INVESTIGATION OF FACTORS ASSOCIATED WITH AUTONOMIC NERVOUS SYSTEM FUNCTION IN PATIENTS WITH RHEUMATOID ARTHRITIS

By

AHMAD OSAILAN

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School of Sports, Exercise and Rehabilitation Sciences

College of life and Environmental Sciences

University of Birmingham

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ABSTRACT

Rheumatoid arthritis (RA) is an autoimmune disease characterised by systemic inflammation, as well as pain, stiffness and swelling of the joints. Patients with RA have an increased risk for cardiovascular diseases (CVD). Poor autonomic nervous system (ANS) function, indicated by increased sympathetic and reduced parasympathetic nervous system activity, is considered to be one of the underlying factors contributing to the increased risk for CVD in RA. This thesis included studies that examined the ANS in patients with RA. The first experimental chapter includes a cross-sectional study in which the association between a novel measure of myocardial ischemia during an exercise tolerance test (ETT) and resting heart rate variability (HRV) was explored in 96 patients with RA. Myocardial ischemia was associated with reduced HRV which was indicated by reduced parasympathetic nervous system and reduced sympatho-vagal balance. The second experimental chapter involves the examination of parasympathetic reactivation using heart rate recovery (HRR) following ETT, and the factors associated with HRR including CVD risk factors, RA-disease related factors, and measures of wellbeing. Despite several factors being associated with HRR, multivariate analyses revealed that none of these was independently associated with HRR, but it was the overall CVD risk and disease related burden that contributed to variability in HRR. In the third experimental chapter, the effects of a three-month semi-supervised exercise intervention on parasympathetic activity using HRR, as well as, CVD risks, RA-disease related inflammation, and measures of wellbeing were investigated in a longitudinal study in 62 patients with RA. The exercise programme was successful in reducing some CVD risk factors and improving some measures of wellbeing, however, parasympathetic activity as well as cardiorespiratory fitness did not improve. In the last experimental study, a cross-sectional study compared parasympathetic activity using HRR between age- and sex-matched RA (N=43) and diabetes mellitus (N=26) patients (a population that has a similar CVD risk profile but lower levels of systemic inflammation), as well as inflammatory markers, CVD risk factors, and measures of wellbeing. There was no difference in HRR or inflammation between the two groups. A sub-analyses looking at the variables associated with HRR in the whole sample together (RA and DM patients) found that cardiorespiratory fitness was an independent predictor of HRR. In conclusion, the findings from the studies in this thesis suggest that parasympathetic activity in RA associate with several CVD risk factors, and that cardiorespiratory fitness is an important factor associated with parasympathetic activity.

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List of Abbreviation

ACPA	Anti-citrullinated protein antibodies
ACR	American college of Rheumatology
ACSM	American college of sports medicine
Anti-CCP	Anti-cyclic citrullinated peptide antagonist
ANS	Autonomic nervous system
APCs	Antigen presenting cells
AV	Atrioventricular
BMI	Body mass index
CAD	Coronary artery disease
COX	Cyclooxygenase
CRF	Corticotropine releasing factor
CRP	C-reactive protein
CVD	Cardiovascular disease
DAS28	Disease activity score
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DMARDs	Disease modifying anti-rheumatic drugs
ECG	Electrocardiograph
ETT	Exercise tolerance test
EULAR	European League Against Rheumatism
EuroQol	European quality of life questionnaire
ESR	Erythrocyte sedimentation rate
FDA	Food and drug administration
FFT	Fast Fourier transform
FRS	Framingham risk score
GH	General health
HADS	Hospital anxiety and depression scale
HAQ	Health assessment questionnaire
HDL	High density lipoprotein
HF	Heart failure
HF $(ms^2)/(nu)$	High frequency
HLA	Human leukocyte antigen
HOMA	Haemostasis model assessment

HR	Heart rate
HRR	Heart rate recovery
HRV	Heart rate variability
HsCRP	High sensitivity C-reactive protein
IgG	Immunoglobulin complex
IHD	Ischemic heart disease
IL	Interleukin
LDL	Low density lipoprotein
LF	Low frequency
MAF	Multidimensional assessment of fatigue
MCP	Metacarpophalangeal
MHC	Major Histocompatibility complex
MI	Myocardial infarction
MTP	Metatarsophalangeal
NSAIDS	Non-steroidal anti-inflammatory drugs
OA	Osteoarthritis
PAD	Peptidyl-arginine deiminase
PIP	Proximal interphalangeal
PTPN22	Protein tyrosine phosphatase
RA	Rheumatoid arthritis
RER	Respiratory exchange ratio
RF	Rheumatoid factor
SA	Sinoatrial
SBP	Systolic blood pressure
SF36	36 Short form survey
SLE	Systemic lupus erythematosus
SW	Swollen Joints
T28	Tender Joints
TNF-α	Tumour necrosis factor alpha
VLF	Very low frequency
VO_2	Volume of oxygen
WBC	White blood cells
WHO	World health organisation

List of Papers

The thesis incorporates a paper that was published which correspond to the experimental Chapter 3:

Osailan A, Metsios GS, Rouse PC, Ntoumanis N, Duda JL, Kitas GD, van Zanten JJ. Factors associated with parasympathetic activation following exercise in patients with rheumatoid arthritis: a cross-sectional study. BMC cardiovascular disorders. 2016 May 10;16(1):1.

In addition, the following abstracts arose from presentations of materials from this thesis:

- Osailan A, Metsios G, Rouse P, Duda J, Ntoumanis N, Kitas G, Zanten JV. OP0163 Predictors of Heart Rate Recovery following Exercise in Patients with Rheumatoid Arthritis. Annals of the Rheumatic Diseases. 2014 Jun 1;73(Suppl 2):123-.

- Osailan A, Veldhuijzen van Zanten JJ, Duda J, Fenton S, Rouse P, Ntoumanis N, Kitas GD, Metsios G. Associations Between Heart Rate Adjusted ST Segment and Heart Rate Variability in Patients with Rheumatoid Arthritis. Arthritis and Rheumatology. 2016; 68 (suppl 10).

Prizes

European league against rheumatism (EULAR) abstract award for clinical research 2014. Paris, France (€1000 cash prize)
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CHAPTER 1

General introduction

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease affecting the joints, causing symptoms such as pain, stiffness and swelling. The disease can be characterised by high-grade systemic inflammation. Patients with RA often experience fluctuations in disease activity, including periods of high disease activity with acute episodes of pain and inflammation, known as flares, and periods of lower disease activity for a certain period of time with partial absence of symptoms, known as remission (1). Despite the advances in treatment, there is no cure for RA. Therefore, treatment is focused on controlling disease activity and reducing comorbidities. Appropriate control of disease activity is important to reduce pain, minimize the articular damage, and maintain mobility (2). The exact aetiology of RA is unknown, however, there is evidence that genetic susceptibility and behavioural factors, such as smoking, could contribute to the development of RA (3). RA does not only affect the joints, there are also extra-articular manifestations in RA, such as an increased risk for cardiovascular disease (CVD), which contributes to up 40% of mortality in RA (4). Patients with RA also have poor wellbeing, such as higher levels of depression and fatigue (5-7). There is an increased healthcare burden of RA compared to another chronic condition (i.e. Diabetes mellitus (DM)) in the United Kingdom. The healthcare burden of RA is estimated to be £8 billion per year for approximately 690,000 RA patients living in the United Kingdom (8), whereas, £9.8 billion per year is the estimated healthcare burden for 3,368,198 DM patients living in the United Kingdom (9). This may suggest that the individual health burden of RA is higher than other chronic conditions such as DM. In the following section, a more detailed overview will be provided of the epidemiology, symptoms, treatment and extra-articular manifestation of RA.

Epidemiology

RA is the most common chronic inflammatory disease and affects ~0.8 % of the adult population of the United Kingdom (10). As with many autoimmune diseases, the majority of patients with RA are women. There are some sex differences in the symptoms of the disease, with more involvement of distal small joints in women (hands and feet) and large

joints involvement in men (hips, knees), and men tend to suffer more from bone erosion damage compared to women (11). Despite the increased bone erosion in men, women tend to have more orthopaedic surgeries than men due to the increased small joint involvement in women and other factors (such as rheumatoid nodules) (11).

Aetiology

Although the exact cause of RA remains unknown, there are multiple factors that can contribute to the onset of RA. A discussion on these factors follows below.

a) Genetic factors

There is evidence implicating genetic factors in RA. For example, it has been found that first degree relatives of patients with RA are more likely to develop RA than the general population (12). Furthermore, monozygotic twins have disease concordance rates of 15 – 30%, and for dizygotic twins this is 5% (13).

There are also certain genomes that have been implicated in RA. For example, major histocompatibility complex (MHC) is an area of genomes that has been linked with RA (14). MHC is divided into three classes: class I, class II, and class III. Human leukocyte antigen (HLA)-DRB1 is the shared epitope found in MHC class II that has been associated with rheumatoid factor (14;15). The function of (HLA)-DRB1 alleles is to encode a sequence of amino acids (14). Normally, recognition of viral peptides is performed by HLA through encoding viral peptides so that they can be destroyed by T-cells (16), but in RA, it was suggested that this function is interrupted due to the structural differences between MHC class II and T-cells receptors (known as the shared epitope hypothesis) (17;18).

There are several other risk alleles that alter the immune regulation functionality such as nuclear factor-dependent signalling ($_{k}$B, TRAF1-C5 and c-REL) (15). Another genome risk for RA susceptibility is the gene-gene interactions between HLA-DRB1 and Protein tyrosine phosphatase type 22 (PTPN22), which could lead to more severe forms of RA (19). Together, these findings provide evidence for a genetic influence in RA (15).

b) Gene-environment interaction

Behavioural factors have also been related to the prevalence of RA. For example, bronchial stressors, such as smoking (20) and silica (21), have been shown to increase the risk for developing RA. Interactions between such behavioural factors and genetic factors have been reported. Even though the mechanism is not fully understood, an interaction has been found between smoking and HLA-DR4 alleles, which may trigger immune reactions against citrullinated proteins (22). The immune reaction against citrullinated proteins is known to precede the development of RA, and results in anti-citrullinated protein antibodies (ACPA) (23). Citrullinated protein is the post-translational modification of arginine proteins into citrulline via an enzyme called peptidyl-arginine deiminase (PAD) (24). The PAD2 enzyme responsible for converting arginine into citrulline has been found to be increased in the alveolar tissues among non-RA smokers (25). More importantly, in patients with RA, it was found that history of smoking and presence of double copies of HLA-DR shared epitope genes increased RA risk 21-fold against non-smokers with no shared epitope genes (22). Similarly, it was found that exposure to silica among older men group (50-70 years old) increased the risk of developing RA more than 2-fold against non-exposed men of the same age group (21). All these findings suggest that inhaled environmental factors may promote alterations and citrullination in the alveolar tissue proteins, and, in interaction with genetic factors, may increase the risk for RA.

c) Hormonal factors

The greater prevalence of RA in women compared to men (women to men ratio: 2 - 3:1) suggest potential hormonal interference in the onset of RA (10). For example, it is known that estrogen has a stimulatory effect on the immune system (26). Further evidence for a role of hormones comes from epidemiological studies which have reported remission or at least amelioration of symptoms during pregnancy. It has been suggested that antibodies are developed during pregnancy to protect against HLA which helps to reduce the severity of the disease (27). Additionally, with contradictive findings, hormonal oral contraception pills may reduce the severity of the disease (28). Together, this provides some evidence for the role of hormones in RA, however, the exact interaction between hormones and the aetiology and progression of RA remains to be determined.

d) Infectious agents

As with other auto-immune diseases, there are several infectious agents that have been linked with development of RA (such as Epstein-Barr virus, cytomegalovirus, proteus species, and Escherichia coli) (15). Periodontal disease, which is an inflammation of the soft and hard structures of the jaw caused by bacterial infection, has been associated with RA (29). Thus, it was postulated that exposure to viruses or bacteria may cause formation of immune complexes that may trigger ACPA. The data in the literature support the hypothesis of a link between infectious agents and development of RA, however, as this area of research is only just emerging, more evidence for this hypothesis and the mechanisms through which these infectious agents could lead to the development of RA is required.

Pathogenesis

Even though the cause of the disease is not known, information is available about the physiological processes occurring just before the diagnosis of RA and during the disease.

During the periarticular phase of RA, autoantibodies such as anti-citrullinated protein antibodies (APCA) have been shown to start to develop in large quantities at the synovium, and potentially also in regional lymph nodes and bone marrow (15;30). As the onset of the disease approaches, the level of these autoantibodies are increased and await to be triggered via certain mechanisms (15). The mechanism can be microbial (via nonspecific infection) or biomechanical damage (via trauma to the structure of the joint) to the joint capsule. Both of these mechanisms trigger an inflammatory cascade (15). Because of the damage to the joint capsule, the vasculature becomes more permeable, which allows immunoglobulin complexes (IgG) to enter the interstitial fluid of the synovial membrane. Due to the humoral adaptive response to antibodies, these immunoglobulin complexes subsequently bind to sentinel cells such as dendritic cells, macrophages and mast cells via Fc receptors (a protein located on the surface of immune cells) (31). The latter contributes to inflammation of the synovium and migration of activated CD4+ T-cells (known as T-helper cells) into the synovial membrane (31). Production of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, IL-17 and tumour necrosis factor-α (TNF-α) are stimulated by T- cells (32). The immune reaction at the synovial membrane starts when the synovial macrophages and dendritic cells function as antigen presenting cells (APCs) by forming complexes with MHC II molecules (31). Furthermore, chemotactic molecules such as IL-8, monocyte-

chemoattractant protein-1 and macrophage inflammatory protein-α are mediators of the recruitment of more inflammatory cells (33). This leads to inflammatory exudate that contributes to the effusion of the synovium causing what is known as synovitis (34). This is followed by an increase in the interaction between leukocytes and the endothelium which is facilitated by complement factors such as C3a and C5, which will cause formation of adhesion molecules along the endothelial lining (35). Presence of pro-inflammatory cytokines also increase the expression of adhesion molecules (36). These series of cellular activities result in hyperplasia of the synovial lining and oedema which is clinically manifested by pain and swelling of the joint (32). The hyperplasia of the synovium continues as the disease progress, leading to a formation of a thick invasive layer termed Pannus (32). Angiogenesis is one of the intra-articular manifestations of the disease, which allow further infiltration of macrophages, T cells and B cells into the synovium (37). The bone and cartilage erosion is caused by macrophages via release of TNF-α, IL-1, and IL-6 which promote osteoclast differentiation and activation of the articular cartilage (15).

Diagnosis of Rheumatoid Arthritis

RA can be difficult to diagnose due to some similarities in disease characteristics with other types of diseases that cause inflammation and joint stiffness, such as osteoarthritis and fibromyalgia. Diagnosis can be complicated as RA can present with various symptoms. According to current guidelines, a referral to a rheumatology specialist should be made when a patient present with three symptoms of RA. These symptoms are early morning stiffness (should last more than 30 minutes), joint swelling (three joints and more), and compression tenderness on squeeze test over the metacarpophalangeal (MCP) or metatarsophalangeal (MTP) (38;39). Early referral is recommended by multiple international guidelines (40;41). Severe joint damage and further complications of RA can be avoided if RA is detected early. This will allow early commencement of pharmacological interventions (e.g. disease modifying anti-rheumatic drugs) which improves disease outcomes and reduces the risk for associated disabilities (39;42). The American College of Rheumatology (ACR) has developed criteria for the diagnosis of RA in 1987 (see Table 1). A patient is diagnosed with RA if at least four of these criteria are fulfilled and present for at least six weeks (43). The following paragraphs discuss the types of investigations implemented in clinical settings to confirm the diagnosis of RA.

Medical history

A medical history is taken in which the patient is asked to describe the symptoms, the start of these symptoms, and changes in these symptoms over time (44). More information about other medical problems such as other comorbidities of the patient may help to make the right diagnosis and impact of the disease on patient's life (44).

Physical examination

Since synovitis (inflammation of the synovial joints) is a common articular symptom of RA, examining tenderness and stiffness of the joints and tendons is important in the process of diagnosis. Palpation of each joint to detect soft tissue thickening, swelling, and tenderness is carried out to investigate the number of joints affected (39). Redness and observation of loss of normal groove between the MCP's can be a sign of inflammation (39). However, this may not always be the case, as some RA patient may have minimal joint swelling, making it difficult to detect during physical examination. Thus, the use of other imaging modalities may be useful in these cases (39). Imaging using X-rays has a limited role in the early stages of RA. However, other types of imaging such as ultrasound with doppler and magnetic resonance imaging are useful to assess disease activity, detection of synovitis, and visualization of erosions (40;41;45).

Laboratory investigation

Systemic inflammation can be assessed with C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and fibrinogen. However, CRP and ESR have low specificity for RA, as some early RA patients may have normal levels due to involvement of only few joints (39;41). CRP and ESR are useful in predicting the radiographic progression, monitoring disease activity, as well as the response to treatment (41).

Another serological factor that can help in the diagnosis of RA is the rheumatoid factor (RF) which has a sensitivity of 65 – 73% and a specificity of 82 – 88 % (46). The majority of patients with RA have positive RF (autoantibodies to the Fc receptors of IgG), however, it may also be positive in other types of chronic inflammatory diseases (such as systemic lupus

erythematosus, hepatitis, and tuberculosis) (41). Therefore, confirming the diagnosis of RA cannot be based only on positive RF, but with confirmation of other symptoms of RA, and especially with another serological factor such as ACPA (41;45).

ACPA or another term used for it is anti-cyclic citrullinated peptide antagonist (anti-CCP) is more useful in the diagnosis of early RA due to its sensitivity of 62 – 72% and specificity of 94 – 97%, which is more than RF (41). ACPA may appear even before the RF or before the development of clinical disease (39) which makes it more useful in the diagnosis of early RA (41).

Assessment of disease activity

Disease activity should be monitored regularly once the diagnosis of RA is confirmed. This not only helps with quantifying the disease activity, but also evaluating treatment effectiveness and disease progression (40). Several disease activity measures have been developed with Disease Activity Score 28 (DAS28) being the most commonly used (40). DAS28 is a composite measure which takes into account the patient's perceptions of their overall health in the previous week as well as markers of inflammation and swelling. DAS28 is composed of the 28 tender joints count and 28 swollen joint count (hand, wrist, elbow, hip and knee) (47). The final DAS28 score takes into account either the CRP or ESR levels and can be calculated online or via downloadable calculators. DAS28 can be calculated using four variables (swollen joints, tender joints, CRP or ESR level, and global assessment of disease activity or visual analogue scale) or three variables (same variables with the exclusion of global assessment of disease activity) with a slight modification when CRP is used instead of ESR (see Figure 1). A DAS28 score of ≤ 3.2 is interpreted as low disease activity, 3.2 to ≤ 5.1 is interpreted as moderate disease activity, and more than 5.1 is interpreted as high disease activity (47). Remission of the disease is indicated by a score of < 2.6 (40).

$$DAS28\,(4) = \left(0.56 * \sqrt{T28} + 0.28 * \sqrt{SW28}\right) + 0.7 * Ln(ESR) + 0.014 * GH$$

$$DAS28\,(3) = \left[0.56 * \sqrt{T28} + 0.28 * \sqrt{SW28} + 0.7 * Ln(ESR)\right] * 1.08 + 0.16$$

$$DAS28\,(4) = 0.56 * \sqrt{T28} + 0.28 * \sqrt{SW28} + 0.36 * Ln(CRP + 1) + 0.014 * GH + 0.96$$

$$DAS\,(3) = \left[0.56 * \sqrt{t28} + 0.28 * \sqrt{sw28} + 0.36 * Ln(CRP + 1)\right] * 1.10 + 1.15$$

Figure 1. Different formulas for calculation of DAS28. T28; tender joints, SW; swollen joint, GH; general health measured via visual analog scale, (4); including four variables, (3); including three variables.

Imaging and radiographic changes

Radiographic changes may be observed after three months' duration from the onset of inflammation (48), and mostly in the hands and feet (49). Despite the limited use of early X-rays, disease progression can be monitored via the use of serial X-rays over years, which may help in monitoring disease activity and revising or changing treatment strategy (41).

Table 1. The 1987 revised criteria for the classification of RA. Information copied from Arnett et al (43)

Criterion	**Description**
1. Morning Stiffness	*Morning stiffness in and around the joints, lasting at least one hour before maximal improvement.*
2. Arthritis of 3 or more joint areas	*At least three joint areas (out of 14 possible; right or left PIP, MCP, wrist, elbow, ankle, MTP joints) simultaneously have had soft tissue swelling or fluid (not bony overgrowth) as observed by a physician*

3. Arthritis of hand joints	*At least one area swollen (as defined in criterion two) in a wrist, MCP, or PIP joint*
4. Symmetric arthritis	*Simultaneous involvement (as in criterion two) of the same joint areas on both sides of the body (bilateral involvement of PIP, MCP, or MTP joints without absolute symmetry is acceptable.*
5. Rheumatoid nodules	*Subcutaneous nodules over bony prominences or extensor surfaces, or in juxta-articular regions as observed by a physician.*
6. Serum rheumatoid factor	*Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects.*
7. Radiographic changes	*Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal body decalcification localized in, or most marked adjacent to, the involved joints (osteoarthritis changes alone do not qualify).*

PIP, proximal interphalangeal; MCP, metacarpophalangeal; MTP, metatarsophalangeal.

Clinical Features of Rheumatoid Arthritis

When the disease is not treated effectively or discovered in later stages, RA can cause progressive and irreversible damage to the synovium of the joints resulting in decreased joint

space, erosion and deformity. This is manifested clinically by swelling, pain and morning stiffness of the affected joints which can eventually lead to limitation of joint motion (50). Typically, the most affected joints in the early stages of RA are the hands and feet joints including the metacarpophalangeal (MCP), proximal interphalangeal (PIP) and wrist joints (50). Other joints, such as distal interphalangeal, sacroiliac joints and lumbar spine can also be affected but with a lower rate of involvement and more commonly during advanced stages of RA. As the disease progresses without appropriate treatment, severe deformities can occur such as "swan neck deformity" as a result of major damage and dislocation of finger joints and tendons (50).

Extra-articular manifestations occur in about 40% of patients during the course of the disease (51) and they are more frequent among patients with high disease activity and longer disease duration (52). There are also extra-articular manifestations of the disease which are more common in men (52). Fever, asthenia, weight loss, rheumatoid cachexia (loss of muscle mass and gaining more fat mass especially trunk adiposity), and anorexia are examples of constitutional symptoms of RA. Other extra-articular manifestation can involve multiple systems including cardiovascular, pulmonary, neurologic, skin, hematologic, renal, and gastrointestinal (52). For the aims of this thesis, the focus will be on cardiovascular diseases (CVD) as a common co-morbidity of RA.

In addition to the articular and extra-articular manifestations of the disease, patients with RA also may experience compromised wellbeing because of the fluctuation of the disease (53;54). Anxiety, depression (55), fatigue, and quality of life can be affected by RA (5).

Although there is no consensus about the definition of depression in RA, depression can be described as a serious mood disorder with a mixture of negative feelings including constant feeling of sadness, loss of interest, and lack of sleep (56). Anxiety is also common in RA and more likely to develop in depressed RA patients (57). Inability or difficulty to perform valued activities can subsequently lead to depression (58). In the literature, depression has been found to be prevalent in 13-20% in patients with RA (59;60), and is associated with many poor outcomes in RA. For example, depression has been linked with increased risk of mortality in RA (61), and has been found to be positively associated with disease activity (62), and pain (60). Depression has also been associated with many physiological changes including higher sympathetic activity, elevated catecholamine, abnormal platelet activation, increased inflammatory markers, and endothelial dysfunction

which subsequently can lead to CVD (63;64). Despite all these associations between depression and poor outcomes in RA, depression remains unrecognized and undertreated in this population (60). This can be due to multiple reasons including the tendency to focus on the physical symptoms of the disease, and the misconception that depression is secondary to pain and disability which then incorrectly implies that no treatment is needed for depression (60). The assessment of depression can be difficult due to the overlap with other symptoms such as fatigue and insomnia (65). However, the use of questionnaires such as the hospital anxiety and depression scale (HADS) (66) can aid the diagnosis of depression in patients with RA (60) and also monitor the psychological wellbeing of RA patients.

Fatigue can be described as a continuous feeling of exhaustion, reduced ability to carry out physical and mental tasks (67), however, there is no consensus about its definition in RA. The perception of chronic fatigue has been described by Piper as "*unpleasant, unusual, abnormal or excessive whole body tiredness, disproportionate to or unrelated to activity or exertion and present for more than one month"* (cited from (67)). Fatigue is often mentioned by patients with RA to be the most debilitating symptom, which has a substantial impact on wellbeing. It is therefore not surprising that is has been considered to be an important patient-focused outcome measure which should be included in clinical trials (68). The prevalence of fatigue has been reported to range between 42 – 80% in patients with RA (67). High levels of fatigue have been found to be associated with depression, pain, lack of sleep (67, 68), and multivariate analyses revealed pain to be an independent predictor of fatigue (69). Quality of life in patients with RA has also been found to be affected by fatigue (70). Considering the impact and the association between fatigue and other mentioned outcomes in RA, the severity of fatigue should be assessed in clinical practice as there is a lack of attention toward assessment or structural discussion about fatigue between health professionals and RA patients (67). Given its subjective nature, it is difficult to assess fatigue. A systematic review reported multidimensional assessment of fatigue (MAF) (71) as one of the six reasonably validated assessments of fatigue in RA (72).

Quality of life can be described as the perception of the patient about his/her ability and limitation to perform activities of daily living and the emotional impact of these limitations on health status (73). It has been found that RA has a substantial impact on health related quality of life (74). For example, limitation in health related activity, which is closely related to quality of life, were twice as common in RA patients compared to a non-RA control group (75). Perhaps not surprisingly, increased levels of pain, disease activity, and

reduced physical function are associated with poorer quality of life (76). There are many questionnaires to assess the quality of life. The European quality of life questionnaire (EuroQol) (77) has been found to be reliable, simple to use, and was shown to be more responsiveness to clinical changes than the 36 short form survey (SF36) (78).

In summary, it is necessary to assess these aspects of wellbeing to monitor and improve the overall wellbeing in patients with RA. There are other types of assessments that can be used to assess measures of wellbeing such as subjective vitality scale (79), which is a measure of positive wellbeing and assesses the perception of the patients to have energy and being able to perform tasks.

Pharmacological approaches in Rheumatoid Arthritis

To date, there is no cure for RA. However, a variety of medication regimes are used to reduce inflammation, alleviate pain, reduce or slow down joint damage and minimize deformity. There are many types of medication that can be used in the management of RA, and patients are often treated with a mix of medication. The administration of the type of medication is based on the concurrent symptoms and severity of the disease. The types of medication commonly used in RA are briefly discussed below.

Non-steroidal anti-inflammatory drugs (NSAIDs)

Classical NSAIDs have been used as a first line of treatment that offer quick onset of symptom relief (80). The therapeutic effect of NSAIDs is limited to reducing the associated symptoms without altering or reversing the disease (80). NSAIDs (such as aspirin and ibuprofen) are prescribed as an adjunct to other types of medications rather than using it solely to treat symptoms of RA (80). The main action of classical non-selective NSAIDs is to inhibit both cyclo-oxygenase enzyme 1 and 2 (COX 1 and COX 2) to prevent synthesis of prostaglandin (81). Inhibition of COX 1 and COX 2 can induce adverse side effects such as gastrointestinal intolerance and bleeding, and platelet inhibition (82;83). To avoid or lessen these side effects, yet provide similar analgesic and anti-inflammatory effect, a selective NSAID class called COX 2 inhibitors has been developed (celecoxib, rofecoxib and valdecoxib) (80). COX 2 inhibitors were developed as they have less gastric intestinal

complications compared to COX 1 (80;81). NSAIDs are less commonly used in RA due to their increased risk of CVD (84;85). Thus, many international guidelines are suggesting that NSAIDs, specifically COX 2 inhibitors, should only be used after careful consideration of the risk of cardiovascular disease (40;41;45).

Glucocorticoids

Glucocorticoids are a type of steroidal medication that is similar to NSAIDs in relieving symptoms of RA but act faster (82). However, glucocorticoids (such as prednisolone) can have more severe adverse side effects including skin atrophy, hyperglycaemia, weight gain, osteoporosis, and hypertension (82). Thus, glucocorticoids are used in low doses and as a therapeutic adjunct to other types of medication during high disease activity (flare) (80). In addition to symptom relief, some studies report the ability of steroids to slow the radiographic changes of RA when combined with other types of medication (86;87). In general, in order to minimize the risks of toxicity, the use of glucocorticoids in RA should follow the recommendations supported by clinical practice (88), which states that oral glucocorticoids should not be administered for routine use, bone density must be monitored to avoid osteoporosis with prolonged use, and the combination of glucocorticoids with NSAIDs must be avoided (41).

Disease-modifying anti-rheumatic drugs (DMARDs)

DMARDs are known to have a better therapeutic effect than NSAIDs and glucocorticoids not only in relieving symptoms but also in alteration of disease progress (80). Compared with NSAIDs and glucocorticoids, DMARDs have a slower onset of action. DMARDs are usually taken as a long-term approach to slow the progression of the disease while the use of NSAIDs and glucocorticoids aid immediate relief of symptoms. There are many types of DMARDs such as Methotrexate, Sulphasalazine, hydroxychloroquine, and Cyclosporin. The exact mechanism of action of DMARDs is not fully known, but it has been reported in-vitro that they modulate distinct facets of the immune system and inflammatory responses which are activated in RA, such as cytokine secretion, macrophage function, and inhibition of IL-2 production and T-cell cytokines (80). Originally, the initial management of RA consisted of NSAIDs, but recently this has been changed. A more aggressive treatment with

DMARDs plus glucocorticoids favour earlier control of the disease progression and less impairment of the functional statues (89). However, not all patients benefit from DMARDs, therefore, some patients may discontinue the course of treatment due to lack of efficacy or development of side effects (80).

