AUTONOMIC FUNCTION IN RHEUMATOID ARTHRITIS

by

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory condition with poorly understood pathophysiology and increased cardiovascular risk. The mechanisms for increased cardiovascular risk are not fully known, however one novel mechanism explored in this thesis is autonomic nervous system (ANS) dysfunction. The thesis comprises of: a systematic literature review; two case-control studies (n=30 RA patients, n=34 controls; a longitudinal case-study (n=1 RA patient); a cohort study (n=112 RA patients); and a randomised placebo-controlled crossover study (n=10 healthy controls). The work presented in this thesis demonstrates that ANS dysfunction is prevalent in ~60 % of RA patients and characterised by heightened sympathetic outflow to the peripheral vasculature (determined by muscle sympathetic nerve activity using microneurography), depressed baroreflex control of heart rate (determined using the modified Oxford technique), depressed heart rate variability and heightened vascular responses to stressors (cold pressor test and mental stress). Inflammation was associated with ANS dysfunction, and may well contribute to the increased cardiovascular risk seen in RA. Further studies are required to: confirm these findings; determine whether therapeutic strategies to restore ANS function improve prognosis in RA; and further explore the precise mechanisms by which inflammatory cytokines may influence ANS function in health and disease.

DEDICATION

In the name of Allah, The Most Merciful, The Most Gracious.

"Read! In the Name of Your Lord Who Created

Created man from a clot

Read! And Your Lord is Most Generous

Who Taught mankind by the pen

He Taught man that which he knew not."

Holy Quran (Surah 96, verses 1-5)

Indeed, all Praises belong to Allah, to Him we worship, to Him we turn to, to Him we ask for Forgiveness and to Him we turn to in repentance. And we seek refuge in Allah from our own selves, from the evil amongst our deeds. He Whom Allah Guides, indeed he is guided. And he Whom Allah Misguides, indeed you will not find a helper to guide. I bear witness that there is none worthy of worship, except Allah and that Muhammad, peace and blessings upon him, is his servant and final Messenger. All Good comes from Allah, and all mistakes are from myself.

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LIST OF ABBREVIATIONS

ACh Acetylcholine

ACE Angiotensin converting enzyme

ACR American College of Rheumatology

ACTH Adrenocorticotrophin hormone

ANOVA Analysis of variation

ANS Autonomic nervous system

Anti-CCP Anti-cyclic citrullinated peptide

BAZ Bazett's formula

BMI Body mass index

BP Blood pressure

BRS Baroreflex sensitivity

cBRS Cardiac/cardiovagal baroreflex sensitivity

CCT Clinical cardiovascular reflex test

CI Confidence interval

COX Cyclo-oxygenase

CPT Cold pressor test

CRH Corticotrophin-releasing hormone

CRP C-reactive protein

DAS Disease activity score

DNA De-oxyribonucleic acid

ECG Electrocardiogram

ELISA Enzyme linked immunosorbent assay

eNOS Endothelial nitric oxide synthase

EPI Epinephrine

ESR Erythrocyte sedimentation rate

FHS Framingham heart study

FVC Forearm vascular conductance

GABA Gamma amino-butyric acid

G_{MOT} Baroreflex gain using MOT

G_{SEQ} Baroreflex gain using sequence method

HAQ Health assessment questionnaire

HCN Hydrocortisone

HERG Human ether-a-go-go related gene

HF High frequency power

HPA Hypothalamic-pituitary-adrenal

HR Heart rate

HRT Heart rate turbulence

HRV Heart rate variability

hs-CRP High sensitivity C-reactive protein

HTN Hypertensive

IFN Interferon

IL Interleukin

IML Intermediolateral

iNOS Inducible nitric oxide synthase

KCNH2 Human ether-a-go-go related gene

LF Low frequency power

LFTF Low frequency transfer function

LPS Lipopolysaccharide

LSD Least significant difference

LVC Leg vascular conductance

MCP Metacarpal joint

MI Myocardial infarction

MOT Modified Oxford technique

mRNA Messenger ribonucleic acid

MSNA Muscle sympathetic nerve activity

MTP Metatarsal joint

NA Noradrenaline

NC Normotensive control

NE Norepinephrine

NICE National institute for health and care excellence

NN50 Number of pairs of adjacent inter-beat intervals differing by more than 50 ms

NO Nitric oxide

NOS Nitric oxide synthase

NPY Neuropeptide-Y

NSAID Non-steroidal anti-inflammatory drug

NTS Nucleus of the solitary tract

PASAT Paced auditory serial addition task

PEP Pre-ejection period

PE Phenylephrine

PIP Proximal interphalangeal joint

PLR Pupillary light reflex

pNN50 NN50 as a percentage of all inter-beat intervals

PRISMA Preferred reporting items for systematic reviews and meta-analyses

PVN Paraventricular nucleus

QIS Quality index score

QTc Corrected QT interval

RA Rheumatoid arthritis

RA-HTN Rheumatoid arthritis hypertensive

RF Rheumatoid factor

rMSSD Square root of the mean of the sum of the squares of differences between adjacent inter-beat intervals

RNA Ribonucleic acid

RR-I inter-beat interval

RVLM Rostral ventrolateral medulla

RVMM Rostral ventromedial medulla

SBP Systolic blood pressure

SD Standard deviation

SD1 Standard deviation of the Poincare plot (short-term HRV)

SD2 Standard deviation of the Poincare plot (long-term HRV)

SD1/SD2 Ratio of SD1/SD2

SDNN Standard deviation of all inter-beat intervals

SEM Standard error of the mean

SFO Subfornical organ

SNP Sodium nitroprusside

SPSS Statistical package for the social sciences

SSR Sympathetic skin response

Th1 T-helper 1

Th2 T-helper 2

TNF-α Tumour necrosis factor-alpha

TP Total power

UK United Kingdom

USA United States of America

VAS Visual analogue scale

VLF Very low frequency power

VPC Ventricular premature complex

VT Ventricular tachycardia

LIST OF PUBLICATIONS

Original articles

- Autonomic function and rheumatoid arthritis: a systematic literature review. **Adlan AM**, Lip GYH, Paton JFR, Kitas GD, Fisher JP. (In print) *Seminars in Arthritis and Rheumatism* 2014; 44(3):283-304.
- Association between QTc interval and inflammatory cytokines in rheumatoid arthritis. **Adlan AM**, Panoulas VF, Smith JP, Fisher JP, Kitas GD. *J Rheumatol* 2015; 42(3): 421-8.

Conference proceedings

- Rheumatoid arthritis and arterial baroreflex sensitivity (abstract). **Adlan A**, Kitas G, Paton J, Lip G, Fisher J. *FASEB J* 2013; 27: 1118.20.
- Rheumatoid arthritis and autonomic function (abstract). **Adlan A**, Shantsila A, Kitas G, Paton J, Lip G, Fisher J. *FASEB J* 2014; 28(1): 1132.10.
- Hydrocortisone acutely reduces cardiovagal baroreflex sensitivity and heart rate variability. **Adlan AM**, Veldhuijzen van Zanten JJ, Lip GYH, Paton JFR, Kitas GD, Fisher JP. *Proc Physiol Soc* 2015.

CHAPTER 1 Introduction and Purpose

Rheumatoid arthritis (RA) is a chronic inflammatory condition predominantly affecting the synovial joints. Other body systems are also affected and it has been recognised that RA carries a significant cardiovascular mortality – estimated at around 50 %. Current understanding is that local inflammation within the synovial joints may lead to systemic inflammatory responses that may affect other body organs and contribute to morbidity and mortality risks. Disease modifying anti-rheumatoid drugs and biologic agents are effective in controlling inflammation in RA patients and there is evidence to suggest improved cardiovascular risk and mortality that parallels reductions in inflammation. Extensive work in RA has not yet been able to identify the underlying mechanisms linking inflammation with increased cardiovascular risk and novel mechanisms are being sought. One such novel mechanism that is explored in this thesis is the role of the autonomic nervous system (ANS). The ANS plays a key role in maintaining homeostasis and in particular blood pressure (BP) and heart rate (HR) regulation, through the parasympathetic and sympathetic nervous systems and a number of neural reflexes including the arterial baroreflex. There is a wealth of evidence to show that ANS dysfunction, in a pattern of increased sympathetic activity, reduced parasympathetic activity and reduced baroreflex sensitivity (BRS), is associated with cardiovascular diseases and predicts increased cardiovascular risk and mortality. Recent work from animal studies have demonstrated reciprocal links between inflammatory molecules and alterations in ANS function which raise the possibility that inflammation may play a role in promoting cardiovascular diseases and increasing mortality risk. Indeed there is evidence that cardiovascular disease such as hypertension, coronary artery disease and chronic heart failure are characterised by low-grade inflammation and that the degree of inflammation relates to cardiovascular and mortality risk. The precise mechanisms by which inflammation can lead to

ANS function are not known. It remains to be shown whether increased concentrations of circulating pro-inflammatory molecules lead to ANS dysfunction in humans.

The central hypothesis in this thesis is that elevated circulating pro-inflammatory cytokines are associated with ANS dysfunction (heightened sympathetic activity, reduced BRS, impaired cardiovascular reactivity) in RA patients. Thesis aims and hypotheses are summarised in Table 1.1.

Chapter 2 provides a literature review covering RA (pathophysiology, immunology, clinical features and management); the immune system; the normal function of the ANS including methods of assessment; autonomic dysfunction and immune interactions. Chapter 3 is a systematic literature review, which aims to (i) determine whether ANS dysfunction is present in RA, (i) determine the pattern of ANS dysfunction, and (iii) determine whether any associations exist between inflammation and ANS dysfunction in RA patients. Chapter 4 outlines the methodology employed in the experimental chapters. There are five experimental chapters (Chapters 5-9) and a synthesis (Chapter 10).

Chapter 5 is a case control study assessing muscle sympathetic nerve activity (MSNA) and BRS in RA patients compared to age-, sex-and body mass index (BMI)-matched hypertensive and normotensive controls. This study is unique and novel as it is the first time MSNA has been recorded directly in RA patients, and the first time BRS has been assessed using the Modified Oxford Technique (MOT, sequential infusions of sodium nitroprusside [SNP] and phenylephrine [PE] one minute apart) in RA patients.

Chapter 6 is a case control study assessing heart rate variability (HRV, marker of parasympathetic activity) and cardiovascular reactivity (responses to cold pressor test, CPT

and paced auditory serial arithmetic task, PASAT mental stress test) in RA patients compared to age-, sex-and BMI-matched hypertensive and normotensive controls.

Chapter 7 is a case report of an RA patient who was assessed prior to, 2 weeks and 3 weeks following anti-inflammatory therapy with tumour necrosis factor-alpha (TNF-α) inhibitor. ANS assessment included MSNA, BRS, HRV and cardiovascular reactivity (responses to mental stress). The hypothesis was that inhibition of the pro-inflammatory cytokine TNF-α would reduce inflammation and restore ANS function (i.e. reduce MSNA, increase HRV and BRS, and normalise cardiovascular reactivity).

Chapter 8 is a cohort study of RA patients assessing for associations between inflammatory cytokines (pro- and anti-inflammatory) and the corrected QT interval (QTc, a proxy marker of ANS function and strong predictor of cardiovascular risk and mortality).

Chapter 9 is a randomised placebo-controlled single-blinded cross-over study assessing the acute effects of intravenous hydrocortisone (HCN, pharmacological preparation of cortisol) on BRS, HRV and cardiovascular reactivity in healthy humans. The study explores important mechanistic links between the hypothalamic-pituitary-adrenal (HPA) axis and the ANS, which are co-activated in acute stress. The study aims to provide insights into autonomic changes during acute stress, which is associated with increased cardiovascular risk and mortality.

The final chapter involve a synthesis and discussion (Chapter 10) summarising the conclusions that can be drawn from the experimental chapters, including limitations and future directions to further advance knowledge and understanding in the field.

Table 1.1 Summary of the aims and hypotheses of the thesis

Chapter	Hypotheses	Aims
3	In patients with RA, elevated concentrations of circulating inflammatory cytokines are associated with autonomic dysregulation (heightened sympathetic activity, reduced parasympathetic activity, reduced BRS and impaired cardiovascular responses to stressors).	A systematic literature review on ANS function in RA was performed to: • investigate whether there is sufficient evidence to determine if patients with RA have altered ANS function; • determine the prevalence and nature of any autonomic dysregulation in patients with RA; • elucidate whether there is a causal relationship between systemic inflammation and ANS dysfunction in RA.
5	Central sympathetic outflow (MSNA) is elevated in RA compared to normotensive controls. Cardiovagal and sympathetic BRS is reduced in RA compared to normotensive controls.	An observational, case-control study in RA patients (with and without hypertension) and controls (with and without hypertension) was undertaken to measure baseline serum inflammatory cytokine concentrations, MSNA and BRS.

	Elevated circulating concentrations of inflammatory cytokines are associated with increased MSNA and reduced BRS.	
6	Reduced HRV is associated with increased inflammatory cytokine concentration. Cardiovascular reactivity to CPT and mental stress are impaired in RA compared to controls, with greater impairments seen in hypertensive RA patients. Cardiovascular responses to CPT and mental stress are associated with serum inflammatory cytokine concentrations.	An observational, case-control study in patients with RA (with and without hypertension) and controls (with and without hypertension) was undertaken to measure baseline serum inflammatory cytokine concentrations, HRV and cardiovascular responses to CPT and PASAT mental stress.
7	In RA, TNF-α inhibition: • reduces sympathetic outflow (MSNA); • increases sensitivity of the baroreflex control of the heart; • increases HRV; • normalises cardiovascular responses to mental stress.	A longitudinal, case study in RA patients was undertaken to measure baseline serum inflammatory cytokine concentrations, MSNA, BRS, HRV and cardiovascular responses to mental stress before and after TNF-α inhibition.

8	In patients with RA, elevated concentrations of circulating inflammatory cytokines are associated with increased QTc interval. Associations between cytokines and QTc interval are affected by the QT correction method used.	An observational cohort study was undertaken to measure baseline serum inflammatory cytokine concentrations, QT interval and relevant clinical parameters.
9	During acute stress, surges in serum cortisol elicit deleterious changes in ANS function that may predispose an individual to adverse cardiac events. HCN: • Reduces cBRS; • Reduces HRV; • Increases BP variability; and • Exaggerates cardiovascular responses to the CPT and PASAT mental stress test.	A randomized placebo-controlled single-blinded cross over study was performed in healthy male volunteers assessing cBRS, HRV, BP variability and cardiovascular reactivity to a CPT and PASAT mental stress test 3 hours after bolus infusion of HCN or placebo.

CHAPTER 2 Background literature review

2.1 Rheumatoid arthritis (RA)

Rheumatoid (adjective, Greek "*rheuma*" meaning "*that which flows*" and "*-oid*" meaning "*to see*") arthritis (noun, Greek meaning "*inflammation of a joint*") is a chronic inflammatory condition characterised by synovial destruction resulting in joint pain, stiffness and swelling. Its name, first proposed by the English Physician A.B Garrod (1819-1907) may reflect the flow of fluid into the inflamed joint or indeed the relapsing/remitting nature of the condition. RA affects patients in a heterogeneous fashion ranging from mild disease to severe advanced disease with marked joint deformity and disability (Figure 2.1). At a given moment a typical RA patient may be in a state ranging from low or high disease activity (remission and flare, respectively).

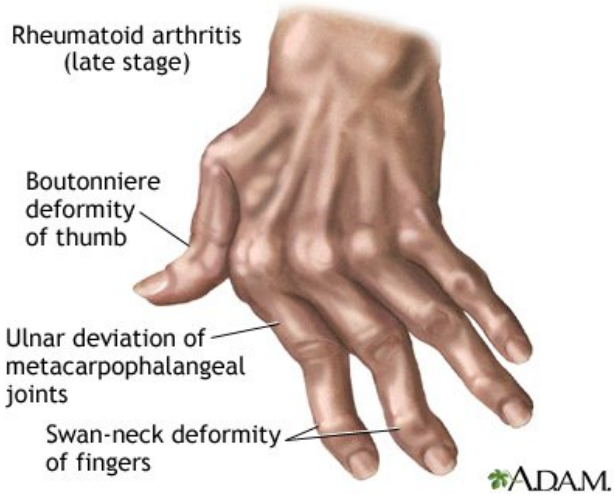


Figure 2.1 Image demonstrating severe joint deformity in a patient with RA.

A.D.A.M. Medical Encyclopedia [Internet]. Atlanta (GA): A.D.A.M., Inc.; ©2005. Rheumatoid arthritis; [updated 22 January 2014; cited September 15 2015]. Available from: http://www.nlm.nih.gov/medlineplus/ency/article/000431.htm

2.1.1 Epidemiology

RA affects approximately 0.8 % of the United Kingdom adult population (1), 0.1-0.5 % of the European population (2, 3) and 0.24 % worldwide (4). RA predominantly affects women (2:1 preponderance) (2, 4) with a higher prevalence in women over 60 years (1).

2.1.2 Pathophysiology

Although the precise pathophysiology remains poorly understood, RA is considered an autoimmune condition thought to be triggered by environmental and lifestyle factors in individuals with a genetic predisposition (5, 6). Hormonal factors, infectious agents and smoking have all been implicated in the development of RA. Autoantibodies bind to and destroy synovial cells within the affected joint leading to activation of the immune system resulting in localised and systemic inflammation. The immune basis of RA is discussed in more detail in section 2.2.3.

2.1.3 Clinical features and diagnosis

RA predominantly affects the small joints of the hands and wrist although other joints may also be affected including the elbows, shoulders, knees, ankles and spine. Symptoms include joint pain, stiffness and swelling which can limit mobility and cause mild to severe disability (4). Clinical examination may reveal tender, swollen joints with reduced range of movement as well as associated muscle wasting and signs of deformity (e.g. ulnar deviation, swan-neck deformity of the fingers, Figure 2.1). In some patients other organs aside from the joints may also be affected giving rise to extra-articular manifestations and their associated signs and symptoms (7) (Table 2.1).

Table 2.1 Extra-articular manifestations of RA (7)

Manifestation	Clinical features
Cardiovascular	
Pericarditis, pericardial effusion, myocarditis, endocarditis, congestive cardiac failure, myocardial infarction, hypertension, arterial stiffness	Chest pain, breathlessness, leg swelling
Pulmonary	
Pulmonary fibrosis, bronchiolitis obliterans, pleural effusions, pulmonary nodules	Breathlessness, haemoptysis, cough
Skin	
Rheumatoid nodules, skin rash, pyoderma gangrenosa, vasculitis	Pain, lumps, rash
Ocular	
Keratoconjunctivitis sicca, scleritis, episcleritis, xerostomia, retinal vasculitis	Visual disturbance, eye redness, soreness, discharge, dry eyes
Oral	
Sjogren's syndrome	Dry mouth

Gastrointestinal Mesenteric vasculitis, drug induced gastritis/gastric ulcer	Abdominal pain, intestinal bleeding, perforation, haematemesis
Renal Glomerulonephritis, vasculitis, amyloidosis	Anuria, oliguria, polyuria, haematuria, breathlessness, swelling
Neurological Neuropathy, carpal tunnel syndrome, cervical myelopathy	Limb weakness, pain, limited mobility/function
Haematological Anaemia (drug induced, chronic disease, nutritional, gastrointestinal bleeding, bone marrow suppression, ineffective erythropoeisis, Felty's syndrome*), neutropenia, thrombocytopenia, thrombocytosis, lymphadenopathy, splenomegaly, hepatomegaly, haematological malignancy	Breathlessness, chest pain, fatigue, bleeding, thrombosis, susceptibility to infections

* Felty's syndrome is a combination of RA, splenomegaly and neutropenia.

The diagnosis of RA is based upon a combination of clinical (history, examination), laboratory and radiographic features (5). The diagnosis of RA can often be difficult to establish as there is no specific test to diagnose RA and often there is an overlap of features with other rheumatological conditions. The American College of Rheumatology (ACR) provide guidelines to help with the classification and diagnosis of RA (8) (Table 2.2). For the classification of RA a patient needs to satisfy at least 4 of the 7 criteria, while criteria 1 to 4 must have been present for at least 6 weeks. Using the ACR 1987 criteria allows distinction of RA from other conditions with a sensitivity and specificity of 94 % and 89 %, respectively (8).

Laboratory investigations are important for the diagnosis of RA, as well as monitoring disease activity and response to treatment (5). Autoantibodies including serum rheumatoid factor (RF, antibodies directed against the Fc portion of immunoglobulin G) and anti-cyclic citrullinated peptide (anti-CCP) may be present in 60-80 % and 65 % of RA patients, respectively (9). The presence of RF and anti-CCP autoantibodies aids in the diagnosis of RA, while providing prognostic information indicating more severe disease (9, 10). Laboratory markers of inflammation include high sensitivity C-reactive protein (hs-CRP, a non specific inflammatory marker) and erythrocyte sedimentation rate (ESR). While plain radiographs of the joints have been traditionally used to diagnose RA, newer modalities have emerged including ultrasound, computed tomography and magnetic resonance imaging which can help with the diagnosis (5).

Table 2.2 The ACR 1987 revised criteria for the classification of RA (8)

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 hour before maximal improvement
2. Arthritis of 3 or more joint areas	At least 3 joint areas* simultaneously have had soft tissue swelling or fluid (not bony overgrowth alone) observed by a physician.
3. Arthritis of hand joints	At least 1 area* swollen in a wrist, MCP, or PIP joint
4. Symmetric arthritis	Simultaneous involvement of the same joint areas* on both sides of the body†
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surfaces, or in juxtaarticular regions observed by a physician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the results has been positive in <5 % of normal control subjects
7. Radiographic changes	Radiographic changes typical of RA on postero-anterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints (osteoarthritis changes alone do not qualify)

* The 14 possible areas are right or left PIP, MCP, wrist, elbow, knee, ankle, and MTP joints. † Bilateral involvement of PIPs, MCPs, or MTPs is acceptable without absolute symmetry. MCP = metacarpal joint, MTP = metatarsal joint, PIP = proximal interphalangeal joint

2.1.4 Management and prognosis

The management of RA requires a holistic multidisciplinary approach (e.g. doctors, nurses, physiotherapists, occupational therapists, and psychologists) and may involve pharmacological agents (e.g. analgesia, disease modifying drugs and biological agents, Table 2.3), non-pharmacological interventions (e.g. aids to improve mobility and disability, lifestyle changes), regular assessment of disease activity and invasive interventions (e.g. orthopaedic surgery, spinal cord nerve stimulator implant). In the United Kingdom (UK) guidelines exist to help aid clinicians in the management of patient with RA (Table 2.4) (11-13).

Assessment of disease activity is crucial in the management of RA, in order to help guide choice of treatment and monitor response to treatment (5). Traditionally, a count of the number of swollen or tender joints on clinical examination was used to assess disease severity. One of the earliest scores used was the Ritchie articular index. Developed in the 1960s, the Ritchie articular index is a composite score based on the number of swollen joints (out of 52 joints) and degree of tenderness (14). The Thompson index, developed in the 1980s, is also based on number of swollen and tender joints (of 38 joints) but weighted according to the surface area of the particular joint (15). The disease activity score 28 (DAS28) is now the most widely accepted measure of disease activity and is a based on a composite of the number of tender or swollen joints (of 28 joints based on clinical examination), the serum ESR (DAS28-ESR) or CRP concentration (DAS28-CRP), and a visual analogue scale (VAS) (16, 17). For the VAS, the patient is asked to make a "global assessment of health" by marking how they feel their disease has been affecting them (usually over the prior week) on a simple scale ranging from 0 (bad) to 10 (good) (16). The composite score provides an assessment of disease activity including disease remission (<2.6), low (2.6-3.2), moderate (3.2-5.1) and high (>5.1) disease activity. In addition, there are a number of

useful questionnaires including the Health Assessment Questionnaire (HAQ) also known as the Stanford HAQ, which provides an assessment of a patient's functional status/disability (i.e. the impact of RA on the patient's ability to carry out normal daily activities) (18). The ACR improvement criteria have been developed for use in clinical trials to help standardise outcome measures with various treatments (5) but are also useful for clinicians as a measure of response to treatment. Falls in number of affected joints, global health assessments, and laboratory markers of inflammation are included as a composite score e.g. ACR 20, ACR 50, ACR 70 indicate 20, 50 and 70 % improvements, respectively.

Patients with RA have a considerably worse prognosis compared to the general population. They have an approximately doubled risk of mortality and 10-year shortening of life span (19). Cardiovascular disease accounts for up to 50 % of the mortality in RA, with a doubled risk of myocardial infarction (MI) and sudden cardiac death (20-23). While traditional cardiovascular risk factors are present in RA (21, 24-33), they do not wholly account for the substantially increased risk seen and hence novel mechanisms are being sought (34-38). One potential mechanism to be explored is ANS dysfunction and its interaction with the immune system.

Table 2.3 Pharmacological agents used in the treatment of RA

Agent	Structure/mechanisms
Analgesics	
Paracetamol	COX inhibitor
Weak opioids	Mu-opioid (e.g. codeine), mu-opioid and monoaminergic (e.g. tramadol)
Strong opioids	Mu-opioid (e.g. morphine, fentanyl)
Antidepressants	Tricyclic antidepressant (e.g. amitriptyline), selective serotonin re-uptake inhibitors (e.g. fluoxetine), serotonin-epinephrine reuptake inhibitors (e.g. venlafaxine)
Anticonvulsants	GABA structural analogue (e.g. gabapentin), iminodibenzyl derivative (e.g. carbamazepine)
Anti-inflammatory	
Non-steroidal anti-inflammatory drugs	Non-selective COX inhibitor (e.g. ibuprofen), selective COX-2 inhibitor (e.g. celecoxib), salicylate (e.g. aspirin)
Steroids	Oral glucocorticoid (e.g. prednisolone), intra-muscular glucocorticoids (e.g. methylprednisolone)
Disease modifying anti-rheumatic drugs	
Methotrexate	Inhibits *dihydrofolate reductase* (enzyme required for DNA, RNA and protein synthesis)

Hydroxychloroquine	Anti-malarial
Sulphasalazine	Mechanism not fully understood but thought to have anti-inflammatory effects due to its metabolites (5-aminosalicylic acid and sulfapyridine)
Leflunomide	Inhibits pyrimidine synthesis (required for DNA and RNA synthesis)
Azathioprine	Inhibits purine synthesis (required for DNA and RNA synthesis)
Cyclosporin	Inhibits calcineurin reducing activity of T-lymphocytes
Mycophenolate	Inhibits B- and T-lymphocyte proliferation
Penicillamine	Inhibits T-lymphocyte, macrophage function
Gold	Unknown mechanism
Biologic agents	
TNF-α inhibitors	
Adalimumab	Human monoclonal antibody
Certolizumab pegol	Pegylated humanised Fab' fragment of an anti-TNF-α monoclonal antibody
Etanercept	TNF-α receptor-Fc fusion
Golimumab	Human monoclonal antibody

Infliximab	Chimeric monoclonal antibody
Interleukin-6 inhibitor	
Toclizumab	Humanised monoclonal antibody
Interleukin-1 inhibitor	
Anakinra	Interleukin-1 receptor antagonist
CD20 inhibitor cell depleting agent	
Rituximab	Chimeric monoclonal antibody
CD80 and CD86 inhibitor	
Abatacept	CTLA4-Ig fusion protein

COX = cyclo-oxygenase, CTLA4-Ig = Cytotoxic T-lymphocyte-associated antigen 4 and the Fc fragment of Immunoglobulin subclass G1, DNA = de-oxyribonucleic acid, GABA = gamma-aminobutyric acid, RNA = ribonucleic acid, TNF = tumour necrosis factor.

Table 2.4 Summary of NICE recommendations for management of symptoms in RA (adapted from (12))

General recommendations
Step 1 Offer oral analgesics if pain control is inadequate
Step 2 Offer an oral NSAID or COX-2 inhibitor at the lowest effective dose for the shortest time with a proton pump inhibitor
Step 3 If NSAIDs or COX-2 inhibitors are not providing adequate control of symptoms then consider disease modifying or biological drugs
For patients with newly diagnosed active disease
Offer combination therapy with disease modifying drugs as first line treatment ideally within 3 months of onset of persistent symptoms (Methotrexate and at least one other, plus short course of glucocorticoids)
If combination therapy not possible then use monotherapy and escalate dose to achieve effective response
Offer short-term oral, intramuscular or intra-articular glucocorticoids to rapidly improve symptoms
For patients with recent onset disease (within last 2 years)
If sustained and satisfactory disease control achieved then attempt to cautiously reduce dose
For patients with established disease (more than 2 years)
If the disease is stable cautiously reduce dose of disease modifying or biological drugs but

return promptly to disease controlling dose at the first sign of a flare

When introducing new drugs to improve disease control consider decreasing or stopping pre-existing drugs once the disease is controlled

If doses of disease modifying or biological drugs are being decreased then arrange a prompt review

Glucocorticoids

Offer short-term glucocorticoid treatment to control flare-ups

Continue long term treatment with glucocorticoids only after discussing with the patient the long term complications and after offering all other treatment options (including biological drugs)

COX = cyclo-oxygenase, NICE = national institute for health and clinical excellence, NSAID = non steroidal anti-inflammatory drug,

2.2 Immune system

2.2.1 Basic overview

The immune system is an important system involved in protection against pathogens (noun, Greek "*pathos*" meaning "*suffering, disease*"). The first line of defence against pathogens involves barriers such as the skin and surface mucus; the innate (adjective, Latin "*innatus*" meaning "*inborn*") and the adaptive (also called the acquired) immune system form the next lines of defence (39). Immune cells of the innate system may recognise molecules within the pathogen resulting in activation. Phagocytes (noun, Greek "*phago*" meaning "*to eat or devour*" and Greek "*kytos*" meaning "*hollow receptacle*" used in biology to describe a "cell") such as neutrophils, monocytes and macrophages devour pathogens (Figure 2.2), natural killer cells destroy damaged host cells, while basophils, mast cells and eosinophils release inflammatory mediators. Inflammatory mediators include cytokines (noun, Greek "*kinesis*" meaning "*movement*"), acute phase proteins and complement that act to promote migration of inflammatory and immune cells, fluid and blood to the site of the pathogen. The inflammatory response results in the clinical features known in Latin as dolor ("*pain"*), calor ("*heat"*), rubor ("*redness*"), tumor ("*swelling*") and functio laesa ("*loss of function*").

The adaptive immune response can be activated directly by the innate response, but also when antigen-presenting cells recognise pathogens or antigens (noun, Green "*anti*" meaning "*against, opposite*" and "*gen*" meaning "*thing that produces*" commonly known in biology as a "*substance that produces an antibody*") within the pathogen. Consequently, antigen-specific B-lymphocytes are stimulated to produce antibodies that act to destroy pathogens, as well as activating macrophages to destroy infected host cells. Additionally, antigen-specific T-lymphocytes are stimulated to produce T-killer cells that destroy infected host cells and T-helper cells which help mediate innate and adaptive immune responses.

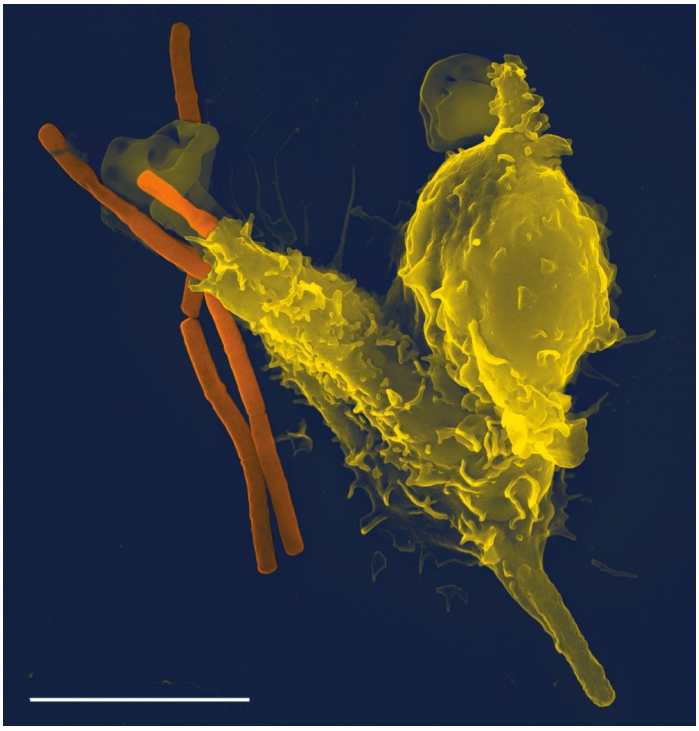


Figure 2.2 Neutrophil engulfing anthrax bacteria, taken with a Leo 1550 scanning electron microscope. Scale bar is 5 micrometers. "Neutrophil with anthrax copy" by Volker Brinkmann - (November 2005).

"Neutrophil engulfing Bacillus anthracis". PLoS Pathogens 1 (3): Cover page. DOI:10.1371. Retrieved on 2009-01-04. Licensed under CC BY 2.5 via Wikimedia Commons - https://commons.wikimedia.org/wiki/File:Neutrophil_with_anthrax_copy.jpg#/media/File:Neutrophil_with_anthrax_copy.jpg

2.2.2 Cytokines

Cytokines are peptides that are released by immune cells to help with modulation of the immune response (39, 40). Cytokines can have pro- or anti-inflammatory properties or both; endothelial functions; or platelet actions. Examples of cytokines include interleukins (IL), TNF, interferon (IFN), cell adhesion molecules and fibrinogen. While cytokines have an essential role in modulation of the immune response during acute inflammation, dysregulation can have deleterious consequences such as in sepsis and chronic inflammatory conditions including RA.

2.2.3 Immunological basis of RA

RA is considered an auto-immune condition (6). Autoantibodies attach to synovial cells within synovial joints causing destruction and activation of the immune system. Localised inflammation ensues which may lead to systemic inflammatory responses. While acute inflammatory responses are protective, chronic inflammation leads to deleterious consequences (41).

The adaptive immune system is thought to play an important role in the early pathogenesis of RA, however both adaptive and innate immune systems are co-activated resulting in a milieu of inflammatory molecules within the synovial joint (6, 42-44). The inflammatory process is regulated by a host of feedback mechanisms and a complex interaction between various structures within the inflamed joint including fibroblasts, synoviocytes, chondrocytes, osteoclasts, macrophages, neutrophils, mast cells, plasma cells and T cells (T helper-1, Th1 and T helper-2, Th2).

Cytokines are key regulators of the inflammatory response in RA (6). The most important cytokines include IL-6, TNF-α, IL-1, IL-17, IFN and IL-10 (Table 2.5). IL-6 has both pro- and anti-inflammatory actions and activates the production of the acute phase molecule CRP from liver cells (40). IL-1 (alpha and beta, IL-1α and IL-1β), IL-17, TNF-α and IFN have pro-inflammatory actions whilst IL-10 has anti-inflammatory properties and hence helps to regulate the inflammatory response (45). Th1 cells secrete the cytokines IL-2, IFN-γ and inhibit Th2 cell activity. Th2 cells secrete the cytokines IL-4, IL-5 and IL-6 while inhibiting Th1 cell activity. Th1 cell activity is mainly related to cell-mediated or innate immunity (i.e. activation of macrophages or cytotoxic T cells) whilst Th2 cell activity is largely related to humoral or acquired immune responses (i.e. production of antibodies by B cells) (39). In RA, Th1 cell (43, 44) and Th17 cell (42) activity predominates within the

inflamed synovium (39). The immune-pathophysiology of RA is complex and not fully understood, however the development of immune targeted therapies which specifically target and inhibit cytokines (IL-6, TNF-α, IL-1) and B cell activity has helped improve understanding (6). The efficacy of TNF-α inhibitors as demonstrated in randomised clinical trials supports the deleterious role of TNF-α in the pathogenesis of RA (46-48).

It has been long known in clinical practice that RA patients are a heterogenous group with differing disease patterns, severity and responses to treatments (1, 5). Indeed, these targeted immune modifying agents (biological therapy) have varying efficacy suggesting non-uniform pathological mechanisms in RA (49, 50). It remains unclear why certain patients appear to have more severe disease activity and more pronounced systemic inflammatory responses. Recent developments suggest that the ANS may play a fundamental part in immune regulation (51) and hence ANS dysfunction may lead to immune dysregulation. Furthermore accumulating animal data has shown that inflammatory cytokines exert deleterious effects on key cardiovascular regulatory sites of the ANS (52-61), which suggests a perpetual cycle of inflammation leading to ANS dysfunction and further inflammation. Given that inflammation and ANS dysfunction are recognised pathological features of numerous cardiovascular diseases including hypertension, atherosclerosis and chronic heart failure (51, 62-71), ANS-immune dysregulation may well contribute to the increased cardiovascular risk in RA. Further work is required to ascertain underlying mechanisms that drive immune-dysregulation in order for appropriate therapies to be developed and targeted to individual patients.

Table 2.5 Key cytokines in the pathophysiology of RA

Cytokine	Important actions
TNF-α	Produced by macrophages, natural killer cells, T cells, B cells and mast cells. Pro-inflammatory actions. Activates leucocytes, endothelial cells and synovial fibroblasts. Stimulates production of cytokines, adhesion molecules and matrix enzymes. Inhibits T cell. Activates osteoclasts. Bone resorption.
IL-1α, IL-1β	Produced by macrophages. Pro-inflammatory actions. Activates leucocytes, endothelial cells and synovial fibroblasts. Stimulates production of matrix enzymes from chondrocytes. Activates osteoclasts. Increases glucose metabolism.
IL-6	Produced by Th2 cells, macrophages. Pro- and anti-inflammatory actions. Activates leucocytes and osteoclasts. Stimulates an acute-phase response with production of CRP in hepatocytes. Regulates lipid metabolism. Mediates fever, chronic anaemia.
IL-17	Produced Th17 cells Activates synovial fibroblasts, chondrocytes and osteoclasts.
IL-10	Produced by monocytes, macrophages, Th1 cells Anti-inflammatory actions. Inhibits synthesis and actions of TNF-α, IL-1β, IL-6. Stimulates B cell maturation and antibody production.

CRP = C-reactive protein, IL = interleukin, Th = T helper, TNF = tumour necrosis factor.

2.3 Autonomic nervous system (ANS)

2.3.1 The sympathetic and parasympathetic nervous systems

The autonomic (adjective, Greek "*auto*" meaning "*self*" and "*nomos*" meaning "*law*") nervous system is the part of the nervous system that regulates physiological processes without conscious control (72). The term "autonomic nervous system" was first coined by the English Physiologist John N. Langley (73). He divided the ANS into three components: sympathetic (adjective, Greek "*sympathos*" meaning "*affected by feelings*"), parasympathetic (adjective, Greek "*para*" meaning "*alongside*") and enteric nervous systems. The ANS is now recognised as a complex network comprising central structures (within the brain and spinal cord), ascending and descending pathways and peripheral nerves (afferent and efferent) that exert their effects on "end-organs" or "effector organs". The purpose of the ANS is to maintain homeostasis (noun, Greek "*homio-*" meaning "*like, resembling, of the same kind*" and "*stasis*" meaning "*standing still*") within the body (e.g. body temperature, BP, brain blood flow). The term "homeostasis" was coined by the American Physiologist Walter B. Canon, whereby he proposed that the brain coordinates the body systems to maintain a set of goal values for internal variables (74). Perturbations in these values, whether by internal or external factors arouse internal nervous and hormonal systems to re-establish homeostasis.

Canon also proposed that the sympathetic nervous system was responsible for the "fight-or-flight" response (74) (peripheral vasodilation, increased HR and BP, increase respiration, mobilisation of glucose into the blood, cutaneous vasoconstriction). During periods of stress (physical or emotional) the sympathetic nervous system is activated to prepare the body to respond to the particular stress and maintain homeostasis. The sympathetic nervous system consists of pre-ganglionic neurons found within the lateral grey column of the spinal cord, the majority within the intermediolateral (IML) cell column

(Figure 2.3), which synapse within ganglia at various sites (paravertebral: cervical, thoracic, lumbar, sacral; prevertebral: celiac, aorticorenal, superior mesenteric and inferior mesenteric; chromograffin cells within the adrenal medulla) and post-ganglionic neurones project to end/effector organs. The myocardium and peripheral vasculature are innervated by sympathetic nerves and can be activated directly or indirectly, increasing cardiac contractility and peripheral vasoconstriction resulting in a rise in arterial BP. The principle post-ganglionic neurotransmitter of the sympathetic nervous system is nor-epinephrine (noun, Greek "*epi*" meaning "*upon*" and "*nephros*" meaning "*kidney*"[1], also known as noradrenaline), with acetylcholine (Ach) as the principle pre-ganglionic neurotransmitter. Noradrenaline exerts its effects by binding to β-adrenoreceptors in the heart and α-adrenoreceptors in the peripheral vasculature. Other important co-transmitters include adrenaline (epinephrine), adenosine triphosphate, neuropeptide-Y (NPY), somatostatin and opioid peptides.

The parasympathetic nervous system is responsible for a number of processes that promote "rest and digest" (75). Activation of the parasympathetic nervous system facilitates numerous activities including digestion (stimulation of gastric acid section and gut smooth muscle contraction), salivation, lacrimation, sexual arousal, urination and defecation. The parasympathetic nervous system works alongside the sympathetic nervous system to maintain homeostasis. In contrast to the sympathetic nervous system the pre-ganglionic cell bodies of the parasympathetic nervous system are found within two sites: the brain stem and sacral spinal cord (72) (Figure 2.3). Important central parasympathetic structures include the dorsal nucleus of the vagus nerve (numerous activities including HR regulation, gastrointestinal motility, thermoregulation), the nucleus ambiguus (HR regulation), the periaqueductal gray (pain modulation) and parabrachial nucleus. Pre-ganglionic neurones project and synapse

[1] so called as the adrenal glands sit upon the kidneys.

within parasympathetic ganglia located within the effector organs. Post-ganglionic neurones are typically very short (1-2 mm in length). The vagus (noun, Latin "*vagus*" meaning "*wandering*"[1]) nerve innervates the heart, lung and visceral organs, and constitutes approximately 75 % of all parasympathetic fibres[2]. Acetylcholine (noun, "*acetyl*" refers to the methyl group single-bonded to a carbonyl, "*choline*" derived from "*cholera*" Greek "*khole*" meaning "*bile*") is the principle neurotransmitter of the parasympathetic nervous system but nitric oxide (NO) can also be used (76). Important co-transmitters include vasoactive intestinal peptide (77), calcitonin gene-related peptide (78), somatostatin (79) and opioid peptides (80).

2.3.2 Integrative control of the cardiovascular system

The ANS plays a critical role in the regulation of HR and arterial BP through a number of reflex neural circuits (72, 81, 82). A multitude of inputs from sensory afferents including arterial baroreceptors, cardiopulmonary receptors, peripheral and central chemoreceptors as well as somatic and visceral receptors (including nociceptors) synapse within the medulla oblongata in the brain stem, and particularly the nucleus of the solitary tract (NTS). The specific pattern of autonomic response that arises as a consequence of the activity of these neural inputs is also dependent upon modulatory inputs from other brainstem regions, the hypothalamus, higher neural structures (e.g. amygdala, cerebral cortex) and circumventricular organs (e.g. area postrema, arcuate [infundibular] nucleus, subfornical nucleus). The circumventricular organs are found within the brain but lack a brain blood barrier. It is thought that circulating molecules (e.g. hormones) (81, 83) can modulate ANS

[1] so called due to its wandering course from the brainstem to the neck, chest and abdomen

[2] the term "vagal" and parasympathetic are used interchangeably

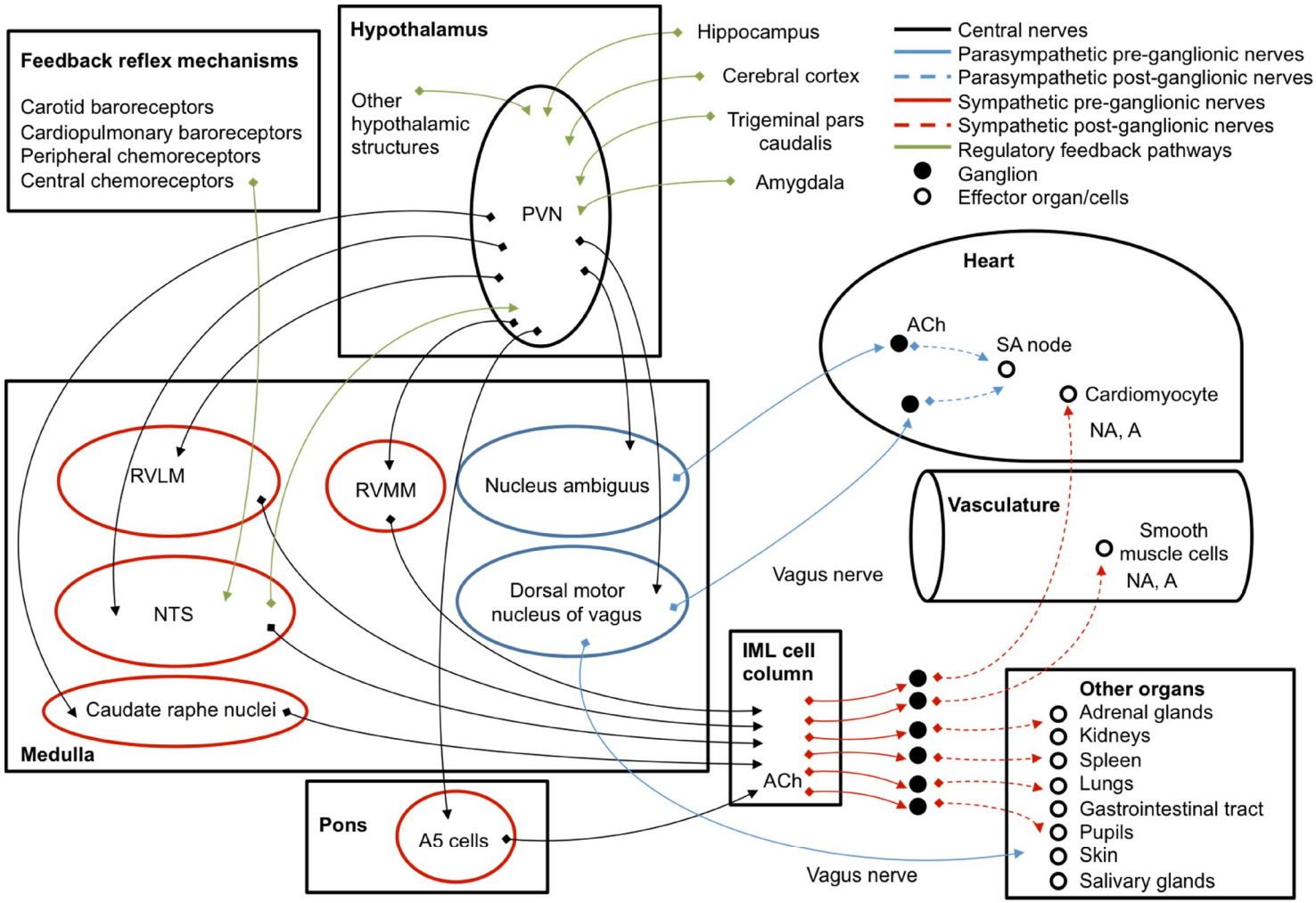


Figure 2.3 Simplified schematic representation of the ANS

This figure is a simplified representation of parasympathetic (blue) and sympathetic (red) activation within the ANS, with regulatory (green) pathways. Central nerve fibres project from the PVN in the hypothalamus to the nucleus ambiguus and dorsal motor nucleus of the vagus within the medulla. Pre-ganglionic parasympathetic fibres project from the nucleus ambiguus and the dorsal motor nucleus of the vagus to synapse with parasympathetic ganglion (closed circles) within the heart. Post-ganglionic parasympathetic nerves project from these ganglia to the SA node to modulate HR. Pre-ganglionic parasympathetic fibres (i.e. the vagus nerve) project from the dorsal motor nucleus of the vagus to other effector organs (open circles). Additionally pre-ganglionic parasympathetic fibres project from the sacral spine to the gastrointestinal and genitourinary tract. Central nerve fibres project from the PVN to nuclei within the medulla (RVLM, RVMM, NTS and caudate raphe nuclei) and pons (A5 cells). Further descending nerve fibres project to the spinal cord (IML). From there pre-ganglionic sympathetic fibres project to and synapse with sympathetic ganglion. The IML cell column contains the most sympathetic ganglia although present in other regions (e.g. coeliac, mesenteric ganglia). Post-ganglionic sympathetic fibres project to cardiomyocytes within the heart, and smooth muscle cells within the peripheral vasculature to allow modulation of BP through changes in cardiac contractility and peripheral vasoconstriction/dilation. The PVN additionally receives input (from the hippocampus, cerebral cortex, trigeminal pars caudalis, amygdala and other hypothalamic structures) to allow modulation of sympathetic and parasympathetic activity. Additionally there are reflex feedback mechanisms that regulate autonomic activity (e.g. carotid and cardiopulmonary baroreceptors, peripheral and central chemoreceptors). Ach, NA and A neurotransmitters are shown. Interconnections between nuclei within the medulla, or between the medulla and pons are not shown. A = adrenaline, Ach = acetylcholine, BP = blood pressure, HR = heart rate, IML = intermediolateral, NA = noradrenaline, NTS = nucleus of the solitary tract, PVN = paraventricular nucleus, RVLM = rostral ventrolateral medulla, RVMM = rostral ventromedial medulla, SA = sino-atrial.

function by binding to receptors within the circumventricular organs, which project to centralANS sites such as the hypothalamus, or to autonomic motor neurons in the spine or brainstem (84). Important mediators include angiotensin II, natriuretic peptides and leptin (83). Collectively, these integrated responses represent the main cardiovascular reflex pathways, i.e. arterial baroreflex, cardiopulmonary reflex, chemoreflex, diving reflex, oculocardiac reflex, startle reflex, somatic nociception and centrally evoked "defence responses" (81, 82, 85).

The hypothalamus, brain stem and limbic structures (including the amygdala, hippocampus, cerebral cortex) are important central structures implicated in the regulation of ANS activity (Figure 2.3) (72, 81). The paraventricular nucleus (PVN), within the hypothalamus receives projections from hypothalamic, brain stem and limbic structures and additionally receives sympathetic and parasympathetic afferent signals from the trigeminal pars caudalis (sympathetic) and the NTS (parasympathetic). Consequently, the PVN is an

important ANS site as it forms connections with both the sympathetic and parasympathetic pathways. Other important central sympathetic structures include the caudal and rostral ventrolateral medulla (RVLM, BP and HR regulation), the pontine and medullary raphe (serotonin mediated control of circadian rhythms) and the lateral hypothalamus (numerous activities including arousal, thermoregulation, gastrointestinal motility, pain modulation, respiration, micturition). The RVLM within the medulla is a key cardiovascular regulatory site, receiving inputs from the hypothalamus and caudal ventrolateral medulla (CVLM) nucleus, while projecting sympathetic pre-ganglionic neurones to the IML cell column allowing sympathetic modulation of the heart and peripheral vasculature (i.e. via arterial baroreflex as discussed in next section).

The heart is innervated by the vagus nerve at the sinus node and within the myocardium (86). Parasympathetic activation and withdrawal results in a fall and rise in HR, respectively. The myocardium and peripheral vasculature are innervated by sympathetic nerves and can be activated directly or indirectly (via noradrenaline or adrenaline acting upon β-adrenoreceptors in the heart and α-adrenoreceptors in the peripheral vasculature), increasing cardiac contractility and peripheral vasoconstriction resulting in a rise in arterial BP. However, this may be an over simplification as responses to efferent impulses are not always uniform. For example Folkow et al. demonstrated that in cats, electrical stimulation of the hypothalamus resulted in differential responses in different vascular beds as well as within the same vascular bed (87).

HR and BP can be influenced by a number of pathways involving reflex (e.g. arterial baroreflex, chemoreflex, diving reflex) and non-reflex (skeletal muscle afferent, respiratory sinus arrhythmia) mechanisms (72, 81, 82, 88, 89). The main components of these reflexes include sensors, afferent pathways, central integration, efferent pathways and effector organs.

Depending on the circumstances there may be reciprocal or non-reciprocal effects on the sympathetic and parasympathetic components of the responses (85), e.g. activation of the arterial baroreceptors results in a reflex parasympathetic activation and sympathetic inhibition resulting in bradycardia and hypotension. Conversely activation of the diving reflex in conscious rabbits results in vagally mediated bradycardia, peripheral vasoconstriction and sympathetically-mediated ventricular arrhythmogenesis (90).

Whilst one can consider each reflex as separate and distinct, in reality there are usually complex interactions between each of these reflexes depending on the clinical situation (e.g. stress, exercise, arrhythmias) (91). For example during ventricular tachycardia (VT) there are conflicting reflex actions. The fall in arterial BP during VT unloads the arterial baroreceptors causing sympatho-excitation, whilst elevations in cardiac filling pressures load the cardiopulmonary baroreceptors resulting in sympatho-inhibition (92). The net outcome is a sympathetic excitation suggesting that arterial baroreceptors have a predominant effect (45, 92, 93).

