) VO_{2max} compared to female patients (measured VO_{2max} = 22.1 ± 6.3 ml kg^{-1} min^{-1}; predicted VO_{2max} = 23.2 ± 5.1 ml kg^{-1} min^{-1}). Similarly, premenopausal women (n = 10, age 33 ± 8 years) had higher measured (23.7 ± 5.9 ml kg^{-1} min^{-1}) and predicted (24.7 ± 4.1 ml kg^{-1} min^{-1}) VO_{2max} compared to postmenopausal women (n = 15, age 57 ± 9 years) (measured VO_{2max} = 21.0 ± 6.5 ml kg^{-1} min^{-1}; predicted VO_{2max} = 22.2 ± 5.5 ml kg^{-1} min^{-1}).

None of the subjects were on beta-blockers at the time of exercise testing. Twelve patients were on oral prednisolone with daily doses ranging between 2 and 10 mg (average dose 6.5 mg/day). All patients completed the first stage of the SST with only three requiring the completion of the second stage to reach their target HR. No subject required the third stage. No adverse event occurred during the study, and patients tolerated well both maximal and submaximal exercise testing.

Validity

The scatterplot and Bland-Altman plot of VO_{2max} predicted by the second visit SST (1.67 ± 0.41 L min^{-1}) against the direct measurement of VO_{2max} during the cycling test (1.57 ± 0.39 L min^{-1}) are displayed in Figure 1 and 2 respectively. There was a moderately high ICC (0.73, $P < 0.001$) between predicted and measured VO_{2max}, and a trend for a positive bias (0.10 L min^{-1}, $P = 0.083$). The residuals in the Bland-Altman plot showed no evidence of significant heteroscedasticity. The 95% LOA were ± 0.58 L min^{-1}, and SEE was 0.28 L min^{-1}. Very similar results were obtained when performing these analyses with the VO_{2max} predicted by the first visit SST (data not shown) suggesting that a familiarization session is not required.

Reliability

The scatterplot and Bland-Altman plot of VO_{2max} predicted by the first (1.66 ± 0.40 L min^{-1}) and the second visit (1.67 ± 0.41 L min^{-1}) SST are displayed in Figure 3 and 4 respectively. There was a very high ICC (0.97, $P < 0.001$) between VO_{2max} predicted by the first and second visit SST, and a non significant negative bias (-0.01 L min^{-1}, $P = 0.500$). The residuals in the Bland-Altman plot showed no evidence of significant heteroscedasticity. The 95% LOA were ± 0.20 L min^{-1}, and CV was 3.2%.

Discussion

The results of this study demonstrate that the SST is well tolerated, reasonably valid and highly reliable in SLE patients with well-controlled disease. Although there was a trend ($P = 0.083$) for the SST to slightly overestimate the VO_{2max} directly measured during the cycling test (0.10 L min^{-1}), this positive bias does not invalidate the SST as it is similar to the bias found by Siconolfi *et al.* in healthy subjects (Siconolfi et al., 1985). The reason of this bias is likely to be the different exercise mode used for the estimation (stepping) and direct measurement (cycling) of VO_{2max}. In fact, treadmill and step tests give similar values and these are typically 5-11% higher than cycling tests (Kasch *et al.*, 1966, Christensen *et al.*, 2004, Maeder *et al.*, 2005). Alternatively, a reduction in measured VO_{2max} might have been caused by fatigue induced by the first SST which was performed in the same visit prior to the maximal bike test. However, the long rest period (30 min or more) between these two tests, and the moderate duration (3 min) and intensity (72% of maximum HR) of the SST argue against this possibility.

The validity of the SST is also supported by an ICC > 0.70 between measured and predicted VO_{2max}, and the moderate SEE (0.28 L min^{-1}). This error is similar to the one reported for other submaximal exercise tests in healthy subjects (Siconolfi *et al.*, 1985, Noonan and Dean, 2000). Furthermore, our results compare well to the ones of Minor et al. (1996) who validated a submaximal walking test in patients with a variety of rheumatic diseases including SLE. Overall, the SST developed in healthy subjects cross-validate well in SLE patients and is well tolerated. However, participants in our study represent a subgroup of SLE patients with well-controlled disease. Therefore, our results can not be

readily generalised to the full SLE population. Furthermore, even a 10-inch high step might present some difficulties to more disabled subjects.

The attractiveness of the SST compared with the treadmill test validated by Minor and colleagues (1996) is that the former requires much less expensive equipment which can be easily transported and stored away. These characteristics should favour its application in clinical practice and epidemiological studies. Nevertheless, health professionals should be aware of the relatively high margin of error of the SST when predicting VO_{2max} in individual patients as revealed by the wide 95% LOA (Figure 2). Therefore, the SST can not be used when precise individual assessment of physical fitness is required.

The very high ICC, low CV, non-significant bias, and relatively narrow 95% LOA (Figure 4) demonstrate that the SST is highly reliable in SLE patients. Again, these results compare well with reliability studies of other exercise tests (Noonan and Dean, 2000) including the one validated by Minor *et al.* (1996). As HR at a fixed submaximal workload is highly sensitive to changes in physical fitness, the SST might be used as an outcome measure in large multi-centre trials of exercise therapy in SLE (Noonan and Dean, 2000). However, further longitudinal validation studies are necessary to confirm this hypothesis.

The importance of physical fitness assessment in SLE is underlined by the mean VO_{2max} (22.4 ml kg^{-1} min^{-1}) measured in our patients. This is similar to Tench *et al.* (2002) (23.1 ml kg^{-1} min^{-1}) and is slightly higher than the mean values found by Forte *et al.* (1999) (17.4 ml kg^{-1} min^{-1}) and Keyser *et al.* (2003) (19.2 ml kg^{-1} min^{-1}). These discrepancies might be due to the exercise mode used for testing, characteristics of patients recruited, and criteria for

maximal effort. Regardless of these differences, all studies clearly demonstrate that physical fitness is poor in SLE patients, including non-anemic, non-obese patients with well-controlled disease such as the ones included in our study. If we consider that moderate activities of daily living require up to 21 ml kg^{-1} min^{-1}(Keyser *et al.*, 2003), it is no surprise that low VO_{2max} contributes to physical disability and fatigue in SLE as patients are forced to use a high percentage of their functional reserve (Keyser *et al.*, 2003, Tench *et al.*, 2003).

Furthermore, we calculated that only 26% of our cohort had a VO_{2max} higher than 85% of the normal predicted value (Gulati *et al.*, 2005). This finding is clinically relevant given that women with an exercise capacity less than 85% of their predicted value have twice as high risk of death from all causes (2.44 for cardiac disease) than women with a normal VO_{2max} (Gulati *et al.*, 2005). Unfortunately, available epidemiological studies on CHD in SLE patients have focused on other traditional risk factors (e.g., high cholesterol) and inflammation (Bruce, 2005), and did not include a measure of VO_{2max}. Therefore, the contribution of reduced exercise capacity towards increased cardiac risk has yet to be determined in this population. Poor physical fitness is a highly modifiable risk factor for CHD, and we hope that the validation of a simple, inexpensive, submaximal exercise test to predict VO_{2max} in SLE patients will facilitate future research in this important area. The association between the HR response to exercise per se and mortality (Cole *et al.*, 1999) also warrants further investigation in this population.

Tables and figures

Table 1. Characteristics of the SLE patients (25 females and 4 males) included in the analysis

Characteristic	Mean or Median	(SD) or [interquartile range]
Age (years)	48	(14)
Height (cm)	164	(9)
Body mass (kg)	71.5	(13.7)
BMI (kg m^{-2})	26.5	(4.5)
Haemoglobin (g dL^{-1})	13.1	(1.3)
VO_{2max} (mL kg^{-1} min^{-1})		
Measured	22.4	(6.0)
Predicted	23.7	(5.3)
Disease Duration (yrs)	10	(9)
SLEDAI (0-105)*	0.0	[0.8]
SLICC damage score (0-46)*	0.0	[0.0]
Fatigue Severity Scale (1-7)*	4.9	[1.2]

BMI = Body Mass Index; VO_{2max} = maximal oxygen uptake; SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; SLICC = Systemic Lupus International Collaborating Clinics.
* These variables are not normally distributed and therefore expressed as median [interquartile range].

Figure 1. VALIDITY: Scatterplot of VO_{2max} predicted by the second visit SST against the direct measurement of VO_{2max} during the cycling test. The diagonal line represents the identity line (slope = 1; intercept = 0).

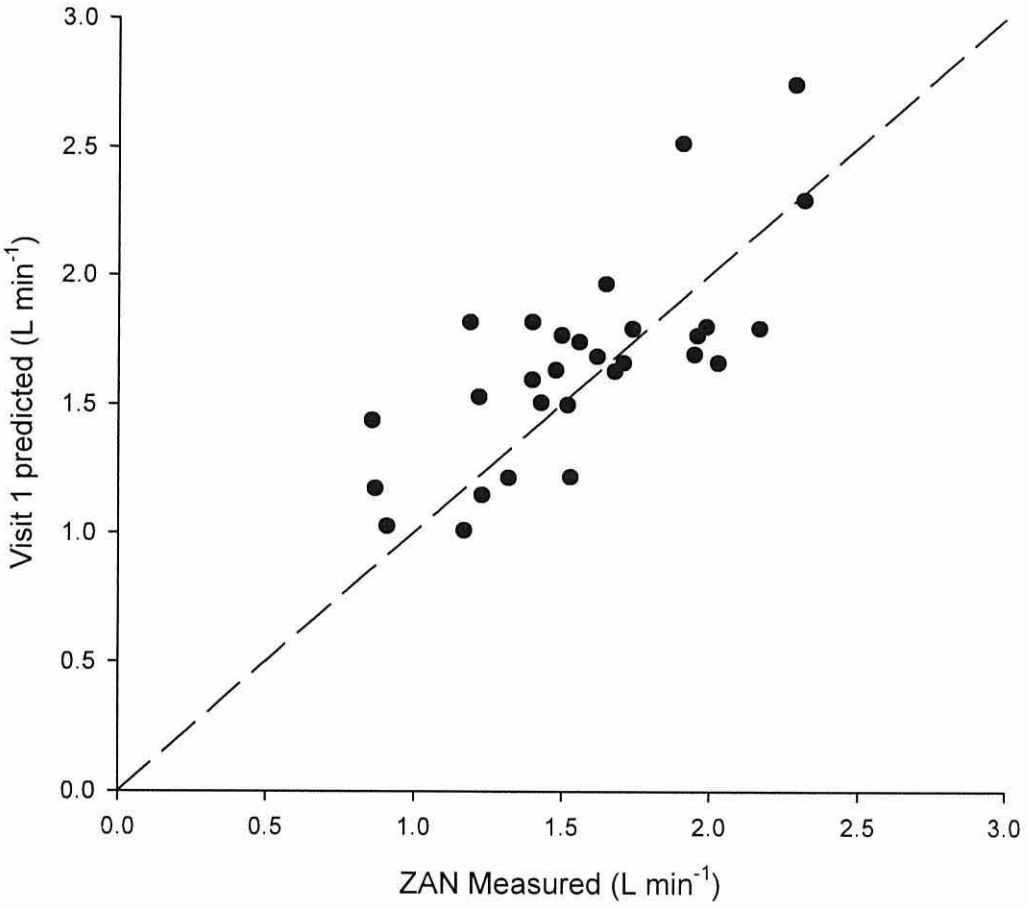


Figure 2. VALIDITY: Bland-Altman plot of VO_{2max} predicted by the second visit SST against the direct measurement of VO_{2max} during the cycling test. The solid bold line within the graph represents the bias, the dashed lines represent the upper and lower 95% limits of agreement.

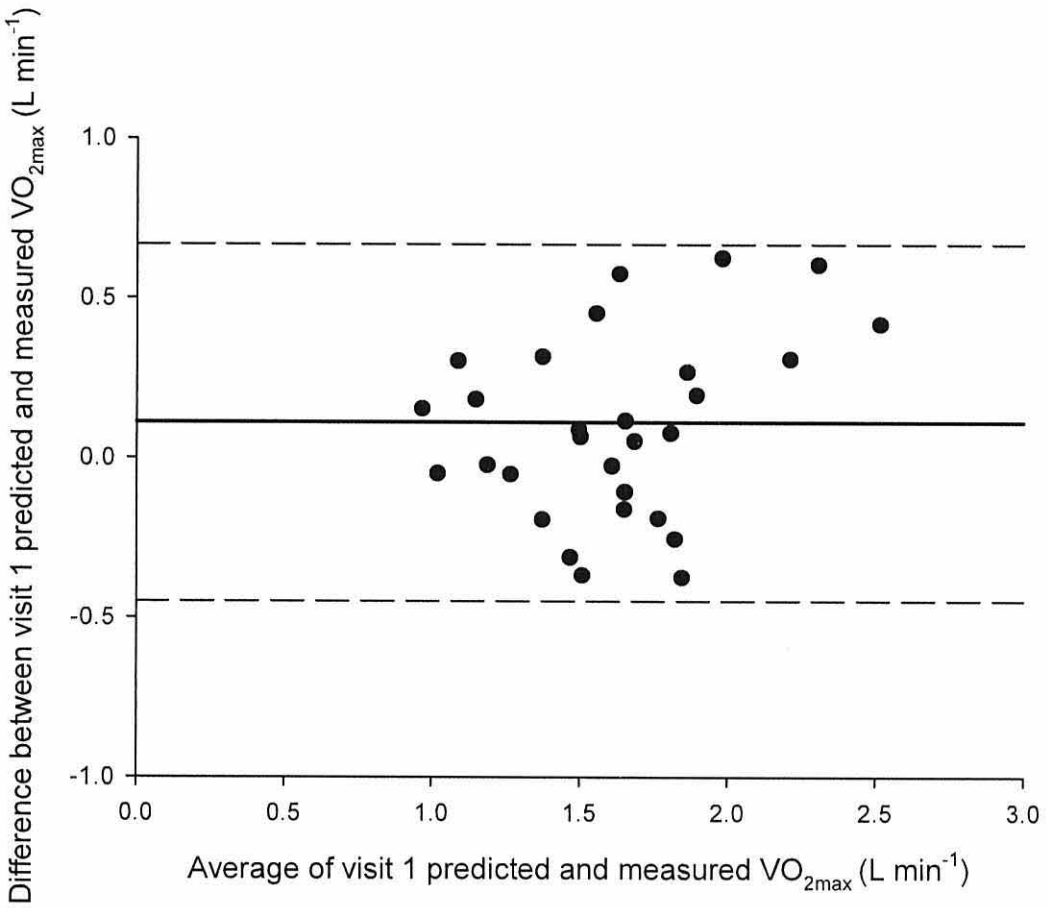


Figure 3. RELIABILITY: Scatterplot of VO_{2max} predicted by the first and the second visit SST. The diagonal line represents the identity line (slope = 1; intercept = 0).