Tumour necrosis factor- α (TNF- α) inhibitors

TNF-α inhibitors are a type of biological drugs that have been developed to block specific components of the immune system, i.e. TNF-α, which contribute to the aetiology of RA. TNF-α over-production by T-cells can have a destructive effect on the cartilage. Thus, the therapeutic effect of TNF-α inhibitors is to reduce inflammation and slow disease progression. There are three Food and Drug administration (FDA) approved TNF-α inhibitors; infliximab, etanercept, and adalimumab (80). Although infliximab (a chimeric monoclonal anti-TNF-α antibody) and adalimumab (a human monoclonal anti-TNF-α antibody) are different in origin, their mechanism of action is similar, while etanercept (a recombinant soluble p75 TNF-receptor-Fc fusion protein) has a different way of inhibiting TNF-α (80). Despite the therapeutic effectiveness of these medications in relieving the symptoms and halting disease progression, there are some safety concerns that have been raised regarding the potential risks and side effects of TNF-α inhibitors such as tuberculosis, increase adiposity (especially trunk fat mass), and infection (80). The combination of these potential side effects and the cost of this treatment TNF-α inhibitors are mainly used for patients with severe RA symptoms who did not benefit from DMARDS agents (40;90).

Non-Pharmacological approaches in Rheumatoid Arthritis

Chronic diseases such as RA impact on many aspects of a person's life that cannot be treated with medical treatment alone. Reduced physical activity, psychological problems, and reduced quality of life are reported to be major consequences of RA (91). Despite the effectiveness of pharmacological management of RA, some of the symptoms or consequences of RA can benefit from non-pharmacological treatment, such as patient education self-management and exercise/physical activity. Non-pharmacological interventions aim is to help the patient in coping with the disease, as well as reducing the impact of the disease. In recent literature reviews, it was a common finding that patient

education/self-management, exercise and physical activity programs (including aerobic, strengthening, and stretching exercise) were the most effective and recommended non-pharmacological management in RA (92-94). For the aims of this thesis, the focus will be on exercise and physical activity as a strategy to reduce the risk of cardiovascular disease in RA, which will be discussed later in this chapter.

Increased risk of cardiovascular disease in Rheumatoid Arthritis

There is ample evidence that patients with RA are at increased risk of cardiovascular disease (CVD) mortality compared to the general population (95;96). Patients with RA may experience unrecognized silent ischemic heart disease (IHD) more frequently than non-RA control participants (97). This increased risk of unrecognized IHD can be due to patients with RA being less likely to report angina symptoms. Indeed, in a follow-up study investigating the reoccurrence of ischemic events or death between 40 RA patients and 40 age- and sex matched patients with acute coronary syndrome, it was found that 82% of RA patients had chest pain on presentation compared to 100% of the control group (98). The risk of developing myocardial infarction (MI) in RA has shown to be double compared with healthy control participants (99). In a large Asian population, the risk of MI in RA was increased by 38% compared to the general population, with an even greater risk for younger RA patients (100). Furthermore, the development of heart failure (HF) has been found to contribute to the increased risk of mortality in RA (101). Similar to MI, the risk of HF is doubled in patients with RA compared to non-RA, which was found to be already increased soon after the onset of RA (102). Even though the CVD risk is increased in RA, the exact reason remains unknown.

The risk of developing CVD in patients with RA is comparable to that of patients with DM and significantly elevated when compared to the healthy control participants (103). Despite the similarity in the increased risk of CVD in RA and DM, the risk in RA is less recognized compared to DM. As a result, CVD prevention strategies are included in clinical guidelines for treatment of patients with DM (104) but less consistently for RA (105), where CVD risk management for RA is currently only a recommendation (106).

There are several traditional CVD risk factors, such as hypertension (107), dyslipidaemia (108), physical inactivity, loss of muscle mass and increase body fatness (i.e. obesity) (109)

that are prevalent in RA which may contribute to the increased risk of CVD mortality in RA (100). In addition, inflammation is now established as a risk factor for CVD, and of course, one of the characteristics of RA is high-grade systemic inflammation. It is therefore not surprising that inflammation, through its influence on the vasculature, has been suggested to contribute towards the increase CVD in RA (110). Autonomic dysfunction is another emerging factor that has been investigated recently and is suggested as an additional factor which contributes to CVD death risk in RA (111). The following paragraphs discuss the prevalence of traditional CVD risk factors and the link between inflammation and CVD in RA. Since autonomic dysfunction in RA is the main topic of the research reported in this thesis, a specific focus will be on the physiology of the autonomic nervous system and its control over the heart will follow.

Traditional cardiovascular disease risk factors

Hypertension

It is known that hypertension is prevalent in people with RA, with the reported rates ranging from 52% to 73% (112). Despite the findings from studies showing no difference in prevalence between hypertension in RA and the general population (113), an increase in arterial stiffness has been found in a study which examined the arterial pliability in RA patients compared with healthy participants (114). This suggests that the arterial system of patients with RA is less able to effectively respond to changes in blood volume, which is termed as arterial stiffness (115;116). Furthermore, increased systemic inflammation in RA has been suggested as a mechanism that contributes to increased prevalence of hypertension in RA, as increased levels of CRP can cause a reduction in nitric oxide in the endothelial cells causing vasoconstriction (112). Although hypertension is considered to be a risk factor for CVD events in the healthy general population, patients with RA tend to suffer from multiple factors that influence control of blood pressure, including physical inactivity, obesity, and most importantly, the anti-inflammatory medications which may increase the chances of developing hypertension (117). It was found that many medications used in the management of RA may cause hypertension or alter its control such as NSAIDs, COX 2 inhibitors, glucocorticoids, and some DMARDs (leflunomide, cyclosporine), even though this was not confirmed in all studies (112). Less is known about the influence of biological drugs, such as TNF-α inhibitors, on blood pressure (112), but biological TNF-α inhibitors

(Etanercept) have been found to reduce arterial stiffness (118;119). Furthermore, there are also reports that found that a specific DMARDs (Hydroxychloroquine) has a positive effect on reduction of lipid profile (120;121), which may reduce the risk of hypertension (122), however, this yet to be proven in patients with RA. Other non-pharmacological treatment such as aerobic exercise has shown to improve blood pressure in patient with RA (123).

Dyslipidaemia

Dyslipidaemia is defined as high levels of low density lipoprotein (LDL) and triglycerides, and a low level of high-density lipoprotein (HDL) (115). Dyslipidaemia is known to be a strong risk factor for the development of CVD in RA as well as the general population (108;124). High levels of cholesterol, in particular LDL, can cause the formation of fatty streaks in the coronary arteries. This can develop into an atherosclerotic plaque, which limits blood flow to the heart and, when the plaque is ruptured, can cause blockage of an artery leading to MI (125). The prevalence of dyslipidaemia in RA is more than 50% (126), which appears to be similar to the prevalence in healthy general population (113;127). Recent meta-analyses revealed that alteration in lipid profile, particularly low HDL (poor lipid profile), are common in RA (128). In addition, an interplay between inflammation and lipid metabolism is suggested to have a role in increasing dyslipidaemia (108). However, the interplay between the effects of medications used in RA on inflammation is complicated, and further studies are required in patients with RA, as certain RA anti-inflammatory medications may also have a favourable effect on their lipid profile (117). Some studies have shown that medications used for RA management including glucocorticoids, DMARDs, and TNF-α inhibitors may affect serum lipid profile (128). The evidence related to the use of glucocorticoids provides an example of the complexity of the influence of medication on the lipid profile. Even though the use of glucocorticoids was not associated with dyslipidaemia (129), when glucocorticoids were mixed with DMARDs (methotrexate, and sulfasalazine) a favourable effect over the total cholesterol/HDL ratio was produced (128;130). The use of TNF-α inhibitors has shown to increase total cholesterol as well as HDL cholesterol, however, there was no improvement in total cholesterol/HDL ratio (131). The latter study also showed that decreased CRP levels after 30 weeks was inversely associated with increase in HDL cholesterol. Based on these studies, it could perhaps be suggested that the more effective the medication is in reducing the inflammation, the better impact on lipid profile,

with more effect that is evident during early stages of the treatment (128). However, more research is needed confirm this hypothesis.

Obesity

Body mass index (BMI) was developed as a clinical measure for estimation of body fat percentage (132). According to world health organization (WHO), category of "obesity" is given to individuals with BMI $\geq$ 30 kg/m^2. However, an adjustment of these cut off points to 28 kg/m^2 has been suggested for patients with RA (133). Using different measurements of body fats percentage such as bioelectrical impedance (133), and abdominal CT scans (134), patients with RA have shown to have more body fat for a given BMI than healthy participants with the same BMI. This may suggest that BMI underestimates the body fat composition and may lead to underestimation of BMI as a CVD risk in RA. In several studies, the difference in BMI between RA patients and healthy general population varied (127;135;136), with ranges between 18-54 % (137-140). However, the biggest problem is the assessment tool used (i.e. BMI vs body component) such as BMI which underestimate fat in patients with RA. Therefore, it is difficult to conclude the actual prevalence of obesity in RA due to the variation between studies in the population considered, disease duration and the type of medications used which may have an influence on weight and body composition (137). In general, most of the studies using BMI are indicating that obesity in RA is similar or slightly higher compared to the healthy general population.

Obesity was found to be a risk factor for the development of RA (20), as it can contribute to low-grade inflammation via accumulated visceral adipose tissue that can release pro-inflammatory cytokines such as IL-6 and adipocytokine (141;142). Eventually, this can lead to increased production CRP (143), which in turn can contribute to development of RA (144). Increased visceral abdominal fats also is known to be strongly associated with cardio-metabolic risk (145), therefore, monitoring the weight and body fat components is recommended in patients with RA, as it constitutes high risk for developing CVD. In addition, a common condition with increased abdominal fats in RA is to have increased fat mass and reduced muscle mass known as rheumatoid cachexia which occurs due to the metabolic alterations associated with increased systemic inflammation (146).

The effect of obesity on the success of anti-inflammatory medication in the management of RA has been investigated. Most of the studies found that obese RA patients have poorer response to TNF-α inhibitors (e.g. infliximab) and are less likely to achieve remission compared to non-obese RA patients (147;148). Similar findings were noticed in patients with early RA, where high BMI was associated with failure to reduce disease activity with treatment (149;150).

Novel cardiovascular disease risk factors

Inflammation

Inflammation is an important factor that contributes to the CVD risk in RA. It is therefore not surprising that a reduction of inflammation lowers CVD risk in RA (151-154), and this has been mentioned as a main strategy for treating CVD risk in RA (106). The exact mechanism of how inflammation contributes to increased CVD risk is not yet fully known. However, it is suggested that inflammation plays a pivotal role in the pathogenesis of atherosclerosis in general population (155-157), which is mediated via pro-inflammatory molecules including cytokines, fibrinogen and CRP (158-160). In patients with RA, the level of these pro-inflammatory cytokines are increased and may induce not only structural vessels abnormalities (known as endothelial dysfunction) but also contribute to changes in lipid levels and insulin resistance (161;162).

Atherosclerosis

Atherosclerosis is a substantial cause of CVD which develops in stages, starting from plaque formation via fatty streaks to plaque rupture and thrombosis. Inflammation contributes to all stages of development of atherosclerosis (163). It is known that endothelial dysfunction can occur at the early stages of atherosclerosis (164). Endothelial dysfunction can lead to increased vascular wall permeability, which allows easy and fast transmigration of monocytes, lipids and T-cells into the vascular wall, which can lead to formation of foam cells (165). Following development of foam cells, pro-inflammatory cytokines such as TNF-α, IL-6, and CRP start to accumulate. This inflammatory pathway in the development of atherosclerosis is very similar to the mechanism leading to development in synovial

inflammation in RA (159). Indeed, an association has been shown in patients with RA between atherosclerosis and IL-6, which was independent of any CVD risk factors (166). As an approach to control or prevent future CVD, two large studies have identified IL-6 receptors, which could be blocked in order to prevent accumulation of IL-6 (167;168). Furthermore, observational studies showed a potential decrease in the risk of CVD in patient with RA using DMARDs (169;170), TNF- α inhibitors (171), and recently, cholesterol lowering drugs (172).

In summary, there are many CVD risk factors which are prevalent in RA. Inflammation has been linked to increased or accelerated atherosclerosis in RA. It is also important to note that most of the traditional CVD risk factors are linked with the increase in pro-inflammatory molecules in RA. Thus, management of traditional CVD risk factors as well as inflammation and ongoing monitoring and management of the CVD risk factors are necessary to reduce the excess risk of CVD in RA (106).

Non-pharmacological strategy to reduce cardiovascular disease risk in Rheumatoid Arthritis

Exercise and physical activity

Patients with RA suffer from severe joint pain, reduced muscle strength and mass (a condition known as rheumatoid cachexia) which may contribute to reduced physical activity (173), and increase the risk for CVD (173). Indeed, physical activity and cardiorespiratory fitness have been inversely associated with CVD risk (174;175). Thus, encouragement of exercise and physical activity are recommended for the management of RA.

The benefits of exercise in general population are widely acknowledged. Exercise and physical activity mainly improve cardiorespiratory fitness, reduce the risk of coronary artery diseases, reduce CVD risk factors, and improve psychological wellbeing (173). According to recommendations from ACSM for people aged from 50 – 64 with chronic condition (such as RA), accumulation of minimum of 30 minutes of moderate intensity exercise on most days of the week is required to gain its benefits with an additional stress on strengthening, balance, and flexibility exercise for this age group with chronic conditions (176). Furthermore, principles of exercise prescription in RA were suggested in a systematic review recommending that exercise should be part of the management of RA, its main aim

should be to maintain optimal musculoskeletal function in order to facilitate exercise for cardiorespiratory fitness, and exercise must be tailored according to baseline fitness and physical ability (177).

Beneficial effects of exercise in patients with RA have been revealed in several randomized controlled trials. For example, aerobic fitness, and psychological wellbeing were improved after 12 weeks' dance-based exercise program (178). Furthermore, high intensity exercise was found to improve functional ability more than routine physical therapy care for patients with RA (179). Restoration of lean muscle mass and improvement in muscle strength were reported in patients with RA after 6 months of high intensity progressive resistance training exercise (180). These studies reported positive effects of exercise without worsening of joint damage or pain.

Despite these positive effects of exercise, the majority of patients with RA are not physically active due to the perception that it may increase the damage to the joints and induce more pain or due to the lack of education from health professionals (181;182). Furthermore, pain and fatigue were found to be important perceived barriers for physical activity and exercise in patients with RA (183). In a systematic review, it was found that patients with RA have lower level of physical activity when compared with healthy control (184). Therefore, increase awareness of the benefits of physical activity and appropriate support for patients with RA to become more physically active needs to be considered as an essential part in the management of RA. Indeed, according to the European League against Rheumatism (EULAR) guideline for the management of CVD risk, exercise training was suggested as a way to manage CVD risk in RA, but it has been also acknowledged that more research was needed in this area (106).

In summary, RA is a chronic inflammatory progressive disease that affects joints and causes decreased functional ability.

Anatomy and physiology of the heart and autonomic nervous system

The cardiovascular system, which includes the heart, lungs and the vasculature, is important in ensuring that organs are supplied with blood. The cardiovascular system is influenced by the autonomic nervous system (ANS). The following section provides an overview of the heart, the ANS and the influence of the ANS on the heart.

Anatomy of the heart

The heart is a muscular organ with the size of a closed fist (185). Its main function is to circulate the oxygenated blood from the lungs, via the arteries throughout the body systems. The heart is composed of four chambers; right atrium, right ventricle, left atrium and left ventricle (185). Between the chambers are valves, which control the flow of the blood in one direction. The right atrium receives the deoxygenated blood from the organs via the superior and inferior vena cava. By contracting the atrium, the blood moves through the tricuspid valve to reach the right ventricle. Once the right ventricle reaches a certain pressure within the chamber, the tricuspid valve closes. The right ventricle then contracts, which allows the opening of the pulmonic valve and the flow of blood through the pulmonary artery to the lungs for re-oxygenation (186). Oxygenated blood travels back via the pulmonary vein to the left atrium. Contraction of the left atrium moves the oxygenated blood through the mitral valve to the left ventricle. By contracting the left ventricle, the oxygenated blood flows through the aortic valve into the aortic artery where it is distributed to all other organs (186). This contraction is orchestrated via the conducting system of the heart. The conducting system is composed of sinoatrial node (SA) node, internodal pathway, atrioventricular (AV) node, atrioventricular bundle or bundle of His, and bundle branches (Purkinji fibres) (186).

The heart's main function is to pump blood to maintain circulation to all organs. Its pumping action relies on the performance of the cardiac muscles. In order to do this efficiently, cardiac cells (myocytes) have to contract in synergy with each other. Cardiac cells are composed of contracting muscle cells and conducting cells (187). The latter is responsible for the heart’s unique ability to generate its own electrical impulses (automaticity) required for the muscle cells to contract (187). The conducting cells deliver the impulse to the contracting cells via intercalated discs. The SA node lies at the junction between superior cava vein and right atrium, whereas the AV node is located at the junction between the atrium and ventricle at

the floor of the right atrium (188). This distribution between the SA and AV node enables systematic cardiac pumping activity from base to apex (189).

A heartbeat is initiated by conducting cells via electrical impulses. The electrical impulse is generated at the SA node, which is known as the pacemaker. The impulse is subsequently distributed through the arterial wall to start the atrial contraction. Then, the impulse travels via the internodal pathway to the AV node, where it is delayed (~ 0.12 seconds) to allow time for the atria to empty blood into the ventricles (186). Once the atrium is empty and the ventricles are filled with blood, the impulse travels from the AV node to the bundle of His through the interventricular septum until it reaches the walls of the ventricles via Purkinji fibres leading to ventricle contraction (186). The blood transfer from inside the heart to the other body cells is done via the vascular system. The following paragraph discusses the mechanism of heart contraction and blood circulation within the cardiovascular system.

Activation of cardiac muscles

There are many similarities between cardiac muscles and skeletal muscles, yet the characteristics of cardiac muscles are unique and different from skeletal muscles. Both have the same type of striated muscle cells, with myofibrils containing actin and myosin filaments, and both contract according to the sliding filament theory (190). An important difference is the reliance on nervous innervation. The activation and contraction of skeletal muscle is dependent on stimulation from the central nervous system, whereas, cardiac muscles have the ability to generate its own activation and contraction without stimulation from the central nervous system. Cardiac muscles and skeletal muscles have similar sodium channels, however, cardiac muscles have slow calcium channels which remain open causing a plateau in action potential of cardiac muscles. The decrease in potassium outflux during this plateau allows a prolonged refractory period in cardiac muscle fibers (0.15-0.30 seconds) compared to skeletal muscle (191). This refractory period in cardiac muscles does not allow re-initiation of the action potential to a cardiac cell, which has not recovered from the previous stimulation (192).

All organs, including the heart muscle (myocardium) require oxygenated blood to survive. Therefore, to maintain constant circulation of blood, the heart pumps continuously. This is possible by the unique ability of the intrinsic conduction system of cardiac cells to generate

and distribute electrical impulses for the cardiac muscle to contract without the need for neural input. However, these cardiac cells can still be regulated extrinsically by the autonomic nervous system to maintain the haemostasis of the body to meet the demands of all organs (i.e. more oxygen during exercise) (193). The following paragraphs discuss the anatomy of autonomic nervous system and its branches to the heart.

Autonomic nervous system

ANS is part of the central nervous system and consists of the sympathetic nervous system and the parasympathetic nervous system. The activity of many organs are regulated by both branches of the ANS, including the cardiovascular system, lungs, gastrointestinal system, and adrenal glands. The sympathetic and parasympathetic nervous system consist of afferent and efferent interneuronal fibres, with the afferent coming from central nervous system, and the efferent from the effector organ (e.g., heart) (189). The sympathetic and parasympathetic nervous systems respond to metabolic and mechanical changes from the heart and other visceral organs via chemoreceptors and baroreceptors, respectively. For example, information about pressure in the arterial system are received by baroreceptors, whereas, information about the level of oxygen and carbon dioxide in the blood are received by chemoreceptors (194). The anatomy of the ANS and its connection to the heart will be described in the next paragraphs.

Cardiac sympathetic innervation

The sympathetic innervation to the heart via neurons originated at the reticular formation of the brain stem and the hypothalamus pituitary axis (195). The sympathetic fibres that innervate the heart branch from the spinal cord between thoracic vertebrae (T1-T4) as preganglionic fibres, then they form synapses with longer postganglionic fibres to reach the heart (196). The axons of the sympathetic nerves are connected to the heart by three branches; superior, middle, and inferior cardiac nerves (196). The fibres of these nerves travel along the epicardial vascular structures of the heart to penetrate the myocardium reaching the endocardium where they become sympathetic nerve terminals (189). The distribution of the sympathetic innervation to the heart is arranged to be from the base to the

apex of the heart, however, there is more sympathetic innervation at the atrium where the pacemaker SA node is (189).

Cardiac parasympathetic innervation

The parasympathetic system innervates the heart through the vagus nerve, which originates from the medulla oblongata (196). The axons of the vagus nerve are connected to the heart with three branches; superior, middle and inferior cardiac nerves (196). These branches merge with postganglionic sympathetic neurons to form the cardiac plexus of the nerves at the base of the heart (189). The parasympathetic neurons have a homogenous distribution of the heart compared to sympathetic neurons, being more concentrated on the SA node and the AV node than on the walls of the atrium or ventricles (189).

Chemical neurotransmitters

The signals of the sympathetic and parasympathetic nervous system are transferred from the neurons to the heart by adrenergic or cholinergic chemical neurotransmitters (193). Noradrenaline and adrenaline are released by postganglionic adrenergic fibres, which are mostly sympathetic, whereas acetylcholine is released by postganglionic cholinergic fibres which are mostly parasympathetic. These chemical neurotransmitters activate specific receptors within the heart. For example, Beta–1 receptors for the sympathetic stimulation and, muscarinic and cholinergic receptors for acetylcholine (193).

Cardiovascular autonomic control

In terms of regulation of heart activity, both arms of the ANS can control the intrinsic activity of the heart's pacemaker (SA node) which modulates the heart rate and the force of the contraction (189). In a healthy state, both systems are functioning in synergy and respond to the physiological state of the body. The sympathetic is known to have an excitatory effect to increase the heart rate, "fight or flight", whereas the parasympathetic have an inhibitory effect to decrease heart rate, "rest and digest", and decrease the inotropic state of the heart. Further discussion on the physiology of ANS modulation of the heart rate will follow later in this chapter.

Cardiac pacemaker activation

The SA node is known as the pacemaker and the primary generator of electrical impulses that induce heartbeats (197;198). Although the SA node and the rest of the conducting cells are autorythmic and do not need a neural axon to receive impulses from the central nervous system, the contraction of the heart can still be regulated by the ANS. The ANS can control how fast or how slow the impulses from the SA node are generated, depending on the physiological requirement of the body. As such, it regulates the rhythm of the heartbeats. During exercise, heart rate tends to increase due to the domination of sympathetic nervous system and withdrawal of the parasympathetic nervous system resulting from the actions of central command in addition to muscle mechanoreflex inhibition of vagal tone (197;199). In this situation, sympathetic nervous system domination is due to the release of adrenaline in response to the increased metabolic demands as a result of increased muscle activity (200;201). Post exercise and during relaxation, heart rate tends to slow down due to the sympathetic withdrawal and parasympathetic reactivation (197;199). As the increased sympathetic activation subsides post exercise, heart rate decreases in a gradual manner to ensure that there is enough blood circulation to meet the metabolic demands after cessation of exercise (202).

The action potential of the SA node is different from action potentials of the other cardiac contracting cells (203), as the SA node cells have fewer myofibrils and organelles (203). Another difference is that conducting cells have faster depolarization compared to contracting cells. The upstroke of depolarization in the SA node starts with the influx of Na^+ through Na^+ channels which account for the slow increase in membrane voltage from ~ -65mv (which is the membrane voltage during resting) up to -40mv (204). Once the membrane reaches ~-40mv this allows influx of Ca^{++} ions through T-type calcium gated channels which subsequently increases the membrane voltage from -40mv to ~-30mv (204). Subsequently, this leads to the activation of the L-type Ca^{++} voltage gated channels, which allows Ca^{++} entry to the membrane through these channels (204). As such, the membrane voltage is further increased until it reaches full depolarization. As the membrane potential reaches 0 mv, repolarization starts by inactivation of Ca^{++} and the opening of K^+ gated channels, which increase the outflux of K^+ channels. Chemical neurotransmitters from the ANS have a significant effect on the action potential of the SA node. Adrenalin or bind with Beta-adrenoceptors to speed up the depolarization period in the SA node which allows entry of Na^+ and Ca^{++} at a faster rate. In contrast, release of Acetylcholine (ACh) leads to slower

depolarization rate and longer time to reach depolarization via the binding of ACh with muscarinic cholinergic receptors in the SA node, which limits fast entry of Na^+ and Ca^{++} ions to the cell (203;205).

Cardiac autonomic balance

Balanced activity from both sympathetic and parasympathetic is necessary to maintain the homeostatic activity of the cardiovascular system. ANS dysfunction, defined by increased activity of sympathetic and reduced activity of parasympathetic, impairs the ability of the ANS to regulate the cardiovascular system (206), which may lead to CVD (189). Thus, a balanced activity of ANS could reduce the risk of developing CVD.

Autonomic dysfunction as a cardiovascular disease risk factor

Autonomic dysfunction has been suggested as a risk factor for CVD (207). Increased sympathetic activity over a long period of time makes it difficult to continue to meet the energy demands for the sympathetic system (208). Increased sympathetic activity can lead to significant arrhythmias, whereas, parasympathetic activity plays a protective role against CVD events (189;209). Major cardiac arrhythmia (e.g. ventricular fibrillation), HF, and MI have been associated with increased chemoreceptor sensitivity and reduced baroreceptor reflex sensitivity (189;210). Sympathetic activation can lead to high blood pressure (210) due to its effect on vasculature (211), and that is a mechanism through which it can lead to CVD (212). In contrast, the protective role of the parasympathetic nervous system for CVD has been demonstrated in animals, where stimulation of the vagal nerve caused a reduction in the secretion of TNF-α (213), which has been implicated in the development of CVD (214). Thus, both sympathetic and parasympathetic activation has been related to CVD risk factors and it is therefore not surprising that autonomic dysfunction is related to CVD risk.

Autonomic dysfunction as a cardiovascular disease risk in Rheumatoid Arthritis

Additional to the prevalence of traditional CVD risk factors in RA mentioned earlier in this chapter, a recent systematic review regarding autonomic dysfunction in RA revealed that this was prevalent in approximately 60 % (range 33% - 86%) of patients with RA (111), and

also contributes to increased risk of CVD in RA (215). Considering the increased risk for CVD in RA, it is perhaps not surprising to find this increased prevalence of autonomic dysfunction. However, the exact mechanism of how autonomic dysfunction contributes to the increased CVD risk in RA is not fully known and still under investigation.

The association between autonomic nervous system and immune system

The ANS is part of the central nervous system, and strong associations have been reported between the central nervous system and the immune system (216;217). The central nervous system responds to increased levels of inflammation to attempt to maintain immune homeostasis (216). For example, during exposure to viral or bacterial infection, IL-1 is released by macrophages to fight the infection that will appear in the systemic circulation. Due to an inflammation-induced increase in permeability of the blood brain barrier, IL-1 can also enter the brain, where it can act on the hypothalamus pituitary axis (217). More specifically, it can stimulate the hypothalamus to release a number of hormones including corticotropin-releasing factor (CRF), and noradrenaline (218). Release of CRF results in secretion of adrenocorticotropic hormone (ACTH), which in turn results in release of glucocorticoids which is known to have a function in the regulation of immune cells (218). This in turn leads to sympathetic system activation which results, for instance, in a rise in body temperature (217) and increase in heart rate (189). In addition, the activation of the sympathetic system results in release of sympathetic neurotransmitters including noradrenaline, adenosine triphosphate, neuropeptide Y, and nitric oxide (219). Since immune cells have adrenergic and cholinergic receptors (220), it is not surprising to find that most of these neurotransmitters can influence immune cells (219). For example, it was found that neuropeptide Y can increase the adhesion of leukocytes to the endothelial cells which is known to be a step in the initiation of inflammation (219;221). Furthermore, the influence of neurotransmitters on immune cells can be facilitated by regulating blood flow, distribution and production of lymphocytes (219). Therefore, during inflammation there is an increased in hypothalamus pituitary axis activity and sympathetic nervous system activity (219). However, if inflammation is chronic, there is still increased sympathetic activity and hypothalamus pituitary axis activity but with less ability or inadequacy of the glucocorticoids to regulate the immune cells (219). Thus, it can be suggested that with

chronic inflammation there is an increased demand from the sympathetic nervous system to be dominant.

Autonomic nervous system assessment

There are several methods to assess ANS non-invasively, and the majority of these assessments involve cardiovascular parameters e.g. heart rate and blood pressure. The most common forms of non-invasive ANS assessment include heart rate variability (HRV), heart rate recovery (HRR) and Ewing's test battery. Due to the complexity of the ANS, it is difficult to obtain full information about all aspects of the ANS with a single assessment. Some assessments reflect both arms of the ANS whereas others focus on one arm only. Ewing's test battery has components that reflect the activation of sympathetic and parasympathetic nervous system, HRV has components that reflect parasympathetic activity and the balance between sympathetic and parasympathetic activity, whereas HRR post exercise testing reflects the reactivation of parasympathetic nervous system following exercise testing.

Heart rate variability

Analysis of HRV is one of the most common non-invasive validated assessments of ANS (222;223). It is based on the analyses of the oscillation in the intervals between consecutive heartbeats at rest (224). This is obtained from R-wave to R-wave intervals between consecutive heartbeats on an electrocardiogram (ECG). The variations in R-R intervals are due to the interaction between sympathetic and parasympathetic nervous system at rest (225). At rest, parasympathetic activity (vagal activity) is more influential for HRV (225). For example, respiratory sinus arrhythmia (shortening of R-R interval during inspiration and prolongation of R-R interval during expiration) which is known to be an index of vagal activity (226), can lead to variability in heart rate at rest (227). The influence of the parasympathetic activity is fast due to the high conduction velocity of the vagus nerve and the rapid high fidelity connections between afferent inputs and central cardiovascular control areas of the brain, which makes the parasympathetic neuron excitation more evident in the next cycle of heartbeat and has therefore more acute influence on heart rate (225). In contrast, the stimulation of the sympathetic activity is slower, and its effect on heart rate is

noticeable after 2-3 seconds at resting state. This results in slower oscillation of heart rhythm (225;228). A phenomenon that explains why sympathetic effect is smaller during respiratory sinus arrhythmia is known as accentuated antagonism, where there is an interaction between the sympathetic nervous system effect and parasympathetic nervous system effect via the vagal tone (229). This interaction allows inhibition of the cardiac effects from the sympathetic nervous system due to presence of background stimulation from the vagal tone at rest. However, during stress (i.e. exercise) there is a withdrawal of the parasympathetic nervous system and reciprocal excitation of the sympathetic nervous system due to the reciprocal innervation of the sympathetic and parasympathetic nervous system.