2.3.3 The arterial baroreflex

The arterial baroreflex modulates the ANS to buffer beat-to-beat fluctuations in BP (94) (Figure 2.4). Baroreceptors (mechanosensitive afferent nerve endings) within the carotid artery or aortic arch (aortic baroreceptors) respond to vessel wall distension which coincide with changes in BP, and when activated transmit afferent signals to the NTS (82). Excitatory neurons project from the NTS to the nucleus ambiguus within the medulla, which transmits signals to the heart via efferent parasympathetic fibres. Vagal pre-ganglionic neurones project to and synapse in the parasympathetic ganglia near the heart, and post-ganglionic fibres

project to the sino-atrial node (82) and myocardium (95). In addition, excitatory neurons from the NTS project to the CVLM, from which inhibitory (GABAergic) neurones project to the RVLM. Pre-ganglionic sympathetic neurones project from the RVLM to the sympathetic ganglion within the IML cell column with further post-ganglionic nerve projections to the effector organs (heart, peripheral vasculature). Therefore, baroreceptor activation results in parasympathetic activation and sympathetic inhibition, resulting in bradycardia and hypotension (91, 96, 97).

Much of our understanding of the contribution of central neural circuits to arterial baroreflex regulation has been derived from animal studies. Kollai and Koizumi (1979) demonstrated that in anaesthetised dogs baroreflex activation (achieved by injecting intravenous noradrenaline) resulted in an immediate increase in cardiac vagal nerve activity with a fall in cardiac sympathetic nerve activity that coincided with a fall in HR (97). A recent human study by Macefield and Henderson (2010) confirmed some of the findings in previous animal studies (98). Functional magnetic resonance imaging of the brain was performed with simultaneous MSNA (peroneal nerve) recordings in healthy humans. They were able to demonstrate that during spontaneous falls in BP there was a fall in NTS activity, decreased CVLM activity and increased RVLM activity. During spontaneous rises in BP the opposite was observed.

In summary, a fall in BP unloads the baroreflex causing parasympathetic inhibition at the sinus node resulting in a reflex tachycardia, and sympathetic activation of the heart and peripheral vasculature resulting in increased cardiac contractility and peripheral vasoconstriction. By contrast a rise in BP activates the baroreflex causing parasympathetic activation at the sinus node, and sympathetic inhibition at the heart and peripheral vasculature.

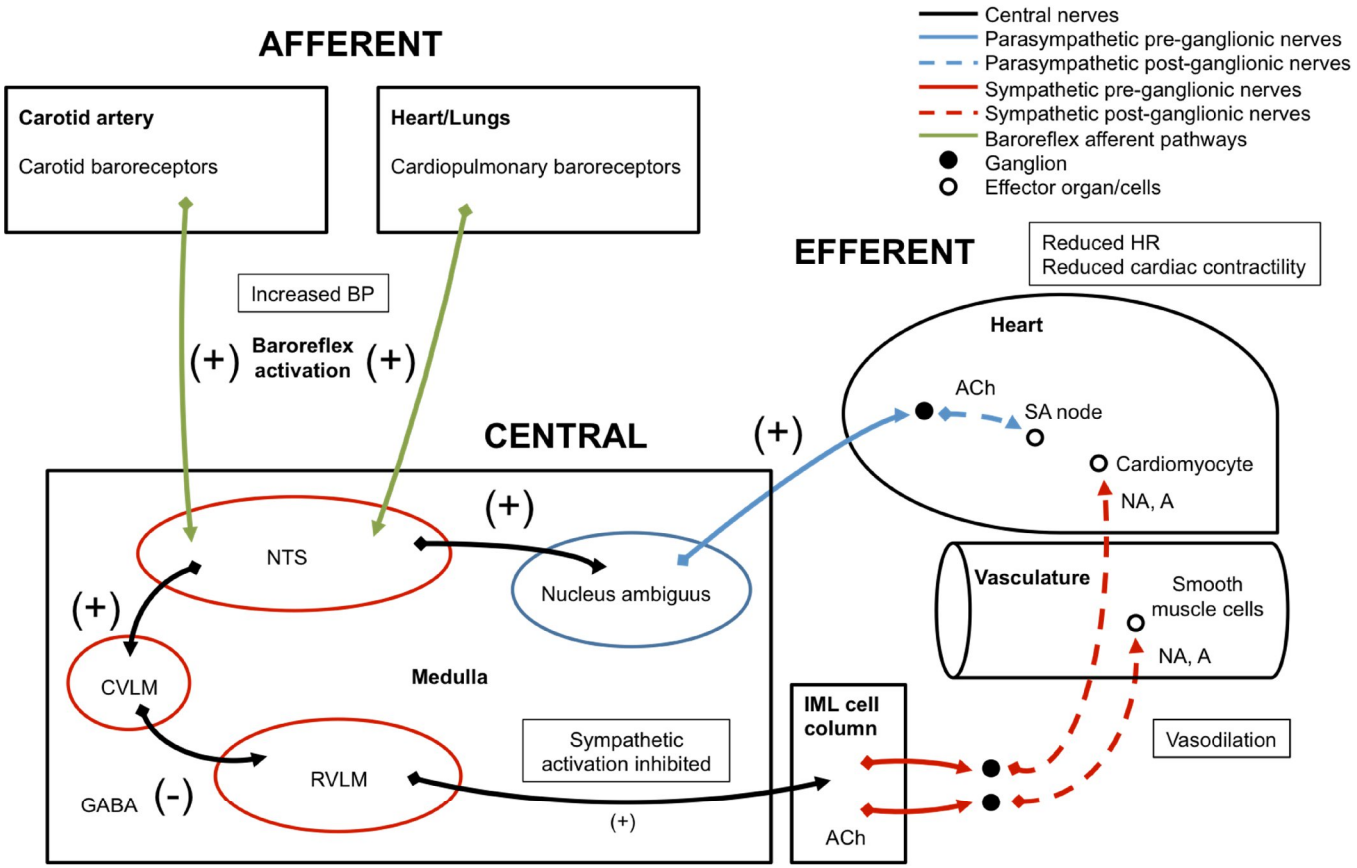


Figure 2.4 Schematic representation of the arterial baroreflex (81, 82)

This figure demonstrates the arterial baroreflex. Baroreceptors (carotid and cardiopulmonary receptors) are activated in response to increased BP. Afferent signals project to the NTS, which stimulate parasympathetic activation via the nucleus ambiguus resulting in slowing of the HR. Sympathetic inhibition occurs via the CVLM, RVLM and ILM cell column resulting in reduced sympathetic outflow to the heart and vasculature causing a fall in BP (reduced cardiac contractility, peripheral vasodilation). Ach, NA and A neurotransmitters are shown. A = adrenaline, Ach = acetylcholine, BP = blood pressure, CVLM = caudal ventrolateral medulla, HR = heart rate, ILM = intermediolateral, NA = noradrenaline, NTS = nucleus of the solitary tract, RVLM = rostral ventrolateral medulla.

BRS, also known as baroreflex gain, can be determined and used as a measure of the autonomic control of the cardiovascular system. Baroreflex gain is defined as the change in autonomic effector responses (e.g. change in HR or sympathetic outflow) for a given change in arterial BP (82). A reduced BRS indicates impairment in the ability to buffer changes in BP. Cardiovagal (or cardiac) BRS (cBRS) is a measure of vagally-mediated HR responses to BP changes whilst sympathetic BRS is a measure of sympathetic nerve activity changes in the peripheral vasculature (81, 82).

2.4 ANS dysfunction

For the body to maintain homeostasis an intact ANS is essential. The consequences of altered sympathetic and parasympathetic activity, manifest as impairments in BRS and cardiovascular reactivity will be outlined in this section. Abnormalities in the QT interval, a proxy marker of autonomic function, and the role of stress and the HPA axis will also be discussed. Evidence regarding the relationships between ANS dysfunction and inflammation will be extensively covered in the relevant sub-sections.

2.4.1 Pathophysiological consequences of sympathetic dysfunction

Failure of the sympathetic nervous system to activate appropriately can lead to deleterious consequences. For example, neuro-cardiogenic syncope is thought to be partly due to failure of sympathetic activation and failure to maintain BP and brain perfusion (99). Conditions such as diabetes or pure autonomic failure can result in debilitating symptoms of postural dizziness and fainting (100, 101).

Heightened sympathetic outflow is a pathogenic feature of numerous cardiovascular conditions including hypertension (62), chronic heart failure (63), cardiac arrhythmias (102) and ischaemic heart disease (64) as well as non-cardiovascular diseases including diabetes mellitus (103), chronic kidney disease (104), obesity (105), metabolic syndrome (106), obstructive sleep apnoea (107), chronic obstructive pulmonary disease (108), pulmonary hypertension (109), depression (110), pre-eclampsia (111) and ulcerative colitis (112). Heightened sympathetic activity has been shown to predict mortality in chronic heart failure (113). Cohn et al. (1984) observed that plasma noradrenaline concentration was an independent predictor of mortality in patients with moderate to severe heart failure, in a dose-dependent manner. The increased mortality risk increased associated with sympathetic activation may be partially explained by arrhythmogenesis (114). In anaesthetised cats, sympathetic stimulation reduced the threshold for cesium chloride-induced VT, whilst β-adrenergic blockade prevented sustained VT (115). There is also evidence to suggest that chronic sympathetic over-activity may contribute to the pathogenesis and progression of hypertension (62, 116-119), although not universally agreed upon (120). In patients with essential hypertension MSNA (62, 119), plasma noradrenaline levels (121, 122) and noradrenaline spillover from the heart and kidneys (123) are reported to be elevated. Smith et al. (2004) found that central sympathetic activity was higher in borderline hypertensives (BP 130/85 – 139/89 mmHg), early stage essential hypertensives (BP 140/90 – 159/99 mmHg) with and without left ventricular hypertrophy compared to normotensives and late stage hypertensive patients (BP ≥ 160/100 mmHg)(119). They also showed that central sympathetic activity was higher in white coat hypertensives compared to normotensive individuals. This data adds to earlier animal evidence suggesting that sympathetic nerve activity may be a causal factor in the development of hypertension. In spontaneously hypertensive rats increases

in sympathetic nerve activity (124), plasma noradrenaline (125) and vascular resistance (126) are seen prior to the development of hypertension. In chronic heart failure, sympathetic activation is thought to be a compensatory mechanism to increase cardiac contractility (cardiac sympathetic activity) and increase circulating volume via peripheral vasoconstriction and co-activation of the renin-angiotensin-aldosterone system (127). However, chronic sympathetic activation places more strain on a failing heart and serves to accelerate the progression of heart failure and can reduce survival (127). The efficacy of β-adrenergic blockade in improving symptoms and mortality in chronic heart failure patients is well established (128-133) and may well relate to the reduction in sympathetic activity (134, 135).

Chronic elevations in BP are known to cause structural and functional remodelling of the heart and vasculature leading to left ventricular hypertrophy (136, 137), vascular smooth cell hypertrophy and hyperplasia (138-140), increased arterial wall stiffness (141, 142) and endothelial dysfunction (143-145). These changes may lead to diastolic dysfunction (146, 147), chronic heart failure (148, 149) and can accelerate the process of atherosclerosis (139, 150). However there is also evidence to suggest that chronic elevations in sympathetic nerve activity may have similar deleterious effects to the vasculature, independent of BP. Bevan (1984) demonstrated that chronic sympathetic activation without a rise in BP caused vascular smooth muscle hypertrophy and proliferation (151). These changes were attenuated by sympathetic denervation (151, 152).

While the deleterious consequences of heightened SNA have been described, the precise mechanisms driving sympathetic over-activity remain to be fully elucidated. Once underlying mechanisms are identified then appropriate therapeutic interventions can be employed on a patient-specific basis.

2.4.2 Sympathetic-immune interactions

There are a multitude of pathways that may result in heightened sympathetic activity (e.g. NO pathways, angiotensin II, respiratory sympathetic coupling etc.), however given the focus of my thesis I will provide an overview of potential mechanisms whereby sympathetic-immune interactions may elevate SNA.

Accumulating evidence suggests the presence of bi-directional links between sympathetic activity and inflammation. Niijima et al. (1991) were the first to report that intravenous administration of recombinant human IL-1β increased splenic, adrenal and renal sympathetic nerve activity in anaesthetised rats (153). Haefeli et al. (1993) were the first to provide evidence that pro-inflammatory cytokines can cause sympathetic activation in humans (154). Infusion of recombinant IL-1β into humans with malignant melanoma increased HR and systolic BP, and decreased hand vein compliance (suggestive of vasoconstriction). The vasoconstriction was sympathetically mediated as it was reversed with local administration of the α-antagonist phentolamine. Subsequent animal studies have demonstrated that cytokines can increase sympathetic activity (52-61). Peripheral administration of TNF-α (58, 61) and IL-1β (54, 55, 153), and central administration of TNF-α (57, 59), IL-1β (53-57, 60) and IL-6 (52) increase sympathetic nerve activity. These studies support the concept that circulating inflammatory cytokines can increase sympathetic activity via centrally mediated mechanisms, although how this occurs is not known. Pro-inflammatory cytokines are lipophobic and too large to pass the blood brain barrier (155). One potential explanation is that circulating cytokines exert their central effects via the circumventricular organs, which lack a blood brain barrier. These organs are important and key regulating sites whereby hormones can modulate autonomic responses e.g. angiotensin, leptin (83). Helwig et al. (2008) administered IL-6 centrally in rats whilst making direct splenic sympathetic nerve

recordings (52). They found that splenic sympathetic activity increased in a dose responsive manner. Interestingly using fluorescent immunohistochemistry they demonstrated that following central infusion IL-6 was distributed within the periventricular areas of the ventricular system and not within the brain parenchyma. Wei et al. (2013) localised the site of action to the subfornical organ (SFO) (58), a forebrain circumventricular organ. In their experiment intra-carotid administration of TNF-α and IL-1β in rats increased HR, mean BP and renal sympathetic nerve activity. These changes were significantly reduced in rats with SFO lesions. Immunofluorescent staining localised a dense distribution of TNF-α and IL-1 receptors within the SFO. Other potential explanations include disruption of the blood-brain barrier (as occurs in bacterial meningitis); blood-brain barrier transporters; activation of cytokine receptors on afferent nerve fibres; or via activation of microglia (inflammatory cells within the brain), which release chemokines that attract immune cells to the brain parenchyma (155, 156). Cytokines may potentially enter the brain via the blood-cerebrospinal fluid barrier. Recent experiments have shown that concentrations of IL-1β within the cerebrospinal fluid of RA patients appear to be elevated (157, 158).

It has long been known that immune organs such as the spleen and bone marrow have sympathetic innervation (159-161). Furthermore, immune cells have been shown to express α- and β-adrenergic receptors on their cell surface. Therefore the immune system has the potential to be modulated by the sympathetic nervous system. There is a growing body of evidence showing that catecholamines can affect the release of cytokines by increasing Th2 responses (humoral) and inhibiting Th1 responses (cellular immunity). Catecholamines inhibit IL-1 (162, 163), IL-2 (164), IFN-γ (165, 166), TNF-α (167-171) and promote release of IL-10 (170, 172-175), IL-6 (168, 176), transforming growth factor-β (177) and IL-8 (163, 178). During acute inflammation there is activation of the HPA axis and sympathetic nervous

system (179), which help equip the body to deal with invading pathogens. From the aforementioned studies it appears that a degree of sympathetic activation may be protective. Current understanding is that sympathetic activation results in a shift from Th1 activity (cellular) to Th2 activity (humoral), resulting in a more specific immune response that limits the inflammatory response to local and specific targets (159). Therefore sympathetic activation may protect the host from the deleterious effects of systemic inflammation. A recent animal study supports this. Martelli et al. measured the inflammatory responses to lipopolysaccharide (LPS) in rats and demonstrated that dissecting the splanchnic nerve (thereby reducing splenic nerve activity) increased the plasma TNF-α responses five-fold (180).

Chronic sympathetic activation however may have pro-inflammatory effects. Studies have consistently shown that conditions associated with chronic sympathetic activation such as hypertension and chronic heart failure are characterised by low-grade inflammation (51, 65-71) although whether this is a cause or effect remains a matter of debate. In animal studies chronic β-adrenergic stimulation with isoproterenol (non selective β-adrenergic agonist) increased plasma concentrations of IL-1β and IL-6 and tissue IL-1β within the pituitary, hypothalamus, hippocampus (181) and increased tissue IL-1β, IL-6 and TNF-α within the myocardium (182). A number of studies have assessed the effects of sympathetic inhibition on inflammation in heart failure and hypertension (183). β-blockers have been shown to reduce concentrations of IL-6 (184-186), TNF-α (184-187), IL-1 (187) and IL-18 (188) (a pro-inflammatory cytokine) whilst increasing IL-10 (188) in chronic heart failure. In one study, renal sympathetic denervation was found to reduce circulating concentrations of IL-6 and hs-CRP in hypertensive patients after 6 months (189). In another study sympathetic blockade with clonidine was found to reduce MSNA and disease activity in patients with

active chronic inflammatory bowel disease however unfortunately the authors did not assess inflammatory cytokines or CRP (112). While these studies suggest that chronic sympathetic activity may have pro-inflammatory effects the exact mechanisms are not known.

The results of these experiments have major implications for RA. It is possible that chronic inflammation as seen in RA may lead to chronic elevations in sympathetic nerve activity leading to deleterious cardiovascular consequences thereby increasing cardiovascular risk and mortality. It is also possible that chronic sympathetic activation in RA may perpetuate inflammation resulting in a "vicious cycle" of sympathetic activation and chronic inflammation. The ANS may therefore be a potential therapeutic target in RA in order to control inflammation and reduce cardiovascular risk. Evidence to date however suggests that a degree of sympathetic activation is needed in order to control inflammatory responses, and therefore reduced sympathetic activity may potentially trigger the onset of RA or exacerbate flares. Chronic sympathetic activation may also cause a down regulation of β2-adrenergic receptors rendering the individual less equipped to deal with acute flares. The relationship between the sympathetic nervous system and inflammation in RA is potentially complex and requires further exploration, to try and gain insights into the pathophysiology of the disease and improve understanding of the increased cardiovascular risk so that therapeutic targets can be developed and effective treatments employed.

A major aim of my thesis is to determine whether elevated sympathetic nerve activity is a pathogenic feature of RA and to explore the relationship between sympathetic activity and inflammation. To achieve this I have performed a systematic literature review (Chapter 4), a case-control study assessing MSNA in RA patients, as compared to healthy and hypertensive controls (Chapter 6) and a case study of an RA patient where MSNA was measured before

and three months following anti-TNF-α therapy (Chapter 8). To my knowledge these are the first MSNA recordings in RA human subjects.

2.4.3 Parasympathetic dysfunction

Inappropriate cardiac parasympathetic stimulation can result in deleterious consequences. The excessive increases in parasympathetic activation that occur in neuro-cardiogenic syncope results in an inappropriate slowing of the HR causing a reduction in cardiac output, BP and brain perfusion that can lead to fainting episodes (99). Conversely, low cardiac parasympathetic activity appears to be associated with worse prognosis. Large epidemiological studies have demonstrated an inverse relationship between resting HR and survival in the general population (190) and in patients with cardiovascular diseases (191-194). Elevated HR predisposes to obesity and diabetes (195); conditions associated with increased cardiovascular risk. A mechanism for such elevations in HR could be low cardiac parasympathetic activity, although this was not directly tested. HRV, a proxy measure of parasympathetic activity based on statistical analysis of HR fluctuations (196, 197), has been found to be reduced in coronary artery disease (198), heart failure (199), diabetes (200), stroke (201, 202) and epilepsy (203). Lower HRV is an independent predictor of death following MI (204-206) and in patients with chronic heart failure (207-210) and chronic renal failure (211). Athletes have a lower resting HR (212, 213) which may be attributed to increased vagal tone as a consequence of exercise training (214). In healthy, individuals exercise training increased resting vagal tone and lowered resting HR (215). This has also been noted in older sedentary individuals (216-218) and patients with chronic heart failure (219-222). The cardiovascular benefits of exercise are well documented (223-225) and

include reductions in BP (226, 227) improved endothelial function (228, 229) and reduced all-cause and cardiovascular mortality in patients with coronary heart disease (230) and chronic heart failure (231). The mechanisms by which exercise improves cardial vagal tone are not fully known (232) but seem to include NO dependent pathways (228).

These studies strongly support the notion that vagal activity has a protective effect against cardiovascular diseases, although the precise mechanisms have not been fully established (233). Suggested mechanisms include antagonising deleterious effects of sympathetic activation (234), inhibiting arrhythmogenesis (235), increasing NO (236) (228), reducing oxidative stress (237) and intracellular calcium (238), improving mitochondrial function (239), and exerting anti-inflammatory effects through the anti-inflammatory pathway (240).

2.4.4 Parasympathetic-immune interactions

Tracey and colleagues stimulated a new understanding of the immune system with the discovery that neural reflex circuits are able to mediate an inflammatory response via nicotinic Ach receptors; a concept that has been described as the cholinergic anti-inflammatory pathway (241-243). In 1984, Blalock put forward the concept that "the immune system may act as a sensory organ" (244). Approximately two decades later Borovikova and associates were able to show that stimulation of the parasympathetic nervous system could attenuate systemic inflammatory responses (241). Ach attenuated the release of inflammatory cytokines (TNF, IL-1β, IL-6 and IL-18) and high mobility group box 1 (a pro-inflammatory nuclear protein with autocrine and paracrine properties (245)) in LPS-stimulated human macrophage cultures (241, 246). Additionally they showed that in rats, electrical stimulation

of the vagus nerve inhibited TNF synthesis within the liver, reduced the peak concentration of serum TNF and prevented the development of shock (hypotension) (241). Interestingly IL-10 concentrations were not affected by Ach suggesting that the anti-inflammatory properties of vagal stimulation could not be attributed to the anti-inflammatory effects of IL-10. Bernik et al. (2002) showed that an intact vagus nerve was required for the anti-inflammatory pathway (247). Injection of a pharmacological vagal stimulator into the brains of rats (intracerebroventricular) suppressed endotoxin-induced release of TNF, however this did not occur in vagotomised rats. Huston et al. (2006) demonstrated that the spleen played a vital role (248) as splenectomy abolished the anti-inflammatory effects of vagal stimulation in their mice model of sepsis. Further work has clarified that Ach acts via the nicotinic Ach receptor α7 subunit (α7nAChR), a ligand gated ion channel (243).

The cholinergic anti-inflammatory pathway has been challenged for a number of reasons (180, 249, 250). Firstly it has been demonstrated that there is no evidence for vagal innervation of the spleen and no synapse between vagal and sympathetic neurons exist within the spleen (179, 249). Secondly, in experimental rat models of sepsis vagotomy had no effect on inflammatory responses (180, 247, 251, 252). The most current proposal for the cholinergic anti-inflammatory reflex involves a vagal afferent arc that relays signals to the brain, which stimulates a complex efferent arc involving vagal efferent neurones with Ach interacting with the α7nAChR expressed on cytokine producing macrophages within the spleen. Activation of this reflex results in attenuated inflammatory responses. How the neural circuit is completed without the vagal innervation of the spleen is not known, but is postulated to result via an interaction with splenic sympathetic neurones (253) or via alternative neural transmission involving Ach-producing T cells (254).

Although the precise pathway remains to be fully elucidated, vagal nerve stimulation has been shown to reduce inflammatory responses in animal experiments (255-258). Vagal nerve stimulation improved local inflammation (ankle swelling, histological joint inflammation) as well as reduced systemic pro-inflammatory cytokine (IL-1α, IL-1β, IL-2, IL-6, IFN-γ and TNF) production in a rat model of collagen-induced arthritis (255). In a mouse model of arthritis vagotomy exacerbated arthritis whilst oral nicotine ameliorated it (258). Cholinergic agonists have been found to reduce clinical signs of arthritis, synovial inflammation, serum cytokine concentrations and bone erosions in animal models of RA (256-258).

Assessing the vagal nerve in humans is problematic primarily due to its anatomical inaccessibility and hence human studies tend to rely on HRV as a proxy (196). A number of studies in healthy individuals (259, 260); the general population (261, 262); populations with increased cardiovascular risk (263-265); and populations with cardiovascular diseases (266-270) have demonstrated inverse relationships between HRV parameters and inflammatory markers. E.g. in inflammatory bowel disease more active disease is associated with higher HR and lower HRV parameters (271). These studies support the hypothesis that increased vagal tone may attenuate inflammation, however the observational nature of these studies carries a major limitation.

Marsland et al. (2007) simulated inflammatory responses in healthy volunteers using LPS and found an inverse relationship between LPS-induced production of IL-6 and high frequency power (HF, frequency domain HRV index of parasympathetic activity), which remained after controlling for age, sex, gender and BP (272). Similar trends were seen between HF power and TNF-α and IL-1β. They also found significant inverse relationships between rMSSD (a time domain HRV index of parasympathetic activity) and TNF-α and a

trend for IL-6. In an animal study infusion of TNF-α caused a depression in HRV with a similar response to that seen in LPS-induced sepsis, suggesting a causal relationship (273).

A few studies in RA patients support the results of animal studies suggesting that vagal nerve stimulation may reduce inflammation. Fibrocyte-like synoviocytes obtained from the synovium of RA patients were pretreated with Ach, nicotine or AR-R1770 (α7nAChR activator) reduced the production of IL-6 and IL-8 (258, 274, 275). These studies in animals and humans led to a trial of vagal stimulation in human RA patients (276, 277). One Bosnian RA patient with severe disease underwent surgical implantation of a vagal stimulator (276). Following 8 weeks of vagal nerve stimulation complete remission of his disease was achieved with reduction of C-reactive protein to normal, and reduced number of swollen, tender joints. Further studies of vagal stimulation are underway in RA and although initial reports suggest they are safe to use, outcome data regarding their efficacy is still awaited (277).

Given this background the major aims of my thesis are to establish whether reduced parasympathetic activity is a feature of RA, and if so to determine whether there is a relationship between parasympathetic activity and inflammation in RA. To achieve this aim I have performed a systematic literature review (Chapter 4) and a case-control study comparing parasympathetic activity (using HRV) amongst RA patients to healthy and hypertensive controls (Chapter 7). In the case report I have assessed parasympathetic activity before and after 12 weeks of anti-TNF-α therapy (Chapter 8).

2.4.5 Impaired baroreflex sensitivity (BRS)

Impairments in BRS have been demonstrated in numerous cardiovascular conditions (278) including hypertension (279, 280), heart failure (281-283), coronary artery disease

(284) as well as normal aging (279, 285-289) and has prognostic implications. Reduced BRS is predictive of decreased survival following MI (206, 284) and in patients with heart failure (290-293) and hypertension (294). Impaired BRS was an independent predictor of new onset hypertension in the elderly (295) and predicted increased BP in healthy adults (296) and patients with type 1 diabetes mellitus (297).

One possible explanation for the increased mortality risk in individuals with reduced BRS is increased risk of cardiac arrhythmia (206, 298, 299). Landolina et al. (1997) assessed cBRS in 24 patients with sustained monomorphic VT and a healed MI (298). They divided patients into those who tolerated the VT well and those who were syncopal or if systolic BP fell <90 mmHg with clinical signs of shock. They found that cBRS was significantly lower in patients who developed haemodynamic compromise or fainted compared to those who tolerated VT well. The arterial baroreflex is a key mechanism in the regulation of beat-to-beat BP, allowing appropriate parasympathetic and sympathetic tone to the heart and peripheral vasculature. Impairments in BRS may result in heightened cardiac sympathetic tone and parasympathetic withdrawal, which can promote arrhythmias (102, 114, 235). Additionally reduced BRS may impair the individual's ability to buffer changes in BP and lead to worsening haemodynamic compromise.

Impairments in BRS may be a consequence of disruption within sites along the reflex arc (300). For example, carotid arterial stiffness or impaired distensibility (287, 288) and abnormalities within the carotid baroreceptor will impair the ability to sense BP changes (301); damage within the baroreceptor afferents may disrupt the transmission of afferent signals (302); and lesions within the NTS (303, 304) or related central areas (305, 306) may impair processing of afferent signals and/or transmission of efferent signals. Finally impaired baroreflex function can be due to abnormalities within vagal or sympathetic efferents (e.g.

denervation, demyelination or nerve damage) (302) or within effector organs (e.g. sinoatrial disease, myocardial or arterial stiffness, endothelial dysfunction). Circulating molecules or hormones may also potentially affect baroreflex function (307-312). Angiotensin (307, 311, 313), melatonin (309), leptin (308), oestrogen (310) and glucocorticoids (312) have all been found to modulate BRS.

2.4.6 BRS and inflammation

In contrast to sympathetic and parasympathetic immune interactions, there is a paucity of literature available assessing interactions between BRS and inflammation. Takagishi and colleagues (2010) provided the strongest evidence for a relationship between inflammation and baroreflex function (314). They found that injection of IL-6 into the NTS reduced cBRS in rats. Prior studies have shown associations between inflammation and reductions in cBRS in humans (315) and experimental models of sepsis (316, 317). Annane et al. (1999) assessed critically ill patients with septic shock and found that hypotension was accompanied with reductions in HRV and cBRS (315). Vaysettes-Courchay et al. (2005) showed that the hypotension induced by sepsis was not a result of baroreflex dysfunction (317). LPS induced septic shock resulted in hypotension, tachycardia and increased sympathetic nerve activity which was similar in rats with intact or denervated baroreceptors. In a study of healthy humans inoculated with the influenza vaccine cBRS remained unchanged (318), although inflammatory markers were not assessed. There is some evidence that improvements in BRS may attenuate inflammatory responses (319, 320). In a canine model of heart failure, 8 weeks of vagal stimulation increased cBRS and attenuated increases in serum CRP (320). In an experimental model of LPS-induced sepsis (319) mice were pre-treated with Ketanserin (5-hydroxytryptamine receptor antagonist), an antihypertensive agent known to increase BRS in

rats (321). Ketanserin reduced serum TNF-α and IL-1β concentrations and increased IL-10 concentrations, whilst improved survival independent of BP and HR (319). In mice with sinoaortic denervation Ketanserin had little effect on reducing shock. In a second experiment Ketanserin prevented the sepsis-induced baroreflex impairment in rats treated with LPS-induced shock and improved survival.

The relationship between BRS and inflammation warrants further investigation. In RA chronic elevations in IL-6 may potentially contribute to reductions in BRS, and partly explain the increased cardiovascular risk and mortality seen in RA. It remains to be seen whether reduced BRS is a pathogenic feature of RA or whether attenuated BRS may exacerbate inflammation. A major aim of my thesis is to establish whether BRS is altered in RA, and if so to determine whether there is a relationship between BRS and inflammation. To achieve this I have performed a systematic literature review (Chapter 4) and a case-control study assessing cardiac and sympathetic BRS in RA patients compared to healthy and hypertensive controls (Chapter 6). In the case report I have also assessed the effects of anti-TNF-α therapy on cardiac and sympathetic BRS (Chapter 8).

2.4.7 Cardiovascular reactivity

Cardiovascular reactivity may be defined as a measure of physiological responses observed in an individual after a physical or mental stressor (322). It is usually reported as a change in a given variable before and after the stressor, or individuals may be grouped into low and high responders (322). Examples of cardiovascular variables include HR, BP and MSNA. Physical stressors include the CPT (immersion of a limb into cold water), isometric handgrip or exercise. Mental stressors include various laboratory-based tasks such as paced

auditory serial arithmetic task, the Stroop test and stressful interviews. The CPT and mental stress tasks can be used to measure sympathetic function (323). Increases in BP, HR and MSNA comprise a normal response to the CPT (324, 325). Mental stress tests also increase BP and HR although sympathetic responses are variable (326-328). Impairments in cardiovascular response can indicate autonomic failure (e.g. in patients with orthostatic hypotension CPT responses are impaired due to sympathetic efferent failure (11)), whilst increased cardiovascular reactivity has prognostic implications. Increased cardiovascular reactivity can predict the development of hypertension (329-338) and may predict future cardiovascular events in selected populations (339) although not necessarily in the general population (329). Mental stress responses may also predict the development of cardiovascular disease. In a study of 756 men, systolic BP responses to mental stress at baseline were independently related to carotid intima-media thickness measured 7 years later (340). This has important implications as carotid intima-media thickness correlates to both systemic (341) and coronary atherosclerosis (342), as well as increased incidence and prevalence of MI and stroke (343-345). There are a number of possible mechanisms that may contribute to an abnormal response. These include: sympathetic and/or parasympathetic dysfunction, impaired BRS, endothelial dysfunction (346), increased arterial stiffness, altered α- and/or β-adrenergic sensitivity, oxidative stress and HPA axis dysfunction. It is also possible that the immune system may have a modulatory effect on cardiovascular responses.

2.4.8 Cardiovascular reactivity and inflammation

Reciprocal links between inflammation and cardiovascular responses to stressors have been identified. Acute stressors induce an inflammatory response (347-354), while inflammation appears to have a modulatory effect on cardiovascular reactivity. The rise in

inflammatory cytokines (IL-6, IL-1β and TNF-α) in sepsis is thought to play a key role in the development of hypotension through vascular hyporeactivity although the precise mechanisms are not fully known. In animal models of sepsis IL-1β induces vascular hyporeactivity via NO-dependent (355) and NO-independent (355-358) mechanisms. Bucher et al. (2003) found that increased tissue concentrations of IL-1β and TNF-α were associated with down-regulation of α-1 receptor messenger ribonucleic acid (mRNA) concentrations within mesangial renal cells (357). The sepsis-induced down-regulation of α-1 receptors was unaffected by nitric oxide synthase (NOS) inhibition suggesting NO-independent mechanisms. Liang et al. (2014) found that IL-1β reduced the vascular reactivity of rabbit superior mesenteric arteries to phenylephrine (an α-1 adrenergic receptor agonist) (358). Administration of IL-1 receptor antagonist partly reversed the LPS-induced decrease in vascular reactivity. IL-1β has also been shown to up-regulate the expression of NO receptors on vascular smooth muscles (355), and downregulate endothelin (359), vasopressin (356) and angiotensin (360). CRP also appears to be involved in the modulation of vascular reactivity (361-363). In a study of 60 males with coronary artery disease, elevated concentrations of serum CRP were independently associated with impaired vascular responses to acetycholine (362). Further evidence from Clapp et al. (2005) suggests that CRP appears to exert a direct effect on vascular function (361). In an in-vitro experiment human CRP applied to human internal mammary arteries induced hyporeactivity to phenylephrine. Taken together these studies suggest that in an acute sepsis paradigm, increased serum concentrations of inflammatory cytokines (IL-6, IL-1β, TNF-α) may promote vascular hyporeactivity through a number of mechanisms. However it remains to be known whether these effects would be seen in chronic inflammatory diseases such as RA.

In summary, cardiovascular reactivity has important prognostic implications and can predict the development of hypertension. Studies to date suggest there are reciprocal mechanistic links between cardiovascular reactivity and inflammation. The pattern of cardiovascular responses to CPT and mental stress in RA patients need to be established and relationships between inflammation and cardiovascular reactivity explored. A major aim of my thesis is to determine whether cardiovascular reactivity is impaired in patients with RA, and to determine whether there is a relationship between inflammation and cardiovascular responses to CPT and mental stress. To achieve this I have performed a case-control study assessing cardiovascular reactivity to CPT and mental stress in RA patients with and without hypertension compared to healthy and hypertensive controls (Chapter 7). I have also assessed the effects of anti-TNF-α therapy on cardiovascular responses to mental stress in a case study (Chapter 8).

2.4.9 QTc interval and inflammation

The QT interval is the time from the onset of ventricular depolarisation (beginning of the Q wave on an electrocardiogram, ECG) to the end of ventricular repolarisation (end of the T wave). The QT interval varies with HR and hence several formulas have been developed to provide a HR-corrected QT measure (QTc) (e.g. Bazett's formula = QT/√RR, Framingham Heart Study formula = QT + 0.154 x [1-RR]). The normal QTc interval is 390-450 ms in males and 390-460 ms in females (364). Prolongation of QTc predicts all-cause mortality in the general population (365, 366) and a number of conditions (367-373) including RA (35). QTc interval prolongation increases the risk of developing arrhythmias including Torsade de Pointes which if left untreated may result in fatal ventricular arrhythmias (374).

QTc interval measurement is a simple, non-invasive and highly specific diagnostic tool for the detection of cardiac autonomic neuropathy (375). Studies have demonstrated associations between QT or QTc prolongation and autonomic dysfunction (376): reduced HRV (377); reduced parasympathetic activity (376, 378-380); and heightened sympathetic activity (381-383). These features of autonomic dysfunction are associated with increased cardiovascular and mortality risk (113, 206, 384, 385). QTc interval prolongation has been shown to be associated with inflammation in the general population (386, 387), in cardiac disease (388) and inflammatory arthritis (35, 377). Accumulating evidence from clinical studies suggests that inflammation may be driving QTc prolongation (35, 377, 386, 388-390) although the precise mechanisms are not fully known. In one animal experiment, TNF-α was found to prolong the cardiomyocyte action potential by inhibiting the human ether-a-go-go related gene (HERG) potassium channel, currently known as KCNH2 (390). The disruption in HERG protein function (either congenital or acquired) is thought to be a primary mechanism for long QT syndrome (391, 392). Few studies have assessed cytokines or inflammatory markers in association with QTc interval. Yue et al. (2007) found that prolonged QTc interval was associated with increased CRP and fibrinogen in patients with coronary artery disease (388). However, the authors found no association between QTc interval and ICAM-1, E-selectin or von Willebrand factor (a marker of endothelial function). Senel et al. (2011) found that 6 months treatment with infliximab (TNF-α inhibitor therapy) reduced QTc interval in patients with ankylosing spondylitis (389). In another study Vadetanib, a vascular endothelial growth factor receptor-2 (VEGFR-2) inhibitor was incidentally found to cause prolongation of QTc interval in patients treated for ovarian cancer (393). In one study of healthy young men prolonged QTc interval was associated with CRP (386). These studies suggest that inflammatory pathways may be implicated in the development of autonomic dysfunction and

prolongation of QTc interval, although further work is required to fully characterise which pathways are affected.

One major aim of my thesis is to determine whether serum concentrations of inflammatory cytokines can predict QTc interval prolongation in patients with RA. To achieve this I have performed an observational cohort study of RA patients to assess for associations between inflammatory cytokines and QTc interval (Chapter 9).

2.4.10 Stress, the HPA axis and autonomic function

Heightened stress is a known cardiovascular risk factor and is associated with an increased mortality (394, 395). In response to stress both the HPA axis and the ANS are activated; however the precise interaction between these systems is not fully understood. At periods of stress, corticotrophin releasing hormone (CRH) is released from the hypothalamus and acts upon the pituitary to stimulate adrenocorticotrophin hormone (ACTH) which acts on the adrenal glands to stimulate the release of glucocorticoids. Cortisol, the principle glucocorticoid in humans has numerous effects: mobilisation of glucose, fatty acids and amino acids; anti-inflammatory; and reducing bone formation. Chronic cortisol excess, as occurs in Cushing's syndrome, has deleterious consequences including hypertension (396), left ventricular hypertrophy, arteriosclerosis and up to four-fold increased mortality (397). However, the cardiovascular consequences of acute rises in cortisol are not fully understood. Acute glucocorticoids have been reported to have differential effects on BP, HR and sympathetic nerve activity (398-401). In healthy subjects, acute HCN has been shown to increase resting HR, without affecting BP or MSNA (398) whereas in other studies a fall in BP and MSNA were observed (399). In an early study, acute glucocorticoid treatment in

healthy subjects increased cardiac output and reduced peripheral vascular resistance, whilst maintaining arterial BP (402). In one study of Pima Indians, cortisol inhibition with metapyrone had no effect on MSNA, however subsequent replacement with HCN reduced MSNA (400). In a study of obese and lean individuals, acute dexamethasone had no effect on HR, BP or MSNA (401). The variability in these studies may be explained by differences in glucocorticoid type, doses administered and time between administration and testing.

Altered baroreflex function may partly explain the observed changes in HR and BP associated with rises in cortisol. BRS has been reported to fall (403, 404) during acute mental stress, an effect that may be due to the central actions of cortisol (403). Animal studies support the hypothesis that glucocorticoids can elicit centrally mediated alterations in BRS (405-407). In rats, administration of glucocorticoids into the RVLM (406) and NTS (407) rapidly alters activity of baroreceptive neurons and depresses baroreflex control of HR. A cortisol-induced fall in BRS may be an important mechanism whereby acute stress increases cardiovascular risk (408). To the author's knowledge only one study has assessed the effects of acute glucocorticoids on BRS (409). Cottin et al. (2015) showed that one-week of oral prednisolone increased HR but did not affect cBRS in healthy volunteers. However the investigators only assessed one parameter of spontaneous BRS, which may have been inadequate given that indices of BRS do not always correlate (410). It still remains to be established whether acute surges in cortisol can reduce BRS and hence provide a substrate for arrhythmogenesis and increased cardiovascular risk.

A major aim of my thesis is to investigate the haemodynamic and autonomic consequences of acute surges in cortisol in healthy humans. A single-blinded cross-over randomized control study was performed to assess the acute effects of HCN bolus infusion on

haemodynamic and autonomic parameters including BRS, HRV and cardiovascular reactivity (Chapter 10).

2.4.11 Autonomic dysfunction in RA

In summary RA is a chronic inflammatory condition with increased cardiovascular mortality that is not fully explained by the presence of traditional risk factors. While it has been established that autonomic dysfunction is a pathogenic feature of cardiovascular disease and is associated with increased cardiovascular and mortality risk, whether autonomic dysfunction is present in RA requires further study. Experimental data supports strong links between inflammation, autonomic dysfunction and cardiovascular disease. Inflammatory cytokines have been shown to increase sympathetic activity and reduce BRS and HRV in animals although whether this occurs in humans is not presently known. Furthermore accumulating evidence suggests that the ANS may have a modulatory effect on inflammatory responses. Given the potential for therapeutic interventions that target the ANS it is important to establish the presence and nature of ANS dysfunction in RA and relationships with inflammation. Additionally, biological agents that specifically target and block inflammatory cytokines (e.g. TNF-α and IL-6 inhibitors) have been shown to improve disease control in RA (411) as well as improve mortality (412) and cardiovascular risk (413). The effects of cytokine inhibition on ANS function need to be determined to provide important mechanistic insights into the pathophysiology of RA, improve our current understanding of autonomic-immune interactions and to potentially pave the way for their use in cardiovascular conditions.

2.5 Assessment of the ANS

There are various clinical and research techniques that can be used to assess ANS function (Table 2.6); each with their relative merits and limitations (89, 196, 323, 414-427).

Clinical cardiovascular reflex tests (CCTs, e.g. HR or BP responses to orthostasis) allow for simple, quick and non-invasive detection of autonomic dysfunction with the additional benefit of grading severity (415). These reflex tests however are unable to diagnose the cause of autonomic dysfunction and hence should be interpreted within the clinical context.

HRV is a useful, non-invasive research tool that provides an indirect assessment of cardiac ANS function (196). Cyclical fluctuations in resting HR are caused by cardiac parasympathetic and sympathetic influences and modulated by baroreflex mechanisms. Statistically derived indices of HRV can indicate the contribution of these parasympathetic and sympathetic influences (422, 428), although the physiological interpretation of HRV metrics is an issue of debate (429). Despite guidelines for HRV assessment and interpretation (Task Force of the European Society and the North American Society of Pacing and Electrophysiology 1996) there is variability in methodology and a lack of normative data (196), which needs to be considered when comparing results between studies.

Plasma or urinary catecholamines provide an estimate of global sympathetic activity but cannot delineate regional variations in sympathetic activity. Measured levels of catecholamines reflect metabolism and clearance, as well as resting sympathetic tone or release and are affected by numerous confounding factors (including medications, diurnal variation and concomitant diseases) that can make interpretation difficult (323, 422). Other blood biomarkers of sympathetic activity (e.g. NPY) have similar limitations (417, 425).

Noradrenaline spillover studies, unlike plasma or urinary measurement, can assess organ-specific sympathetic activity but are invasive, expensive and technically challenging (323, 422). Pharmacological agents (e.g. adrenoreceptor antagonists or sympathomimetics) interrogate the ANS system to characterise the precise mechanisms of ANS dysfunction but are invasive and carry inherent risk (323).

Cardiovagal BRS assesses cardiovascular control mechanisms that are important for beat-to-beat regulation of BP. Baroreflex assessment involves simultaneous measurement of HR and BP while subjects are resting quietly (e.g. spontaneous methods), and during perturbations of BP either by non-invasive procedures (e.g. Valsalva's manoeuvre, lower body negative pressure or neck suction pressure) or pharmacological agents (e.g. phenylephrine infusion) (323, 414). The relative strengths and weakness of spontaneous versus dynamic methods used for assessing baroreflex function have been debated (410, 430-432) (Table 2.5). Dynamic assessments have the advantage of assessing baroreflex function across a wide BP range, and allow a baroreflex function curve to be determined. The MOT (sequential infusion of SNP and PE one minute apart) is thought to be a "gold standard" assessment of baroreflex function (433). The technique was first developed by Smyth et al. (1969)(434) and later improved by Ebert et al. (1992)(435) and Rudas et al. (1999)(433). The main limitations are the invasive nature, risk of complications during the infusions and a concern that the vasoactive drugs may exert unwanted effects on baroreflex structures that may influence results. Spontaneous baroreflex assessments provide measures of baroreflex function at rest. They have the advantage of being non-invasive and there is no risk of unwanted side effects of BP perturbation. In addition they allow baroreflex assessment over a 24-hour period with the use of ambulatory ECG monitors to provide a "real world" measure of BRS (426). The Sequence method uses an automated computer model to identify

spontaneous fluctuations in BP and HR (426, 427) while the transfer function gain is calculated using cross-spectral analysis (436). The main limitations are that the Sequence method relies on the presence of sequences of 3 or more consecutive cardiac cycles with changes in BP (of at least 1 mmHg), which may vary from patient to patient, and that these indices do not always correlate with each other or with barororeflex determined using pharmacological measures (410, 430-432, 437).

The microneurography technique uses tungsten microelectrodes to make intra-neural recordings (typically from the peroneal nerve) of sympathetic outflow to the muscle (blood vessel vasoconstrictor impulses) or skin (323, 422). MSNA correlates well with cardiac sympathetic activity; is reproducible and well-tolerated in numerous disease populations; and allows quantification of resting activity and response to various stimuli. Its technically challenging nature is the main limitation of this procedure (422).

Cardiac sympathetic imaging is a minimally invasive research technique that allows for visualisation of various imaging agents (e.g. radio-labelled sympathomimetic amines) using single photon emission computed tomography (323, 422). This technique has been used in cardiovascular disease and demonstrated prognostic significance; however its use is limited due to expense and lack of availability (323).

Other assessments such as pupillary light reflex responses (420) or sympathetic skin responses (418, 421) can provide an estimation of autonomic dysfunction; however their significance in cardiac autonomic function is not clear .

Table 2.6 ANS assessments

Parameter	Definition	Abnormalities
PARASYMPATHETIC FUNCTION		
Clinical Cardiovascular Tests		
HR response to orthostasis (415)	HR response to standing up unaided following a period of lying quietly on a couch. Normal response is an immediate increase in HR (around the 15th beat) after standing followed by a nadir in HR (around the 30th beat). The 30:15 ratio (of the longest inter-beat (RR) interval around the 30th beat to the shortest RR-interval around the 15th beat) forms part of the Ewing battery of cardiovascular tests.	30:15 ratio ≤1 indicate parasympathetic dysfunction
HR response to Valsalva's manoeuvre (415)	HR response to straining against a closed glottis at a pressure of 40mmHg for 15 seconds. The Valsalva ratio (of the longest RR- interval shortly after the manoeuvre followed by a rebound bradycardia after release) forms part of the Ewing's battery of cardiovascular tests.	Valsalva ratio ≤1.1 indicates parasympathetic dysfunction
HR variation to deep breathing	HR variation to deep breathing at a rate of 6 breaths per minute. The	HR difference ≤10 indicates

(415)	mean differences between the maximum and minimum heart rates during each breathing cycle forms part of the Ewing's battery of cardiovascular tests.	parasympathetic dysfunction
Strengths: Simple, bedside tests; non-invasive; inexpensive; normative values available; allows grading of severity when tests used in combination (415). **Weaknesses**: Indirect measures of parasympathetic activity; some parameters also influenced by sympathetic and baroreflex activity (e.g. Valsalva's manoeuvre)(414); relies on experienced practitioners; multiple factors can affect responses to Valsalva's maneouvre (volume and rate of pre-strain breath, strain pressure, depth and duration, standing v supine) and deep breathing (rate and depth of breathing)(323); provides limited information about the mechanism of autonomic dysfunction; single tests are not reliable in detecting autonomic dysfunction as there is a poor correlation between the various indices (323).		
HRV		
rMSSD (196)	Square root of the mean of the sum of the squares of difference between adjacent inter-beat (NN) intervals. Time domain estimate of short-term components of HRV.	Reduced levels indicate low HRV and parasympathetic dysfunction
NN50 (196)	Number of pairs of adjacent NN intervals differing by more than 50 milliseconds in the entire recording. Time domain measure.	

pNN50% (196)	NN50 as a percentage of the total number of all NN intervals. Time domain measure.	
SDNN (196)	Standard deviation of all NN intervals. Estimate of overall HRV. Time domain measure.	
SDANN (196)	Standard deviation of the averages of NN intervals in all 5 minute segments of the entire recording. Time domain estimate of long-term components of HRV.	
SDSD (196)	Standard deviation of differences between adjacent NN intervals. Time domain measure.	
HF power (196)	High frequency power of pulse interval in the range 0.15-0.4 Hz. Frequency domain measure.	Reduced levels indicate reduced parasympathetic activity
SD1, SD2 (423)	Standard deviation of the Poincare plot (non linear technique). Estimate of short (SD1) and long-term (SD2) HRV.	Reduced levels indicate reduced HRV
Strengths: Non-invasive; inexpensive; reproducible; automated analysis; resting activity and responses to stimuli can be measured; Task Force guidelines (196) exist for the optimum utility of this technique; 24-hour ambulatory ECG monitoring provides a measure of		

autonomic function in "real life" environment therefore a good clinical technique to monitor responses to interventions. **Weaknesses**: Indirect measure of autonomic activity; no normative values exist; despite the availability of guidelines the variability in methodology makes it difficult to compare values between studies.		
Heart rate turbulence (HRT) – turbulence onset (424)	Early acceleration of the heart rate immediately following a ventricular premature beat is a result of parasympathetic withdrawal.	Impaired HRT represent reduced parasympathetic activity
Strengths: Non-invasive; inexpensive; automated analysis; 24-hour holter monitoring provides a measure of autonomic function in "real life" environment therefore a good clinical technique to monitor responses to interventions. **Weaknesses**: Indirect measure of autonomic activity; no normative values exist; relies on the presence of premature ventricular beats (424).		
Respiratory sinus arrhythmia (89)	Rhythmical fluctuations in heart rate periods during inspiration (rise) and expiration (fall) represent parasympathetic activity.	Reduced represents reduced parasympathetic activity
Strengths: Non-invasive; inexpensive; selective index of vagal control of the heart (89). **Weaknesses**: Results can be affected by rate and depth of breathing; provides a measure of resting autonomic activity only.		
Pupillary Light Reflex		
Constriction latency (420)	Measure of the onset of pupillary constriction in response to light	A delay can reflect

	stimulus.	parasympathetic dysfunction
Maximum velocity latency (420)	Measure of the maximum constriction velocity in response to light stimulus.	A reduced velocity indicates parasympathetic dysfunction
Strengths: Non-invasive; inexpensive; validated (420); normative values available; allows grading of severity. **Weaknesses**: Provides limited information about the mechanism of autonomic dysfunction; can be confounded by impairments in ocular muscle function and retinopathy (420).		
SYMPATHETIC FUNCTION **Clinical Cardiovascular Tests**		
Systolic BP response to orthostasis (415)	Systolic BP response to standing up unaided following a period of lying quietly on a couch. The postural drop in systolic BP forms part of the Ewing's battery of cardiovascular tests.	Decrease in systolic BP≥20 mmHg indicates sympathetic dysfunction
BP response to sustained handgrip (415)	BP response to sustained handgrip (30% of the maximum voluntary contraction using a handgrip dynamometer for up to 5 minutes). The	Increase in diastolic BP≤10 mmHg indicates

	difference between diastolic BP before starting and just prior to releasing handgrip forms part of the Ewing's battery of cardiovascular tests.	sympathetic dysfunction
BP response to CPT (323)	BP response to immersion of hand in a container of ice water for 1-3 minutes, which results in sympatho-excitation.	Diminished responses indicate sympathetic dysfunction, increased responses indicate exaggerated sympatho-excitation
BP and HR response to mental stress (323)	BP and HR response to mental stress tasks (e.g. mental arithmetic or the Stroop colour-word naming test) which results in sympatho-excitation.	
Strengths: Non-invasive; inexpensive; normative values available for responses to orthostasis and handgrip (415); allows grading of severity (415). **Weaknesses**: Relies on experienced practitioners; difficult to standardise muscle effort during sustained handgrip; wide variability in inter-subject responses to CPT and mental stress; provides limited information about the mechanism of autonomic dysfunction; single tests are not reliable in detecting autonomic dysfunction(415); CPT, mental stress and handgrip responses have a low sensitivity and specificity for detecting sympathetic dysfunction (323).		
HRV		
LF power (196)	Low frequency power of pulse interval in the range 0.04-0.15 Hz.	Increased levels indicate

	Frequency domain measure indicating sympathetic activity (but also has a significant parasympathetic component).	heightened sympathetic activity
LF/HF ratio (196)	Ratio of low frequency / high frequency power of pulse intervals. Frequency domain measure of sympatho-parasympathetic balance.	Increased levels indicate predominantly heightened sympathetic activity
Strengths: Non-invasive; cheap; reproducible; automated analysis; resting activity and responses to stimuli can be measured; Task Force guidelines(196) exist for the optimum utility of this technique; 24-hour ECG holter monitoring provides a measure of autonomic function in "real life" environment therefore a good clinical technique to monitor responses to interventions. **Weaknesses**: Indirect measure of autonomic activity; no normative values exist; despite the availability of guidelines the variability in methodology makes it difficult to compare values between studies; LF power has contributions from the parasympathetic nervous system and hence not purely a measure of sympathetic activity (430).		
Pre-ejection period (PEP) (416, 419)	The interval from the onset of the Q wave (on an ECG) to the left ventricular ejection (detected using impedence cardiography). PEP is inversely related to myocardial contractility and can represent sympathetic influences on the heart.	Reduced PEP indicates increased sympathetic activity
Strengths: Non-invasive; provides a reliable measure of systolic time intervals; can provide a measure of resting activity and response to		

<table>
<tr><td colspan="3">stimuli (419).