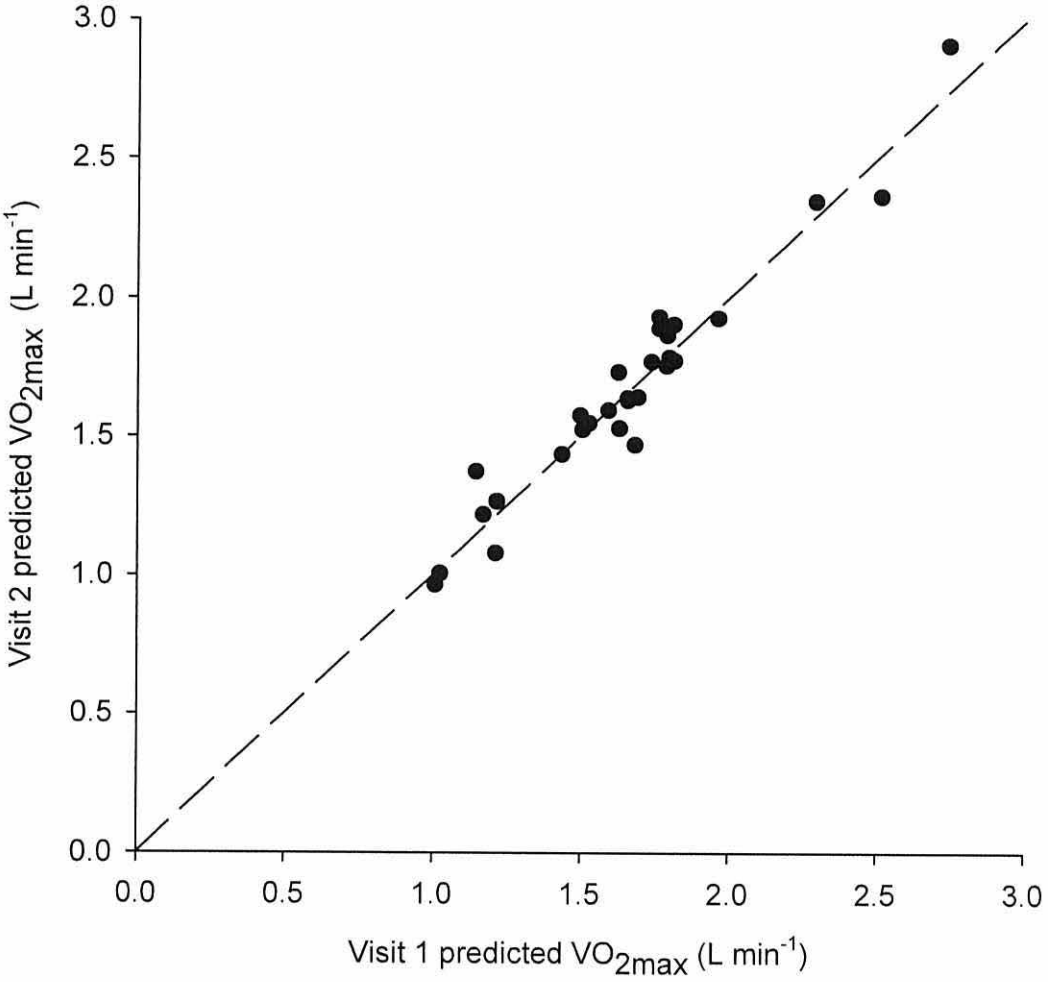
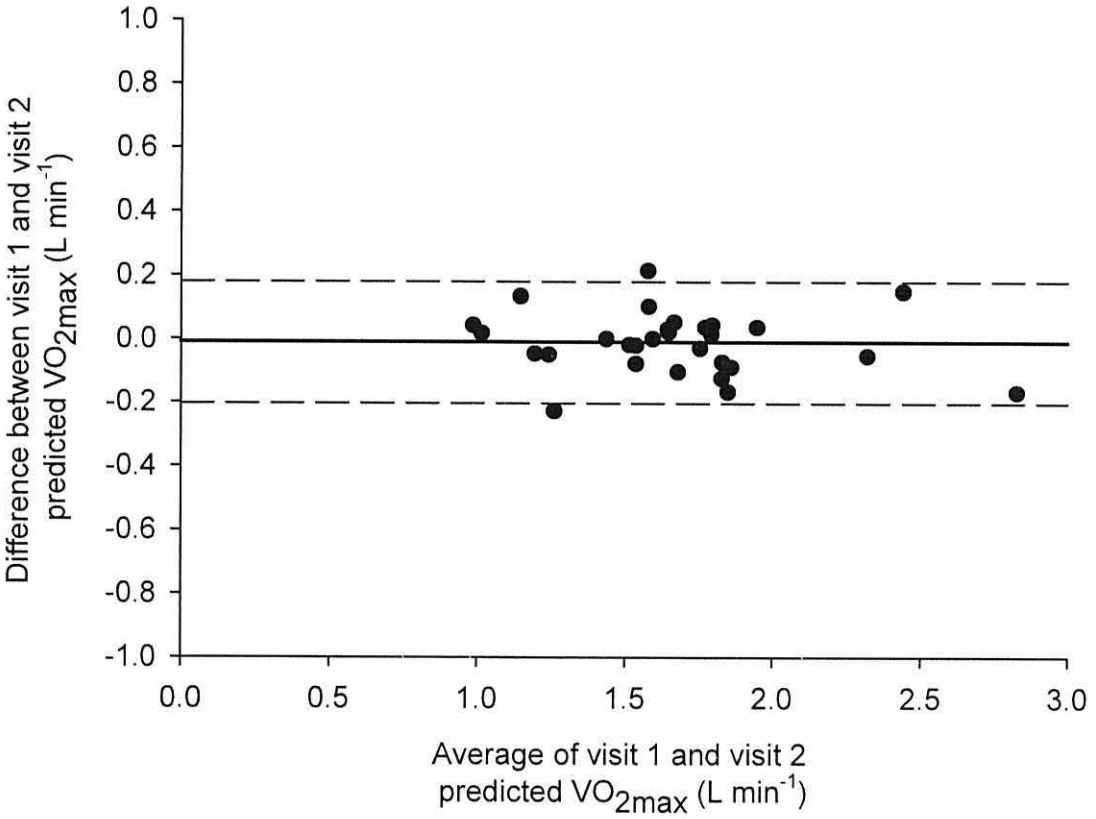


Figure 4. RELIABILITY: Bland-Altman plot of VO_{2max} predicted by the first and the second visit SST. The solid bold line within the graph represents the bias, the dashed lines represent the upper and lower 95% limits of agreement.



CHAPTER 4

MECHANISM OF REDUCED MAXIMAL OXYGEN CONSUMPTION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Introduction

Systemic lupus erythematosus (SLE) is a chronic, inflammatory, autoimmune disease that can affect various different organs such as the heart, lungs, skin and muscles. Lupus patients demonstrate a high incidence of cardiovascular mortality (Manzi *et al.*, 1997, Bruce *et al.*, 2003, Frostegard, 2005), physical disability (Bostrom *et al.*, 2008) and fatigue (Krupp *et al.*, 1990).

Maximal oxygen consumption (VO_{2max}), a physiological measure of physical fitness, has recently received great attention in this population because of its value in predicting cardiovascular mortality in other clinical populations (Cohen-Solal *et al.*, 1997, Sietsema *et al.*, 2004) and its ability to explain part of the variance in physical function and fatigue measures (Tench *et al.*, 2002). All studies measuring VO_{2max} in subjects suffering with SLE found a significant reduction compared to healthy controls (Robb-Nicholson *et al.*, 1989, Daltroy *et al.*, 1995, Sakauchi *et al.*, 1995, Forte *et al.*, 1999, Ramsey-Goldman *et al.*, 2000, Tench *et al.*, 2002, Keyser *et al.*, 2003, Carvalho *et al.*, 2005, Clarke-Jenssen *et al.*, 2005, Bostrom *et al.*, 2008). In these studies, VO_{2max} in SLE patients ranged between 17.4 and 25.1 ml $kg^{-1}min^{-1}$. These values are indicative of a severe impairment of the oxygen transport and utilisation systems and they are associated with poor functional capacity and increased risk of cardiovascular problems (Gulati *et al.*, 2005).

Given the serious implications of low VO_{2max}, it is important to understand why aerobic fitness is so poor in patients with SLE as many different factors could be involved. Any step of the oxygen pathway from ambient air to its utilisation in the muscles can potentially

limit VO_{2max}. These include pulmonary function and diffusion, haemoglobin concentration in the blood, cardiac function, peripheral oxygen extraction and its utilisation in the mitochondria. The importance of each potential limiting factor can be summarised mathematically by using the Fick equation which states that:

$$VO_{2max} = HR_{max} \times SV_{max} \times A\text{-}V\ O_2diff_{max}$$

where HR is heart rate, SV is stroke volume (the amount of blood ejected by the ventriculum during each cardiac cycle) and A-V O_2diff is the difference in oxygen content between arterial and mixed venous blood. From the Fick equation it follows that VO_{2max} can be limited by: 1) cardiac impairment affecting HR and/or SV, 2) pulmonary dysfunction and/or anaemia decreasing the arterial oxygen content and 3) blood flow distribution and/or oxygen extraction/utilization at muscle level affecting mixed venous blood oxygen content. In the healthy population it is generally believed that at sea level VO_{2max} is limited by SV (Levine, 2008).

In SLE, processes directly related to the disease such as organ damage, autoantibodies and inflammation could be causing and/or contributing to reduction in VO_{2max}. Additionally, components indirectly related to the disease such as physical inactivity could be also involved in the aetiology of this decrease in VO_{2max}. Indeed, sedentary lifestyle is associated with a lower physical fitness in the healthy population (Wagner *et al.*, 1992).Unfortunately, physical activity of SLE patients and healthy controls involved in previously published studies (Tench *et al.*, 2002, Keyser *et al.*, 2003) was self reported and

no objective measure of this variable was used. Therefore, the direct effect of SLE on VO_{2max} and related physiological functions is still unclear.

Two pathological pathways (peripheral reduction in muscle oxygen uptake and impaired lung function) have been suggested in previous studies (Sakauchi *et al.*, 1995, Forte *et al.*, 1999, Tench *et al.*, 2002) but the techniques used in these investigations limit the validity of these speculations. In fact, to the best of our knowledge no previous investigation have measured cardiac output during exercise in SLE patients. Therefore, the aim of this study was to investigate cardiac, respiratory and muscle factors potentially affecting maximal aerobic capacity in SLE patients.

Methods

Subjects and eligibility

After ethics approval from the local Research Ethics Committee, twelve patients fulfilling the American College of Rheumatology revised criteria for SLE (Hochberg, 1997) and twelve gender and age (± 5 years) matched healthy controls took part in this investigation. Patients were excluded if they had severe cardio-pulmonary pathology, history of myocardial infarction or ischemic heart disease, anaemia (defined as haemoglobin level < 10 g/L), severe myositis, active nephritis, active neurological disease or pregnancy. Eligibility to undertake the study was assessed by a specialist clinician. Healthy subjects were excluded if they reported having cardio-pulmonary pathology, history of myocardial infarction, ischemic heart disease, musculoskeletal disorders, renal disease, or if they have been involved in regular physical training (defined as moderate to high intensity exercise more than two times a week) in the previous six months. The investigation involved three visits to the School of Sport, Health and Exercise Sciences in Bangor.

First visit: familiarisation and physical activity assessment

In the first visit, all subjects were familiarised with all the procedures to be used in the study. Before the practice session, height, weight, body composition, resting blood pressure and total blood cholesterol were measured. Height and weight were measured using a wall mounted stadiometer (Body Care, Warwickshire, UK) and balance scale (Seca, Hamburg, Germany), resting blood pressure was measured by manual sphigmomanometry and body composition by whole body bioelectrical impedance (Bodystat 1500, Isle of Man, UK). For

bioelectrical impedance measures, proximal electrode sites were the dorsal surfaces of the wrist and ankle and the distal sites were the base of the third metacarpo- and metatarsal-phalangeal joints of the hand and foot. Fat free mass was then calculated from electrical data (impedance), height and weight using the equation developed by Sun *et al.* (2003). Finally, total blood cholesterol was measured from 5 μL of arterialised blood taken from the finger using a photometry technique (Reflotron, Boehringer Mannheim UK, East Sussex, UK).