The required duration for the heart rate recording to be used for HRV calculation ranges from 24 hours to shorter periods (e.g. two to five minutes) (224). Assessment of HRV is based on either time domain or frequency domain analyses.

Time domain heart rate variability

Time domain analysis of HRV is based on the mathematical analyses on R-R interval (224). Measures of time domain HRV include standard deviation of R-R intervals (SDNN), the square root of mean successive R-R intervals (rMSDD), and the percentage of R-R interval that differ by more than 50 ms (pNN50) in the assessed period (224;225).

Frequency domain heart rate variability

Frequency domain analyses of HRV is based on the spectral analyses of a series of R-R intervals (225). Spectral analysis of HRV involves the calculation and measurement of the power and magnitude of the oscillation in R-R intervals in separate frequency ranges, which is most commonly done with a fast fourier transform (FFT) or autoregressive approach (225). According to the Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, frequency domain HRV indices are recommended for calculation from short-term recordings (two to five minutes) because of the number of R-R intervals (See table 2) (228). These HRV indices include low frequency (LF, 0.04 to 0.15 Hz) which is modulated by sympathetic activity and a component of parasympathetic activity, high frequency (HF, 0.15 to 0.4 Hz) which is modulated by the parasympathetic activity, and LF/HF ratio which reflects the balance between the

sympathetic and parasympathetic activity (111). There is an additional component termed very low frequency (VLF, below 0.04 Hz) which is used less in studies due to the unclear interpretation of its physiological meaning (e.g. hibernation and hormone change) (225). Apart from using LF and HF in absolute value of power (ms^2), LF and HF can be measured in normalized units (nu) which represent the relative power of each component to the total power minus the VLF component (228). The advantage of representation of LF and HF in (nu) is the minimization of the effect of change in total power on the values of LF and HF (228) meaning less fluctuations in readings (e.g. very high numbers then followed by smaller numbers) reflected by sympathetic and parasympathetic activity. It should be noted that the parasympathetic component has a reflection not only in HF but also in other HRV parameters including LF. This was shown with the use of atropine as a parasympathetic blockade, which caused decreases in all frequency domain HRV indices. In healthy participants, a significant reduction in LF and HF power have been observed following atropine injection (230;231). Due to the complexity in LF and its representation of both sympathetic activity and parasympathetic components, it cannot be interpreted as a reflection of sympathetic activity only (231).

Time domain analyses provides substantial information; the frequency domain analysis is easier to obtain because it can be measured retrospectively from short-term recordings of resting ECG, and therefore is commonly used in laboratory based studies. Thus, the study in this thesis utilised frequency domain analyses.

Table 2. HRV Indices definition

HRV parameter	Definition
LF power (ms^2)	Recorded peaks between (0.04-0.15 Hz). Reflects sympathetic effect and parasympathetic component
HF power (ms^2)	Recorded peaks between (0.15-0.40 Hz). Reflects the parasympathetic effect
LF/HF	LF power (ms^2) divided by HF power (ms^2). Indicates autonomic balance
LF (nu)	Reflects the relative power of LF (normalized unit) in proportion to the total power minus the very low frequency (VLF)
HF (nu)	Reflects the relative power of HF (normalized unit) in proportion to the total power minus the very low frequency (VLF)

Heart rate recovery

HRR reflects cardiac parasympathetic function post exercise. During exercise, sympathetic activity is increased and parasympathetic activity is decreased resulting in an increase in heart rate (232;233). During post exercise recovery, there is a gradual decrease of heart rate, which is mediated by parasympathetic nervous system reactivation and withdrawal of the sympathetic nervous system (234). This period of HRR can be measured up to the six minutes, however, one minute HRR (HRR1) and two minutes HRR (HRR2) are validated, and commonly used (202;235).

HRR1 is known to reflect the reactivation of parasympathetic nervous system, whereas, HRR2 reflects parasympathetic reactivation and sympathetic withdrawal (234). HRR1 can be measured as the absolute change from peak heart rate during exercise testing to heart rate one minute post peak heart rate, and HRR2 can be measured as the absolute change from peak heart rate during exercise testing to heart rate two minutes post peak heart rate (235;236). The greater drop in heart rate is a reflective of better parasympathetic and sympathetic activity. For example, HRR1 $\leq$ 12 beats·min^{-1} was a predictor of overall mortality in a population with no history of CVD, independent of CVD risk factors, age, sex or medication (199;237). HRR2 $\leq$ 22 beats·min^{-1} has been reported to predict mortality in a mix of population with and without CVD (235). These studies provide good evidence that greater values of HRR are predictive of lower risk for CVD events. However, care should be taken when comparing these different cut off points as methodological difference can influence the HRR assessments. For example, some studies include a cooling down period after the test (199;237), whereas in other studies the assessments were taken with the participant in supine position during the recovery period (238). Therefore, utilising these cut off points is difficult to generalise to all populations, and there are no established cut off points or normative reference values for HRR1 or HRR2. Many CVD risk factors have been found to be associated with poor or slow HRR including atherosclerosis (239), pre hypertension (240), diabetes mellitus (241), high body mass index (242), and inflammatory markers (243). However, in RA patients, the association between HRR and CVD risk factors is currently unknown.

Due to the prognostic value of HRR and its association with many CVD risk factors, it has been recommended to be incorporated in the results of exercise testing assessment (244) as it can be easily used and no sophisticated equipment or interpretation techniques are needed.

This would help to risk stratify patients who are at high risk for CVD and further evaluate the effect of treatment intervention (244).

Ewing's test battery (cardiovascular autonomic reflex tests)

Ewing's test battery consists of five simple tests examining the two arms of the autonomic nervous system. These tests include heart rate response to standing, Valsalva manoeuvre, heart rate response to deep breathing, blood pressure response to standing, and blood pressure response to sustained handgrip. These tests were originally developed by Ewing and colleagues for the assessment of cardiovascular autonomic reflex in diabetic patients (245), and has been found to be a valid measure for autonomic function in diabetic patients (246). Even though some tests are more reflective of sympathetic or parasympathetic activity, together, the test battery provides a good overall reflection of ANS. For example, heart rate and blood pressure response to standing are known to reflect sympathetic activity and parasympathetic activity, and Valsalva manoeuvre and deep breathing exercise reflect parasympathetic activity (247). Heart rate can be monitored via ECG or tachogram to obtain information about heart rate as well as R-R intervals. The following paragraphs explain each task and the physiological reaction to it.

Heart rate response to standing

During this test, the response of heart rate to postural change is assessed. Before standing, the participant is quietly resting in a supine position for five minutes. Then at the end of fifth minute, the participant is asked to stand up unaided. The immediate change in posture from supine position to standing causes an increase in blood flow to the lower extremities, and distend the veins which decreases the venous return to the heart and reduces stroke volume (225). A physiological reaction from the heart is initiated, which is mediated by the baroreceptors detecting the drop in blood pressure at the start after standing, which causes an immediate vagal response at earlier stages before the 15^{th} beat (1^{st} beat to the 14^{th} beat after standing), which is followed by sympathetic nervous system activation to accelerate heart rate normally at the 15^{th} beat from standing, then followed by a period of stabilization or reduction of heart rate at the 30^{th} beat from standing (225;245). Thus, the changes in heart rate are quantified as 30:15 ratio using the shortest R-R interval at the 15^{th} beat and longest

R-R interval at the 30th beat (245). The greater the ratio is reflective of better sympathetic activation.

Valsalva manoeuvre

During this test, the participant is seated on a chair holding a mouthpiece with a pressure bar. The participant is asked to blow into the mouthpiece with closed glottis at a pressure of 40 mmHg for 15 seconds. During this manoeuvre, heart rate increases due to elevated intrathoracic pressure, followed by a decrease in heart rate after the manoeuvre (225;245). The changes in heart rate are quantified as the ratio of the longest R-R interval after the manoeuvre to the shortest R-R interval during the manoeuvre. Usually, the average of three successive valsalva manoeuvres is used. The greater the ratio is reflective of better parasympathetic activation.

Heart rate response to deep breathing

During this test, the participants is asked to conduct six deep breaths in a minute while seated. Participant is asked to do this at a rate of five seconds for inspiration and five seconds for expiration. The test is repeated three times and the mean difference between maximum heart rate and minimum heart rate during each minute is calculated (245). Deep breathing at the rate of six breaths per minute makes the phenomenon of respiratory sinus arrhythmia more prominent (225), and this test is therefore a good reflection of parasympathetic activity. The greater the difference between the maximum and minimum heart rate is a reflection of better parasympathetic activity.

Blood pressure response to standing up

At the start of this assessment, the participant is lying quietly in a supine position for five minutes, before being asked to stand up. Blood pressure is measured while the participant is lying down (preferably between the third or fourth minute) and measured again after standing up. The difference in systolic blood pressure between standing up and lying down is calculated (245). The gravitational change in this test causes an immediate response from vascular system with a decrease in systolic blood pressure. The test reflects the activity of

balance between sympathetic and parasympathetic nervous system, with a lesser drop reflective of better sympathetic activity.

Blood pressure response to sustained handgrip

To start this assessments, resting blood pressure is measured while the participant is seated on a chair. With the aid of a dynamometer, maximum voluntary handgrip force is measured. Subsequently, the participant is asked to maintain 30% of maximum voluntary handgrip force for five minutes, while blood pressure is measured each minute. The response is measured by calculating the difference in diastolic blood pressure just before the start of the test and before the release of the handgrip (245). The test induces an increase in diastolic blood pressure (often used as a reflection of total peripheral resistance) which results from both an initially vagally mediated increase heart rate and cardiac output, as well as a progressive increase in vascular resistance. (225). A greater difference between the diastolic pressure measurements is reflective of higher sympathetic activity.

Analyses of the results of Ewing's test battery (cardiovascular reflex test)

Ewing et al. (244) suggested multiple ways of categorizing the results of the tests. However, the most commonly used way in clinical research is by scoring each test based on the reference values. For example, 0 is given for a normal score, 1 is given for an abnormal score, and 0.5 is given to a borderline score (245). Therefore, the total score ranges from 0 – 5, with higher numbers indicating greater abnormality. Some of these tests are associated with age including heart rate response to standing, heart rate response to deep breathing, and blood pressure response to sustained handgrip in women only, therefore, during analyses of these tests, age must be considered as a confounding variable (245).

Similar to the other methods of ANS assessment, ANS dysfunction assessed by Ewing's test battery has been associated with increased risk of CVD, mortality, and sudden cardiac events (248).

In summary, non-invasive assessment of autonomic function can be simple and easily implemented in clinical research, however, the interpretation of what these tests reflect precisely is complex. Therefore, knowledge of these tests and their technical application is

important for successful implementation in clinical practice (225). Clear interpretation may contribute to better understanding of the role of ANS dysfunction in the development of CVD, which would improve screening strategies to control the risk of CVD (224).

Autonomic nervous system in Rheumatoid Arthritis

In RA, the majority of the studies assessing ANS have used the methods mentioned above. However, a few other assessments have been used including: skin conductance (249), cold pressor (250), pre-ejection period (251), blood assessment for chemical neurotransmitters (e.g. adrenaline) (252), and pupillography (light reaction) (253). Some of these tests were used in conjunction with the aforementioned ANS assessments (HRV, Ewing's test battery) or used with individual or multiple tasks of Ewing's test battery. Table 3 provides an overview of all the studies exploring ANS in RA.

As is evident from Table 3, HRV is most frequently used to assess ANS in RA. Some studies have used both time domain and frequency domain (215;254;255), whereas some have used time domain only (256) or frequency domain only (252;257). In general, these studies showed that patients with RA have significantly reduced HRV indicating poorer ANS compared with healthy control participants (215;254-256) or patients with spondyloarthritis (an inflammatory condition of the vertebrae of the spine) (257).

Only two studies have explored HRR in patients with RA (see Table 3). Both studies reported on the changes in HRR following an exercise programme and both showed improvements in HRR. The first study showed greater improvements in HRR following aerobic exercise compared to anaerobic exercise in a large group of RA and osteoarthritis patients (258), and the other study revealed also an improvement in HRR following ten weeks of high intensity exercise in a small group of women with RA (259). The high intensity exercise programme improved cardiorespiratory fitness and BMI, but associations between the changes were not reported.

There are numerous studies that used all the five tasks of Ewing's test battery to compare ANS between RA and healthy control participants (260;261) or other types of autoimmune diseases (262-264). The comparisons between RA and healthy control participants revealed equivocal results, with some showing poorer ANS in RA (261;262) whereas others showed no difference (260;264). A similar pattern was found when only some tests, e.g. Valsalva

manoeuvre, were used in the assessments (249-251;253;265;266), with some showing differences (249;250;266) and others reporting no differences (251;265). It is worth noting that age did influence the differences between RA and healthy control participants and could perhaps provide an explanation for the equivocal findings. For example, a difference was found between RA and healthy participants in younger participants (younger than 60 years), whereas those older than 60 years had similar ANS function (261).

ANS was also compared between patients with RA and other autoimmune conditions, with systemic lupus erythematosus (SLE) being the most commonly used comparison group. Similar to the comparison with healthy control participants, the findings of these studies are equivocal. Some have reported poorer ANS in RA compared to SLE (262), others no differences (264) and some poorer ANS in SLE (263). It is difficult to explain the reason why there was a difference in findings with regard to the comparison of ANS between RA and SLE, as the samples included in these studies vary with regards to age, disease duration, and sample size, which could have all contributed to these findings.

Several studies have used more than one assessment to investigate ANS in the same participants. Poorer ANS in RA was reported based on HRV and Ewing test battery compared to healthy participants in some studies (267;268). Contradicting findings were reported in a comparison study between RA patients and healthy participants using HRV and measures of adrenaline, a sympathetic neurotransmitter (252). Whereas no differences were observed in HRV between the groups, adrenaline levels indicated higher resting sympathetic activity in patients with RA (252). The lack of difference in the HRV may be due to the smaller sample size than previously mentioned studies, although differences in adrenaline levels were detectable despite the sample size.

As mentioned before, some studies compared RA patients and healthy control participants using some of the tasks of Ewing's test battery and other forms of ANS assessments (249-251). Poorer ANS in RA compared to healthy control participants was reported based on some of tasks of Ewing's test battery and skin conductance (249), a measure that reflects the sympathetic activation of sweat glands in the palms of the hands or soles of the foot via electrical stimulation (225). Both types of assessments indicated that RA patients have poorer sympathetic and parasympathetic activity than healthy control participants (249). Similar findings were reported in another study using some tasks of Ewing's test battery and cold pressor, which reflects the activity of the sympathetic nervous system via the changes

in heart rate or blood pressure resulting from immersion of one hand into cold water (225), compared to healthy control participants (250). Again, all types of assessments indicated poorer ANS in patients with RA than healthy participants. Contradictory findings were reported in a comparison study between RA and healthy participants using heart rate response to deep breathing (as a reflection of parasympathetic activity) and pre-ejection period, which measures the time duration between stimulation of the heart and the start of ejection as a reflection of sympathetic activity (251). Whereas no difference was observed in parasympathetic activity between the two groups, lower pre-ejection period indicated higher sympathetic activity in patients with RA (251).

Not all the studies compared RA with healthy control participants. An observational study investigated the prevalence of ANS dysfunction using some tasks of Ewing's test battery as well as pupillary autonomic function (253). The pupillary autonomic function was measured using the latency time in pupillary reflex to the light as an indication of parasympathetic function, and the maximal pupillary area in the darkness as an indication of sympathetic function (253). The results of the study showed that 60% of RA patients demonstrated abnormal response either in the tasks of Ewing's test battery or pupillary autonomic function. Moreover, in longitudinal analyses, difference were in autonomic function were found between those who died during the 8-year follow up period and those who did not. More specifically, parasympathetic activity indicated by heart rate response to deep breathing, Ewing's test battery and pupillary autonomic function were all poorer among those who died due to heart failure, immune deficiency, and sudden cardiac death during a follow-period compared to those who stayed alive (253).

Association between autonomic nervous system and cardiovascular disease risk factors

It is surprising that only limited studies have investigated the association between HRV and CVD risk factors (see Table 3). Age was related to reduced HRV in some (254;257), but not all studies (256) and also not with ANS assessed with Ewing's test battery (268). It is difficult to conclude if obesity is related to ANS, as one study reported no association (268), whereas another did (263). In separate studies, smoking (256) and blood pressure (249;263) were related to ANS, but no association was found with sex (268).

Even though little is known about the association between ANS and CVD in RA, more information is available in other populations. A study has reported CVD risk factors

associated with reduced HRV including triglycerides, systolic blood pressure, BMI, and waist to hip ratio in both diabetic and age-sex matched non-diabetic participants (269). Using HRR, many CVD risk factors including systolic blood pressure, triglycerides, and waist to hip ratio were inversely associated with HRR in healthy children and adolescents (270). Poor HRR was also found to be associated with higher BMI, and higher blood pressure in apparently healthy participants (271). In patients with DM, an inverse association between HRR and triglycerides was reported (272). These studies suggest that there is an association between CVD risk factors and ANS in different populations; however, this association is less investigated in RA.

Association between autonomic nervous system and inflammation

Several studies explored the associations between inflammation and ANS with equivocal findings. Reduced HRV was found to be associated with higher level of CRP (257), leukocytes (256), and Rheumatoid factor (254) but no association was reported between HRV and ESR. Measures of disease activity, which includes inflammation as well as the patients' perception of pain or general health and swelling, were related to HRV in some (254;256) but not all (268) studies. Most of the studies that utilised Ewing's test battery reported no association between this measure of ANS and inflammatory markers, disease duration, and CRP (249;261-263;265;266).

In summary, the literature review described above tends to show that ANS is poorer in patients with RA compared to healthy control participants. There are many studies that investigated ANS in RA using HRV, and Ewing's test battery (all tasks or multiple individual cardiovascular reflex tasks). However, the results from these studies are controversial and contradictory. Furthermore, in general, the studies do not provide enough information about the association of ANS with CVD risk, and inflammation.

Table 3. Overview of studies assessing ANS in patients with RA.

Authors	participants	Type of ANS test	Main Findings	Association between measure of ANS and inflammation	Association between measure of ANS and CVD risks
Yadav et al. (254)	45 RA (39 ♀), and 45 HCP (39 ♀)	HRV (time domain and frequency domain)	Reduced HRV in RA	HRV inversely associated with DAS28 and Rheumatoid factor	HRV inversely associated with age
Evrengul et al. (215)	42 RA (35 ♀), and 44 HCP (33♀)	HRV while recumbent (time domain and frequency domain)	Reduced HRV in RA	HRV not associated with inflammation or disease duration	NA
Anichkov et al. (256)	23RA, and 23 HCP (all ♀)	HRV (time domain)	Reduced HRV in RA.	HRV inversely associated with DAS28, and Leucocytes.	HRV inversely associated with smoking
Lazzerini et al. (257)	101 (50 ♀) with chronic inflammation including 25 with RA	HRV (frequency domain)	Reduced HRV in RA with high inflammation	HRV inversely associated with CRP	HRV inversely associated with age
Van Rensburg et al. (255)	45 RA, and 39 HCP (all ♀)	HRV (time domain and frequency domain) in supine and standing position	Reduced HRV in RA	NA	NA
Vlcek et al. (252)	8 RA, and 8 HCP (all ♀)	HRV (frequency domain) in different positions supine, tilted, blood assessment for noradrenaline and adrenaline and neuropeptide	No difference in HRV. Higher baseline levels of noradrenaline in RA	NA	NA

Milovanovic et al. (267)	52 SLE (46 ♀), 38 RA (32 ♀, and 41 HCP (23 ♀)	HRV (time domain and frequency domain and Ewing's test (all tasks except blood pressure response to standing)	Reduced HRV and poorer ANS response for Ewing's test in RA than SLE and HCP	NA	NA
Aydemir et al. (268)	36 RA (30 ♀), 38 SLE (32 ♀), and 40 HCP (31 ♀)	HRV (frequency domain) and Ewing's test (except blood pressure response to sustained handgrip)	Reduced HRV and poorer ANS response for Ewing's test in SLE than RA and HCP	No association	Both measures of ANS not associated with and age, or BMI
Minor et al. (258)	120 (40 RA 85% ♀, 80 OA 80% ♀)	HRR change in 5 minutes	RA patients and OA patients had significant improvement in HRR after three times/week of moderate to high intensity aerobic exercise training for 12 weeks compared to the same population who had three times/week anaerobic exercise training for 12 weeks	NA	NA
Sandsted et al. (259)	7 RA and 11 Juvenile idiopathic arthritis (all ♀)	HRR one minute	RA patients, as well as juvenile idiopathic arthritis, had improvement in HRR, cardiorespiratory fitness, and BMI after two times/week of high intensity training for ten weeks	NA	NA
Bekkelund et al. (260)	43 RA, and 61 HCP (all ♀)	Ewing's test	No difference in Ewing test between RA and HCP	NA	NA
Maule et al. (264)	34 connective tissue (17 SLE 17 RA), and 25 HCP (all ♀)	Ewing's test	No difference in Ewing's test between RA patients and SLE patients or between patients (RA and SLE) and HCP	NA	NA
Sandhu and Allen. (261)	62 RA (39 ♀), and 41 HCP (21 ♀)	Ewing's test	Poorer ANS response for Ewing's test in RA	No association between ANS measures and ESR or CRP	NA

Louthrenoo et al. (262)	34 RA (30 ♀), 37 SLE (34 ♀, and 62 HCP (50 ♀)	Ewing's test	Poorer ANS response for Ewing's test in RA than SLE and HCP	No association between ANS measures and ESR, disease duration, and rheumatoid factor	NA
Stojanovich et al. (263)	125 Autoimmune disease [39 RA (33♀), 54 SLE (49 ♀), 20 primary Sjögren syndrome (19 ♀), 8 polymyalgia rheumatic (6 ♀), 4 scleroderma (3 ♀)] and 35 HCP (19 ♀)	Ewing's test	Poorer ANS response for Ewing's test in SLE than RA and HCP	No association between ANS measures and inflammation	Abnormal responses of Ewing's test associated with obesity in RA
Piha et al. (265)	34 RA, 76 DM, and 67 HCP (all ♀)	Valsalva manoeuvre, heart rate response to deep breathing, and heart rate response to standing.	Poorer ANS responses in DM than RA and HCP. No difference in the ANS responses between RA and HCP	No association between ANS measures and ESR	NA
Toussirot et al. (266)	50 RA (32 ♀), and 82 HCP (49 ♀)	Valsalva manoeuvre, heart rate response to deep breathing, and heart rate response to standing	Poorer ANS response in RA than HCP	No association between ANS measures and inflammation, or disease duration	NA
Saba and Sultan. (249)	25 RA (22 ♀), and 30 HCP (27 ♀)	Heart rate and blood pressure response to standing and sympathetic skin response	Poorer ANS response in RA than HCR	No association between ANS measures and inflammation or DAS28	Blood pressure response to standing inversely associated with systolic blood pressure
Dekkers et al. (251)	25 RA (19 ♀) 28 HCP (20 ♀)	Respiratory sinus arrhythmia (heart rate response to	No difference in heart rate response to standing, Lower pre-ejection period in RA with active disease	NA	NA

		deep breathing) and pre-ejection period			
Bidikar et al. (250)	50 RA (46 ♀), and 50 HCP (46 ♀)	Blood pressure response to standing, and sustained handgrip, and Cold pressor	Poorer ANS response in RA	NA	NA
Schwemmer et al. (253)	30 RA (17 ♀) (observational and follow up study)	Ewing's test battery and Pupillography test (light reaction)	60% had poor ANS response. During follow up, most of the non-survivors had either abnormal response in Ewing's test or pupillography test	NA	NA

ANS; autonomic nervous system, RA; rheumatoid arthritis, HCP; healthy control participants, SLE; systematic lupus erythematosus, OA; osteoarthritis, NA; not assessed, HRV; heart rate variability, HRR; heart rate recovery, DAS; disease activity score, BMI; body mass index, CRP; C-reactive protein, ESR, erythrocyte sedimentation rate.

Overview and aims of thesis

RA associated with increased risk of CVD and increased risk of premature mortality from CVD. A factor that may contribute to that increased risk of CVD is ANS dysfunction, an imbalance between the activity of the ANS manifested by increased sympathetic and decreased parasympathetic activity. Even though studies have reported ANS dysfunction in RA, little is known about the associations between ANS with CVD risk factors, disease-related factors and measures of wellbeing in this particular population. Therefore, the overarching aim of this thesis is exploring factors related to ANS in RA. In the separate chapters of this thesis, this aim is addressed using different hypotheses and study designs, including cross-sectional and longitudinal designs.

Specific aims and hypotheses

Chapter 2

The aim of the study presented in chapter 2 was to investigate the association between a marker of myocardial ischemia during an exercise tolerance test and 2-min short term resting HRV. Induced ST segment depression during exercise testing is known as an identification of ischemic changes of the heart (273). However, this conventional method requires high workload intensities to detect significant ST segment depression that defines clinically positive myocardial ischemic changes, and patients with RA may not be able to work at this high intensity due to joint problems. Therefore, heart rate adjusted ST segment (i.e. the ST/HR index) was used as a novel method to detect ischemic changes during exercise testing (274). The advantage of this method is that it does not rely on the workload intensity achieved, and changes in ST segment are taken into account from resting to the maximum induced ST segment depression during exercise testing. Since reduced HRV has been found to be associated with ischemic changes of the heart, it was hypothesized that ST/HR index will associate with reduced HRV, an indication of reduced parasympathetic activity and increased sympatho-vagal imbalance.

Chapter 3

The aim of the study in chapter 3 was to explore the factors associated with parasympathetic reactivation following an exercise tolerance test. The study explored the association between HRR, as a measure of parasympathetic activity, and a range of factors reflecting CVD risk, RA-related disease activity, and measures of wellbeing. HRR is a predictor of CVD and has been associated with inflammation in healthy population. Therefore, it was hypothesized that HRR was associated with CVD risk factors, RA-related disease activity, and measures of wellbeing.

Chapter 4

The data presented in chapter 4 involves an exercise intervention study. Exercise has been shown to be one the best behavioural interventions to reduce the risk of CVD in many clinical populations, including RA (275). However, little is known about the effect of exercise training on ANS, in particular, parasympathetic nervous system using HRR, in patients with RA. To the best of our knowledge, a pilot study investigated the effect of exercise training on parasympathetic function in a relatively small group of women (259) is the only investigation of the effect of this. Therefore, this study aimed to investigate the effects of three months semi-supervised exercise training programme on parasympathetic activity (HRR) in patients with RA. Secondary aims involved the investigation of the effects of the exercise programme on CVD risk factors (including cardiorespiratory fitness), RA-related disease activity and measures of wellbeing, as well as the associations between the changes in these measures with the changes in HRR in response to the exercise programme. It was hypothesized that 3-month semi-supervised exercise intervention would improve HRR. It was further hypothesised that the exercise programme would induce changes in CVD risk factors, RA-related disease activity and measures of wellbeing, and that the changes in these factors would be related to the expected changes in HRR.

Chapter 5

In the final experimental chapter of this thesis, a comparison is made between HRR in patients with RA and patients with DM. As reported above, the CVD risk in patients with RA is comparable to that of patients with DM. However, a difference between the two populations is

the level of systemic inflammation. Whereas patients with RA can be characterised by high-grade systemic inflammation, patient with DM have low-grade systemic inflammation. Therefore, the comparison between ANS in patients with RA and patients with DM allows for the assessment of the impact of inflammation on ANS and in particular the parasympathetic nervous system. Therefore, the aim of the study was to compare parasympathetic activity using HRR between age- and sex-matched RA and DM patients. The secondary aim of the study was to compare inflammation as well as CVD risk factors and measures of wellbeing and its association with HRR in these populations. It was hypothesized that HRR would be poorer in patients with RA, and that this difference could be attributed to higher levels of inflammation.

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CHAPTER 2

Association between heart rate adjusted ST segment and heart rate variability in patients with Rheumatoid arthritis: a cross-sectional study

Abstract

Introduction Coronary artery disease (CAD) is one of the causes of increased mortality in patients with rheumatoid arthritis (RA). CAD can be undetected, especially in RA, and can lead to myocardial infarction (MI). Heart rate adjusted ST segment (ST/HR index) is a novel method used to assess ischemic changes of the heart during exercise testing. Autonomic dysfunction, reflected by increased sympathetic activity and reduced parasympathetic activity, measured by heart rate variability (HRV) while resting, has been suggested as factor contributing to the risk of developing MI. The associations between autonomic function, assessed by HRV, and ST/HR index are currently not known in RA. Therefore, the study aimed to explore the association between resting HRV indices and ST/HR index in patients with RA.

Methods 96 RA patients (54.4±12.6 years, 68% women) completed a treadmill exercise tolerance test (ETT) as part of a baseline assessment for an exercise intervention study. While resting, two minutes beat to beat R-R interval data from ECG were recorded. Low frequency (LF; 0.04-0.15 Hz), and high frequency (HF; 0.15-0.40 Hz) spectral powers, LF/HF ratio, and the normalized units (nu) of LF and HF were used as HRV indices to measure cardiac autonomic control. In addition, ST/HR index was measured as the difference between ST segment depression at rest and maximum ST segment depression during ETT divided by the absolute change from resting heart rate (HR) to the corresponding HR of maximum ST depression during ETT.

Results Mean ST/HR index was 2.6 ± 1.7v $\mu V \cdot bm^{-1} \cdot min^{-1}$. Mean or median values for HRV indices were LF (nu) [60.4 (41.7-76.1)], HF (nu) (43.0 ± 24.4), LF/HF ratio [1.5 (0.7-3.1)] Pearsons moment correlation revealed that LF/HF ratio and LF (nu) were correlated with ST/HR index, whereas, HF (nu) was inversely correlated with ST/HR index. However, when these correlated variables entered in a linear regression model using enter method, the variation in ST/HR index was not explained ($R^2 = 0.08$, $p = 0.08$), and no individual HRV variable was independently associated with ST/HR index.