Weaknesses: Indirect measure of cardiac autonomic influences; lack of standardised methodology; derived values of stroke volume and cardiac output are less reliable (416); PEP may be confounded by changes in preload or afterload (419).</td></tr>
<tr><td colspan="3">Microneurography</td></tr>
<tr><td>MSNA (323, 422)</td><td>Intra-neural recordings of MSNA using tungsten microelectrodes inserted percutaneous into a peripheral nerve (typically peroneal nerve) allow direct measurement of vasoconstrictor sympathetic outflow.</td><td>Increased levels indicate sympathetic over-activity</td></tr>
<tr><td colspan="3">Strengths: Direct and continuous measure of muscle sympathetic outflow; correlates with cardiac sympathetic activity; reproducible; well tolerated in healthy disease populations; can record for several hours at a time; allows quantification of resting activity as well as response to stimuli (422).

Weaknesses: Invasive; technically challenging procedure.</td></tr>
<tr><td colspan="3">Catecholamines or Biomarkers of Sympathetic Activity</td></tr>
<tr><td>Catecholamines (323)</td><td>Catecholamines such as adrenaline, noradrenaline and their metabolites detected in the plasma or urine (24-hour collection) may represent sympathetic activity. Confounding factors include medications, diurnal variations and concomitant diseases.</td><td>Increased levels may indicate sympathetic over-activity</td></tr>
</table>

Plasma NPY (425)	Peripheral marker peptide released with noradrenaline following sympathetic activation.	
Serum chromogranin A (417)	Acidic, soluble proteins with widespread neuroendocrine distribution in secretory vesicles, co-released with catecholamines by exocytosis from vesicles in adrenal medulla and sympathetic nerve endings.	
Strengths: Minimally invasive; inexpensive; plasma levels allow measurement of resting activity and response to stimuli. **Weaknesses**: Difficult to measure; represents global sympathetic activity and cannot delineate regional variances; plasma levels of catecholamines reflect uptake, release and clearance whilst urinary levels are dependent on renal function; can be confounded by medications, diurnal variations and concomitant diseases (422).		
Noradrenaline spillover (323, 422)	Regional or organ-specific noradrenaline spillover measurements can characterise regional sympathetic activity.	Increased spillover rates indicate regional sympathetic over-activity
Strengths: Allows direct measurement of organ specific sympathetic activity. **Weaknesses**: Invasive; considerable costs; technically challenging (422).		
Cardiac sympathetic imaging (323, 422)	Imaging agents (e.g. radio-labelled sympathomimetic amines) can be detected using single photon emission computed tomography, providing	Provides images showing areas of sympathetic over-

	visual representation of sympathetic activity. Has been used to demonstrate cardiac sympathetic denervation in cardiovascular disease and has prognostic significance.	or under-activity
Strengths: Allows direct measurement of organ specific sympathetic activity; provides structural and functional assessment of the sympathetic nervous system; can provide quantification of organ specific noradrenergic uptake (422). **Weaknesses**: Minimally invasive; considerable costs; limited availability; assessing sympathetic activity in the heart can be technically difficult (422).		
Other Assessments		
Pupillary light reflex maximal pupillary area in darkness (420)	Measure of maximal pupillary area in response to darkness.	A reduced area indicates sympathetic dysfunction
Strengths: Non-invasive; inexpensive; validated; normative values available; allows grading of severity (420). **Weaknesses**: Provides limited information about the mechanism of autonomic dysfunction; can be confounded by impairments in ocular muscle function and retinopathy (420).		
Sympathetic skin responses (418, 421)	Changes in skin electrical conductance in response to various stimuli (such as electrical, acoustic) represent sympathetic cholinergic function.	Absent responses indicates sympathetic dysfunction
Strengths: Non-invasive; simple; fast; inexpensive.		

Weaknesses: Wide intra- and inter-subject variability in sympathetic skin responses due to confounding factors (e.g. ambient temperature, skin temperature, mental or emotional state, habituation with repeated stimuli); low sensitivity and specificity; poor correlation with other autonomic assessments (e.g. sudomotor dysfunction).(421)		
BAROREFLEX SENSITIVITY **cBRS**		
Sequence technique (426, 427)	Spontaneous assessment involving simultaneous recording of BP and RR-interval whilst the patient rests quietly. A computer is used to identify sequences of three or more consecutive beats characterised by a progressive increase or decrease in BP which results in lengthening or shortening of the RR interval (consecutively). Regression slope of Systolic BP and RR interval provides a measure of BRS	Reduced slope indicates impaired cBRS
Strengths: Non-invasive; simple; inexpensive; automated analysis; reliable; provides distinct measurements for rising and falling BPs (206, 427). **Weaknesses**: No normative values exist; relies on the presence of sequences; marked within subject variation in BRS (possibly due to haemodynamic, temporal and behavioural factors) (427).		

Pharmacological agents (323, 414)	Phenylephrine (vasoconstrictor) causes increase in BP, which results in baroreflex-mediated slowing of the HR. Regression slope of SBP and RR interval or HR provides a measure of cBRS.	
Strengths: Inexpensive; usually produces a high correlation between BP and RR interval suggesting it is a good indicator of arterial baroreflex gain (323). **Weaknesses**: Invasive; no normative values exist; only assesses the response to rises in BP which may be reduced in subjects with low resting sympathetic outflow (typically young healthy individuals) (323).		
Heart rate turbulence - turbulence slope (424, 438)	Rate of late deceleration (after early acceleration) of the HR immediately following a ventricular premature beat represents cBRS	Reduced turbulence slope indicates impaired cBRS
Strengths: Non-invasive; inexpensive; automated analysis; 24-hour ECG holter monitoring provides a measure of autonomic function in "real life" environment therefore a good clinical technique to monitor responses to interventions. **Weaknesses**: Indirect measure of autonomic activity; no normative values exist; relies on the presence of premature ventricular beats (424).		

BP = blood pressure, BRS = baroreflex sensitivity, cBRS = cardiac baroreflex sensitivity, ECG = electrocardiogram, HR = heart rate, HRV = heart rate variability, MSNA = muscle sympathetic nerve activity, NN = inter-beat, NPY = neuropeptide-Y, PEP = pre-ejection period, RR interval = inter-beat interval, SBP = systolic blood pressure.

CHAPTER 3 Systematic literature review

3.1 Abstract

Objectives RA is a chronic inflammatory condition with increased all-cause and cardiovascular mortality. Accumulating evidence indicates that the immune and ANS are major contributors to the pathogenesis of cardiovascular disease. The first systematic literature review was performed to determine the prevalence and nature of ANS dysfunction in RA and whether there a causal relationship between inflammation and ANS function exists.

Methods Electronic databases (Medline, PubMed Central and Cochrane Library) were searched for studies of RA patients where autonomic function was assessed.

Results Forty studies in total were included. ANS function was assessed by CCTs (n=18), HRV (n=15), catecholamines (n=5), biomarkers of sympathetic activity (n=5), sympathetic skin responses (n=5), cBRS (n=2) and pupillary light reflexes (n=2). 9 small studies reported a ~60% (median, range 20-86%) prevalence of ANS dysfunction (defined by abnormal CCTs) in RA. 73% of studies (n=27/37) reported at least one abnormality in ANS function: parasympathetic dysfunction (n=20/26, 77%), sympathetic dysfunction (n=16/30, 53%) or reduced cBRS (n=1/2, 50%). An association between increased inflammation and ANS dysfunction was found (n=7/19, 37%) although causal relationships could not be elucidated from the studies available to date.

Conclusions ANS dysfunction is prevalent in ~60% of RA patients. The main pattern of dysfunction is impairment of cardiovascular reflexes and altered HRV indicative of reduced cardiac parasympathetic (strong evidence) and elevated cardiac sympathetic activity (limited evidence). The literature to date is underpowered to determine causal relationships between inflammation and ANS dysfunction in RA.

3.2 Introduction

RA is a chronic inflammatory condition predominantly affecting the synovial joints but leading to extra-articular manifestations. The increased cardiovascular mortality in RA patients (by up to 50%) (20-23) is not fully explained by the presence of traditional risk factors and remains an important research focus (21, 24-29, 31, 33, 41).

The ANS plays a critical role in the normal regulation of cardiovascular disease through its effects on the heart, peripheral vasculature and kidneys (Figure 3.1) (385). The ANS is broadly comprised of the sympathetic and parasympathetic branches, which work independently, or in counter-balance to ensure homeostasis is maintained. Accumulating evidence indicates that altered ANS function contributes to the pathogenesis of cardiovascular disease (51, 439) and is an important predictor of cardiovascular mortality (113, 206, 384, 385). Indeed, recent animal studies have demonstrated mechanistic and reciprocating links between inflammation and ANS dysfunction (52, 153, 273, 314, 440-442). Elevations in circulating pro-inflammatory cytokines increase sympathetic activity (52, 153), reduce cBRS (314) and reduce HRV derived indices of cardiac parasympathetic activity (Figure 3.1) (273); these are all features of ANS dysfunction associated with cardiovascular disease and increased mortality in humans (113, 206, 384, 385). Therefore, determining ANS function in RA may provide prognostic benefit as well as improve understanding of underlying pathological mechanisms, and hence new improved therapeutic strategies.

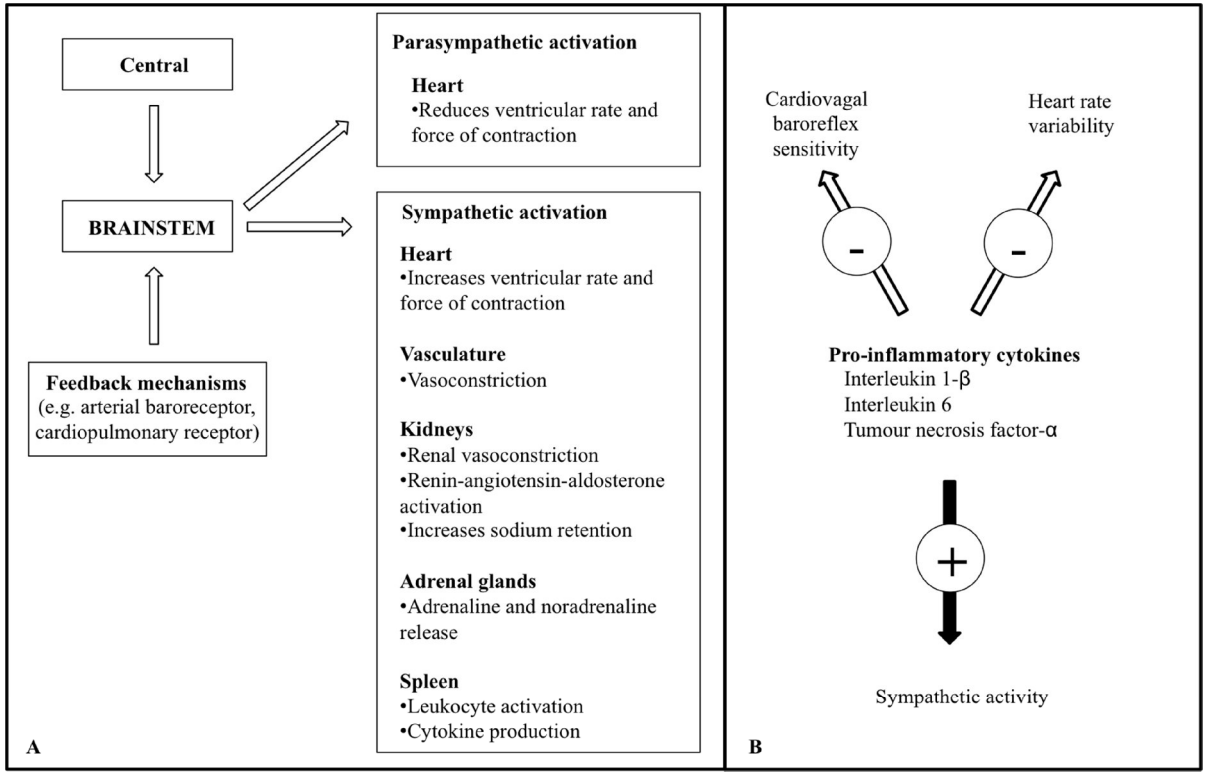


Figure 3.1 Simplified schematic showing ANS regulation and the effects of inflammatory cytokines on the ANS.

A. Nerve signals from the brain stem are relayed to various organs in the ANS. Parasympathetic activation results in slowing of the HR, whereas sympathetic activation causes increased ventricular contraction, peripheral and renal vasoconstriction, activation of the renin-angiotensin-aldosterone system, increased sodium retention (kidneys), adrenaline and noradrenaline release (adrenal glands) and increased inflammation (leukocyte activation and increased cytokine production in the spleen). Central and peripheral feedback mechanisms are in place (e.g. arterial and cardiopulmonary baroreceptors, chemoreceptors) to ensure homeostasis is maintained. B. Experimental studies have shown that pro-inflammatory cytokines (e.g. IL1-β, IL-6 and TNF-α) attenuate (-) cardiovagal baroreflex sensitivity and heart rate variability, as well as heighten (+) sympathetic activity. ANS = autonomic nervous system, HR = heart rate, IL = interleukin, TNF = tumour necrosis factor.

3.3 Aims and hypothesis

Central hypothesis: In patients with RA, elevated concentrations of circulating inflammatory cytokines are associated with autonomic dysregulation (heightened sympathetic activity, reduced parasympathetic activity, reduced BRS and impaired cardiovascular responses to stressors).

Aim: To begin to test this, the first systematic literature review on ANS function in RA was performed to:

i) investigate whether there is sufficient evidence to determine if patients with RA have altered ANS function;

ii) determine the prevalence and nature of any autonomic dysregulation in patients with RA;

iii) elucidate whether there is a causal relationship between systemic inflammation (e.g. clinical markers of disease activity, elevated concentrations of specific circulating pro-inflammatory molecules) and ANS dysfunction in RA.

3.4 Methods

3.4.1 Search criteria

Using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (443), electronic databases (Medline, PubMed Central and Cochrane Library) were searched to identify articles between January 1974 and June 2013, in English. The search term "rheumatoid arthritis" was used in combination with each of the following terms (incorporating common assessments of ANS function, Table 2.4): "autonomic", "sympathetic", "parasympathetic", "vagal", "heart rate variability", "baroreflex", "catecholamine", "epinephrine", "norepinephrine", "adrenaline", "acetylcholine", "noradrenaline", "cardiovascular battery", "Ewing", "Valsalva", "hand grip", "cold pressor", "orthostasis" and "tilt".

6350 citations were identified and the summaries and/or abstracts were screened for relevance; clinical studies of adults with RA where at least one aspect of ANS function was assessed were deemed relevant. Following removal of duplicate and irrelevant articles 44 full articles were accessed. Irrelevant articles included those that were non-original research (e.g. review articles, editorials, letters etc.), non-RA and animal studies. The following eligibility criteria were applied: articles written in the English language; involving adults with RA; at least one known parameter of ANS function assessed and reported; and an attempt to assess the association between inflammation and ANS function either by inclusion of a non-RA control group, by statistical analysis within a cohort of RA patients, or by intervention with anti-inflammatory therapy. Four articles were excluded as they failed to meet the eligibility criteria (association between inflammation and ANS function not assessed and did not include a non-RA control group). In total 40 articles were included in the review (Figure 3.2).

3.4.2 Quality assessment

Data extraction was performed and a quality assessment was made for each study by adapting a known quality assessment tool (Appendix 1) (444). The following indices were assessed: study design, inclusion/exclusion criteria, disease characteristics, standardised testing conditions (e.g. time of test, subject position), standardised methodology for autonomic assessment (e.g. adhering to published guidelines), quality of autonomic assessment tool (e.g. more than one technique used), appropriate sample size (e.g. use of power calculations to determine sample size), appropriate statistical tests (e.g. adjustment made for group differences), and associations between ANS function and inflammation tested. Each index was graded between 0-2, and the total points added to give a final score between 0-18. A percentage was calculated to give a Quality Index Score (QIS). The quality assessments were performed by two investigators (Ahmed Adlan and James Fisher) and disagreements were discussed until a consensus was reached. Each study was placed into one (or more) category representing parasympathetic function, sympathetic function and cBRS and scored as either normal or abnormal. At least one abnormal parameter of autonomic function was required to qualify as an abnormal study (i.e. no studies could be classified as both normal and abnormal in a single domain). Furthermore each study was classified according to the type of autonomic function test performed and placed into one category if comparisons were made between RA patients and controls: RA worse than control, no difference or RA better than control. Due to the large heterogeneity in the patient characteristics, tools of ANS assessment employed and parameters reported, no meta-analysis was performed.

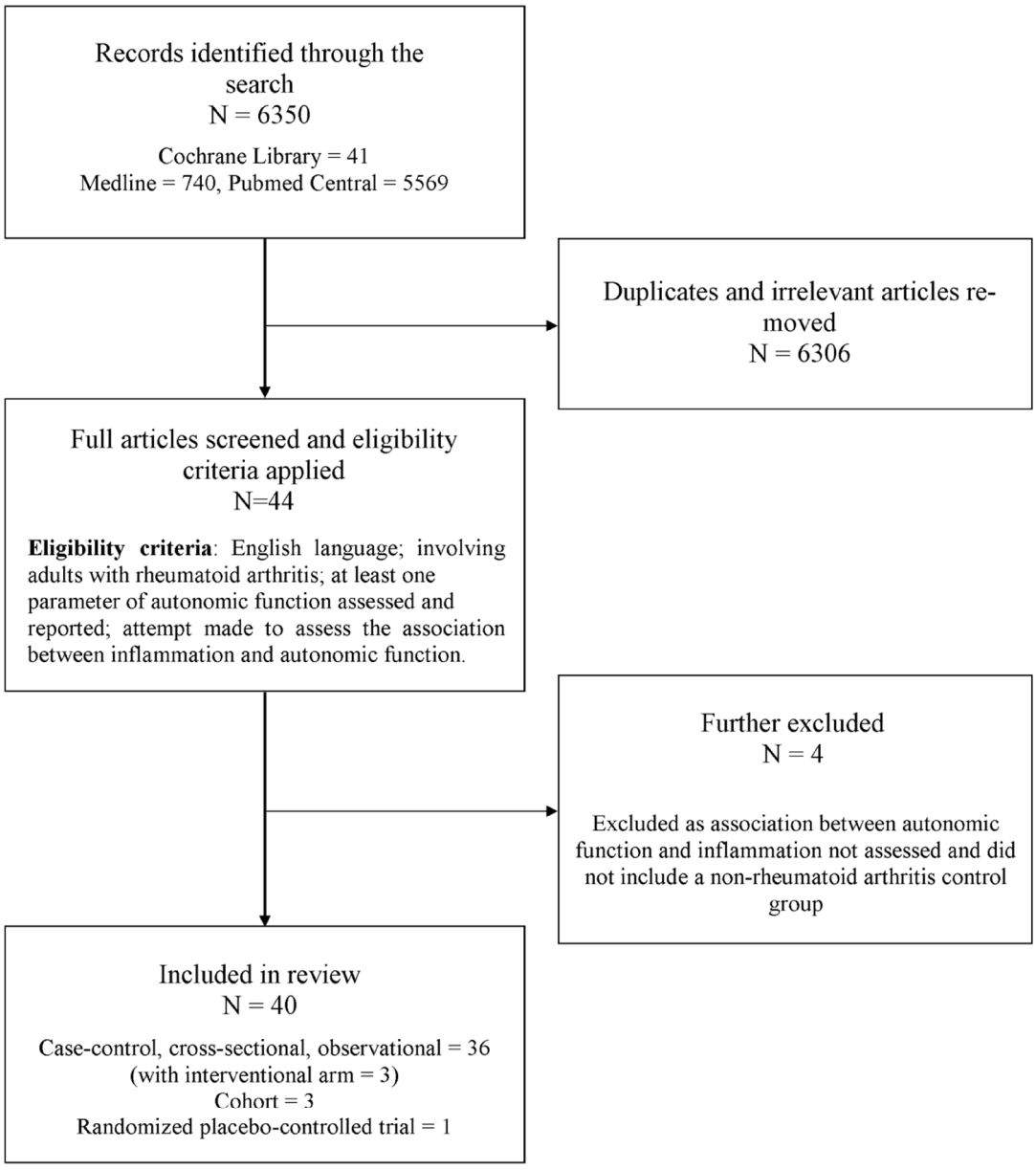


Figure 3.2 Flow diagram showing literature search

3.5 Results

Forty articles were included in the review (445-484). Thirty-six studies were case-control, cross-sectional, observational (Appendix 2), of which 3 had an interventional arm (Appendix 3); 3 were cohort studies, of which 1 was cross-sectional; and 1 study utilized a randomized, placebo-controlled, single-blind, cross-over design (Appendix 3).

In all but six studies the diagnosis of RA was based on the 1987 revised criteria of the American Rheumatism Association (8). Approximately 80 % of patients studied were female with a mean age of ~50 years (estimated calculation from reported values). Mean reported disease duration (from 26 studies) was ~9 years; 4 studies included RA patients diagnosed <2 years. Twenty three (of forty) studies reported RA medications which included disease modified anti-rheumatic drugs of which methotrexate was the most common. Other medications and co-morbidities were only reported in a few studies; but most studies (30 of 40) excluded patients with conditions or medications affecting the ANS (e.g. diabetes mellitus, neurological disease, hypertension, heart failure, vaso-active drugs).

3.5.1 Assessment of ANS Function

Eighteen studies utilized CCTs of ANS function (447, 449, 450, 454, 456, 458, 465, 470-479, 481); 15 studies assessed HRV (445-447, 451, 453, 455, 457, 461, 464, 467, 469, 473, 482-484) of which 5 assessed HRV in combination with clinical cardiovascular reactivity (447, 464, 473, 482, 483); and 16 studies used other methods of assessing ANS function including catecholamines (n=5) (462, 463, 480, 482, 483), biomarkers (n=5) (452, 459, 460, 482, 483), sympathetic skin responses (SSR)(n=5) (456, 458, 465, 466, 478), cBRS

(n=2)(446, 447) and pupillary light reflexes (PLR)(n=2) (448, 476). Studies assessed one (n=30), two (n=8) or three (n=2) parameters of ANS function.

Assessments of ANS function undertaken in RA patients can be broadly categorised into parasympathetic activity (414), sympathetic activity (422) and cBRS (323). Resting activity was assessed in addition to the response to stimuli. For the purposes of this review ANS dysfunction is defined as either: abnormality in CCTs; impaired HRV and/or disrupted sympatho-vagal balance; reduced cBRS; altered concentrations of catecholamines or biomarkers of sympathetic activity; impairment in SSR; impairment in PLR; abnormalities in the above parameters occurring either at rest or following various stimuli.

3.5.2 Prevalence of ANS dysfunction

Seventy three percent of studies (n=27/37) reported at least one abnormality in ANS function in RA patients. Nine studies reported the prevalence of ANS dysfunction, determined from abnormal CCTs, in RA patients with varying results (median prevalence 60 %, range 33-86 %) (Appendix 4) (447, 450, 454, 456, 458, 473, 476, 477, 479). The wide range in prevalence is reflective of variations in criteria for ANS dysfunction, numbers of patients included in studies (n=10-50), and assessments of ANS function performed. CCTs, unlike many others assessments of ANS function have validated reference values and established criteria for detection of abnormalities and classification of the severity of dysfunction (mild, moderate or severe) (415).

3.5.3 Parasympathetic dysfunction

Parasympathetic activity in RA patients was assessed by 25 case-control, cross-sectional observational studies and 1 cohort study using: CCTs (n=14) with HR responses to deep breathing (447, 449, 454, 458, 470-479) and/or orthostasis (447, 449, 454, 470-477, 479) and/or Valsalva's manoeuvre) (447, 449, 454, 472, 474-477, 479); HRV (n=13) with time domain (445, 455, 457, 464, 467, 473, 484) and/or frequency domain parameters (447, 451, 455, 457, 464, 482-484), RSA (453) or heart rate turbulence (HRT) (446); and the PLR (n=2) (448, 476) with constriction and/or maximum velocity latency (Appendix 2, 3).

Of the 26 cross-sectional studies assessing parasympathetic activity, approximately 77 % reported parasympathetic dysfunction (Table 3.1). The main pattern of parasympathetic dysfunction included impaired clinical cardiovascular reflexes (85 %) and abnormal HRV indices (62 %) (Table 3.2). When studies of low quality were excluded (QIS less than 50 %) most studies using CCTs found parasympathetic dysfunction (7 of 8), which was supported by abnormal HRV in most studies (7 of 12). Most of the studies that failed to demonstrate an abnormality in parasympathetic function assessed females only (n=5/7) who were relatively young (mean age range 31-56 years); a demographic known to have elevated HRV indices of parasympathetic activity possibly reflecting the effects of oestrogen (485-488).

For example, Piha et al. (474) found a higher resting HR in 43 female RA patients (mean age 49 years) compared to 69 female controls (mean age 43 years) which may suggest reduced resting parasympathetic activity in the RA group. They reported impaired HR (parasympathetic) responses to orthostasis and Valsalva's manoeuvre in RA patients, which was statistically non-significant when age and resting HR were used as covariates. Although elevations in resting HR may be a result of autonomic dysfunction other factors are known to contribute (e.g. anaemia, infection, anxiety, medications).

Table 3.1 Number of studies with abnormal ANS function in RA patients from cross-sectional studies

	Abnormal studies		Quality Index Score %; range		
	Number/Total	**%**	**Normal**	**Abnormal**	**Total**
Parasympathetic	20/26	77	65%; 44-78	65%; 33-89	65%; 33-89
Sympathetic	16/30	53	59%; 28-89	66%; 44-89	63%; 28-89
Cardiac BRS	1/2	50	56%	89%	73%; 56-89

Avsar et al. (446) reported no difference in HRT in 26 RA patients (18 females, mean age 56 years) compared to 26 age- and gender-matched healthy controls. HRT assesses the autonomic response to ventricular premature complexes (VPC) and hence there is a selection bias inherent to this technique; the ANS function of subjects without VPCs cannot be assessed. Secondly, no power calculation was reported and larger studies (>100 patients) were required to predict cardiovascular risk using HRT (424).

Table 3.2 Abnormal ANS tests in RA patients from observational studies

PARASYMPATHETIC	**RA worse than control**	**No difference**	**RA better than control**
Clinical Cardiovascular Tests, N (refs)			
HR responses to deep breathing	8 (447, 458, 470, 471, 473, 475, 477, 478)	5 (449, 454, 472, 474, 479)	0
HR responses to orthostasis	7 (447, 454, 470, 473-475, 477)	4 (449, 471, 472, 479)	0
HR responses to Valsalva's Manoeuvre	5 (447, 474, 475, 477, 479)	4 (449, 454, 471, 473)	0
Total N (QIS %; range)	**11 (63; 39-89)**	**2 (61; 44-78)**	**0 (NA)**
Pupillary light reflex, N (refs)			
Maximum constriction velocity	1 (448)	0	0
Total N (QIS %; range)	**1 (61)**	**0 (NA)**	**0 (NA)**

PARASYMPATHETIC	**RA worse than control**	**No difference**	**RA better than control**
HRV, N (refs)			
Frequency domain	5 (451, 455, 457, 464, 473)	4 * (447, 482-484)	0
Time domain	7 (445, 455, 457, 464, 467, 473, 484)	0	0
Heart rate turbulence	0	1 (446)	0
Respiratory sinus arrhythmia	0	1 (453)	0
Total N (QIS %; range)	**8 (70; 33-89)**	**5 (71; 56-89)**	**0 (NA)**

SYMPATHETIC	RA worse than control	No difference	RA better than control
Clinical Cardiovascular Tests N (refs)			
BP responses to orthostasis	5 (447, 450, 470, 473, 477)	4 (449, 471, 472, 475)	0
BP responses to hand grip	4 (447, 450, 475, 477)	0	0
BP responses to cold pressor test	1 (450)	0	0
BP responses to mental stress	2 (456, 465)	1 (481)	0
Total N (QIS %; range)	**8 (67; 44-89)**	**4 (61; 44-78)**	**0 (NA)**
Catecholamines			
Plasma	2 (463, 482)	1 (483)	1 (463) †
Urinary	0	1 (462)	0
Total N (QIS %; range)	**2 (64; 61-67)**	**2 (61; 44-78)**	**1 † (63)**

SYMPATHETIC	**RA worse than control**	**No difference**	**RA better than control**
Biomarkers			
NPY	2 (460, 468)	2 (459, 482)	0
Chromogranin	1 (452)	0	0
Total N (QIS %; range)	**3 (61; 50-67)**	**2 (44; 28-61)**	**0 (NA)**
HRV			
Frequency domain	2 (447, 464)	7 (451, 455, 457, 473, 482-484)	0
Pre-ejection period	1 (453)	0	0
Total N (QIS %; range)	**3 (80;72-89)**	**7 (71; 61-89)**	**0 (NA)**
Skin sympathetic responses			
	2 (456, 465)	3 (458, 466, 478)	
Total N (QIS %; range)	**2 (58; 50-67)**	**3 (46; 39-56)**	**0**

BRS	RA worse than control	No difference	RA better than control
	N (refs)	N (refs)	N (refs)
Cardiac BRS			
Spontaneous	1 (447)	0	0
Heart rate turbulence	0	1 (446)	0
Total N (QIS %; range)	**1 (89)**	**1 (56)**	**0 (NA)**

* This study (484) is included in two categories as the authors reported abnormal time domain HRV parameters (worse than control) but normal frequency domain (no difference).

** This study (463) is included in two categories as the authors reported lower resting sympathetic activity (better than control) but with an impaired response (worse than control).

BP = blood pressure, BRS = baroreflex sensitivity, HR = heart rate, HRV = heart rate variability, NPY = neuropeptide-Y, QIS = quality index score, RA = rheumatoid arthritis.

3.5.4 Sympathetic dysfunction

Sympathetic activity in RA patients was assessed by 29 case-control, cross-sectional observational studies and 1 cohort study using CCTs (n=13) with BP responses to orthostasis (447, 449, 450, 470-473, 475-477) and/or handgrip (447, 450, 475, 477) and/or CPT (450) and/or mental stress (456, 465, 481); HRV (n=10) with frequency domain parameters (447, 451, 455, 457, 464, 473, 482-484), pre-ejection period (PEP) (453); biomarkers of sympathetic activity (n=5) with plasma NPY (459, 460, 468, 482), serum chromogranin (452); SSR (n=5) (456, 458, 465, 466, 478); catecholamines (n=4) with plasma (463, 482,

483) or urinary (462) adrenaline, noradrenaline; PLR (n=1) with maximal area in darkness (476).

Of the 30 studies assessing sympathetic activity over half reported sympathetic dysfunction (Table 3.3). The main pattern of sympathetic dysfunction included impaired clinical cardiovascular reflexes (67 %), whilst HRV parameters of sympathetic activity were normal in the majority of studies (70 %) (Table 3.2). When studies of low quality were excluded (QIS less than 50%) most studies using CCTs found sympathetic dysfunction (6 of 9) however this was not supported by abnormal HRV in the majority of studies (2 of 10).

The majority of studies that failed to demonstrate sympathetic dysfunction in RA patients were of predominantly pre-menopausal women, which as discussed previously may cause confounding results. Other possible explanations for negative findings include: failure to control for medications that are known to have an effect on the ANS (481); underpowered studies (459, 471); selection bias when matching controls to RA patients (471); and limitations inherent to ANS assessments for example lack of standardised testing conditions.

3.5.5 BRS

Of the two cross-sectional, case-control, observational studies (446, 447) assessing cBRS one reported abnormality in RA compared to controls (447) (Tables 3.1, 3.2). Aydemir et al. (447) reported a lower resting cBRS (sequence technique) in 36 RA patients (30 females, mean age 49 years) compared to 40 age and gender matched controls (447). In the other study (previously discussed in section 4.5.3) Avsar et al. found no difference in HRT (446).

3.5.6 Time course of ANS dysfunction

Three studies assessed patients with early RA (duration<2years) (453, 456, 459); and in 2 studies sympathetic dysfunction was reported (increased resting sympathetic activity and impaired sympathetic responses to mental stress) (453, 456). Dekkers et al. (453) found no difference in parasympathetic activity (respiratory sinus arrhythmia) but increased sympathetic activity (PEP) in 25 RA patients (19 females, mean age 55 years) compared to well matched healthy controls. RA patients included in this study had a low ESR (mean 15 mm/1st hour) and disease duration <2 years. These few studies suggest that ANS dysfunction in RA may not necessarily be a consequence of long-term disease and inflammatory burden, and that heightened sympathetic activity may be an early phenomenon that precedes parasympathetic dysfunction.

3.5.7 Inflammation and ANS dysfunction

Observational studies

Twenty four studies reported at least one marker of disease severity including ESR (n=19; range 14-61 mm/1st hour) (445, 447, 449, 453, 455, 456, 459, 462, 463, 467, 469, 471, 473, 474, 476-478, 480, 481), CRP (n=12; 5-380 mg/L) (447, 449, 455, 457, 463, 464, 467, 469, 476, 478, 481, 483) and DAS/DAS28 (n=8; 6 moderate and 2 severe) (445, 447, 451, 457, 461, 464, 481, 483). ANS dysfunction was reported more frequently in those studies with higher CRP values (5 v 2; CRP≥14.5 v <14.5 mg/L) and mainly comprised of parasympathetic dysfunction: reduced HRV indices of cardiac parasympathetic control (n=3) (455, 457, 467); and impaired HR responses to deep breathing, orthostasis and Valsalva's manoeuvre (n=1) (476). Approximately one third of studies (n=7/19) reported an association

between ANS function and inflammation: CCTs (n=2/9); HRV (n=3/5); biomarkers of sympathetic activity (n=1/2); and PLR (n=1/1) (Table 3.3). When low quality studies were excluded (QIS less than 50 %) only 5 of 14 studies found an association. In 7 more recent studies (≥1993) using CCTs (447, 456, 471, 474, 475, 477, 479), no significant correlation was found in RA patients between ANS function and any of the following: ESR, CRP, the Ritchie articular index (assessment of joint tenderness and swelling), the presence of an inflammatory syndrome (not defined), DAS28 (an updated version of DAS with clinical assessment of 28 joints), disease duration, presence of rheumatoid factor or articular damage on radiograph.

Studies demonstrating associations between inflammation and parasympathetic function are conflicting. Yadav et al. (484) studied 45 RA patients (41 females, mean age 41 years) and found a significant positive correlation between DAS28 and a parasympathetic index of HRV. Anichkov et al. (445) in contrast found an inverse correlation between 24-hour HRV parameters of parasympathetic function and markers of disease severity and inflammation (number of swollen joints, Ritchie articular index, DAS). Barendregt et al. (448) found that ESR levels were higher in the group with parasympathetic dysfunction (abnormal PLR in the RA group with ocular dryness) compared to those without (although significance values were not reported). Two studies found no significant correlation between catecholamines and inflammatory indices. Vlcek et al. (483) found no significant correlation between plasma catecholamines and inflammation (CRP, DAS28-CRP). Van Middendorp et al. (480) found no correlation between 24-hour urinary noradrenaline excretion and markers of inflammation (ESR or IL-6) in a cohort of 60 RA patients (38 females, mean age 59 years). In contrast, Dekkers et al. (453) (described earlier) reported that higher sympathetic activity (PEP) was associated with higher disease activity (ESR and Thompson joint score). In another

Table 3.3 Associations between autonomic function and inflammation in RA

	Outcome	
	Association found	**No association**
Clinical cardiovascular tests (n=9)	2 (42; 39-44)	7 (73; 56-89)
Heart rate variability (n=5)	3 (77; 72-88)	2 (72; 56-89)
Catecholamines (n=2)		2 (67; 56-78)
Biomarkers (n=2)	1 (67)	1 (67)
Pupillary light reflex (n=1)	1 (61)	
TOTAL	**7 (64; 39-88)**	**12 (71 56-89)**

Values are number of studies (% quality index score; range).

study, Igari et al. (462) found that 24-hour urinary adrenaline and noradrenaline significantly decreased 2 weeks following synovectomy in 6 RA patients. Although the investigators did not assess inflammatory markers following synovectomy, it may be postulated that local joint inflammation could have been reduced following synovectomy and hence possibly removing the stimulus for sympathetic activation.

Interventional studies

Two studies investigated HRV in RA patients receiving TNF-α inhibitor therapy (461, 469). Holman et al. (461) studied 33 patients (25 with RA, 8 with psoriatic arthritis) before treatment with TNF-α inhibitor therapy and assessed clinical response to treatment (using ACR criteria ACR20/50/70 and DAS28) at various time points up to one year. They found

that low HRV indices, reduced parasympathetic and increased sympathetic activity were predictors of poor response to TNF-α inhibitor therapy. However the study may have been underpowered as they found no direct correlation between baseline autonomic function and change in DAS28 score following TNF-α inhibitor therapy. Despite limitations of the study (one third of patients discontinued therapy by one year; use and dosage of other medications were not controlled; small numbers of RA patients) these results suggest that HRV and sympatho-parasympathetic balance may play an important role in disease activity. In another study Lazzerini et al. tested the acute effects of infliximab (TNF-α inhibitor) on HRV and arrhythmia risk in RA patients (461, 469). They found that TNF-α inhibition acutely reduced HRV, and that patients who developed new-onset arrhythmia (infliximab-induced) tended to have lower HRV and a higher CRP. While the acute effects reported may relate to a direct drug effect as opposed to cytokine inhibition, this study suggests an increased arrhythmia risk in RA patients with lower resting parasympathetic activity and increased inflammation.

Two studies assessed plasma NPY levels before and after TNF-α inhibitor therapy. In a study of 16 female RA patients Kopec-Medrek et al. (468) found that infliximab significantly reduced inflammation (CRP, ESR) but did not reduce sympathetic activity (plasma NPY). In fact, plasma NPY concentrations rose to a peak after 6 infusions of infliximab and fell to baseline levels 8 weeks after the ninth (final) infusion. The authors did however report a positive correlation between plasma NPY concentrations and CRP (Kendall tau coefficient=0.506, $P<0.006$) and DAS28 (Kendall tau coefficient=0.393, $P<0.033$) at baseline, indicating that plasma NPY may reflect inflammatory status. Harle et al. (460) found that in a cohort of RA patients, adalimumab (TNF-α inhibitor) had no effect on serum NPY levels despite good clinical response. They reported higher plasma NPY concentrations in RA patients with previous prednisolone use only, indicating a possible HPA axis interaction.

3.6 Discussion

3.6.1 Epidemiology of ANS dysfunction in RA

The results of this systematic literature review indicate that ANS dysfunction is prevalent in ~60 % (33-86 %) of RA patients as determined from observational studies of small sample size (10-50 patients). Stronger evidence (from large prospective cohort studies) is required to confidently determine the true prevalence of autonomic dysfunction in RA. HRV is probably the most feasible ANS assessment in such a large population. Few studies have assessed patients with early RA (duration<2years) but have shown that ANS dysfunction occurs early in RA and is not necessarily an effect of long-term disease and inflammatory burden. More studies of RA patients with early disease are clearly needed and if possible ANS assessment preceding the onset of RA, to determine whether altered ANS function predisposes to developing RA.

3.6.2 Pattern of ANS dysfunction in RA

Studies using CCTs in RA have shown reduced resting parasympathetic activity and impairment in both sympathetic and parasympathetic reflex responses. Strong evidence from good quality HRV data supports these findings with the majority demonstrating low HRV reflecting reduced resting parasympathetic activity. In addition there is limited evidence for elevated resting sympathetic activity with the majority of good quality HRV data failing to detect abnormal sympathetic function in RA. Studies employing other methods of ANS assessment have shown conflicting results, which may reflect their inherent limitations. There is a lack of evidence from the literature to date to determine causal relationships between systemic inflammation and autonomic dysfunction. The available literature is too small to be

clear whether the lack of evidence represents a lack of relationship or simply inadequate power. Only two studies assessed the effects of anti-inflammatory therapy on ANS function and failed to demonstrate an effect. However, their results suggest that plasma NPY may not be a reliable method of assessing sympathetic activity particularly as the effects of steroids on NPY are not known. Further interventional studies are needed to elucidate causation. The most feasible and ethical study design would be to assess ANS function in RA patients prior to and after anti-inflammatory therapy. This could be achieved for example with HRV assessments using a 24-hour ECG holter. Although HRV is not routinely used in clinical practice one study suggested a possible clinical role. Holman et al. (461) found that low HRV in RA patients predicted a poor response to TNF-α inhibitor therapy indicating a possible benefit in determining ANS status prior to initiation of biologic agents. What remains unknown however is whether therapy to improve HRV in these patients would improve their response to anti-inflammatory agents.

3.6.3 Associations between ANS and inflammation

Less than half the studies demonstrated an association between increased inflammation and ANS dysfunction (mainly CCTs and HRV), consistent with the results of recent animal studies (52, 153, 314). The lack of associations in the remaining studies may be simply due to a lack of statistical power; the majority of studies in our review did not report a power calculation. Another possible explanation may be the relatively low inflammatory status of patients tested. CRP, ESR and DAS (reported in less than two thirds of studies) were only modestly elevated although it is unclear whether cumulative inflammatory burden can be determined from assessment at a single time point. Another explanation for a lack of association between inflammation and ANS function in the studies included in our review

may be the subtle nature of autonomic dysfunction present in RA or simply the inappropriate choice of immune markers assessed.

3.6.4 Limitations

The main limitations of this review are the types and number of ANS tests employed in RA patients, with the majority of studies making only one assessment of ANS function. ANS function is complex and multi-faceted and hence a comprehensive assessment is required in order to fully categorise the presence of dysfunction. Future studies should include a greater variety of tests including arterial baroreflex assessment, with attempts to measure resting ANS function and response to stimuli. Larger sample sizes are required to confirm the prevalence of ANS in RA, and in order to ensure that statistical power is achieved.

3.6.5 Future directions

Future studies in RA should aim to characterise the inflammatory profile of patients studied so that causal links between inflammation and ANS dysfunction can be determined. The effects of RA medications on ANS function is not fully known and is a difficult confounding factor to control for, especially as RA patients often require medications to induce and maintain remission of disease. One study showed that infliximab infusion (TNF-α inhibitor therapy) caused an acute reduction in HRV and sympathetic activity compared to a placebo. The effects of other RA medications on the ANS tests employed to date are unknown although studies of healthy subjects may be the most ethically acceptable way to investigate this.

Another difficulty is discerning between the effects of RA and concomitant co-morbidities or medications on ANS function. Although many studies excluded RA patients with conditions or medications affecting the ANS system, cardiovascular disease remains under-diagnosed in this population (25, 27, 29). Cardiac imaging (e.g. echocardiography or magnetic resonance imaging) to identify such patients and the possible inclusion of a cardiovascular disease control group may help tackle this problem.

3.7 Conclusion

In conclusion, the evidence to date supports that ANS dysfunction is a feature of RA, although not universally found in all patients. The profile of ANS dysfunction found in RA patients (low HRV, reduced parasympathetic activity and elevated sympathetic activity) is associated with increased cardiovascular and mortality risk and may help to explain the increased risk in RA patients. Furthermore, this pattern of ANS dysfunction supports the findings from animal studies and may be a consequence of high inflammatory burden. Although associations between inflammation and ANS dysfunction are present in RA patients, the available literature is too small and underpowered to be clear about causality. Further studies are required to: determine the true prevalence of ANS dysfunction in RA, characterise RA patients who have altered ANS function; determine the prognostic role of ANS assessments in predicting cardiovascular and mortality risk; assess the effects of biologic agents on ANS function; consider the role of therapeutic strategies targeting the ANS in RA patients to help control disease activity or improve response to biologic agents.

CHAPTER 4 Methodology

4.1 Subjects

Patients with a diagnosis of RA based on the 1987 ACR criteria (8) were recruited from the rheumatology clinics at Russells Hall Hospital, Dudley, UK and Sandwell General Hospital, West Bromwich, UK. Exclusion criteria included: age <18 or >75 years; atrial fibrillation or other heart rhythm disorder, significant valvular disease, coronary artery disease, diabetes, ischemic stroke, chronic renal failure, liver impairment, hormone replacement therapy and those who are pregnant or who might be pregnant. Age-, sex- and BMI-matched control participants were recruited from the hospitals and surrounding areas. Normotensive control (NC) participants were free from major illnesses, whilst hypertensive controls (HTN) either had a prior diagnosis of hypertension or BP≥140/90 mmHg. In addition 10 healthy young male controls (HC) free from major illnesses or medications were recruited from the University of Birmingham, UK and surrounding areas. Figure 4.1 shows a flow-chart of recruitment and group allocation for the experiments. Ethical approval was obtained by the local Research Ethics Committee (National Research and Ethics Service Committee West Midlands - Edgbaston, 11/WM/0298). Written informed consent was received from participants prior to inclusion in the study, in accordance with the Declaration of Helsinki 2013.

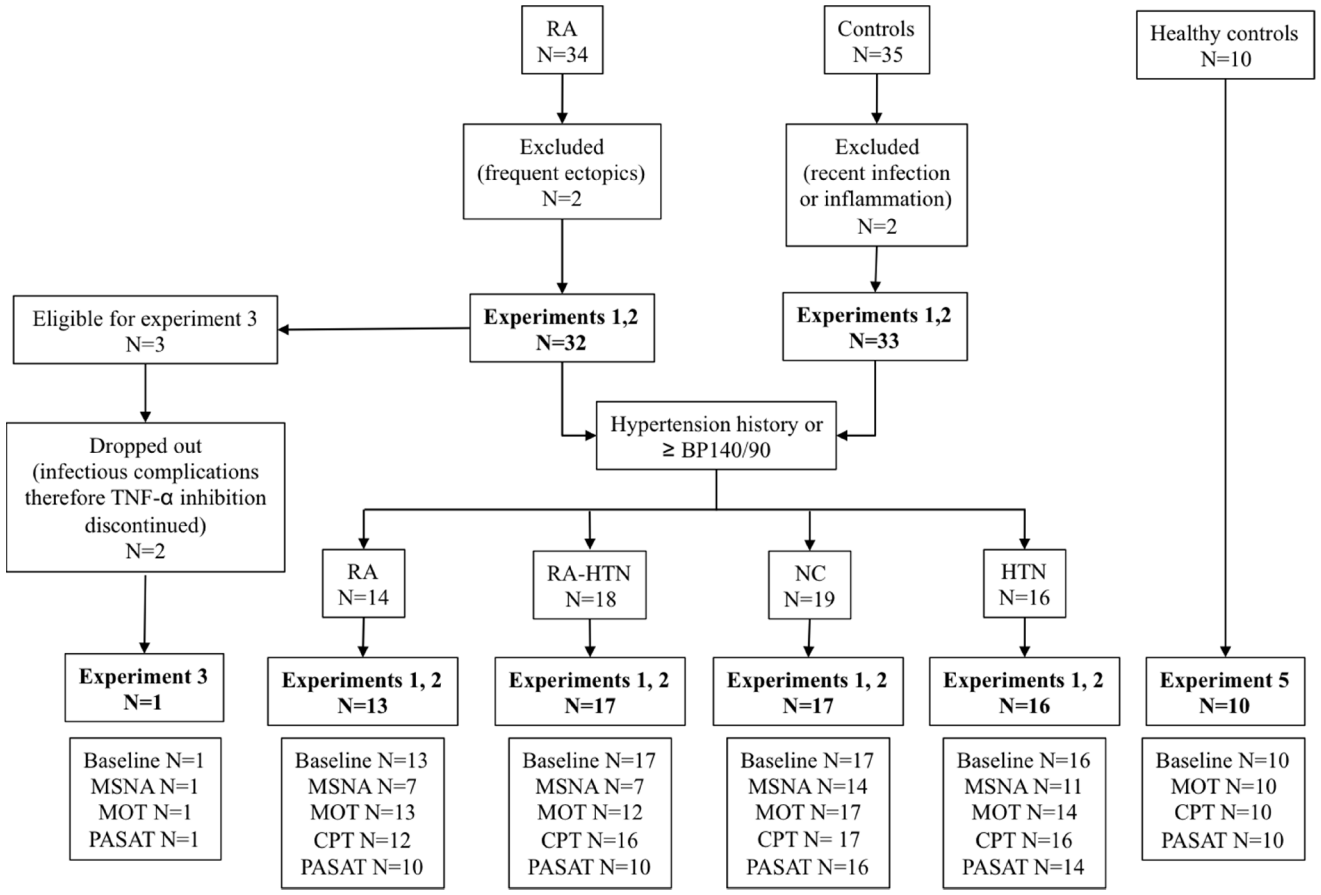


Figure 4.1. Flow chart summary showing subject recruitment and group allocation.

CPT = cold pressor test, HTN = hypertension, MOT = modified Oxford technique, MSNA = muscle sympathetic nerve acticity, NC = normotensive control, PASAT = paced auditory serial arithmetic task, RA = rheumatoid arthritis, TNF = tumor necrosis factor

4.2 Experimental protocol

Experiments 1, 2 and 3

Participants attended the research laboratory at 09:00 h following an overnight fast (from food, caffeine and alcohol). Additionally all medications were withheld on the morning of testing. A detailed clinical history was obtained and physical examination performed in RA patients to count the number of swollen and tender joints in order to determine the DAS28-CRP (489). Pain VAS was used as a measure of pain (490). Participants were instructed to "mark the point on the line that best describes your overall pain at the moment" ranging from a score of 0 indicating "no possible pain" to 100 indicating "worst possible pain". Height and weight was measured, and BMI index was determined (weight/height^2). All subsequent measurements were performed under uniform conditions in a temperature-controlled room whilst resting quietly in the supine position. Measurement equipment (HR, BP, leg blood flow, MSNA) was attached to the participant and an intravenous catheter was inserted into a superficial vein in the antecubital fossa for blood sampling and injections (Figure 4.1). Data was recorded during the protocol, which involved a resting 10-minute baseline period followed by the MOT, CPT and PASAT mental stress test, consecutively.

Experiment 5

Each participant was investigated on two occasions separated by one week, according to a placebo-controlled, single-blinded, cross-over design. Participants were randomised to receive either placebo or HCN on the morning of the first visit using a randomisation method in two groups of five (491). The choice was blinded to the participant throughout the duration of the study but not the investigator. Computer generated random sequences were used to

determine the order of drug administration. None of the subjects reported any side effects and were unable to distinguish between HCN and placebo administration.

Participants attended the research laboratory at 09:00 h. An intravenous catheter was inserted into a superficial vein at the antecubital fossa for blood sampling and injections. Baseline blood samples were taken for biochemistry including renal function (serum creatinine), electrolytes (sodium, potassium), plasma osmolality, baseline cortisol and ACTH. All participants were then administered either 200 mg hydrocortisone (Solu-Cortef ®) or placebo (9 % saline solution) intravenously. Following this, subjects were given a standardised light breakfast (a single sachet of Quaker Oats So Simple ® porridge Apple & Blueberry, Golden Syrup or Original with 200 ml of semi-skimmed milk, one banana and 200 ml carton of Vitafit © Apple or Orange juice) and observed in a quiet, temperature-controlled room. At 12:00 h height and weight were measured and BMI was determined (weight/height^2). The participants assumed a comfortable supine position and were instrumented with the measurement equipment (HR, BP, leg blood flow). Further blood samples were taken (cortisol, ACTH, catecholamines, serum electrolytes, serum osmolality and haematocrit) following which participants rested quietly for a further 30 min. During the final 10 min of this period data were recorded. The protocol involved a resting 10-minute baseline period followed by the MOT, CPT and PASAT mental stress test, consecutively.