Finally, the patients were given a pedometer (Digi-Walker SW-200 Yamax, Japan) to assess routine physical activity in the following week by counting the number of steps taken during a day. The subjects wore the pedometer on the right hip for a week recording the number of steps taken at the end of each day in a diary. The average number of steps taken over a day was used as a measure of physical activity. This method gives an accurate estimation because average step count over a week including weekend has been found to provide an acceptable estimation of physical activity (Gretebeck and Montoye, 1992).

Second visit

At the start of visit two, the physical activity diaries were collected and patients sat down for a resting period before the maximal exercise test. During this resting period 5 μL of arterialised blood were collected from the finger tip to measure blood haemoglobin (Hb) concentration using a B-hemoglobin photometer (Hemocue, Sweden).

Maximal oxygen consumption was determined during an incremental exercise protocol on a Corival electro-magnetically braked cycle ergometer (Lode, The Netherlands). The protocol started with the subjects exercising for 2 minutes at 0W followed by 25W increments every 2 minutes until exhaustion (operationally defined as a pedal frequency of less than 50 revolutions per minute for more than 5 s despite strong verbal encouragement). Expired gases were analysed continuously for ventilatory equivalent of oxygen (VO_2), carbon dioxide (VCO_2) and minute ventilation (V_e) using a breath-by-breath monitoring system (ZAN 600, ZAN Messgerate GmbH, Germany). This automated device was calibrated before each test using certified gases of known concentration (12.0 % O_2 and 5.1% CO_2) and a 3.0 L calibration syringe (Series 5530, Hans Rudolph Inc, Kansas City, Missouri). All data were averaged over 1 min periods before statistical analysis. After exhaustion, a 5 µl sample of arterialised blood was taken from the right earlobe and analysed for lactate concentration (mMol L^{-1}) using a portable analyser (Lactate Pro LT-1710, Arkray, Shiga, Japan). From expired gasses data, anaerobic threshold (AT) was calculated using the V-slope method (Gaskill *et al.*, 2001). Using this method, in a plot of production of CO_2 over utilization of oxygen, AT are defined as the intensity where the slope of the regression line changes from less than 1 to more than 1. The ATs were determined by two independent assessors blinded to the condition and the average of the two values obtained was used for the analysis.

A transthoracic bioimpedance device (Physioflow PF05L1, Manatec, Petit-Ebersviller, France) was used to measure HR, SV and cardiac output (CO) during exercise. Two sets of two electrodes (Ambu Blue Sensor VL, Ambu A/S, Ballerup, Denmark), one transmitting and the other one receiving a low amperage alternating electrical current, were applied on

the supraclavicular fossa at the left base of the neck and along the xiphoid. Another set of two electrodes was used to monitor a single ECG lead in the V1/V6 position. Wires connected to the electrodes were fixed on the body using tape to reduce movement artefacts. Stroke volume (ml) is estimated by this computerised device from changes in transthoracic impedance during cardiac ejection according to the method described in detail by Charloux *et al.* (2000). Cardiac output (L min^{-1}) is then automatically calculated as:

$$CO = (HR \times SVi \times BSA) / 1000$$

where BSA is body surface area (m^2) calculated according to the Haycock formula [BSA = 0.02465 x body mass $(kg)^{0.5378}$ x stature $(cm)^{0.3964}$] and SVi (ml m^{-2}) = SV / BSA. Heart rate (beats min^{-1}) is based on the R-R interval determined from the first derivative of the ECG. These data were averaged over 1 min periods before statistical analysis. Before each test, the Physioflow was autocalibrated using a procedure based on 30 consecutive heartbeats recorded whilst the participant was resting in a seated position on the cycle ergometer, anthropometric data and resting systolic and diastolic blood pressure values (mmHg) (Charloux *et al.*, 2000). These were the averages of two separate blood pressure recordings taken before and after the Physioflow autocalibration using an automated blood pressure monitor (Tango, SunTech Medical Ltd, Morrisville, North Carolina). The Tango device was interfaced to the Physioflow by an analog cable for the ECG trigger. Blood pressure was also monitored at the end of warm-up and at the end of each incremental stage. From all the previously collected data oxygen pulse (O_2 pulse; ml/beat) and mean arterial pressure (MAP; mmHg) were calculated respectively as:

O_2 pulse = VO_2 / HR

MAP = 1/3 x (systolic pressure – diastolic pressure) + diastolic pressure

Finally, throughout all the exercise test arterial haemoglobin saturation was measured by finger infrared oximetry (ZAN 820, ZAN Messgerate GmbH, Germany) and rate of perceived exertion was recorded at the end of each incremental stage and at exhaustion using the 6-20 Borg Scale (Borg, 1982).

Third visit

In the third visit, subjects performed a battery of pulmonary function tests and an isokinetic muscle strength and endurance test. Pulmonary function was tested using a Keystone3 spirometer (Ferraris Group Plc, U.K.) according to the procedures recommended by the American Thoracic Society (ATS, 1995). Standard tests included forced vital capacity (FVC) and one second forced expiratory volume (FEV_1). Every subject performed three acceptable FVC manoeuvres and the best performance was recorded. Maximal voluntary ventilation (MVV) was calculated from FEV_1 as FEV_1 x 40. Additional pulmonary function tests included single-breath carbon monoxide diffusing capacity (D_{LCO}, ml $min^{-1}mmHg^{-1}$) and respiratory muscle strength tests. Single-breath carbon monoxide diffusing capacity was performed by maximally inspiring a mixture of gas containing a known concentration of carbon monoxide (0.3%) and a tracer (helium). The maximal inspiration was followed by a 10 s breath holding period and expiration. Diffusion capacity was then automatically calculated by the Keystone3 spirometer as the product of the uptake of carbon monoxide and the alveolar volume calculated from the tracer.

Respiratory muscle strength tests were assessed by measuring maximal static inspiratory and expiratory pressure. The pressure measured at the mouth by a pressure transducer reflects the inspiratory and expiratory muscle strength. For this test, patients forcefully breathed into a disposable tube mouthpiece attached to the Keystone3 spirometer and the measure was accepted when the maximum pressure was maintained at least for 1 s. Measures were repeated at least three times and the highest value recorded for the analysis.

The isokinetic muscle strength and endurance test was performed on a isokinetic dynamometer (Humac Norm, CSMi, Stoughton, MA, USA) following a validated protocol previously described (Franssen *et al.*, 2005). Briefly, subjects were seated upright on the chair of the dynamometer with straps around their waist, shoulders and thighs. The lever arm was attached to the distal part of the tibia and the axis of rotation was visually aligned with the centre of rotation of the knee joint. The isokinetic protocol consisted of 15 consecutive voluntary maximal contractions at a constant speed of 90° sec^{-1}. Maximal isokinetic strength was defined as the highest torque measured during the 15 repetitions while isokinetic muscle endurance was defined as the slope of the linear curve fitted through the peak torque measured in each repetition expressed as a percentage of the maximal isokinetic strength. Therefore, a more negative slope indicates lower muscle endurance.

Clinical measures

Global disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI; Bombardier *et al.*, 1992). The SLEDAI is a valid measure of SLE

activity (Ward *et al.*, 2000) and generates a total score between 0 and 105 with high scores indicating higher disease activity. Organ damage was assessed by the Systemic Lupus International Collaborating Clinics (SLICC) damage index (Gladman *et al.*, 1996a). The SLICC produces a score between 0 and 46 with high scores indicating more severe damage. All these measures were completed by each patient's consultant rheumatologist within a maximum of three weeks from exercise testing. Finally, all subjects were asked to fill the Fatigue Severity Scale (FSS), a validated questionnaire to measure fatigue experienced in their life (Krupp et al., 1990).

Statistics

Data are reported as mean (SD) for normally distributed variables; otherwise, data are reported as median [interquartile range]. Differences between the groups during exercise tests were analysed using a 2 way mixed model ANOVA (between-within; group x load). Differences between the two groups at maximal exercise were assessed by two tailed paired t-tests. Significance level was set at 0.05 for all analyses.

Results

Subjects characteristics

Twenty four subjects (22 female, 2 males) were recruited and performed all tests. The characteristics of these subjects are presented in Table 1. The two groups were well matched for age, weight, height, body composition and there were no differences in physical activity resting BP, blood cholesterol levels and haemoglobin concentration.
The clinical characteristics of the SLE patients are presented in table 2. Patients had long standing disease, low disease activity and very little damage but yet disabling fatigue.
At the time of exercise testing no patients were receiving beta-blockers, 7 patients were receiving oral prednisolone with daily dosages ranging between 2 and 5 mg, 7 were receiving hydroxychloroquine with daily dosage ranging between 200 and 400mg, 5 were receiving non steroidal anti-inflammatory drugs, 2 were receiving immunosuppressants (1 Azathioprine 150mg and 1 Mycophenolate 500mg) and 2 were receiving antidepressants. None of these drugs are known to affect VO_{2max} directly.

Exercise test

The results of the physiological measures at VO_{2max} during the exercise test are reported in figure 1 and table 3. The values for RPE, RER, venous blood lactate concentration and HR at VO_{2max} showed that both SLE patients and healthy controls exercised to exhaustion and reached a level of exercise intensity that can be considered maximal.
Cardiac data during submaximal exercise are reported in figure 2. For CO, there was no significant interaction ($p = 0.111$), no significant main effect for group ($p = 0.359$) and a

significant main effect for load ($p < 0.001$). For SV, there was no significant interaction ($p = 0.332$), a significant main effect for load ($p = 0.005$) and a trend towards a significant main effect for group ($p = 0.087$). For HR, there was no significant interaction ($p = 0.117$), no significant main effect for group ($p = 0.629$) and a significant main effect for load ($p < 0.001$).

At maximal exercise SLE patients had a significantly lower VO_{2max}, CO and a trend towards a significant reduction in SV and oxygen pulse (table 3). No significant differences at maximal exercise were observed for maximal MAP, oxygen saturation and no difference was found in AT expressed as a percentage of VO_{2max}.

Respiratory function

The results of the spirometry tests as well as the ventilatory results during exercise and all the other lung function tests are reported in table 4. There was a significant difference between SLE and controls in FVC, FEV_1 and maximal exercise Vt while there was no significant difference in any other respiratory parameters.

Isokinetic muscle test

Results of the muscle function test demonstrated a significant reduction in isokinetic muscle endurance in the SLE group as demonstrated by a more negative value of the slope (SLE = -1.91 ± 0.5, controls = -1.38 ± 0.69, $p = 0.046$) and no significant difference in isokinetic muscle strength (SLE = 80.5 ± 22.3 N m, controls = 95.3 ± 20.8 N m, $p = 0.103$).

Discussion

VO_{2max}

The results of the maximal exercise test in this study confirm previous investigations (Robb-Nicholson *et al.*, 1989, Sakauchi *et al.*, 1995, Tench *et al.*, 2002, Keyser *et al.*, 2003, Bostrom *et al.*, 2008) measuring VO_{2max} in SLE patients. In fact, our cohort of SLE patients reached an average VO_{2max} of 22.16 ml $kg^{-1}min^{-1}$ which compares very well with previously published values ranging between 17.4 and 25.1 ml $kg^{-1}min^{-1}$. This is not surprising because the group of subjects recruited for this investigation share similar characteristics in terms of disease state and organ damage with subjects previously involved in exercise studies. In fact, all patients recruited in previous and present investigations suffer from long standing, well controlled SLE. It is therefore possible to speculate that patients with more active SLE might have an even lower VO_{2max}.

Importantly, our data clearly demonstrate that this reduction is caused by the disease itself rather than being secondary to reduced physical activity. Sedentary lifestyle has been demonstrated to be related to a decrease in cardiovascular function (Myers, 2003) but our measure of physical activity shows that patients in this study were as active as the subject in our control group. Moreover, both groups in this study can be considered as sedentary but still having a normal physical activity because average daily step count positively compares to the result of a one year pedometer monitoring in a group of sedentary healthy adults (Tudor-Locke *et al.*, 2004).

Respiratory limitations

Patients recruited for this investigation demonstrated no sign of restrictive or obstructive spirometric patterns at rest. In fact, measured FVC in our cohort was 98.3 % of the predicted value and FEV_1 to FVC ratio was above the clinically accepted cut off value (70%) for obstructive pattern (Vandevoorde *et al.*, 2006). Moreover, there was no difference in "FEV_1 to FVC ratio" between SLE patients and healthy controls. Taken together this data suggest that, despite the significant reduction in FVC and FEV_1 found in this study, lung function was not clinically affected in our cohort. This is not surprising given that patients with pulmonary disease were excluded from the study.

Based on the analysis of arterial oxygen content and the ventilatory patterns during exercise tests we are able to exclude a pulmonary limitation to VO_{2max} in our group of SLE patients. There was in fact no arterial desaturation and breathing reserve was similar between patients and controls at maximal exercise. Arterial desaturation occurs when the lungs are unable to maintain homeostasis of arterial blood oxygen content and it is believed to affect VO_{2max} only when arterial saturation falls below 95% (Dempsey and Wagner, 1999). Therefore, both SLE patients and controls' VO_{2max} in the present investigation does not appear to be limited by a decrease in arterial blood oxygen content. This finding is supported by similar results presented by Forte *et al.* (1999). Moreover, we found that breathing reserve was normal in both groups; breathing reserve relates ventilatory response during maximum exercise (V_{emax}) to the maximal ability to breathe (MVV) and usually low breathing reserve (< 11 L min^{-1} or 20% MVV) suggests exercise limited by ventilatory capacity (Myers, 2005).