Conclusion ST/HR index was associated with reduced parasympathetic tone (as indicated by HF) and reduced sympatho-vagal balance (as indicated by LF/HF). However, these variables did not explain the variance in ST/HR index and none was independently associated with ST/HR index.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that causes progressive damage to the joints, eventually leading to functional disability (1). In addition to the disease-related manifestations, RA is also associated with increased risk of mortality from cardiovascular disease (CVD), which accounts for more than 40% of deaths in RA (2). Moreover, coronary artery disease (CAD) has been suggested as a major cause of increased cardiovascular mortality in patients with RA (3;4). This may be further supported by the fact that the risk of hospitalization due to CAD is three times higher in RA compared to non-RA cohorts, and the risk of unrecognized myocardial infarction is more than five times higher in RA compared to non-RA (5). CAD is most commonly caused by the development of atherosclerosis in coronary arteries of the heart (6), which is driven by inflammation (7). As such, it seems reasonable to suggest that one of the main reasons that RA patients experience increased risk for CAD, may be due to inflammation.

Myocardial ischemia is caused by decreased oxygenated blood flow to the heart muscles, most likely due to stenosis in the coronary arteries or arterial stiffening. The twelve lead electrocardiogram (ECG) is the most commonly used non-invasive method to detect obstructive changes in the coronary arteries of the heart. Conventionally, these changes are identified via exercise induced ST segment depression. In the absence of existing pathology, there are no or minimal changes in ST segment before exercise testing, but as the intensity of test increases, the myocardial demands increase, and thus, any limitations in coronary blood flow will be manifested by further changes in ST segment depression. However, due to the great dependence of this conventional method on workload intensity (8), it has been debated that it has poor sensitivity to detect ischemic changes in certain circumstances, especially in patients with RA who may have early termination of the exercise test due to joint problems. Thus, adjusting the ST segment against a dynamic factor that causes ST segment depression to change during progressive workload may help to distinguish the ischemic changes during the test, regardless of the ability to achieve maximum workload intensity. Adjusting ST segment against the changes in heart rate (i.e. the ST/HR index) is a novel method which has been established to increase the sensitivity in detection of ischemic changes during exercise testing (8;9). Furthermore, predictability of ST/HR index has been reported to be better than the conventional method in prediction of CVD events in a low risk population (women and men) from the Framingham offspring study, and prediction of CVD mortality in high risk asymptomatic individuals (10).

One of the factors that can cause myocardial ischemia is the imbalanced activity of the autonomic nervous system (ANS) which manifests as increased sympathetic and decreased parasympathetic tone, commonly termed as ANS dysfunction (11-13). Heart rate variability (HRV) is a valid, reliable, non-invasive measure for the activity of these two arms of the ANS (14-16), with different aspects of HRV reflecting different information about ANS. The high frequency component of HRV is known to be a reflection of parasympathetic/vagal activity (e.g. increased higher frequency indicates increased parasympathetic activity), whereas, the low frequency component and the ratio of low and high frequency component are proposed as a reflection of sympathetic and parasympathetic balance (16-18). Generally, increased HRV is an indication of better ANS modulation of the heart. Previous studies have shown that reduced HRV (characterized by less vagal activity indicated by HF component and reduced sympatho-vagal balance indicated by LF and LF/HF) is associated with sudden cardiac death in patients with RA (19-21), and patients with CAD (22). Due to the increased risk of CAD and cardiovascular mortality in patients with RA, identification of autonomic factors associated with myocardial ischemia may lead to better screening and strategies in CAD prevention in this population. Given the dearth of relevant studies, this study's main aim is to investigate the association between resting HRV and myocardial ischaemia (via heart rate adjusted ST segment) during treadmill exercise testing in RA patients. It was hypothesized that heart rate adjusted ST segment (ST/HR index) associates with sympatho-vagal imbalance indicated by LF and LF/HF ratio and reduced parasympathetic activity indicated by HF.

Methods

Study population

Ninety-six RA patients (fulfilling the revised American College of Rheumatology criteria (23)) were recruited from outpatient clinics of the Dudley Group NHS Foundation Trust, UK from October 2011 to 2014 to participate in an exercise intervention study Trial registration number: ISRCTN04121489). Exclusion criteria were: fibromyalgia, recent joint surgery (in the preceding six months), current flare of the disease, comorbidity incompatible with exercise as per American College of Sports Medicine (ACSM) guidelines (24), atrial fibrillation, and established CVD. The study was approved by the National Research Ethics Service and all patients provided written informed consent prior to participation.

Protocol

Patients were invited to the research laboratory of the Russell's Hall Hospital, Dudley Group of Hospitals NHS Foundation Trust, for a treadmill exercise tolerance test (ETT). During this visit, all participants were measured for: height (Seca 214 Road Rod), weight and BMI (Tanita BC 418 MA Segmental Body Composition Analyser, Tanita Corporation, Tokyo, Japan). An electronic sphygmomanometer (Datascope Accutor, Mahwah, NJ, USA) was used to measure brachial blood pressure while the patient was seated using standardised procedures (25). Then, every participant was fitted with a 12-lead electrocardiography (ECG) (12-channel ECG custo cardio 200, Custo Med, Liepzig, Germany). While patients were seated, resting heart rate was recorded for two minutes. This was followed by an ETT and six minutes' post ETT recovery period during which heart rate was continuously monitored.

Exercise tolerance test

All patients performed an individualized treadmill (HP Cosmos Mercury, Nussdoerf-Traunstien, Germany) ETT. As per current practise, all patients were familiarised with the ETT testing procedures. The ETT protocol was modified according to each patient's fitness and physical abilities (26), an approach previously utilised in RA patients (27). The test was started at a speed of the patient's preference (approximately three mph) and 0% inclination for two minutes; the starting speed was identified and agreed with the patient during the familiarisation phase with the ETT. Thereafter, according to the patient's ability, the speed was increased gradually to the level of maximum brisk walking.

The test started at the end of the two minutes warm up with the preferred speed, and the inclination of the treadmill was increased by 1% every minute while maintaining the same speed. ECG was recorded throughout the ETT and during recovery period. The test was terminated if volitional exhaustion was reached, or any of the relative or absolute contraindications of ACSM's guidelines were met (24). Six minutes of recovery were given to patients while seated after termination of the test. All tests were supervised throughout by an exercise physiologist as well as a cardiac physiologist.

Outcome measures

Heart rate variability indices

The acquisition of HRV was done by extraction of the resting beat-to-beat R-R interval duration from the ECG recorded during the two minutes before the start of the test while the patient was seated. R-R interval was determined manually during 2-min baseline resting and then entered manually into a notepad after deletion of artefacts (i.e. ectopic beats) encountered during ECG reading. Spectral analysis of R-R intervals was made using version 2.2 Kubios software (University of Eastern Finland, Kubio, Finland). Two patients did have less than 2-minutes of resting R-R interval data and were therefore excluded from the statistical analyses. Mean R-R interval and standard deviation of all normal R-R interval (SDNN) were not used as they require longer resting ECG recording to be valid, according to guidelines and literature in this field (16;28). Frequency domain results of the 2-min resting HRV parameters were used including, low frequency (LF; 0.04-0.15 Hz), and high frequency (HF; 0.15-0.40 Hz) spectral powers, LF/HF ratio, and the normalized units of LF and HF were used as the HRV indices to measure cardiac autonomic control. The Fast Fourier transform (FFT) algorithm was used to compute the frequency domain measures including LF, HF and LF/HF. An example of the frequency domain spectrum is shown in Figure 1. The standard duration for HRV measurement for frequency domain analyses is five minutes, however, as per guidelines, it has been suggested that the minimum requirement to assess HF component is one minute, and for LF minimum of two minutes (16). The validity and reliability of short term HRV were tested including 10 seconds (29), and one minute in healthy (30;31), and patient with diabetes (32).

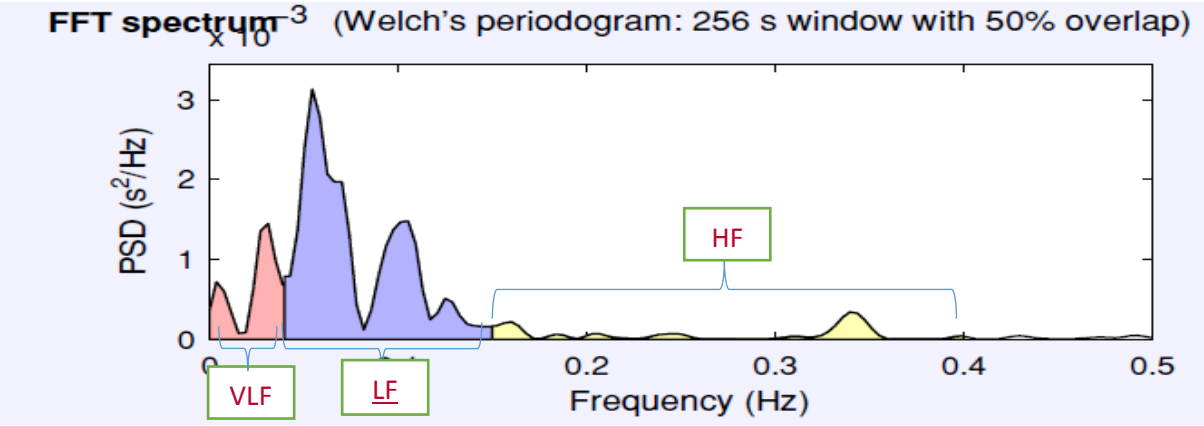

Figure 1. A typical example from one of our RA patients showing the frequency domain spectrum using Fast Fourier Transform method. VLF; very low frequency, LF; Low frequency, HF; high frequency.

ST/HR index

The ST segment was measured during the two minutes resting and throughout the ETT in five ECG leads (I, aVF, V_2, V_4, and V_5) (9). Resting ST segment was calculated by taking the average of ST segment from these five leads every 10 seconds. During ETT, the lead with the maximal ST segment depression was used as the maximum during ETT. ST/HR index in $\mu V \cdot bm^{-1} \cdot min^{-1}$ was calculated by dividing the difference between the maximum ST segment depression and resting ST segment depression of the same lead divided by the difference between resting HR and the exercise-induced changes in HR (the correspondent maximum HR to the maximum ST segment depression). The ST segment depression was measured by computer at 60 ms after the j-point (34). The formula used for calculation is

ST/HR index = (Maximum ST [(Exercise ST-Resting ST) over five leads])/((exercise induced changes HR-Resting HR)) (9).

Statistical analyses

The normality of all variables was tested using the Kolmogorov-Smirnov test. All non-normally distributed variables were log-transformed [LF power (ms^2), HF power (ms^2), LF/HF, and LF (nu)] and were presented as median and interquartile range. Normally distributed variables were presented as mean and standard deviation. The relationship between HRV indices and ST/HR index was tested using bivariate correlation (correcting for sex as a possible confounder) using Pearsons moment correlation coefficients. Normally distributed variables and log-transformed variables were included in bivariate correlation (correcting for sex). To identify if HRV parameters were independently associated with ST/HR index, linear regression was used via enter method (correcting for sex). ST/HR index was used as the dependent variable while the HRV indices that were significantly associated with ST/HR index in the previous correlation analysis, were the independent variables. Additional analyses were conducted to explore the association between age and HRV indices using Pearsons moment correlation coefficients was used to determine if age was a confounding variable. The level of significance for all analyses was set at .05. Statistical analyses was performed using SPSS20 (Chicago, IL, USA).

Results

Patient characteristics

Demographic characteristics of the 96 RA patients (66 women, 30 men) are presented in Table 1. The results of the spectral analysis of baseline 2-min HRV and the ST/ HR index calculated during the exercise test are presented in Table 2. According to the established cut-off points for ST/HR index, which indicate the value of ST/HR index ≥ 1.6 μV/beats/min as abnormal (8;35), a majority (69.4%) of the patients showed abnormal ST/HR index 2.6 ± 1.7 $\mu V \cdot bm^{-1} \cdot min^{-1}$.

Table 1. Demographic characteristics of rheumatoid arthritis patients (N=96).

Characteristics	Value
Age (years)	54.4 ± 12.6
Height (m)	1.7 ± 0.1
Weight (kg)	79.3 ± 18.5
BMI ($kg \cdot m^{-2}$)	27.8 (23.9 – 31.0)
Heart rate Rest (bpm)	80 ± 13
Resting SBP (mmHg)	134 ± 17
Resting DBP (mmHg)	81 ± 10
Disease duration (years)	7.9 ± 9.1
Hypertension	33%
Diabetes	8%
Current smokers	9%
Previous smokers	44%
Medication	
Methotrexate	74%
Biologic DMARDs	52%
Non-biologic DMARDs	3%
Anti TNF-α	12%
Predinsolone	16%
NSAID	35%
Analgesics	37%
Cholesterol Lowering	27%
Anti-hypertension	24%

Values are presented as means ± standard deviation, or median (25^{th} to 75^{th} percentile values) as appropriate or percentage. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DMARDs, disease modifying anti-rheumatic drugs; TNF-α, tumour necrosis factor- alpha; NSAID, non-steroidal anti-inflammatory drugs.

Table 2. Results of HRV parameters and ST/HR index of rheumatoid arthritis patients (N= 96)

Variable	Value	Normative values (36)			
Frequency domain parameters		Mean	SD	Median	Range
LF power (ms^2)	227.5 (86.0 – 523.5)	519	291	458	193 - 1009
HF power (ms^2)	146 (49.0 – 503.0)	385	777	657	82 - 3630

LF/HF	1.5 (0.7 – 3.1)	2.8	2.6	2.1	1.1 – 11.6
LF (nu)	60.4 (41.8 – 76.1)	52	10	54	30 – 65
HF (nu)	43 ± 24.4	40	10	38	16 - 60
Marker of ischemia		Percentage of normal and abnormal values			
		Normal < 1.6		Abnormal ≥ 1.6	
ST/HR index ($\mu V \cdot bm^{-1} \cdot min^{-1}$)	2.6 ± 1.7	N= 26 (30.6 %)		N= 59 (69.4%)	

Values are presented as means ± standard deviation, or median (25^{th} to 75^{th} percentile values) as appropriate. Normative values for HRV are obtained from healthy participants. SD; standard deviation, LF; low frequency, HF; high frequency. (nu); normalized units.

Correlations

Higher sympatho-vagal imbalance (i.e. both LF/HF ratio and normalized units of LF) were positively associated with ST/HR index (r (81) = .28, p = .01, and r (81) = .23, p = .03, respectively), whereas, parasympathetic activity (i.e. normalized units of HF) (r (81) = -.27, p = .01) were inversely associated with ST/HR index (Table 3).

Table 3. Correlation of ST/HR index with HRV indices of rheumatoid arthritis patients (N=96).

	ST/HR index	
Variable	***r***	***p***
LF power (ms^2)	.18	.11
HF power (ms^2)	-.04	.69
LF/HF	.28	**.01**
LF (nu)	.23	**.03**
HF (nu)	-.27	**.01**

All correlations are corrected for sex. *P* value in bold indicate statistical significance

Linear regression

Linear regression was used to identify if the HRV variables were independently associated with ST/HR index. Only the variables were correlated in the bivariate analyses; LF/HF, LF (nu), and HF (nu) were included as independent variables while ST/HR index was the dependent variable in the linear regression analyses. Due to the lack of significant correlation between HRV indices and age (data not reported), the linear analyses were corrected for sex only as a possible confounding factor. A model which included, LF/HF, LF (nu), and HF (nu) did not

explain the variation in ST/HR index (F (4, 78) = 2.23, p = .08), and none of the variables were independently associated with ST/HR index (Table 4).

Table 4. Linear regression model for HRV indices associated with ST/HR index in rheumatoid arthritis patients (N=96).

	ST/HR index		
Variable	**β**	**t-value**	***p***
Sex	0.62	0.15	.88
LF/HF	0.61	0.61	.55
LF (nu)	0.43	0.48	.63
HF (nu)	0.001	-0.04	.97
R2 and p-value of the model	R^2		*p*
	.08		.08

All variables corrected for sex.

Discussion

This cross sectional study explored the association between ANS using resting HRV and ST/HR index as a marker of myocardial ischemia during exercise testing. As hypothesized, reduced HRV characterized by increased sympatho-vagal imbalance (i.e. both LF/HF ratio and normalized units of LF) and reduced parasympathetic (i.e. normalized units of HF) activity was associated with ST/HR index. However, the linear regression revealed that variance in ST/HR index was not explained by the studied HRV variables used in the current study, and none of the HRV variables were independently associated with ST/HR index. The results of this study suggest that it may be the overall increased sympatho-vagal imbalance indicated by LF and LF/HF combined with the reduced parasympathetic activity indicated by HF was associated with myocardial ischemia during exercise testing in patients with RA.

Relative to normative values obtained from healthy participants, HRV values in our RA patients tend to be slightly different from normative values (i.e. LF power, HF power, LF/HF are lower than normative values, whereas, LF (nu), HF (nu) are slightly higher). It is worth mentioning when these normative values were established, there was a variation between males and females which make these normative values not a generalizable reference. Therefore, the current findings suggest that some HRV parameters demonstrated higher value, but whether this is associated with increased or reduced CAD risk remains to be investigated in future

studies as the interpretation of these data is difficult considering the absence of normative data for clinical populations. Our data suggests that there is an association between ANS and CVD, but no definite conclusion can be drawn from it.

To our knowledge, this is the first study to investigate the association between HRV as a measure of ANS and ST/HR index as a marker of myocardial ischemia in patients with RA. Many studies have shown that reduced HRV is known to predict the development of CAD (37;38). In the current study, ST/HR index was associated with sympatho-vagal balance as indicated by the ratio between LF and HF (LF/HF), and LF (nu) collectively, whereas, reduced parasympathetic, indicated by HF (nu) was inversely associated with higher ST/HR index. This is in line with relevant findings suggesting that heightened sympathetic activity and reduced parasympathetic activity is linked with CAD (19;22). However, some of these studies included multiple populations with autoimmune diseases and used long term end points of cardiovascular disease or death. Most of the previous studies associating reduced HRV with CAD in asymptomatic healthy individuals reported no association between LF/HF with CAD (37;39), whereas, Miyase et al. (40) concluded that LF/HF is a predictor of CAD. LF/HF ratio is known as an indicator of sympatho-vagal balance (16). The lower LF/HF ratio is considered to be a better indication of sympatho-vagal balance (e.g. lower number indicate dominance of parasympathetic) (16). It is difficult to compare the current study with other studies due to either the differences in methodology (using short term and long term HRV) or the use of long term end points (i.e. established CAD). Although LF is a representation of both sympathetic and parasympathetic activity, the current study showed that LF/HF and LF (nu) are positively associated with ST/HR index; based on this it can be speculated that the sympatho-vagal balance is disrupted due to increase sympathetic activity. There is a possible explanation to the relationship between symbatho-vagal imbalance and myocardial ischemia. It is known that sympathetic activation increases vasoconstriction of blood vessels, which makes the vascular wall vulnerable to mechanical injuries, a process aiding the development of atherosclerosis (41). This may be especially relevant in patients with RA who are known to be vulnerable for endothelial dysfunction (42). In contrast, the parasympathetic nervous system has shown a protective role from significant arrhythmia and experimental acute MI in animals (43). This protective mechanism was mediated via stimulation of muscarinic cholinergic receptors to inhibit the release of chemical neurotransmitters of sympathetic nerve terminals (i.e. noradrenaline) (43) (commonly known as accentuated antagonism).

Another noteworthy finding in this study is that none of the HRV indices were independently associated with ST/HR index. Furthermore, the model created with associated HRV parameters including LF/HF, LF (mu), and HF (nu) in the bivariate analyses with ST/HR index did not significantly explain the variance in ST/HR index. Possible reasons for this include the existence of other factors that contribute to the variance in the ST/HR index that were not included in the model, or the lack of adequate sample size to perform this investigation, or the use of 2-min short term HRV (i.e. too few intervals). This warrants further research in a larger sample using long term HRV in this area to explore the other factors along with HRV associated with ST/HR index.

There is some controversy about the diagnostic use of ST/HR index. Although, some studies are suggesting heart rate adjusted ST segment analyses can add substantial diagnostic benefits in exercise ECG more than conventional method (44;45), other studies did not confirm this(46;47). Furthermore, the clinical usefulness of ST/HR index in detection and assessment of CAD has been found to be better than the conventional method (measuring ST-segment depression) (34). Moreover, a study showed that abnormal ST/HR index can identify individuals with no symptoms of CAD who can benefit from risk factors reduction programs (10). To our knowledge, this is the first study to utilise ST/HR index as a measure of myocardial ischemia in patient with RA. Using such a novel method in clinical practice may help early detection of myocardial ischemic changes especially in patients with RA who are more likely to develop silent MI (5). However, this has to be proven in follow up clinical trials where RA patient can be followed up for a period of time, or by investigating patients with more invasive assessments for CAD.

The study has some limitations. Conventionally, HRV is based on several hours or minutes of heart rate recording, which can provide a wealth of information. However, this might be difficult in population-based studies and epidemiological research (48). Published recommendations indicated that one minute is the minimum requirement for heart rate recording to obtain HF power and two minutes is the minimum duration required for LF power (16). During the two minutes' assessment before exercise testing, breathing rate was not controlled which may have influenced HRV indices. Assessment of the causality between variables is not possible due to the cross-sectional design of the study. For ethical reasons, patients continued taking their medication on the day of assessment, which may interfere with HRV. Due to the variety of medications taken from our RA patients, it was difficult to achieve

appropriate statistical power to explore the influence of medication on HRV. Furthermore, the participants included in this study had well-controlled disease activity, which makes it difficult to generalize the findings of this study to RA patients with worse disease activity. Unfortunately, DAS28 was not available for all the participants, therefore, was not included in the analyses. Future research should explore the association of HRV with ST/HR index in patient with RA with various levels of disease activity.

In conclusion, the results of the study showed that depressed HRV, reflective of increased sympatho-vagal imbalance and reduced parasympathetic activity, associate with ST/HR index, reflective of myocardial ischemia during ETT. This may suggest that patients with RA may benefit from early pharmacological interventions (49) or non-pharmacological interventions to improve parasympathetic activation (50). Using ST/HR index could be particularly useful for RA patients in early detection of ischemic changes during ETT. In addition, LF/HF ratio, LF (nu), HF (nu) did not explain the variance in ST/HR index, and none of these variables was independently associated with ST/HR index. This suggests that other factors may contribute to the variance and this requires further investigation.

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CHAPTER 3

Factors associated with parasympathetic reactivation following exercise in patients with Rheumatoid Arthritis: a cross-sectional study

Abstract

Introduction Patients with rheumatoid arthritis (RA) have an increased risk for cardiovascular disease (CVD) with poor parasympathetic function being implicated as an underlying factor. Factors related to parasympathetic function, commonly assessed by heart rate recovery (HRR) following maximal exercise, are currently not known in RA. The study aimed to explore the association between HRR with CVD risk factors, inflammatory markers, and wellbeing in patients with RA.

Methods 96 RA patients (54.4±12.6 years, 68% women) completed a treadmill exercise test, during which heart rate (HR) was monitored. HRR1 and HRR2 were defined as the absolute change from HR peak to HRR 1 minute post HR peak and 2 minutes post HR peak, respectively. Cardiorespiratory fitness, CVD risk factors, and serological markers of inflammation were measured in all patients. The Framingham Risk Score (FRS) was used as an assessment of global risk for CVD events, and wellbeing was assessed by questionnaires.

Results Mean HRR1 and HRR2 were 29.1±13.2 bpm and 46.4±15.3 bpm, respectively. CVD risk factors as well as most inflammatory markers and measures of wellbeing were inversely correlated with HRR1 and HRR2. Multivariate regression analyses revealed that 27.9% of the variance in HRR1 and 37.9% of the variance in HRR2 was explained collectively by CVD risk factors, measures of inflammation, and wellbeing (p=0.009, p=0.001 respectively), however no individual measure was independently associated with HRR1 or HRR2.

Conclusion Parasympathetic reactivation was associated with overall CVD risk, arthritis-related burden and wellbeing in patients with RA.

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic systemic inflammation affecting approximately 1% of the general population (1). The main symptoms are pain, stiffness and swelling of joints. However, there are also extra-articular aspects in RA, such as an increased risk for cardiovascular disease (CVD), which causes more than 40% of the deaths in RA (2). Although the mechanisms for the increased risk for developing CVD in RA remain to be elucidated, several risk factors for CVD are prevalent in RA, such as hypertension (3) dyslipidemia (4), physical inactivity (5), inflammation (6) and obesity (7). This increased prevalence of CVD risk factors has been associated with abnormalities in vascular function and morphology (8), which could lead to CVD and even CVD-related death (9). More recently, in a systematic review by Adlan et al. (10), autonomic dysfunction has been suggested as another factor which contributes to the CVD death risk in RA.

The autonomic nervous system (ANS) is divided into the sympathetic and the parasympathetic nervous systems, which, in a healthy state, work together to maintain cardiovascular autonomic balance. Autonomic dysfunction, an increased activity of the sympathetic tone and less parasympathetic tone at rest (11), has been associated with increased risk of CVD (12) and all-cause mortality (13). Inflammation has been related to autonomic dysfunction in clinical (14), and healthy populations (15). However, little is known about the factors that contribute to autonomic dysfunction in a population with a high long-term inflammatory burden, such as patients with RA (16).

A common method to assess autonomic function, and in particular parasympathetic tone, is heart rate recovery (HRR). The rapid fall in heart rate immediately following an exercise tolerance test (ETT) is suggested to represent reactivation of the parasympathetic tone (17). HRR is a predictor of CVD and all-cause mortality (17), and has been associated with inflammation levels in a healthy population (15). HRR is commonly assessed one or two minutes post exercise, which is thought to reflect the parasympathetic activity and withdrawal of sympathetic activity, respectively (18). Even though autonomic function has been explored in RA using different methods (16;19-41), to our knowledge, HRR following exercise has only been investigated in a pilot study involving RA and Juvenile idiopathic RA (42). However, the study was looking at the improvement post exercise intervention and there was no investigation of factors associated with HRR. Therefore, the aim of this cross-sectional study was to assess

HRR post ETT and explore factors associated with HRR in RA. It was hypothesized that HRR is associated with markers of CVD risk, as well as markers of inflammation and wellbeing.

Methods

Study population

Ninety-six RA patients (fulfilling the revised American College of Rheumatology criteria (43)), were recruited from outpatient clinics of the Dudley Group NHS Foundation Trust, UK from October 2011 to 2014 to participate in an exercise intervention study (Trial registration number: ISRCTN04121489). Exclusion criteria were: fibromyalgia, recent joint surgery (in the preceding six months), current flare of the disease, comorbidity incompatible with exercise as per American College of Sports Medicine (ACSM) guidelines (44), atrial fibrillation, and established CVD. Ethical approval was obtained by the National Research Ethics Service and all patients provided written informed consent prior to participation.

Protocol

Participants were invited to visit the research laboratory on two different occasions. During visit one, a fasted blood sample was taken and questionnaires were completed. During visit two, brachial blood pressure was taken using an electronic sphygmomanometer (Datascope Accutor, Mahwah, NJ, USA) while the patient was seated. Height was measured to the nearest 0.5 cm using a standard height measure (Seca 214 Road Rod), and weight and BMI were measured using a Tanita BC 418 MA Segmental Body Composition Analyser (Tanita Corporation, Tokyo, Japan). Subsequently, the patients were fitted with an appropriate size mask to cover the nose and mouth securely for the purpose of inspired and expired gas analysis, and electrocardiography (ECG) (12-channel ECG custo cardio 200, custo med, Liepzig, Germany) electrodes were attached. Resting heart rate and volumes of O_2 consumption were recorded for two minutes while seated, followed by an ETT and six minutes' post ETT recovery period.

Exercise Tolerance Test (ETT)

ETT was performed on a treadmill (HP Cosmos Mercury, Nussdoerf-Traunstien, Germany). All patients performed an individualized treadmill ETT which was modified according to their fitness and physical abilities (45). The starting speed of the test was set based on the patient's preference (approximately three mph) and 0% inclination. Thereafter, the speed was gradually increased until a maximum brisk walking was reached, again based on the patient's ability.

After two minutes warming up at the preferred speed, the test started. The inclination increased by one percent every minute while keeping the speed constant. Breath by breath gas analyses (Metalyzer 3B, Cortex, Liepzig, Germany) were recorded throughout the exercise task, which was used to calculate peak volume of O_2 uptake (VO_2 peak). ECG was recorded throughout the exercise task and recovery period. The test was terminated if patients reached volitional exhaustion, or any of the relative or absolute contraindications of ACSM's guidelines (44) were met. Following the termination of the test, patients were asked to rest on a chair for a minimum of six minutes recovery while blood pressure was measured every two minutes.

Outcome Measures

Heart Rate Recovery

HRR was measured at 1 minute and 2 minutes following peak heart rate during exercise. Heart rate recovery 1 minute (HRR1) was defined as the absolute change from peak heart rate to heart rate 1 minute post peak heart rate (HRR1 = peak heart rate – heart rate at 1 minute post peak heart rate). Similarly, heart rate recovery 2 minutes (HRR2) was calculated as the absolute change from peak heart rate to heart rate 2 minutes post peak heart rate. Peak heart rate was identified as the maximum heart rate during the exercise protocol.

VO_2 peak

Aerobic capacity (VO_2 peak) was measured during treadmill ETT via a breath by breath gas analyser. The inspired and expired gases data from the analyser were averaged every two seconds. To avoid spikes and fluctuations of the VO_2 $ml.min^{-1}$ readings, these data were smoothened by taking the average of VO_2 every 28 second (taking the average of 14 readings

of VO_2ml.min^{-1}). VO_2 peak was defined as the highest VO_2 during the test and was expressed asml.kg^{-1}.min^{-1}.

Serological assessments

The blood samples were analysed for serological risk factors for CVD: total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides. Homeostasis models assessment (HOMA) was utilized to assess insulin resistance (46). Inflammatory markers were assessed including erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hsCRP), fibrinogen, and white blood cells (WBC). Analyses were carried out using routine laboratory procedures in the hospital's laboratory.

Global cardiovascular risk

In order to measure the probability of occurrence of cardiovascular events within 10 years period, the Framingham risk score (FRS) was utilized (47).

Measures of wellbeing

Functional ability, psychological and general wellbeing were assessed using health assessment questionnaire (HAQ) (48), hospital anxiety and depression scale (HADS) (high score is indicative of worse anxiety or depressive symptoms) (49), subjective vitality scale (high score is indicative of better vitality) (50), multidimensional assessment of fatigue (MAF) (high score is indicative of greater levels of fatigue) (51), and the European quality of life questionnaire (EuroQol) (low score is indicative of poor quality of life) (51).