4.3 Variables

Data was acquired using the Powerlab 16/35 data acquisition system and a personal computer equipped with Labchart Pro software (ADInstruments, Bella Vista, Australia). All analyses were performed offline. Cardiovascular variables were sampled at 1 kHz, and beat-

to-beat values of HR, systolic BP, diastolic BP and mean BP calculated. MSNA signals were sampled at 10 kHz. Using a macro (Microsoft Office Excel 2007, Microsoft Corporation, Washington, USA) beat-to-beat values were averaged over 1 minute intervals.

4.3.1 Haemodynamic

HR was continuously recorded using a lead II ECG (BioAmp, ADInstruments, Bella Vista, Australia). Beat-to-beat BP was recorded using finger photoplethysmography (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands) and was calibrated with brachial BP recordings using an automated sphygmomanometer (Omron 705IT, Omron Corporation, Hoopddorp, The Netherlands).

4.3.2 MSNA

Multi-unit recordings of MSNA were obtained (FE185 NeuroAmp EX, ADInstruments, Bella Vista, Australia) from the peroneal nerve using tungsten microelectrodes (200 μm, 1-3 μm uninsulated tip; UNA32F2S, FHC, Bowdoin, USA) via the microneurography technique (sampling frequency 10 kHz). Electrical stimulation on the skin surface was used for nerve mapping, and a reference electrode was inserted subcutaneously 2 to 3 cm away from the recording electrode which was inserted into the nerve fascicle (Figure 4.1). The neural signals were amplified, filtered (2000 Hz high pass, 100 Hz low pass, 60 Hz notch), rectified and integrated (absolute value, time constant decay 0.1 s) to obtain a mean voltage of sympathetic nerve activity. MSNA was confirmed by: listening to the amplified signal on speakers; observing the characteristic bursting pattern on a computer screen; palpating the skin and muscle fibres; and performing a breath hold (422, 492). Sympathetic

bursts were identified and scored by a trained observer (Ahmed Adlan – author) using a semi-automated macro created using Spike2 (Cambridge Electronic Design, Cambridge, UK). Identifiable features of sympathetic bursts include: a characteristic spike (rapid rise and fall in signal) which can be seen on the screen and heard from the speaker; cardiac cycle linked; larger amplitude compared to background noise; increase in activity following breath hold. MSNA burst frequency (bursts/minute) and incidence (bursts/100 heart beats) was determined (Figure 4.2).

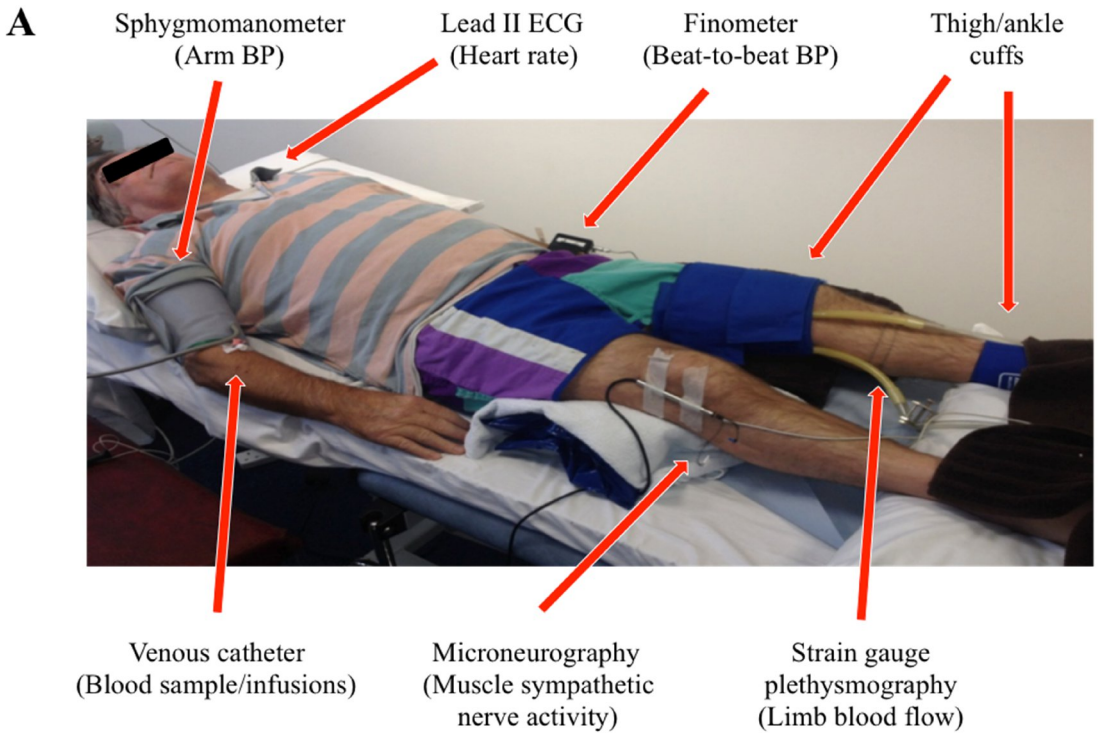


Figure 4.2 Experimental setup.

(A) Participants lay in the supine position with the measurement equipment as shown. MSNA was recorded using the microneurography technique whereby a tungsten microelectrode (200 µm length, 1-3 µm tip) was placed subcutaneously approximately 2 to 3 cm away from the recording electrode that was inserted into the peroneal nerve (magnified in B). BP = blood pressure, ECG = electrocardiogram, MSNA = muscle sympathetic nerve activity

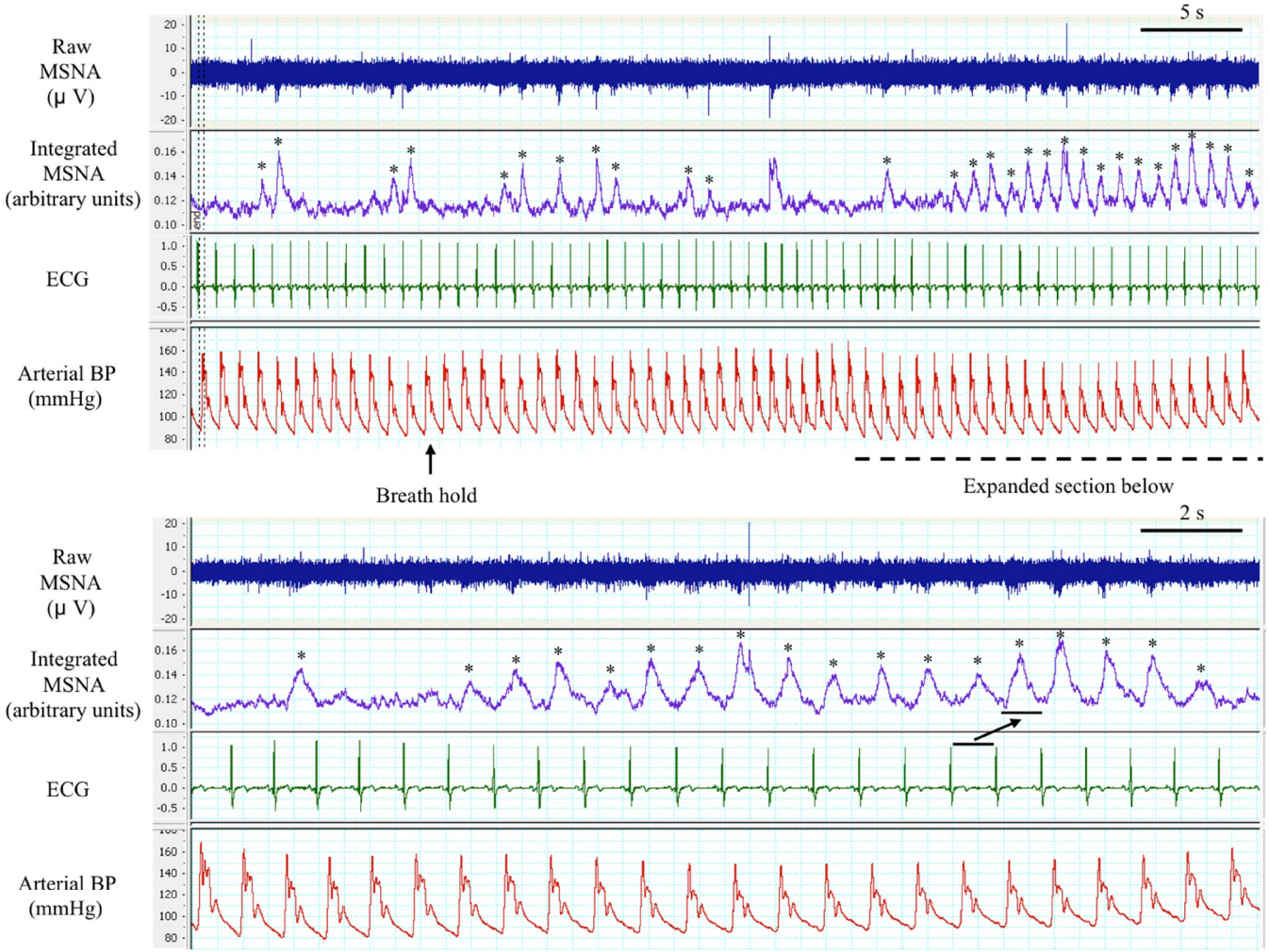

Raw MSNA (μ V)
Integrated MSNA (arbitrary units)
ECG
Arterial BP (mmHg)
5 s
Breath hold
Expanded section below
2 s

Figure 4.3 Original record showing MSNA burst identification.

Original record of an RA patient showing raw MSNA, integrated MSNA, ECG and arterial BP during a breath hold with expanded section below (dashed line). MSNA bursts (*) can be identified by the following features: characteristic spikes (rapid rise and fall in signal); cardiac cycle linked (see arrow); activity greater than the background noise; and increased burst frequency following breath hold. Muscle sympathetic nerve activity can be quantified by dividing the number of MSNA bursts by time to give MSNA burst frequency (MSNA bursts/min), and by dividing the number of MSNA bursts by (number of heart beats x 100) to give MSNA burst incidence (MSNA bursts/100 heartbeats).

4.3.3 Cardiovagal BRS (cBRS)

The MOT involves sequential intravenous bolus infusions of sodium nitroprusside (100 micrograms) and phenylephrine (150 micrograms) one minute apart, during which arterial BP and RR-interval are simultaneously recorded (433). Infusion of these agents results in a fall and subsequent rise in arterial BP that activates and deactivates the arterial baroreceptors (Figure 4.3). The relationship between the arterial BP and RR-interval was analysed during the phenylephrine-induced rise in systolic BP. Analysis began at the first concordant change in systolic BP and RR-interval after phenylephrine infusion until the systolic BP and RR-interval changes were discordant (433). Baroreflex delays were accounted for by associating systolic BP with concurrent (resting RRI greater than 800 ms) and subsequent RR-intervals (resting RRI between 500 and 800 ms) (493, 494). RR-interval values were averaged over 3 mmHg pressure bins to account for respiratory-related variations (495). Saturation and threshold regions of the baroreflex curve were excluded and the linear relationship between systolic BP and RR-interval was determined using piecewise regression (G_{MOT}) (Figure 4.5) (496). A minimum of 10 points was required and only values with $r^2 \geq 0.6$ were accepted.

The 'sequence technique' was used to provide a spontaneous measure of cBRS (427). Commercially available computer programs (CardioSeries v2.4, CardioSeries, Sao Paulo, Brazil) were used to identify sequences of three or more consecutive cardiac cycles where increases/decreases in systolic BP (of at least 1 mmHg) were related to lengthening/shortening in RR-interval of the following cardiac cycle (i.e. lag +1). The slope of the regression line (RRI v systolic BP) was calculated individually and all slopes were averaged to provide an overall sensitivity gain (G_{SEQ}) (497). A minimum threshold of 1

mmHg for systolic BP and 1 ms for RRI was applied, and only sequences with an R^2>0.8 were accepted.

Low frequency transfer function gain (G_{LFTF}) was determined using cross-spectral analysis of systolic BP (input) and subsequent RR-interval (output) in the low frequency range (0.047-0.156 Hz) (436). Only values with a coherence ≥ 0.5 were included. The low frequency range has the advantage of representing spontaneous oscillations in BP and RR-interval without the effects of breathing (206).

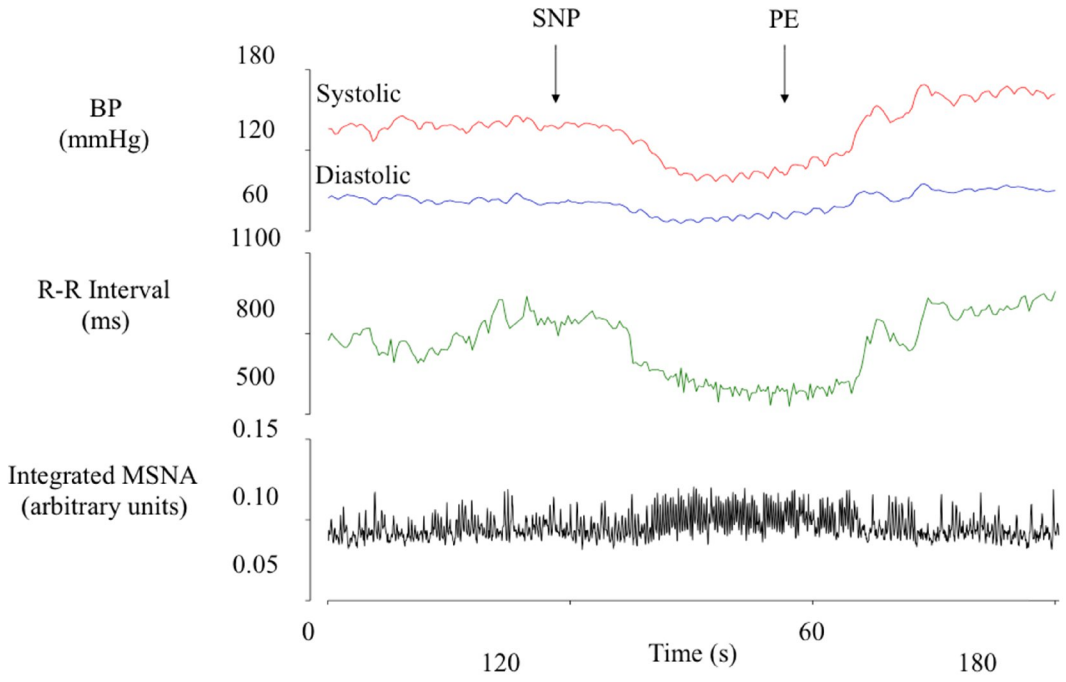


Figure 4.4 Original record showing the modified Oxford technique.

Original record of an RA patient showing HR, BP (systolic and diastolic) and MSNA responses during the modified Oxford technique. Following a period of rest sequential infusions of sodium nitroprusside (100 µg) and phenylephrine (150 µg) one minute apart induce a fall then rise in BP, a rise then fall in HR and MSNA.

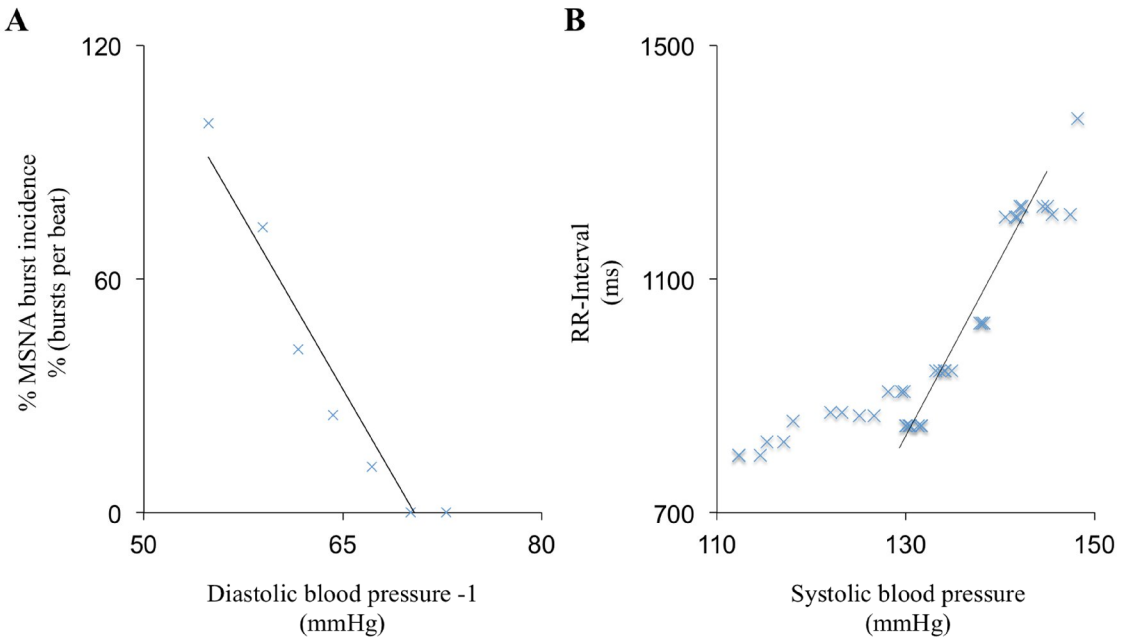


Figure 4.5 Original record showing baroreflex function curves derived from the modified Oxford technique.

Original record of a healthy control showing (A) sympathetic and (B) cardiovagal baroreflex function curves derived from the modified Oxford technique.

4.3.4 Sympathetic BRS

Assessment of baroreflex control of MSNA was determined using linear regression (diastolic BP v MSNA burst activity) during the phenylephrine-induced rise in BP (498). Briefly, each cardiac cycle was scored according to the presence or absence of a sympathetic burst. Data was binned according to the diastolic BP of the previous cardiac cycle (DBP-1, 3 mmHg bins). Linear regression analysis was then performed on the relationship between the mean of each diastolic BP bin and MSNA activity (% MSNA bursts/beat). All data was weighted for the number of cardiac cycles in each diastolic BP bin (498). The slope of the linear regression (DBP-1 mmHg v % [MSNA bursts/beat]/mmHg) was used as an index of

sympathetic BRS (Figure 4.5). Sympathetic BRS was also determined from 10-minute resting period (spontaneous).

4.3.5 Blood flow and vascular conductance

Leg and forearm blood flow was measured using venous occlusion strain gauge plethysmography (Hokanson EC-6 plethysmograph, D E Hokanson, Bellevue, United States of America, USA) (499). A lightweight indium-in-silastic strain gauge was positioned around the right calf and the right forearm at the point of greatest circumference. The length of the strain gauge was 2 cm less than the widest girth of the calf or forearm. Calibration was performed by measuring the change in voltage produced by a 1 % change in length of strain gauge. Cuffs were placed around the ankle and wrist and inflated to a pressure of 200 mmHg and maintained for 1 minute to achieve arterial occlusion. Sixty seconds later cuffs placed around the thigh and forearm were rapidly inflated to 50 mmHg (Hokanson E20 rapid cuff inflator and AG101 air source, Hokanson, Bellevue, USA) to evoke venous occlusions. Venous occlusion was repeated three times during 1 min, with the thigh and forearm cuffs inflated for 5 s and then deflated for 10 s each time. LabChart software (ADInstruments, Bella Vista, Australia) was used to measure the slope during venous occlusion. The following formulae were then used to calculate leg and forearm blood flow and vascular conductance using a Microsoft Office Excel 2007 (Microsoft Corporation, Washington, USA):

Leg/forearm blood flow (ml/100ml/min) = ([slope (V/s) / 1 % calibration (ml/100 ml)] x 60)

Leg/forearm vascular conductance (LVC/FVC, arbitrary units, AU) = ([Blood flow (ml/100 ml/min) / Mean BP (mmHg)] x 1000))

Blood flow measurements were obtained three times during the 10 minute resting baseline period, five times during the CPT and seven times during the PASAT (Table 4.1).

Table 4.1 Blood flow measurements obtained during resting baseline, CPT and PASAT

	Phase	Duration (mins)	Limb blood flow recorded (min)
Baseline	Rest	10	2, 5 and 8
CPT	Rest	4	1, 3
	Test	2	2
	Recovery	4	1, 3
PASAT	Rest	4	1, 3
	Test	6	1, 3 and 5
	Recovery	4	1, 3

CPT = cold pressor test, PASAT = paced auditory serial mental arithmetic task

4.3.6 HRV

Time domain, frequency domain and non-linear indices of short-term HRV were determined from a 10 min resting period (Kubios HRV, Kuipio, Finland) in accordance with guidelines from the Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology (196). Data was pre-screened for ectopics and these were corrected using the Kubios software (accounted for <1 % of all recordings) (196). Time domain indices included square root of the mean sum of the squares of difference between adjacent inter-beat intervals (rMSSD), number of pairs of adjacent inter-beat intervals differing by more than 50 ms in the entire recording expressed as an absolute value

(NN50) and a percentage of all inter-beat intervals (pNN50 %). Frequency domain indices (using fast Fourier transform) of R-R intervals included total power (TP), very low frequency power (VLF, range 0-0.04 Hz), low frequency power (LF, range 0.04-0.15 Hz) and high frequency (HF, range 0.15-0.4 Hz). Absolute values for TP, VLF, LF and HF were determined in addition to normalised units for LF, HF and ratio of LF/HF power. In addition non-linear indices of HRV were determined; standard deviation of the Poincare plot SD1, SD2 and the SD1/SD2 ratio. rMSSD, NN50, pNN50 % and HR fluctuations in the HF power range are suggested to be principally indicative of cardiac parasympathetic activity (196). The physiological correlates to LF power fluctuations and the LF/HF ratio remain uncertain (429). The Poincare plot is a scatter diagram where each RR-interval is plotted against the previous RR-interval (500). SD1 provides an estimate for short term HRV whilst SD2 is representative of long term HRV (501) and is influenced by both parasympathetic and sympathetic activity (502). The SD1/SD2 ratio provides a measure of the relationship between SD1 and SD2.

4.3.7 Cardiovascular reactivity

For the CPT participants lay still in a supine position whilst baseline measurements for 4 minutes were obtained. The right hand was then immersed completely in a container of cold water at 4 ^{o}C for 2 minutes (324). Following this recovery measurements were obtained for 4 minutes. Brachial BP and leg flow measurements were obtained during minutes 1 and 3 (baseline and recovery) and in the second minute of cold-water immersion. During the PASAT test a series of single digit numbers were presented to the participants for 6 min using a pre-recorded audio file on a computer. Participants were instructed to add each number they heard to the previous number presented to them, and retain the last number to add to the next number they heard (481, 503). In order to make the task progressively more challenging the

numbers were presented every 3.5 s, 3.0 s and 2.5 s respectively, in three consecutive blocks each lasting two minutes. An experimenter checked their responses against the correct answers and alerted the participant with a loud buzzer noise with each incorrect answer, hesitation or once during every 10 additions if no mistakes were made. Finally, in order to increase social evaluation participants were instructed to view themselves in a mirror for the duration of the mental stress test. A 10-point scale was used to obtain perceived pain and stress ratings from the subjects following the CPT and the PASAT mental stress test, respectively. Cardiovascular variables were averaged during rest, test and recovery phases to provide absolute values. Differences between baseline, test and recovery phases of CPT and PASAT were reported as absolute or percentage change.

4.3.8 BP variability

Time and frequency domain parameters of systolic and diastolic BP variability were determined from a 10 min resting period (CardioSeries v2.4, CardioSeries, Sao Paulo, Brazil). Time domain indices included standard deviation (SD) and variation of coefficient (VC = SD/BP x 100). Frequency domain indices (using fast Fourier transform) included LF power (range 0-0.04 Hz) and HF power (range 0.15-0.4 Hz). BP oscillations in the LF range are believed to principally represent vasomotor activity whilst HF oscillations represent respiratory influences (497).

4.3.9 QT interval

QT interval (time between beginning of a Q wave and then end of a T wave) was manually measured by two experts and corrected for HR using Bazett's formula (QT_{BAZ});

methods described in detail elsewhere (35). QT interval, HR and QT_{BAZ} data was obtained from a previous study (35). Additionally QT was corrected using the Framingham Heart Study formula (QT_{FHS} = QT + 0.154x [1-RR]) (504). The Minnesota Code Classification System was used to code for the presence of ST abnormality on the ECG (505).

4.3.10 Blood sampling

Blood samples for inflammatory markers were centrifuged immediately and the plasma stored at -80 ^{o}C. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to determine hs-CRP (MP Biomedicals, California, USA) and cytokines (IL-6, TNF-α, IL-10; BioSupply UK, Bradford, UK). The intra- and inter-assay coefficients of variations were 7.5 % and 4.1 % respectively for hs-CRP, 4.9 % and 6.0 % for IL-6, 8.5 % and 9.8 % for TNF-α and 6.8 % and 7.5 % for IL-10. Local routine clinical laboratories were used to analyse full blood count, renal function, fasting lipid profile and fasting glucose.

4.3.11 Statistical analyses

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software, version 19 (SPSS Inc, Chicago, USA). Continuous variables were assessed for normality using the Kolmogorov-Smirnov test. Unless otherwise specified data are expressed as mean± SD, geometric mean (95% confidence interval, CI) or frequency (%). Significance levels were set at $P \leq 0.05$. Further details are found within the specific experimental chapters.

CHAPTER 5 Experiment One. Heightened sympathetic nerve activity and reduced BRS in RA: a case control study

5.1 Abstract

Objectives To test the hypothesis that MSNA was increased and BRS was decreased in RA, a chronic inflammatory condition associated with increased cardiovascular mortality and poorly understood pathophysiology.

Methods MSNA (microneurography), beat-to-beat BP (Portapres), and HR (lead II ECG) were recorded in age- and sex-matched RA normotensive (n=13), RA hypertensive patients (RA-HTN; n=17), normotensive (NC; n=17) and hypertensive controls (HTN; n=16). BRS was determined with sequential bolus infusions of SNP and PE (MOT). Inflammation and pain were determined using serum hs-CRP and a pain VAS, respectively.

Results MSNA was elevated similarly in RA, RA-HTN and HTN patients (32±9, 35±14, 37±8 bursts/min) compared to NC (22±9 bursts/min; p=0.004). Sympathetic BRS was not different between groups (p=0.927), while cBRS was reduced in RA, RA-HTN and HTN patients (geometric mean 5, 95 % CI [3-8], 4[2-7], 6[4-9] ms/mmHg) compared to NC (11[8-15] ms/mmHg; p=0.002). hs-CRP was independently associated with HR (positive), but associations with MSNA (positive) and cBRS (inverse) were confounded in this study. Pain VAS was independently associated with MSNA burst frequency, cBRS and HR.

Conclusions This study provides the first direct evidence for heightened central sympathetic outflow and reduced cBRS in RA that can be independent of hypertension. ANS dysregulation in RA is strongly associated with pain and to a lesser extent with inflammation. Further studies are required to determine whether anti-inflammatory therapy can restore ANS function.

5.2 Introduction

A heightened central sympathetic outflow to the heart and/or vasculature has been identified in numerous cardiovascular diseases (439), and is associated with the pathogenesis of hypertension (439), cardiac arrhythmias (506) and increased mortality (113, 384). Reciprocal links between inflammation and the sympathetic nervous system have been identified (52, 61, 153). Central sympatholytic agents have anti-inflammatory actions in human hypertension (183), whereas infusion of inflammatory cytokines elevated in RA patients, such as IL-6 (52), TNF-α (61) and IL-1β (153), increase sympathetic nerve activity in rats. Thus, the chronic activation of inflammatory cytokines in RA may elicit deleterious increases in sympathetic neural outflow that further promote activation of pro-inflammatory cytokines. In addition, microinjection of IL-6 into the NTS is reported to reduce BRS (314). Depressed BRS in RA could precipitate further increases in central sympathetic outflow, reduce cardiac electrical stability and contribute to the recognised increase in cardiovascular risk (21, 507).

Whether sympathetic activity is increased and BRS is reduced in RA is presently equivocal, partly owing to the indirect methods of assessment used to examine this (508). Elevations in plasma noradrenaline have been reported in RA patients (482), but it is not clear if this is a consequence of increased central sympathetic outflow, an enhanced release from peripheral adrenergic stores, or from an altered local reuptake mechanisms (509). Such limitations are overcome by the microneurography technique, which provides a direct assessment of sympathetic nerve activity to the skeletal muscle vasculature (MSNA), although such measures have not been undertaken previously in RA. It is also unknown whether baroreflex regulation of MSNA is altered in RA. Although a reduction in cBRS has

been suggested in RA patients (447, 510), baroreflex control of the heart and vasculature do not always function in a parallel fashion (433).

5.3 Aims and hypothesis

Central hypothesis: In patients with RA, elevated concentrations of circulating inflammatory cytokines are associated with increased MSNA and reduced BRS.

Aim: To test this, an observational, case-control study in RA patients (with and without hypertension) and controls (with and without hypertension) was undertaken to measure baseline serum inflammatory cytokine concentrations, MSNA and BRS.

Hypothesis:

i) central sympathetic outflow (MSNA) is elevated in RA compared to normotensive controls;

ii) sensitivity of the baroreflex control of the heart and MSNA is reduced in RA compared to normotensive controls;

iii) elevated circulating concentrations of inflammatory cytokines are associated with increased MSNA and reduced BRS.

5.4 Methods

5.4.1 Subjects

Thirty patients with a diagnosis of RA based on the 1987 ACR criteria (8) were recruited from the rheumatology clinics at Russells Hall Hospital, Dudley, UK and Sandwell General Hospital, West Bromwich, UK. Thirty three age-, sex- and BMI-matched control participants were recruited from the hospitals and surrounding areas.

5.4.2 Experimental protocol

Participants were studied according to an observational, case-control study that included four groups: 13 RA patients, 17 RA-HTN patients, 16 HTN and 17 NC. Participants attended the research laboratory at 09:00 h following an overnight fast (from food, caffeine and alcohol). Data was recorded during the protocol which involved a resting 10 minute baseline period followed by the MOT: sequential infusion of SNP (100 μg) and PE (150 μg) one minute later (433).

5.4.3 Measurements

HR (lead II ECG), beat-to-beat BP (finger photoplethysmography) and MSNA (microneurography) were continuously recorded during baseline and the MOT. Brachial BP recordings (automated sphygmomanometer) were obtained for calibration. Leg blood flow (venous occlusion strain gauge plethysmography) was measured during the resting baseline.

5.4.4 Data analysis

Data analysis was performed offline. MSNA burst frequency (bursts/minute) and incidence (bursts/100 heart beats) was determined. cBRS was determined during the phenylephrine induced rise in BP (G_{MOT}) (433, 496) and during the 10 minute rest period using the Sequence Technique (G_{SEQ}). The slope of the linear regression (DBP-1 mmHg v % [MSNA bursts/beat]/mmHg) was used as an index of sympathetic BRS during the MOT and at rest (spontaneous). Some patients were unsuitable for microneurography (e.g. discomfort laying still for an extended period, or declined microneurography) and it was not possible to obtain sufficiently high quality MSNA recordings in all patients, thus these were omitted from the MSNA analyses. Participant numbers are stated in the legend of each Table and Figure.

5.4.5 Blood sampling

Commercially available ELISA kits were used to determine hs-CRP and cytokines (IL-6, TNF-α, IL-10). Local routine clinical laboratories were used to analyse full blood count, renal function, fasting lipid profile and fasting glucose.

5.4.6 Statistics

Statistical analysis was performed using SPSS software, version 19 (SPSS Inc, Chicago, Ilinois). Continuous variables were tested for normality using the Kolmogorov-Smirnov test. Non-parametric data was (naturally) logarithmically transformed. Group differences were assessed using a one way analysis of variance (ANOVA) with least significant difference (LSD) post-hoc for continuous variables, and Pearson Chi-square test for categorical data. Differences between RA normotensive and RA-HTN group were

assessed using an independent t-test. Associations between autonomic parameters and inflammation were assessed before (Pearson product/Spearman's rank correlation coefficient) and after adjustment for potential confounders. Data expressed as mean±SD for parametric data; geometric mean (95 % CI) for non-parametric data; and frequency (%) for categorical variables. A P value of less than 0.05 was considered statistically significant.

5.5 Results

5.5.1 Subject characteristics

Baseline subject characteristics are represented in Table 5.1. Aside from hypertension there were no significant differences in other cardiovascular risk factors between the groups. Compared to RA normotensive patients and age-matched NC, RA RA-HTN and HTN patients had a higher prevalence of osteoarthritis (RA 15 %; RA-HTN 53 %; NC 0 %; HTN 25 %; p=0.003) and statin therapy (p=0.032). RA and RA-HTN patients had a higher prevalence of proton pump inhibitor (p=0.016) and folic acid (p<0.001) therapy, whilst calcium/ vitamin D supplementation tended to be highest in the RA-HTN group (p=0.06). Similar anti-hypertensive agent use was noted in RA-HTN and HTN patients (Table 5.1).

RA disease-related characteristics are represented in Table 5.2. RA normotensive and RA-HTN patients had similar disease duration, sero-positivity and RA drug therapy. RA-HTN patients had a greater number of swollen joints (p=0.045) and a trend for higher disease activity (DAS28-CRP, p=0.063) compared to RA patients.

Table 5.1 Subject characteristics

	RA	**RA-HTN**	**NC**	**HTN**	**P value**
N	13	17	17	16	
Age, years	55.9±11.7	60.5±9.6	53.6±13.0	59.6±10.1	0.257
Female, n (%)	8 (62)	12 (71)	10 (59)	11 (69)	0.876
BMI, kg/m^2	27.8 (25.4-30.4)	29.6 (26.4-33.3)	26.4 (23.8-29.2)	25.8 (24.5-27.2)	0.130
Total cholesterol, mmol/L	4.8±1.1	5.1±1.0	5.1±0.9	5.1±1.0	0.804
Triglycerides, mmol/L	1.1 (0.9-1.5)	1.0 (0.8-1.4)	1.1 (0.8-1.3)	1.1 (0.9-1.3)	0.968
HDL, mmol/L	1.3 (1.1-1.5)	1.5 (1.3-1.8)	1.4 (1.2-1.6)	1.4 (1.2-1.7)	0.498
LDL, mmol/L [a]	2.9±0.9	2.9±0.9	3.1±0.8	3.0±0.9	0.866
eGFR, ml/min/1.73 m^2	100.4±20.3	89.9±14.6	88.0±19.6	83.5±17.9	0.099
Haemoglobin, g/L	126±12 *†	133±14	138±10	138±12	0.019
Smoking, n (%)	4 (31)	1 (6)	3 (18)	0	0.064
Osteoarthritis, n (%)	2 (15)	9 (53)	0	4 (25)	0.003
Antihypertensive	-	7 (41)	-	11 (69)	0.112

agent, n (%)					
ACEi or ARB, n (%)	-	5 (29)	-	5 (31)	0.909
Calcium channel blocker, n (%)	-	4 (24)	-	7 (44)	0.218
Thiazide, n (%)	-	2 (12)	-	5 (31)	0.171
β-blocker, n (%)	-	2 (12)	-	1 (6)	0.582
α-blocker, n (%)	-	0	-	2 (13)	0.133
Aspirin, n (%)	1 (8)	1 (6)	0	1 (6)	0.748
Statin, n (%)	1 (8)	6 (35)	0	4 (25)	0.032
Proton pump inhibitor, n (%)	4 (31)	6 (35)	0	1 (6)	0.016
Folic acid, n (%)	8 (62)	12 (71)	0	0	<0.001
Adcal D3, n (%)	2 (15)	5 (29)	1 (6)	0	0.060

Values expressed as mean±SD (parametric) for continuous variables and frequency (%) for discrete variables. Non parametric data was (natural) log transformed and displayed as geometric mean (95% CI). One way ANOVA with post-hoc LSD. Pearson Chi-Square for categorical data. Significance P≤0.05. Post hoc P≤0.05 * v HC, † v RA-HTN. [a] calculated using the Friedewald formula.

ACEi = angiotensin converting enzyme inhibitor, ARB = angiotensin-renin blocker, BMI = body mass index, CVD = cardiovascular disease, eGFR = estimated glomerular filtration rate, HDL = high-density lipoprotein, HTN = hypertensive, LDL = low-density lipoprotein, NC = normotensive control, RA = rheumatoid arthritis.

Table 5.2 RA-related characteristics

	RA	**RA-HTN**	**P value**
N	13	17	
RA duration, years	7.6 (4.6-12.4)	5.5 (2.9-10.5)	0.443
RF positive, n (%)	9 (69)	10 (59)	0.768
Swollen joints, n (%)	5.2 (2.9-9.0)	2.6 (1.6-4.0)	0.045
Tender joints, n (%)	6.7 (2.8-14.7)	3.5 (1.7-6.7)	0.194
DAS28-CRP	4.8±1.9	3.7±1.2	0.063
Disease activity, n (%)			
Remission	4 (31)	5 (31)	0.088
Low	3 (23)	9 (56)	
High	6 (46)	2 (13)	
Pain VAS, %	36.8 (21.7-62.0)	12.9 (5.3-30.1)	0.047
DMARD, n (%)	11 (85)	15 (88)	0.773
No of DMARDs	1.8 (1.2-2.4)	1.8 (1.3-2.2)	0.850
Methotrexate, n (%)	8 (62)	12 (71)	0.602
Hydroxychloroquine, n (%)	5 (38)	10 (59)	0.269
Sulfasalazine, n (%)	4 (31)	3 (18)	0.400
Leflunomide, n (%)	2 (15)	1 (6)	0.390

Glucocorticoid, n (%)	1 (8)	6 (35)	0.077
Prednisolone, n (%)	1 (8)	4 (24)	0.249
Prednisolone dose, mg	3	5±3	0.534
Intra-muscular, n (%)	0	1 (6)	0.374
Intra-articular, n (%)	0	1 (6)	0.374
NSAID, n (%)	5 (38)	6 (35)	0.858
Opioid, n (%)	6 (46)	7 (41)	0.785
Weak, n (%)	5 (38)	7 (41)	0.880
Strong, n (%)	1 (8)	0	0.245
Biologic agent, n (%)	4 (31)	3 (18)	0.400
TNF-α inhibitor, n (%)	4 (31)	1 (6)	0.070
Certolizumab, n (%)	2 (15)	0	0.094
Etanercept, n (%)	2 (15)	0	0.094
Golilumab, n (%)	0	1 (6)	0.374
Rituximab, n (%)	0	2 (12)	0.201

Values expressed as mean±SD for continuous variables (parametric) frequency (%) for discrete variables. Non parametric data was (natural) log transformed and displayed as geometric mean (95% CI). Statistical differences were tested using an independent t-test for continuous variables and Pearson Chi-Square for categorical data. Significance $P \leq 0.05$.

CRP = C-reactive protein, DAS = disease activity score, DMARD = disease modifying anti-rheumatic drug, HTN = hypertensive, NSAID = non-steroidal anti-inflammatory drug, RA = rheumatoid arthritis, RF = rheumatoid factor, TNF = tumour necrosis factor, VAS = visual analogue scale.

5.5.2 Haemodynamic parameters

MSNA (burst frequency) was higher in RA, RA-HTN and HTN groups compared to NC ($p=0.004$; Figure 5.1). When adjusted for HR, there was a significant difference in MSNA (burst incidence) between the groups ($p=0.029$). Post-hoc analysis showed higher MSNA burst incidence in HTN ($p<0.05$) and RA-HTN (trend, $p=0.111$) compared to NC, and higher burst incidence in HTN compared to RA normotensives (trend, $p=0.056$). Mean BP was higher in HTN and RA-HTN compared to RA patients and NC ($p<0.001$), with no significant difference between RA-HTN and HTN, or between RA and NC (Figure 5.2). Resting HR was higher in RA and RA-HTN patients compared to NC and HTN groups ($p=0.008$). Leg blood flow was significantly higher in the RA and RA-HTN groups compared to NC and tended to be higher than in the HTN group (RA geometric mean 2.0, 95 % CI 1.5-2.6; RA-HTN 2.0, 1.4-2.8; NC 1.2, 0.9-1.7; HTN 1.4, 1.0-1.8 ml/100ml/min; $p=0.047$; post-hoc analysis RA v HTN $p=0.101$, RA-HTN v HTN; $p=0.086$). LVC was similar between all groups (RA 21, 15-27; RA-HTN 18, 12-26; NC 14, 10-19; HTN 13, 10-17 arbitrary units; $p=0.148$).

5.5.3 BRS

cBRS (G_{MOT}) was lower in RA, RA-HTN and HTN groups compared to NC ($p=0.002$). Spontaneous arterial baroreflex control of MSNA was slightly lower in RA and RA-HTN groups (although not significant $p=0.315$) however during MOT there were no differences between all groups ($p=0.927$; Figure 5.3). G_{SEQ} was higher in NC compared to RA, RA-HTN and HTN groups although post-hoc analysis showed only significant differences between RA-HTN and NC (RA 9.2, 7.0-11.9; RA-HTN 6.4, 4.5-9.0; NC 13.0, 10.9-15.4; HTN 8.1, 5.8-11.4 ms/mmHg; $p=0.004$).

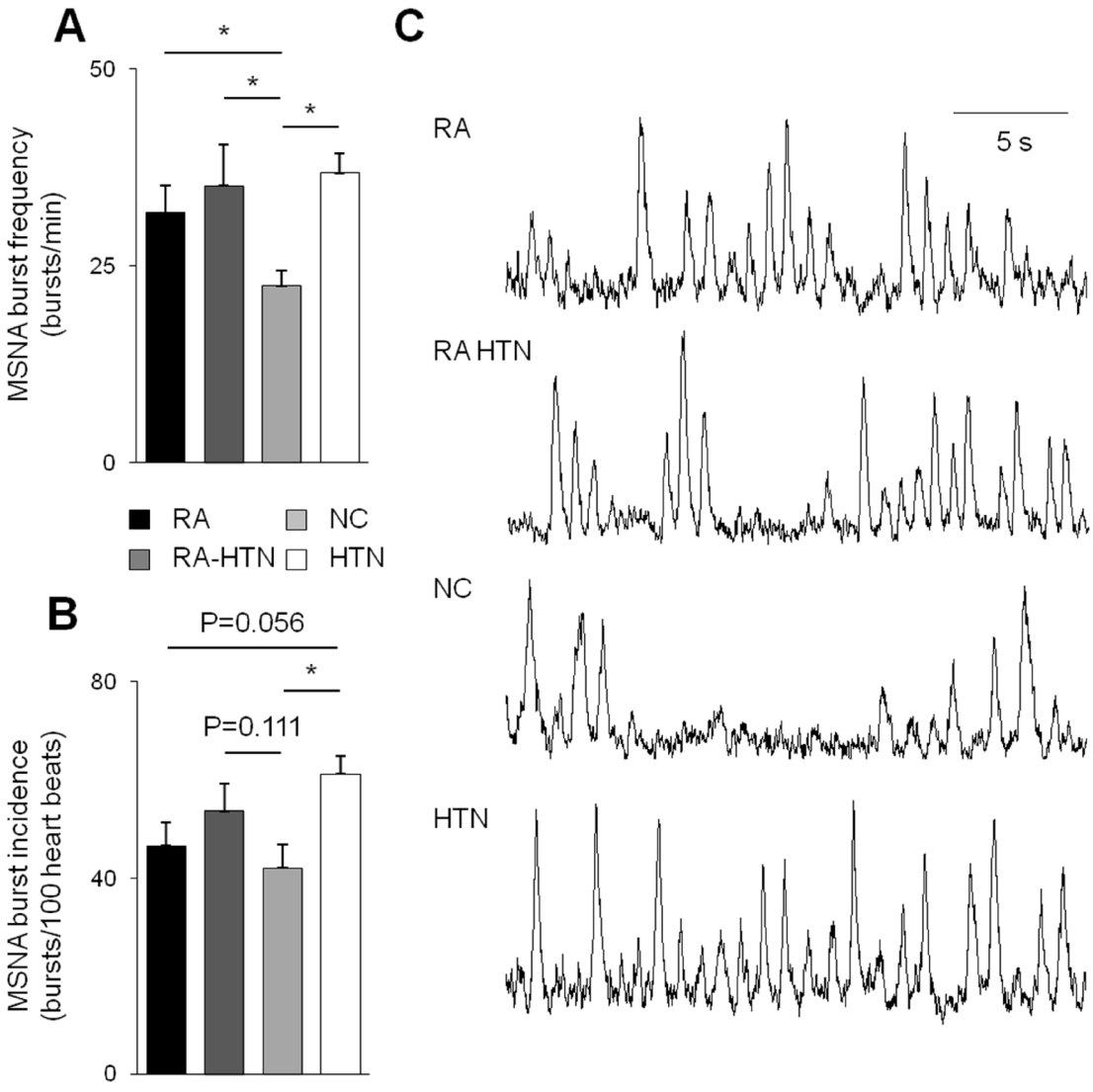


Figure 5.1 Muscle sympathetic nerve activity.

Bar charts showing group mean±SEM data for MSNA burst frequency (A) and MSNA burst incidence (B) and sympathetic neurograms showing MSNA (C) in individual subjects from RA, RA-HTN, NC and HTN groups. Overall effect $P<0.05$. Post hoc * $P<0.05$.

BP = blood pressure, HTN = hypertensive, MSNA = muscle sympathetic nerve activity, NC = normotensive control, RA = rheumatoid arthritis. A and B, RA n = 7, RA-HTN n = 7, NC n = 13, HTN n= 11.

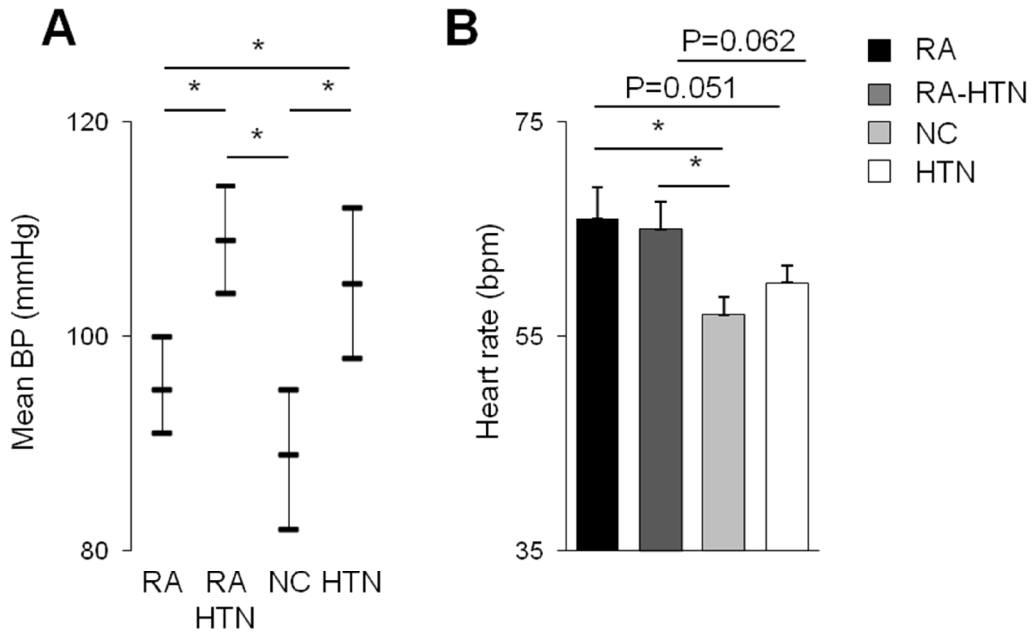


Figure 5.2 Resting BP and HR.

Box and whisker plot showing mean BP (A, geometric mean and 95 % CI) and bar chart showing HR (B, mean±SEM) in RA, RA-HTN, NC and HTN groups. Overall effect P<0.05. Post hoc * P<0.05. BP = blood pressure, HR = heart rate, HTN = hypertensive, NC = normotensive control, RA = rheumatoid arthritis. A and B, RA n = 13, RA-HTN n = 17, NC n = 17, HTN n= 16.

5.5.4 Inflammation and pain

RA and RA-HTN patients had higher hs-CRP compared to NC and HTN ($p<0.001$; Figure 5.4) with no significant difference between RA and RA-HTN. IL-6 was higher in RA patients compared to RA-HTN and NC, and higher in RA-HTN patients compared to NC ($p<0.001$). Additionally, RA patients had a higher IL-6 compared to RA-HTN and HTN patients (post hoc $p<0.05$). RA patients had higher TNF-α compared to NC and HTN (ANOVA $p=0.027$; post hoc RA vs. NC and RA vs. HTN $p<0.05$), and numerically (but not statistically significantly) higher TNF-α than RA-HTN patients (post hoc RA v RA-HTN $p>0.05$). IL-10 tended to be higher in RA patients compared to the other groups ($p=0.159$).

RA and RA-HTN groups had more pain than NC and HTN groups (as measured by pain VAS), and RA patients had more pain than RA-HTN (RA 37, 22-62; RA-HTN 13, 5-30; NC 1, 0-2; HTN 1, 0-3; $p<0.001$).

5.5.5 Associations between inflammation and autonomic function

Inflammatory cytokines (IL-6, TNF-α, and IL-10) were positively associated with each other, whilst hs-CRP was only associated with IL-6 (Appendix 5). Both hs-CRP and IL-6 were positively associated with HR although TNF-α and IL-10 were not. MSNA burst frequency was positively associated with hs-CRP but not with inflammatory cytokines; however this association was not evident when MSNA was adjusted for HR (MSNA burst incidence). cBRS (G_{MOT}) was inversely associated with inflammation (hs-CRP, IL-6 and TNF-α), whilst arterial baroreflex control of MSNA was not. Spontaneous cBRS (G_{SEQ}) was also inversely associated with inflammation (hs-CRP)

Table 5.3 shows the association between inflammation markers and autonomic function before and after multivariable adjustment. Following multivariable adjustment (i.e. age, sex, BMI, haemoglobin, presence of hypertension, RA) hs-CRP remained positively associated with HR (adjusted R squared 0.375, $p<0.001$, Appendix 6) while the associations with MSNA burst frequency and cBRS were attenuated. Similarly, the associations between inflammatory cytokines and autonomic parameters disappeared following multivariable adjustment. In patients with RA, disease activity (DAS28-CRP) was independently associated with HR (adjusted R squared 0.204, $p=0.034$) after multivariable adjustment.

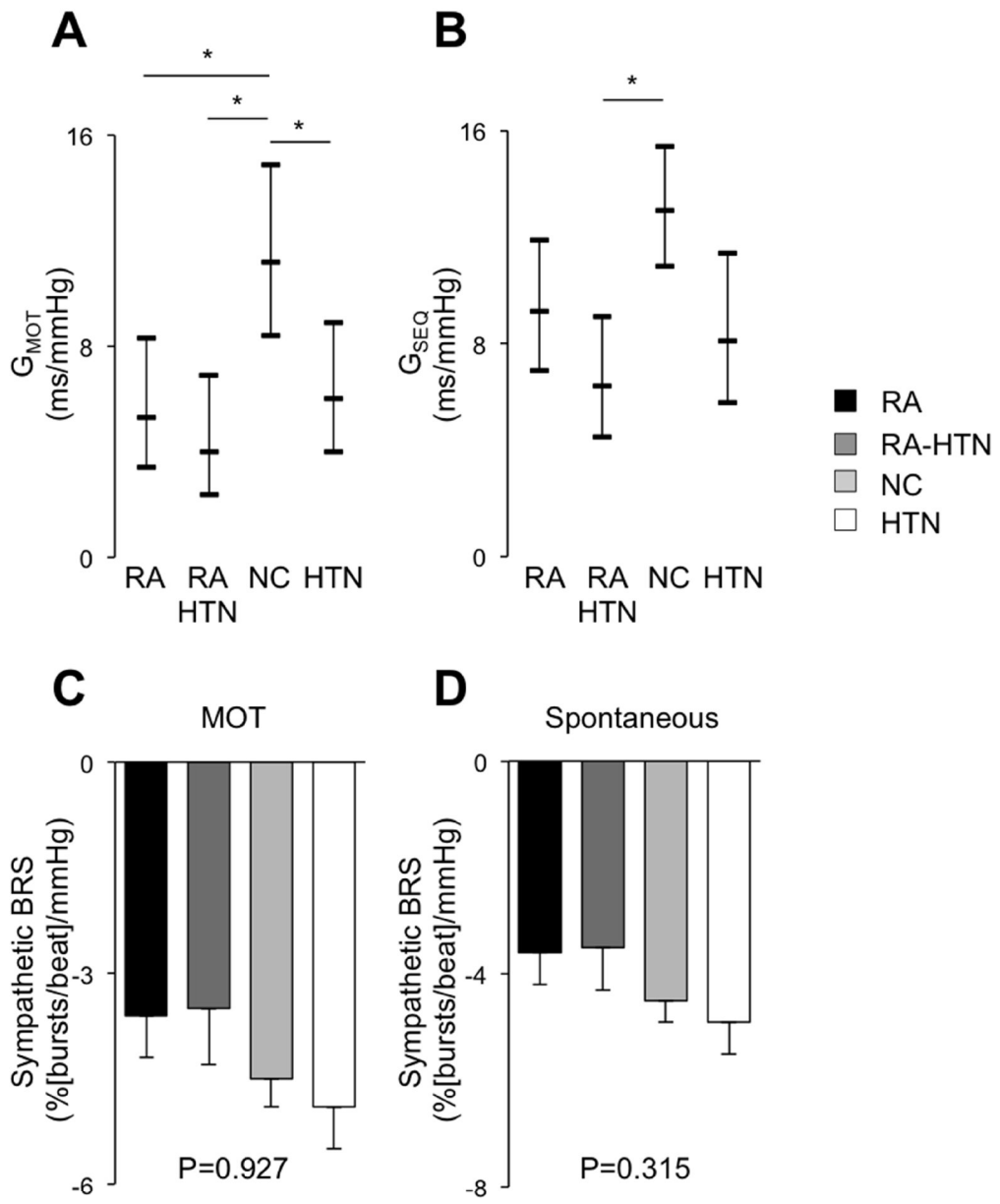


Figure 5.3 Cardiovagal and arterial sympathetic BRS.