This evidence is in contrast with the hypothesis formulated by Tench *et al.* (2002) who, based on a significant reduction in FVC and FEV_1, cautiously suggested pulmonary function as a limiting factor of SLE patients' exercise capacity.

It is worth highlighting that, in our study, SLE patients appear to obtain an overall V_{emax} equal to the healthy controls by increasing breathing frequency to compensate for a lower V_t. This respiratory pattern is usually referred to as "shallow rapid breathing" and has been demonstrated in patients with CHF (Dimopoulou *et al.*, 2001, Witte *et al.*, 2003) where some authors suggest it might be related to the severity of the cardiac impairment (Weber *et al.*, 1982). A similar breathing pattern has been reported in COPD patients (Matthews *et al.*, 1989) but, in this population, patients with more severe obstruction seem to rely less on this mechanism and V_t appears to be the principal mean of augmenting minute ventilation (Vaz Fragoso *et al.*, 1993). Despite the fact that pulmonary function does not appear to limit maximal oxygen consumption in our cohort, it is possible that respiratory pattern changes might develop after respiratory muscle fatigue as it is believed to minimise respiratory sensation (Gallagher *et al.*, 1985). We did not find any evidence of a reduction in respiratory muscle strength but this does not exclude a possible early development of respiratory muscle fatigue during exercise because no measures of respiratory muscle endurance were performed.

Finally, the analyses of FVC, D_{LCO} and the ratio between these two pulmonary functional parameters have been used in other autoimmune diseases, such as systemic sclerosis, as a predictor of pulmonary hypertension (Steen, 2003). In this clinical context, a FVC to D_{LCO} ratio higher than 1.4 has been considered to increase the likelihood of having pulmonary

hypertension (Steen *et al.*, 1992), a condition that could lead to a decrease in SV (Nootens *et al.*, 1995, Holverda *et al.*, 2006). Our patients group demonstrates a very low FVC to D_{LCO} ratio (0.45 ± 0.06) and therefore a pulmonary hypertension seem to be an unlikely mechanism to explain the reduction in SV in our cohort (see next paragraph).

Cardiac limitations

The present study reveals for the first time that a decrease in CO is limiting VO_{2max} in SLE patients. This reduction in CO appears to be caused by a reduced ability to increase SV rather than an inability to increase HR during exercise. In fact, during submaximal exercise stages patients demonstrate no difference in CO compared to the healthy controls. This is because reduced stroke volume at each stage was compensated by a non significant increase in HR (Fig. 2) as demonstrated by the trend towards a significant main effect for group for SV and a visual inspection of HR, given the lack of main effect for group for the latter variable. A larger number of patients are needed to confirm these findings which are commonly observed in healthy subjects and patients with low VO_{2max} during submaximal exercise at the same absolute workload (Ogawa *et al.*, 1992). Both groups show, as expected, a significant increase in SV at each stage (main effect for load) but SLE group appears to reach an early plateau in SV (between 25 and 50W) while the control group exhibit an increase without signs of a plateau. This response is not unusual and it has previously reported in untrained healthy individuals (Vella and Robergs, 2005).

Similarly to submaximal stages, the reduction in maximal CO found in this investigation appears to be caused by the reduced SV rather than an inability of the heart to increase its

pumping rate. This idea is supported by the lack of significant difference in maximal HR together with clear a trend toward significance for SV in our cohort.

Taken together, the analysis of submaximal and maximal data from the present study seem to indicate that SV is impaired in a group of SLE patients with no diagnosed cardio-pulmonary pathology. Therefore, in our cohort of SLE patients CO appears to limit VO_{2max} by limiting maximum systemic oxygen delivery to the muscles because all other factors determining this variable (haemoglobin concentration and haemoglobin saturation) are not different in this study.

The pathophysiological mechanism causing our results remain unknown. It can be excluded, though, that cardiac function is reduced because of existing clinically relevant cardiac pathologies (exclusion criteria) and lack of physical activity (step counting data). Consequently, cardiac limitation in our SLE cohort could only be caused by subclincal cardiac disease such as pericardial constrain, diastolic and/or systolic dysfunction. Even if a consensus does not exist (Bahl *et al.*, 1992), various investigations have found a reduction in either systolic or diastolic function at rest in SLE patients (Sasson *et al.*, 1992, Astorri *et al.*, 1997, Kalke *et al.*, 1998) but, to the best of our knowledge, no data are available on cardiac response during exercise. Both diastolic and systolic dysfunction can lead to a reduction of stroke volume during exercise as demonstrated by investigations in patients with heart failure (Sullivan and Cobb, 1992).

Alternatively, a mechanical restriction of the heart expansion due to subclinical pericarditis can lead to impaired cardiac function. This cardiac complication is common in SLE patients (Doria *et al.*, 2005) and the pericardium is regarded by many as one of the main factors

limiting SV in healthy subjects (Levine, 2008). Moreover, Levine (2008) highlights how the higher VO_{2max} in athletes is primarily due a more compliant cardiac chamber, and pericardium in particular, allowing a larger SV to be obtained.

These results contradict previous investigations which, based on speculations, suggested peripheral limitations to exercise (Sakauchi *et al.*, 1995, Forte *et al.*, 1999, Keyser *et al.*, 2003). Keyser *et al.* (2003) based their hypothesis simply on the fact that patients in their study, like our cohort, had no clinical evidence of cardiopulmonary disease and no reduction in haemoglobin concentration while Forte *et al.* (1999) and Sakauchi *et al.* (1995) based their hypothesis on O_2 pulse measurements. In a group of SLE patients with no history of cardiovascular problems Sakauchi and colleagues (1995) suggested a peripheral limitation to exercise based on a reduction in the rate of increase in O_2 pulse and a similar argument was used by Forte *et al.* to confirm Sakauchi's findings. The Fick equation shows that a decrease in O_2 pulse can be caused either by a reduction in SV and/or a reduction in A-V O_2diff. The conclusions of the authors are not based on their data but only on the assumption that SV is unchanged because in their cohort there were no cardiopulmonary complications. This is clearly not a valid argument as demonstrated by our data as well as by many studies demonstrating a very high incidence of subclinical heart disease in SLE (Doherty and Siegel, 1985). Furthermore, our data at maximal exercise show a very similar trend towards significance for O_2 pulse and SV, therefore suggesting that the reduction in O_2 pulse is primarily due to a reduction in SV.

Muscle endurance

The results of the isokinetic muscle function test demonstrate a reduction in performance even in an exercise where cardiac output is not the limiting factor (Saltin 1985). Therefore, these data suggest that in SLE patients there is an intrinsic reduction in muscle endurance. This reduction is similar to the one demonstrated by Franssen *et al.* (2005) using the same protocol in patients with COPD. This is the first time that a decrease in muscle endurance has been demonstrated in SLE and it may contribute to the reduction in performance observed during whole-body exercise such as cycling (Marcora *et al.*, 2008, Gagnon *et al.*, 2009). Therefore, it is important to understand its mechanisms. In other patient populations, reduced muscle endurance has been associated with a reduction in mitochondrial function and muscle oxidative capacity (Allaire *et al.*, 2004, Brassard *et al.*, 2006). Future investigations should assess peripheral muscle function in SLE patients to explain the reduction in muscle endurance found in this study.

Conclusions

In conclusion this study demonstrated that in a group of sedentary SLE patients with long standing, well controlled disease, very little organ damage and no clinical evidence of cardio-pulmonary disease, VO_{2max} is limited by CO. The exclusion of patients with clinical evidence of cardiopulmonary disease together with the results of the physical activity analysis indicate that in our cohort only subclinical changes could be causing the results we demonstrated. Certainly, patients with clinically evident cardiovascular disease or more severe pulmonary impairment could demonstrate a different pathological pathway to

exercise limitation. Moreover, our conclusions might underestimate the true extent of cardiac limitations to exercise in SLE; in fact, the cohort recruited for this investigation represent a healthier subgroup of SLE population. Further studies are needed to address this issue.

It is interesting to note that the fact that CO limits VO_{2max} in SLE patients supports the need to measure VO_2 in future studies as an indicator of cardiovascular health and potentially as a predictor of cardiovascular mortality. Submaximal test such the step test **[chapter 2]** previously validated are suitable for this kind of studies requiring large number of participants. Further studies should clarify the mechanism of reduced muscle endurance and investigate the potential role of peripheral muscle oxidative system as a limiting factor during exercise.

Tables and figures

Table 1. Characteristics of SLE patients and healthy controls

Characteristic	SLE (n =12)	Controls (n =12)	*p*
Age (yrs)	51.4 ± 15.4	50.7 ± 15.3	0.583
Height (cm)	162.7 ± 9.4	165.2 ± 5.0	0.372
Body mass (kg)	66.8 ± 12.6	67.0 ± 5.8	0.962
BMI (kg/m^2)	25.1 ± 3.5	24.6 ± 2.0	0.575
Fat free mass (kg)	44.2 ± 6.8	46.1 ± 3.5	0.321
Fat mass (kg)	22.7 ± 7.1	20.9 ± 4.7	0.435
Total cholesterol ($mMol\ L^{-1}$)	4.23 ± 0.98	3.88 ± 0.71	0.289
Systolic Resting BP (mmHg)	124.3 ± 15.1	129.6 ± 9.3	0.325
Diastolic Resting BP (mmHg)	79.8 ± 8.5	81.8 ± 7.7	0.342
Haemoglobin ($g\ dL^{-1}$)	14.0 ± 1.2	14.4 ± 1.2	0.413
Physical activity (steps/day)	10037 ± 3824	9794 ± 2966	0.876

BMI = Body Mass Index; BP = Blood Pressure

Table 2. Clinical characteristics of SLE patients included in the study

Characteristic	Mean or Median	(SD) or [interquartile range]
Disease Duration (yrs)	13.8	(10.5)
SLEDAI (0-105)	0	[2]
SLICC damage score (0-46)	0	[2]
Fatigue Severity Scale (1-7)	4.5	(1.3)

SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; SLICC = Systemic Lupus International Collaborating Clinics.

Table 3. Physiological measure at maximal exercise.

	SLE (n =12)	Controls (n =12)	*p*
VO_{2max} (ml min^{-1} kg^{-1})	22.16 ± 5.88	28.36 ± 8.84	0.017
Cardiac Output (L min^{-1})	13.91 ± 3.41	16.07 ± 2.75	0.025
Stroke Volume (ml)	85.50 ± 18.39	96.37 ± 12.49	0.067
Heart Rate (beats min^{-1})	160.7 ± 20.9	166.3 ± 12.5	0.308
Oxygen pulse (ml min^{-1} kg^{-1} $beats^{-1}$)	9.23 ± 2.70	11.42 ±3.52	0.069
RER	1.23 ± 0.09	1.14 ± 0.05	0.262
MAP (mmHg)	121.4 ± 11.	115.6 ± 17.9	0.294
Oxygen saturation (%)	96.3 ± 1.1	96.7 ± 1.0	0.240
RPE	17.5 ± 1.6	18 ± 1.5	0.484
Blood Lactate (mMol L^{-1})	6.1 ± 2.2	6.5 ± 2.2	0.688
AT (%VO_{2max})	53.9 ± 5.9	52.9 ± 6.4	0.758

RER = Respiratory equivalent ratio; MAP = mean arterial pressure; RPE = rate of perceived exertion; AT = anaerobic threshold.

Table 4. Respiratory function of SLE patients and healthy controls

Variable	SLE (n = 12)	Controls (n = 12)	*P*
FVC (liters)	3.11 ± 0.62	3.70 ± 0.76	0.041
FVC (% of predicted value)	98.3 ± 22.2	106.4 ± 18.7	0.390
FEV_1 (liters)	2.40 ± 0.52	2.94 ± 0.70	0.035
FEV_1/FVC (%)	78 ± 10	79 ± 9	0.778
MVV (L min^{-1})	96.1 ± 21.0	117.6 ± 28.1	0.035
D_{LCO} (ml $min^{-1}mmHg^{-1}$)	7.16 ± 1.36	7.82 ± 1.54	0.263
Inspiratory strength (cm H_2O)	76.3 ± 28.8	81.4 ± 19.6	0.639
Expiratory strength (cm H_2O)	106.1 ± 39.4	117.6 ± 29.6	0.131
$V_{e\,max}$ (L min^{-1})	57.6 ± 12.3	66.83 ± 14.7	0.112
$V_{t\,max}$ (L min^{-1})	1.46 ± 0.27	1.80 ± 0.33	0.015
BF_{max} (breaths min^{-1})	40.1 ± 6.3	37.4 ± 6.3	0.262
Breathing Reserve (L min^{-1})	38.7 ± 20.1	50 ± 19,6	0.166
% MVV at max exercise	63.0 ± 17.3	59.1 ± 13.6	0.584

FVC = forced vital capacity, FEV_1 = forced expiratory volume in 1 second, MVV = maximal voluntary ventilation, $V_{e\,max}$ = minute ventilation at maximal exercise, $V_{t\,max}$ = tidal volume at maximal exercise, BF_{max} = breathing frequency at maximal exercise, Breathing reserve = (MVV- V_{emax})

FIGURE 1. Results of VO_{2max} (top left), HR_{max} (top left), CO_{max} (bottom left) and SV_{max} (bottom right); SLE (white bars) and healthy controls (grey bars).