Statistical analysis

Statistical analysis was performed using SPSS20 (Chicago, IL, USA). Kolmogorov-Smirnov test was used to test the normality of all variables. Log-transformation was performed for non-normally distributed variables (BMI, triglycerides, LDL, hsCRP, ESR, HOMA, FRS, HADS depression, EuroQol). All normally distributed variables were presented as mean and standard deviation, whereas, non-normally distributed variables were presented as median and interquartile range. To assess the relationship between HRR at both time points and other

normally distributed variables, bivariate correlation (correcting for sex as a possible confounder) using Pearsons moment was used. Bivariate correlation was performed on normally distributed variables and after log transformation for the skewed variables. To assess factors associated with HRR at both time points, two multivariate linear regression were used using enter method (correcting for sex) where HRR (HRR1 and HRR2 separately) was the dependent variable, while the independent variables were all the variables that came out significantly associated with HRR1 and HRR2 separately in our correlation analysis. The level of significance for all analyses was set at .05.

Results

Patient characteristics

Demographic characteristics as well as CVD risk, markers of inflammation and wellbeing of the 96 RA patients (66 female, 30 male, disease duration 7.9 ± 9.1 years) are presented in Table 1. The most common comorbidities were hypertension (33%) and diabetes (8%), and 9% were current smokers whereas 44% of the patients were previous smokers. The types of medication used included: Methotrexate (74%), other non-biologic disease modifying anti-rheumatic drugs (DMARDs) (52%), anti-tumor necrosis factor (12%), other biologic DMARDs (3%), prednisolone (16%), non-steroidal anti-inflammatory drug (35%), analgesics (37%), cholesterol-lowering (27%), and anti-hypertensive (24%). Patients were requested to maintain their course of treatment during the study. The results from the exercise tests, including HRR1 and HRR2, are presented in Table 2. On average, the overall fitness level of the participants was low as indicated by their mean VO_2 peak 20.6 ± 5.1 $ml \cdot kg^{-1} \cdot min^{-1}$ (53).

Table 1. Demographic Characteristics, CVD risk, inflammatory markers, and measures of wellbeing in rheumatoid arthritis patients (N=96)

Characteristics	Value
Cardiovascular risk factors	
Age (years)	54.4 ± 12.6
Height (m)	1.7 ± .09
Weight (kg)	79.3 ± 18.5
BMI ($kg \cdot m^{-2}$)	27.8 (23.9 – 31.0)
Heart rate Rest (bpm)	79.7 ± 12.5
Resting SBP (mmHg)	134.2 ± 17
Resting DBP (mmHg)	81 ± 9.7
Total Cholesterol ($mmol \cdot L^{-1}$)	5.1 ± 1.05

Triglycerides ($mmol \cdot L^{-1}$)	1.1 (0.8 – 1.4)
HDL ($mmol \cdot L^{-1}$)	1.4 ± 0.38
LDL ($mmol \cdot L^{-1}$)	3.1 (2.5 – 3.6)
HOMA	1.5 (0.94 – 2.05)
Framingham Risk score	7.0 (4 – 13)
Inflammatory markers	
WBC ($10^9 \cdot L^{-1}$)	6.8 ± 2.4
Fibrinogen ($g \cdot L^{-1}$)	4.7± 1.1
HsCRP ($mg \cdot L^{-1}$)	4.6 (1.6– 8.8)
ESR ($mm \cdot hr^{-1}$)	10 (5 – 21.5)
Wellbeing	
Vitality	4.1 ± 1.6
HADS anxiety	6.7 ± 4.2
HADS depression	4.0 (2 – 7)
Global Fatigue	25.8 ± 11.8
EuroQol	0.7 (0.6 – 0.8)

Values are presented as means ± standard deviation, or median (25^{th} to 75^{th} percentile values) as appropriate. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; WBC, white blood cells; HsCRP, high sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; HOMA, homeostasis model assessment insulin resistance; Vitality, score ranges 1 (low vitality) – 7 (high vitality); HADS, hospital anxiety and depression scale (0-21); Global fatigue, score ranges from 0 (no fatigue) – 50 (severe fatigue) EuroQol, European assessment for quality of life.

Table 2. Exercise related variables in rheumatoid arthritis patients (N=96)

Variable	**Value (Mean ± SD)**
Heart Rate Peak (bpm)	153.4 ± 19.7
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	20.6 ± 5.1
RER ($VCO_2 \cdot VO_2^{-1}$)	1.2 ± 0.1
HRR1 (bpm)	29.1 ± 13.2
HRR2 (bpm)	46.4 ± 15.3

VO_2 peak, highest volume of oxygen; RER, respiratory exchange ratio; HRR1, the difference between HR peak and heart rate 1 minute post HR peak; HRR2, the difference between HR peak and heart rate 2 minute post HR peak.

Correlations

Correlational analyses were conducted to explore the associations between HRR and risk factors for CVD and wellbeing. With regards to CVD risk factors, results revealed that HRR1 and HRR2 were inversely associated with age and serum triglycerides, whereas resting SBP was only inversely associated with HRR2 (Table 3). HRR2 was inversely associated with HOMA (r (87) = – .27, p = .009). VO_2 peak was positively associated with HRR1 and HRR2 (r (93) = .33, p = .001 and r (93) = .41, p < .001, respectively). HRR1 and HRR2 were both inversely related to FRS. WBC, Fibrinogen, hsCRP, and ESR were all inversely associated with HRR1, and, albeit somewhat weaker, with HRR2 with the exception of CRP (Table 3).

With regards to measures of wellbeing, EuroQol and vitality were positively associated with HRR1 (r (90) = .26, p = .01 and r (88) = .30, p = .004), respectively, whereas, only vitality was significantly associated with HRR2 (r (88) = .21, p = 0.05). Additional non-parametric correlational analyses (Spearman correlation) were conducted between HRR and those variables which were not normally distributed. These analyses revealed similar associations as the Pearsons correlations reported on the log-transformed data (data not reported).

Table 3. Correlation of HRR1 and HRR2 with CVD risk, inflammatory markers, and wellbeing in rheumatoid arthritis patients (N=96)

	HRR1		**HRR2**	
Variable	***r***	***p***	***r***	***p***
Cardiovascular risk factors				
Age	-.27	**.006**	-.44	**<.001**
BMI	-.15	.14	-.08	.43
Resting SBP	-.18	.08	-.28	**.005**
Resting DBP	-.09	.38	-.10	.33
Triglycerides	-.31	**.003**	-.27	**.008**
HOMA	-.14	.18	-.27	**.009**
VO_2 peak	.33	**.001**	.41	**<.001**
Framingham risk score	-.29	**.005**	-.45	**<.001**
Inflammatory markers				
WBC	-.23	**.02**	-.22	**.04**
Fibrinogen	-.32	**.001**	-.27	**.009**
hsCRP	-.22	**.03**	-.15	.13
ESR	-.26	**.01**	-.21	**.03**
Wellbeing				
Vitality	.30	**.004**	.20	**.05**
HADS depression	-.18	.07	-.03	.81
HADS Anxiety	-.02	.87	.12	.21
Global fatigue	-.19	.054	-.03	.75
EuroQol	.26	**.01**	.12	.24

HADS, hospital anxiety and depression scale; EuroQol, European assessment for quality of life. All variables were corrected for sex.

Linear regression

In order to identify if the variables were independently associated with HRR, all variables which were significantly correlated with HRR were subsequently entered in multivariate linear regression analyses (see Table 4). A model (based on the correlated variables in the univariate analyses) which included age, triglycerides, VO_2 peak, FRS, WBC, CRP, ESR, fibrinogen, vitality, and EuroQol accounted for 29.7% of the variation in HRR1 (F (11, 67) = 2.57, p = .009, R^2 = 0.29). However, even though almost a third of the variance in HRR1 was explained by these variables, none of the variables were independently associated with HRR1. For HRR2, using the same method, a model which included age, resting SBP, triglycerides, HOMA, VO_2 peak, FRS, WBC, ESR, fibrinogen and vitality accounted for 37.9% of the variation in HRR2 (F (11, 65) = 3.6, p = .001, R^2 = 0.37). Similar to HRR1, none of the variables were independently associated with HRR2. Data was checked for collinearity, but was revealed not to influence the data. Additionally, in a different analyses, using age as a confounding factor along with sex did not change the model presented in table 4 (data not reported).

Table 4. Linear regression model for factors associated with HRR1 and HRR2 in rheumatoid arthritis patients (N=96)

	HRR1		**HRR2**	
Variable	**β**	**t (*p*)**	**β**	**t (*p*)**
Cardiovascular risk factors				
Age	-0.26	-1.25 (.21)	-0.46	-1.91 (.06)
Triglycerides	-21.69	-1.48 (.14)	-6.03	-0.37 (.71)
VO_2 peak	0.05	0.11 (.91)	0.45	-0.98 (.32)
Framingham risk score	0.09	0.01 (.99)	-2.72	-0.25 (.79)
Resting SBP *			-0.07	-0.69 (.48)
HOMA *			-2.51	-0.16 (.86)
Inflammatory markers				
WBC	-0.57	-0.66 (.51)	-0.01	-0.02 (.98)
Fibrinogen	-3.18	-1.36 (.17)	-2.24	-0.94 (.34)
hsCRP **	0.86	0.16 (.87)		
ESR	4.49	0.70 (.48)	6.62	0.99 (.32)
Wellbeing				
Vitality	1.27	0.98 (.32)	2.09	1.78 (.07)
EuroQol **	33.65	1.09 (.27)		

R^2 and p-value of the model	R^2	*p*	R^2	*p*
	0.297	**.009**	0.379	**.001**

* Indicates variable that was not correlated with HRR1 in univariate analyses, therefore, was not included in model 1. ** Indicates variable that was not correlated with HRR2 in univariate analyses, therefore, was not included in model 2.

Discussion

To our knowledge, this is the first study to assess the relationship between HRR and CVD risk factors, disease-related measures and indicators of wellbeing in RA patients. As expected, many CVD risk factors, inflammatory markers and some measures of wellbeing were correlated with HRR. However, multivariate analyses revealed that the variance in HRR was explained by a group of factors including CVD risk factors, inflammatory markers and some measures of wellbeing, but none of the variables tested were independently associated with HRR. This may suggest that it is the overall CVD risk and disease-related burden that contributes to changes HRR, instead of one or several individual factors.

The current study is the first to reveal that HRR was associated with individual CVD risk factors in RA. In line with other populations (54), an association was found between triglycerides and HRR, which is not surprising given the association between lipid metabolism and the ANS (55). As expected based on findings from other populations (56), cardiorespiratory fitness was also correlated with HRR, a phenomenon mainly attributed to improved baroreflex sensitivity (57). Moreover, our results revealed that age was inversely associated with HRR which is in line with some (31;36), but not all studies (28;32) in this field. Similar equivocal findings have been found between autonomic function and body composition (25;28). The variation in findings of the available studies is probably due to the differences in the ANS assessment.

To our knowledge, this is the first study to report an association between global CVD risk (FRS) and HRR in RA. The effect size of the associations between global CVD risk and HRR were greater than the effect sizes of the associations between individual risk factors and HRR. Given that FRS comprises of multiple variables, this association, together with the finding that none of the individual risk factors were independently associated with HRR in multivariate analyses, suggests that rather than individual risk factors, the overall RA- and CVD related burden is an important factor that associates with parasympathetic function.

In line with cross-sectional and experimental studies in other populations (15;58), disease-related inflammation was inversely associated with HRR. In RA patients, however, the results are equivocal. As recently reviewed, the majority of studies do not find an association between inflammation or disease activity and autonomic function in RA, even though there are exceptions (10). For example, CRP (36), and leukocytes (35) were reported to be related to autonomic dysfunction, but more studies reported no such association (16;20;24;25;34). Similar to inflammation, disease activity (DAS28) was found to be associated with autonomic function in some (21;31;35), but not all studies (16;20;24;25;32;34). Comparison between the different studies is difficult given the different methods used to assess autonomic function (i.e. heart rate variability (HRV) (37), pupillary response to light (32), Ewing test (23), and HRR as in the current study). When exploring the available studies in more detail, it is worth noting that studies that reported no association between autonomic function and inflammation used the Ewing test battery as a method of assessment (16), whereas those who did report an association measured autonomic function using HRV (36). The Ewing test battery consists of five separate assessments with a dichotomized response to each test and the total score is the sum of all the responses (59). HRV and HRR in contrast, use continuous heart rate readings to derive a measure of autonomic function. Therefore, it could be argued that statistically HRV and HRR are more sensitive tests for exploring associations compared to the Ewing test battery. However, without making a direct comparison between factors associated with the Ewing test battery and HRV or HRR in the same population, this suggestion remains speculative.

The associations between inflammation and HRR1 and HRR2 in the current study were slightly different; CRP was associated with HRR1 which is related to parasympathetic reactivation, but not HRR2, which is reflective of sympathetic withdrawal (18). Similarly, inducing inflammation (e.g. via flu vaccination) reduced HRR1 but not HRR2 in healthy participants (15). Interestingly, the evidence for parasympathetic dysfunction is also more evident in RA compared to sympathetic dysfunction (10). From a theoretical standpoint, the association between inflammation and the parasympathetic nervous system is not unexpected. This could be explained via the mechanism of the cholinergic anti-inflammatory pathway, where release of inflammatory markers such as tumor necrosis factor-α and interleukin-1 is controlled by the vagus nerve which is part of the parasympathetic system (60). However, future research is needed to explore this association in more detail in patients with RA.

The first two minutes of HRR are the most validated (61) and commonly used method in studies, and have been suggested to be a better predictor of mortality and coronary artery disease (CAD) (62). The subtle differences in the other factors related with HRR1 and HRR could be again due to the different aspects of ANS which are reflected by HRR1 and HRR2: HRR1 is known to represent reactivation of parasympathetic nervous system, whereas, HRR2 represent reactivation of parasympathetic and withdrawal of sympathetic nervous system (63). In the current study, SBP and insulin resistance were significantly associated with HRR2 but not HRR1. Interestingly, insulin has been found to have a stimulatory effect on the sympathetic nervous system (64), and metabolic risk factors have been more strongly related to HRR2 than HRR1 in healthy participants. However, this complex interaction between the two systems in controlling post exercise HRR still needs further clarification.

To our knowledge, this is the first study to explore associations between markers of psychological wellbeing and autonomic function in RA. This is surprising given that psychological factors have been associated with CVD, and autonomic function has been implicated as an underlying pathway by which psychological wellbeing contributes to the pathogenesis of CVD (65). Indeed, history of anxiety and depression (66), as well as emotional stress (67), have been associated with lower parasympathetic activity. In the current study, even though no significant relationship was found between either depression or anxiety and HRR, vitality and quality of life (EuroQol) were related to HRR. This may suggest that maintaining a good quality of life and the perception of feeling energized and capable of doing tasks may favor a balanced activity of the ANS.

There are number of limitations in this study. The cross-sectional design does not allow for the assessment of causality between variables. The current study did not have a comparison group, however, as the aim of the current study was to explore factors associated with parasympathetic activity in RA, the focus was specifically on RA patients. Without a control group, a direct comparison between parasympathetic activity in RA and other populations is not possible. Comparing HRR in this well-controlled cohort of RA patients from this study to HRR reported in healthy participants in other studies (68;69), reveal similarities in parasympathetic function. Caution should of course be taken when comparing different studies as variations in the methods are likely to influence the findings. Therefore, future studies should examine the differences between HRR in RA and other populations as well as exploring factors associated with HRR in other populations. For ethical reasons, the patients did not discontinue their

medication prior to the assessments. As is common in this population, the patients included in this study were on a mix of medications, which means that we did not have appropriate statistical power to explore the influence of medication on HRR. Further research should investigate the potential influence of medications on HRR in patients with RA. The disease activity of most of the RA patients involved in this study was well-controlled; therefore, it is difficult to generalize these findings to RA patients with higher disease activity. Unfortunately, DAS28 data was not available for all participants, therefore, it was not included in the analyses. It is possible that patients were not able to exercise to their maximum cardiorespiratory ability due to problems with their joints, which could have influenced their HRR (18). However, based on respiratory exchange ratios (RER) which reflect the amount of energy metabolized to provide energy, we are confident that most of the participants in our study were able to exercise until exhaustion. Future research should also explore the association between CVD risk, inflammation, and wellbeing with other measures of ANS, such as HRV.

In conclusion, the results of this study showed that CVD risk, disease related inflammation, and wellbeing associate with HRR in RA. As there is ample evidence that HRR is related to CVD mortality in other populations (17), further research should explore if HRR is also predictive of hard CVD endpoints in RA. In addition, given that exercise interventions can reduce CVD risk in RA (70), and improve parasympathetic reactivation (71), further research should also examine the effects of an exercise intervention on HRR in this population. The results of this study suggest that reducing CVD risk in RA is likely to require interventions to improve classical CVD risk factors, inflammation, physical fitness, and psychological well-being. HRR could be used as an overall marker of patients at risk of developing CVD and, therefore, can be used as a marker to see whether an intervention is effective in this cohort. However, this will need further research to investigate the effectiveness of an intervention using HRR.

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CHAPTER 4

Effect of three months exercise training on parasympathetic function– heart rate recovery in patients with Rheumatoid Arthritis: a longitudinal study

Abstract

Introduction Cardiovascular disease (CVD) is the leading cause of death in patients with rheumatoid arthritis (RA). Poor parasympathetic function has been suggested as a factor contributing to the increased risk of CVD in RA. Heart rate recovery (HRR) following maximal exercise testing is a non-invasive assessment for parasympathetic function. Exercise has been shown to reduce the overall risk for CVD in RA, but the effects on HRR are unknown. Therefore, this study aimed to investigate the effect of 3-month exercise programme on HRR in patients with RA.

Methods 97 RA patients enrolled in a 3-month exercise programme consisting of two semi-supervised sessions and one session at home. Out of 97, 62 RA patients (55.5 ± 12.7 years, 67.7% women) completed baseline and post intervention assessments including exercise tolerance test (ETT). At baseline and post intervention, heart rate recovery (HRR) was measured: HRR1 and HHR2 were defined as the absolute change from heart rate (HR) peak during ETT to HR 1 minute post HR peak and 2 minutes post HR peak, respectively. Cardiorespiratory fitness (VO_2 peak), individual CVD risk factors, 10 years CVD risk, serological markers of inflammation, and measures of wellbeing were measured at baseline and post intervention.

Results ANOVA revealed no significant changes in HRR1 (p=.31), HRR2 (p=.67), and VO_2 peak (p=.17) after the 3-month exercise training programme. There were significant improvements in systolic blood pressure (p=.01), diastolic blood pressure (p=.001), Qrisk2 (p=.04) and vitality (p=.02) post intervention. Additional analyses revealed that patients who improved HRR at both time point post intervention were the ones with poorer HRR as well as CVD risk profile at baseline.

Conclusion Three semi-supervised exercise training sessions per week for three months was not sufficient to improve parasympathetic function and cardiorespiratory fitness in patients with RA, however, there were improvement in blood pressure, Qrisk2, and vitality post the intervention.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease which affects approximately 1% of the United Kingdome adult population (1). RA impacts on the joints of the body, causing pain, stiffness, and swelling of the joints, but can also extend to other organs. Cardiovascular disease (CVD) is a common extra articular manifestation of RA, which contributes to more than 40% of mortality in RA (2). The mechanisms underlying the increased risk for CVD in RA remain to be fully understood, however, several CVD risk factors are prevalent in people with RA such as hypertension (3), inflammation (2), dyslipidaemia (4) and lack of physical activity (5). These factors do not explain the reason why RA associates with increased risk of CVD, as all of these factors may contribute individually and/or collectively to increase the risk of CVD in patients with RA. Furthermore, autonomic dysfunction (unbalanced activity of the autonomic nervous system) has recently been identified recently in a systematic review as a factor to contribute to the development of CVD in RA (6).

The autonomic nervous system consists of the sympathetic and parasympathetic nervous system. These systems work in synergy to regulate and coordinate multiple body functions such as maintaining cardiovascular autonomic balance. Autonomic dysfunction occurs when this balance is disrupted, which is manifested by increased sympathetic activity and decreased parasympathetic activity (7), and this has been recognized as a predictor of CVD and cardiovascular mortality (8;9). It is therefore not surprising that autonomic dysfunction is prevalent in RA (6;10) and it has been related to the overall cardiovascular and arthritis related disease burden (11). Therefore, interventions aimed to reduce CVD risk and disease burden are hypothesized to improve autonomic function in patients with RA.

Exercise is a commonly used behavioural intervention to reduce CVD risk (12). Exercise programmes have also been shown to improve autonomic function in clinical populations with increased levels of inflammation, such as chronic obstructive pulmonary disease (13), coronary artery disease (14), and chronic heart failure (15). Both physical activity (5), which is lower in patients with RA compared to the general population (16), and physical fitness (17) have been related to CVD risk in RA, and exercise has been shown to be successful in decreasing CVD risk in patients with RA (18;19). However, little is known about the effect of exercise on autonomic function in this population.

A common method to assess autonomic function is heart rate recovery (HRR) which reflects parasympathetic activity and can be easily conducted in a clinical setting, as no sophisticated equipment or analyses techniques are necessary (20). Following maximal exercise testing, the reactivation of the parasympathetic activity is represented by the rapid fall in heart rate (21). (22). Two previous studies have shown positive effects of supervised exercise on autonomic function using different ways of measurement in a relatively small group of women with RA (23;24). However, little is known about the effect of semi-supervised exercise interventions on HRR. Given that the costs associated with a semi-supervised exercise programme would be less, it is suggested that this would be more feasible to incorporate in the healthcare system. Therefore, the primary aim of this study was to investigate the effect of a 3-month semi-supervised exercise intervention on heart rate recovery one minute post peak heart rate (HRR1), heart rate recovery two minutes post peak heart rate HRR2, and cardiorespiratory fitness as indicated by the peak volume of oxygen (VO_2 peak) during exercise. The Secondary aim was to investigate the effect of the exercise intervention on CVD risks, markers of inflammation and measures of wellbeing in a sample of men and women with RA. It was hypothesized that exercise training will improve the HRR1, HRR2, and VO_2 peak, which will be associated with changes in CVD risk, markers of inflammation, and wellbeing.

Methods

Study population

Ninety-seven RA patients were recruited from rheumatology outpatient clinics of the Dudley Group NHS foundation Trust. All patients met the revised criteria of the American College of Rheumatology (25). Patients were recruited to participate in a 3-month exercise intervention study (ISRCTN04121489) (26). Patients were assessed before enrolment into exercise intervention programme, then re-assessed within 1 month after the completion of the exercise programme. Exclusion criteria were joint surgery in the preceding 6 months, fibromyalgia, established CVD, current flare of disease activity, comorbidity incompatible with exercise as per American College of Sports Medicine (ACSM) guidelines (27). Ethical approval was obtained from the appropriate National Research Ethics Service and all patients provided written informed consent prior to participation.

Exercise intervention

The exercise intervention was conducted at two separate exercise centres in close vicinity of the hospital and with comparable equipment (Action Heart and Dudley Leisure Centre). The instructors of both centres received training related to RA and the exercise programme. All patients received the same exercise programme; however, those who exercised in Action Heart also received personal consultations. As the primary aim of this study is to investigate the effect of effect of a 3-month semi-supervised exercise intervention on HRR, data for the participants was treated as one group.

The exercise programme started with an induction session to familiarise the patient with the equipment and exercises. Following this, the patient started the 3-month exercise programme in a semi-supervised setting, with instructors available when needed. The 3-month individualized exercise programme was based on the baseline assessment of the cardiorespiratory fitness, and all available equipment (e.g., treadmill, cycle ergometer, hand ergometer, rowing machine) were used based on patient's preference and abilities. The total duration of each exercise session was 55 minutes which included 10 minutes' warm-up, 30-40 minutes' main session, and 5-10 minutes cooling down. The intensity of exercise was monitored by the patients using a heart rate monitor, and patients were asked to exercise at a heart rate corresponding to 70% of VO_2 peak for a minimum of 30 minutes during each exercise session. The frequency of exercise sessions was three per week, two sessions were conducted in a semi-supervised setting within the centres and patients were encouraged to complete one session in non-supervised setting (e.g., patient's home). Attendance to the exercise programme was monitored throughout the three months.

Assessment Protocol

All patients were assessed before starting the intervention and upon completion of the exercise programme. The assessment protocol was the same for both time points, and included two separate visits to the laboratory, at least 1 week apart. During the first visit, a fasted blood sample was taken and questionnaires were given to the patients to complete at home. During the second visit, blood pressure was measured using electronic sphygmomanometer on the arm (Datascope Accutor, Mahwah, NJ, USA). Height was measured to the nearest 0.5cm using a standard height measure (Seca 214 Road Rod), weight and BMI were measured using a Tanita BC 418 MA Segmental Body Composition Analyser (Tanita Corporation, Tokyo, Japan). Once

the patient was fitted with the appropriate equipment, the patient sat quietly for two minutes, which was followed by the exercise tolerance test and a post exercise recovery period in a sitting position. Heart rate was recorded throughout these periods.

Exercise tolerance test

Before commencing exercise tolerance test, the patient was fitted with an appropriate size mask for the purpose of inspired and expired gas analyses, and 12 leads electrocardiography (ECG) (12-channel ECG custo cardio 200, custo med, Liepzig, Germany) were attached. To measure cardiorespiratory fitness, treadmill exercise testing was performed on all patients (HP Cosmos Mercury, Nussdoerf-Traunstien, Germany). An individualized ramp protocol test was used which was modified according to the patient's fitness and physical abilities (28). For the purpose of familiarization, two minutes warm up was used which also informed the initial speed of the test. The starting speed was set based on the patient's preference typically (approximately 3 mph) and 0% inclination. Thereafter, speed was gradually increased to the level of maximum brisk walking based on each patient's ability. Following the two minutes warming up, the test started. During the test, speed was kept constant (at maximum brisk walking) whereas the inclination was set up to increase by 1% every minute. To calculate peak oxygen consumption (VO_2 peak) throughout the exercise test, breath by breath gas analyses were recorded (Metalyzer 3B, Cortex, Liepzig, Germany). ECG was recorded throughout the exercise task and recovery period. The test was terminated when patients reached volitional exhaustion, or any of the relative or absolute contraindications of ACSM's guidelines (27) were met. Following termination of the test, patients were asked to rest on a chair for the minimum of six minutes' recovery while blood pressure was measured every two minutes. The test was supervised by a cardiac physiologist and an exercise physiologist.

Outcome measures

Heart rate recovery

HRR was measured at two time points: at one minute and two minutes following cessation of exercise. Heart rate recovery one minute (HRR1) was defined as the difference between peak heart rate and heart rate one minute post peak heart rate (HRR1 = peak heart rate – heart rate at one minute post peak heart rate). Similarly, heart rate recovery two minutes (HRR2) was

defined as the difference between peak heart rate and heart rate two minute post peak heart rate (HRR2 = peak heart rate – heart rate at two minutes post peak heart rate). Peak heart rate was defined as the maximum heart rate during the exercise test.

VO_2 Peak

Maximal aerobic capacity was determined using maximal oxygen uptake (VO_2 peak) which was measured via a calibrated breath by breath gas analyzer. The inspired and expired gases data from the analyser were averaged every two seconds. These data were smoothened by taking the average of VO_2 every 28 seconds (taking the average of 14 readings of VO_2 $ml \cdot min^{-1}$). VO_2 peak was defined as the maximum VO_2 during the test and was expressed as $ml \cdot kg^{-1} \cdot min^{-1}$.

Serological assessments

Blood samples were analyzed for total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides. To assess insulin resistance, Homeostasis models assessment (HOMA) was utilized (29). Erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hsCRP), fibrinogen, and white blood cells (WBC) were assessed and categorized as markers of inflammation. Analyses were carried out using routine laboratory procedures in the hospital laboratory.

Global cardiovascular risk

Two global cardiovascular risk scores were used to measure the probability of developing cardiovascular events within 10 years period: Framingham risk score (FRS) (30), and Qrisk2 (31). FRS, and Qrisk2 scores were calculated via entering the data online into the main website of these algorithms.

Measures of wellbeing

Multiple assessments were used to subjectively assess functional ability, psychological and general wellbeing; including hospital anxiety and depression scale (HADS) (high score is indicative of worse anxiety or depressive symptoms) (32), subjective vitality scale (high score is indicative of better vitality) (33), multidimensional assessment of fatigue (MAF) (high scores is indicative of greater level of fatigue) (34) and the European quality of life questionnaire (EuroQol) (low scores is indicative of poor quality of life) (35).

Statistical analyses

Statistical analysis was performed using SPSS22 (Chicago, IL, USA). Normality of variables was tested using Kolmogorov-Smirnov test. All normally distributed variables were presented as mean and standard deviation. Non-normally distributed variables (BMI, triglycerides, HDL, LDL, HOMA, FRS, hsCRP, ESR, vitality, HADS Anxiety, HADS Depression, global fatigue index, and EuroQol) were used after log-transformation and were presented as median and interquartile range. As patients exercised in two separate locations, two groups (Action Heart, Dudley Leisure Centre) by two time (baseline, 3-months) repeated measures analyses of variance were conducted to explore group differences in the responses to the exercise intervention. As no group differences were evident for any of the outcome measures, data is presented for all patients as one group. Differences in variables between baseline and post exercise intervention were evaluated using two time (baseline, post intervention) ANOVAs. Pearsons moment correlation was used to assess the relationship between attendance and HRR, as well as the relationship between changes in HRR and changes in other variables. In order to explore the potential influence of age on the effects of the exercise, Pearsons moment correlational analyses were conducted. ANOVAs were used to explore the difference in means of outcome measures between males and females. Given the aim of the study to explore the factors contributing to changes in autonomic function, further analyses were conducted to compare those patients who showed an improvement in autonomic function and those who did not show such improvement. Two group (improver, non-improver) ANOVAs were conducted to explore differences in baseline between improvers and non-improvers in HRR1 and HRR2. The level of significance for all analyses was set at .05.