Box and whisker plots showing cardiovagal BRS (A, G_{MOT}; B, G_{SEQ} geometric means and 95 % CI) and bar charts showing baroreflex control of MSNA (C, MOT; D, spontaneous means±SEM) in RA, RA-HTN, NC and HTN groups. Overall effect $P<0.05$. Post hoc * $P<0.05$.

BRS = baroreflex sensitivity, HTN = hypertensive, MOT = modified Oxford technique, MSNA = muscle sympathetic nerve activity, NC = normotensive control, RA = rheumatoid arthritis. SEQ = sequence method.

A, RA n = 13, RA-HTN n = 17, NC n = 17, HTN n= 16. B, RA n = 6, RA-HTN n = 5, NC n = 9, HTN n= 7.

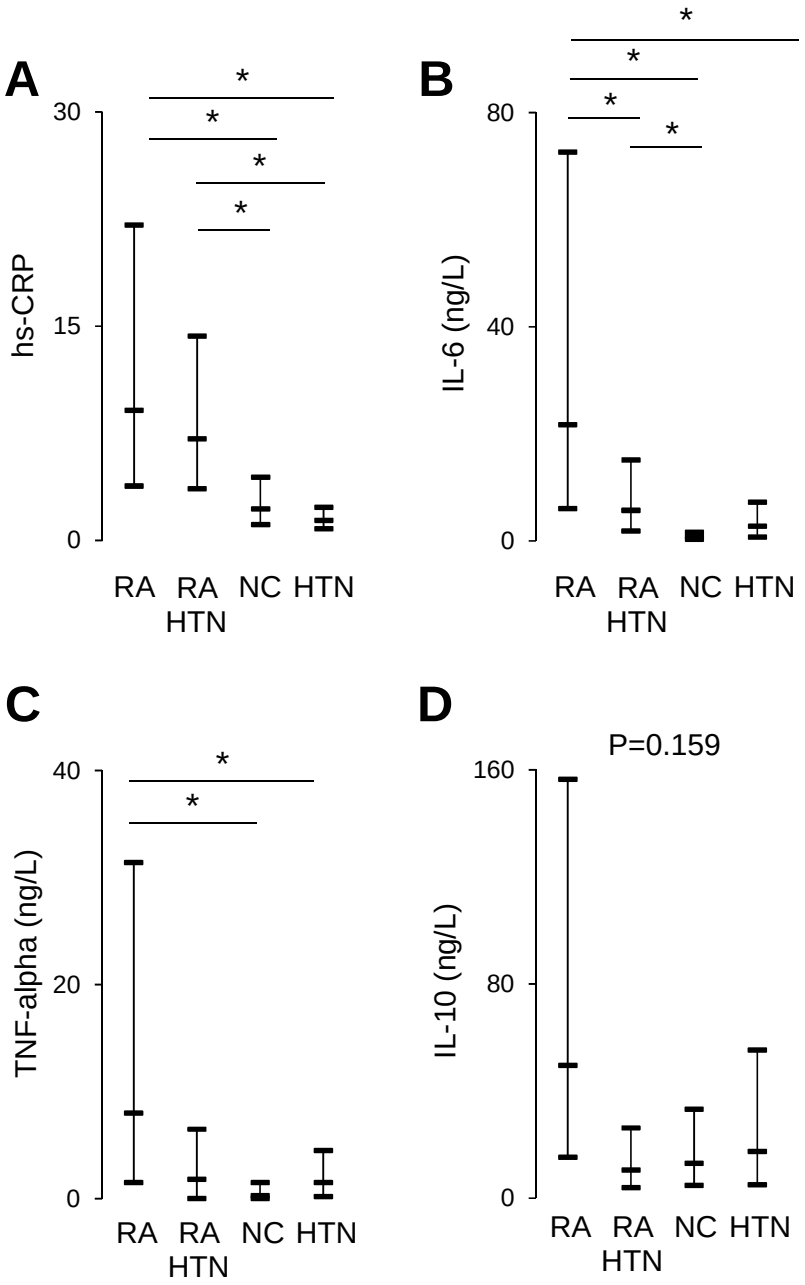


Figure 5.4 Inflammatory markers

Box and whisker plots showing concentrations (geometric mean and 95 % CI) of hs-CRP (A), IL-6 (B), TNF-α (C) and IL-10 (D) in RA, RA-HTN, NC and HTN groups. Overall effect $P<0.05$. Post hoc * $P<0.05$.

hs-CRP = high sensitivity C-reactive protein, HTN = hypertensive, IL = interleukin, NC = normotensive control, RA = rheumatoid arthritis, TNF = tumour necrosis factor

RA n = 13, RA-HTN n = 17, NC n = 17, HTN n= 16.

Table 5.3 Associations between inflammation, pain and ANS function

	N	Univariable [a]		Multivariable [c]		
		Rho	P	R^2	F	P
Dependent variable: MSNA burst frequency						
hs-CRP	36	0.418	0.011	0.222	1.452	0.238
Pain VAS	38	0.238	0.150	0.345	**7.237**	**0.012**
Dependent variable: cBRS (G_{MOT})						
hs-CRP	50	-0.332	0.019	0.364	0.683	0.413
IL-6	55	-0.408	0.002	0.364	0.039	0.845
TNF-α	55	-0.322	0.016	0.364	0.055	0.815
Pain VAS	55	-0.506	<0.001	0.417	**4.279**	**0.044**
Dependent variable: cBRS (G_{SEQ})						
hs-CRP	57	-0.338	0.010	0.252	2.591	0.114
Pain VAS	63	-0.318	0.011	0.162	**5.113**	**0.028***
Dependent variable: HR						
hs-CRP	62	0.362	0.006	0.366	**13.705**	**0.001**
IL-6	62	0.339	0.010	0.208	0.230	0.634
DAS28-CRP [d]	29	0.499 [b]	0.006	0.204	**5.123**	**0.034**

Pain VAS	63	0.464	<0.001	0.526	**36.661**	**<0.001**

[a] Spearman's rank or [b] Pearson's correlation. [c] After adjustment for age, sex, BMI, presence of hypertension, RA diagnosis and haemoglobin concentration. [d] After adjustment for age, sex, BMI, presence of hypertension, haemoglobin concentration and RA duration.

cBRS = cardiovagal baroreflex sensitivity, DAS = disease activity score, hs-CRP = high sensitivity C-reactive protein, IL = interleukin, MSNA = muscle sympathetic nerve activity, RA = rheumatoid arthritis, TNF = tumour necrosis factor, VAS = visual analogue scale.

5.5.6 Associations between pain and autonomic function

Pain VAS was independently associated with HR (positively), MSNA burst frequency (positively) and cBRS (inversely) after multivariable adjustment (Table 5.3).

5.6 Discussion

This study provides the first direct evidence for heightened central sympathetic outflow and reduced arterial baroreflex control of the heart in RA, whilst baroreflex control of MSNA remains preserved. These autonomic alterations could occur independently of hypertension and were associated with increased pain and inflammation.

5.6.1 Increased MSNA in RA

Heightened sympathetic outflow to the heart and/or vasculature can have a multitude of deleterious consequences (439) and is associated with increased mortality risk (113, 384, 506). Experimental data from animals showing that inflammatory cytokines (IL-6, TNF-α, IL-1β) can increase sympathetic nerve activity (52, 61, 153) led to the hypothesis that elevated circulating cytokine concentrations are associated with increased MSNA in RA. By using microneurography to provide a direct assessment of central sympathetic outflow limitations associated with the measurement of plasma catecholamines which reflect tissue clearance and uptake as well as production (509), were circumvented. This study demonstrated that RA is characterised by increased MSNA, which may contribute to the increased cardiovascular risk and mortality seen in RA. Further interventional studies that inhibit cytokines and reduce inflammation in RA are needed to determine whether the relationship between MSNA and inflammation is causal.

5.6.2 Reduced cBRS in RA

Reduced cBRS is present in cardiovascular diseases such as hypertension, chronic heart failure and predicts mortality risk following MI (284, 294). Using the MOT, which

provides an assessment of BRS control across a wide BP range (433), we observed that cBRS was reduced in patients with RA. The underlying mechanisms may relate to altered central baroreflex modulation, disruptions in afferent or efferent pathways. Nevertheless, this reduced cBRS may contribute to the increased cardiovascular and mortality risk seen in RA (21, 507). Interestingly, the baroreflex dysfunction was specific to the cardiovagal limb as the control of MSNA was not different between groups. The prognostic significance of alterations in sympathetic BRS has not been studied, however baroreflex activation therapy has been shown to reduce MSNA, improve symptoms and increase ejection fraction in chronic heart failure patients (511). Studies of elderly individuals (512) and patients with HTN (62) have previously reported a discrepancy between baroreflex control of the heart and MSNA as observed in the present study. Such observations may reflect disparate changes in vagal and sympathetic motoneuronal modulation, and/or cholinergic signalling at the sinoatrial node (433, 513, 514).

5.6.3 Pain and autonomic interactions

In the present study, pain was independently associated with MSNA burst frequency and cBRS. These findings are in agreement with work showing that experimentally evoked chronic pain can heighten sympathetic outflow (515-518) and reduce cBRS (519). Although the precise mechanisms are not fully understood interactions between pain and cardiovascular control may be explained by an overlap in anatomical structures and pathways including afferent pathways (e.g. baroreceptor and nociceptor projections), central modulation (e.g. nucleus of the solitary tract) and efferent pathways (e.g. descending pain inhibitory, sympathetic and parasympathetic projections) (520). In the present study RA patients had greater self reported pain than RA-HTN patients despite similar serum concentrations of hs-

CRP. This may reflect the higher pain threshold observed in hypertensive individuals (i.e., hypertensive hypoalgesia) (521) or possibly an effect of the higher concentration of inflammatory cytokines in the RA group. Evidence from animal (522, 523) and human experiments show that inflammatory cytokines have a direct role in modulation of pain perception (524). TNF-α inhibition acutely blocked both central nociceptive activity and activation of the limbic system in RA patients (524). While disease control improves survival in RA (412, 525, 526), further clarification is needed to distinguish between the effects of pain management and disease control on autonomic function.

5.6.4 Elevated HR in RA

Elevated HR is an independent predictor of mortality in the general population (527) and in other conditions (e.g., chronic heart failure, coronary artery disease, MI, hypertension and diabetes mellitus) (528-532). Patients with RA were observed to have an elevated HR in the present study, independent of hypertension. Aside from an increase in cardiac sympathetic nerve activity and decrease in parasympathetic activity there are a several potential mechanisms: chronic anaemia (533, 534), increased metabolic rate due to chronic inflammation (41) or concomitant thyroid dysfunction (535, 536), medication (534, 537); anxiety or depressive illness (538) and low physical activity (539). Attempts to lower HR with the use of β-blockers following MI or in chronic heart failure has been shown to improve survival (540). In an experimental model of arthritis the use of carvedilol (non selective β-blocker) was associated with reduction in markers of oxidative stress and release of inflammatory cytokines (TNF-α, IL-6) (541). Whether such benefits would be manifest in RA patients treated with β-blockers requires further study. In this present study, MSNA burst frequency (bursts per minute) was higher in RA patients but MSNA burst incidence (bursts

per 100 heart beats) was not, likely on account of the elevated HR. Nevertheless, the observation that bursts per unit time are elevated in RA and RA-HTN compared to NC may be interpreted as being indicative of heightened sympatho-excitation. The relatively small sample size is a potential limitation of the present study however this did not prevent a significantly elevated MSNA burst frequency being detected in the RA, RA-HTN and HTN groups compared to control.

5.6.5 Strengths and limitations

An important strength of this study design is the inclusion of hypertensive control groups allowing to control for the presence of hypertension in RA. Whilst increased MSNA and reduced cBRS are known to be present in hypertension (542), this study shows that in RA these autonomic alterations can occur independently of hypertension suggesting an alternative mechanism. Although the cross-sectional design precludes establishment of causality, it may be postulated that heightened sympathetic outflow can occur prior to the development of hypertension and may possibly contribute to the development of hypertension in some patients with RA. Autonomic dysfunction may be a causal agent in the pathogenesis of RA or a consequence of the disease, or both. This study could not discern between the influence of pain and inflammation on autonomic dysfunction.

5.6.6 Future directions

Future randomised controlled interventional studies are needed to clarify whether autonomic dysfunction is a cause or consequence of RA and in particular to discern between the contributions of inflammation and pain to autonomic dysfunction in RA. In light of the

evidence that heightened sympathetic outflow can evoke T cell activation (159) and may reduce blood flow (543) causing cytokine release and increased oxidative stress thus compounding problems of inflammation and afferent sensitisation, it should be examined whether therapeutic lowering of central sympathetic outflow in RA has anti-inflammatory actions.

5.7 Conclusion

In conclusion, this study is the first to demonstrate heightened MSNA and reduced cBRS using the MOT in RA. Heightened sympathetic outflow and reduced baroreflex control of HR in RA may potentially contribute to the recognised increase in cardiovascular risk. Further studies in RA are warranted to determine whether anti-inflammatory agents restore autonomic balance, whether sympatholytic agents reduce inflammation, and whether this translates into improved morbidity and mortality. Such interventional studies may help improve understanding of the pathophysiology of RA, and in particular shed light on the interplay between inflammation, pain and the ANS.

CHAPTER 6 Experiment Two. HRV and cardiovascular reactivity in RA: a case control study

6.1 Abstract

Objectives RA is a chronic inflammatory condition characterised by reduced HRV and impaired cardiovascular reflexes. It is unknown whether cardiovascular reactivity to stress is altered in RA. This study sought to determine HRV and cardiovascular reactivity in RA, and to assess for associations with inflammation.

Methods Beat-to-beat BP (Portapres), HR (ECG), LVC and FVC (strain gauge plethysmography) were recorded in age- and sex-matched RA-normotensive (n=13), RA-HTN (n=17), NC (n=17) and HTN (n=16) controls during rest, CPT and PASAT mental stress test. Short-term HRV (rMSSD) and inflammation (serum hs-CRP, TNF-α and IL-10) were determined.

Results. HRV was reduced in RA, RA-HTN and HTN groups compared to NC (p=0.001). CPT increased HR and BP and reduced LVC while PASAT increased HR, BP and FVC in all groups. During CPT: ΔHR was greater in HTN compared to NC and RA-HTN (p=0.05), but similar to RA; ΔLVC was greater in RA and RA-HTN compared to controls (p=0.065). During PASAT: ΔBP tended to be higher in RA compared to RA-HTN patients (p=0.183); ΔFVC was greater in RA, RA-HTN and HTN groups compared to NC (p=0.110). Multivariable analysis revealed independent positive associations between: IL-10 and ΔHR CPT (F=6.192, p=0.016), ΔHR PASAT (F=5.831, p=0.02), ΔFVC PASAT (F=4.555, p=0.039); TNF-α and ΔHR CPT (F=4.715, p=0.034); and a trend for inverse association between hs-CRP and rMSSD (F=3.036, p=0.088).

Conclusions. Vascular responses to stress are exaggerated in RA although further work is required to determine the prognostic significance. Independent relationships are present between inflammatory cytokines and cardiovascular reactivity (positive) but not HRV.

6.2 Introduction

RA is a chronic inflammatory condition associated with increased cardiovascular mortality and risk (20-23). Low HRV predicts mortality risk following MI (204-206) and hence may contribute to the increased cardiovascular risk seen in RA. Studies to date have shown that HRV indices of parasympathetic function are reduced in RA, compared to healthy controls (445, 451, 455, 457, 464, 467, 473, 484)(Chapter 4), although the mechanisms are not known. One possible mechanism is the effect of circulating inflammatory cytokines. Reciprocal relationships have been demonstrated between HRV and inflammatory cytokines in animals. Cholinergic stimulation has been shown to inhibit inflammatory cytokines (IL-1, IL-6 and TNF) (255-258), whilst TNF-α reduced HRV in animals (273). In one human study of inflammatory arthritis, hs-CRP was independently inversely associated with HRV although cytokines were not measured (377). To the author's knowledge no human studies have assessed for relationships between cytokine concentrations and HRV.

The ANS is responsible for cardiovascular regulation at rest and in response to stressors (72, 81, 82). Exaggerated cardiovascular responses to a CPT (immersion of a limb into cold water) or mental stress can predict the development of cardiovascular disease (329-338) (339, 340). In RA, one prior study demonstrated impaired diastolic BP responses to CPT (450) while cardiovascular responses to mental stress appear conflicting (456, 465, 481, 544, 545), although limb blood flow was not measured. Evidence from animal studies suggests that inflammatory cytokines released during sepsis (IL-6, IL-1β and TNF-α) may play an important role in mediating vasodilatory responses (355-360). It remains unknown whether chronic elevations of inflammatory cytokines as occurs in RA would impair cardiovascular reactivity to stressors.

6.3 Aims and hypothesis

Central hypothesis: In patients with RA, elevated concentrations of circulating inflammatory cytokines are associated with reduced HRV and impaired cardiovascular reactivity. Cardiovascular responses to the CPT and PASAT mental stress would be impaired in RA.

Aim: To test this, an observational, case-control study in patients with RA (with and without hypertension) and controls (with and without hypertension) was undertaken to measure baseline serum inflammatory cytokine concentrations, HRV and cardiovascular responses to CPT and PASAT mental stress.

Hypothesis:

i) Reduced HRV is associated with increased inflammatory cytokine concentrations;

ii) Cardiovascular reactivity to CPT and mental stress are impaired in RA compared to controls;

iii) Cardiovascular responses to CPT and mental stress are associated with serum inflammatory cytokine concentrations.

6.4 Methods

6.4.1 Subjects and experimental protocol

Participants and study design was the same as in Chapter 5, 6. The protocol involved a resting 10-minute baseline period followed by CPT, and PASAT mental stress test.

6.4.2 Measurements

HR (lead II ECG) and beat-to-beat BP (finger photoplethysmography) were continuously recorded at rest and during the cardiovascular tests. Brachial BP recordings (automated sphygmomanometer) were used to calibrate BP. Leg blood flow (venous occlusion strain gauge plethysmography) was recorded during rest, test and recovery phases of the CPT and PASAT. In addition forearm blood flow was recorded during the PASAT. Pain and stress ratings (10 point scale) were taken after the CPT and PASAT, respectively.

6.4.3 Data analysis

Time domain, frequency domain and non-linear indices of HRV were determined during the 10-minute resting baseline period (Kubios HRV software). Some participants declined or were unable to tolerate the cardiovascular reactivity tests, and blood flow signals were not analysable in a number of cases due to movement artefacts, thus these were omitted from the analyses. Participant numbers are stated in the legend of each Table and Figure. BP, HR, forearm and leg blood flow and FVC, LVC were averaged during rest, test and recovery phases, and change from rest calculated.

6.4.4 Blood sampling

Blood samples for inflammatory markers were centrifuged immediately and the plasma stored at -80 ^{o}C. Commercially available ELISA kits were used to determine hs-CRP (MP Biomedicals, California, USA) and cytokines (IL-6, TNF-α, IL-10; BioSupply UK, Bradford, UK).

6.4.5 Statistics

Statistical analysis was performed using SPSS software, version 19 (SPSS Inc, Chicago, Ilinois). Continuous variables were tested for normality using the Kolmogorov-Smirnov test. Non-parametric data was logarithmically transformed. Group differences were assessed using an ANOVA (LSD post-hoc) for continuous variables. For cardiovascular reactivity an ANOVA with repeated measures (Bonferroni adjustments for multiple comparisons) was used to test for significant differences between groups and phase (rest, test, recovery) during CPT and PASAT. Post-hoc LSD analysis was performed if significant group x phase interactions were found. Differences in changes from baseline (Δtest, Δrecovery) in HR, BP, leg and forearm blood flow, LVC and FVC were tested using a one-way ANOVA. Associations between autonomic parameters and inflammation were assessed before (Pearson product/Spearman's rank correlation coefficient) and after adjustment for potential confounders. Data expressed as mean±SD for parametric data; geometric mean (95 % CI) for non-parametric data; and frequency (%) for categorical variables. A P value of ≤0.05 was considered statistically significant.

6.5 Results

6.5.1 HRV

Time domain (rMSSD, pNN50 %), frequency domain (HF, LF absolute powers) and non-linear parameters (SD1, SD2) of HRV were lower in RA, RA-HTN and HTN groups compared to NC (Table 6.1). VLF and TP were also lower in RA and RA-HTN groups compared to NC. There was a trend for difference in normalised frequency domain parameters of HRV. RA normotensive patients had higher normalised HF power (p=0.053), but lower normalised LF power (p=0.053) and LF/HF ratio (0.059) compared to RA-HTN.

6.5.2 Cold pressor test

As expected, HR and BP rose during the CPT while LVC fell (Figure 6.1). HTN controls had a significantly higher rise in HR compared to NC and RA-HTN (RA +6±5, RA-HTN +5±4, NC +3±5, HTN +9±8 beats/min, p=0.05). There was no statistically significant difference in BP responses between the groups. The fall in LVC tended to be highest in RA and RA-HTN groups compared to HTN and NC (p=0.065). Leg blood flow rose in the HTN group but was unchanged in the remaining groups, although this difference was not statistically significant. RA, RA-HTN and HTN patients tended to have higher pain rating (RA geometric mean 8.3, 95 % CI 6.1-9.5; RA-HTN 7.8, 6.1-9.5; NC 5.6, 4.3-7.2; 7.4, HTN 7.4, 5.4-8.6 max score 10; p=0.10) compared to NC.

Table 6.1 HRV

	RA	RA-HTN	NC	HTN	P value
N	13	17	17	16	
rMSSD, ms	25 (18-36) *	20 (13-29) *	48 (38-60)	29 (20-40) *	0.001
pNN50, %	5 (2-12) *	3 (1-7) *	18 (11-29)	5 (2-12) *	0.005
SD1	18 (13-26) *	14 (9-21) *	38 (27-43)	20 (14-29) *	0.001
SD2	48 (36-63) *	46 (35-61) *	79 (67-94)	58 (45-76)	0.005
HF, ms^2	256 (131-500) *	116 (52-260) *	759 (436-1322)	240 (116-496) *	<0.001
LF, ms^2	272 (153-482) *	245 (132-456) *	1097 (746-1612)	357 (186-683) *	<0.001
VLF, ms^2	556 (304-1020) *	567 (315-1017) *	1431 (951-2151)	946 (552-1621)	0.025
TP, ms^2	1135 (631-2042)*	1014 (569-1807) *	3104 (2010-4793)	1665 (943-4793)	0.010
HF, nu	49±15 †	34±14	42±15	41±15	0.053
LF, nu	51±15 †	67±14	58±15	59±15	0.053
LF/HF ratio	1.1 (0.7-1.5)	2.1 (1.5-3.0)	1.5 (1.0-2.1)	1.5 (1.0-2.1)	0.059

Values expressed as mean±SD (parametric). Non-parametric data was (natural) logarithmically transformed and displayed as geometric mean (95% CI). Statistical differences were tested using a one-

way ANOVA with post-hoc LSD. Significance P≤0.05. Post hoc P≤0.05 * v healthy control, † v RA hypertensive.

BMI = body mass index, BP = blood pressure, HF = high frequency power (0.15-0.4Hz), HR = heart rate, LF = low frequency power (0.04-0.15), RA = rheumatoid arthritis, pNN50 = NN50 as a percentage of all NN intervals, RMSSD = root mean square of successive differences, TP = total power, VLF = very low frequency power (0-0.04 Hz)

6.5.3 PASAT mental stress

As expected HR, BP, leg blood flow, forearm blood flow and FVC rose during the PASAT in all groups. There were no significant differences in HR responses between the groups (Figure 6.2). There was a trend for higher BP responses in HTN controls compared to RA-HTN patients (p=0.183). RA, RA-HTN and HTN groups tended to have higher forearm blood flow (p=0.110) and FVC (p=0.107) responses compared to NC. LVC and leg blood flow responses were higher in RA, RA-HTN and HTN groups compared to NC although the differences were not statistically significant. No difference in self-reported stress was found between groups (4.9±3.7, 5.3±3.7, 5.3±3.3, 4.4±2.7 max score 10; p=0.962).

6.5.4 Associations between inflammation and autonomic function

Time domain (rMSSD and pNN50), frequency domain (TP, LF power, HF power) and non-linear (SD1, SD2) parameters of HRV were inversely associated with hs-CRP (Appendix 5). Following adjustments for multiple variables (i.e. age, sex, BMI, presence of hypertension, RA diagnosis and serum haemoglobin concentration) associations between rMSSD, pNN50, SD1 and hsCRP were attenuated (Table 6.2). Inflammatory cytokines were inversely associated with HRV parameters (IL-6 and rMSSD, LF power, SD1, SD2; IL-10 and LF/HF ratio; trend for TNF-α and LF power) although these associations disappeared after multivariable analysis.

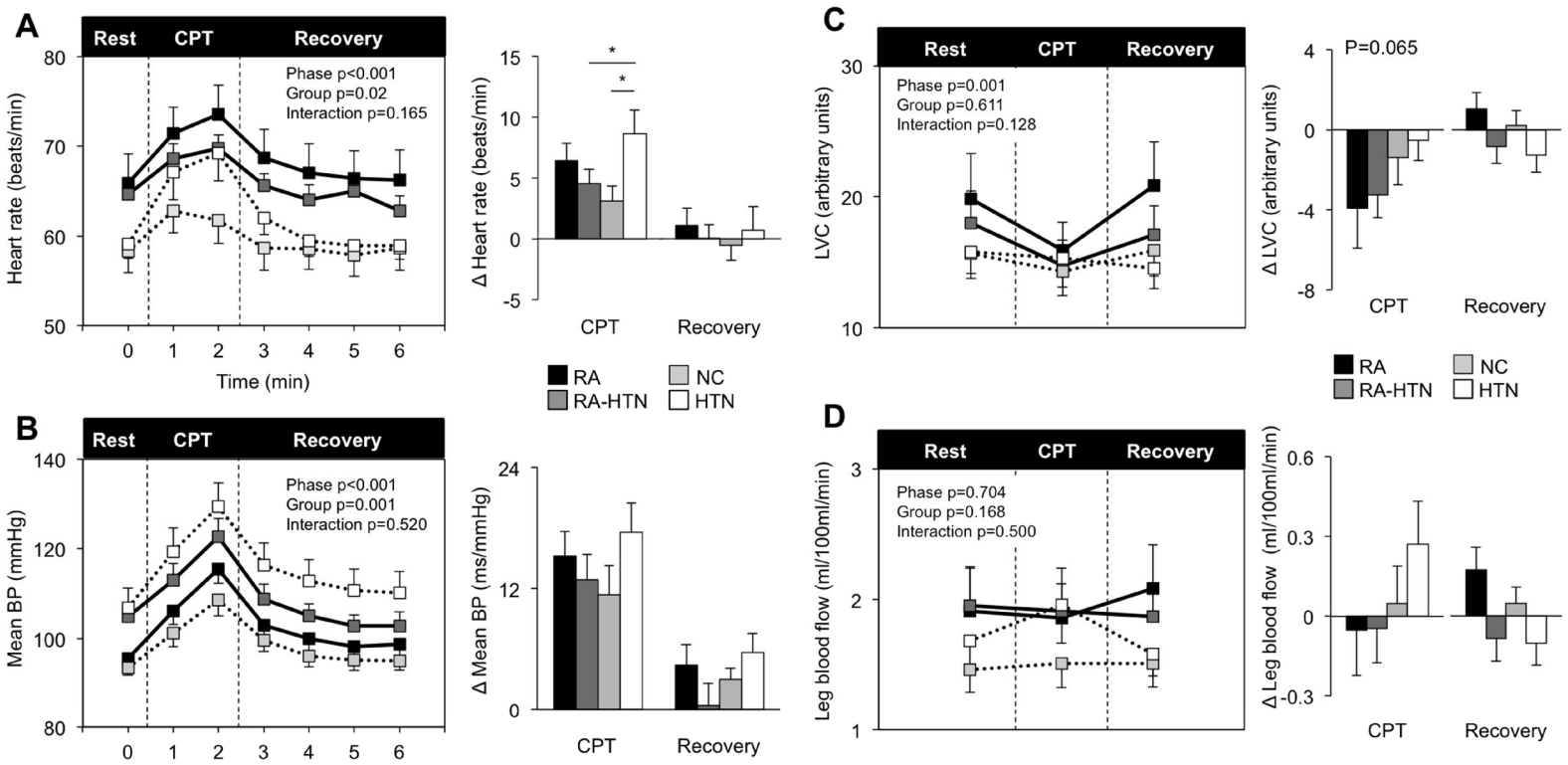


Figure 6.1 Cardiovascular reactivity to the CPT.

HR (Panel A), mean BP (Panel B), LVC (Panel C) and leg blood flow (Panel D) during rest, CPT and recovery. Data represented as group means±SEM. Times series is shown on the left. Significance for phase (rest, CPT and recovery), group (RA, RA-HTN, NC and HTN) and interaction were assessed using ANOVA with repeated measures. Bar charts on the right represent changes from baseline. Significant group differences were assessed using a one-way ANOVA. * P≤0.05. BP =blood pressure, CPT = cold pressor test, HR = heart rate, HTN = hypertensive, LVC = leg vascular conductance, NC = normotensive control, RA = rheumatoid arthritis.

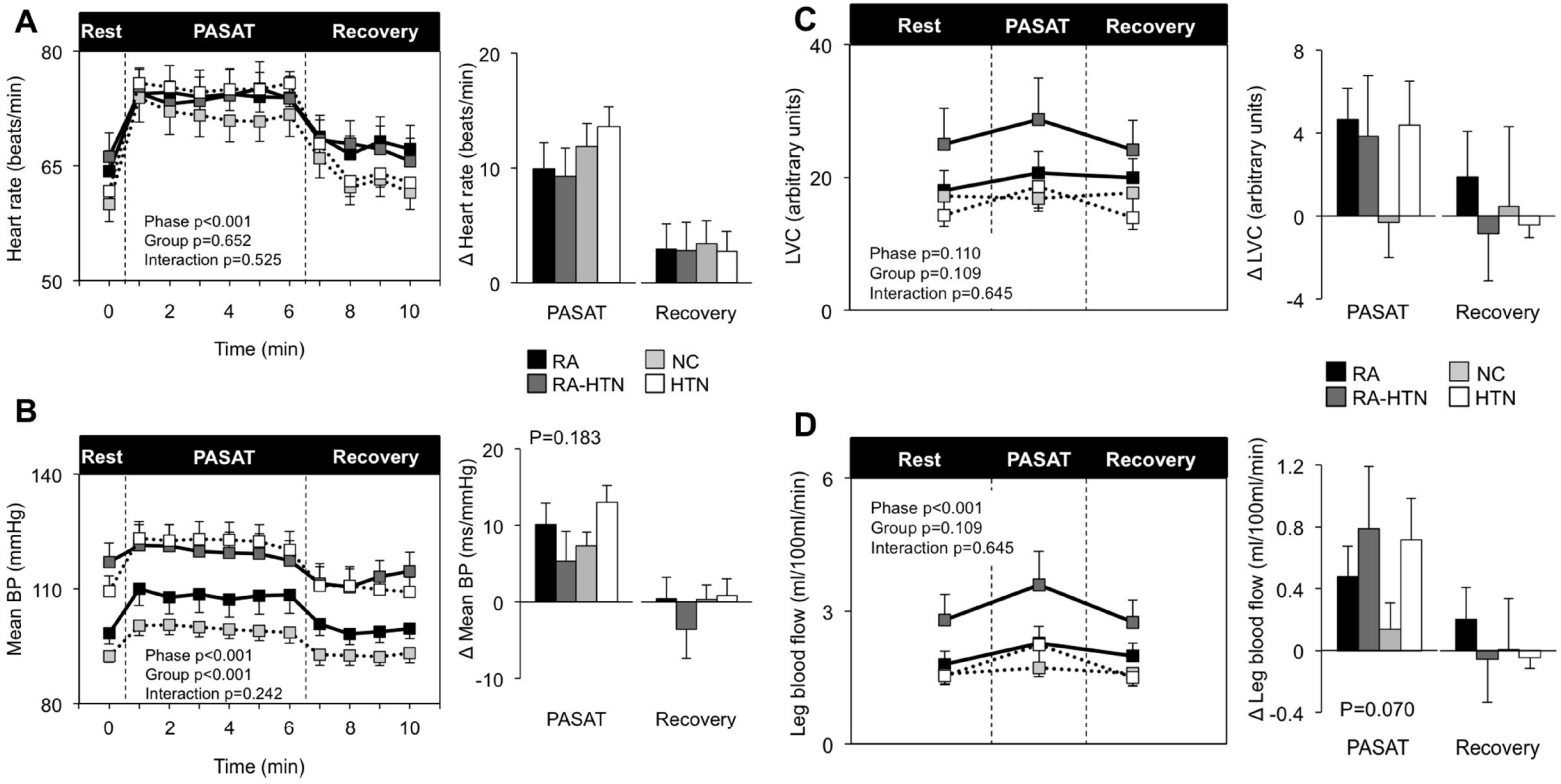
RA n = 12, RA-HTN n = 16, NC n = 16, HTN n = 16

ΔHR CPT was positively and independently associated with the inflammatory cytokines TNF-α and IL-10 (Appendix 5 and 6). There were trends for positive association between Δmean BP CPT and TNF-α, and ΔHR CPT and DAS28-CRP although these disappeared after multivariable adjustment. There was a trend (p=0.057) for an inverse association between IL-10 and Δmean BP CPT following multivariable analysis (Table 6.2). There were no associations seen between leg vascular responses to CPT and inflammation.

ΔSystolic BP PASAT was inversely associated with IL-6 (Appendix 5), while there were trends for positive association between leg vascular responses to PASAT and inflammation (hs-CRP, IL-6). Following multivariable analysis IL-10 was independently positively associated with ΔHR PASAT (p=0.020) (Appendix 6), while Δmean BP PASAT was independently associated with the number of tender joints (p=0.029, Table 6.2). IL-10 was independently positively associated with ΔFVC PASAT (p=0.039), and Δforearm blood flow PASAT (p=0.025) (Appendix 6). Trends for association between leg vascular responses to mental stress and inflammation (hs-CRP and IL-6) disappeared after multivariable adjustment.

6.5.5 Associations between pain and autonomic function

Pain was independently and inversely associated with time domain (rMSSD, pNN50) and non-linear (SD1, SD2) parameters of HRV (Table 6.2). There was a trend for an inverse association between pain and HR responses to mental stress however this disappeared after adjustment for multiple variables (Table 6.5). No other relationships were found between pain and cardiovascular responses to CPT or mental stress (Appendix 5).


A
Rest
PASAT
Recovery
Heart rate (beats/min)
Time (min)
Phase p<0.001
Group p=0.652
Interaction p=0.525
Δ Heart rate (beats/min)
PASAT
Recovery
RA
RA-HTN
NC
HTN
B
Mean BP (mmHg)
Phase p<0.001
Group p<0.001
Interaction p=0.242
P=0.183
Δ Mean BP (ms/mmHg)
C
LVC (arbitrary units)
Phase p=0.110
Group p=0.109
Interaction p=0.645
Δ LVC (arbitrary units)
D
Leg blood flow (ml/100ml/min)
Phase p<0.001
Group p=0.109
Interaction p=0.645
P=0.070
Δ Leg blood flow (ml/100ml/min)

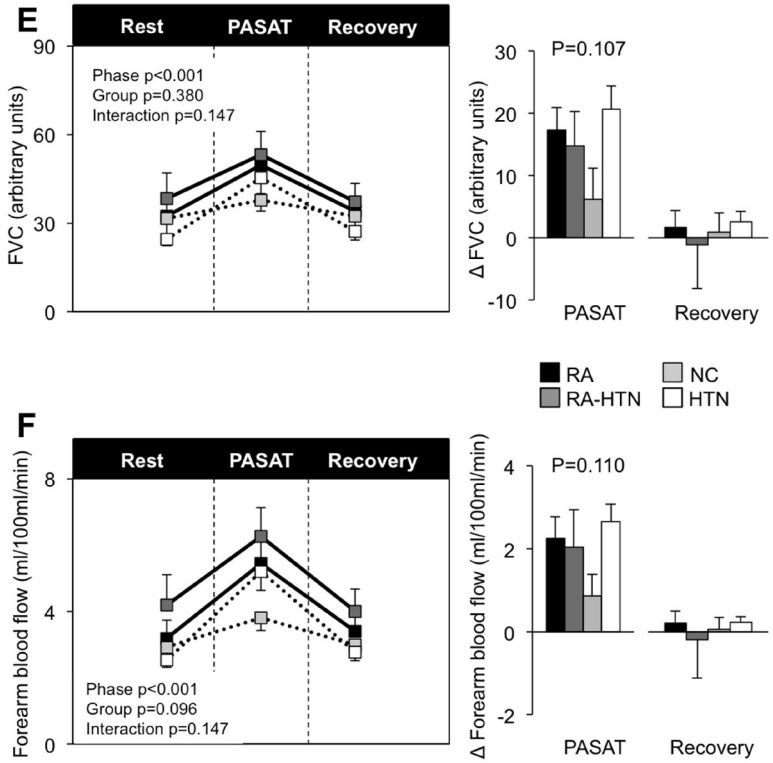


Figure 6.2 Cardiovascular reactivity to the PASAT mental stress test.

HR (Panel A), mean BP (Panel B), LVC (Panel C), leg blood flow (Panel D), FVC (Panel C), forearm blood flow (Panel D) during rest, PASAT mental stress test and recovery. Data represented as group means±SEM. Times series is shown on the left. Significance for phase (rest, PASAT and recovery), group (RA, RA-HTN, NC and HTN) and interaction were assessed using ANOVA with repeated measures. Bar charts on the right represent changes from baseline. Significant group differences were assessed using a one-way ANOVA. * P≤0.05. BP =blood pressure, CPT = cold pressor test, FVC = forearm vascular conductance, HR = heart rate, HTN = hypertensive, LVC = leg vascular conductance, NC = normotensive control, PASAT = paced auditory serial arithmetic task, RA = rheumatoid arthritis.

RA n = 10, RA-HTN n = 10, NC n = 16, HTN n = 14

Table 6.2 Association between inflammation, pain and ANS function

	N	Univariable [a]		Multivariable [c]		
		Rho	P	R^2	F	P
HEART RATE VARIABILITY						
Dependent variable: rMSSD						
hs-CRP	57	-0.420	0.001	0.334	3.036	0.088
IL-6	62	-0.258	0.043	0.216	0.230	0.633
Pain VAS	63	-0.437	<0.001	**0.303**	**7.015**	**0.011***
Dependent variable: pNN50						
hs-CRP	57	-0.430	0.001	0.388	3.270	0.077
Pain VAS	63	-0.419	0.001	**0.356**	**7.179**	**0.010***
Dependent variable: SD1						
hs-CRP	57	-0.420	0.001	0.334	3.038	0.088
Pain VAS	63	-0.437	<0.001	**0.303**	**7.017**	**0.011***
Dependent variable: SD2						
Pain VAS	63	-0.390	0.002	**0.227**	**4.531**	**0.038***
Dependent variable: LF/HF ratio						
IL-10	62	-0.262	0.040	-0.01	2.639	0.110

	N	Univariable [a]		Multivariable [c]		
		Rho	P	R^2	F	P
COLD PRESSOR TEST RESPONSES						
Dependent variable: ΔHR CPT						
TNF-α	60	0.254	0.050	**0.175**	**4.715**	**0.034 ***
IL-10	60	0.299	0.020	**0.196**	**6.192**	**0.016 ***
DAS28-CRP [d]	28	-0.308	0.111	0.399	0.901	0.354
Dependent variable: ΔMean BP CPT						
TNF-α	60	0.232	0.074	0.094	0.724	0.399
IL-10	60	-0.179	0.171	0.143	3.783	0.057
Dependent variable: ΔSystolic BP CPT						
IL-10	60	0.112	0.395	0.133	3.396	0.071
Dependent variable: ΔDiastolic BP CPT						
TNF-α	60	0.245	0.059	0.077	1.664	0.203
IL-10	60	0.212	0.103	0.109	3.536	0.066

	N	Univariable [a]		Multivariable [c]		
		Rho	P	R^2	F	P
MENTAL STRESS TEST RESPONSES						
Dependent variable: ΔHR PASAT						
IL-10	50	0.187	0.194	**0.203**	**5.831**	**0.020 ***
Pain VAS	50	-0.244	0.088	0.119	1.257	0.269
Dependent variable: ΔMean BP PASAT						
Number of tender joints	20	-0.376	0.102	**0.610**	**5.996**	**0.029 ***
Dependent variable: ΔSystolic BP PASAT						
Number of tender joints	20	-0.403	0.078	0.529	4.455	0.055
Dependent variable: Δleg blood flow PASAT						
hsCRP	45	0.287	0.056	0.133	0.451	0.506
Dependent variable: ΔLVC PASAT						
hsCRP	45	0.246	0.103	0.083	0.337	0.565
IL-6	49	0.237	0.101	0.085	0.473	0.495
Dependent variable: Δforearm blood flow PASAT						
IL-10	50	0.198	0.168	**0.220**	**5.435**	**0.025**
DAS28-CRP [d]	20	-0.394	0.086	0.434	2.008	0.182

Tender	20	-0.394	0.110	0.407	3.253	0.095
Dependent variable: ΔFVC PASAT						
IL-10	50	0.250	0.080	**0.197**	**4.555**	**0.039**
DAS28-CRP [d]	20	-0.402	0.079	0.115	0.219	0. 642

[a] Spearman's rank or [b] Pearson's correlation. [c] After adjustment for age, sex, BMI, presence of hypertension, RA diagnosis and haemoglobin concentration. [d] After adjustment for age, sex, BMI, presence of hypertension, haemoglobin concentration and RA duration.

BP = blood pressure, CPT = cold pressor test, DAS = disease activity score, FVC = forearm vascular conductance, HF = high frequency, HR = heart rat, hs-CRP = high sensitivity C-reactive protein, IL = interleukin, LF = low frequency, LVC = leg vascular conductance, NN50 = number of pairs of adjacent NN intervals differing by more than 50 ms, pNN50 = NN50 as a percentage of all NN intervals, PASAT = paced auditory serial arithmetic test, RA = rheumatoid arthritis, rMSSD = root mean square of successive differences, SD = standard deviation of the Poincare plot, TNF = tumour necrosis factor, VAS = visual analogue scale.

6.6 Discussion

The main findings in this study were that RA patients had reduced resting HRV, increased leg vasoconstrictor responses to CPT and increased vasodilatory responses to mental stress compared to normotensive controls. Inverse associations were found between HRV and inflammation (hs-CRP, IL-6) although confounded, while independent associations were found between cardiovascular reactivity and inflammation (IL-10, TNF-α). Additionally independent inverse associations were found between pain and HRV, while differences in cardiovascular reactivity between RA and non-RA patients with hypertension were observed.

6.6.1 Reduced HRV in RA

This study demonstrated reduced HRV in RA, consistent with prior studies, and weak inverse associations between HRV indices and inflammation (hs-CRP, IL-6, TNF-α) although confounded. In one prior animal study, TNF-α reduced HRV in a pattern similar to LPS-induced sepsis although the mechanism is not clear (273). One potential mechanism for the reduced HRV is via altered baroreflex modulation of HR, which is impaired in RA (Chapter 6). Administration of the inflammatory cytokine IL-6 directly into the NTS (a key ANS cardiovascular regulatory site) reduced BRS in rats (314) suggesting a direct effect of inflammatory cytokines. The strong independent inverse relationship found between pain and HRV in this study suggests that pain may have a significant contribution to the reduced HRV seen in RA and other chronic pain conditions (546). Another possible explanation for reduced HRV in RA is due to the effects of respiration, which has an important contribution to short-term HR fluctuations (88, 89). Unfortunately one limitation of this study is that respiration was not measured. Further interventional studies with agents that inhibit cytokines are needed

to establish the mechanisms for reduced HRV in RA, and to distinguish between the influence of inflammation and pain.

6.6.2 Altered cardiovascular reactivity in RA

The present study demonstrated altered cardiovascular reactivity to CPT and mental stress in RA patients, which include increased HR (although not statistically significant) and leg vasoconstrictor responses to CPT and increased vasodilatory responses to mental stress in RA. BP responses to CPT and HR and BP responses to mental stress in RA were not significantly different compared to controls. The exaggerated leg vasoconstrictor responses observed in RA patients suggest a heightened MSNA response or increased sensitivity to MSNA within the peripheral vasculature. Resting MSNA was reported to be elevated in RA (Chapter 6) however no studies have assessed MSNA responses to CPT or indeed adrenergic sensitivity in the peripheral vasculature in RA. Another explanation is increased sensitivity to cold stimuli (547) due to chronic pain sensitisation. In this present study self reported pain ratings were higher in RA, RA-HTN and HTN patients compared to normotensive controls however no associations between pain and cardiovascular responses to the CPT were found, suggesting alternative mechanisms. This study is the first to demonstrate exaggerated forearm vasodilatory responses to mental stress in RA. The mechanisms underlying mental stress-induced vasodilation are not fully understood and include: regional sympathetic withdrawal (548), β-adrenergic mediated vasodilation(548), flow (shear stress) and nitrate mediated vasodilation (549), acetycholine-mediated (550) and circulating factors (e.g. including adrenaline (551). One potential factor is the influence of inflammatory cytokines.

6.6.3 Inflammation and vascular reactivity

In this study, serum concentrations of IL-10 were positively and independently associated with forearm vasodilatory responses to mental stress whilst no relationship was found with other inflammatory cytokines. The release of inflammatory cytokines during acute sepsis is thought to contribute to hypotension via vascular hyporeactivity though a number of suggested mechanisms (355-360). However, while prior studies have shown that TNF-α and IL-1β reduce vascular reactivity in animals (357, 358), no studies have assessed the effects of IL-10 on the vasculature. During acute inflammation the release of serum pro-inflammatory cytokines stimulates the production and release of IL-10 (45). Although IL-10 inhibits the synthesis and actions of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) elevated circulating IL-10 is likely to represent inflammation. This is likely given the strong positive association between IL-10 and other cytokines (TNF-α, IL-6) found in this study. The results of the present study implicate a role for IL-10 in exaggerated vasodilation although further studies are required to determine the precise mechanisms.

6.6.4 Strengths and limitations

The inclusion of a hypertensive control group was a major strength in this study and provided insights into the mechanisms of cardiovascular disease in RA. The differences in cardiovascular profiles between RA and non-RA hypertensive individuals, namely that RA-HTN patients had reduced HR and leg vasoconstrictor responses to CPT, and reduced BP responses to mental stress (trend) compared to non-RA HTN, suggest alternative pathophysiological mechanisms for hypertension in RA. Another major strength is the inclusion of limb blood flow measurements, which were absent in prior studies. The major limitations of this study include the cross-sectional design, which prevents establishment of

causality and the relatively small numbers, which increases the risk of a type II error. Nevertheless despite this strong links between inflammation and cardiovascular responses were demonstrated suggesting a true relationship. The lack of strong associations between HRV and inflammation probably relate to insufficient sample size. Another important limitation is that respiration and the HPA system (co-activated along with the sympathetic nervous system during stress) were not assessed and therefore may have been a potential confounder.

6.6.5 Future directions

Further interventional studies are required in RA using biological agents to target and inhibit cytokines to confirm whether elevated concentrations of inflammatory cytokines contribute to the altered cardiovascular reactivity seen in RA. In addition further larger studies are required to confirm the presence of altered cardiovascular reactivity in RA, explore the underlying mechanisms and to establish the prognostic implications. Further studies are also needed to determine the interactions between autonomic and HPA systems during stress and their influence on cardiovascular responses.

6.7 Conclusion

In conclusion, this study is the first to demonstrate increased forearm vasodilatory responses to mental stress and increased leg vasoconstrictor responses to CPT in RA compared to controls. Independent associations were found between inflammation and cardiovascular reactivity however further studies are needed to determine causal relationships, and to determine their prognostic significance.

CHAPTER 7 Experiment Three. Effects of TNF-α inhibitor on autonomic function in RA: a longitudinal case-study

7.1 Abstract

Objectives RA is a chronic inflammatory condition associated with increased cardiovascular mortality. In animal studies, inflammatory cytokines increase sympathetic activity and reduce cBRS. This study sought to determine the effects of TNF-α inhibition on autonomic function in an RA patient.

Methods Whilst resting supine, beat-to-beat BP (Portapres), HR (lead II ECG) and MSNA (microneurography) were continuously recorded at rest (10 mins) and following sequential bolus infusions of SNP and PE (MOT) in a chronic RA male patient (age 60 years). MSNA burst incidence (burst/100 heartbeats) and short-term HRV (rMSSD) were determined at rest. Sympathetic and cBRS were determined during the MOT. Inflammation (serum hs-CRP) and pain (Pain VAS) were assessed. Experiments were undertaken before and after (2 weeks and 3 months) initiation of TNF-α inhibitor therapy.

Results Inflammation (Δhs-CRP -18.8 mg/L) and pain (Δpain VAS -30 mm) fell within 2 weeks. MSNA (+6.8 bursts/100 heart beats) increased after 3 months, whilst sBRS remained unchanged (+0.5 %[bursts/beat]/mmHg). At 2 weeks cBRS (+3.2 ms/mmHg) and rMSSD (+4.4 ms) rose, while mean BP (-18 mmHg) and HR (-5 beats/min) fell although these changes were not sustained at 3 months.

Conclusion These results suggest that TNF-α inhibition in RA elicits acute improvements in autonomic function in parallel with reductions in inflammation and pain, although not sustained. Further studies are warranted to confirm these findings.

7.2 Introduction

RA is a chronic inflammatory condition associated with synovial joint destruction, systemic inflammation and elevated cytokine concentrations (6). Traditionally disease-modifying drugs were the mainstay agents in controlling disease activity during acute flares (11, 13). In recent years biologic agents have been developed to target and counteract specific immune cells and cytokines (411). TNF-α is one such example; a pro-inflammatory cytokine that plays a key role in regulating inflammatory responses in RA (6). TNF-α has multiple actions including activation of leucocytes, endothelial cells and synovial fibroblasts leading to production of further cytokines, adhesion and inflammatory molecules (552). TNF-α also has a role in promoting atherosclerosis (553), angiogenesis, endothelial dysfunction (554, 555) and induction of pain (524). Recent evidence suggests that TNF-α has deleterious effects on the ANS. In animal studies peripheral (58, 61) and central (57, 59) administration of TNF-α increased sympathetic activity whilst central administration of IL-6 (an inflammatory cytokine elevated in RA patients and stimulated by TNF-α (556) reduced BRS (314). Heightened sympathetic activity and reduced BRS are pathogenic features of hypertension (62, 279, 280), ischemic heart disease (64, 284), chronic heart failure (63, 281-283) and RA (Chapter 6), and are associated with increased mortality risk (113, 206, 284, 290-294, 384). While studies have demonstrated that TNF-α inhibition effectively reduces disease activity (411) and improves cardiovascular (557) and mortality risk (412, 558) in RA patients, the mechanisms are unknown. Prior studies assessing the effects of TNF-α inhibition on autonomic function showed conflicting results. In one study, TNF-α inhibition reduced BP and serum noradrenaline concentrations after 2 weeks (559) whilst in two other studies no effect on serum NPY (biomarker of sympathetic activity) concentrations were seen (468).

Previous studies have suggested that increased inflammation is associated with increased MSNA (Chapter 6), reduced BRS (Chapter 6), reduced HRV (Chapter 7) and exaggerated vascular responses to mental stress (Chapter 7). This is the first study to report the acute and medium-term effects of TNF-α inhibitor therapy on MSNA as measured using the microneurography technique, a direct measure of sympathetic outflow, in a patient with RA. In addition BRS, resting HRV and cardiovascular reactivity to mental stress before and after TNF-α inhibition was assessed.