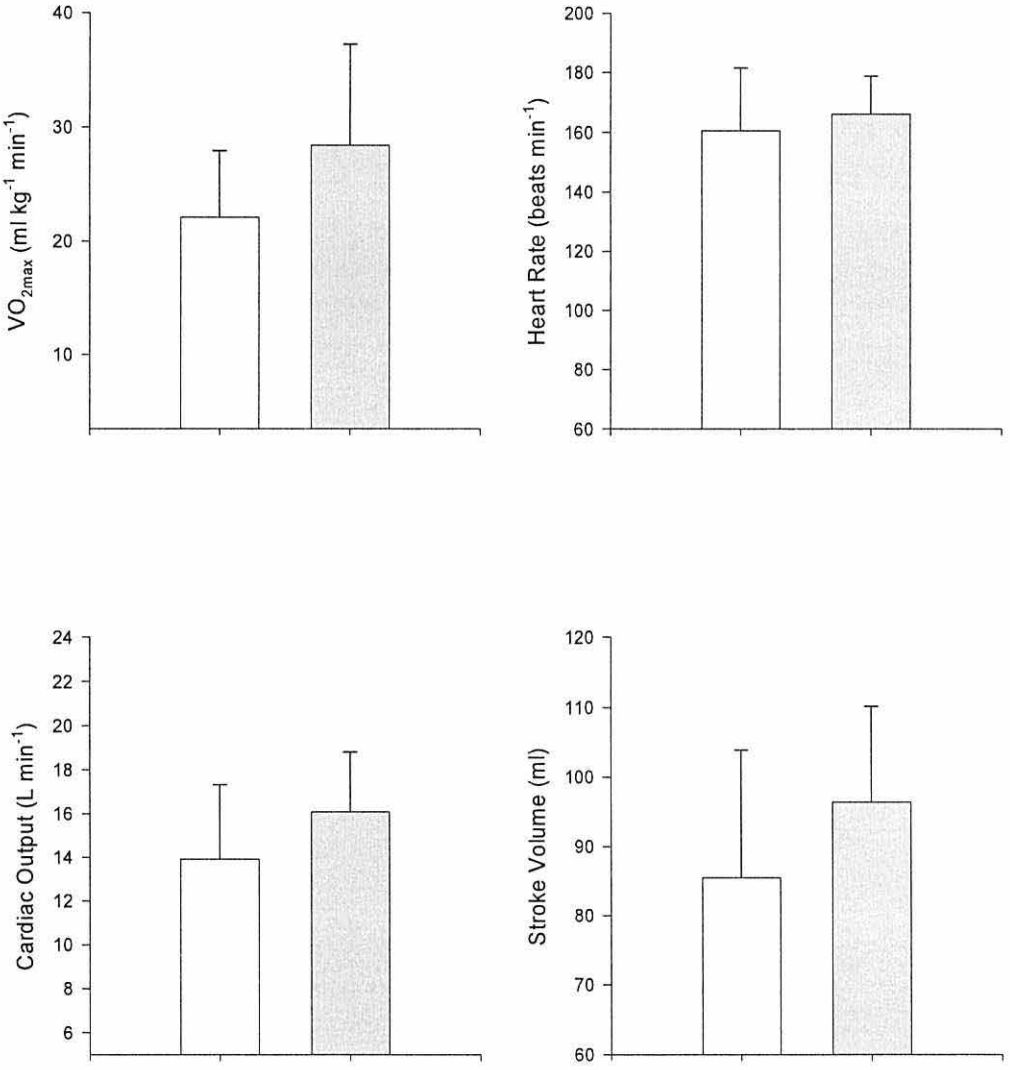
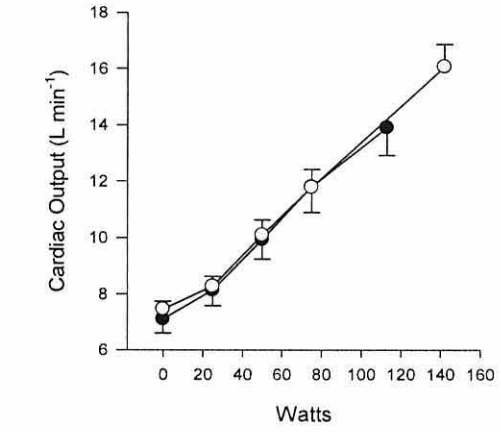
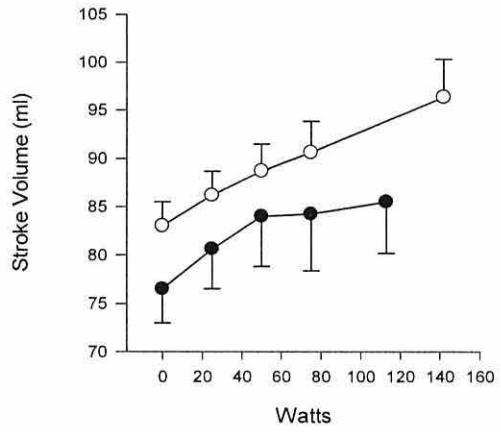
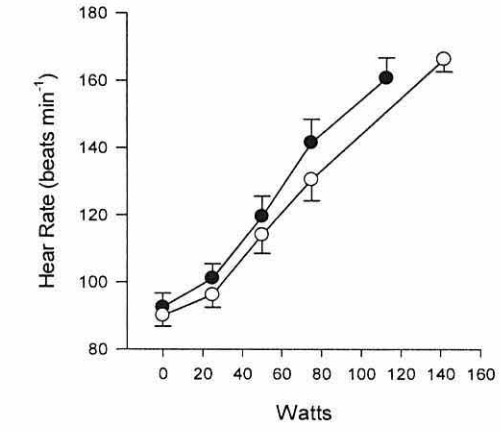


FIGURE 2: Cardiac response to exercise. Data presented are all stages completed by all subjects and maximal data. Error bars represent standard error.







CHAPTER 5

MUSCLE OXYGEN CONSUMPTION IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS MEASURED BY NEAR INFRARED SPECTROSCOPY

Introduction

Systemic lupus erythematosus (SLE) is a chronic, autoimmune, inflammatory disease that can manifest itself in many different organs with different degrees of severity (Isenberg and Ehrenstein, 2004). Many investigations have demonstrated a reduction in maximal oxygen consumption (VO_{2max}) in this population (Sakauchi *et al.*, 1995, Tench *et al.*, 2002, Keyser *et al.*, 2003, Bostrom *et al.*, 2008): the maximal values available in the literature range between 17.4 and 25.1 ml $kg^{-1}min^{-1}$ thus demonstrating a severe impairment of exercise capacity.

In a previous study **[chapter 4]** we confirmed this impairment and we demonstrated for the first time that cardiac function limits whole body VO_{2max} in SLE patients; however, in the same investigation we found a reduction in a single-leg muscle endurance test that can not be limited by cardiac function because of the relatively low cardiac output required to perfuse a small muscle mass (Calbet *et al.*, 2009).

The role of this reduced muscle endurance towards the reduction in exercise capacity is unknown but locomotor muscle dysfunction per se can decrease exercise tolerance (Marcora *et al.*, 2008). It is, therefore, important to understand the mechanisms of reduced muscle endurance in SLE patients.

Performance in this test could be limited by a wide range of different mechanisms such as inadequate peripheral blood flow regulation (Wernbom *et al.*, 2009), poor muscle oxygen extraction and utilisation (Mortensen *et al.*, 2005), as well as ionic and metabolic changes at muscle cell level (Allen, 2009). Reduced neuromuscular activation, known as "central

fatigue", may also contribute to poor muscle endurance during voluntary contractions (Gandevia, 2001).

Of particular interest is the potential impairment of muscle oxygen extraction and utilisation. Such impairment has been related to mitochondrial dysfunction (Van Beekvelt *et al.*, 1999) which is a likely complication of SLE. In fact, systemic inflammation is associated with mitochondrial dysfunction (Perl, 2009). Furthermore, SLE patients have anti mitochondrial antibodies and reduced lymphocyte mitochondrial function (Perl *et al.* 2004). Whether such impairment occurs at muscle level and is large enough to reduce muscle oxygen extraction and utilisation is unknown.

Muscle oxygen consumption in vivo can be measured invasively using the Fick method by sampling blood from relevant arteries and veins combined with a measure of blood flow, normally obtained through the use of the thermodilution method (Calbet *et al.*, 2005). This method is considered to be the gold standard for this measurement but some limitations have been highlighted (Rowell, 2007). Firstly, measures are limited by the fact that some muscle have no convenient measuring site like a single collecting vein; moreover, it requires great expertise and its invasive nature carries an increased risk of infections.

Alternative non invasive methods exist and these are commonly based on haemoglobin spectroscopic measurements. Near infra-red spectroscopy (NIRS) is a non invasive method to indirectly assess local muscle oxygen consumption (Van Beekvelt *et al.*, 2001b). This technique has been used in a number of studies on different clinical populations (Van Beekvelt *et al.*, 1999, Brunnekreef *et al.*, 2006, Abozguia *et al.*, 2008) and appears to

provide useful information as well as being well tolerated by the patients. Moreover, NIRS technology has been used non invasively to discriminate between patients with mitochondrial myopathies and healthy controls (Van Beekvelt, *et al.*, 1999).

Near infra-red spectroscopy technology is based on the transparency of tissue to light in the near infra red spectral region combined with the fact that human tissue contains chromophores, such as haemoglobin. In the spectral range 700–1000 nm, light can be used to assess muscle concentration of these chromophores and their relative change. There is a wide range of NIRS technologies available on the market. These different machines calculate chromophores concentrations using various algorythms based on specific physical principles.

NIMO is new a continuous source wave instrument that utilises a three laser diode sources and a low-noise detection system and performs quantitative assessments exploiting precise optical absorption measurements close to the absorption peak of the water (Rovati *et al.*, 2004). Utilising this principle NIMO offers physiological information about the oxygenation state of the tissue by noninvasively determining oxyhaemoglobin concentration (HbO_2), deoxyhaemoglobin concentration (HHb) and total haemoglobin concentration (HbO_2 + HHb) (Rovati *et al.*, 2004). NIMO has been previously validated but it has never been used to investigate local muscle oxygen consumption.

Therefore, the first aim of this study was to assess the responsiveness of the NIMO to changes in muscle oxygen consumption induced by light isometric exercise in a group of healthy subjects. If found sensitive, the second aim of the study was to use this technique to investigate the effect of SLE on muscle oxygen consumption by comparing patients with a group of age and sex matched sedentary controls.

Methods

This investigation consisted of two separate studies. Methods used in each study will be presented separately.

Study 1: Subjects

For the first study, twelve healthy volunteers (6 males, 6 females) participated in this study conducted at the School of Sport, Health and Exercise Sciences in Bangor. Subjects were required to attend one testing session lasting approximately 1 hour.

Measurements

The protocol used in this study has been developed and validated by Van Beekvelt *et al.* (2001). After signing the informed consent to take part in the study, the participant sat in a comfortable position for 10 min before the test, right hand rested on a handgrip dynamometer with the upper arm at heart level and the forearm in an upward angle of 30°. Pneumatic cuffs were placed around the upper arm and the wrist.
During this resting period, haemoglobin concentration (Hb) was measured from 5 μL of arterialised blood collected from the finger tip using a B-hemoglobin photometer (Hemocue, Sweden).

Each subject's maximum voluntary contraction (MVC) force and subcutaneous fat thickness were determined before the test. After 3 submaximal warm up contractions, the subjects

performed three maximal contractions using a hand held dynamometer (MLT003A, Powerlab, Adi Instruments Pty Ltd, Bella Vista, Australia) and the highest value recorded was used as MVC for the analysis. Maximal voluntary contraction force was measured during 3 seconds contractions separated by 1 min resting periods. Adipose tissue thickness (ATT) was measured by mean of a Harpenden skinfold calliper (John Bull British Indicators Ltd, West Sussex, England) by dividing by 2 the measure of a double fold of skin and adipose tissue. Three consecutive measurements were performed overlaying the flexor digitorum superficialis muscle and the average of the three measures was used for the study.

Near infra red spectroscopy measures were performed on the forearm of the subjects by placing the probe of a continuous wave NIRS instrument (NIMO, Nirox, Brescia Italy) on the skin in correspondence with the flexor digitorum superficialis muscle using palpation and anatomical reference points to locate the muscle. The distal tendon of the biceps brachii muscle was used as a reference point: the probe was placed distally (1-2 cm according to subject's arm length) from the point where the tendon can be palpated in the antecubital fossa. Moreover, probe was placed in the medial half of the forearm (with the subject in the standard neutral anatomical position) with the optdoes placed on the muscle's belly. In order to ensure good positioning, subjects were required to perform gentle muscle contraction to locate the flexor digitorum superficialis muscle. Interoptode distance for this investigation was 30mm and therefore sampling depths was approximately 15mm.
The protocol used in this study started with 4 min of rest after which the wrist cuff was inflated (260 mmHg). One minute later, an arterial occlusion (260 mmHg) was obtained by inflating the upper arm cuff. Inflation and deflation were controlled by an automated cuff

inflator system (Hokanson AG 101 air source and Hokanson E 20 rapid cuff inflator, PMS Instruments, Maidenhead, UK). The arterial occlusion lasted 45 s.

After 5 min of recovery, the subjects were asked to perform sustained isometric handgrip exercise at 10% MVC using the same device utilised for the maximal strength measurement. The 10% level was maintained with the help of a visual feedback system (LabChart, Powerlab, Adi Instruments, Bella Vista, Australia). The wrist cuff was inflated at the start of exercise. After 50 s of exercise, when NIRS signals reached steady state a rapid inflation of the upper arm cuff was applied in order to create an arterial occlusion which lasted 45 s. When this time was elapsed the exercise ended and all cuffs released.