Results

Ninety-seven patients completed baseline ETT and 62 completed the second ETT assessment after the 3-month exercise programme. No differences in baseline variables were found between those who completed the second assessment (N = 62) and those who did not (N = 35) (data no reported). The most common reasons for drop out were medical reasons and being unable to contact for follow up assessment as indicated in Figure 1. Baseline characteristics of the 62 patients (42 female, mean age 55.5 ± 12.7 years) included in these analyses are reported in Table 1. Patients continued their course of treatment throughout the duration of the study. The average number of attended exercise sessions in the exercise facility was 13.4 ± 8.5 out of 24 (55.8 % attendance). Unfortunately, due to some practical complications, it was not possible to obtain full information about adherence and compliance to the exercise programme from the participants.

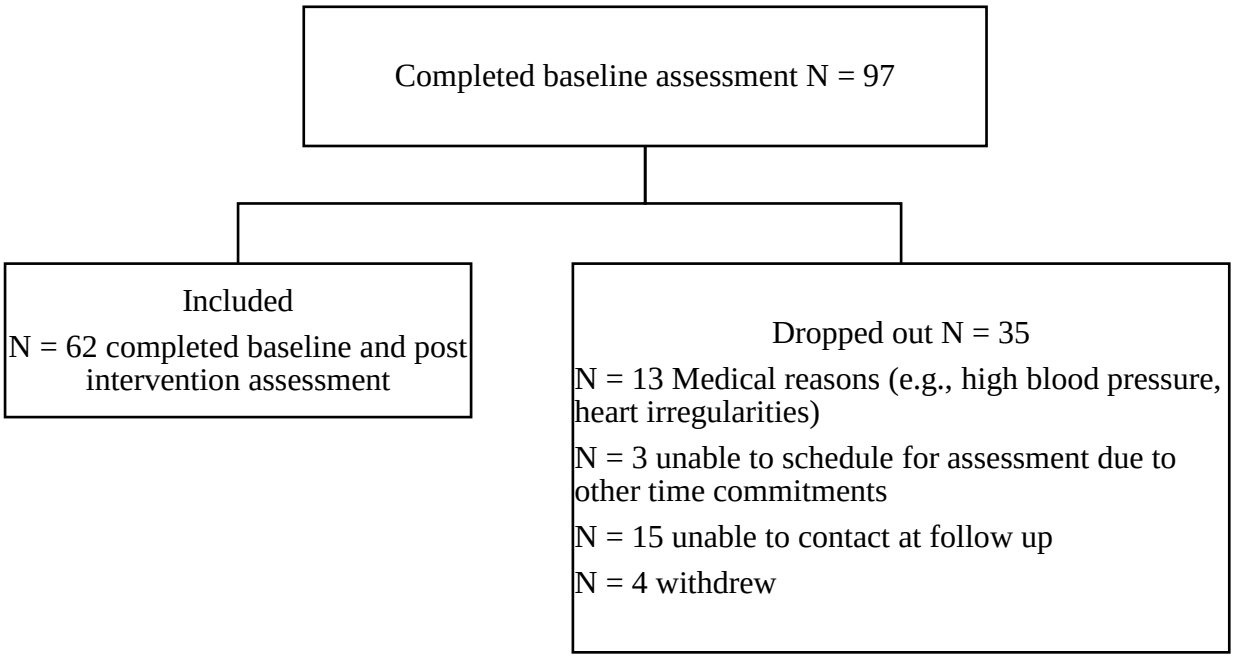

Figure 2. Flow diagram of the rheumatoid arthritis patients of the study

Table 1. Characteristics of rheumatoid arthritis patients at baseline and post intervention

Variable	Baseline	Post intervention	P value
Cardiovascular risk factors			
Weight (kg)	77.7 ± 16.3	78.4 ± 16.9	.60
BMI ($kg \cdot m^{-2}$)	27.5 ± 4.9	26.7 (24.4 – 31.2)	.45
Resting SBP (mmHg)	133 ± 17	128 ± 15	**.01**

Resting DBP (mmHg)	81 ± 10	78 ± 9.0	**.001**
Total Cholesterol (mmol.L^{-1})	5.1 ± 1.0	4.9 ± 1.0	.16
Triglycerides (mmol·L^{-1})	1.1 (0.8 – 1.4)	1.00 (0.8 – 1.5)	.83
HDL (mmol·L^{-1})	1.3 (1.1 – 1.)	1.4 ± 0.4	.69
LDL (mmol·L-1)	3.1 ± 0.8	3.0 ± 0.8	.50
HOMA	1.46 (1.1 – 2.1)	1.49 (1.1 – 2.3)	.09
Framingham Risk score	3.0 (1.0 – 8.5)	2.0 (1.0 – 6.8)	.51
Qrisk2	16.9 ± 13.6	15.1 ± 13.1	**.04**
Inflammatory markers			
WBC (10^9·L^{-1})	6.4 ± 2.3	6.2 ± 2.1	.33
Fibrinogen (g·L^{-1})	4.6 ± 0.8	4.6 ± 0.9	.84
hsCRP (mg·L^{-1})	3.8 (1.5 – 8.4)	3.8 (1.1 – 7.5)	.36
ESR (mm·hr^{-1})	10.5 (5.0 – 21.0)	12.0 (5.0 – 19.5)	.48
Wellbeing			
Vitality	4.0 (2.6 – 5.9)	4.8 (3.4 – 5.6)	**.02**
HADS anxiety	7.0 (5.0 – 10.0)	6.0 (3.3 – 8.0)	.15
HADS depression	4.0 (2.0 – 7.0)	3.3 (2.0 – 5.8)	.09
Global Fatigue	27.4 ± 10.8	24.2 (15.0 – 35.1)	.41
EuroQol	0.7 (0.6 – 0.8)	0.7 (0.6 – 0.8)	.08

Values are presented as means ± standard deviation, or median (25th to 75th percentile values) as appropriate. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; Qrisk, is an algorithm used to predict 10 years' probability of cardiovascular diseases; WBC, white blood cells; HsCRP, high sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; HOMA, homeostasis model assessment insulin resistance. HADS, hospital anxiety and depression scale EuroQol, European assessment for quality of life. *P* in bold indicate statistical significance.

Table 2. Heart rate recovery and VO_2 peak at baseline and post intervention.

Variable	Baseline	Post intervention	P value
Outcome measures			
HRR1 (bpm)	29.6 ± 11.4	27.8 ± 10.1	.31
HRR2 (bpm)	48.8 ± 12.1	49.4 ± 11.5	.67
VO2 peak (ml·kg^{-1}·min^{-1})	21.3 ± 4.5	21.7 ± 4.4	.17

Values are presented as means ± standard deviation. HRR1, the difference between HR peak and heart rate 1 minute post HR peak; HRR2, the difference between HR peak and heart rate 2 minute post HR peak. VO_2 peak, highest volume of oxygen.

Effects of Exercise on HRR and VO_2

HRR at baseline and post intervention are presented in Table 2. Results revealed no increase in HRR1 (F (1, 52) = 1.04, p = .31), HRR2 (F (1, 49) = .18, p = .67) and VO_2 (F (1, 54) = 1.93, p = .17) after the 3-month exercise programme.

Effects of exercise on CVD risk, inflammation, and measures of wellbeing

Baseline and post intervention values of the CVD risk factors, markers of inflammation and wellbeing are displayed in Table 1. Only SBP (F (1, 53) = 6.96, p = .01), DBP (F (1, 54) = 11.47, p = .001), and Qrisk2 (F (1, 51) = 4.52, p = .04) were significantly lower, and subjective vitality higher (F (1, 51) = 5.66, p = .02) post intervention compared to baseline.

Given that the aim of the study was to explore the effects of exercise on autonomic function and factors related to these changes, subsequent analyses were conducted to explore if there were differences at baseline between those who improved HRR following exercise (N=24 for HRR1, N=25 for HRR2) and those who did not (N=29 for HRR1, N=24 for HRR2). There was no difference in attendance between those who improved HRR and those who did not. As can be seen from Table 3 (HRR1) and Table 4 (HRR2), those who showed an improvement in HRR1 and HRR2 after the intervention had poorer baseline HRR than non-improvers. Additionally, patients who improved HRR1 had significantly lower HDL (F (51) = 6.97, p = .011), higher FRS (F (50) = 12.92, p = .001) and Qrisk2 scores (F (49) = 4.48, p = .039). Those who showed an improvement in HRR2, only had significantly higher resting DBP (F (47) = 5.54, p = .02).

Factors associated with heart rate recovery

Given the individual differences in changes in HRR1 and HRR2 after exercise intervention, further analyses were conducted to explore if changes in HRR were related to changes in other outcome measures. These analyses yielded no significant correlation between attendance and HRR1 or HRR2. Correlation analyses between changes in HRR1 and HRR2 and changes in other variables revealed no significant associations (data not reported). Additional analyses revealed no difference between males and females in changes from baseline to post intervention in any outcome measure (data not reported). Correlation analyses between age and change in scores revealed that age was positively associated with the difference in anxiety and fatigue (r (50) = .35, p = .01, and r (38) = .33, p = .04, respectively), and was inversely associated with change in scores in vitality and Qrisk2 (r (52) = -.29, p = .03, and r (52) = -.29, p = .04, respectively). Thus, being older was related to attenuated improvements in anxiety, fatigue, and vitality.

Table 3. Baseline characteristics of those who improved and those who did not improve HRR1

Variable	Improvers (N=24)	Non-improvers (N=29)	P value
Cardiovascular risk factors			
HRR1 (bpm)	22.3 ± 8.7	35.7 ± 9.6	**<.001**
HRR2 (bpm)	41.0 ± 9.8	55.0 ± 10.1	**<.001**
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	21.1 ± 3.9	21.7 ± 5.0	.59
Age (years)	58.7 ± 11.0	51.3 ± 13.4	**.04**
Resting SBP (mmHg)	134 ± 18	133 ± 17	.76
Resting DBP (mmHg)	82 ± 8	80 ± 11	.58
BMI ($kg \cdot m^2$)	27.3 ± 4.6	26.7 ± 4.7	.67
Total Cholesterol ($mmol \cdot L^{-1}$)	5.1 ± 1.2	5.0 ± 0.9	.78
Triglycerides ($mmol \cdot L^{-1}$)	1.2 (0.8 – 1.7)	1.0 ± 0.5	.06
HDL ($mmol \cdot L^{-1}$)	1.2 (1.1 – 1.5)	1.5 ± 0.3	**.01**
LDL ($mmol \cdot L^{-1}$)	3.2 ± 1.0	3.1 ± 0.7	.71
HOMA	1.5 (1.1 – 2.3)	1.4 (0.9 – 1.9)	.27
Framingham risk score	5.5 (2.3 – 13.5)	1 (1.0 – 3.0)	**.001**
Qrisk2	20.6 ± 13.9	6.7 (3.3 – 24.7)	**.04**
Inflammatory markers			
WBC ($10^9 \cdot L^{-1}$)	6.7 ± 2.1	5.8 ± 2.2	.14
Fibrinogen ($g \cdot L^{-1}$)	4.6 ± 0.9	4.5 ± 0.8	.66
hsCRP ($mg \cdot L^{-1}$)	5.0 ± 4.2	3.6 (1.2 – 6.7)	.58
ESR ($mm \cdot hr^{-1}$)	9.0 (5.0 – 15.0)	10.0 (5.5 – 15.8)	.76
Wellbeing			
Vitality	4.0 (2.6 – 6.0)	4.1 ± 1.5	.83
HADS Anxiety	6.9 ± 3.8	8.1 ± 3.9	.28
HADS Depression	5.9 ± 4.1	4.3 ± 2.6	.29
Global fatigue index	25.7 ± 11.8	29.5 ± 10.5	.19
EuroQol	0.7 ± 0.2	0.7 (0.6 – 0.8)	.72

Values are presented as means ± standard deviation, or median (25^{th} to 75^{th} percentile values) as appropriate. HRR1, the difference between HR peak and heart rate 1 minute post HR peak; HRR2, the difference between HR peak and heart rate 2 minute post HR peak.. VO_2 peak, highest volume of oxygen,; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA, homeostasis model assessment insulin resistance; Qrisk2, is an algorithm used to predict 10 years probability of cardiovascular diseases ;WBC, white blood cells; HsCRP, high sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate;. HADS, hospital anxiety and depression scale EuroQol, European assessment for quality of life. *P* in bold indicate statistical significance.

Table 4. Baseline characteristics of those who improved and those who did not improve HRR2

Variable	Improvers (N=25)	Non-improvers (N=24)	P value
Cardiovascular risk factors			
HRR1 (bpm)	26.4 ± 12.3	33.6 ± 9.9	**.03**
HRR2 (bpm)	43.9 ± 11.3	53.8 ± 11.1	**.003**
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$ **)**	21.4 ± 4.1	21.8 ± 5.2	.77

Age (years)	56.2 ± 10.6	52.3 ± 15.0	.30
Resting SBP (mmHg)	132 ± 17	133 ± 17	.81
Resting DBP (mmHg)	84 ± 10	77 ± 9	**.02**
BMI ($kg \cdot m^{-2}$)	26.7 ± 4.8	27.5 ± 4.8	.58
Total Cholesterol ($mmol \cdot L^{-1}$)	5.2 ± 1.2	4.9 ± 0.9	.44
Triglycerides ($mmol \cdot L^{-1}$)	1.0 (0.8 – 1.4)	1.1 (0.6 – 1.3)	.47
HDL ($mmol \cdot L^{-1}$)	1.4 ± 0.3	1.5 ± 0.3	.30
LDL($mmol \cdot L^{-1}$)	3.3 ± 1.0	3.0 ± 0.7	.34
HOMA	1.0 (0.9 – 2.0)	1.4 (1.1 – 2.2)	.98
Framingham risk score	3.5 (1.0 – 11.5)	1.0 (1.0 – 4.0)	**.**06
Qrisk2	16.3 ± 11.2	7.9 (1.6 – 24.9)	.70
Inflammatory markers			
WBC ($10^9 \cdot L^{-1}$)	6.1 ± 2.2	6.1 ± 2.1	.99
Fibrinogen ($g \cdot L^{-1}$)	4.4 ± 0.9	4.6 ± 0.9	.37
hsCRP ($mg \cdot L^{-1}$)	3.0 (1.2 – 6.3)	3.6 (1.2 – 7.9)	.77
ESR ($mm \cdot hr^{-1}$)	9 (2.0 – 13.0)	11 (7.0 – 21.0)	.19
Wellbeing			
Vitality	4.2 ± 1.6	4.1 ± 1.4	.93
HADs A	7.3 ± 4.1	7.7 ± 3.8	.63
HADS D	4 (2.0 – 9.0)	4.5 ± 3.0	.97
Global fatigue index	24.6 ± 12.1	29.8 ± 9.5	.07
EuroqOol	0.7 (0.5 – 0.8)	0.7 (0.6 – 0.8)	.51

Values are presented as means ± standard deviation, or median (25^{th} to 75^{th} percentile values) as appropriate. HRR1, the difference between HR peak and heart rate 1 minute post HR peak; HRR2, the difference between HR peak and heart rate 2 minute post HR peak.. VO_2 peak, highest volume of oxygen,; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; HOMA, homeostasis model assessment insulin resistance; Qrisk2, is an algorithm used to predict 10 years probability of cardiovascular diseases ;WBC, white blood cells; HsCRP, high sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate;. HADS, hospital anxiety and depression scale EuroQol, European assessment for quality of life. *P* in bold indicate statistical significance.

Discussion

This study explored the effect of a 3-month semi-supervised exercise intervention on parasympathetic function in patients with RA, using HRR as a measure of parasympathetic activity. HRR and VO_2 peak did not improve after the exercise intervention. The exercise intervention did not influence the majority of the CVD risk factors, or measures of wellbeing, except for blood pressure, Qrisk2, and vitality, which were all improved post intervention. None of the inflammatory markers were changed by the exercise intervention. Additional analyses revealed that those who improved HRR, had lower baseline HRR, HDL, global CVD risk, resting diastolic blood pressure and were older.

To our knowledge, only two studies investigated the effects of exercise training on autonomic function in patients with RA. Ten weeks of high intensity exercise training induced an

improvement in HRR1 (24) and three months of moderate intensity exercise induced an improvement in heart rate variability (HRV) (23). There are notable differences between these studies and the current one which could perhaps explain the different findings. Previous studies used a supervised exercise setting in a relatively smaller sample of women with RA (23;24), whereas, the current study employed a semi-supervised setting in a larger group of men and women with RA. There have also been variations in the intensity of the exercise programme, with a decrease in HRR seen following a high-intensity programme (24), which is in line with the suggestion that the intensity of exercise during an intervention may influence HRR improvements (36). It is noteworthy to mention that an improvement in VO_2 peak was gained in Sandstad et al study (24), whereas, in the current study no improvement in VO_2 was found. Even though not confirmed in all studies (37), the positive effects of exercise training on autonomic function are commonly found to be accompanied with an improvement in fitness in other clinical populations such as patients with congestive heart failure (38) and patients with coronary artery disease (39). In patients with RA, a previous study found a significant improvement in VO_2 peak and many CVD risks, and disease related risk factors after six months individualized aerobic and resistance high intensity exercise training (40). More importantly, it was found that the change in VO_2 was the strongest predictor for improvement in the assessed CVD risk factors, and disease related risk factors. Thus, it can be speculated that the differences between the programmes in patients with RA with regards to intensity and compliance to the intensity, and the level of supervision (supervised and semi-supervised) have contributed to the lack of improvements in VO_2 peak, which on its turn resulted in a lack of improvement in HRR. It is important to note that prescribed exercise intensity in the current study was the same as a previous exercise study in patients with RA (40). However, there were notable differences between the level of supervision in the current study and the previous study. In the previous study, all participants had two exercise instructors who were dedicated to the project. In the current study, participants were supervised in a community-based leisure centre. It is likely that this difference in supervision and attention, has had an impact on the compliance with the exercise programme. However, without data regarding adherence and compliance, this remains speculative.

It is not just the exercise programme which is different between the current study and the previous work, there are also differences in the patient characteristics which could contribute the different findings. Apart from the inclusion of males, the most obvious difference is the age of the participants. The participants in the current study were older (55.5 ± 12.7 years)

compared to the previous studies (46.81 ± 9.23 years) (23) and (32.4 ± 8.3 years) (24). The associations between age and the effectiveness of the intervention are interesting. Those who showed an improvement in HRR were older, but age was also related to attenuated benefits in anxiety, fatigue, vitality and global CVD risk. Therefore, the associations between age and the effects of an exercise programme warrant further examination.

Additional analyses comparing improvers and non-improvers in HRR revealed baseline differences between the two groups, i.e. poorer HRR, lower HDL, higher FRS, Qrisk2, and blood pressure. This could indicate that those with poorer parasympathetic function and CVD risk at baseline are more likely to benefit from the exercise intervention. In concordance with the current study, Yayali et al. (41), reported improvement in HRR1 only in patients with poor abnormal baseline HRR (<12 bpm). It is also worth noting that baseline HRR in the current population was similar to that reported in healthy populations (42;43). This may suggest that patients with poorer clinical characteristics could benefit more from therapeutic interventions.

The exercise programme was successful in improving blood pressure, which is in line with healthy normotensive, hypertensive (44) and clinical populations (45), as well as patients with RA (40). Hypertension is common in RA and has been implicated as a factor contributing to the increased risk for CVD (3). Therefore, interventions which can reduce blood pressure are needed. In a population who already takes a mix of medications, a behavioral intervention like exercise could be an appealing alternative to treating high blood pressure. A possible mechanism underlying reduction of blood pressure post exercise training is through decreased total peripheral resistance of the vascular system, which is mediated by a reduction in both sympathetic nervous system and renin-angiotensin II production (46). Future studies are needed to tests these hypotheses.

The current study explored the effect of exercise intervention on two CVD risk algorithms (FRS and Qrisk2) in patients with RA, and only Qrisk2 was significantly improved after the exercise intervention. Both of these algorithms predict the 10 year probability of CVD events, and both incorporate age, sex as well as individual CVD risk factors like blood pressure, smoking, cholesterol, and HDL levels. Qrisk2 also incorporates other comorbidities such as diabetes, history of angina in relatives, chronic kidney diseases, atrial fibrillation, and RA in the calculation of global CVD risk. Thus, it can be argued that Qrisk2 is more suitable as a tool of measuring the risk of CVD in RA. Surprisingly, to our knowledge, no study has explored the effect of exercise on Qrisk2. FRS is more commonly used, and a reduction in FRS has been

reported after exercise training in other clinical populations (47), but not in all studies (48). The current study suggests that the use of CVD risk algorithm, in particular Qrisk2, may be a useful tool to assess the impact of an intervention on reduction of CVD risk in patients with RA.

As expected, the exercise intervention caused an improvement in subjective vitality. Vitality has been consistently reported to improve after exercise training in people with medical conditions (49) and in cancer patients (50;51). The current study suggests that an exercise programme can impact on positive psychological wellbeing, even without inducing a significant increase in cardiorespiratory fitness.

The current study is not without limitations. The power calculation for the study was done for the main outcome of the initial study which is VO_2 peak, therefore, it is unknown whether the sample was adequate to investigate HRR. Another limitation is the absence of a control or a comparison group. However, given the known benefits of exercise, it was deemed unethical to have a no-exercise control group. It should also be acknowledged that our sample consisted of well-controlled RA patients, which makes it difficult to generalize the results of this study to RA patients with more severe disease activity. Unfortunately, this limitation is not restricted to this exercise intervention, as with many exercise intervention, only those participants with controlled disease activity tend to participate. Therefore, it would be interesting to explore suitable programmes for patients with more disease activity. Furthermore, DAS28 was not available for all the participants, therefore, it was not included in the analyses to investigate the effect of the exercise intervention on DAS28.

In conclusion, the results of this study showed that a 3-month exercise programme was not sufficient to produce positive changes on parasympathetic activity in patients with RA, but did improve blood pressure and subjective vitality. Given the differences in the characteristics between those who improved and those who did not improve, future research should explore the impact of these factors on the effectiveness of an intervention to improve autonomic function in patients with RA.

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CHAPTER 5

Inflammation and parasympathetic activity: a comparison between patients with rheumatoid arthritis and diabetes mellitus: a cross-sectional study

Abstract

Introduction Rheumatoid arthritis (RA) and diabetes mellitus (DM) share relatively similar risk factors that may contribute to increased risk of cardiovascular disease (CVD), but differ with regards to the levels of inflammation. Autonomic dysfunction implicated by increased sympathetic activity and reduced parasympathetic activity contribute to the risk of CVD. Poor parasympathetic activity, measured by heart rate recovery (HRR) associates with CVD. Given that inflammation contribute to the development of CVD and it associates with parasympathetic activity, the aim of this study was to compare parasympathetic activity using HRR in RA and DM.

Methods 26 DM patients (60.8 ± 10.4 years, 38.5% female) were matched 1:2 for age and sex with 46 RA patients (59.8 ± 9.6 years, 39.5% female) who completed a treadmill exercise tolerance test (ETT), during which heart rate (HR) was monitored. The parasympathetic function was measured at one minute post peak exercise (HHR1) and two minutes post peak exercise (HHR2). Parasympathetic activity (HRR), cardiorespiratory fitness (VO_2 peak), markers of inflammation, CVD risk factors, 10 years CVD risk (Qrisk2), and measures of wellbeing (via questionnaires), were compared between RA and DM patients.

Results ANOVA revealed no differences in HRR1 (p=.79), HRR2 (p=.83), and in any of the inflammatory markers (p's>.05) between RA and DM patients. Total cholesterol (p=.05) and high density lipoprotein (p=.02) were higher in RA, whereas, weight (p=.04), BMI (p=.001), HOMA (p=.001), and overall CVD risk measured by Qrisk (p<.001) were significantly higher in DM patients. Patients with RA reported lower levels of wellbeing including vitality (p=.005), and quality of life (p<.001), and higher depression (p=.02), and greater fatigue (p<.001). With the two groups combined, multivariate linear regression revealed that VO_2 peak was an independent predictor for HRR1 and HRR2.

Conclusion Although, parasympathetic activity and inflammatory markers were similar between RA and DM patients, DM patients had some higher CVD risks factors, whereas, RA patients had poorer measures of wellbeing. Cardiorespiratory fitness was an important factor related to parasympathetic activity in both RA and DM patients.

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by increased systemic inflammation (1). There is increasing evidence that patients with RA are at risk of developing CVD, with more than 40% of patients dying due to CVD (2-4). The reason for this increased CVD risk is currently unknown. Several CVD risk factor, such as hypertension (5), dyslipidaemia (6), lack of physical activity (7), low cardiorespiratory fitness (8), and systemic inflammation (9) are prevalent in patients with RA and are likely to contribute to the increased risk for CVD mortality. More recently, autonomic dysfunction, which is another potential CVD risk factor, has been shown to be prevalent in RA patients (10).

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated levels of blood glucose (hyperglycaemia) and disturbances of fat, protein and carbohydrate metabolism (11). Patients with DM have an increased risk of CVD (12), which accounts for 60% of deaths in this population (13). Similar to RA, a cluster of CVD risk factors including hypertension, insulin resistance, dyslipidaemia, and obesity (14) are prevalent in patients with DM. Interestingly, low grade inflammation was found to be involved in the pathogenesis of DM (15), which indicate presence of subclinical inflammation in this population. In addition, autonomic dysfunction, which is associated with inflammation in patients with DM (16), is also prevalent and contribute to the increased risk of CVD in this population (17;18).

Autonomic nervous system (ANS) is part of the central nervous system. ANS consist of two branches, the sympathetic nervous system and the parasympathetic nervous system, which play a major role in regulatory processes amongst multiple physiological systems (19). For example, there is ample evidence that ANS can mediate the interaction between the brain and the immune system (19-21). The activity of both the sympathetic and the parasympathetic nervous system is influenced by levels of inflammatory cytokines, such as interleukin-1, interleukin-6, and tumour necrosis factor-α in the blood (19). In the case of chronic inflammation, there is constant increased activity of the sympathetic nervous system with inadequate regulation of the immune cells (22). Autonomic dysfunction is implicated when there is increased activity of the sympathetic and less activity from the parasympathetic nervous system. Inflammation associates with autonomic dysfunction not only in conditions with chronic inflammation such as RA (23) or patients with diabetes mellitus (DM) (24) but also in healthy population (25). In patients with RA, an inverse association has been identified between various inflammatory markers and measures of ANS, such as heart rate recovery (26).

There are many non-invasive methods to assess autonomic function. Heart rate recovery (HRR) following an exercise tolerance test (ETT) is a method to assess parasympathetic activity that can be easily incorporated in clinical practice. The parasympathetic activity is represented by the immediate fall in heart rate following an ETT (27). Epidemiological studies have shown that HRR strongly associates with CVD and all-cause mortality (27;28). HRR measured after one (HRR1) or two minutes (HRR2) post ETT are the most validated and commonly used in research (29), with HRR1 reflecting parasympathetic reactivation and HRR2 reflecting further parasympathetic reactivation as well as sympathetic withdrawal (30).

We have shown that parasympathetic activity using HRR in patients with RA was associated with the overall burden of inflammation, CVD risk factors, and wellbeing (26). To explore more specific associations of inflammation with parasympathetic activity, it would be interesting to compare two groups who are similar with regards to CVD risk profile, but differ with regards to inflammation. This allows for an examination of the additional impact of inflammation on the parasympathetic activity. The majority of CVD risk factors are relatively similar between patients with RA and patients with DM including systolic and diastolic blood pressure, however, some factors are different including BMI, total cholesterol and the level of inflammation which is higher in RA (31;32). Although, autonomic dysfunction is prevalent in RA patients (33 to 86%) (10) as well as in DM patients (20 to 80%) (33) and contributes to increased CVD risk in both populations, to our knowledge, only one study has compared autonomic function between these populations. This study compared autonomic function using cardiovascular reflex tests (e.g. orthostatic stress test, valsalva manoeuvre, and deep breathing) in females of 34 RA, 76 DM (including both type 1 and type 2), and 67 healthy controls (34). This study revealed that responses in valsalva manoeuvre and deep breathing test (both tests reflect the activity of the parasympathetic nervous system) were significantly poorer in the DM group more than in the RA group. Unfortunately, in thisstudy, no information was provided about the impact of inflammation on these findings. Therefore, the main aim of this study was to compare parasympathetic activity using HRR following an exercise tolerance in patients with RA with age- and sex-matched patients with type 2 DM/pre-diabetes. A second aim was to compare inflammatory markers as well as other CVD risk factors and measures of wellbeing between these two groups. A third aim was to investigate the factors associated with parasympathetic activity in both groups. The study provides comparison between two groups with similar CVD risk but

differences in inflammation. It was hypothesized that HRR will be poorer in patients with RA and inflammation will be higher in RA patients.

Methods

Study population

Twenty-six patients with type 2 DM (HbA1C > or = 48 mmol·mol^{-1}, and fasting glucose > 7.0 mmol) or pre-diabetes (HbA1C 42-47 mmol·mol^{-1} or fasting glucose 6.1-7.0) recruited from diabetes educational classes at Brierley Hill Health and Social Care Centre, Dudley, UK. The 26 DM patients were matched in 1:2 ratio for age (within five years range) and sex with 46 patients with RA (meeting the revised American College of Rheumatology criteria (35)) from an existing cohort of RA patients (N=97). Patients in this cohort were recruited from Rheumatology outpatient clinics of the Dudley Group NHS Foundation Trust and they were on stable medications as they were about to participate in an exercise intervention study (Trial registration number: ISRCTN04121489). The data presented in this chapter relate to the baseline assessment of this cohort. Patients with a comorbidity incompatible with exercise as per American College of Sports Medicine (ACSM) guidelines (36), atrial fibrillation, current flare of disease in RA, and established CVD were excluded. Established DM for RA patients and established RA with DM patients were additional exclusion criteria. The study was approved by National Research Ethics Committee (IRA ID: 169234, Ref: 15/EM/0138) and all patients provided written informed consent prior to participation.

Protocol

Participants were invited to visit the research laboratory on two different occasions. During visit one, height was measured to the nearest 0.5 cm using a standard height measure (Seca 214 Road Rod), weight and BMI were measured using a Tanita BC 418 MA Segmental Body Composition Analyser (Tanita Corporation, Tokyo, Japan), brachial blood pressure was taken using electronic sphygmomanometer (Datascope Accutor, Mahwah, NJ, USA) while the patient was seated a fasted blood sample was taken. On the second visit, both patient groups (RA and DM/pre-diabetes) undertook an exercise tolerance test following the same protocol. An appropriate mask was fitted to the patient covering the nose and mouth for analysis of inspired and expired gases, and ECG (12-channel ECG custo cardio 200, custo med, Liepzig,

Germany) electrodes were attached. Two minutes' baseline measurement was used to measure resting heart rate and O_2 consumption while seated, followed by an exercise tolerance test (ETT), and six minutes' post ETT recovery period.