7.3 Aims and hypothesis

Central hypothesis: In patients with RA, elevated concentrations of circulating inflammatory cytokines are associated with increased MSNA, reduced BRS, reduced HRV and impaired cardiovascular reactivity to stressors.

Aim: To test this, a longitudinal, case study in RA patients was undertaken to measure baseline serum inflammatory cytokine concentrations, MSNA, BRS, HRV and cardiovascular responses to mental stress before and after TNF-α inhibitor therapy.

Hypothesis: In RA, TNF-α inhibition

i) reduces sympathetic outflow (MSNA);

ii) increases sensitivity of the baroreflex control of the heart;

iii) increases HRV;

iv) normalises cardiovascular responses to mental stress.

7.4 Methods

7.4.1 Subjects

Patients fulfilling the ACR 1987 diagnostic criteria for RA were recruited from the Rheumatology clinic at Russells Hall Hospital, Dudley Group of Hospitals, Dudley, UK to take part in an observational longitudinal study. Patients were assessed by a qualified Rheumatologist and deemed eligible to receive TNF-α inhibitor therapy for control of an acute RA flare as part of routine clinical care. Ethical approval was obtained from the local research ethics committee and informed written consent was obtained in accordance with the Declaration of Helsinki 2013. Patients were invited to the research laboratory for assessments prior to initiation of TNF-α inhibition and again at 2 weeks and 3 months after starting treatment. Patients were contacted by telephone to check they remained on the treatment, and those who discontinued treatment were excluded from the study. Clinical history and examination was performed at each visit to record changes in medications, the number of swollen and tender joints and to record pain (pain VAS).

7.4.2 Measured variables

HR, beat-to-beat BP and MSNA were recorded continuously during a 10 minute resting baseline and during the MOT as described in Chapters 3, 5 and 6. MSNA activity was reported as burst frequency and incidence. Cardiac baroreflex gain was determined using the MOT (G_{MOT}) and rest using the "sequence method" (G_{SEQ}) and low frequency transfer function (G_{LFTF}). Sympathetic BRS was also determined using the MOT and during rest (spontaneous). HRV was assessed using time domain, frequency domain and non-linear indices. In addition SDNN was also used as a time domain index of HRV. HR, BP, leg blood

flow, LVC, forearm blood flow and FVC responses to the PASAT mental stress test were also recorded.

7.4.3 Blood sampling

Commercially available ELISA kits were used to determine hs-CRP and cytokines (IL-6, TNF-α, IL-10). ESR (Starrsed compact Mechatronics BV, The Netherlands) was also analysed.

7.5 Results

Of 4 patients recruited into the study, 2 dropped out due to discontinuation of TNF-α inhibitor therapy, 1 was excluded due to frequent ectopics on their ECG and 1 completed the study (60 year old Caucasian male, RA disease duration 4.0 years, RF antibody negative, anti-CCP antibody positive, DAS28-CRP 6.6) receiving TNF-α inhibitor therapy (Adalimumab 40 mg subcutaneous every 2 weeks) for at least 3 months without interruption. There were no changes in concomitant medications, which included hydroxychloroquine, leflunomide, an NSAID, weak opioid and a proton pump inhibitor. BMI fell at 2 weeks but returned to pre-treatment level at 3 months (Pre v 2 weeks v 3 months 27.2 v 26.5 v 27.8 Kg/m^2).

7.5.1 Inflammation and pain

Inflammation and pain were reduced after 2 weeks of TNF-α inhibitor therapy and remained low at 3 months (Table 7.1). Of the cytokines tested serum concentrations of TNF-α rose at 2 weeks and returned to pre-treatment levels at 3 months.

7.5.2 MSNA and haemodynamic parameters

Following 3 months of treatment MSNA increased (burst frequency pre v 3 months 23.0 v 27.4 bursts/min; burst incidence 36.0 v 42.8 bursts/100 heart beats). HR and BP fell at 2 weeks but returned to pre-treatment levels at 3 months (Table 7.2). LVC increased at 2 weeks and fell to below pre-treatment level at 3 months, while leg blood flow did not change at 2 weeks and fell at 3 months.

7.5.3 BRS

Spontaneous and pharmacological measures of BRS demonstrated different responses. At 3 months sympathetic BRS during the MOT increased whilst spontaneous sympathetic BRS fell (Figure 7.1). G_{MOT} and G_{LFTF} increased at 2 weeks but returned to pre-treatment levels at 3 months (Figure 7.2) while G_{SEQ} fell at 2 weeks and remained lower at 3 months.

7.5.4 HRV

Time domain and non-linear indices of HRV increased at 2 weeks and fell to pre-treatment levels at 3 months (Table 7.3). Absolute VLF and LF powers increased at 2 weeks and fell to pre-treatment levels at 3 months. Absolute HF power fell at 2 weeks and increased to higher than pre-treatment levels at 3 months.

Table 7.1 Effects of TNF-α inhibition on pain and inflammation

	Pre	2 weeks	3 months
Pain VAS, mm	64	34	24
DAS28-CRP	6.6	5.1	4.9
Swollen joints, n	8	5	4
Tender joints, n	24	17	1
hs-CRP, mg/L	26.0	7.2	2.1
ESR, mm 1st hour	37	8	8
TNF-α, pg/ml	63	71	63
IL-6, pg/ml	92	73	72
IL-10, pg/ml	135	125	108

DAS = disease activity score, ESR = erythrocyte sedimentation rate, hs-CRP = high sensitivity C-reactive protein, IL = interleukin, TNF = tumour necrosis factor, VAS = visual analogue scale

7.5.5 PASAT mental stress test

As expected HR increased during the PASAT (Figure 7.3). Pre-stress resting HR was lower at 2 weeks, as were HR responses. Prior to treatment BP responses to the PASAT were blunted, however these increased at 2 weeks and again at 3 months following TNF-α inhibitor therapy. Leg vasodilatory responses were blunted prior to treatment and although improved at 2 weeks this was not sustained at 3 months. Prior to treatment forearm vasodilation was seen in response to mental stress. Forearm vasodilatory responses were increased at 2 weeks but were blunted at 3 months (Figure 7.3).

Table 7.2 Effects of TNF-α inhibition on haemodynamic parameters

	Pre	**2 weeks**	**3 months**
MSNA burst frequency (bursts/min)	23.0	-	27.4
MSNA burst incidence (bursts/100 heart beats)	36.0	-	42.8
Systolic BP, mmHg	136	108	128
Diastolic BP, mmHg	87	73	83
Mean BP, mmHg	103	85	98
HR, beats/min	64	59	64
Leg blood flow, ml/100ml/min	3.0	3.0	2.4
LVC, arbitrary units	29	35	24

BP = blood pressure, HR = heart rate, LVC = leg vascular conductance, MSNA = muscle sympathetic nerve activity

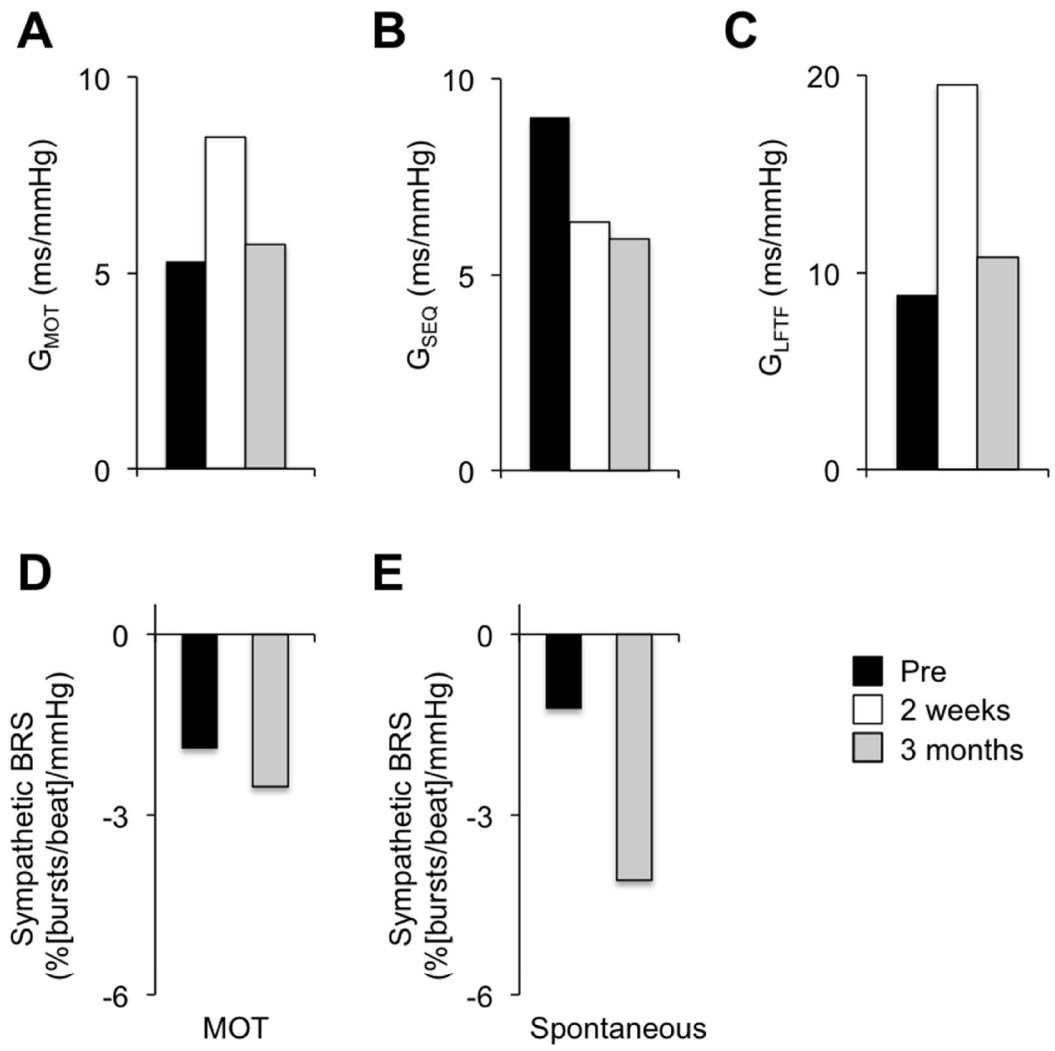


Figure 7.1 Effects of TNF-α inhibition on BRS.

Plot displaying cardiac and sympathetic BRS prior to (pre, black) and 2 weeks (white) and 3 months (grey) after TNF-α inhibitor therapy. Cardiac baroreflex gain determined using the MOT (G_{MOT}, Panel A), the sequence technique (G_{SEQ}, Panel B) and low frequency transfer function gain (G_{LFTF}, Panel C). Sympathetic baroreflex gain determined using MOT (Panel D) and spontaneous measures (Panel E).

BRS = baroreflex sensitivity, LFTF = low frequency transfer function, MOT = modified Oxford technique, TNF = tumour necrosis factor.

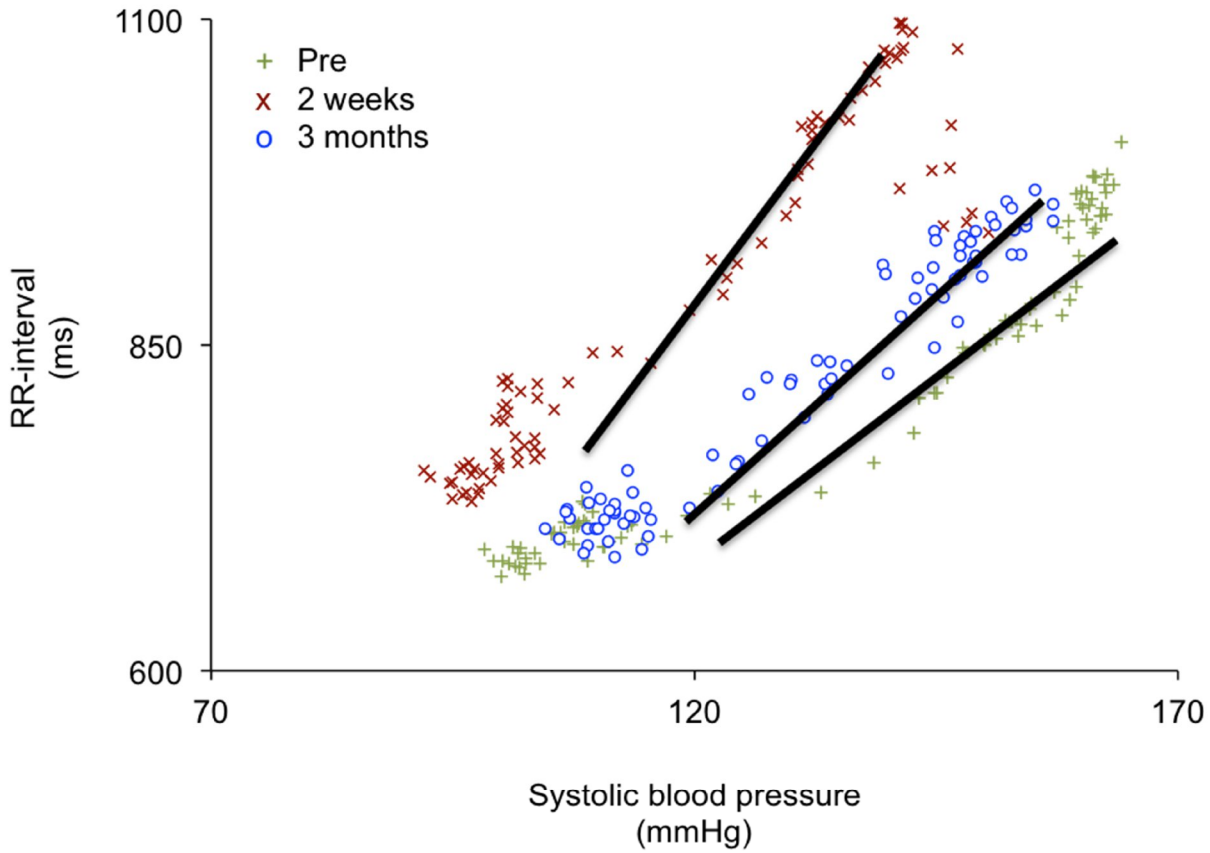


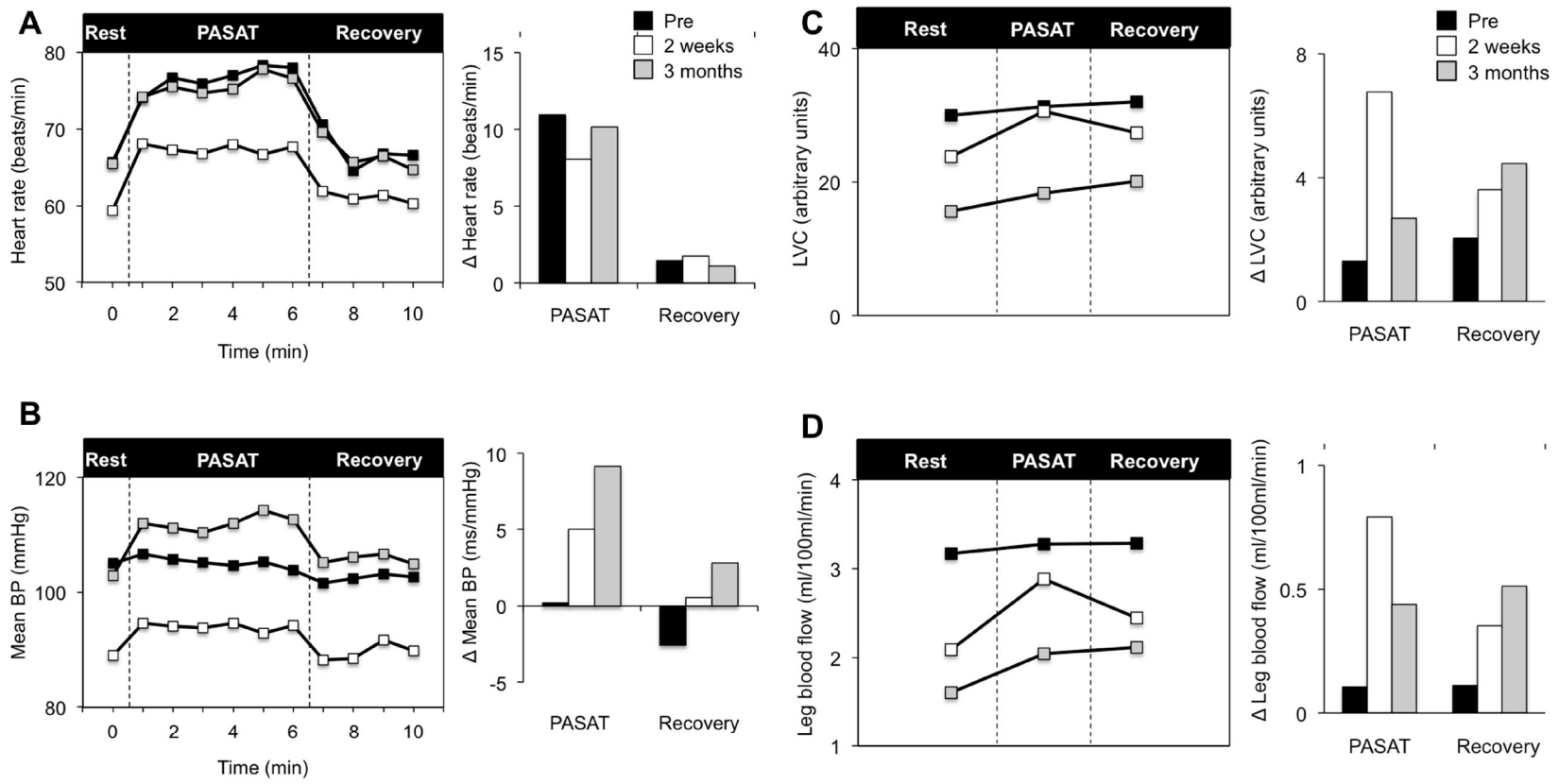
Figure 7.2 Effects of TNF-α inhibitor therapy on cBRS (original record).

Original record showing baroreflex function curves during the MOT prior to (pre, +), 2 weeks (X) and 3 months (O) after TNF-α inhibitor therapy. At 2 weeks, the slope of the baroreflex slope increased and shifted upwards and to the left but this was not sustained at 3 months. BRS = baroreflex sensitivy, TNF = tumor necrosis factor

Table 7.3 Effects of TNF-α inhibition on HRV

	Pre	2 weeks	3 months
SDNN, ms	23.6	49.1	33.2
rMSSD, ms	16.1	20.5	16.7
NN50, count	0	21	0
pNN50, %	0	3.7	0
VLF abs, ms^2	262	875	288
LF, ms^2	114	344	83
HF, ms^2	150	83	175
TP, ms^2	264	427	258
LF, nu	43.3	80.6	32.2
HF, nu	56.7	19.4	67.8
LF/HF ratio	0.8	4.1	0.5
SD1	11.4	14.5	11.8
SD2	31.4	67.9	45.4

HF = high frequency (0.15-0.4 Hz), LF = low frequency (0.04-0.15 Hz), NN50 = number of pairs of adjacent NN intervals differing by more than 50 ms, pNN50 = NN50 as a percentage of all NN intervals, nu = normalised units, rMSSD = root mean square of successive differences, SD = standard deviation of the Poincare plot, SDNN = standard deviation of all NN intervals, TP = total power, VLF = very low frequency (0-0.04 Hz).


A
Rest
PASAT
Recovery
Heart rate (beats/min)
Time (min)
Δ Heart rate (beats/min)
Pre
2 weeks
3 months
PASAT
Recovery
B
Mean BP (mmHg)
Δ Mean BP (ms/mmHg)
C
LVC (arbitrary units)
Δ LVC (arbitrary units)
D
Leg blood flow (ml/100ml/min)
Δ Leg blood flow (ml/100ml/min)

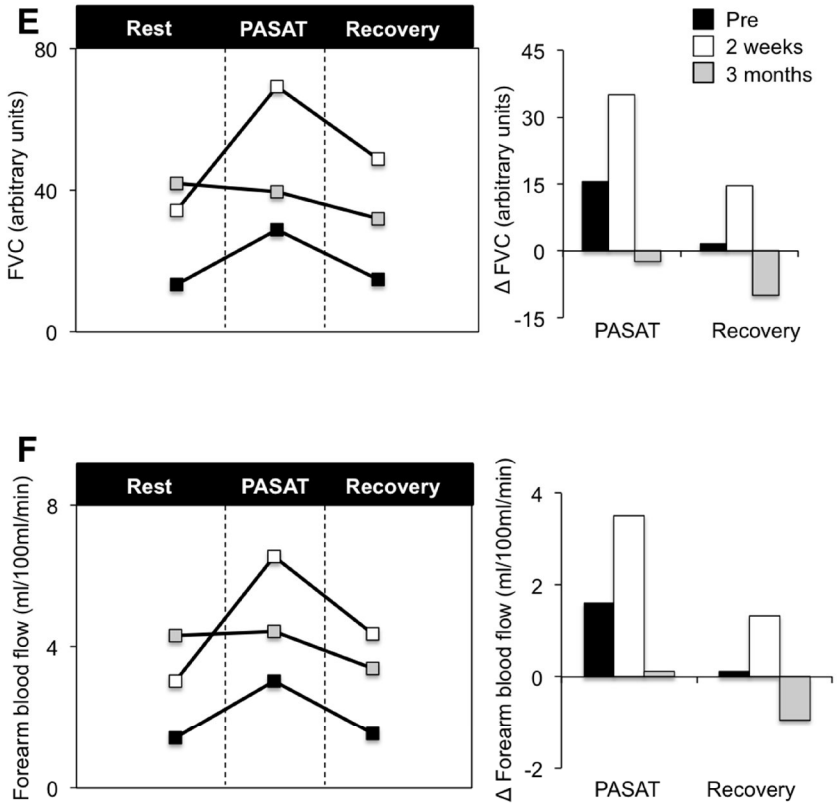


Figure 7.3 Effects of TNF-α inhibition on PASAT mental stress responses

HR (Panel A), mean BP (Panel B), LVC (Panel C), leg blood flow (Panel D), FVC (Panel E) and forearm blood flow (Panel F) during rest, PASAT mental stress test and recovery prior to (pre, black), and after 2 weeks (white) and 3 months (grey) of TNF-α inhibitor therapy. Times series is shown on the left. Bar charts on the right represent changes from baseline.

BP = blood pressure, FVC = forearm vascular conductance, HR = heart rate, LVC = leg vascular conductance, PASAT = paced auditory serial arithmetic task

7.6 Discussion

This study, to the author's knowledge is the first to describe the effects of TNF-α inhibition on MSNA and BRS in RA. A modest increase in MSNA was seen following 3 months of treatment with TNF-α inhibitor that was accompanied by a small reduction in resting BP, increased resting leg vasoconstriction and reduced forearm vasodilatory responses to mental stress. In addition BP responses to mental stress were increased. With the exception of serum TNF-α concentrations all other markers of inflammation and pain improved after 3 months of treatment; TNF-α concentrations however remained unchanged.

Animal studies showing that peripheral and central administration of inflammatory cytokines (TNF-α, IL-6) increase sympathetic nerve activity (52, 57-59, 61) led to the hypothesis that anti-inflammatory agents would reduce MSNA. However in this study cytokine inhibition increased MSNA. One simple explanation for the apparent rise in resting MSNA is a shift in the operational point of the baroreflex function curve as a result of a lower resting BP (Figure 7.4), an effect seen with chronic anti-hypertensive medication use (560). This is possible given that sympathetic BRS was relatively unchanged. In prior studies plasma concentrations of NPY were unchanged in RA patients after 2 months of infliximab (468) and 3 months of Adalimumab (460). NPY, a co-transmitter released with noradrenaline, may be used as a plasma biomarker of sympathetic activation (425). Plasma biomarkers of sympathetic activation however may be confounded by numerous factors (including medications and diurnal variation) and are affected by metabolism and clearance, which can make interpretation difficult (323, 422). In contrast, MSNA using the microneurography technique provides a direct measure of sympathetic outflow that appears to be relatively stable (422) and correlates with cardiac sympathetic activity (422). Prior studies have also shown that plasma biomarkers of sympathetic activity do not always correlate with MSNA (122,

561, 562). Unfortunately MSNA was not measured at 2 weeks therefore it is not possible to comment on the effects of TNF-α on sympathetic activity in this study.

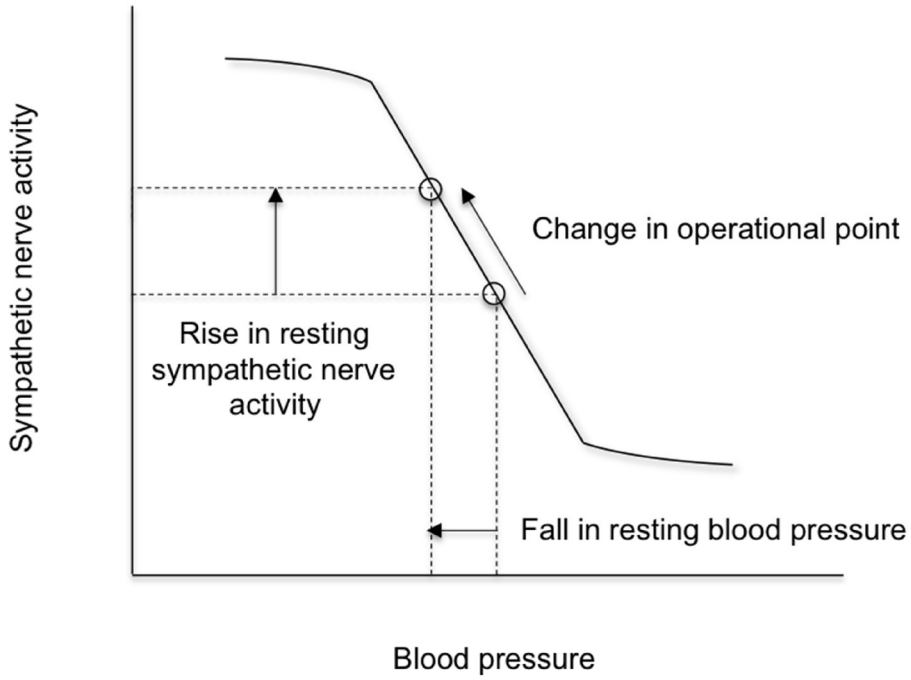


Figure 7.4 Baroreflex function curve.

A change in the operational point (open circle) can occur when resting BP falls causing a rise in resting MSNA, whilst the slope of the baroreflex function curve (BRS) remains unchanged.

BP = blood pressure, BRS = baroreflex sensitivity, MSNA = muscle sympathetic nerve activity

In this study Adalimumab effectively reduced pain and inflammation within 2 weeks of treatment. This was accompanied by reductions in resting BP and HR, and improvements in parasympathetic activity and cBRS. These results build upon the results of prior work, which demonstrated associations between inflammatory cytokines and HR, cBRS (Chapter 6) and HRV (Chapter 7). The present study suggests that inflammation and pain are two important mechanisms involved in autonomic dysregulation in RA, although it was not

possible to discern between them. This is consistent with a prior study showing that TNF-α inhibition acutely reduces pain in RA patients by blocking central nociceptive activity and limbic system activation (524). To the author's knowledge this is the first study to assess the effects of TNF-α inhibition on cBRS. TNF-α inhibition acutely altered the baroreflex curve in two ways. Firstly there was a shift in the position of the curve and secondly the slope of the linear portion of the curve was changed (Figure 7.2). This supports the hypothesis that inflammatory cytokines have a role in the central modulation of baroreflex function. The mechanisms by which inflammatory cytokines exert their effects are not fully known, as they are too large to pass the blood brain barrier. Wei and colleagues have performed a series of animal experiments suggesting that cytokines can exert central effects via the circumventricular organs and in particular the SFO (48, 49). Intra-carotid artery injection of TNF-α and IL-1β dramatically increased BP, HR and sympathetic activity in rats with an intact SFO however these responses were significantly reduced in rats with SFO lesions (49). Additionally using immunofluorescent techniques they were able to demonstrate a dense distribution of TNF-α receptor within the SFO. In a subsequent experiment they directly injected the SFO with TNF-α, which increased BP, HR and sympathetic responses. Pre-treatment of the SFO with angiotensin II type-1 receptor blocker, ACE inhibitor and COX-2 inhibitor attenuated the TNF-α responses. Furthermore they demonstrated that 4 hours following direct injection of TNF-α into the SFO, mRNA expression of angiotensin II type-1 receptor blocker, ACE inhibitor and COX-2 inhibitor were increased within the SFO and downstream within the hypothalamic PVN (a key autonomic regulatory site). While these experiments suggest that inflammatory cytokines can modulate autonomic control further studies are required to demonstrate whether these mechanisms occur in RA. In this study acute improvements in autonomic function were not sustained following 3 months of TNF-α

inhibitor therapy despite adequate control of inflammation and pain. This finding is surprising and may reflect the development of resistance to therapy (e.g. via upregulation of TNF-α receptors) or the effects of other pro-inflammatory cytokines not measured (e.g. IL-1β).

This study found that TNF-α inhibition evoked transient vascular changes. At rest, compared to pre-treatment leg vasodilation occurred at 2 weeks, but at 3 months there was vasoconstriction. Forearm and leg vasodilatory responses to mental stress were increased at 2 weeks but blunted at 3 months. These findings suggest an acute increase in vasodilatory capacity, which may be related to a fall in sympathetic vasoconstriction or alterations in endothelial function. In one study of RA patients TNF-α inhibition resulted in a significant improvement in microvascular endothelial-dependent function after 2 weeks however this was not sustained at 3 months (563). One possible explanation is an improvement in bioavailability of NO. TNF-α has deleterious consequences on the endothelium (555) resulting in reduced NO availability via down-regulation of endothelial NOS expression (554). In healthy humans, TNF-α acutely inhibited acetylcholine-mediated vasodilation with no further effect seen with NOS inhibition. In another study of metabolic syndrome patients, TNF-α inhibition acutely improved acetylcholine-mediated vasodilation. These studies suggest that TNF-α has an important role in mediating vascular reactivity likely via increasing NO bioavailability. Increased NO availability may also contribute to the reduction in BP seen at 2 weeks; although does not account wholly as HR would have expected to rise (via baroreflex activation). Further work is required to explain why the increased vascular responses were not sustained 3 months following TNF-α treatment.

The main limitation of this study is that it is a case study of one individual. Whilst interesting insights may be gleaned from this study further studies with larger sample size are needed to confirm these findings before firmer conclusions may be drawn. Another limitation

is that MSNA was not recorded 2 weeks post-treatment, due to the nature of the microneurography technique.

7.7 Conclusions

In conclusion this case study demonstrates that TNF-α inhibition induces transient improvements in autonomic function that parallels a reduction in inflammation and pain. Adalimumab acutely reduces BP and HR likely via central modulation of baroreflex function resulting in increased BRS and parasympathetic activity. Despite adequate control of inflammation and pain these changes were not sustained in the long term and sympathetic activity increased. Further larger studies are required to confirm these findings.

CHAPTER 8 Experiment Four. Associations between cytokines and QTc interval in RA: a cohort study

8.1 Abstract

Objectives QTc interval predicts all-cause and cardiovascular mortality and may contribute to the increased mortality risk in RA. Animal experiments have shown that pro-inflammatory cytokines (TNF-α and IL-1) can prolong cardiomyocyte action potential. This study sought to determine whether elevations in circulating inflammatory cytokines were independently associated with QTc prolongation in RA patients.

Methods One hundred and twelve patients (median age 62 [inter-quartile range 17] years; 80 [71%] women) from a well-characterised RA cohort underwent baseline 12-lead electrocardiograms for QT interval measurement and contemporary blood sampling to assess concentrations of inflammatory markers including CRP, TNF-α, IL-1α, IL-1β, IL-6 and IL-10. QTc was calculated using Bazett's (QT_{BAZ}=QT/√RR) and Framingham Heart Study (QT_{FHS}=QT+0.154[1-RR]) HR correction formulae.

Results Inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10) were positively correlated with QT_{BAZ} (spearman's rank correlation coefficient rho=0.199, 0.210, 0.222, 0.333; all $p<0.05$). In multivariable regression analysis these associations were all confounded by age except IL-10, where higher tertile groups were independently and positively associated with QT_{BAZ} (β=0.202, p=0.023) and QT_{FHS} (β=-0.223, p=0.009) when compared to the lower tertile. CRP (per unit increase) was independently associated with QT_{BAZ} (β=0.278, p=0.001) but not QT_{FHS}.

Conclusions This is the first study demonstrating a contemporary link between inflammatory cytokines and QT interval in humans. These results suggest that a lower inflammatory burden may protect against QTc prolongation in RA patients, however further studies are required to confirm the effects of pro- and anti-inflammatory cytokines on QTc interval.

8.2 Introduction

The QT interval represents the time from onset of ventricular depolarization (beginning of the Q wave) to completion of repolarization (end of the T wave). QTc prolongation is a weak independent predictor of all-cause and cardiovascular mortality in the general population (564) but a more significant one in patients with RA (35). Risk of sudden cardiac death is almost doubled in RA compared to the general population (21), which may in part be explained by prolonged QTc interval, a likely surrogate of sudden arrhythmic death (35, 565).

Given the variability of the QT interval in relation to HR, several formulae are used to yield a corrected measure (QTc). Bazett's formula ($QT_{BAZ} = QT/\sqrt{RR}$), one of the earliest and most commonly used HR correction formulae, has been criticised due to an over- and under-correction of QT interval at high and low heart rates respectively (364). To overcome this problem, a linear regression model using data from the Framingham Heart Study ($QT_{FHS} = QT + 0.154 \times [1-RR]$) was recently developed (504).

Prior studies (including one in RA patients) have suggested that QTc prolongation (QT_{BAZ}) may be driven by increased inflammatory burden (35, 377, 386, 388-390), however few studies have assessed the direct effects of inflammatory cytokines on QT interval. In one animal experiment TNF-α prolonged the cardiomyocyte action potential by inhibiting the hERG (the human *Ether-à-go-go*-Related Gene) gene (KCNH2) that codes for a protein known as $K_v11.1$, the alpha subunit of a potassium ion channel (390). In another animal study IL-1 was found to prolong cardiomyocyte action potential via its effects on calcium channels (566). QTc prolongation may occur as a consequence of structural heart disease (i.e. ischemic heart disease, heart failure), which is more prevalent in RA patients (567) and the development of which may be accelerated by systemic inflammation (565). Alterations in

ANS function may also result in QTc prolongation (377, 565). Reduced cardiac parasympathetic activity (568) and increased sympathetic activity (569), a pattern of ANS dysfunction prevalent in RA patients (445, 464, 468, 484, 570), have been shown to contribute to QTc prolongation. The relationship between inflammation and ANS remains to be fully elucidated and is a focus of current research (51, 242, 571, 572). Evidence from animal experiments demonstrates that IL-1β (153), IL-6 (52) and TNF-α (573) cause sympatho-excitation, which potentially may prolong QT interval. Collectively, these studies in animal models suggest several potential mechanisms by which pro-inflammatory cytokines may lead to prolongation of the QT interval but it remains unclear whether such pathways are involved in *humans*.

8.3 Aims and hypotheses

Central hypothesis: In patients with RA, elevated concentrations of circulating inflammatory cytokines are associated with increased QTc interval.

Aim: To test this, an observational cohort study was undertaken to measure baseline serum inflammatory cytokine concentrations, QT interval and relevant clinical parameters.

Hypothesis:

i) Serum inflammatory cytokine concentrations are correlated with QTc interval

ii) Associations between cytokines and QTc interval are affected by the QT correction method used (QT_{BAZ} vs. QT_{FHS}).

8.4 Methods

8.4.1 Subjects

One hundred and forty six subjects from a well-characterised RA patient cohort (29, 30) were assessed with 12 lead ECG for QTc interval and contemporary blood samples, which were analysed for biochemical markers of inflammation. Patients with an RA diagnosis, based on the revised 1987 ACR classification criteria (8), were recruited from routine outpatient clinics at the Department of Rheumatology of the Dudley Group of Hospitals, Dudley, UK. The study was approved by the local ethics committee and all subjects gave written informed consent. Clinical information was obtained from history, medical records and clinical examination (joint swelling and tenderness) performed to determine co-morbidities, medications, disease duration and DAS (16). Weight and height were measured to determine BMI.

8.4.2 QT measurements

QT interval (time between beginning of a Q wave and then end of a T wave) was manually measured by two experts and corrected for HR using Bazett's formula (QT_{BAZ} = QT/√RR)(574) and the Framingham Heart Study formula (QT_{FHS} = QT + 0.154x [1-RR]) (504).

8.4.3 Blood sampling

Serum was collected following an overnight fast and frozen immediately at -70^{o}C until analysed. Concentrations of IL-6, TNF-α, IL-1β and IL-10 were analysed by flow cytometry using FlowCytomix kits (Bender MedSystems GmbH, Vienna, Austria). CRP (Vitros®5.1-

FS, USA) and ESR (Starrsed compact Mechatronics BV, The Netherlands) were also analysed.

8.4.4 Statistics

Statistical analysis was performed using SPSS software, version 19 (SPSS Inc, Chicago, Illinois). Continuous variables were tested for normality using the Kolmogorov-Smirnov test. Correlations between QTc (QT_{BAZ}, QT_{FHS}) interval and continuous variables was assessed using Pearson's correlation for parametric and Spearman's correlation coefficient (rho) for non-parametric data. Associations between QTc (QT_{BAZ}, QT_{FHS}) interval and dichotomous categorical variables were assessed using a two-tailed, independent t-test. In addition cytokines and CRP were divided into tertiles. Differences in QTc interval between tertiles were assessed using an ANOVA with Tukey's post-hoc test. Step-wise multi-variable linear regression analysis was performed to determine independent associations between QTc (QT_{BAZ} and QT_{FHS} separately) interval and other pertinent measured variables (variables considered clinically significant or those with a univariate association with QTc demonstrating a p-value<0.1 were included in the model). Cytokine tertile groups were entered into the regression models as continuous variables (calculating a beta per tertile increase). In order to minimise the risk of collinearity, variables that are known to interact were entered into separate models (e.g. β-blocker, ischemic heart disease, cytokines). Values expressed as mean±SD for parametric data; median (inter-quartile range) for non-parametric data; and frequency (%) for categorical variables. A P value of less than 0.05 was considered statistically significant throughout.

8.5 Results

8.5.1 Subject characteristics

Of the 146 patients studied 34 (23%) were excluded due to the presence of bundle branch block precluding QT interval assessment. Analysis was performed on the remaining 112 patients. Baseline patient characteristics are summarised in Table 8.1. Median age (inter-quartile range) was 62.4 (17.2) years with 80 (71%) females.

8.5.2 HR correction

Mean QTc interval was 423.2±22.5 ms (QT_{BAZ}) and 412.5±18.0 ms (QT_{FHS}). QT_{BAZ} was positively correlated with HR whilst QT_{FHS} was not (Appendix 7).

8.5.3 Associations between QTc and demographic variables

QTc interval (QT_{BAZ} and QT_{FHS}) was weakly positively correlated with age and was higher in women compared to men (QT_{BAZ} = 12.5ms and QT_{FHS} = 7.8ms higher) (Table 8.2). Patients with history of ischemic heart disease had a higher QTc interval (QT_{BAZ} = 16.8ms and QT_{FHS} = 20.4ms higher). Hydroxychloroquine use was associated with a higher QTc interval (QT_{BAZ} = 12.9ms, QT_{FHS} = 9.8ms higher). There was a trend for β-blockade to lower QT_{BAZ} but not QT_{FHS}. Aside from hydroxychloroquine, no other medications, including those with a known association with risk of Torsades de Pointes (thiazide diuretics, amitriptyline and other anti-depressants, http://www.crediblemeds.org/), were associated with QTc interval.

Table 8.1 Baseline characteristics for RA patients

Characteristic	(n=112)
Demographic	
Age, years	62.4 (51.5-68.6)
Female, n (%)	80 (71%)
BMI*, kg/m^2	27.9±5.2
Co-morbidities and medications	
Osteoarthritis, n (%)	44 (39%)
Hypertension†, n (%)	88 (79.5%)
Ischemic heart disease, n (%)	12 (10.7%)
Hypercholesterolaemia, n (%)	16 (42%)
Antihypertensive agent, n (%)	47 (42%)
ACE inhibitor, n (%)	29 (25.9%)
β -blocker, n (%)	22 (19.6%)
Diuretic, n (%)	18 (16.1%)
RA-related	
Disease duration, years	9 (3-15)
RF positive, n (%)	87 (78%)

Anti-CCP positive‡, n (%)	77 (69%)
Disease Activity Score §	4.2±1.3
Methotrexate, n (%)	98 (88%)
Sulphasalazine, n (%)	80 (71%)
Hydroxychloroquine, n (%)	22 (20%)
Gold, n (%)	20 (18%)
NSAID, n (%)	26 (23%)
Biological agent, n (%)	10 (9%)
ECG features	
QT interval, ms	393.6±31.0
Corrected QTc interval, ms	
Bazett's correction (QT_{BAZ})	423.2±22.5
Framingham Heart Study correction (QT_{FHS})	412.5±18.0
HR, beats/min	71.0±13.1
Left ventricular hypertrophy (Sokolow-Lyon)	7 (6%)
Q wave abnormality	6 (5%)
ST abnormality ¶	6 (5%)

Inflammatory markers

CRP, mg/L	9 (5-19.8)
ESR, mm/hr	21.5 (10-38.8)
IL-6, pg/ml	16.8 (4-52.7)
TNF-α, pg/ml	8.3 (5-35.8)
IL-1α, pg/ml	2.7 (0-24.1)
IL-1β, pg/ml	1.1 (0-7.4)
IL-10, pg/ml	1.0 (0-5.9)

Data expressed as mean ± standard deviation for parametric data, median (inter-quartile range) for non-parametric data or frequency (%) for categorical data. * n=109, † blood pressure ≥ 140/90 or on anti-hypertensives, ‡ n=105, § n=111, ¶ coded using the Minnesota Code Classification System.

ACE = angiotensin converting enzyme, anti-CCP = anti-cyclic citrullinated peptide. CABG = coronary artery bypass graft, CRP = C-reactive protein, CVD = cardiovascular disease, ESR = erythrocyte sedimentation rate, IL = interleukin, NSAID = non-steroidal anti-inflammatory drug, RF = rheumatoid factor, TNF = tumour necrosis factor.

Patients with hypercholesterolaemia had a higher QT_{FHS}, whilst there were trends for higher QT_{FHS} in patients with co-existent osteoarthritis, those receiving proton pump inhibition and in (anti-CCP, RF) sero-positive patients. There were no significant associations between QTc interval and ECG abnormalities, other co-morbidities, medications and RA-related characteristics (Table 8.2).

Table 8.2 Significant associations between QTc and selected variables

Variable	N	QTc interval, ms	t	P-value
QT_{BAZ}				
Sex				
Woman	80	426.7±21.0	2.733	0.007**
Man	32	414.2±23.9		
Ischemic heart disease				
Yes	12	438.2±22.3	2.503	0.014*
No	100	421.4±22.0		
β-blocker				
Yes	22	415.6±19.5	-1.775	0.079
No	90	425.0±22.9		
Hydroxychloroquine				
Yes	22	433.5±22.1	2.448	0.016*
No	90	420.6±21.8		

Variable	N	QTc interval, ms	t	P-value
QT_{FHS}				
Sex				
Woman	80	414.8±17.3	2.103	0.038
Man	32	407.0±18.8		
Ischemic heart disease				
Yes	12	430.8±17.3	3.948	<0.001
No	100	410.4±16.9		
Hypercholesterolaemia				
Yes	16	424.0±23.9	2.840	0.005
No	96	410.6±16.2		
Osteoarthritis				
Yes	44	416.1±18.0	1.719	0.088
No	68	410.2±17.7		
Proton pump inhibitor				
Yes	22	418.8±18.9	1.847	0.067
No	90	411.0±17.5		
Hydroxychloroquine				

Yes	22	420.4±16.6	2.336	0.021
No	90	410.6±17.9		
RF positive				
Yes	87	414.1±18.7	1.749	0.083
No	25	407.0±14.2		
Anti-CCP positive				
Yes	77	413.7±18.5	2.016	0.053
No	28	406.1±18.5		

Data expressed as mean±standard deviation. Associations between QTc interval and dichotomous categorical variables were tested using two-tailed, unpaired t test. Significance = $P<0.05$.

Anti-CCP = anti-cyclic citrullinated peptide, df = degrees of freedom, QT_{BAZ} = QT corrected using Bazett's formula, QT_{FHS} = QT corrected using Framingham Heart Study formula, RF = rheumatoid factor.

8.5.4 Associations between QTc and inflammation

Correlation analysis showed that CRP, TNF-α, IL-6, IL-1β and IL-10 were all significantly associated with QT_{BAZ} (weakly positive) whilst IL-1α showed only a trend (Appendix 7). However, when QT_{FHS} was used, only IL-1β and IL-10 were significantly associated (weakly positive) while there was a trend with TNF-α and IL-1α. There was no significant association between QT_{FHS} and CRP or IL-6.

QT_{BAZ} and QT_{FHS} were significantly lower in the first tertile for IL-10 compared to the second and third; this was attenuated after multivariable adjustment for age, sex, presence of ischemic heart disease or hypercholesterolaemia, hydroxychloroquine and β-blocker use (Table 8.3). QT_{FHS} was significantly lower in the first tertile for TNF-α compared to the third

tertile however this was insignificant after adjustment for age and other variables (Table 8.3). QT_{BAZ} tended to be lower in the first tertile for CRP compared to the third tertile. There was no significant association between IL-6, IL-1α, IL-1β tertile group and QTc interval.

8.5.5 Multivariable linear regression

Step-wise linear regression analysis showed that age, sex and CRP were independently associated with QT_{BAZ} interval, accounting for 22% of the variation (Table 8.4). The associations (and trends) found between the cytokines and QT_{BAZ}/QT_{FHS} in univariate analysis were all confounded by age with the exception of IL-10 (Table 8.4). IL-10 tertile group (when entered into the model as a continuous variable) was independently associated with QT_{BAZ} and QT_{FHS} interval independent of other factors (including age, sex, β-blocker or hydroxychloroquine use and presence of ischemic heart disease) (Figure 8.1).

Table 8.3 Associations between QTc and inflammatory markers according to tertiles before and after mulitivariable adjustments

	Tertile			Univariate		Mulitivariable					
	1	2	3			Age		A		B or C[3]	
CRP, mg/ml	**≤7.0**	**8-13**	**>14**	**F**	**P**	**F**	**P**	**F**	**P**	**F**	**P**
N	44	31	37								
QT_{BAZ}, ms	417.4±22.9	425.5±18.4	428.0±24.2	2.552	0.083	2.675	0.073	4.078	0.020*†	2.497	0.087
QT_{FHS}, ms	410.9±19.2	414.5±17.0	412.8±17.6	0.362	0.697	0.375	0.688	1.760	1.155	0.177	0.319
IL-6, pg/ml	**≤6.3**	**6.31-37.23**	**>37.24**								
N	38	37	37								
$QT_{BAZ,}$ ms	417.0±20.7	426.3±21.4	426.3±24.6	2.214	0.114	0.954	0.388	0.972	0.382	0.615	0.543
QT_{FHS}, ms	409.5±16.0	415.1±17.5	413.1±20.2	0.942	0.393	0.892	0.413	0.505	0.605	0.245	0.783

	Tertile			Univariate				Mulitivariable			
	1	2	3			Age		A		B or C[3]	
TNF-α, pg/ml	**≤6.14**	**6.15-23.99**	**>24.0**								
N	38	37	37								
QT_{BAZ}, ms	419.1±23.5	421.4±20.8	429.2±22.5	2.109	0.126	1.174	0.313	0.883	0.417	1.320	0.271
QT_{FHS}, ms	408.6±15.7	410.8±17.3	418.4±19.7	3.153	0.047†	1.823	0.167	1.381	0.256	1.570	0.213
IL-1α, pg/ml	**0.0**	**0.01-9.45**	**>9.46**								
N	41	34	37								
QT_{BAZ}, ms	422.9±27.4	419.1±18.1	427.1±19.9	1.123	0.329	1.036	0.358	1.214	0.301	0.860	0.426
QT_{FHS}, ms	410.8±17.9	410.1±16.7	416.8±18.9	1.549	0.217	0.248	0.781	1.141	0.323	0.907	0.407

	Tertile			Univariate			Mulitivariable				
	1	2	3			Age		A		B or C[3]	
IL-1β, pg/ml	**≤0.45**	**0.46-3.51**	**>3.52**								
N	38	37	37								
QT_{BAZ}, ms	418.4±26.2	423.9±21.2	427.2±19.2	1.460	0.237	0.860	0.426	0.507	0.604	0.838	0.435
QT_{FHS}, ms	408.2±17.1	411.7±18.0	412.5±18.0	2.806	0.065	2.004	0.140	1.584	0.210	1.800	0.170
IL-10, pg/ml	**0.0**	**0.01-2.0**	**>2.01**								
N	40	35	37								
QT_{BAZ}, ms	413.4±20.8	429.8±23.7	427.4±19.9	6.506	0.002*†	5.555	0.005*†	3.255	0.042*†	4.212	0.017*†
QT_{FHS}, ms	405.6±14.9	415.6±18.2	417.1±19.0	4.989	0.008*†	3.976	0.022*†	2.498	0.087	3.344	0.039†

Univariate analysis performed using one way analysis of variance (ANOVA) with Tukey's post-hoc test. Multivariable analysis performed with adjustments for age alone; model A = age, sex, presence of ischemic heart disease and hydroxychloroquine use; and [3]either model B = age sex, β-blocker use and hydroxychloroquine use (for QT_{BAZ}) or model C = age, sex, presence of hypercholesterolaemia and hydroxychloroquine use (for QT_{FHS}), with LSD post-hoc test. Post-hoc Significance P<0.05 *Tertile 1 v 2, †Tertile 1 v 3, ‡Tertile 2 vs. 3

CRP = C-reactive protein, IL = interleukin, QT_{BAZ} = QT corrected using Bazett's formula, QT_{FHS} = QT corrected using Framingham Heart Study formula, TNF = tumour necrosis factor.

Table 8.4 Step-wise multivariable linear regression with QTc interval as dependent variable

QT_{BAZ} as dependent variable

Step-wise linear regression					
Variable	**beta**	**B**	**95% CI**		**P**
Model 1	$R^2 = 0.220$				
Age	0.263	0.487	0.175	0.800	0.003
Sex (woman)	0.236	11.719	3.354	20.083	0.006
CRP	0.278	0.300	0.118	0.483	0.001
Model 2	$R^2 = 0.211$				
Age	0.264	0.489	0.169	0.808	0.003
Sex (woman)	0.210	10.441	1.894	18.987	0.017
β-blocker	-0.177	-9.973	-19.647	-0.298	0.043
IL-10 (Tertiles, per unit increase)	0.202	5.457	0.754	10.160	0.023

QT_{FHS} as dependent variable

Step-wise linear regression					
Variable	**beta**	**B**	**95% CI**		**P**
Model 3	$R^2 = 0.238$				

Heart disease	0.351	20.321	10.562	30.080	<0.001
HCQ	0.248	11.197	3.650	18.744	0.004
IL-10 (Tertiles, per unit increase)	0.223	4.830	1.205	8.455	0.009

The following variables were entered into the step-wise multivariable regression analysis: Model 1, 2: Age, sex (woman), CRP, IL-6, TNF-α, IL-1α, IL-1β, IL-10, heart disease, β-blocker, hydroxychloroquine; Model 3: Age, sex (woman), TNF-α (Tertiles), IL-1β (Tertiles), IL-10 (Tertiles), heart disease, hypercholesterolaemia, hydroxychloroquine, RF positive, anti-CCP positive. In order to minimise risk of collinearity β-blocker, heart disease and hypercholesterolaemia as well as anti-CCP positive and RF positive were entered into separate models. Standardised (beta) and unstandardised (B) coefficients, with 95 % CI shown. Significance = P <0.05.

Anti-CCP = anti-cyclic citrullinated peptide, CRP = C-reactive protein, IL = interleukin, QT_{BAZ} = QT corrected using Bazett's formula, QTc = corrected QT interval, QT_{FHS} = QT corrected using Framingham Heart Study formula, RF = rheumatoid factor, TNF-α = tumour necrosis factor-α.