Muscle oxygen consumption was derived from NIRS using arterial occlusion by evaluating the rate of increase in HHb. During arterial occlusion, muscles can utilise only oxygen available in the tissue because no arterial inflow or venous outflow is permitted by the pressure imposed by the cuffs. In this condition changes in HHb trace reflects MVO_2; moreover, when using NIRS technology, HHb is commonly considered as an indicator of muscle oxygen extraction (DeLorey *et al.*, 2003, Ferreira *et al.*, 2007) and it is therefore the most suited NIRS derived variable for this analysis. Concentration changes in HHb were expressed in micromolars per second and converted to milliliters of oxygen per minute per 100 grams (ml O_2 min^{-1} $100g^{-1}$). A value of 1.04 kg L^{-1} was used for muscle density according to the procedures described by Van Beekvelt *et al.* (2001).

Statistical analysis

Data are reported as mean (SD) for normally distributed variables; otherwise, data are reported as median [interquartile range]. Significant change in MVO_2 from rest to exercise was analysed using a two tailed paired t-test. Responsiveness of the measure was evaluated by using effect size calculated by Cohen's D (change scores normalised by pooled standard deviation)(Husted *et al.*, 2000, Terwee *et al.*, 2003). Significance level was set at 0.05 for all analyses.

Study 2: Subjects

For the second study, after ethical approval from the local Research Ethics Committee 10 SLE patients fulfilling the American College of Rheumatology 1997 revised criteria for SLE (Hochberg, 1997) and 10 healthy age (±5 years) and gender matched controls were recruited for this study. The test was conducted during one visit at the School of Sport, Health and Exercise Sciences in Bangor and lasted approximately one hour.

Measurement

Muscle oxygen consumption was measured using the same procedures described in study 1.

Clinical measures

Global disease activity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Bombardier *et al.*, 1992). The SLEDAI is a valid measure of SLE activity (Ward *et al.*, 2000) and generates a total score between 0 and 105 with high scores indicating higher disease activity. Organ damage was assessed by the Systemic Lupus International Collaborating Clinics (SLICC) damage index (Gladman *et al.*, 1997). The SLICC produces a score between 0 and 46 with high scores indicating more severe damage. All these measures were completed by each patient's consultant rheumatologist within a maximum of three weeks from exercise testing

Statistical analysis

Differences in subject's characteristics between the two groups were assessed by two tailed paired t-tests. A two way ANOVA for repeated measures (group by condition) was used to compare the response between group (SLE, controls) and condition (rest, exercise) in MVO_2 derived from HHb trace. Significance level was set at 0.05 for all analyses.

Results

Study 1

Subjects characteristics

In Study 1, twelve subjects volunteered for this investigation, the descriptive characteristics of the subjects recruited are reported in table 1. Importantly, subjects in this study were not overweight or anaemic, thus excluding potential error due to very high subcutaneous fat and very low haemoglobin concentration (van Beekvelt *et al.*, 2001a).

NIRS measurements

For study 1, values for MVO_2 are presented in figure 1. There was a significant increase ($p = 0.008$) in MVO_2 from rest (0.05 ± 0.02 ml O_2 min^{-1} $100g^{-1}$) to isometric handgrip exercise (0.10 ± 0.08ml O_2 min^{-1} $100g^{-1}$). The effect size of the change between rest and exercise was very large (Cohen's D = 0,87).

Study 2

Subjects characteristics

In study 2, twenty subjects (18 female, 2 males) were recruited for this investigation. The anthropometric, demographic and descriptive characteristics of these subjects are presented in Table 2. The two groups were well matched for age, weight, height, and there were no significant differences in haemoglobin concentration, skinfold thickness and handgrip strength. The results of the clinical measurement are shown in Table 3. Patients recruited in this study had long standing disease, low disease activity and very little damage.

NIRS measurements

For study 2, values for MVO_2 are presented in Table 4 and Figure 2. There was no significant difference in changes from rest to exercise between the groups (no group by condition interaction $p = 0.879$), no significant difference between groups (no main effect for group $p = 0.307$) and a significant change from rest to exercise (significant main effect for condition $p = 0.005$).

Discussion

These studies demonstrate that NIMO infra-red spectroscopy is able to detect significant changes in MVO_2 during arterial occlusion in healthy volunteers (study 1). Moreover, study 2 demonstrates that there is no significant difference in MVO_2 between healthy controls and a group of SLE patients with long standing disease, low disease activity and limited organ damage.

NIMO sensitivity

In study one we investigated the possibility to use NIMO to detect significant changes in MVO_2 induced by light isometric exercise. The significant increase measured in our cohort clearly demonstrate that NIMO technology is suitable to measure MVO_2. This conclusion is also supported by the analysis of Cohen's D which is commonly used as an accepted measure of responsiveness. In fact effect size was 0.87 in this study thus showing good internal responsiveness, i.e. that the ability of the measure to change in response to exercise was acceptable (Husted *et al.* 2000). No previously published data on responsiveness of NIRS are available and therefore no comparison can be made on this variable; moreover, responsiveness of previous studies cannot be calculated as no data on the standard deviation of the change scores are available.

In this investigation there was an 100% (from 0.05 to 0.1 ml O_2 min^{-1} $100g^{-1}$) increase in MVO_2 from rest to exercise. Comparison of our results on absolute resting values and exercise induced changes with previously available studies is difficult because published

data vary largely according to the muscle group measured, the depth of measurement and the NIRS technology adopted. For example, Van Beekvelt *et al.* (2001), in the validation of the protocol used in these studies, reported resting values between 0.09 and 0.13 when measuring flexor digitorum superficialis and brachio-radialis muscles at different depths (1.75 and 2.5mm for flexor digitorum superficialis and 1.75mm for brachio-radialis). In the same investigation changes induce by exercise ranged between 54 and 600% according to probe position and depth of measurement. Using the same protocol Brunnekreef *et al.* (2006) reported a resting value of 0.06 ml O_2 min^{-1} $100g^{-1}$ and a 350% increase with exercise when measuring the extensor carpi radialis brevis muscle. Moreover, Van Beekvelt *et al.* (2002) reported that published values for resting MVO_2 measured by NIRS and 31P magnetic resonance spectroscopy ranged between 0.05 and 0.23 ml O_2 min^{-1} $100g^{-1}$ thus supporting the validity of NIMO as measuring device. This great variety of results might be surprising but it has to be noted that local muscle metabolism is highly variable irrespective of the mean used for the assessment (Larson-Meyer *et al.*, 2000). An additional explanation of the large range of results obtained for NIRS derived MVO_2 measurement could be the different physical principles used by the machines available on the market. NIMO, for example, performs quantitative assessments exploiting precise optical absorption measurements close to the absorption peak of the water (Rovati *et al.*, 2004). On the contrary, Van Beevelt and colleagues in their studies used a continuous-wave near infra-red spectrometer (Oxymon) using a range of wavelength (frequency resolved) to obtain quantitative measurement of the choromophores. To the best of our knowledge, no direct comparison has ever been done between different NIRS devices so no definitive conclusion can be done on the technological explanation behind the range of published values.

Overall, NIMO appears to provide useful MVO_2 data that are in agreement with published data, despite being in the lower end of the range. From this consideration it can be concluded that it is helpful to use a control group when measuring MVO_2 using NIRS technology because comparison with published data could be meaningless given the wide range found in the literature.

MVO_2 in SLE

Results of study 2 demonstrate that muscle oxygen extraction and utilisation at rest and during exercise do not appear to be affected in our group of SLE patients. To the best of our knowledge this is the first time that local oxygen consumption has been evaluated in SLE patients. Using indirect measures it has previously been suggested that SLE patients might have a reduced muscle oxygen uptake (Sakauchi *et al.*, 1995) but the result of our investigation excludes this hypothesis. Sakauchi and colleagues (1995) speculated that a reduction in the rate of increase in oxygen pulse (whole body oxygen consumption / hear rate) during whole body dynamic exercise could be caused by impaired oxygen extraction. A decrease in oxygen pulse can be caused either by a reduction in stroke volume and/or a reduction in arterial-venous oxygen difference, a variable that can be influenced by muscle oxygen uptake. The authors failed to measure stroke volume and therefore their conclusion remains speculative. Data from the current study provide a more direct measure of oxygen extraction; the reason for this is that MVO_2 measurement using NIRS were done during arterial occlusion and consequently independent from blood flow, therefore influenced only by muscle oxygen uptake and utilization

The rate of increase in MVO_2 from rest to exercise in both SLE patients and healthy controls appears to be slightly lower than previously reported. The reason for this smaller response could be, as mentioned before, technical differences between the NIRS machines used in these studies. Alternatively, aging could partially explain our limited increase in MVO_2. In fact, our groups of SLE patients and healthy controls were older than the groups studied in the aforementioned investigations (48 yrs in our cohort vs approximately 30 yrs, where stated, in other studies). Aging has been found to cause a decrease in muscle metabolism and mitochondrial function (Nair, 2005) even if some authors (Kutsuzawa *et al.*, 2001) argue that this is not a consistent finding. Importantly, Parker and colleagues (2008) have recently demonstrated, using NIRS measurements, that elderly patients have a reduced increase in muscle oxygen extraction in the first few minutes of dynamic exercise thus supporting the role of ageing as an explanation for our results.

In conclusion, this study using non invasive quantitative NIRS technology demonstrated that there was no significant difference in MVO_2 at rest and during exercise between a group of SLE patients with long standing disease, low disease activity, limited damage and a group of age and gender matched healthy controls. This findings seem to exclude oxygen extraction and utilisation as mechanisms for the reduce muscle endurance found in **chapter 4**. Further studies are needed to investigate other possible mechanism of diminished muscle endurance such as ionic and metabolic changes at muscle cell level (Allen *et al.*, 2008) and reduced neuromuscular activation (Gandevia, 2001).

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Tables and figures

Table 1. Characteristics of the participants in study 1

Variable	
Age (yrs)	26.5 ± (4.7)
Height (cm)	171 ± (9)
Body mass (kg)	70.8 ± (12.9)
BMI (kg m^{-2})	24.1 ± (2.5)
Forearm ATT (mm)	3.24 ± (0.9)
Haemoglobin (g dL^{-1})	14.2 ± (1.5)
Grip Strength (N)	43.5 ± (9.7)

BMI = Body Mass Index, ATT = adipose tissue thickness

Table 2. Characteristics of the SLE patients and healthy controls in study 2

Characteristic	SLE (n =10)	Controls (n =10)	*p*
Age (yrs)	48.3 ± 15.5	48.6 ± 15.1	0.498
Height (cm)	162.4 ± 10	163.4 ± 6	0.679
Body mass (kg)	68.8 ± 12.1	67.9 ± 10.6	0.930
BMI (kg m^{-2})	26.0 ± 3.0	25.4 ± 3.7	0.812
ATT (mm)	5.8 ± 2.3	5.2 ± 2.6	0.525
Haemoglobin (g dL^{-1})	12.9 ± 1.5	13.9 ± 1.1	0.127
Handgrip strength (N)	27.9 ± 10.6	30.2 ± 9.5	0.616

BMI = Body Mass Index. ATT = adipose tissue thickness

Table 3. Clinical characteristics of the SLE patients included in the study

Characteristic	Mean or Median	(SD) or [interquartile range]
Disease Duration (yrs)	13.1	(10.6)
SLEDAI (0-105)	0.0	[0.7]
SLICC damage score (0-46)	0.1	[0.2]

SLEDAI = Systemic Lupus Erythematosus Disease Activity Index; SLICC = Systemic Lupus International Collaborating Clinics.

Table 4. NIRS muscle oxygen consumption in SLE patients and healthy controls

	MVO_2 ($ml\ O_2\ min^{-1}\ 100g^{-1}$)	
	Rest	Exercise
SLE	0.056 ± 0.06	0.083 ± 0.10
Controls	0.028 ± 0.07	0.058 ± 0.04

FIGURE 1. Muscle oxygen consumption measured from changes in the HHb traces at rest and during 10% MVC exercise in study 1.