Exercise Tolerance Test (ETT)

Before commencing ETT, a mask was fitted and 12 leads ECG were attached to the patient. ETT was performed on a treadmill (HP Cosmos Mercury, Nussdoerf-Traunstien, Germany). A minimum of two minutes was given to the patient for familiarization purposes before commencing the individualized ramp protocol test, which was modified according to the patient's fitness and physical abilities (37); an approach that we have previously used in RA patients (38). During this familiarization period, the speed was set at the patient's preference (approximately three mph) with 0% inclination for two minutes. Thereafter, the speed of treadmill was increased up to the level of maximum brisk walking based on patient's ability. Then, the speed was set to be constant (maximum brisk walking) throughout the test, while increasing the inclination by 1% every minute. Peak oxygen consumption (VO_2 peak) was calculated using breath by breath gas analyses (Metalyzer 3B, Cortex, Liepzig, Germany). Heart rate was recorded before the commencement, throughout the ETT and during recovery period using ECG. If volitional exhaustion was reached during ETT, or any of the relative or absolute contraindication of ACSM's guidelines were met (36), the test was immediately terminated. Upon termination of the test, the patient was seated on the chair for up to six minutes as a recovery period. All the tests were supervised by a cardiac physiologist who monitored the relative and absolute contraindications. The cardiac physiologist was blinded from the aim of the study.

Outcome measures

Heart rate recovery

The rapid fall in heart rate (HRR) after peak exercise was measured at two time points. Heart rate recovery one minute (HRR1) was defined as the absolute difference between peak heart rate and one minute post ETT heart rate (HRR1 = peak heart rate – one minute post peak heart rate). Similarly, heart rate recovery two minutes (HRR2) was defined as the absolute difference between peak heart rate and two minutes' post ETT heart rate. (HRR2 = peak heart rate – two

minutes post peak heart rate). Peak heart rate was defined as the maximum heart rate reached during ETT.

VO_2 peak

Peak aerobic capacity (VO_2 peak) was measured via calibrated breath by breath gas analyser during treadmill ETT. Inspired and expired gas data were averaged every two seconds. VO_2 readings data were further smoothened by taking the average of VO_2 every 28 seconds (taking the average of 14 readings of VO_2 $ml \cdot min^{-1}$). VO_2 peak was defined as the highest VO_2 during the test and was expressed as $ml \cdot kg^{-1} \cdot min^{-1}$.

Serological assessments

Blood samples were analysed for erythrocyte sedimentation rate (ESR), high sensitivity C-reactive protein (hsCRP), fibrinogen, and white blood cells (WBC) as markers of inflammation. Total cholesterol, triglycerides, high density lipoprotein (HDL), and low density lipoprotein (LDL) were analysed and categorised as risk factors for CVD. Insulin resistance was analysed using homoeostasis models assessment (HOMA) (39).

Global cardiovascular risk

Qrisk2 score was used to measure the probability of developing cardiovascular events within 10 years period via entering the data online into the main website (40). Data required for the calculation of the score include age, sex, smoking status, diabetes diagnosis, diagnosis of angina or heart attack in first degree relative <60, chronic kidney disease (stage 4 or 5) diagnosis, atrial fibrillation diagnosis, rheumatoid arthritis diagnosis, cholesterol/HDL ratio, systolic blood pressure, and BMI.

Measures of wellbeing

Hospital anxiety and depression scale (HADS) (high score is indicative of worse anxiety or depressive symptoms) (41), subjective vitality scale (high score is indicative of better vitality) (42), multidimensional assessment of fatigue (MAF) (high score is indicative of greater levels of fatigue) (43), and the European quality of life questionnaire (EuroQol) (low score is

indicative of poor quality of life) (44) were used to assess psychological wellbeing and quality of life by questionnaire.

Statistical analysis

Statistical analyses were performed using SPSS20 (Chicago, IL, USA). Kolmogorov-Smirnov test was used to test variables for normality. Normally distributed variables were presented as means and standard deviation. Skewed variables (weight, BMI, total cholesterol, triglycerides, HOMA, HDL, ESR, hsCRP, HADS depression, and EuroQol) were log-transformed and presented as median and interquartile range. The comparison between the two populations was performed using analysis of variance (ANOVA). Bivariate Pearsons moment correlation (correcting for patient group, and sex) for normally distributed and log-transformed variables were used to assess the relationship between HRR1, HRR2 separately and other variables. To identify independent factors associated with HRR at both time points in both groups joined together, two models were analysed using linear regression (using enter method) in which patient group, and sex were entered in the first block. Thereafter, another block included only the significantly correlated variables in the univariate analyses as an independent variable and HRR1 and HRR2 as dependent variables, separately. The level of significance for all analyses was set at .05.

Results

Patient characteristics

26 DM patients [(60.8 ± 10.4 years, 38.5% female) type 2 DM or pre- DM] were matched with 46 RA patients based on age and sex. Three RA patients were excluded due to presence of DM, thus, the final RA group consisted of (N=43) (59 ± 9.6 years, 39.5% female). The demographic characteristics of the two groups are reported in Table 1. There was no difference in HRR, CVD risk factors, inflammatory markers or measures of wellbeing between the matched (N=43) RA and the remainder of the cohort of RA patients from the exercise intervention study (N=54) (data not reported).

CVD risk factors, inflammation, and measures of wellbeing of all participants, and the comparison between the two groups are reported in Table 1. Weight (F (1, 67) = 4.39, p = .04), BMI (F (1, 67) = 13.03, p = .001), HOMA (F (1, 66) = 12.61, p = .001) and Qrisk2 (F (1, 66)

= 40.03, p = <.001) were significantly higher in patients with DM, whereas total cholesterol (F (1, 65) = 4.19, p = .05) and HDL (F (1, 65) = 5.96, p = .02) were higher in patients with RA. Vitality (F (61, 1) = 8.60, p = .005) and EuroQol (F (1, 64) = 5.78, p = .02) were significantly poorer in patients with RA, HADS depression (F (1, 63) = 5.41, p = .02), global fatigue index (F (1, 61) = 47.01, p = <.001) were significantly higher in patients with RA. No differences were observed in inflammatory markers between the two groups.

Table 1. Demographic characteristics, CVD risk, inflammatory markers, and measures of wellbeing of RA and DM patients.

Variable	RA (N=43)	DM (N=26)	P value
Cardiovascular risk factors			
Age (years)	59.2 ± 9.6	60.8 ± 10.4	.65
Sex, F (%)	17 (39.5)	10 (38.5)	.57
Height (m)	1.7 ± 0.1	1.6 ± 0.1	.11
Weight (kg)	77.1 (70.4 – 93.9)	91.9 (70.8 – 103.7)	**.04**
BMI ($kg \cdot m^{-2}$)	27.3 (23.8 – 30.3)	30.8 (29.1 – 36.7)	**.001**
Heart rate rest (bpm)	79 ± 14	71 ± 12	**.05**
Resting SBP (mmHg)	137 ± 18	132 ± 16	.26
Resting DBP (mmHg)	82 ± 10	80 ± 10	.34
Total cholesterol ($mmol \cdot L^{-1}$)	4.9 (4.4 – 6.0)	4.4 (3.9 – 5.3)	**.05**
Triglycerides ($mmol \cdot L^{-1}$)	1.2 (0.8 – 1.9)	1.4 (0.9 – 1.9)	.89
HDL ($mmol \cdot L^{-1}$)	1.4 (1.1 – 1.6)	1.2 (1.0 – 1.3)	**.02**
HOMA	1.5 (1.0 – 2.3)	2.3 (1.5 – 4.5)	**.001**
Qrisk2	20.2 ± 12.7	38.7 ± 10.9	**<.001**
Inflammatory markers			
WBC ($10^9 \cdot L^{-1}$)	6.9 ± 2.1	6.3 ± 1.8	.20
Fibrinogen ($g \cdot L^{-1}$)	4.8 ± 1.1	4.5 ± 0.8	.39
hsCRP ($mg \cdot L^{-1}$)	5.4 (1.9 – 10.5)	3.1 (1.4 – 7.4)	.18
ESR ($mm \cdot hr^{-1}$)	11 (5.0 – 18.8)	9.5 (5.0 – 19.8)	.89
Wellbeing			
Vitality	3.9 ± 1.5	4.9 ± 1.3	**.005**
HADS anxiety	6.5 ± 3.7	5.1 ± 4.1	.16
HADS depression	4.5 (2.6 – 7.0)	3.0 (1.0 – 6.0)	**.02**
Global Fatigue	27.6 ± 11.1	9.9 ± 7.1	**<.001**
EuroQol	0.7 (0.6 – 0.8)	0.8 (0.7 – 0.8)	**.02**

Values are presented as means ± standard deviation, or median (25^{th} to 75^{th} percentile values) as appropriate. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; Qrisk2, is an algorithm used to predict 10 years probability of cardiovascular diseases;WBC, white blood cells; HsCRP, high sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; HOMA, homeostasis model assessment insulin resistance; Vitality, score ranges 1 (low vitality) – 7 (high vitality); HADS, hospital anxiety and depression scale (0-21); Global fatigue, score ranges from 0 (no fatigue) – 50 (severe fatigue) EuroQol, European assessment for quality of life scores range between 0 (worst quality of life) to 1 (best quality of life)

Table 2. HRR and exercise-related variables in RA and DM patients.

Variable	**RA (N=43)**	**DM (N=26)**	**P value**
Heart Rate Peak (bpm)	147 ± 20	145 ± 19	.69
VO_2 peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	21.1 ± 4.8	22.3 ± 4.5	.35
HRR1 (bpm)	24 ± 11	24 ± 7	.79
HRR2 (bpm)	42 ± 13	42 ± 9	.83

Values are presented as means ± standard deviation, or median (25^{th} to 75^{th} percentile values) as appropriate. LF, low frequency; HF, high frequency; VO_2 peak, highest volume of oxygen; HRR1, the difference between HR peak and heart rate 1 minute post HR peak; HRR2, the difference between HR peak and heart rate 2 minute post HR peak.

Comparison in parasympathetic function between RA and DM patients

No differences between RA and DM were observed in HRR1 (F (1, 64) = 0.07, p = .79) or HRR2 (F (1, 64) = 0.05, p = .83) following ETT, as presented in Table 2. In addition, overall fitness was similar in both groups as indicated by VO_2 peak (F (1, 64) = 0.88, p = .35).

Factors associated with HRR

Correlational analyses were used to assess the factors associated with both measures of HRR including HRR1 and HRR2 with adjustments for patient group and sex. Age was inversely associated only with HRR2 (r (62) = -.28, p = .03). VO_2 peak was associated with HRR1 and HRR2 [(r (62) = .31, p = .01), (r (62) = .41, p = .001) respectively]. Thus, older patients had poorer HRR, and better levels of fitness were related to better HRR.

To identify independent associations between variables and HRR as well as investigating if the patient group associates with HRR, significantly correlated variables with HRR were entered in linear regression analyses (see Table 4). Analyses revealed that a significant model which included patient group and sex as a first entry then VO_2 peak as independent variables and HRR1 as a dependent variable accounted for 9.5% of the variation of HRR1 (F (62, 1) = 6.53, p = .01, R^2 = .09). VO_2 peak was observed as an independent variable for the variation in HRR1 in this model (see Table 4). A significant model for HRR2 which included patient group and sex as independent variables, then age and VO_2 peak as independent variables and HRR2 as a dependent variable accounted for 19.3% of the variation of HRR2 (F (61, 2) = 7.74, p = .001, R^2 = .19). In HRR2 model, sex and VO_2 peak were the independent variable for the variation in HRR2 in this model (p = .005, p = .003, respectively) (see Table 4).

Table 3. Factors associated with HRR1 an HRR2 in RA and DM patients

Variable	**HRR1**		**HRR2**	
	r	***p***	***r***	***p***
Cardiovascular risk factors				
Age	-0.22	.08	-0.28	**.03**
Weight	0.12	.34	0.09	.49
BMI	0.01	.96	0.02	.87
Resting SBP	-0.10	.43	-0.12	.34
Resting DBP	-0.12	.35	-0.13	.33
VO_2 peak	0.31	**.01**	0.41	**.001**
Total cholesterol	0.08	.54	0.23	.07
Triglycerides	-0.11	.42	-0.72	.58
HDL	-0.04	.74	-0.06	.64
HOMA	-0.10	.42	-0.03	.82
Qrisk2	-0.22	.08	-0.19	.13
Inflammatory markers				
WBC	-0.11	.38	-0.07	.57
Fibrinogen	-0.22	.09	-0.23	.07
hsCRP	0.05	.71	0.004	.97
ESR	-0.02	.87	-0.02	.89
Wellbeing				
Vitality	0.24	.07	0.21	.11
HADS anxiety	-0.19	.13	-0.04	.75
HADS depression	-0.07	.61	0.04	.75
Global fatigue index	0.04	.78	0.12	.37
EuroQol	-0.16	.22	-0.00	.99

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; VO_2 peak, highest volume of oxygen; HDL, high density lipoprotein; WBC, white blood cells; HsCRP, high sensitivity C-reactive protein; ESR, erythrocyte sedimentation rate; HOMA, homeostasis model assessment insulin resistance; Qrisk2, is an algorithm used to predict 10 years probability of cardiovascular diseases; Vitality, score ranges 1 (low vitality) – 7 (high vitality); HADS, hospital anxiety and depression scale (0-21); Global fatigue, score ranges from 0 (no fatigue) – 50 (severe fatigue), EuroQol, European assessment for quality of life

Table 4. Linear regression model for factors associated with HRR1 and HRR2

Variable	**HRR1**		**HRR2**	
	β	**t (*p*)**	**β**	**t (*p*)**
Group	-1.27	-0.51 (.61)	-0.02	-0.01 (.99)
Sex	4.04	1.54 (.13)	8.77	2.94 (**.005**)
Age			-0.24	-1.74 (.09)
VO_2 peak	0.71	2.56 (**.01**)	0.99	3.12 (**.003**)
R^2 and p-value of the model	R^2	*P*	R^2	*p*
	0.09	**.01**	0.19	**.001**

Discussion

This study showed that parasympathetic function, assessed by post exercise HRR, was similar in patients with RA and patients with DM. Level of inflammation in RA patients was not significantly different from DM patients. CVD risk factors varied and were slightly higher in DM patients, but measures of wellbeing were poorer in RA. Multivariate analyses revealed that cardiorespiratory fitness was the only factor which was independent of other variables associated with HRR1, fitness and age were the independent predictors of HRR2.

HRR is a simple, valid method to assess the ANS, and in particular parasympathetic activity (29). To our knowledge, this is the first study to compare parasympathetic activity between patients with RA and DM using HRR. Contrary to expectations, HRR was not different between RA patients and DM patients. The only other study to compare autonomic function in patients with RA and DM showed that compared to RA patients, DM patients had a poorer response to the valsalva manoeuvre and deep breathing test, which both reflect parasympathetic function (34). In the current study, we had type 2 DM or pre- DM patients, whereas, in the previous study, both type 1 and type 2 DM patients were included. Given that autonomic function has been reported to be poorer in type 1 DM (45) this could explain the different findings. Another notable difference between the current study and the previous study is the method of assessment. We assessed parasympathetic function following ETT, but the previous study used the valsalva and deep breathing test. This means that the conditions of the assessment are different with regards to the cardiovascular activation, as valsalva maneuverer and deep breathing test reflect the parasympathetic activity while resting whereas HRR reflects the parasympathetic reactivation after an exercise bout. However, in the absence of relevant studies, this remains a speculation.

Contrary to our expectations, inflammation was not significantly different between RA and DM. In contrast, a significant difference in inflammation between RA and DM patients using CRP was reported in some studies (31;32). One of the studies had larger populations of 294 RA patients and 194 DM patients (31), whereas, in the other study, the number of RA patients (48 RA) was similar to the sample size in the current study, but the number of DM patients was larger (48 DM) (32). Another similarity between the latter study and the current study is that patients were matched based on age and sex. An important difference perhaps is that the participants in the current study were recruited for an exercise trial. Therefore, a possible reason is that patients with RA in the current study had well-controlled disease activity as these RA patients were selected from a trial involving participation in an exercise intervention study.

Cardiorespiratory fitness was the only outcome measure which was independently associated with both HHR1 and HHR2. The association between cardiorespiratory fitness and parasympathetic activity is well established in both healthy and clinical populations (46), and this association was also apparent in older men (47). This moderate association between cardiorespiratory fitness can be attributed to the improved baroreflex sensitivity (46). The baroreflex sensitivity has an important role in response to the increased blood pressure, which occurs during ETT. Increased stimulation from the baroreceptors via the vagus nerve to the cardioregulatory and vasomotor centres in the medulla oblongata (origin of the parasympathetic nervous system) causes stimulation of the parasympathetic nervous system, which decreases the heart rate (48). Thus, the better the cardiorespiratory fitness, the better parasympathetic modulation of the heart after exercise. Given the association between cardiorespiratory fitness indicated by VO_2 peak and HRR, additional analyses were made to identify independently associated variables as well as to explore if patients group was a predictor for HRR. The results indicated that cardiorespiratory fitness was an independent predictor for HRR1 and HRR2, however, sex had an effect only in HRR2 and was a significant predictor for HRR2.

This difference in the variables associated with HRR1 and HRR2 has been reported (49). . This difference can be due to the difference in information reflected by HRR1, which reflects parasympathetic reactivation whereas HRR2 reflects parasympathetic reactivation in combination with sympathetic withdrawal (30).

The number of studies comparing ANS, inflammation, and CVD risks between RA and DM as two separate conditions is very limited. To our knowledge, only three studies compared RA and DM patients including one study that compared ANS (34), and two studies that compared inflammation and CVD risks only between RA and DM patients (31;32). In line with previous research, we reported similar CVD risk factors in patients with RA and DM. For example, Stamatelopoulos et al. (32) also reported lower weight and BMI and higher total cholesterol and HDL in RA. In contrast, Van Halm et al. (31), reported higher total cholesterol but lower HDL in DM relative to RA, and no differences was observed in weight or BMI of RA and DM patients. The differences in total cholesterol and HDL reported in the latter study could be explained due to the higher use of lipids lowering drugs (i.e. statins) in RA than DM patients which have resulted in lower levels of cholesterol in RA patients than DM patients. RA patients demonstrated higher resting heart rate in the current study which is not reported in many comparison studies between RA and DM patients. A contrary finding was reported by Piha et

al. (34), showing no difference in resting heart rate between RA and DM patients. It is difficult to explain why there was no difference in resting heart rate, however, it could be due to differences in inclusion criteria. For example, apart from inclusion of women only, the range of age included in the DM group was between 12-80 years, whereas, for RA it was 25-66 years. The variations in the results from studies suggest the need for further studies in larger populations to confirm these findings. However, overall, these studies suggest that some CVD risk factors in RA are comparable to DM patients, which suggests that CVD risk factors in RA patients need to be addressed clinically in a similar way to DM patients. Qrisk2 was used to compare the 10 years CVD risk in both groups. Qrisk2 was used due to its more suitability for the comparison, as it includes diagnosis of RA or DM as an independent risk factor (40;50). The current study found higher Qrisk2 in DM patients than RA patients. The higher Qrisk2 in DM patients is due to the higher individual CVD risk in DM patients, as DM patients in the current study had higher weight and BMI which are incorporated in the risk algorithm, resulting in higher Qrisk2 scores in DM patients.

Most of measures of wellbeing were poorer in RA patients than DM patients, except for anxiety. Similar findings using different forms of psychological wellbeing assessments were reported in a large population-based study comparing different chronic diseases including chronic obstructive pulmonary disease, RA, and DM patients (51). Comparing RA and DM, this study reported that RA patients had poorer wellbeing than DM patients (51). This included higher prevalence of anxiety and depression, poorer quality of life, impaired psychological wellbeing, and higher levels of fatigue (51). It is worth emphasising that these differences in wellbeing were found in the absence of differences in the physiological measures that were taken. Therefore, the current study adds to the suggestion that patients with RA need attention with regard to the measures of wellbeing to minimise the burden of the disease (52). Despite the finding that none of these measures of wellbeing were related to parasympathetic activity, long term burden of poor wellbeing such as depression associate with high risk of CVD (53). The management of RA is mostly focused on treating the symptoms and reducing progression of the disease with less focus given to addressing the psychological burden of the disease (52). It is worth noting that even though our RA sample were well-controlled, measures of wellbeing were poorer than DM. This can suggest that RA patients with severe disease activity are more likely to have poorer measures of wellbeing than our RA sample. However, without making a direct comparison in measures of wellbeing between RA patients with high disease activity and RA patients with low disease activity, this must remain speculative.

The study is not without limitations. It is possible that the small sample size could have contributed to the lack of difference in inflammation. However, it should be emphasised that even though the sample size was smaller than previous studies, the participants were matched for age and sex. Another limitation is the absence of a healthy control group, which would have added information about the difference between RA patients and DM patients in comparison to the healthy control group. Therefore, future studies should examine the influence of inflammation on HRR in patients with RA, DM, as well as a healthy control group. Furthermore, RA patients that were matched with DM had well-controlled disease activity to enable them to participate in an exercise intervention study, therefore, it would be interesting to compare DM patients with RA patients with severe disease activity. Thus, further research should compare HRR between different levels of disease activity in patients with RA. In order to determine the disease activity of RA patients, DAS28 is helpful to determine the disease activity for patients with RA, however, DAS28 was not available for all of our RA patients.

In conclusion, the results of the current study indicated that there were no differences in parasympathetic activity or inflammation between RA and DM patients. Cardiorespiratory fitness was an independent factor for parasympathetic activity in both groups of the patients. Despite the higher CVD risks in DM patients, some of the CVD risk factors in patients with RA were comparable to DM patients, which also mandate the need for CVD management in RA similar like what is established in DM patients (31). Future research should investigate the effect of CVD management on CVD risk and compare it between RA and DM patients. Given the association between CVD and poor measures of wellbeing, the current study also showed that measures of wellbeing were poorer than for DM, which emphasises the need for psychological management of patients with RA. Future research should investigate the effect of such management on measures of wellbeing.

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CHAPTER 6

General Discussion

The studies presented in this thesis focused on the autonomic nervous system (ANS) in patients with rheumatoid arthritis (RA). ANS consists of the sympathetic and the parasympathetic nervous systems, two systems that work in synergy to maintain cardiovascular autonomic balance. Autonomic dysfunction, which is a disruption of the balance in the activity of sympathetic and parasympathetic nervous system (i.e. increased sympathetic and reduced parasympathetic), is known to be associated with increased risk for cardiovascular diseases (CVD) (1;2). The risk of CVD in RA is increased compared to the general population and multifactorial, with autonomic dysfunction being implicated as one of the risk factors for CVD in this population (3). As discussed in the first chapter, there are various non-invasive methods to assess ANS: heart rate variability (HRV), heart rate recovery (HRR), and Ewing's test battery are the most commonly used methods.

HRV was used in the study presented in Chapter 2 to explore the association between induced myocardial ischemia during exercise tolerance test (ETT) (using ST/HR index), and the overall activity of ANS while resting. Subsequently, the factors associated with parasympathetic reactivation reflected by heart rate recovery (HRR) following ETT including a range of factors reflecting CVD risk, RA-related disease activity, and measures of wellbeing were explored (Chapter 3). In the next chapter, the effects of a 3-month exercise intervention on parasympathetic activity (using HRR), as well as the CVD risk factors (including cardiorespiratory fitness), markers of inflammation and measures of wellbeing were investigated (Chapter 4). Finally, since inflammation was one of factors associated with parasympathetic activity in RA patients (Chapter 3), parasympathetic activity (using HRR) was compared between two populations [RA and diabetes mellitus (DM)] with similar CVD risk profiles but different levels of inflammation (Chapter 5). The study presented in Chapter 5 compared inflammatory markers, a range of CVD risk factors, and measures of wellbeing in these populations and the association of these factors with HRR. The following paragraphs provide a summary of the findings reported in this thesis, the implications of these findings, limitations, and suggestions for future research.

Cardiovascular disease risk factors associated with autonomic nervous system

Association between a marker of ischemia and autonomic nervous system

Chapter 2 aimed to investigate the association between resting HRV using frequency domain analyses and ST/HR index as a marker of ischemia during ETT and in patients with RA. As hypothesized, there was an association between this marker of ischemia and ANS. This may suggest that poor parasympathetic function at rest indicated by high frequency (HF) component, as well as a disrupted balance between the sympathetic and parasympathetic nervous system at rest (i.e. LF/HF), may associate with myocardial ischemia (indicated by ST/HR index) during ETT.

Chapter 3 revealed associations between parasympathetic activity following ETT and factors that can contribute to myocardial ischemia. Myocardial ischemia is caused by atherosclerosis. Accumulation of triglycerides on the arterial wall (4) as well as high blood pressure (5) are known as factors that contribute to the development of atherosclerosis. Inverse association between resting parasympathetic tone and myocardial ischemia was reported in Chapter 2, and the inverse association between parasympathetic activity following ETT and factors that contribute to myocardial ischemia (triglycerides, and systolic blood pressure) were reported in Chapter 3. Therefore, it was not surprising to find the same inverse association between parasympathetic activity and Framingham risk score, which includes total cholesterol, blood pressure, as well as other CVD risks.

The study presented in Chapter 2 was the first to use heart rate adjusted ST segment (i.e. ST/HR index) as marker of ischemia during exercise testing in RA, and to report an association between this marker and reduced HRV. Other studies in autoimmune diseases including RA have suggested that coronary artery disease and sudden cardiac death are associated with reduced HRV (6-8). Therefore, given the high risk of unrecognized myocardial infarction (MI) in RA compared to patients without RA (9), it could be beneficial to use ST/HR index during an exercise tolerance test in patients with RA for the early detection of myocardial ischemia. The advantage of using the ST/HR index is that it takes the achieved HR into account, therefore even if a patient is not able to exercise until exhaustion because of joint problems, this measurement will still provide information regarding ischemia. However, further investigation is needed to investigate the association between ST/HR index and CVD events in RA. Furthermore, it was found that ST/HR index has good accuracy to detect ischemic changes in comparison to angiogram in other populations (10), thus, similar studies can be implemented

in patients with RA which will identify if ST/HR index is indeed related to clinically relevant CVD measures.

Individual cardiovascular risk factors associated with autonomic nervous system

Chapter 3 aimed to explore the association between a range of CVD risk factors, RA-related disease activity, and measures of wellbeing with the ANS, in particular parasympathetic activity following exercise testing using HRR. As hypothesized, many CVD risk factors including age, resting systolic blood pressure, triglycerides, insulin resistance (i.e. HOMA), and Framingham risk score, were inversely associated with HRR, whereas cardiorespiratory fitness indicated by VO_2 peak was positively associated with HRR. RA-related disease activity variables including higher WBC, fibrinogen, CRP, and ESR, were associated with poorer HRR. Associations were also found for measures of wellbeing; poor vitality was associated with poor HRR and better quality of life was associated with better HRR. Interestingly though, none of these factors were independently associated with HRR. This may suggest that it is the overall CVD risk and disease-related burden that contributes to changes HRR, instead of one or several individual factors.

The study presented in Chapter 3 was the first study to investigate HRR and the factors related to it in a sample containing men and women with RA. Furthermore, the study also explored the association between a wide range of CVD risk factors and psychological wellbeing in a systematic manner, which has not been done before in other studies (an overview of these studies is given in Chapter 1). It is important to know which factors are related to ANS, in particular, the parasympathetic nervous system, given that poor parasympathetic activity has been suggested to contribute to increased risk of CVD (1;2). Therefore, knowing which factors are associated with ANS would help with the development of interventions to improve ANS. Based on the findings in this chapter, it can be suggested that reducing the overall CVD risk and disease-related burden will have a positive effect on ANS in RA.

Comparison between rheumatoid arthritis and diabetes mellitus (impact of inflammation on autonomic nervous system)

In the last experimental chapter, a different approach was taken to specifically explore the impact of inflammation on ANS in RA. This was done by comparing RA and DM patients as

both conditions share similar CVD risk profile but have different levels of inflammation (Chapter 5). RA is a condition characterised by systemic inflammation, whereas, DM has low-grade systemic inflammation. Therefore, the study aimed to compare parasympathetic activity using HRR following exercise tolerance between age- and sex-matched RA and DM patients. The secondary aim was to compare inflammation as well as CVD risk factors and measures of wellbeing and the association of these variables with HRR in both populations. No difference in HRR was observed and despite inflammation being increased in RA, this difference did not reach statistical significance.

The studies investigating the difference in the ANS between RA and DM patients are limited. Therefore, the study presented in Chapter 5 investigated the difference in ANS between two chronic conditions which are prone to develop CVD and have similar CVD risk factors. To the best of my knowledge, only one study has investigated the difference in ANS between RA, DM, and healthy control participants using cardiovascular autonomic reflex tests, where DM patients were reported to have poorer ANS than RA or healthy participants (11). Thus, further studies are required to investigate the difference in ANS between RA and DM patients using difference measures of ANS.

In Chapter 3 an inverse association between parasympathetic activity and inflammation was reported, which was not confirmed in Chapter 5, where none of the inflammatory markers were associated with parasympathetic activity. It is difficult to explain the reason why there was no association in the study presented in Chapter 5. Both populations have a similar CVD risk profile, and therefore, it could be speculated that the overall CVD risk profile has a stronger association with parasympathetic reactivation than inflammation. For example, cardiorespiratory fitness was associated with parasympathetic reactivation in both Chapter 3 and 5. It should also be acknowledged that the cross-sectional designs of these studies only allow for the examination of associations but do not provide information about cause and effect. Therefore, in order to get a better understanding of the role of inflammation in ANS, additional intervention or experimental studies are needed to explore the impact of changing inflammation on ANS. To our knowledge, this has not been done in patients with autoimmune disease.