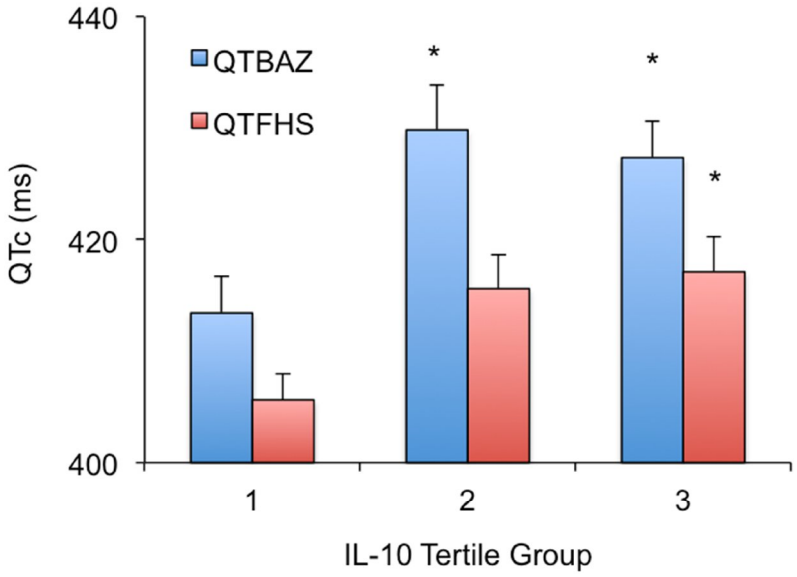


Figure 8.1 Association between QTc and IL-10

Bar chart demonstrating lower QTc interval (QT_{BAZ} = blue, QT_{FHS} = red) in patients in the lowest tertile for serum IL-10 following multivariable analysis. * P<0.05 v Tertile 1.

8.7 Discussion

This study is the first to report a correlation between QTc interval and contemporary serum concentrations of pro- and anti-inflammatory cytokines in humans. CRP and IL-10 demonstrated a positive correlation with QTc suggesting that a higher inflammatory burden associates independently with longer duration of QTc interval. Given that QTc prolongation increases risk of potentially life threatening arrhythmias (e.g. Torsades de Pointes) these results suggests a possible mechanistic link between increased inflammatory burden in RA and cardiovascular mortality.

8.7.1 Interleukin-10 and QTc interval

Of the cytokines analysed, IL-10 was independently associated with QTc interval prolongation. IL-10, the major anti-inflammatory cytokine is released by monocytes, macrophages and B-cells in response to inflammatory stimuli and is known to inhibit the production and release of pro-inflammatory cytokines (including TNF-α, IL-1β and IL-6) (45, 575-577). In addition IL-10 reduces the surface expression of TNF receptors and promotes the release of TNF receptors into the circulation (578, 579). There are a number of possible explanations for the positive correlation between IL-10 concentration and QTc prolongation found in this study. The surge of IL-10 production is known to follow that of pro-inflammatory cytokines (45) and hence increased levels of IL-10, as seen in RA (580), may represent increased inflammation. Conversely, undetectable levels of IL-10 may indicate lower circulating concentrations of TNF-α and IL-1 and hence may result in a lower QTc interval; given that both TNF-α (390) and IL-1 (566) were shown to prolong cardiomyocyte action potential duration in animal studies. This is supported by the finding that IL-10 was strongly correlated with other pro-inflammatory cytokines including TNF-α (rho=0.775,

p<0.001), IL-1α (rho=0.853, p<0.001) and IL-1β (rho=0.902, p<0.001). Additionally, given that the release of IL-10 is stimulated by adrenaline (45) and that sympatho-excitation may be associated with QTc prolongation (569), lower levels of IL-10 may reflect lower resting sympathetic nerve activity. Finally, the direct effects of IL-10 on cardiomyocyte action potential are currently unknown and further studies are warranted to explore the mechanisms by which IL-10 and other inflammatory cytokines may affect QTc interval.

8.7.2 CRP and QTc interval

In this study a significant and independent association between CRP and QT_{BAZ} was found but no association between CRP and QT_{FHS}. This is the first study to report such a discrepancy between QT_{BAZ} and QT_{FHS}; to the author's knowledge prior studies showing associations between QTc interval and CRP used the Bazett's HR correction formula. In one study of 101 patients with inflammatory arthritis elevated concentrations of hs-CRP was associated with higher QT_{BAZ} but also a higher resting HR (377). One possible explanation is that HR may be confounding the relationship between QTc interval and CRP; once HR is adequately corrected with the stricter formula (QT_{FHS}) this relationship disappears. This is further supported by the finding that CRP was correlated with HR.

8.7.3 Immune and autonomic interactions

Interactions between the immune and ANS could also explain the associations found between QTc interval and inflammation in this study. Both the sympathetic and parasympathetic nervous systems contribute to QTc interval (568, 569). Left cardiac sympathetic denervation shortens QTc interval in patients with the congenital long QT

syndrome (569), while cholinergic stimulation with pyridostigmine shortens QTc interval in patients with coronary artery disease (568). In patients with RA an increased sympathetic and reduced parasympathetic activity has been identified (445, 464, 468, 484) and could contribute to QTc interval prolongation. Recent evidence suggests a bi-directional relationship between the immune system and ANS. For example, IL-6 has been shown to increase sympathetic activity in rats (52), while the ANS has been implicated in regulation of the immune response through cholinergic nerve fibres, known as the cholinergic inflammatory reflex (242). Indeed, the principle vagal neurotransmitter Ach inhibits the release of cytokines TNF-α, IL-1β, IL-6 but not IL-10 (241) and direct electrical stimulation of the vagus nerve in rats during endotoxaemia inhibited synthesis of TNF-α (241).

8.7.4 Limitations

One of the major limitations of the present study is the cross-sectional nature of the associations between inflammatory cytokines and QTc interval. These results cannot prove causality and should be viewed as hypothesis generating. Another limitation is the one off blood sampling, which does not allow for cytokine fluctuations over time to be accounted for. Finally, the variables included in the regression models explained approximately 21-24% of the variation in QTc interval suggesting the presence of other confounders not being accounted for. These may include autonomic dysfunction, electrolyte abnormalities, structural heart disease and the presence of genetic polymorphisms causing congenital long QT syndrome.

8.7.5 Future directions

Future studies are needed to further explore the relationship between inflammatory cytokines and QTc prolongation. It is likely that concurrent assessments of ANS function and QTc interval, with multiple methods of HR correction can help provide greater understanding of the precise mechanisms by which inflammatory cytokines contribute to QTc prolongation in RA.

8.8 Conclusion

In conclusion this study is the first to report a contemporary association between higher serum pro- and anti-inflammatory cytokine concentrations and QTc prolongation in RA patients. Undetectable concentrations of IL-10 were independently associated with shorter QTc interval while pro-inflammatory cytokines (TNF-α, IL-1β) were positively associated with prolonged QTc interval - although this association was attenuated when adjusted for age. Further studies are needed to confirm the effect of circulating inflammatory cytokine levels on QTc interval and assess whether this effect is contemporary or cumulative.

CHAPTER 9 Experiment Five. Effects of hydrocortisone on BRS, HRV and cardiovascular reactivity in healthy humans: a randomised placebo-controlled cross-over study

9.1 Abstract

Objectives Surges in cortisol concentration during acute stress may increase cardiovascular risk, however the interaction between cortisol and the ANS remains poorly understood. This study sought to determine the acute effects of cortisol, induced by administration of HCN, on cBRS, HRV and cardiovascular reactivity.

Methods In a randomised placebo-controlled single-blinded crossover study ten healthy males received either a single intravenous bolus of saline (placebo) or 200 mg of HCN, one week apart. HR, BP and limb blood flow were monitored 3 h later, at rest and during the MOT (sequential infusion of SNP and PE), a CPT and a PASAT mental stress test. Short-term HRV was assessed using rMSSD.

Results HCN markedly increased serum cortisol 3 h following infusion (1771±598 vs. 94±37 nmol/L, $p<0.001$; HCN vs. placebo). Compared to placebo, HCN elevated resting HR (+7±4 beats/min; $p<0.001$) and systolic BP (+5±5 mmHg; $p=0.008$); lowered cBRS (geometric mean, 95 % CI 15.6, 11.1-22.1 v 26.2, 17.4-39.5 ms/mmHg, $p=0.011$) and HRV (rMSSD 59±29 v 84±38 ms, $p=0.004$); and increased leg vasoconstrictor responses to CPT (ΔLVC -45±20 v -23±26 %; $p=0.023$).

Conclusions An acute cortisol surge is accompanied by increases in HR and BP, and reductions in cBRS and HRV, potentially providing a pro-arrhythmic milieu that may precipitate ventricular arrhythmia or MI and increase cardiovascular risk.

9.2 Introduction

Cortisol is the principle glucocorticoid in humans and has important metabolic (e.g. mobilisation of glucose, fatty acids and amino acids), immune (e.g. anti-inflammatory) and cardiovascular (e.g. maintenance of normal BP) functions. The HPA axis is activated during periods of stress and stimulates the production and release of cortisol. The surge in cortisol during acute stress may precipitate left ventricular dysfunction, ventricular arrhythmia and MI (408). Although, the interaction between cortisol and the ANS has been implicated in the increased cardiovascular risk of acute stress (408), the modulatory effects of cortisol on cardiac autonomic control remain incompletely understood.

In healthy humans exogenous administration of cortisol acutely (within 6 hours) increases HR (398, 399) via a number of suggested mechanisms including parasympathetic withdrawal; reduced cBRS; central baroreflex resetting; increased cardiac sympathetic (adrenergic) activity; changes in cardiac ion channel properties; and, alterations in hypothalamus-regulated circadian rhythms. Reduced cBRS has been identified in a variety of cardiovascular diseases including coronary artery disease, hypertension and heart failure (278) and has been shown to predict mortality following MI (206) and in patients with chronic heart failure (292). BRS has been reported to fall during acute mental stress (403, 404) and may, in part, be due to the central effects of cortisol (403). Animal studies support the hypothesis that glucocorticoids can elicit centrally mediated alterations in BRS (405-407, 581). In rats, administration of glucocorticoids in the RVLM (406) and the NTS (407) rapidly alters activity of baroreceptive neurons and depresses baroreflex control of HR. A cortisol-induced fall in BRS may be an important mechanism whereby acute stress increases cardiovascular risk (408). However, to the authors' knowledge there have been no prior studies assessing the direct acute effects of cortisol on resting cardiovagal BRS in humans. HCN acutely augments

the diastolic BP response to a CPT (398). However, whether this is attributable to an exaggerated peripheral vasoconstrictor response is unclear.

9.3 Aims and hypothesis

Central hypothesis: During acute stress, surges in serum cortisol elicit deleterious changes in ANS function that may predispose an individual to adverse cardiac events. Glucocorticoid-induced elevations in HR are mediated by changes in autonomic cardiovascular control.

Aim: To test this, a randomized placebo-controlled single-blinded cross over study was performed in healthy male volunteers assessing cBRS, HRV, BP variability and cardiovascular reactivity to a CPT and PASAT mental stress test 3 hours after bolus infusion of HCN or placebo.

Hypothesis: HCN

(i) reduces cBRS;

(ii) reduces HRV;

(iii) increases BP variability; and

(iv) exaggerates cardiovascular responses to the CPT and PASAT mental stress test.

9.4 Methods

9.4.1 Subjects

Ten male participants (median age 27.0 interquartile range 23.8-34.5 years, BMI 24±3 Kg/m^2) were recruited from the University of Birmingham, UK and surrounding areas. Participants comprised of university students and members of staff. All participants were free from cardiovascular, pulmonary, renal, metabolic and neurological conditions, and none were taking any prescription or over-the-counter medications.

9.4.2 Experimental protocol

Detailed methods are described in Chapter 3.2. Using a placebo-controlled, single-blinded, cross-over design participants were randomised to receive either placebo or HCN on the morning of the first visit. Blood tests were taken prior to infusion and three hours later. The protocol began 3 hours after infusion and involved rest (10 min), MOT, CPT and PASAT mental stress test.

9.4.3 Measurements

HR (lead II ECG), beat-to-beat BP (finger photoplethysmography) were recorded continuously. Brachial BP (automated sphygmomanometer) was measured for calibration. Limb blood flow (venous occlusion strain gauge plethysmography) was measured during CPT (leg blood flow) and PASAT (leg and forearm blood flow). LVC and FVC were calculated (described in Chapter 5).

9.4.4 Data analysis

Beat-to-beat values of HR and BP were calculated offline, and 1 min averages were used to calculate HR and BP responses during CPT and PASAT. Percentage changes in limb blood flow and vascular conductance (LVC, FVC) were used to determine vascular responses to CPT and PASAT. BRS was assessed using three methods: G_{MOT}, G_{SEQ} and G_{LFTF}. Short term HRV were determined from a 10 min resting period (Kubios HRV, Kuipio, Finland) using time domain (rMSSD, NN50 and pNN50 %), frequency domain (TP, VLF, LF, and HF absolute powers, LF, HF normalised power, LF/HF ratio) and non-linear indices (SD1, SD2, SD1/SD2 ratio) as described in the methods section. Systolic and diastolic BP variability (CardioSeries v2.4, CardioSeries, Sao Paulo Brazil) were determined from a 10-min resting period using time (SD, VC) and frequency domain parameters (LF, HF power).

9.4.5 Blood sampling

Blood samples for analysis of hormones (ACTH, cortisol) were centrifuged immediately and the plasma was stored at -80 ^{o}C. Plasma ACTH levels were determined using an ELISA (Abnova, Taiwan). The sensitivity was <1 pg/ml and the intra-assay and inter-assay coefficients of variation were ≤4.2 and ≤6.2 % respectively. Serum cortisol levels were determined using ELISA (Abcam, Cambridge, UK). The sensitivity was 2.44 ng/ml and the intra-assay and inter-assay coefficients of variation were ≤9.0 and ≤9.8 % respectively. Serum electrolyte concentrations, glucose, serum osmolality and haematocrit were determined according to standard laboratory methods.

9.4.6 Statistics

Statistical analyses were performed using SPSS software, version 19 (SPSS Inc, Chicago, USA). Continuous variables were assessed for normality using the Kolmogorov-Smirnov test. Baseline differences in parameters were tested using a two-tailed paired Student's t-test. Non-parametric data was log-transformed (natural). The effect of treatment (with HCN or placebo) during each of the tasks was tested using repeated measures ANOVA with Bonferroni adjustments for multiple comparisons. Differences between the percentage changes (%Δ) in parameters from baseline during each CPT and PASAT mental stress test were assessed using a two-tailed paired Student's t-test. Based on a previous study (398), a sample size calculation indicated that 7 patients were required in order to show a mean difference in HR of 7±5 beats per minute before and after HCN. Data are expressed as mean± SD or geometric mean (95% CI). Significance levels were set at $P \leq 0.05$.

9.5 Results

9.5.1 Biochemical and haemodynamic parameters

There was no significant difference in baseline concentrations of serum cortisol, ACTH, glucose, electrolytes (sodium, potassium), creatinine and plasma osmolality in the placebo and HCN trials (Table 9.1). HCN elevated serum cortisol concentrations 3 h after infusion and suppressed ACTH compared to placebo. HCN did not influence serum glucose, electrolytes, creatinine, plasma osmolality (Table 9.1) or haematocrit (HCN 42±2 v placebo 41±2 %; p=0.215).

Resting HR was elevated following HCN administration compared to placebo (+7±4 beats/min; $p<0.001$) (Table 9.2). HCN also elevated resting systolic BP (+5±5 mmHg;

p=0.008) but did not alter resting diastolic or mean BP, leg blood flow and LVC. Compared to placebo there was a trend for increased forearm blood flow (p=0.095) and FVC (p=0.065) following HCN.

9.5.2 cBRS

cBRS was reduced with HCN administration (G_{MOT} geometric mean HCN 15.6, 95% CI 11.1-22.1 v placebo 26.2, 17.4-39.5 ms/mmHg; t=-3.165, p=0.011; G_{SEQ} 17.1±5.9 v 23.2±12.6 ms/mmHg; t=-2.290, p=0.048; G_{LFTF} 12.6±7.6 v 17.2±9.0 ms/mmHg; p=0.05) (Figure 9.1). SNP and PE infusion induced a similar fall and rise in systolic BP in the HCN and placebo conditions (fall in systolic BP after SNP geometric mean 26, 95% CI 21-34 v 26, 19-36 mmHg; t=0.107, p=0.917; rise in systolic BP after PE 26±8 v 24±11 mmHg; t=0.628, p=0.546).

9.5.3 HRV

Time domain parameters of HRV (rMSSD, NN50, pNN50 %) were reduced following HCN compared to placebo (Table 9.3). Absolute HF power was reduced following HCN while there was a tendency for LF power to decrease (p=0.057). There was no significant difference in normalised values of HF power, LF power and LF/HF ratio between trials. SD1 was reduced with HCN whilst the numerical fall in SD2 was not statistically significant (p=0.215). The ratio of SD1/SD2 fell following HCN administration (p=0.042).

Table 9.1 Effect of hydrocortisone on biochemical parameters

	Placebo	HCN	ANOVA		
			Time	Drug	Interaction
Cortisol, nmol/L					
9am	133 (97-181)	155 (108-222)	<0.001	<0.001	<0.001
12pm	94±37 *	1771±598 *†			
ACTH, mmol/L					
9am	3.0±0.7	3.6±1.5	0.004	0.035	<0.001
12pm	3.4±1.6	0.7±0.4 *†			
Glucose, mmol/L					
9am	4.5 (4.3-4.8)	4.3 (3.9-4.7)	0.325	0.772	0.158
12pm	4.3±1.1	4.9±1.1			
Na+, mmol/L					
9am	141±2	142±2	0.623	0.799	0.079
12pm	142±3	141±1			
K+, mmol/L					
9am	4.4 (4.1-4.6)	4.3 (4.2-4.5)	0.360	0.348	0.087
12pm	4.2±0.2	4.4±0.4			
Creatinine, μmol/L					
9am	83 (75-92)	82 (74-91)	0.062	0.003	0.207
12pm	79 (70-90)	76 (68-84)			
Plasma osmolality, mmol/Kg					
9am	291±4	292±3	0.589	0.833	0.564

12pm	292±6	290±7

Values represent mean±SD or geometric mean (95% CI). Comparisons were made using a repeated measures one way analysis of variance (ANOVA) with Bonferroni correction.
Post hoc significance P<0.05 * compared with 9am, † compared with placebo.
ACTH = adrenocorticotrophin hormone.

9.5.4 BP variability

Time domain parameters of systolic BP variability tended to increase with HCN whilst frequency domain parameters remained unaffected (Table 9.4). HCN had no effect on diastolic BP variability.

9.5.5 Cardiovascular reactivity

CPT

As expected, HR and BP rose during the CPT while leg blood flow and LVC fell (Figure 9.2). HCN administration resulted in greater reduction in leg blood flow (-33±24 v -7±28 Δ %; t=2.713; p=0.027) and LVC (-45±20 v -23±26 Δ %; t=2.798; p=0.023) during the CPT. Although there was a trend for increased systolic BP responses with HCN compared to placebo (+21±10 v +14±15 Δ mmHg; t=1.640; p=0.135), there were no differences in diastolic BP (+11±4 v 10±5 Δ mmHg; t=1.562; p=0.153) or HR (+3±4 v 4±4 Δ beats/min; t=-0.657; p=0.528) responses. There was no difference in the perceived pain rating during the CPT between HCN and placebo (6.6±2.2 v 7.1±2.0; t=-0.785, p=0.453).

Table 9.2 Effect of hydrocortisone on resting haemodynamic parameters

	Placebo	HCN	t	df	P Value
HR, beats/min	51±10	58±9 *	6.117	9	<0.001
Systolic BP, mmHg	114±8	118±7 *	3.395	9	0.008
Diastolic BP, mmHg	65 (61-69)	64 (62-67)	-0.270	9	0.793
Mean BP, mmHg	81±6	83±4	0.971	9	0.357
Leg blood flow, ml/100 ml/min	1.9±0.8	1.9±0.7	-0.492	9	0.635
LVC, AU	25±12	23±8	-0.023	9	0.982
Forearm blood flow, ml/100 ml/min	2.4±1.1	3.3±1.2	1.866	9	0.095
FVC, AU	29±14	40±13	2.099	9	0.065

Values represent mean± standard deviation or geometric mean (95% CI). Non-parametric data was transformed and comparisons made using paired t-test. * Significance P≤0.05 compared with placebo.
AU = arbitrary units, BP = blood pressure, FVC = forearm vascular conductance, HR = heart rate, LVC = leg vascular conductance

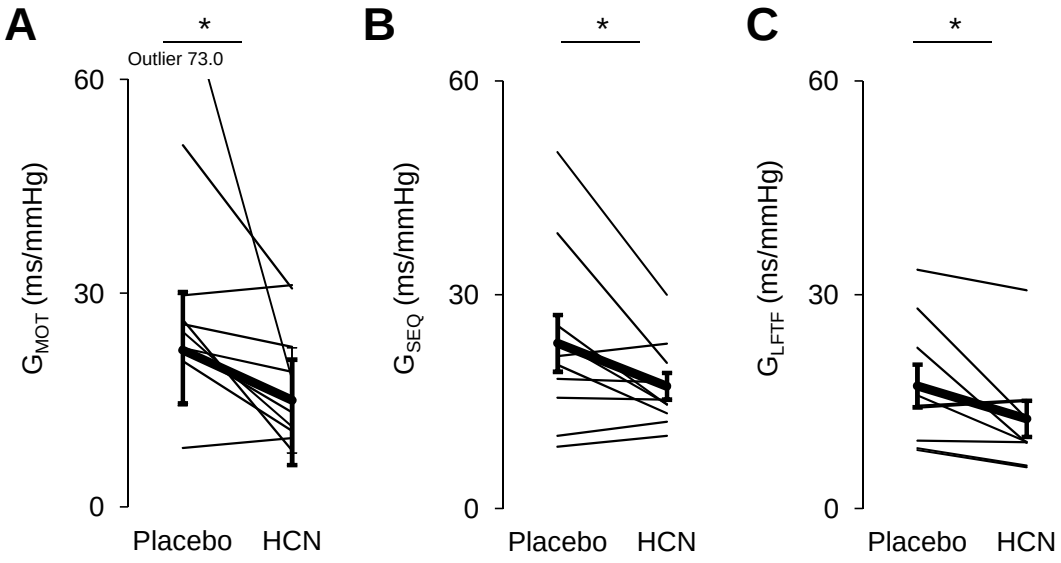


Figure 9.1 Effect of hydrocortisone on cBRS

Plot displaying baroreflex gain determined using the MOT (G_{MOT}) (Panel A), sequence technique (G_{SEQ}) (Panel B) and low frequency transfer function (G_{LFTF}) (Panel C). Individual participants data are shown as thin lines. Bold lines indicate geometric mean (95% CI) (Panel A) or group arithmetic mean±SEM (Panels B and C). Significance * $P \leq 0.05$.

cBRS = cardiac baroreflex sensitivity, HCN = hydrocortisone, MOT = modified Oxford technique.

Table 9.3 Effect of hydrocortisone on resting HRV parameters

	Placebo	HCN	t	df	P Value
rMSSD, ms	84±38	59±29 *	-3.801	9	0.004
NN50, count	242±81	188±101 *	-2.491	9	0.034
pNN50, %	49±17	34±19 *	-4.453	9	0.002
TP, ms^2	5627 (3358-9429)	4258 (2757-6576)	-1.318	9	0.220
VLF power, ms^2	2089 (1210-3605)	2176 (1308-3620)	0.143	9	0.890
LF power, ms^2	1523 (816-2842)	982 (647-1491)	-2.180	9	0.057
HF power, ms^2	1587 (900-2800)	887 (502-1566) *	-3.192	9	0.011
LF power, nu	49.0±13.8	52±14	0.711	9	0.495
HF power, nu	51.0±13.8	48±14	-0.711	9	0.495
LF/HF ratio	1.12±0.66	1.28±0.71	0.640	9	0.538
SD1	60±27	42±20 *	-3.801	9	0.004
SD2	105 (79-139)	90.1 (74-110)	-1.333	9	0.215
SD1/SD2	0.55±0.18	0.44±0.13 *	-2.368	9	0.042

Values represent means±SD or geometric mean (95% CI). Non-parametric data was transformed and comparisons made using paired t-test. * Significance $P \leq 0.05$ compared with placebo.

HF = high frequency (0.15-0.4 Hz), LF = low frequency (0.04-0.15 Hz), NN50 = number of pairs of adjacent NN intervals differing by more than 50 ms, pNN50 = NN50 as a percentage of all NN intervals, nu = normalised units, rMSSD = root mean square of successive differences, SD = standard deviation of the Poincare plot, TP = total power, VLF = very low frequency (0-0.04 Hz).

Table 9.4 Effect of hydrocortisone on resting BP variability

	Placebo	HCN	t	df	P Value
Time domain					
Systolic BP					
SD, mmHg	6.4±1.4	7.4±1.2	1.997	9	0.077
VC, %	5.5 (4.8-6.1)	6.3 (5.5-7.2)	1.731	9	0.117
Diastolic BP					
SD, mmHg	3.5±0.8	3.1±0.8	-1.337	9	0.214
VC, %	5.4±1.4	5.0±1.4	-0.752	9	0.471
Frequency domain					
Systolic BP					
LF power, ms^2	7.3±4.7	7.4±3.2	0.061	9	0.953
HF power, ms^2	1.5 (0.8-2.7)	1.4 (0.9-2.3)	-0.625	9	0.548
LF power, %	29.9±7.6	27.7±5.9	-0.887	9	0.398
HF power, %	12.2±7.3	9.0±5.4	-1.163	9	0.275
Diastolic BP					
LF power, ms^2	2.9 (1.9-4.4)	2.2 (1.6-3.1)	-1.472	9	0.175
HF power, ms^2	0.5±0.2	0.5±0.3	-0.079	9	0.939
LF power, %	36.4±6.6	36.9±6.5	0.252	9	0.807
HF power, %	6.7±1.6	9.1±4.1	1.776	9	0.110

Values represent means±SD or geometric mean (95% CI). Non-parametric data was transformed and comparisons made using paired t-test. Significance $P \leq 0.05$.

BP = blood pressure, HF = high frequency power range 0.15-0.4 Hz, LF = low frequency power range 0.05-0.15 Hz, SD = standard deviation, VC = variation of coefficient (SD/BP x100).

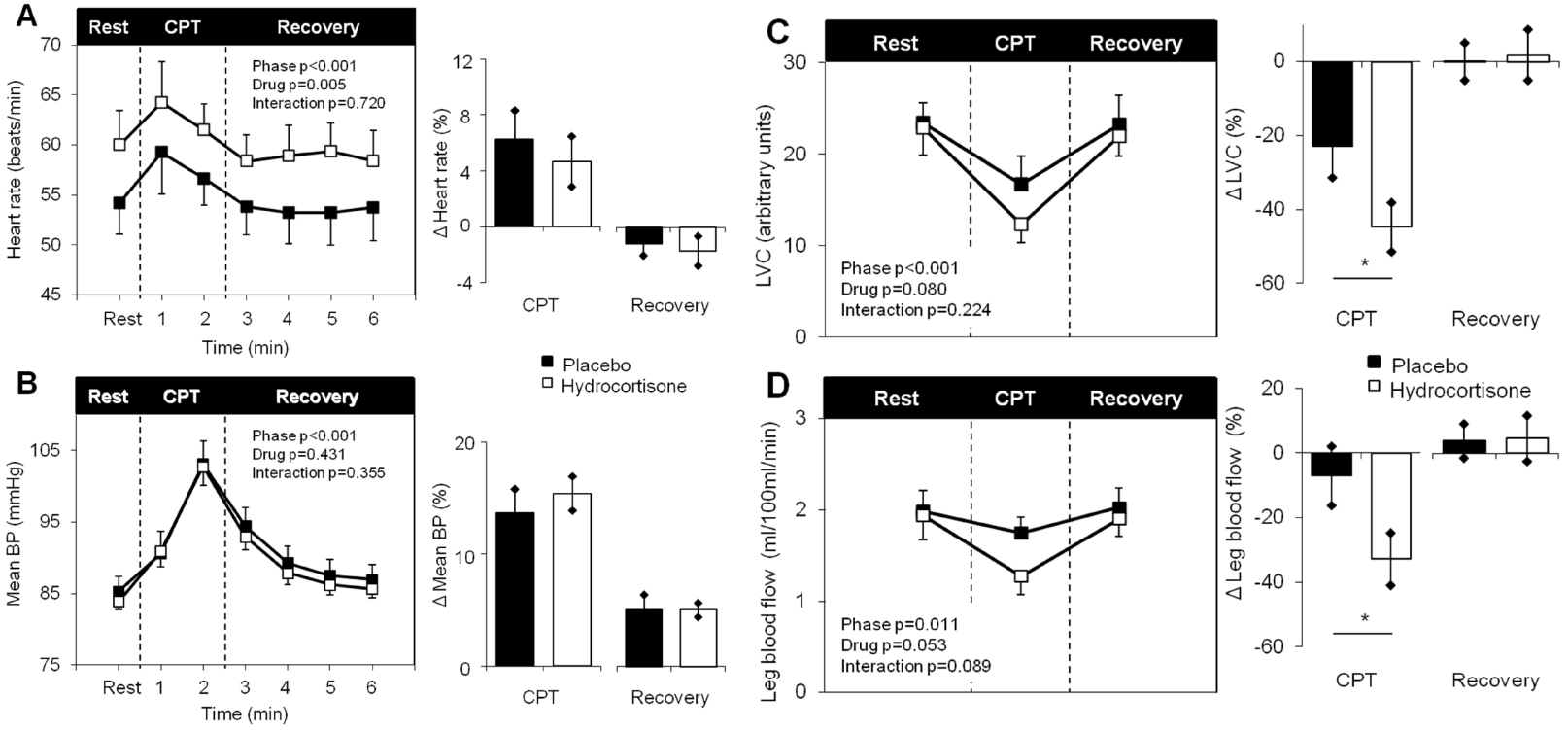


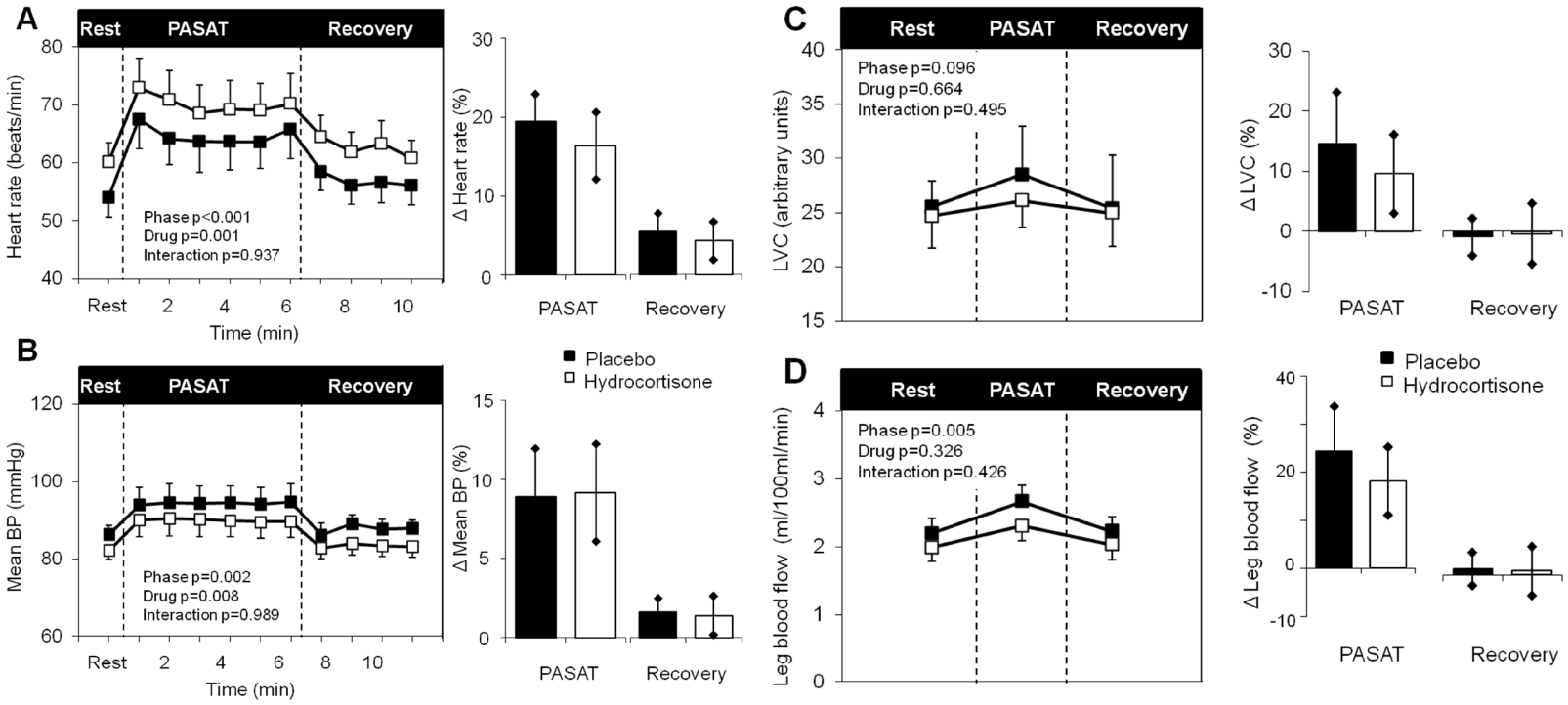
Figure 9.2 Effects of hydrocortisone on cardiovascular responses to CPT

HR (Panel A), mean BP (Panel B), LVC (Panel C) and leg blood flow (Panel D) during rest, CPT and recovery following pre-treatment with intravenous placebo (black) and hydrocortisone (white). Data represented as group means±SEM. Times series is shown on the left. No significant interactions were found between time (rest, CPT and recovery) and drug (placebo or hydrocortisone) conditions when assessed using ANOVA with repeated measures. Bar charts on the right represent changes from baseline. Significance was determined using paired Student's t-test * P≤0.05 compared to baseline.

BP = blood pressure, CPT = cold pressor test, HR = heart rate, LVC = leg vascular conductance.

PASAT mental stress test

As expected HR, BP, leg blood flow, forearm blood flow and FVC rose during the PASAT, while LVC tended to rise (Figure 9.3). There was no difference in HR (+10±8 v +11±7 Δ beats/min; t=-0.296; p=0.774), systolic BP (+10±13 v +11±14 Δ mmHg; t=-0.279; p=0.787), diastolic BP (+6±6 v +6±8 Δ mmHg; t=-0.098; p=0.924), leg blood flow (+19±20 v +25±27 Δ %; t=-0.935; p=0.185) and LVC (+10±19 v +16±25 Δ %; t=-0.934; p=0.375) responses to the PASAT between HCN and placebo trials. However, there was a trend for reduced forearm blood flow (geometric mean +32, 95 % CI 14-69 v +56, 29-107 Δ %; t=-1.685; p=0.126) and FVC (geometric mean +21, 95 % CI 8-59 v +50, 23-108 Δ %; t=-2.103; p=0.069) responses following HCN administration. There was a tendency for reduced perceived stress (10 point scale) during the PASAT with HCN (4.8±1.9 v 5.7±1.8 points out of 10; t=-0.785, p=0.095); however, there was no difference in performance (geometric mean 74, 95 % CI 53-103 v 82, 74-91 %; t=-0.828, p=0.429).


A
Rest
PASAT
Recovery
Heart rate (beats/min)
Phase p<0.001
Drug p=0.001
Interaction p=0.937
Rest 2 4 6 8 10
Time (min)
Δ Heart rate (%)
PASAT
Recovery
B
Mean BP (mmHg)
Phase p=0.002
Drug p=0.008
Interaction p=0.989
Time (min)
Δ Mean BP (%)
Placebo
Hydrocortisone
C
LVC (arbitrary units)
Phase p=0.096
Drug p=0.664
Interaction p=0.495
Δ LVC (%)
D
Leg blood flow (ml/100ml/min)
Phase p=0.005
Drug p=0.326
Interaction p=0.426
Δ Leg blood flow (%)

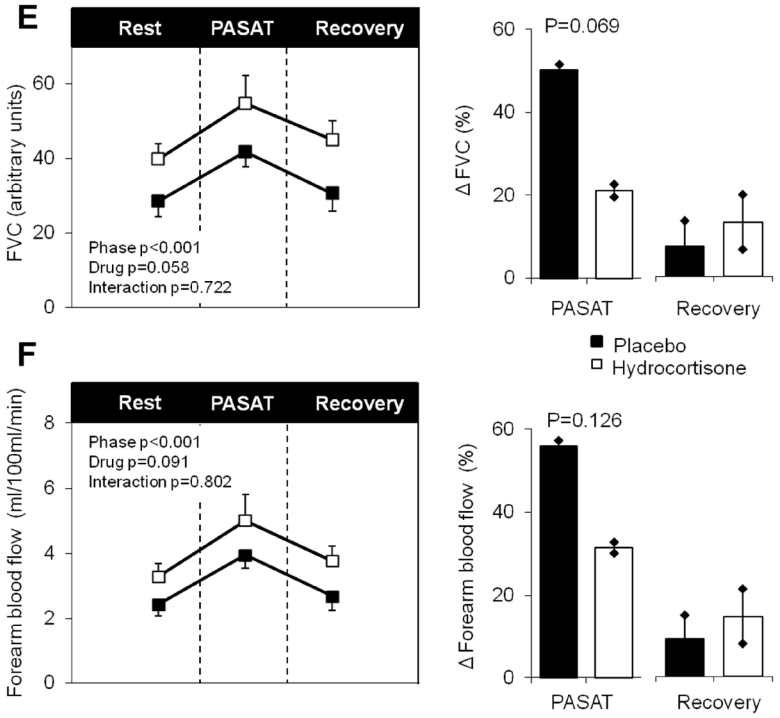


Figure 9.3 Effects of hydrocortisone on cardiovascular reactivity to the PASAT

HR (Panel A), mean BP (Panel B), LVC (Panel C), leg blood flow (Panel D), FVC (Panel E) and forearm blood flow (Panel F) during rest, PASAT mental stress test and recovery following pre-treatment with intravenous placebo (black) and hydrocortisone (white). Data represented as group means±SEM. Times series is shown on the left. No significant interactions were found between time (rest, PASAT and recovery) and drug (placebo or hydrocortisone) conditions when assessed using ANOVA with repeated measures. Bar charts on the right represent changes from baseline. Significance was determined using paired Student's t-test P≤0.05 compared to baseline.

BP = blood pressure, FVC = forearm vascular conductance, HR = heart rate, LVC = leg vascular conductance, PASAT = paced auditory serial addition task.

9.6 Discussion

This study is the first to report that acute HCN administration elicits an increase in HR that is associated with a reduction in cBRS and HRV derived indices of cardiac parasympathetic nerve activity. In addition, it was observed that HCN administration significantly increased systolic BP and exaggerated the leg vasoconstrictor responses to CPT, whilst vasodilatory forearm responses to mental stress tended to be reduced. Reduced cBRS and HRV are known to have deleterious cardiovascular consequences and are both predictive of increased mortality (204, 206, 292, 582). Thus these findings may help explain the known associations between acute stress-induced increases in cortisol and cardiovascular risk (408).

9.6.1 Central mechanisms for reduced BRS

Animal studies suggest that cortisol may elicit central effects in areas of the central nervous system important for modulation of the baroreflex (e.g. RVLM, NTS) (405-407, 581, 583). However, it is possible that cortisol acts upon other structures within the baroreflex arc i.e. afferent structures (e.g. vascular structures, afferent terminals), efferent target-organ structures (e.g. sino-atrial node, or smooth muscle) or the efferent nerves (584). The cortisol-induced increased HR and systolic BP found in this study and others (398-400, 585, 586) may suggest a centrally-mediated resetting of the baroreflex. A rise in systolic BP would be expected to increase baroreceptor firing resulting in a compensatory fall in HR and inhibition of sympathetic outflow to the peripheral vasculature. Thus, it appears that an upwards and rightwards resetting of the baroreflex curve accompanies the observed reduction in cBRS with acute HCN administration, as occurs in hypertension and heart failure. A reduction in cardiac parasympathetic activity may also explain the cortisol-induced increased HR reported in this study. HCN acutely reduced HRV in a pattern consistent with reduced cardiac

parasympathetic activity. Reduced HRV is a pathogenic feature of cardiovascular diseases including hypertension and heart failure, and has been shown to be of prognostic significance after MI (196, 204, 582).

9.6.2 Sympathetic activation

BP responses to the CPT and mental stress have previously been used as an index of sympathetic reactivity of the vasculature (323), and if exaggerated can predict the development of hypertension (336). In this study cortisol acutely increased leg vasoconstrictor and systolic BP (trend) responses to cold water and inhibited forearm vasodilatory (trend) responses to mental stress. Cortisol-induced increases in pressor responsiveness have been demonstrated in prior studies (398, 587) and may reflect potentiation of catecholamines (adrenaline/noradrenaline) by cortisol in vascular smooth muscle (588). The altered vascular responsiveness following cortisol may also be explained by changes in endothelial function. Glucocorticoids may alter endothelial function via stimulation and production of endothelin (circulating peptide with vasoconstrictor properties) (589) and inhibition of the NOS isoforms inducible NOS (iNOS) and endothelial NOS (eNOS) (590, 591). In one study cortisol inhibition blocked mental-stress induced impairments in endothelial function as assessed by flow mediated dilatation (403).

During acute stress there is co-activation of the HPA axis and the sympathetic nervous system. While the effects of heightened sympathetic activity are well documented the acute cardiovascular effects of a cortisol surge is not fully understood. The increased HR and systolic BP noted in this study may represent heightened sympathetic activity, although this may not be due to increased central sympathetic outflow. Glucocorticoids can up-regulate

cardiac β_1-adrenergic receptor sensitivity (592) which can potentiate the effects of circulating adrenaline and noradrenaline. In prior studies resting MSNA decreased (399) or did not change (398, 400) following acute glucocorticoid administration. Peripheral vasoconstriction is unlikely to be contributing to the rise in systolic BP as LVC remained unaffected in this study and diastolic BP did not increase. The short time course of this study did not allow the mineralocorticoid actions of cortisol (i.e. increased sodium and water retention) to take effect and cannot explain the rise in systolic BP, especially as haematocrit and plasma osmolality were unchanged.

9.6.3 Strengths and limitations

The study design (single-blinded, placebo-controlled crossover) is a strength allowing a direct assessment of the effects of HCN. Additionally, a comprehensive assessment of cBRS was made using a variety of methods that corroborate one another. The main limitation of this study is the difficulty in distinguishing between the direct effects of cortisol and subsequent compensatory mechanisms (e.g. cortisol suppresses ACTH through negative feedback of the HPA axis). It therefore cannot be ruled out that some of the findings may be due to ACTH suppression. Secondly, these findings can only be applied to healthy young males. Additionally, the autonomic assessments used in this study have inherent limitations due to the inaccessibility of the parasympathetic nervous system (e.g. HRV is used as an indirect measure of parasympathetic influences on the sinus node) and sympathetic activity was not measured.

9.6.4 Future directions

The precise mechanisms and time course by which cortisol elicits the alterations in ANS observed in this study and others needs further investigation. For example, further assessments of BRS (cardiovagal and sympathetic) are warranted in healthy humans to determine whether there is a central resetting of the baroreflex as hypothesised. In addition, further studies are also required to determine whether the autonomic responses to HCN are affected by the dose and type of glucocorticoid, sex, age and presence of pathological diseases (such as RA). During acute stress there is concomitant activation of the HPA and sympathetic nervous system therefore assessing autonomic function following cortisol administration *and* concomitant sympathetic activation (possibly by administration of sympathomimetics) may provide additional insight into the pathophysiology underlying stress induced increases in cardiovascular risk.

9.7 Conclusion

Activation of the HPA axis during acute stress results in a surge in serum cortisol. While acute stress is associated with increased cardiovascular risk the effects of acute surges in cortisol is not known. The findings in the present study might suggest a possible mechanism by which acute stress increases cardiovascular risk (408). During acute stress the surge in cortisol may result in acute depression in BRS and HRV, along with increased peripheral vasoconstrictor responsiveness, increased sympathetically-mediated positive cardiac inotropic activity and impairment in vasodilatory mechanisms. Thus could provide a pro-arrhythmic milieu that precipitates ventricular arrhythmia, MI, left ventricular dysfunction and increased cardiovascular mortality.

CHAPTER 10 Synthesis

10.1 Overall aims of thesis

RA is a chronic autoimmune condition characterised by synovial destruction with associated local and systemic inflammatory responses. The pathophysiology of RA is poorly understood but has improved with the advent of biological agents that block specific immune targets effectively reducing inflammation (5, 6). Drugs that inhibit the inflammatory cytokines IL-6 and TNF-α have demonstrated remarkable efficacy in reducing inflammatory burden and achieving disease control in RA patients (6, 47, 48). RA is not a benign condition. Epidemiological data demonstrates that RA is associated with increased cardiovascular risk with up to 50 % cardiovascular mortality, including a doubled risk of MI and sudden cardiac death (20-23). Moreover RA has recently been included in a cardiovascular risk prediction algorithm as per NICE guidelines (593) as an independent cardiovascular risk factor, in addition to diabetes mellitus, hypertension, hypercholesterolaaemia, smoking and others (594). The reason for the increased cardiovascular risk in RA is not known with numerous studies consistently showing that traditional risk factors do not wholly account for the increased risk seen in RA (37, 38). The observation that cytokine inhibition improves mortality in RA (412, 557, 558) coupled with the increasing recognition of chronic inflammation in cardiovascular diseases (51) raises the possibility that inflammation may play a major role in the increased cardiovascular and mortality risk in RA. The evidence from recent animal studies demonstrating that inflammatory cytokines (TNF-α, IL-6, IL-1β) directly affect key cardiovascular regulatory centres in the brain resulting in cardiovascular dysregulation suggests that ANS dysfunction may be an important mechanism. In animals inflammatory cytokines increased sympathetic activity (52) and reduced BRS (314) and HRV (273), a pattern of ANS dysfunction that is prevalent in cardiovascular conditions (e.g. hypertension, chronic heart failure) (62, 63, 198, 199, 279-283) but also associated with

increased mortality risk (113, 204-206, 284). It is in this context that led to the hypothesis that inflammation may lead to ANS dysfunction and increased cardiovascular and mortality risk in RA. Therefore the aims of the thesis were to firstly determine whether ANS dysfunction is a recognised pathogenic feature of RA, and if so to then determine the prevalence and nature of ANS dysfunction as well as the presence of known associations between ANS dysfunction and inflammation. This was achieved with the systematic literature review (Chapter 4), which demonstrated that ANS dysfunction was prevalent in ~60% of RA patients predominantly manifesting as impaired cardiovascular reflexes and reduced HRV indices of parasympathetic activity. The review showed that there was limited data regarding sympathetic activity or baroreflex function in RA and insufficient data to determine causal relationships between inflammation and ANS dysfunction in RA. Consequently the overall aims of the thesis were to carry out a series of experiments in RA patients to comprehensively assess the ANS and to explore for relationships between ANS dysfunction and inflammation. Observational case-control studies were performed to assess sympathetic activity, BRS (Chapter 6) and HRV and cardiovascular reactivity to stressors (Chapter 7) in RA patients compared to normotensive and hypertensive controls. The rationale behind the inclusion of hypertensive controls was to control for the presence of high BP and medications (important confounders) and to characterise whether RA patients had similar profiles to hypertensive patients. A longitudinal interventional case study was performed assessing the effects of TNF-α-inhibitor therapy on sympathetic activity, BRS, HRV and cardiovascular reactivity to mental stress (Chapter 8). An explorative observational cohort study of RA patients was performed to assess for associations between the corrected QT interval (proxy marker of ANS function) and inflammation (Chapter 9). Finally a randomised-controlled cross-over study was performed in

healthy individuals to assess the acute effects of an exogenous cortisol surge (mimicking HPA axis activation) on autonomic function (Chapter 10).

10.2 General discussion

The systematic literature review (Chapter 4) was the first to be performed and demonstrated that autonomic dysfunction was prevalent in RA. The review delineated that the pattern of ANS dysfunction from prior studies predominantly included abnormal cardiovascular reflex tests and reduced HRV indices of parasympathetic function. The review highlighted that there was insufficient evidence to support the presence of sympathetic or baroreflex dysfunction in RA. Finally there was insufficient evidence to support the presence of associations between inflammation and ANS dysfunction in RA. In the 1980s Ewing and colleagues developed a battery of clinical cardiovascular reflex tests that could be performed at the bedside (415). These tests were quick, cheap, and reproducible, and allowed categorisation of the severity of ANS dysfunction and the system involved (i.e. parasympathetic, sympathetic or both). Their work allowed great progress to be made in the in recognition of ANS dysfunction particularly in diabetes, a disease characterised by autonomic neuropathy (415). However a major limitation of the reflex tests was their inability to distinguish the mechanisms underlying ANS dysfunction. The literature review demonstrated that cardiovascular reflex tests were impaired in RA although the mechanisms were not known. In the mid-1990s a Task Force guideline was developed for the assessment of HRV allowing a standardised method for measuring the variability of HR fluctuations detected on an ECG recording (196). Following these guidelines a number of studies in RA emerged which consistently demonstrated that HRV indices of parasympathetic activity were reduced in RA, although the mechanisms still remained unknown. Baroreflex control of HR is an

important mechanism however the literature review demonstrated that this was only studied in two experiments; cBRS was reported as reduced in one study (447) and normal in another (446). Using the MOT, which allows BRS to be determined across a wide BP range it was demonstrated that reduced cBRS is indeed a pathogenic feature of RA, whilst arterial baroreflex of MSNA is unchanged (Experiment 1, Chapter 6). The MOT, a semi-invasive test requiring intravenous access that carries inherent risk during infusions of vasoactive drugs (433), had not been performed in RA and hence this was a novel element to the thesis. The experiments in this thesis demonstrated that cBRS (Chapter 6) and HRV (Chapter 7) are reduced in RA independent of the presence of HTN and furthermore that inverse relationships exist between cBRS and serum concentrations of inflammatory markers (hs-CRP, IL-6 and TNF-α) and between HRV and inflammatory markers (hs-CRP, IL-6). Although confounded by other factors, these associations are consistent with findings from animal studies that demonstrated causal relationship. In rats, administration of inflammatory cytokines (IL-6) depressed HRV (273) and cBRS (314). The results of Experiment Three (Chapter 8) support a causal relationship by demonstrating acute improvements in HRV and cBRS in an RA patient following two weeks of TNF-α inhibition. These experiments are novel as it is the first time that cBRS was assessed before and after TNF-α inhibition in RA, and collectively provide evidence that elevations in circulating cytokines can depress HRV and cBRS. While reductions in cBRS and parasympathetic activity contribute to higher resting HR, other factors are also involved. In Experiment One (Chapter 6) a higher resting HR was observed in RA patients compared to controls, which has been reported previously (447, 464, 474). Strong independent associations were demonstrated following multivariable adjustment between HR and inflammation (hs-CRP, R^2 =0.366, F=13.7, p=0.001) and pain (pain VAS, R^2=0.526, F=36.7, p<0.001, Chapter 6). In addition to inflammation, studies have shown that

experimentally evoked pain can also reduce cBRS (519) however another important contributory factor is the HPA axis. During stress there is co-activation of the sympathetic and HPA systems, resulting in a surge of cortisol and adrenaline released from the adrenal glands. The acute effects of cortisol on HR are not clear with two studies showing an acute rise in HR in healthy humans, following hydrocortisone (398, 399). Animal studies showed that glucocorticoids can induce centrally mediated changes in baroreflex function (406, 407). In this thesis an experiment was performed in young healthy male volunteers that demonstrated an acute rise in HR, with reductions in HRV and cBRS (Chapter 10). This study was the first to demonstrate such an acute effect of cortisol on cBRS in humans. In one other recent study one week of prednisolone treatment did not appear to significantly affect cBRS (409), although only one spontaneous index of cBRS was assessed. Taken together these findings suggest that acute surges in cortisol are required to elicit significant changes in cBRS in humans. In summary this thesis demonstrates elevated resting HR, depressed HRV and depressed cBRS in RA that are mediated in part by pain and inflammation and provides a novel mechanism for the influence of acute cortisol excesses. These findings are relevant as they provide novel potential mechanisms for the increased cardiovascular mortality risk seen in RA.