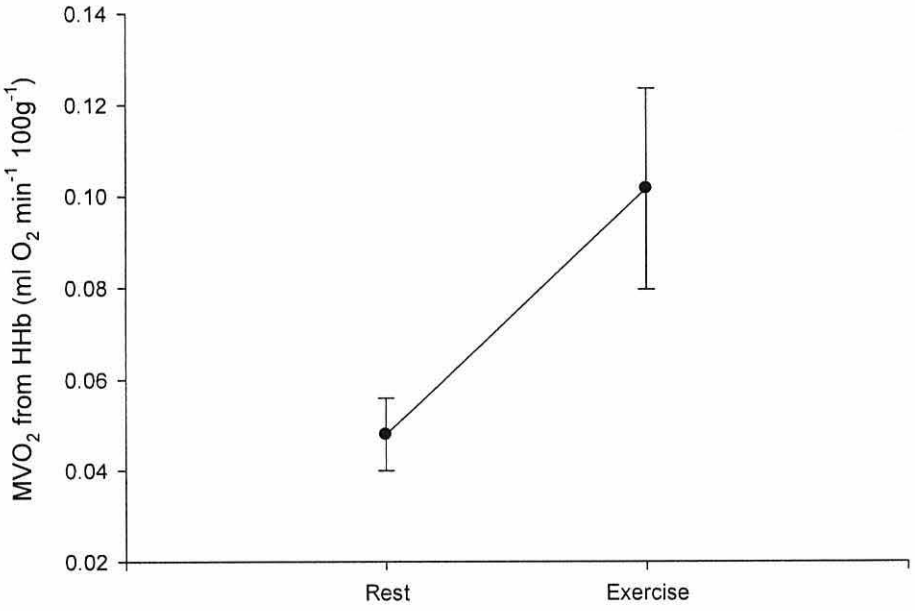
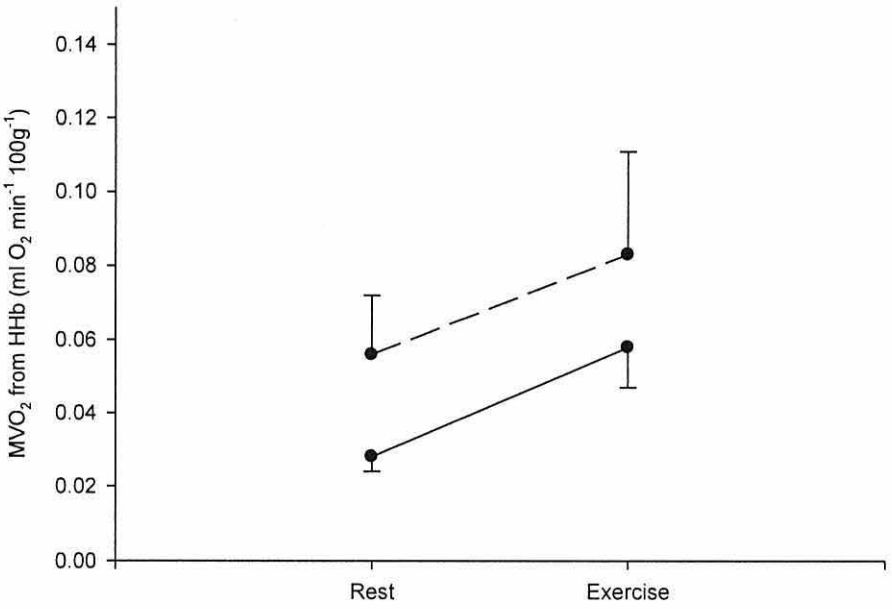


FIGURE 2. Muscle oxygen consumption measured from changes in the HHb traces at rest and during 10% MVC exercise in SLE patients (dashed line) and healthy controls (solid line).



CHAPTER 6

GENERAL DISCUSSION

This chapter brings together and discusses the findings that emanate from the various studies within this thesis. The chapter comprises six main sections. The first section provides a brief summary of individual findings of each chapter. The second section discusses direct and indirect measurements of maximal oxygen consumption (VO_{2max}) in patients affected by systemic lupus erythematosus (SLE). The third section analyses the relative impact of factors limiting VO_{2max} by combining different studies within this thesis. The fourth and fifth sections present potential limitations of the thesis and suggestions for future research. Finally, brief concluding remarks are presented.

Summary of main findings

The first study of this thesis **[chapter two and three]** proves the feasibility of using prediction equations based on heart rate (HR) to predict VO_{2max} in patients affected by SLE. In **chapter two** we showed that prediction of VO_{2max} using the Siconolfi bike test was as accurate in our group of SLE patients as it was in the healthy population used for the original validation (Siconolfi *et al.* 1982). The same finding was replicated using the step test in **chapter three** thus showing that the relationship between VO_2 and HR, on which this test is based, is the same in SLE and in the general population independently of exercise modality.

The validation of these two submaximal tests offers the possibility of choosing between two different methods of prediction. Both these two tests have advantages and some limitations that can influence the choice between them. The validation of the step test offers the possibility to predict VO_{2max} in clinical settings and in large epidemiological studies because of the short time required to complete it (3 min in most patients) and the low cost of the equipment. However, the stepping exercise might be impossible for

patients with musculoskeletal problems. On the other hand, the submaximal bike test might be feasible in this kind of patients but its use in a clinical setting might be limited by the availability of the cycle ergometer and the longer time required for the test.

The focus of the maximal exercise test in **chapter four** was to investigate the mechanisms of reduced VO_{2max} in SLE by combining a maximal cycle ergometer test with cardiovascular, pulmonary and peripheral function assessments. The result of our investigation shows for the first time that VO_{2max} is limited by cardiac output (CO) with no other apparent contribution from the other factors. This reduction in CO appeared to be caused by an inability of the heart to increase stroke volume (SV). Similarly to Tench *et al.* (2002), we found changes in pulmonary function but we were able to demonstrate that these changes do not contribute to reduction in VO_{2max} by analysing respiratory pattern and haemoglobin saturation at maximal exercise. We also demonstrated for the first time a significant reduction in muscle endurance, which seems to indicate that some peripheral impairment is present in SLE patients.

Study three was designed to further understand the reduction in the muscle endurance test found in study two by analyzing muscle oxygen consumption (MVO_2) **[chapter four]** and to verify the feasibility of using NIMO infrared technology to implement laboratory protocols previously published using different NIRS technologies. By using a validated protocol we were able to demonstrate that NIMO technology is suited to detect changes in MVO_2 between rest and light isometric exercise. Furthermore, by using the same non invasive technique we demonstrated that MVO_2 in our cohort of SLE patients was not different from a healthy control group.

Measurements of VO_{2max} in SLE patients

The first two studies of this thesis **[chapter two to four]** directly measured VO_{2max} in SLE patients using an incremental protocol on a cycle ergometer. Our results show that the maximal values in our studies are in agreement with previously published investigations (Robb-Nicholson *et al.*, 1989, Sakauchi *et al.*, 1995, Tench *et al.*, 2002, Keyser *et al.*, 2003, Bostrom *et al.*, 2008). In fact, we found an average VO_{2max} of 22.4 ml kg^{-1} min^{-1} in study one and an average of 22.2 ml kg^{-1} min^{-1} in study two, demonstrating high consistency of our results. Importantly, no adverse events resulted from maximal exercise tests in these studies.

Other investigators (Tench *et al.*, 2002, Keyser *et al.*, 2003) previously reported the results of a maximal exercise test in SLE patients as VO_{2peak} rather than VO_{2max}. The difference between these two values measured during incremental exercise is that the latter definition can be used only when a true maximal limit of exercise capacity is reached, while the former does not necessitate this requirement. In **chapter two** and **chapter three** we used the criteria for the definition of maximal effort adopted in the original validation studies (Siconolfi *et al.* 1982, Siconolfi *et al.* 1985). Specifically, these criteria were 1) a plateau, defined as a difference less than 0.25 L min^{-1} between the two final VO_2 measurements taken every 30s, and 2) a respiratory quotient (RER: VO_2/VCO_2) for the last measurement equal to or greater than 1.0.

Some researchers might question such criteria as defining a true VO_{2max} but our choice of criteria was constrained by the primary aim of the study. In fact, the validation of the test required that the criteria originally used by Siconolfi *et al.* (1982) were applied to our data. Using these predefined criteria we excluded one patient from the final analysis for failing to reach VO_{2max} as previously defined.

By contrast, in study two **[chapter four]** we were not compelled to use criteria used by Siconolfi *et al.* (1982) and therefore a range of different criteria was used. As discussed in the introduction to the thesis [Maximum oxygen consumption-What is it?], primary and secondary criteria for achieving a true maximum during incremental exercise are subject to criticism and no clear definition has been found in the literature (Duncan *et al.*, 1997). In study two, we used a combination of secondary criteria that were more strict than those used in study one. Specifically, these criteria were 1) achievement of an HR higher than 90% of maximal predicted HR (220-age), 2) a RER equal to 1.15 or higher, 3) a blood lactate concentration equal to 6 mMol L^{-1} or higher, 4) a rate of perceived exertion of 17 or higher on the Borg 6-20 scale. The achievement of these criteria gave us clear evidence that a true maximal effort determined the end of exercise in the SLE patients and healthy controls. However, it is interesting to note that the average value achieved in study one and two are almost identical, supporting the idea that a true maximum was achieved in all studies of this thesis.

Previous investigations reporting their data as VO_{2peak} described values consistent with their conclusion. For example, Tench *et al.* (2002) reported that 33% of their cohort could not reach a RER higher than 1.1 and therefore correctly defined their result VO_{2peak}. All but one patients recruited in study one and all patients in study two were able to reach an RER higher than 1.1, demonstrating that a true maximum can be achieved in SLE patients with low disease activity and limited organ damage.

Another important finding demonstrated by **chapter two and chapter three** is the validity of submaximal tests to predict VO_{2max} in SLE patients. The previous discussion about achieving a true maximal result highlights how the direct measure of oxygen consumption, despite being the gold standard in this area, is not always practical and

can potentially carry a small risk. In fact, vigorous exercise such as maximal exercise performed by patients and controls in this thesis is associated with an increase risk of cardiovascular problems and sudden death in healthy subjects with no self reported cardiovascular disease and in patients with CHF (Albert *et al.*, 2000, Thompson *et al.*, 2007b). This risk of fatal cardiovascular complications is highly decreased by the availability of an automatic defibrillator and trained personnel (Thompson *et al.*, 2007b). In all laboratory sessions of this thesis a defibrillator was accessible and a researcher trained in resuscitation techniques present in the laboratory. Furthermore, in many situations direct measure of VO_{2max} might not be practical because it requires expensive, cumbersome equipment and trained personnel.

The validation of Siconolfi's submaximal tests for the prediction of VO_{2max} in SLE overcomes all the previously mentioned problems by requiring only a moderate effort. **Chapter two and chapter three** established that the relationship between VO_2 and HR is as valid in SLE as it is in the healthy population. This is a novel finding as no other published studies have validated submaximal tests in SLE. The study by Minor and Johnson (1996) used a group of patients with a range of rheumatic diseases and included only a minor percentage of SLE patients therefore only giving partial information in the context of SLE.

In our view, the work in this thesis gives a definitive answer concerning the validity of the VO_2 /HR relationship in SLE patients so that there is no particular need for validation of other submaximal exercise tests. There is, in fact, no reason to suggest that this relationship would not hold for different exercise modalities considering that it has been proven valid for stepping and cycling. Of course, for research purposes and when an accurate estimation of VO_{2max} is needed, only the gold standard direct measure

should be employed when one considers the relatively wide limits of agreement and standard error of estimate found in **chapter two and chapter three**.

Mechanism of reduced VO2max in SLE patients

The second and third study of this thesis **[chapter four and five]** investigated the factors limiting VO_{2max} in SLE patients. In exercise physiology, cardiac function, pulmonary function and arterial blood oxygen content are usually referred to as "*central factors*" whereas blood flow distribution, muscle oxygen extraction and utilisation are referred to as "*peripheral factors*". **Chapter four** demonstrated for the first time that central factors were limiting VO_{2max} in SLE patients. This finding was in contrast with previous investigations using other methods [see discussion **chapter four**].
Of the central factors, CO was measured for the first time and emerged as the only limiting factor in our cohort. There was in fact no sign of pulmonary limitation to exercise (see **chapter four** for a thorough discussion). Indeed, CO was found to be reduced in SLE patients and this reduction appears to be due to SV rather than HR. This conclusion is supported by data presented in **chapter two, three and four**. In fact, in **chapters two and three**, as well as in **chapter four**, HR appeared to reach a level indicating maximal effort and, therefore, the heart chronotropic ability does not appear to limit VO_{2max}. This finding is consistent with previous studies reporting maximal HR during exercise in SLE patients (Robb-Nicholson *et al.*, 1989, Sakauchi *et al.*, 1995; Bostrom *et al.* 2008). Moreover, in **chapter four**, during all submaximal stages HR was found to be elevated (although not significantly) in order to obtain an adequate CO in view of a decreased SV. This pattern of cardiac response to exercise is not unusual and it s commonly observed in other clinical populations or in sedentary subjects when exercising at the same absolute workload (Ogawa *et al.*, 1992).

Cardiac output, consequently, is limited by SV although the aetiology of this impairment cannot be explained on the basis of our data. In our cohort of patients we excluded individuals with clinically relevant cardiovascular disease and we obtained a good match for physical activity therefore only subclinical cardiac impairment could be the cause of the reduction in SV. In this thesis, we did not perform any other investigation of cardiac function and therefore we can only speculate which subclinical cardiac dysfunction might have caused the reduction in SV. Previous investigations in SLE showed that subclinical pericardial disease, systolic and/or diastolic dysfunction could be demonstrated in these patients. These conditions could potentially limit SV (Kitzman *et al.*, 1991, Sullivan and Cobb, 1992, Schlant and Roberts, 2001) and further research is needed to investigate this hypothesis.

Finally, the finding that CO is limiting maximal exercise in SLE patients supports the need to study the potential role of VO_{2max} as a predictor of cardiovascular mortality. In fact, in healthy subjects VO_{2max} is limited by CO (Levine, 2008) and it is a excellent predictor of cardiovascular mortality (Carnethon *et al.*, 2005, Gulati *et al.*, 2005, Kallinen *et al.*, 2006). Consequently, it is plausible to hypothesise that VO_{2max} might demonstrate a similar predictive power in SLE patients.