Cardiorespiratory fitness and parasympathetic activity

The association between cardiorespiratory fitness and parasympathetic activity was found in two cross-sectional studies (Chapter 3 and 5). VO_2 peak was associated with HRR in RA

patients in Chapter 3. In Chapter 5, VO_2 peak was an independent factor associated with HRR in a sample combining RA and DM patients. Behavioural interventions, including exercise, have been found to improve autonomic function , in particular, in many clinical populations (12;13). Furthermore, exercise has been shown to reduce CVD risk and inflammation in RA patients (14;15). Therefore, the study presented in chapter 4 aimed to investigate the effects of a 3-month semi-supervised exercise intervention on parasympathetic reactivation-HRR, CVD risk, RA-related disease activity, and measures of wellbeing. However, the intervention was not successful in improving HRR or VO_2 peak. It was suggested that the lack of improvement in VO_2 peak could have resulted in the lack of improvement in HRR. These findings suggest that without improvement in VO_2 peak, HRR is less likely to improve.

A sub-analysis of the data revealed that when the sample was split into two groups based on improvement and no-improvement in HRR, it was found that those with poorer parasympathetic activity-HRR and CVD risk at baseline were more likely to gain benefits from exercise intervention (Chapter 4).

Autonomic function has been reported to improve after exercise programmes. The improvement in autonomic function following exercise programmes is suggested to be due to improvement in cardiorespiratory fitness which subsequently improves the vagal modulation of the heart making the parasympathetic effect on the heart effective on reducing the heart rate after exercise (16). The improvements in HRR following an exercise intervention has been reported in a group of women with RA following 10 weeks of supervised high intensity exercise training (17). Despite the difference in the sample sex, and level of supervision, , an improvement in VO_2 peak was reported in the latter study, which may have prompted the improvement in HRR. Therefore, this may suggest that exercise interventions should focus on improvement of cardiorespiratory fitness to gain beneficial effect on parasympathetic reactivation. Furthermore, the study reported in Chapter 4 also suggested that the exercise programme might have an impact on positive psychological wellbeing, without the improvement in cardiorespiratory fitness.

Limitations

There were some limitations in the studies presented in this thesis, which were mostly discussed in the individual chapters. Overall, the data of the RA cohort used in this thesis was obtained

from an exercise intervention study, which involved patients with well-controlled disease activity. This makes it difficult to generalise the results presented in this thesis to RA patients with severe disease activity. However, the studies in this thesis still highlight some interesting findings (i.e. association between ANS and CVD risk factors) and open up further questions related to parasympathetic activity in RA and how it compares to another condition with similar CVD risk profiles such as DM Furthermore, the cross-sectional design of Chapter 2, 3, and 5 do not allow investigations around causality between the associated variables. The lack of a comparison group in Chapter 2 and 3 is another limitation that would have allowed a direct comparison between RA and another population. In addition, DAS28 would have added more information about the disease activity of our RA cohort, however, DAS28 was not available from all RA patients to be able to include it into our analyses.

In Chapter 2, two minutes short-term HRV is a method that is not often used in clinical research. Although a wealth of information can be obtained from HRV based on several hours or minutes of heart rate recording, this may not always be possible in population-based studies or epidemiological research (18). Furthermore, the study utilised the minimum requirement (which is two minutes) for heart rate recording to obtain HRV parameters (19), which is a method that was previously used in epidemiological studies (18;20). In Chapter 4, it was unknown whether the RA sample size was enough to investigate the effect of exercise on HRR, as the power calculation performed initially was for the main outcome of the main study (i.e.VO_2 peak).. In Chapter 5, the lack of healthy control participants to investigate the difference in parasympathetic activity as well as inflammatory markers between RA patients/DM patients and healthy individuals.

Recommendations for future research

A number of future studies can arise from the findings reported in Chapter 2. There is evidence that ST/HR index has shown good sensitivity with clinical measures of coronary artery disease such as angiography in other populations (10). It is therefore very likely that this is also the case for patients with RA, however, this remains to be confirmed. Thus, subsequent research could investigate the association or the sensitivity of ST/HR index with a clinically established measure of coronary artery disease in patients with RA. Although, the difference in HRV between RA patients and healthy control participants is known, the difference in ST/HR index would be interesting to investigate. In addition to these cross-sectional studies, longitudinal

designs should be employed to examine the relationship between ST/HR index and coronary artery disease (e.g., MI), which will help to explore if ST/HR index can predict future CVD events in patients with RA.

Due to the similarity in the values of HRR reported in Chapter 3 with HRR recovery of healthy participants reported in other studies (21;22), it would be interesting to compare HRR between RA patients and healthy participants in one study, as direct comparison between the HRR reported from different studies is difficult due to the difference in methodology. Another interesting comparison group would be patients with established CVD. The current data only allows for the examination of associations, by adding comparison groups without and with established CVD, it will be possible to examine if HRR in RA is indeed different from these populations. Furthermore, numerous studies have indicated that HRR is a predictor of CVD mortality in other populations (23-25), therefore, similar longitudinal design should be employed to investigate if HRR is a predictor of CVD mortality in patients with RA. Such a study would also allow for the establishment of clinical cut off points specific for patients with RA, as the current clinical cut off point were obtained from other populations and difficult to be generalised to patients with RA.

In Chapter 5, the impact of inflammation on ANS was explored by using two different populations. The impact of inflammation on ANS can also be investigated in a different way by stimulating the vagus nerve e.g., the cholinergic anti-inflammatory pathway via the stimulation of α7 nicotinic acetylcholine receptor (α7nAChR) of the vagus nerve has been suggested as a method to reduce inflammation in RA (26). Stimulating these receptors via non-invasive transcutaneous electrical stimulation of the vagus nerve has been shown to effectively increase the parasympathetic activity in a healthy population (27). Therefore, it can be used as a method to investigate if treatment using this method can reduce inflammation as well as improving ANS over a period of time in RA patients.

Another method is to investigate the influence of inflammation on ANS is by examining the effects of pharmacological treatment on ANS. For example, by exploring the changes in ANS following successful treatment of tumour necrosis factor-α inhibitors (TNF-α inhibitors). Given the mixture of medications taken by patients with RA, future studies could also compare the effects of different commonly used treatments on ANS in RA. This may help to identify the individual and combined effects of these medications on parasympathetic activity.

In addition to the inverse association between parasympathetic activity and blood pressure reported in the cross-sectional study in Chapter 3, one of the interesting findings in the study presented in Chapter 4 is the improvement in blood pressure after the exercise intervention. Given the benefits of exercise training for the treatment of hypertension (28;29), it would be interesting to investigate the effect of exercise intervention in comparison to the effect of anti-hypertensive medications in patients with RA. Previous studies which reported positive effects of exercise training on autonomic function have used supervised exercise training in patients with RA (17;30). Future studies should investigate the difference in the effects of semi-supervised versus supervised exercise training on HRR as well as cardiorespiratory fitness. Furthermore, perhaps an additional study should investigate the difference between high intensity and moderate intensity of exercise training on HRR and cardiorespiratory fitness in RA patients. Furthermore, as it was assumed that in Chapter 4 that the lack of improvement in HRR is due to the lack of improvement in cardiorespiratory fitness indicated by VO_2 peak, further study should confirm this hypothesis. In order to test this hypothesis, a longitudinal study can be deployed to investigate the effect of exercise intervention on VO_2 peak as well as HRR using high intensity aerobic exercise versus low intensity aerobic exercise in patients with RA. This would identify if exercise targeting improvement in cardiorespiratory fitness can result in a subsequent improvement in parasympathetic activity.

The study presented in Chapter 5 revealed that several CVD risk factors are comparable between RA and DM patients. Unlike DM, CVD management receives less attention in RA (31), therefore, future studies should investigate the effects of CVD management on ANS in patients with RA. This would be helpful in establishing guidelines similar to thosefor DM. Although it was not associated with the parasympathetic activity in Chapter 5, poorer measures of wellbeing reported in this study in comparison to DM indicate that they need to be addressed in RA patients to reduce the burden of the disease. It has been reported that improvement of psychological wellbeing can reduce CVD mortality in healthy populations and reduce death rates in other clinical populations (32). Therefore, future studies need to investigate the effect of interventions aimed at improving psychological wellbeing in RA and the effect of improvement of wellbeing on the reduction of CVD risk in RA patients.

Conclusion

This thesis identified associations between ANS with CVD risk factors, RA-disease related activity and measures of wellbeing. Parasympathetic activity following exercise testing was not improved after 3-month semi-supervised exercise intervention. However, the exercise intervention improved some of the CVD risk factors, and the improvement was more noticeable in patients with poorer CVD risk profiles. When ANS was compared with DM, the parasympathetic activity following exercise testing was similar, inflammatory markers were not different, some CVD risk factors were comparable, and measures of wellbeing were poorer in RA. Cardiorespiratory fitness was an independent predictor for parasympathetic activity in both populations, which suggests that by improving cardiorespiratory fitness, the parasympathetic activity can also be improved.

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Appendices

STANFORD HEALTH ASSESSMENT QUESTIONNAIRE

For the items below, please tick the <u>ONE</u> response which best describes your usual ability over the <u>PAST TWO WEEKS</u>.

	Without ANY Difficulty	With SOME Difficulty	With MUCH Difficulty	Unable to do
1. DRESSING AND GROOMING *Are you able to:* - Dress yourself, including tying shoelaces and doing buttons?	☐	☐	☐	☐
- Shampoo your hair?	☐	☐	☐	☐
2. RISING *Are you able to:* - Stand up from an armless straight chair?	☐	☐	☐	☐
- Get in and out of bed?	☐	☐	☐	☐
3. EATING *Are you able to:* - Cut your meat?	☐	☐	☐	☐
- Lift a full cup or glass to your mouth?	☐	☐	☐	☐
- Open a new carton of milk (or powder)?	☐	☐	☐	☐
4. WALKING *Are you able to:* - Walk outdoors on flat ground?	☐	☐	☐	☐
- Climb up five steps?	☐	☐	☐	☐

Please tick any aids and devices that you usually use for any of these activities:	Please tick any categories for which you usually need help from another person:
Cane ☐ Walking frame ☐ Crutches ☐ Wheelchair ☐ Special or built-up chair ☐ Devices used for dressing ☐ (button hook, zipper pull, long handled shoe horn etc.) Built-up or special utensils ☐ Other (please specify) ☐	☐ Dressing and grooming ☐ Rising ☐ Eating ☐ Walking

For the items below, again please tick the <u>ONE</u> response which best describes your usual ability over the <u>PAST TWO WEEKS</u>.

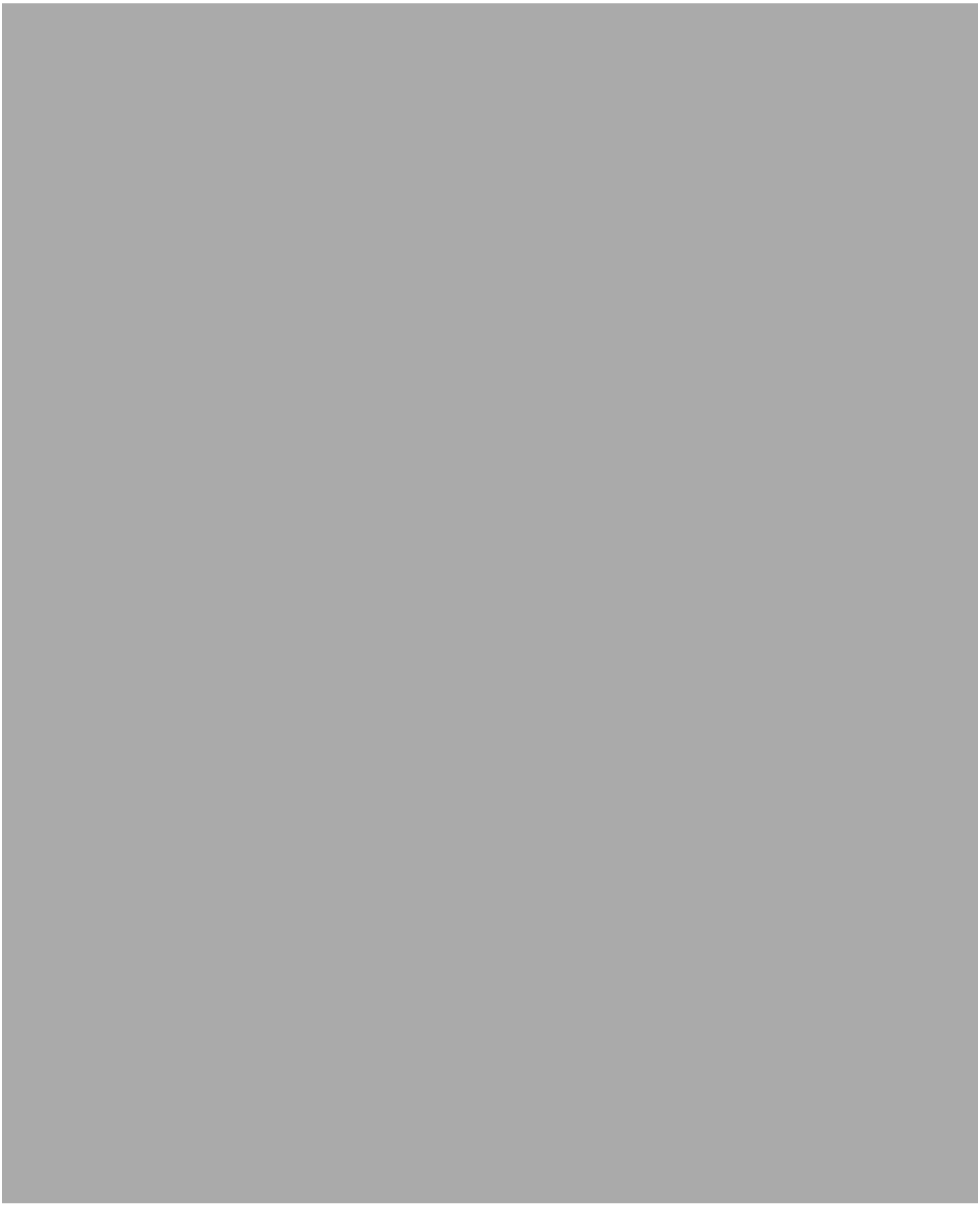
	Without ANY Difficulty	With SOME Difficulty	With MUCH Difficulty	Unable to do
5. HYGIENE *Are you able to:* - Wash and dry your entire body?	☐	☐	☐	☐
- Take a bath?	☐	☐	☐	☐
- Get on and off the toilet?	☐	☐	☐	☐
6. REACH Are you able to: - Reach and get down a 5lb object (e.g. a bag of potatoes) from just above your head?	☐	☐	☐	☐
- Bend down to pick up clothing from the floor?	☐	☐	☐	☐

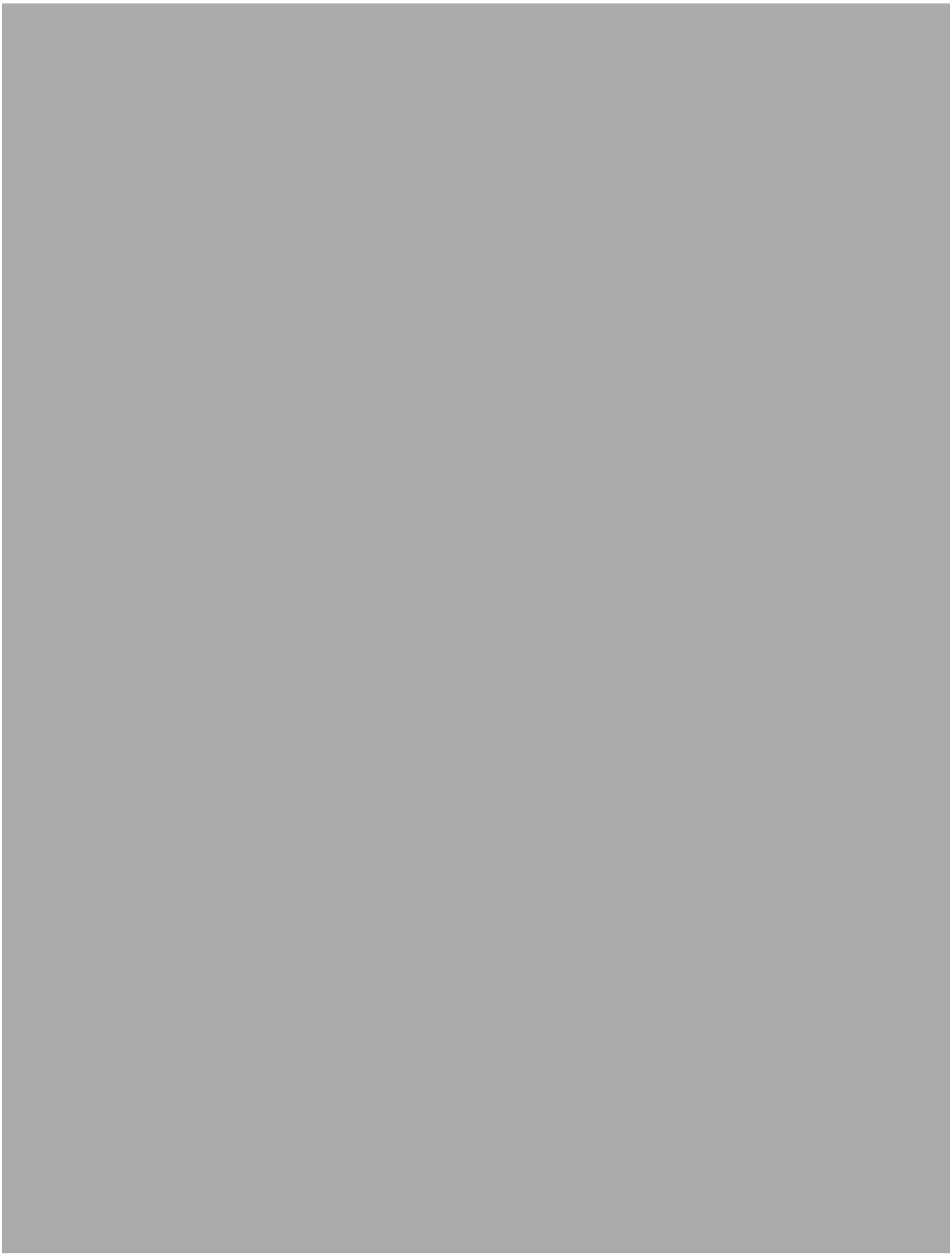
Continued over…

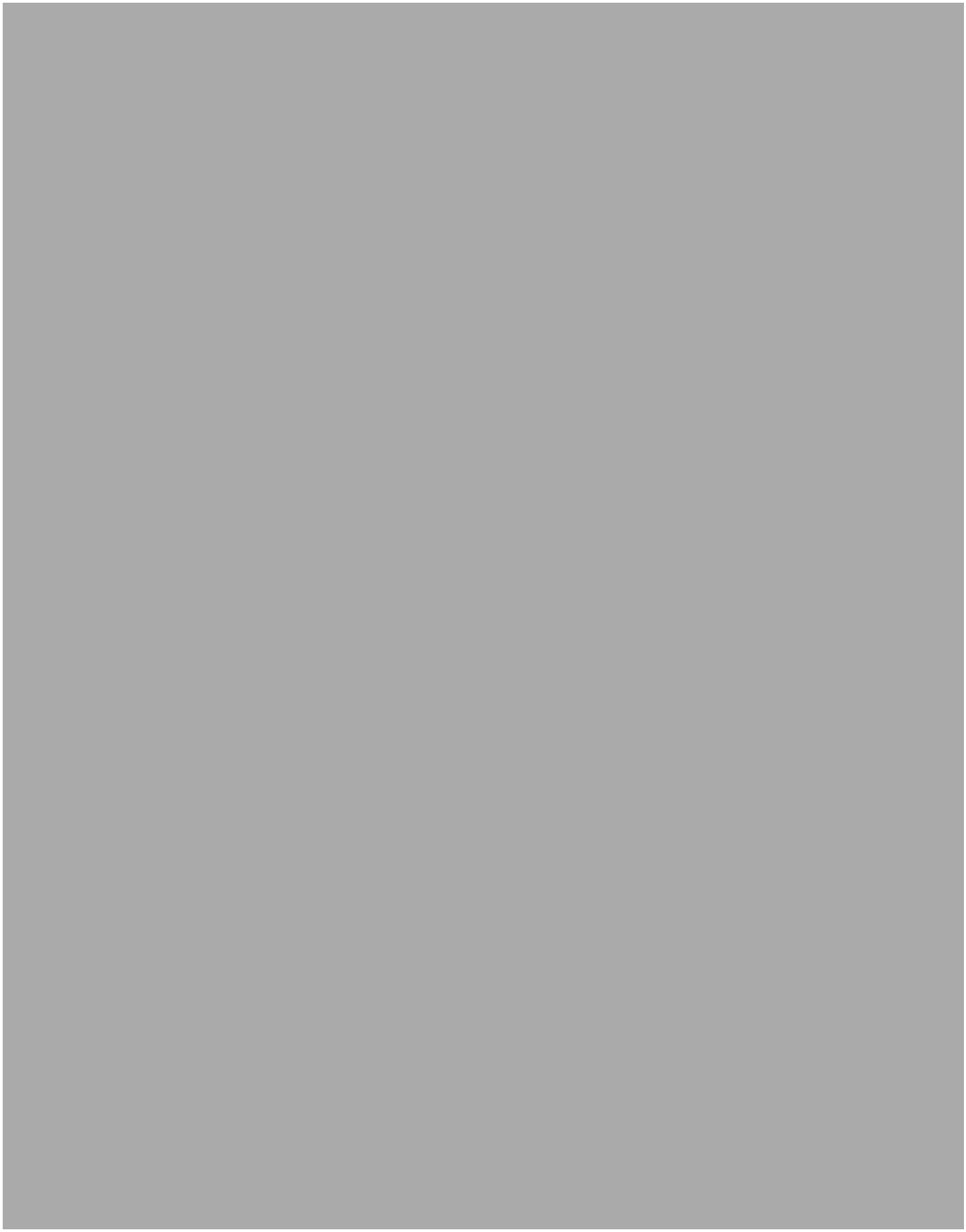
	Without ANY Difficulty	With SOME Difficulty	With MUCH Difficulty	Unable to do
7. GRIP *Are you able to:* - Open car doors?	☐	☐	☐	☐
- Open jars which have been previously opened?	☐	☐	☐	☐
- Turn taps on and off?	☐	☐	☐	☐
8. ACTIVITIES *Are you able to:* - Run errands and shop?	☐	☐	☐	☐
- Get in and out of a car?	☐	☐	☐	☐
- Do chores such as vacuuming, housework or light gardening?	☐	☐	☐	☐

Please tick any aids or devices that you usually use for any of these activities:	Please tick any categories for which you usually need help from another person:
☐ Raised toilet seat ☐ Bath seat ☐ Jar opener (for jars previously opened) ☐ Bath rail ☐ Long handled appliances for reach ☐ Other (please specify)	☐ Hygiene ☐ Reach ☐ Gripping and opening things ☐ Errands and housework

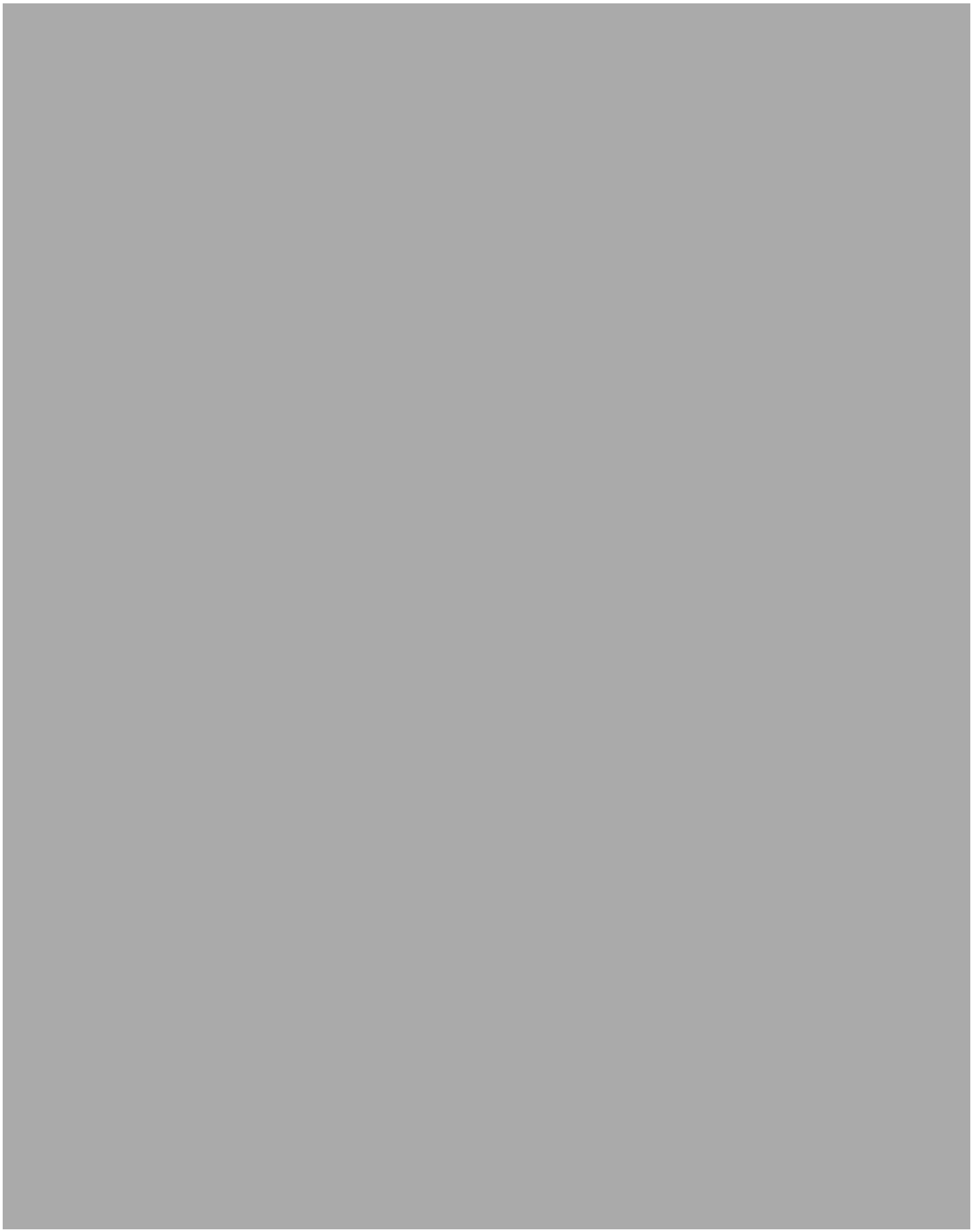
MULTIDIMENSIONAL ASSESSMENT OF FATIGUE (MAF) SCALE



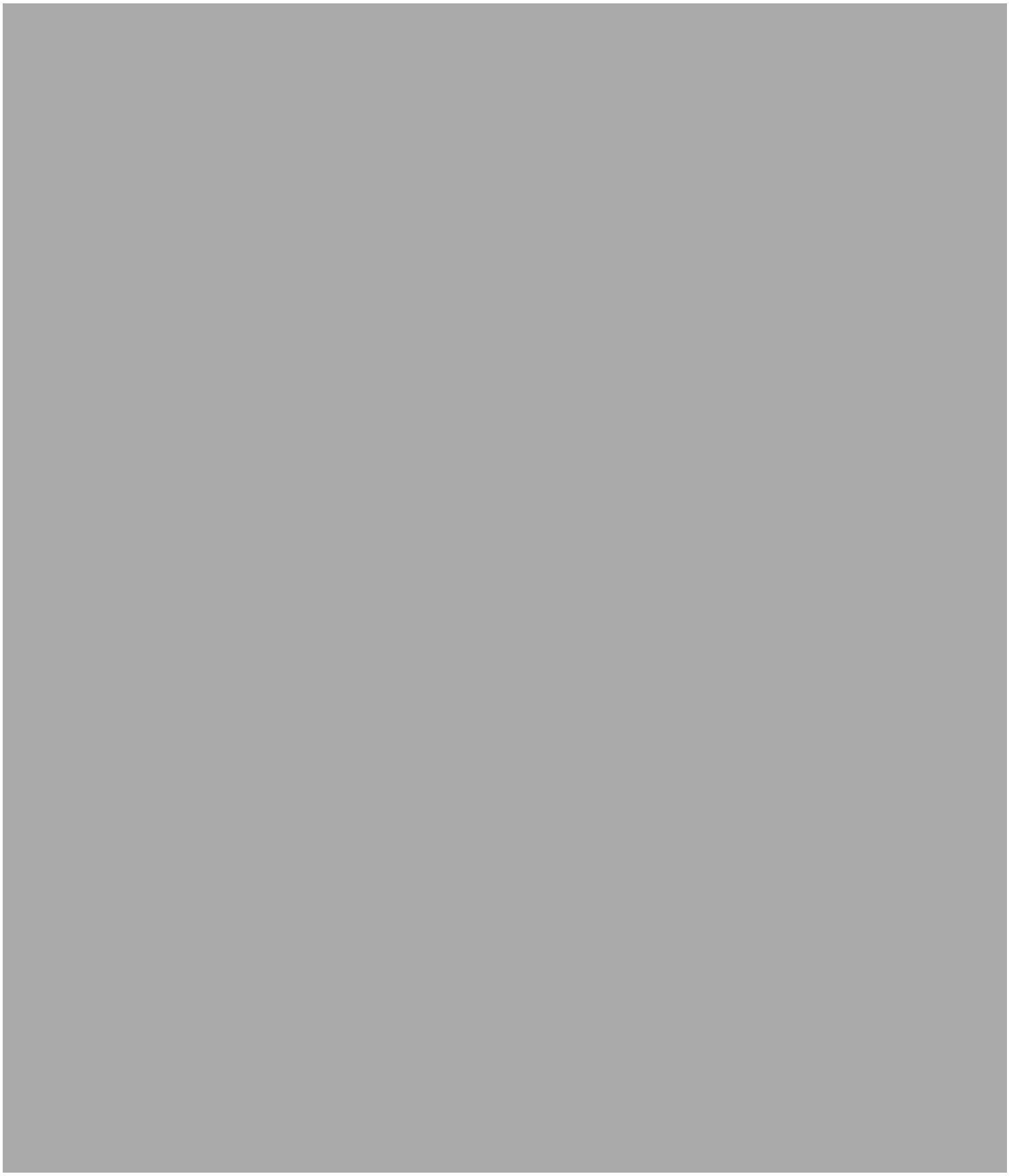




HOSPITAL ANXIETY AND DEPRESSION SCALE



EUROQUOL EQ 5D



Subjective Vitality Scale