No definitive conclusion on the contribution of peripheral factors towards the reduction in VO_{2max} can be drawn from this thesis. It appears, though, that peripheral factors play only a minor role, if any, in limiting oxygen consumption in SLE patients. In fact, when assessing peripheral oxygen consumption measured by NIRS in the forearm **[chapter five]** no difference was found. This measure is independent of circulatory factors and reflects oxygen extraction and utilisation in skeletal muscles. In the introduction of this thesis **[chapter one]** we described how basal membrane thickening could reduce muscle

oxygen extraction and possible mitochondrial dysfunction could diminish oxygen utilisation. The measurement of MVO_2 in chapter five indicate that those factors are not majorly affected by SLE in our cohort. The finding of **chapter five** also demonstrates that metabolic changes does not appear to be causing the limitation in the muscle endurance test in **chapter four**. We demonstrated for the first time a reduction in muscle endurance using a one legged test requiring 15 consecutive maximal contractions of the quadriceps muscle. The relative small muscle mass recruited for the test excluded that CO could be limiting muscle endurance. In fact, has been previously demonstrated that CO is not a limiting factor in one legged exercises (Saltin, 1985). Therefore, we originally hypothesised that impairment in muscle oxygen extraction and/or utilisation could have explained the decreased muscle endurance and designed a study to address this question **[chapter five]**. However, MVO_2 measured by NIRS in chapter five suggests that other factors are causing this reduction.

From data in this thesis it is not possible to conclude what causes the reduction in muscle endurance. We can only speculate, based on other publications, what other factors might lead to reduced muscle endurance. At the cellular level, for example, it has been suggested that accumulation of potassium, reduced sarcoplasmic reticulum calcium release in the muscle cells and low pH can lead to muscle fatigue during repetitive contractions in healthy subjects (Allen *et al.*, 2008). Alternatively, the role central nervous system in reduced neuromuscular activation ("central fatigue"; Gandevia, 2001) during repetitive muscle contraction has also been studied in healthy subjects. An interesting possible explanation for the reduction in muscle endurance found in this study comes from the studies of Reid and co-workers. In fact, they demonstrated that inflammatory mediators such as TNF-α could decrease muscle's contractile function (Reid *et al.*, 2002). Moreover, Lundberg and Grundtman (2008)

suggest that other inflammatory cytokines such as IL-1 might demonstrate the same effect on muscles. Patients with SLE are exposed to low grade chronic inflammation and therefore it is possible to speculate that reduction in muscle endurance could be the direct consequence of inflammation on muscle's contractile ability. All these factors could explain the reduction in muscle endurance and further studies are therefore needed to clarify our findings in SLE.

Limitations of the research program

Patients recruited for these studies were comparable to patients recruited in previously reported investigations assessing VO_{2max} in SLE. In fact, our inclusion and exclusion criteria were very similar to those used in studies by Robb Nicholson *et al.*, (1989), Tench *et al.*, (2002) and De Carvalho *et al.*, (2005). This similarity explains why our results reflect those in other studies. The selection of patients, however, means that this thesis shares the same limitations with these previously mentioned investigations.

Firstly, the generalisability of our results is questioned by the fact that our sample was not representative of SLE population as a whole in terms of disease activity and damage severity. Thus, selection bias might have affected all the studies of this thesis since patients are self selected to undertake the study procedures and are likely to have less morbidity and disability compared to the general SLE population. Patients with more active disease or severe organ damage are likely to have more relevant impairment of exercise capacity.

Secondly, the fluctuant nature of disease activity in SLE implies that the conclusions of these studies are applicable only to patients with minimal inflammation. The presence of inflammation could potentially exacerbate the findings of this thesis. Indeed, previously published data suggest that active disease can directly affect the oxygen transport chain. Data from Sasson *et al.* (1992), Kalke *et a*l. (1998) and a very interesting study by Singh *et al.* (2005) demonstrated that, at rest, SLE patients with active disease exhibit abnormalities in myocardial function such as high diastolic relaxation times compared to SLE patients with inactive disease and healthy controls. In Singh *et al.* (2005) these changes returned to normal after clinical improvement of disease activity. Changes in myocardial function during exercise where not evaluated in these studies but it can be hypothesised that cardiac function could be affected during active disease. Furthermore, inflammation is known to disrupt endothelial function (Bhagat and Vallance, 1997, Cleland *et al.*, 2000) by decreasing nitric oxide production. Nitric oxide is one of the main vasodilatory stimuli released by endothelium during exercise and a decrease of this chemical compound could affect blood flow. Therefore, it is reasonable to speculate that raised level of inflammation will affect VO_{2max}.

Thirdly, although all our patients were not involved in regular formal exercise, they were just as active as the sedentary healthy controls **[chapter four]**. It is likely that SLE patients with more morbidity and organ damage might have reduced physical activity compared with our cohort, which would decrease their physical fitness even further.

This thesis could be also limited by some technical issues. We used thoracic bioimpedance cardiography to assess cardiac function **[chapter four]**, a technique that has been criticised in the past (Warburton *et al.*, 1999b). Thoracic bioimpedance cardiography measures changes in impedance through the thorax during systole and

diastole by measuring electrical resistance and electrocardiography (for details see methods section in **chapter four**). From these values SV and CO are then calculated using different formulas according to the specific equipment used (Warburton *et al.*, 1999b).

Catheterization (direct Fick method) and dye dilution are the gold standards in measuring CO but these techniques have got limitations because of their highly invasive nature (Warburton *et al.*, 1999a). Of the other non invasive techniques available, carbon dioxide rebreathing requires multiple tests to provide useful information and therefore increases the discomfort of the subjects. By contrast, thoracic bioimpedance cardiography offers the possibility of measuring cardiac function during the same test in which VO_{2max} is determined.

In this thesis we used a recently developed Physioflow PF-05 impedance cardiograph which employs a new method to estimate SV from changes in thoracic impedance that overcomes some of the limitations of earlier impedance cardiographs (Charloux *et al.*, 2000). Therefore, we are confident that our results reflected real changes in the SLE group. In fact, this device has been found to be valid in healthy subjects (Charloux *et al.*, 2000) and reproducibility during intense cycling is high (coefficient of variation 3.4%;Hsu *et al.*, 2006). Similarly, in a study conducted by our group, reliability was high (5% test re-test coefficient of variation) in a group of sedentary middle aged women performing incremental exercise (unpublished data).

By contrast, Bougault *et al.* (2005) and Kemps *et al.* (2008) found that Physioflow overestimated CO compared to the direct Fick method in patients with COPD and CHF respectively. Bougault *et al.* (2005) suggested that problems related to dynamic

hyperinflation related to low FEV_1 could explain these differences. Alternatively, Kemps *et al.* (2008) suggested that this overestimation is due to inaccuracy in the SV measurement at rest when very low values are calculated (approximately 60 ml).

To the best of our knowledge no direct comparison between thoracic bioimpedance cardiography and other methods of assessing cardiac function has been published in SLE patients and, consequently, no definitive conclusion can be made. It can be added that patients recruited for these studies had no clinically relevant cardiovascular disease or obstructive/restrictive respiratory patterns and therefore the conclusion by Kemp *et al.* and Bougalt *et al.* might not apply to our cohort.

Moreover, even considering the hypothetical argument that an overestimation might be present in our data, we are confident that this would not lead us to wrong conclusions. In fact, if we consider that the hypothetical CO overestimation was due to low FEV_1, as suggested by Bougault *et al.* (2005), than the conclusion of our investigation would not be changed. In fact, FEV_1 was reduced in the SLE group compared the control group and therefore, in this hypothetical scenario, the real CO in the SLE could be even lower (definitively not higher) than the one found in **chapter four**.

On the contrary, if the hypothetical CO overestimation was due the resting SV as suggested by Kemps *et al.* (2008), then the fact that we did not find any difference at rest in SV [chapter four, data not presented; SLE = 72.7 ± 9.5 ml, controls = 68.1 ± 9.7, $p = 0.196$] demonstrates that our maximal data are equally effected in both groups, thus confirming our conclusion. Moreover, both groups at rest demonstrate a higher SV than the one found by Kemps *et al.*(2008) and therefore their hypothesis would not apply to our data.

Another possible technical limitation of this thesis could be due to the use of a newly developed NIRS technology to assess MVO_2. A description of the working principles of NIMO can be found in **chapter five** and we believe that a more in depth discussion of the technical characteristics of this machine is behind the scope of this thesis. In **chapter five** we presented data from a group of healthy volunteers showing that NIMO infrared technology was able to significantly detect changes in MVO_2 induced by exercise. Comparison of our results with previously published data is difficult because values of MVO_2 varies hugely between muscles in the same individual and between different depths in the same muscle (Van Beekvelt *et* al., 2001). It can be observed, though, that data obtained using NIMO are within the published range of data, although on the lower end of the spectrum, and therefore we believe that data obtained using NIMO are suited for research purposes. It need to be added that given the wide range of MVO_2 published data (0.05 and 0.23 ml O_2 min^{-1} $100g^{-1}$) it is necessary to use a control group when assessing this variable because comparison with other published data can be meaningless.

Potential areas of future research

The results of this thesis open up a wide range of potential areas for future research. We believe that the results from study one **[chapter two and three]** provide the instruments to increase understanding of cardiovascular disease in SLE. In particular, the validation of the step test **[chapter three]**, offers a simple submaximal test that can be easily used in large epidemiological studies.

As highlighted in the discussion of **chapter two, chapter three** and previously in this **general discussion**, the relative contribution of poor aerobic fitness to the increased risk

of cardiovascular mortality found in SLE patients is unknown but likely to be substantial. A large multicenter study measuring at the same time traditional Framingham risk factors, lupus specific risk factors (Manzi *et al.*, 1997, Bessant *et al.*, 2006), markers of cardiovascular disease and VO_{2max} (using one of the submaximal tests validated in this thesis) could address the relative contribution of physical fitness to cardiovascular and overall mortality in SLE. A study of this kind would be difficult to conduct using gold standard measures of VO_{2max} because of the expertise, time and cost required.

The step test could also be a valuable tool to be used in the clinics to monitor changes in physical fitness over time, although this requires further validation as training in SLE might change the VO_2/HR relationship. Time in the clinic dedicated to each visit is a major problem and the step test would fit such a busy schedule because, in most patients, it would require only three minutes to be administered.

The step test validated in this thesis, could also be used for investigation of the effect of active disease on physical fitness. It has previously been reported that, during "flares", patients report decreased physical health (Dobkin *et al.*, 1999) and often patients report physical limitations in performing activities of daily living. Moreover, the previously discussed results by Sasson *et al.* (1992), Kalke *et al.* (1998) and Singh *et al.* (2005) suggest that cardiac impairment could be exacerbated during active disease. The step test requires a limited amount of energy and could, potentially, be conducted at home. This test, possibly accompanied by some other portable equipment such as an ultrasound imaging device, would increase the understanding of the physiological changes during active disease. However, patients might not be able to perform the step test during flares and alternative approaches might be more practical. Near infrared

spectroscopy could be used, for example, to provide information about the muscle metabolism during active disease. The level of exercise required for NIRS testing are minimal and we believe that most of the patients would be able to perform the test even during "flares".

The results of **chapter four** demonstrated for the first time a decrease in CO during exercise in SLE patients. The most likely mechanism for this reduction appears to be a reduction in SV. Further studies should confirm this novel finding and investigate the factors causing the reduction in SV. Based on data collected for this thesis we were only able to speculate on the mechanism of reduced SV. Investigations using cardiac imaging techniques, such as Doppler ultrasound or MRI, to assess systolic and diastolic function might provide further insights concerning the reduction in cardiac response to exercise in SLE patients. In recent years, MRI has been successfully applied to investigating cardiac function during exercise using a MRI compatible bicycle ergometer (Roest *et al.*, 2001). This technique has a great potential because it allows accurate measures of left and right ventricular function during exercise and therefore could provide interesting data on the mechanism causing a reduction in SV in SLE patients.

Magnetic resonance imaging techniques could also be used to understand the progression of cardiac dysfunction by testing newly diagnosed SLE patients. In fact, the studies mentioned before would give information on the mechanisms leading to reduced SV but different investigations are needed to understand the progression of cardiac dysfunction over time. A recent small study by Keenan *et al.* (2008) in SLE demonstrated that MRI can be used to assess cardiac and endothelial function in SLE patients. This approach could be used with newly diagnosed SLE patients and, by

repeating the measure in subsequent years, information could be gathered on the aetiology of reduced SV.

Further research is needed to explain the results of the endurance test in **chapter four**. Based on the data presented in **chapter five**, factors other than muscle oxygen consumption seem to be implicated. Studies need to be purposely designed to address muscle endurance in SLE. Ideally, these investigations should employ methodologies independent of participant motivation. For example, endurance tests using voluntary contractions combined with electrical muscle stimulation could explain the contribution of the aforementioned central fatigue to the development of fatigue. In addition, biopsy and electromyographic studies would also be required to investigate impairment at the muscle cell level.

Conclusions

This thesis demonstrated for the first time that maximal exercise is limited by cardiac output in SLE patients with low disease activity, limited organ damage, normal physical activity and no previous history of cardiovascular disease. We accept that the results are like to be different (and probably worse) in SLE patients with severe organ damage, high disease activity and a more sedentary lifestyle.

The reduction in cardiac output appears to be due to a reduction in SV with more research needed in this area to explain the mechanism leading to this finding.

With the validation of the submaximal tests we developed tools to predict VO_{2max} in epidemiological studies and clinical practice. By using these assessment methods it will be possible to determine if VO_{2max} can be used as a physiological marker of cardiovascular disease and mortality. Finally, this thesis also provided evidences that

muscle oxygen metabolism does not appear to be impaired in our cohort of SLE patients. The cause of limited exercise endurance in our SLE patients (endurance test **chapter four**), which is unrelated to cardiac output, remains unknown and needs further investigation.

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