One important finding from the systematic literature review was the insufficient evidence for sympathetic dysfunction in RA. This probably relates to the methods employed. The majority of studies assessed sympathetic activity using clinical cardiovascular reflex tests and HRV, and reported impaired reflexes but normal HRV (Chapter 4). A major limitation of clinical reflexes is that impairments may not just reflect sympathetic dysfunction but also impaired baroreflex mechanisms and it is difficult to distinguish between them. Additionally subtle impairments will be missed given the way that they are scored (categorically based on

Ewing's criteria). The main limitation of HRV is that there is no parameter that directly and solely reflects sympathetic activity. LF power and LF/HF ratio are often purported to indicate sympathetic activity however these are heavily influenced by parasympathetic activity (196, 429). The few studies that assessed catecholamines or sympathetic biomarkers were also conflicting. Urinary or plasma catecholamines are not reliable for multiple reasons: difficulty in measurement, represent global sympathetic activity, cannot delineate regional variances, plasma levels reflect uptake, release and clearance whilst urinary levels are dependent on renal function, and can be confounded by medications, diurnal variations and concomitant diseases (422). In one study pre-ejection period was used as an indirect marker of sympathetic activity and reported to be lower in RA patients suggesting increased sympathetic activity (453). Using the microneurography technique for the first time in RA it was demonstrated that resting MSNA was heightened in RA patients, independent of hypertension (Experiment One, Chapter 6). In RA patients MSNA burst frequency was significantly increased compared to age- sex- and BMI-matched normotensive controls. These results were able to provide direct evidence for a heightened sympathetic outflow to the peripheral vasculature in RA that supports the findings of Dekkers et al (453). Furthermore this work adds to the literature by showing positive associations between MSNA and hs-CRP, although confounded. Given the evidence from animals that central administration of inflammatory cytokines increases sympathetic activity, a further experiment was performed to assess the effects of TNF-α inhibition on MSNA (Experiment Three, Chapter 8). It was demonstrated that 3 months of TNF-α inhibition resulted in an unexpected rise in MSNA with a fall in BP, whilst arterial baroreflex control of MSNA remained unchanged. In light of the fall in BP and unchanged sympathetic BRS it may be interpreted that the modest rise in MSNA simply reflects a change in the operational point rather than heightened sympathetic outflow, although the latter is

possible. The autonomic changes after TNF-α inhibition coincided with satisfactory reductions in pain and inflammation (disease activity, serum hs-CRP and cytokines fell although inflammatory cytokine concentrations remained relatively high) whilst HR remained unchanged. It is not clear whether temporal changes in ANS function following TNF-α inhibition relate to inflammation, pain or unknown factors. It may also be possible that a longer duration of treatment may restore autonomic balance in a more favourable profile, particularly as biologic anti-inflammatory agents are associated with improved prognosis (412). The results of the case study (Chapter 8) are hypothesis generating and require further studies to confirm these findings, and delineate the temporal changes in ANS function.

In addition, the systematic review demonstrated that there was insufficient literature regarding the cardiovascular responses to CPT and mental stress in RA. Only one study assessed CPT responses in RA and reported impaired diastolic BP, however blood flow measurements were not made. Similarly data regarding mental stress responses in RA have also been conflicting and without limb blood low measurements (Chapter 4). This thesis assessed cardiovascular responses to CPT and mental stress in RA with concomitant limb blood flow measurements for the first time (Experiment Two, Chapter 7). It was demonstrated that HR and BP responses to CPT and mental stress in RA patients were not significantly altered compared to normotensive controls. However significant differences in vascular responses were seen. In RA leg vasoconstriction during the CPT and forearm vasodilation during mental stress were exaggerated compared to normotensive controls. Additionally the study showed that forearm vasodilatory responses to mental stress were independently associated with inflammation (IL-10). In the case study (Experiment Three, Chapter 8) TNF-α inhibition altered forearm vasodilatory responses to mental stress, causing an increased response at 2 weeks and an attenuated response at 3 months. BP responses to mental stress

were blunted prior to starting treatment, and progressively increased at 2 weeks and 3 months post treatment. These observations are fascinating and suggest complex mechanisms that may involve alterations within the endothelium, circulating factors (e.g. adrenaline, endothelin, cortisol), NO bioavailability, central or peripheral sympathetic inhibition, altered α- or β-adrenergic sensitivity, central dysregulation or a combination of the above. Of course it is also possible that the vascular changes may be a direct side-effect of the drug rather that the effects of TNF-α cytokine inhibition. One study showed that TNF-α inhibitor infusion acutely improved vasodilation during hyperinsulinaemia in patients with metabolic syndrome, suggesting a rapid direct mechanism (595).

Finally, this thesis sought to investigate a potential novel mechanism for increased cardiovascular risk in RA, QTc interval prolongation (35). The cohort study (Experiment Four, Chapter 9) performed in RA patients is the first study to show associations between inflammatory cytokines and QTc interval, a surrogate marker of autonomic function and independent predictor of mortality risk. In this study undetectable concentrations of IL-10 were independently associated with shorter QTc interval. Positive associations were demonstrated between QTc prolongation and serum concentrations of pro-inflammatory cytokines (TNF-α, IL-1β), although confounded by age. These results suggest that low levels of inflammation may protect against QTc prolongation, which may theoretically improve survival. However further interventional studies are required to confirm whether the mortality benefit of anti-inflammatory agents can be attributed to reductions in QTc interval.

10.3 Limitations

The main limitation of the presented studies is that the observational nature of the cross-sectional studies precludes causality but are hypothesis generating. The interventional study was of one individual RA patient and hence should be interpreted with caution. The cohort study and case-control studies may be affected by sample bias and hence further studies are required to confirm these findings. Another limitation is the relatively small sample size in the case-control studies which may introduce type I and type II errors, however the invasive nature of the studies makes them less tolerable to diseased groups. The negative findings may be due to a lack of statistical power particularly given that cytokine concentrations are highly variable, as indeed are HRV parameters. A larger sample size may be necessary to demonstrate significant independent relationships between ANS function and inflammatory markers. Other limitations include not measuring respiration or cortisol (important potential confounders), and limitations inherent to the methods employed. For example, the microneurography technique provides a quantitative assessment of sympathetic activity but does not provide a qualitative one (i.e. the nature and strength of the signals or indeed whether appropriate muscular transduction occurs as a result of nerve firing are not known). Similarly given the cyclical nature of inflammatory markers (e.g. hs-CRP and inflammatory cytokines) a single laboratory measurement may not necessarily reflect cumulative inflammatory burden.

10.4 Future directions

Further interventional studies with larger numbers are required to assess the effects of cytokine inhibition on ANS function, ideally over several time points. The changes observed in ANS function following cytokine inhibition (Chapter 8) suggest that ANS dysfunction in RA may be due to functional rather than structural abnormalities. However further studies,

including pathological analysis of ANS nerves and associated structures, are required to exclude structural aetiologies for the ANS dysfunction observed in RA. Large epidemiological studies are required to determine whether ANS dysfunction precedes the development of RA, is predictive of the disease's natural course, and what the prognostic significance of these ANS alterations are for patients with RA. Larger case-control studies are needed to reproduce the findings from the present studies, ideally with concomitant measures of the HPA, respiratory and renin-angiotensin-aldosterone systems to control for their influences. Further interventional studies are required to assess whether therapies that target the ANS are effective in reducing inflammation in RA (e.g. sympathomimetic agents, β-adrenergic blockers, vagal nerve stimulation, chronic baroreflex activation therapy). Further work is required to investigate the precise mechanisms by which cortisol acutely alters baroreflex function in humans. It remains to be determined whether the cortisol-induced ANS changes are affected by the dose, type or route of glucocorticoid administration or by the presence of pathological diseases (e.g RA). In addition more work is required to determine the effect of concomitant sympathetic activation (as occurs during acute stress) on cortisol-induced ANS changes in health and disease, to further understand the mechanisms underlying increased cardiovascular risk associated with acute stress.

10.5 Conclusion

The present work demonstrates that ANS dysfunction is prevalent in RA and characterised by depressed HRV indices of parasympathetic function, heightened sympathetic outflow to the peripheral vasculature, depressed baroreflex control of HR and heightened vascular responses to stressors. These autonomic changes may contribute to the increased cardiovascular risk seen in RA, and may well be mediated by inflammation with QTc

prolongation as one potential mechanism. Further studies are required to confirm these findings, determine whether therapeutic strategies to restore ANS function improve prognosis in RA, and to further explore the precise mechanisms by which inflammatory cytokines may influence ANS function in health and disease.

APPENDICES

Appendix 1 Quality index score assessment tool criteria

Index	Criteria Assessed		
	High = 2 points	**Medium = 1 point**	**Low = 0 points**
1. Study Design	Case-control study with appropriate matching (e.g. age, sex, body mass index); and/or interventional with assessment before and after biologic agent	Case-control study but inappropriately matched	Cohort study or other design with inappropriate or no control group
Rationale	A case-control study with appropriate matching is the best study design to answer the principle question of the study – is autonomic dysfunction present in rheumatoid arthritis? An interventional study with assessment before and after biologic agent is the best study design to answer another principle question – is there a link between inflammation and autonomic function in RA?		
2. Inclusion/Exclusion Criteria	Patients included with a formal rheumatoid arthritis diagnosis according to recognised criteria and those with conditions or medications that interfere with autonomic function excluded	Patients included with a formal rheumatoid arthritis diagnosis according to recognised criteria but those with conditions or medications that interfere with autonomic function not excluded	Criteria for rheumatoid arthritis diagnosis not mentioned
Rationale	In order to establish meaningful conclusions from the study patients included must have the correct diagnosis according to recognised criteria and to prevent confounding factors those with condition or medications affecting autonomic function should be excluded		
3. Disease characteristics	Mentioned in detail (i.e. at least 2): disease duration, inflammatory marker e.g. C-reactive protein or erythrocyte sedimentation rate, swollen or tender joints, medications, functional capacity	Mentioned only 1: disease duration, inflammatory marker e.g. C-reactive protein or erythrocyte sedimentation, swollen or tender joints, medications, functional capacity	Not mentioned
Rationale	Disease characteristics are necessary to determine the inflammatory status of the rheumatoid arthritis patients tested at the time of the study. They allow for meaningful interpretation and comparison between different studies.		
4. Standardised testing condition	Mentioned in detail (i.e. at least 2): e.g. testing room temperature, time of testing, fasting status, subject position	Mentioned only 1: e.g. testing room temperature, time of testing, fasting status, subject position	Not mentioned or not standardised
Rationale	Testing conditions can affect the results of autonomic function assessments and hence unwanted bias can be avoided by standardising the testing conditions for each subject.		
5. Autonomic assessment – standardised protocol	Mentioned that the study adhered to published guidelines or protocols and comprehensive details	Mentioned that the study adhered to published guidelines and protocols but important details	No mention of guidelines or protocols

	provided	missing; or mentioned that study was adapted from guidelines or protocols	
Rationale	Adhering to published guidelines or protocols ensures that testing is performed to the highest standard available and allows for meaningful comparison between different studies.		
6. Autonomic assessment – quality of test	Autonomic function assessed using a recognised and validated tool, and a comprehensive assessment performed (i.e. more than one technique employed) Gold standard or close to gold standard assessment of autonomic function	Autonomic function assessed using a recognised tool but a basic assessment performed (i.e. only one technique) Reasonable assessment of autonomic function	Unrecognised tool to measure autonomic function such as a novel or non-established method Unknown or poor indicator of autonomic function
Rationale	A comprehensive assessment of autonomic function involves using the best validated tools with numerous aspects of autonomic function tested		
7. Statistics – appropriate sample size	Power calculation performed to determine sample size and sample size achieved	Power calculation performed to determine sample size but sample size not achieved	No mention of power calculation
Rationale	In order to prevent type 2 errors the correct sample size should be calculated in advance and reached.		
8. Statistics – appropriate tests used	Appropriate statistical test applied and comprehensive details mentioned with adjustment made for co-variables/confounders when necessary	Appropriate statistical test applied but lacking details with no adjustment made for co-variables/confounders when necessary	Inappropriate statistical test used
Rationale	Choosing the most appropriate statistical test ensures accurate results and adjusting for co-variables helps to minimise the bias, allowing meaningful and accurate interpretation and conclusions.		
9. Associations between autonomic function and inflammation made	Associations made (e.g. using regression analysis) and adjustments made for co-variables/confounders (e.g. multiple regression) when necessary	Associations made (e.g. using regression analysis) but no adjustment made for co-variables/confounders when necessary	Not mentioned or no associations made
Rationale	To determine whether links between inflammation and autonomic function in RA exist associations between indices of inflammation and parameters of autonomic function need to be made.		
Each index was graded between 0-2, and the total points added to give a final score between 0-18. If an index was found to be inappropriate (or irrelevant) to a particular study then the index was omitted and the total score reduced to 16. This occurred in studies employing 24-hour home assessments (e.g. 24-hour electrocardiogram monitor or urinary testing) where the index "standardised test conditions" did not apply. For all studies a percentage was calculated to give a Quality Index Score (QIS). The quality assessment was performed by two researchers (Ahmed Adlan and James Fisher) and disagreements were discussed until a consensus was reached.			

Appendix 2 Characteristics of studies included in the review (cross-sectional, observational, case-control studies)

A. Cross-sectional, observational, case-control studies

Study	Year	N	Characteristics	Inclusion Exclusion	Assessment	Key findings	QIS
Clinical cardiovascular tests (n=17)							
Aydemir et al (447)	2010	RA 36	30 female, 49 years Disease duration 11.2 years DAS28 4.1 CRP 11mg/L ESR 33 mm/1st hour	I: ARA 1987 criteria E: Condition or medication affecting ANS	Ewing HR variation response to DB, O, VM BP response to HG, O	Abnormal cardiovascular tests in 61-75% of RA patients Impaired sympathetic and parasympathetic responses Higher resting HR in RA patients No association between inflammation (DAS28, CRP, ESR) and ANS function	89%
		HC 40	31 female, 43 years				
Bidikar et al (450)	2010	RA 50	46 female, 38 years	I: ARA 1987 criteria, age 20-60 yrs E: Condition or medication affecting ANS	Ewing BP response to CP, HG, O	Higher resting HR and SBP in RA patients Abnormal cardiovascular tests in RA Impaired sympathetic responses	56%
		HC 50	46 female, 38 years				
Milovanovic et al (473)	2010	RA 38	32 female, 56 years 25 RF positive ESR 14.3 mm/1st hour	I: ARA 1987 criteria E: Condition or medication affecting ANS	Ewing HR variation response to DB, O, VM	Abnormal cardiovascular tests more prevalent in RA than controls	67%

		HC 41	17 female, 37 years		BP response to O	Impaired sympathetic and parasympathetic responses	
Stojanovich et al (477)	2007	RA 39	33 female, 58 years Disease duration 9.5years 64% RF positive ESR 14.3 mm/1st hour	I: ARA 1987 criteria E: Condition or medication affecting ANS	Ewing HR variation response to DB, O, VM BP response to HG, O	Abnormal cardiovascular tests more prevalent in RA Impaired sympathetic and parasympathetic responses in RA patients No correlation between inflammation (CRP, ESR, Ritchie score) and ANS function	78%
		HC 35	19 female, 52 years				
Veldhuijzen van Zanten et al (481)	2005	RA 21	18 females, 57 years Disease duration 12 years CRP 10.4 mg/L ESR 27.5 mm/1st hour DAS28 4.57	I: ARA 1987 criteria, able to stand for 15 minutes E: Previous acute coronary syndrome, diabetes mellitus, serious psychiatric disease	HR and BP (sympathetic) responses to mental stress	Normal sympathetic responses to mental stress seen in RA compared to osteoarthritis controls	61%
		DC 10	6 females, 47 years (osteoarthritis)				
Sandhu et al (475)	2004	RA 62	39 female, median 63 years Steinbrocker's class 1 or 2 76% RF positive None had evidence of current flare in joint 7 had peripheral nerve damage	I: ARA 1987 criteria E: Condition or medication affecting ANS	Ewing HR variation response to DB, O, VM BP response to HG, O	Abnormal cardiovascular tests in RA – worse in patients with peripheral neuropathy or RF positive Impaired parasympathetic and sympathetic (only	83%

		HC 41	21 females, median 50 years			DBP response to HG) responses in RA patients No correlation between inflammation (CRP, ESR) and ANS function	
Gozke et al (458)	2003	RA 10 HC 14	10 females, 49 years 14 females, 45 years	I: ARA 1987 criteria E: Symptoms of clinical ANS dysfunction	RR interval variation at rest and in response to DB	Abnormal cardiovascular tests in RA Impaired parasympathetic responses in RA patients	39%
Johannes et al (465)	2003	RA 13 HC 30 DC 53	No females, 64 years No females, 39 years No females, 49 years (Hypertensive)	I: RA (no criteria), male E: None reported	HR and BP (sympathetic) responses to mental stress	Higher resting HR in RA patients and hypertensive controls, compared to healthy Lower resting DBP in RA patients compared to hypertensive and healthy controls Higher BP (sympathetic) response to mental stress in RA patients compared to hypertensive and healthy controls	50%
Louthrenoo et al (471)	1999	RA 34	30 females, 47 years Disease duration 5.1years 15.5 swollen joints Ritchie articular index 11.6 56% RF positive ESR 35.2 mm/1st hour	I: ARA 1987 criteria E: Condition or medication affecting ANS	Ewing HR variation in response to DB, O SBP response to O	Abnormal cardiovascular tests in RA Parasympathetic dysfunction in RA patients.	61%

		HC 62	50 females, 47 years 34 age and gender match controls used in analysis			No correlation between inflammation (ESR, number of swollen joints) and ANS function	
Bekkelund et al (449)	1997	RA 43	43 females, 44 years Disease duration 13.6 years 24.1 arthritic joints Ritchie articular index 22.6 CRP 10.8mg/L ESR 23.2 mm/1st hour	I: ARA 1987 criteria, females, aged 16-55 years E: Known somatic or psychiatric disease, concomitant systemic connective tissue disease or primary neurological disease, alcoholism, atlantodental space>5mm	Ewing HR variation in response to DB, O, VM BP response to O	Normal cardiovascular tests in RA	78%
		HC 61	61 females, 42 years				
Maule et al (472)	1997	RA 17	17 females, 37 years Disease duration 9.3 years	I: ARA 1987 criteria E: Diabetes, obesity, renal failure, chronic liver disease, arrhythmia, anaemia, anti-hypertensive therapy	Ewing HR variation in response to DB, O, VM BP response to O	Normal cardiovascular tests in RA	44%
		HC 25	25 females, 32 years				
Geenen et al (456)	1996	RA 21	17 females, 56 years Disease duration 4-12months, VAS pain 26mm ESR 23 mm/1st hour	I: ARA 1987 criteria E: Any other serious disease. Controls were free from chronic pain, cardiovascular complaints or disease.	HR and BP (sympathetic) responses to mental stress	Abnormal cardiovascular tests in RA Impaired HR and BP (sympathetic) responses in RA patients No correlation between inflammation (ESR) and ANS function	67%
		HC 20	16 females, 53 years				

Piha et al (474)	1993	RA 34 HC 69 DC 76	34 females, 49 years Disease duration 15 years ARA functional class: I = 6, II = 20, III = 8 28 had arthritis in 3 or more joint areas and positive findings on hand radiographs ESR 23 mm/1st hour 69 females, 43 years 76 females, 43 years (diabetic)	I: ARA 1987 criteria, females E: Condition or medication affecting ANS.	HR variation response to DB, O, VM	Higher resting HR in RA Impaired HR variation (parasympathetic) responses to O and VM (which were statistically insignificant when age and HR used as co-variants) No correlation between inflammation (ESR) and ANS function	78%
Tan et al (478)	1993	RA 30 HC 30	27 females, 51 years Disease duration 90.2months Steinbrocker function class: II = 25, III = 5 CRP 380 mg/L ESR 61 mm/1st hour 26 females, 50 years	I: ARA 1987 criteria E: Control subjects were healthy with no symptoms or signs of neurological disease	RR interval variation at rest and in response to DB	Abnormal cardiovascular tests in 27% of RA patients Impaired parasympathetic activity (RR interval variation in response to DB)	56%
Toussirot et al (479)	1993	RA 50 HC 82	31 females, 56 years Disease duration 6 years 52% RF positive 52% inflammatory syndrome (not clearly defined) 53 females, 47 years	I: ARA 1987 criteria, patients hospitalized with a flare or for therapeutic adjustment E: Condition or medication affecting ANS	HR response to DB, O, VM	Abnormal cardiovascular tests in 60% of RA patients Impaired parasympathetic responses (HR response to VM only) in RA patients No correlation between	56%

						inflammation (inflammatory syndrome, articular damage on radiograph) and ANS function	
Leden et al (470)	1983	RA 17 HC 24	12 females, 56 years Disease duration 20 years 14 seropositive Steinbrocker's function class: II = 6, III = 8, IV = 2. All had erosions 8 females, 53 years	I: ARA 1987 criteria admitted for reconstruction joint surgery E: Respiratory disease, abnormal creatinine or proteinuria	BP response to O HR variation response to DB, O	Normal cardiovascular tests in RA patients overall Sub-group showed significant impairment in cardiovascular tests in RA patients with a high v low (7 v 10) disease severity score Impaired parasympathetic (HR variation response to DB and O) and sympathetic responses (BP response to O) found in RA patients with high disease severity score v controls	44%
Edmonds et al (454)	1979	RA 27 HC 13 HC 15 DC 13	55 years 51 years (old healthy) 25 years (young healthy) 54 years (osteoarthritis)	I: Ropes et al 1958 criteria, normotensive E: Cardiac failure, anaemia, medications affecting cardiac rhythm	Ewing HR variation response to DB, O, VM	Higher proportion of abnormal cardiovascular tests in RA Impaired parasympathetic responses in RA patients Mean ESR higher in RA patients with abnormal HR variation response to O	39%

HRV tests (n=13)							
Janse van Rensburg et al (464)	2012	RA 45 HC 39	45 females, 47 years Disease duration 4.3 years DAS28 3.3 CRP 8.6 mg/L 39 females, 45 years	I: ARA 1987 criteria, classification of global functional status = class I or II, female, aged 30-60 years, controlled disease E: Condition or medication affecting ANS.	Short term HRV Parasympathetic (pNN50%, SDNN, rMSSD, HF, SD1), sympathetic (LF, LF/HF ratio) balance at rest and in response to O	Higher resting HR in RA Lower HRV in RA Increased sympathetic tone and decreased parasympathetic activity Reduced response to O in RA	78%
Vlcek et al (483)	2012	RA 22 HC 15	22 females, 31 years Disease duration 7.4 years DAS28-CRP 3.4 CRP 7.5 mg/L 15 females, 30 years	I: ARA 1987 criteria, female, age<40years, normal BMI E: Any disease	Short term HRV Parasympathetic (HF), sympathetic (LF, LF/HF ratio) balance at rest and in response to O	Normal HRV at rest and in response to O in RA	78%
Yadav et al (484)	2012	RA 45 HC 45	39 females, 41 years 39 females, 37 years	I: ARA 1987 criteria E: Condition or medication affecting ANS	Short term HRV Parasympathetic (SDNN, SDSD, rMSSD, NN50, HF), sympathetic (LF, LF/HF) balance	Lower HRV in RA Reduced parasympathetic activity Positive correlation between inflammation (DAS28) and parasympathetic tone (SDSD only)	72%
Avsar et al (446)	2011	RA 26	18 females, 56 years	I: ARA 1987 criteria E: Condition or medication affecting	Heart rate turbulence from 24 hour holter ECG monitor at home.	Normal heart rate turbulence (parasympathetic activity	56%

		HC 26	18 females, 55 years	ANS	Parasympathetic and arterial baroreflex sensitivity	and arterial baroreflex sensitivity) in RA patients	
Aydemir et al (447)	2010	RA 36	30 females, 49 years	I: ARA 1987 criteria E: Condition or medication affecting ANS	Short term HRV Parasympathetic (HF), sympathetic (LF, LF/HF ratio) balance at rest and in response to O	Reduced sympathetic activity (LF) in RA	89%
		HC 40	31 females, 43 years				
Bruchfeld et al (451)	2010	RA 13	9 females, 52 years Disease duration 13.2years 11 RF positive DAS28-CRP 3.9	I: ARA 1987 criteria E: Smoking, diabetes mellitus	Short term HRV Parasympathetic (HF) and sympathetic (LF, LF/HF ratio) balance	Reduced parasympathetic activity (HF) in RA	61%
		HC 10	3 females, 32 years				
Milovanovic et al (473)	2010	RA 38	32 females, 56 years 25 RF positive ESR 14.3 mm/1st hour	I: ARA 1987 criteria, stable condition E: Condition or medication affecting ANS	Short term HRV Parasympathetic (pNN50%, SDRR, rMSSD, HF), sympathetic (LF, LF/HF ratio) balance Long term HRV Parasympathetic, sympathetic activity	Lower HRV in RA Reduced parasympathetic (SDNN, pNN50%, rMSSD) activity in RA	67%
		HC 41	17 females, 37 years				
Vlcek et al (482)	2008	RA 8	8 females, 31 years	I: ARA 1987 criteria E: None reported	Short term HRV Parasympathetic (HF) and sympathetic (LF, LF/HF) balance at rest and in response to O	Normal HRV at rest and in response to O in RA patients	61%
		HC 8	8 females, 31 years				

Anichkov et al (445)	2007	RA 23	23 females, 48 years Disease duration 4 years 19 RF positive DAS 4.2 ESR 24mm/1st hour Ritchie articular index 16	I: ARA 1987 criteria, female, aged 18-65 yrs, disease duration ≥12 months E: Condition or medication affecting ANS	Long term HRV Parasympathetic (SDNN, SDANN, rMSSD, SD1) activity	Lower HRV in RA patients Reduced parasympathetic activity (SDNN, SDANN, rMSSD, SD1) Negative correlation between inflammation (number of swollen joints, Ritchie articular index, DAS, leucocyte count) and HRV, parasympathetic activity (SDNN, SDANN)	88%
		HC 23	23 females, 47 years				
Goldstein et al (457)	2007	RA 13	9 females, median 52 years Disease duration 13 years, 11 RF positive DAS28 4.5 CRP 14.5mg/L	I: ARA 1987 criteria E: None reported	Short term HRV Parasympathetic (rMSSD, HF) and sympathetic balance (LF, LF/HF ratio)	Lower HRV in RA patients Reduced parasympathetic activity (rMSSD, HF) in RA patients	72%
		HC 11	6 females, median 38 years				
Kamal(467)	2007	RA 52	49 years Disease duration 8.4 years CRP 51.4 mg/L ESR 42.6 mm/1st hour	I: RA (no criteria) E: Condition or medication affecting ANS	Short term HRV. Parasympathetic activity (SDNN).	Low HRV in RA patients Reduced parasympathetic activity (SDNN) in RA patients	33%
		HC 51	46 years				

Dekkers et al (453)	2004	RA 25 HC 28	19 females, 55 years Disease duration <2years Thompson joint score 31 ESR 15 mm/1st hour 20 females, 56 years	I: ARA 1987 criteria, minimum age 18 yrs E: Any other serious disease. For healthy controls: chronic disease, chronic pain, hypertension or heart problems	ECG, impedence cardiogram Parasympathetic (respiratory sinus arrhythmia), sympathetic (pre-ejection period) activity.	Lower pre-ejection period found in RA patients (indicating higher sympathetic activity) Normal respiratory sinus arrhythmia (parasympathetic activity) in RA patients Association between inflammation (ESR, Thompson joint score) and increased sympathetic activity	72%
Evrengul et al (455)	2004	RA 42 HC 44	31 females, 48 years Disease duration 6.5 years 35 RF positive Steinbrocker's function class: I = 16, II = 18, III = 8 CRP 50.3 mg/L ESR 41.7 mm/1st hour 31 females, 45 years	I: ARA 1987 criteria, stages I-IV of Steinbrocker's functional classification E: Condition or medication affecting ANS	Short term HRV Parasympathetic (SDNN, pNN50%, rMSSD, HF), sympathetic (LF, LF/HF ratio) balance	Low HRV in RA patients Reduced parasympathetic activity (SDNN) in RA patients No correlation between inflammation (ESR) and HRV parameters	89%
Biomarkers (n=5)							
Kopec-Medrek et al (468)	2012	RA 16	16 females, post-menopausal	I: RA (no criteria) treated with infliximab (TNF alpha inhibitor), post menopausal females, active disease and not received	Plasma NPY (sympathetic activity)	Plasma NPY (sympathetic activity) was higher in RA patients Positive correlation between inflammation	67%

		HC 16	16 females, post-menopausal Age and BMI matched	remission after treatment with at least two DMARDs E: HRT, smoking, conditions known to affect ANS		(CRP, DAS28) and plasma NPY (sympathetic activity)	
Capellino et al (452)	2008	RA 24	14 females, 58 years	I: ARA 1987 criteria E: None reported	Serum chromogranin A (sympathetic activity)	Serum chromogranin A (sympathetic activity) was higher in RA patients	50%
		HC 37	26 females, 38 years				
Vlcek et al (482)	2008	RA 8	8 females, 31 years	I: ARA 1987 criteria E: None reported	Plasma NPY (sympathetic activity) at rest and in response to O.	Normal plasma NPY (sympathetic activity) in RA patients	61%
		HC 8	8 females, 31 years				
Harle et al (460)	2006	RA 62	52 females, 58 years Disease duration 9.7 years 9 tender joints 7.5 swollen joints ESR 27.7 mm/1st hour.	I: ARA 1987 criteria, fertile women were not taking contraceptives and tested in the early to mid-follicular phase of the menstrual cycle E: None reported	Serum NPY (sympathetic activity)	Higher NPY found only in RA patients with previous prednisolone use	67%
		HC 23	12 females, 52 years				
Grimsholm et al (459)	2005	RA 7	51 years (early RA) Disease duration <1 year	I: ARA 1987 criteria E: None reported	Serum NPY (sympathetic activity)	NPY higher in long-standing RA patients but not statistically significant NPY in early RA patients comparable to healthy controls	28%
		RA 28	59 years (long-standing RA) Disease duration >1year				
		HC 11	39 years Note: 25/35 female RA patients				

Skin sympathetic responses (n=5)							
Gozke et al (458)	2003	RA 10	10 females, 49 years	I: ARA 1987 criteria E: Symptoms of clinical ANS dysfunction	Sympathetic skin responses to nerve stimulation	Normal sympathetic skin responses in RA	39%
		HC 14	14 females, 45 years				
Johannes et al (465)	2003	RA 13	No females, 64 years	I: RA (clinical diagnosis), male E: None reported	Skin temperature and conductance responses to mental stress	Sympathetic skin responses to mental stress higher in RA patients	50%
		HC 30	No females, 39 years				
		DC 53	No females, 49 years (hypertensive)				
Geenen et al (456)	1996	RA 21	17 females, 56 years Disease duration 4-12months VAS pain 26mm ESR 23 mm/1st hour	I: ARA 1987 criteria E: Any other serious disease. Controls were free from chronic pain, cardiovascular complaints or disease	Sympathetic skin conductance to mental stress	Normal resting skin conductance in RA patients Reduced sympathetic skin responses to mental stress	67%
		HC 20	16 females, 53 years				
Jolliffe et al (466)	1995	RA 40	57 years	I: ARA 1987 criteria E: Diabetes mellitus, vasoactive medication, skin conditions affecting the wrist	Sympathetic skin responses to intra-dermal nicotine	Normal sympathetic skin responses in RA patients	44%
		HC 46	57 years				
Tan et al (478)	1993	RA 30	27 females, 51 years Disease duration 90.2months Steinbrocker function class: II = 25, III = 5 ESR 61 mm/1st hour CRP 380 mg/L	I: ARA 1987 criteria E: Control subjects were healthy with no symptoms or signs of neurological disease	Sympathetic skin responses to nerve stimulation	Normal sympathetic skin responses in RA	56%

		HC 30	26 females, 50 yrs				
Catecholamines (n=4)							
Vlcek et al (483)	2012	RA 22	22 females, 31 years Disease duration 7.4 years DAS28-CRP 3.4 CRP 7.5 mg/L	I: ARA 1987 criteria, female, age<40yrs, normal BMI E: Any disease	Plasma EPI and NE (sympathetic activity) at rest and in response to O	Normal EPI and NE (sympathetic activity) at rest and in response to O in RA	78%
		HC 15	15 females, 30 years				
Vlcek et al (482)	2008	RA 8	8 females, 31 years	I: ARA 1987 criteria E: None reported	Plasma EPI and NE (sympathetic activity) at rest and in response to O	Baseline plasma NE (sympathetic activity) was higher in RA patients There was a trend for reduced plasma NE (sympathetic activity) in RA patients	61%
		HC 8	8 females, 31 years				
Imrich et al (463)	2005	RA 15	15 females, 41 years Disease duration 8.2 years CRP 15.4mg/L ESR 20.3 mm/1st hour	I: ARA 1987 criteria, female E: Diabetes, impaired glucose tolerance	Serum EPI and NE (sympathetic activity) at rest and in response to insulin-induced hypoglycaemia	Basal and cumulative levels of EPI were reduced (but not statistically significantly) in RA patients Basal levels of NE were normal in RA patients, but cumulative levels were	67%

		HC 14	14 females, 44 years			reduced (reduced basal sympathetic activity). Serum EPI response to insulin-induced hypoglycaemia was normal in RA however serum NE response was reduced in RA (impaired sympathetic response)	
Igari et al (462)	1977	RA 22	20 females, 45 yrs ESR 44.8mm/1st hr, Steinbrocker class 2.5	I: Ropes et al 1958 criteria for classical or definite RA E: None reported	24 hour urinary adrenaline and noradrenaline (sympathetic activity)	Baseline 24 hour urinary adrenaline was reduced in RA patients	44%
		HC 6	2 females, 33 years				
Arterial baroreflex sensitivity (n=2)							
Avsar et al (446)	2011	RA 26	18 females, 56 years	I: ARA 1987 criteria E: Condition or medication affecting ANS	Heart rate turbulence from 24 hour holter ECG monitor at home Parasympathetic and arterial baroreflex sensitivity	Normal heart rate turbulence (parasympathetic activity and arterial baroreflex sensitivity) in RA patients	56%
		HC 26	18 females, 55 years				
Aydemir et al (447)	2010	RA 36	30 females, 49 years	I: ARA 1987 criteria E: Condition or medication affecting ANS	Sequence method (arterial baroreflex sensitivity) at rest and in response to O	Reduced arterial baroreflex sensitivity at rest in RA patients	89%
		HC 40	31 females, 43 years				
Pupillary light reflex (n=1)							
Barendregt et al (448)	1996	RA 18	18 females, 64 years (with ocular dryness)	I: ARA 1987 criteria, with or without dryness of eyes or mouth	Pupillary light reflexes: constriction latency and maximum constriction	Parasympathetic dysfunction (prolonged constriction latency and	61%

	RA 18 HC 33	18 females, 59 years (without ocular dryness) 33 females, 56 years	E: Condition or medication known to affect ANS	velocity (parasympathetic activity)	elevated maximum constriction velocity) found in RA patients with ocular dryness

Mean values given unless otherwise indicated.

ANS = autonomic nervous system, ARA 1987 criteria = American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis,(8) BP = blood pressure, BMI = body mass index, CP = cold pressor test, CRP = C reactive protein, DAS28 = disease activity score 28, DB = deep breathing, DBP = diastolic blood pressure, DC = disease controls, DMARD = disease modifying anti-rheumatic drug, E = exclusion, ECG = electrocardiogram, EPI = epinephrine, ESR = erythrocyte sedimentation rate, HC = healthy controls, HF = high frequency power in the range 0.15-0.40 Hz, HG = handgrip, HR = heart rate, HRT = hormone replacement therapy, HRV = heart rate variability, I = inclusion, LF = low frequency power in the range 0.04-0.15Hz, LF/HF ratio = low frequency to high frequency ratio, N = number of subjects, NE = norepinephrine, NN = inter-beat interval, NN50 = number of pairs of adjacent NN intervals differing by more than 50 milliseconds in the entire recording, NPY = neuropetide Y, O = orthostasis, pNN50% = NN50 as a percentage of the total number of all NN intervals, QIS = quality index score (%), RA = rheumatoid arthritis, RF = rheumatoid factor antibody, rMSSD = square root of the mean of the sum of the squares of difference between adjacent NN intervals, Ropes et al 1958 criteria = 1958 Revision of diagnostic criteria for rheumatoid arthritis,(596) SBP = systolic blood pressure, SD1 = standard deviation of the Poincare plot, SDANN = standard deviation of the averages of NN intervals in all 5 minute segments of the entire recording, SDNN = standard deviation of all NN intervals, SDSD = standard deviation of differences between adjacent NN intervals, TNF = tumour necrosis factor, VAS = Visual Analogue score, VM = Valsalva's manoeuvre

Appendix 3 Characteristics of studies included in the review (cohort and interventional studies)

B. Cohort and interventional studies

Study	Year	N	Characteristics	Inclusion Exclusion	Assessment	Key findings	QIS
Interventional studies (n=3)							
Kopec-Medrek et al (468)	2012	RA 16	16 females, post-menopausal	I: RA (no criteria) treated with infliximab (TNF alpha inhibitor), post menopausal females, active disease and not received remission after treatment with at least two DMARDs E: HRT, smoking, conditions known to affect ANS	Plasma NPY (sympathetic activity) at week 0, 2, 14, 54 and 62.	Plasma NPY (sympathetic activity) was higher in RA patients at baseline and with infliximab infusion. Positive correlation between inflammation (CRP, DAS28) and plasma NPY (sympathetic activity)	67%
		HC 16	16 females, post-menopausal Age and BMI matched Cross-sectional, case-control, observational study with longitudinal interventional component. Intervention: TNF alpha inhibitor therapy (infliximab) in 16 RA patients. 1 year follow up				

Harle et al (460)	2006	RA 62	52 females, 58 years Disease duration 9.7 years 9 tender joints 7.5 swollen joints ESR 27.7 mm/1st hour	I: ARA 1987 criteria, fertile women were not taking contraceptives and tested in the early to mid-follicular phase of the menstrual cycle E: None reported	Serum NPY (sympathetic activity) at week 0 and 12	Higher serum NPY found only in RA patients with previous prednisolone use TNF alpha inhibitor therapy had no effect on serum NPY levels, despite a good clinical response	67%
		HC 23	12 females, 52 years Cross-sectional, case-control, observational study with longitudinal interventional component Intervention: TNF alpha inhibitor therapy (adalimumab) in 32 RA patients Follow up 12 weeks post therapy				
Igari et al (462)	1977	RA 22	20 females, 45 years ESR 44.8 mm/1st hour Steinbrocker class 2.5	I: ARA 1987 criteria for classical or definite RA E: None reported	24 hour urinary adrenaline and noradrenaline (sympathetic activity) before and after synovectomy	Baseline 24 hour urinary adrenaline was reduced in RA patients 24 hour urinary adrenaline and noradrenaline significantly decreased two weeks after synovectomy in RA patients	44%
		HC 6	2 females, 33 years Cross-sectional, case-control, observational study with longitudinal interventional component Intervention: synovectomy performed in 6 RA patients				

Cohort studies (n=3)							
Holman et al (461)	2008	ALL 33 RA 25	RA 25, Psoriatic arthritis 8 Disease duration 7.6 years 14 RF positive Baseline DAS28 4.9 Remission DAS28 2.0 Prospective, double-blind, exploratory study to investigate HRV as a predictor of TNF alpha inhibitor therapy in patients with inflammatory arthritis.	I: Inflammatory arthritis including 25 RA (no criteria) undergoing TNF alpha inhibitor therapy.	Short term HRV. Parasympathetic (HF), sympathetic (LF) and overall HRV (total power).	Low HRV (total power), low parasympathetic (HF) and high sympathetic function (LF) was predictive of poor response to TNF alpha inhibitor therapy. No correlation between baseline autonomic function (HRV parameters) and change in DAS28 score.	56%
Schwemmer et al (476)	2006	RA 30	17 females, 52 years Disease duration 6.7 years 9 swollen joints 9 tender joints 63% RF positive CRP 31 mg/L ESR 30.2 mm/1st hour Prospective, cohort study with longitudinal survival Follow-up: 8 years	I: ARA 1987 criteria E: Condition or medication affecting ANS	Clinical cardiovascular tests (Ziegler et al 1992) HR variation at rest and responses to DB, O, VM SBP responses to O Pupillary light reflex: latency time, area in darkness	Cardiac and pupillary ANS dysfunction in 60% of RA patients 3 of 4 deaths were due to cardiac causes Non-survivors had higher HR variation response to DB, but lower HR variation to O	61%
van Middendorp et al (480)	2005	RA 60	38 females, 59 years Disease duration 13 years Thompson joint score 21 ESR 16 mm/1st hour Cross-sectional, cohort, observational study	I: RA (no criteria) E: Receiving glucocorticoid therapy	24 hour urinary noradrenaline excretion (sympathetic activity)	No correlation found between sympathetic activity and inflammation (ESR or IL-6)	56%

Other studies (n=1)

Lazzerini et al (469)	2008	RA 20	Disease duration 10.4 years 16 erosive disease CRP 4.8 mg/L ESR 22.9 mm/1st hour Randomized, placebo-controlled, single-blind cross-over to investigate the arrhythmia risk during acute infliximab therapy in patients with chronic arthritis	I: RA (ARA 1987 criteria) or Spondyloarthritis E: coronary artery disease, no alterations in cardiac enzymes or serum electrolytes, ECG or echocardiographic abnormalities	Short and long term HRV Parasympathetic (rMSSD, pNN50%, SDNN, SDANN, HF power), sympathetic (LF, LF/HF ratio) activity and overall HRV (total power) during infliximab and placebo infusions (2 hour recordings)	TNF alpha inhibitor therapy (infliximab) acutely reduced HRV (total power) and sympathetic activity (LF, LF/HF) Patients who developed new-onset arrhythmia had reduced HRV (total power) and parasympathetic activity (rMSSD, pNN50%, HF), reduced sympathetic activity (LF) and tended to have a higher CRP	61%

Mean values given unless otherwise indicated.

ANS = autonomic nervous system, ARA 1987 criteria = American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis,(8) BMI = body mass index, CRP = C reactive protein, DAS28 = disease activity score 28, DB = deep breathing, DMARD = disease modifying anti-rheumatic drug, E = exclusion, ESR = erythrocyte sedimentation rate, HC = healthy controls, HF = high frequency power in the range 0.15-0.40 Hz, HR = heart rate, HRT = hormone replacement therapy, HRV = heart rate variability, I = inclusion, IL-6 = interleukin-6, LF = low frequency power in the range 0.04-0.15Hz, LF/HF ratio = low frequency to high frequency ratio, N = number of subjects, NN= inter-beat interval, NPY = neuropetide Y, O = orthostasis, pNN50% = NN50 as a percentage of the total number of all NN intervals, QIS = quality index score (%), RA = rheumatoid arthritis, RF = rheumatoid factor antibody, rMSSD = square root of the mean of the sum of the squares of difference between adjacent NN intervals, SBP = systolic blood pressure, SDANN = standard deviation of the averages of NN intervals in all 5 minute segments of the entire recording, SDNN = standard deviation of all NN intervals, TNF = tumour necrosis factor, VM = Valsalva's manoeuvre

Appendix 4 Prevalence of ANS dysfunction in RA

Study	N	Criteria for ANS dysfunction	Prevalence (%)
Aydemir et al. 2010	36	Ewing test.(415) Two of five abnormal tests from:	61
		HR response to Valsalva's manoeuvre (Valsalva ratio≤1.1)	
		HR variation during deep breathing (inter-beat interval maximum-minimum ≤10)	
		HR response to standing (30:15 ratio≤1.0)	
		BP response to standing (fall in systolic BP≥20)	
		BP response to handgrip (diastolic BP rise ≤10mmHg)	
		Modified (by authors) Ewing test.(447) Two abnormal and one borderline from:	75
		Ewing test + inspiration/expiration heart rate ratio ≤1	
		BP response to orthostasis (fall in diastolic BP≥10mmHg)	
Bidikar et al. 2010	50	Fall in systolic BP in response to orthostasis ≥10mmHg	44
Milovanovic et al. 2010	50	Two of three positive tests from:	86
		BP response to orthostasis	
		HR response to deep breathing	
		HR response to orthostasis	
Stojanovic et al. 2007	39	Two of three positive tests from:	74
		BP response to orthostasis	
		BP response to handgrip	
		HR response to deep breathing	
		HR response to orthostasis	
		HR response to Valsava's manoeuvre	
		Moderate to severe autonomic nervous system (ANS) dysfunction: Ewing score≥4	
Schwemmer et al. 2006	30	Ewing test (result below 5th percentile)	43
		Two of five abnormal (below 5th centile from normal healthy control subjects) tests from:	20
		RRI variation at rest	
		RRI variation difference between deep breathing and rest	
		RRI variation difference between deep breathing and rest	
		Valsalva's manoeuvre (RRI maximum/RRI minimum)	
		HR response to orthostasis, BP fall ≥25mmHg	
		One of two abnormal (below 5th centile from normal healthy control subjects) tests from:	50
		Latency time of pupillary reflex	
		Maximal pupillary area	
		Cardiovascular and pupillary dysfunction (both of the above abnormal)	60
Gozke et al. 2003	10	Inter-beat interval (RRI) variation difference between deep breathing and rest	50
		RRI variation ratio of deep breathing to rest	80

Geenen et al. 1996	13	Lower mean response to cognitive discrimination than the least responding control	38
Tousirrot et al. 1993	50	Two of three abnormal tests from: HR response to deep breathing HR response to orthostasis HR response to Valsalva's manoeuvre	60
Edmonds et al. 1979	27	HR response to orthostasis, RRI ratio<1	33

N = number of RA patients. Prevalence (%) values given are means either quoted or calculated from the study.
ANS = autonomic nervous system

Appendix 5 Associations between inflammation and autonomic function

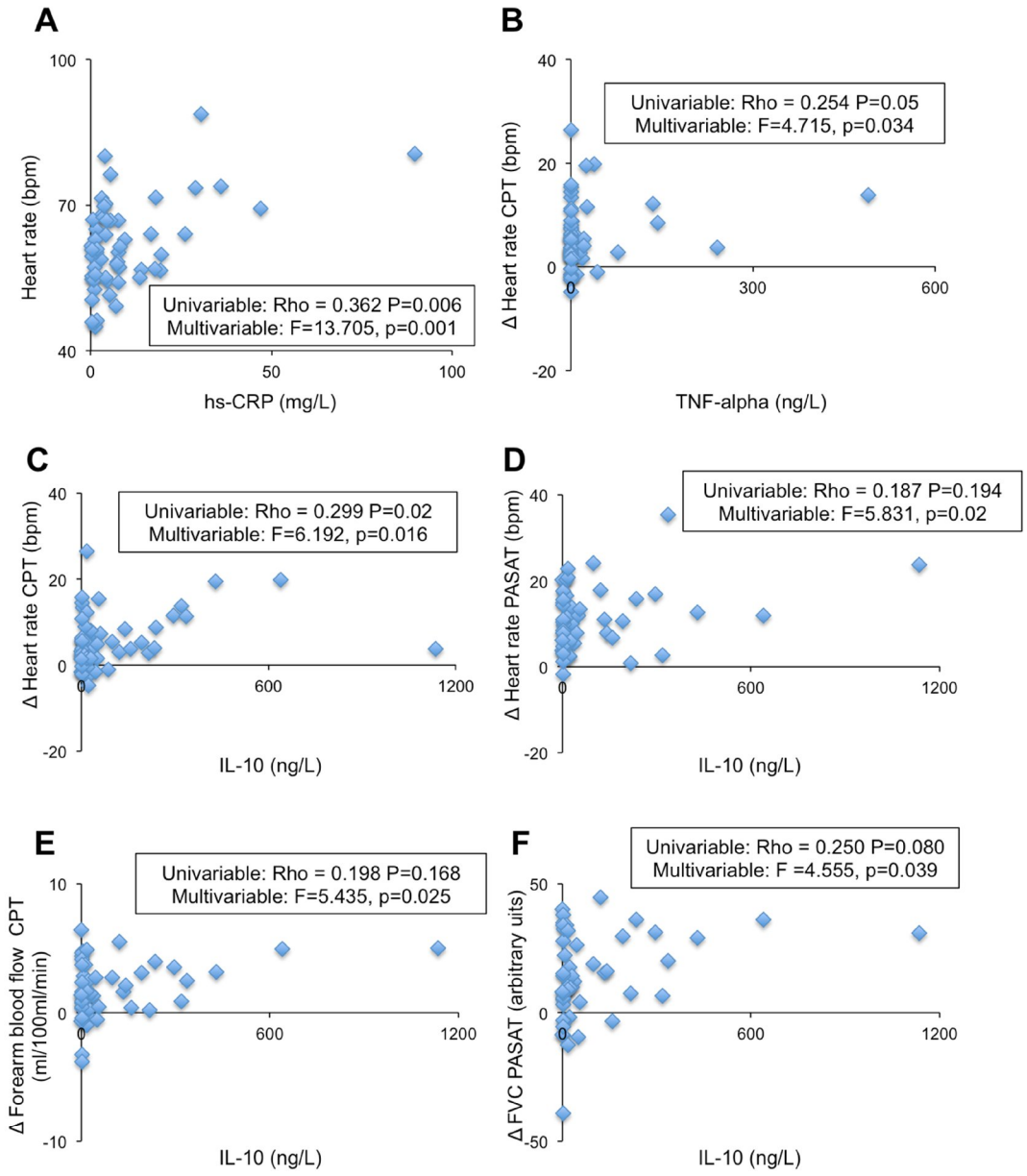
	Pain VAS	hs-CRP	IL-6	TNF-α	IL-10
hs-CRP	0.586 * <0.001 57	-	-	-	-
IL-6	0.519 * <0.001 62	0.339 * 0.010 57	-	-	-
TNF-α	0.306 * 0.016 62	0.010 0.939 57	0.753 * <0.001 62	-	-
IL-10	0.114 0.378 62	-0.068 0.616 57	0.537 * <0.001 62	0.612 * <0.001 62	-
HR	0.464 * <0.001 63	0.362 * 0.006 57	0.339 * 0.010 57	0.059 0.646 57	-0.068 0.616 57
Mean BP	0.224 0.077 63	0.253 0.057 57	0.073 0.572 62	0.025 0.844 62	-0.138 0.286 62
MSNA burst frequency	0.238 0.150 38	0.418 * 0.011 36	0.266 0.107 38	0.039 0.816 38	-0.044 0.794 38
MSNA burst incidence	-0.032 0.849 38	0.193 0.260 36	0.039 0.815 38	-0.033 0.844 38	-0.109 0.516 38
G_{MOT}	-0.506 * <0.001 55	-0.332 * 0.019 50	-0.408 * 0.002 55	-0.322 * 0.016 55	-0.080 0.561 55
G_{SEQ}	-0.318 * 0.011 63	-0.338 * 0.010 57	-0.132 0.306 62	-0.024 0.853 62	-0.087 0.500 62

	Pain VAS	hs-CRP	IL-6	TNF-α	IL-10
rMSSD	**-0.437 ***	**-0.420 ***	**-0.258 ***	-0.137	0.018
	<0.001	**0.001**	**0.043**	0.289	0.891
	63	**57**	**62**	62	62
pNN50	**-0.419 ***	**-0.430 ***	-0.226	-0.074	0.040
	0.001	**0.001**	0.077	0.568	0.759
	63	**57**	62	62	62
LF power	**-0.367 ***	**-0.371 ***	**-0.270 ***	-0.224	-0.098
	0.003	**0.004**	**0.034**	0.080	0.451
	63	**57**	**62**	62	62
HF power	**-0.371 ***	**-0.348 ***	-0.205	-0.138	-0.098
	0.003	**0.008**	0.110	0.285	0.451
	63	**57**	62	62	62
LF/HF ratio	0.126	0.112	-0.060	-0.168	**-0.262 ***
	0.325	0.407	0.643	0.192	**0.040**
	63	57	62	62	**62**
SD1	**-0.437 ***	**-0.420 ***	-0.258 *	-0.137	0.018
	<0.001	**0.001**	0.043	0.289	0.891
	63	**57**	62	62	62
SD2	**-0.390 ***	**-0.344 ***	**-0.313 ***	-0.192	-0.055
	0.002	**0.009**	**0.013**	0.134	0.670
	63	**57**	**62**	62	62
Systolic BP response to CPT	-0.057	-0.036	0.081	0.185	0.112
	0.664	0.793	0.536	0.156	0.395
	60	55	60	60	60
Diastolic BP response to CPT	-0.103	-0.023	0.143	**0.245**	0.212
	0.432	0.865	0.277	**0.059 (trend)**	0.103
	60	55	60	**60**	60
Mean BP response to CPT	-0.093	0.017	0.139	**0.232**	0.179
	0.479	0.900	0.289	**0.074 (trend)**	0.171
	60	55	60	**53**	60
HR response to CPT	-0.100	-0.147	0.113	**0.254 ***	**0.299 ***
	0.447	0.285	0.388	**0.050**	**0.020**
	60	55	60	**60**	**60**

	Pain VAS	hs-CRP	IL-6	TNF-α	IL-10
Leg blood flow response to CPT	-0.202 0.129 58	-0.022 0.874 55	-0.086 0.520 60	-0.0574 0.670 58	-0.118 0.380 58
LVC response to CPT	-0.100 0.447 60	-0.086 0.543 55	-0.082 0.543 60	-0.040 0.765 58	-0.142 0.287 58
Systolic BP response to PASAT	-0.120 0.408 50	0.018 0.905 46	**-0.287 *** **0.044** **50**	-0.107 0.461 50	-0.141 0.328 50
Diastolic BP response to PASAT	-0.120 0.408 50	0.017 0.912 46	-0.177 0.220 50	-0.056 0.699 50	-0.145 0.315 50
Mean BP response to PASAT	-0.084 0.561 50	0.010 0.949 46	-0.151 0.296 50	-0.017 0.909 50	-0.088 0.541 50
HR response to PASAT	**-0.244** **0.088 (trend)** **50**	-0.195 0.193 46	-0.133 0.355 50	-0.009 0.950 50	0.187 0.194 50
Leg blood flow response to PASAT	0.171 0.240 49	**0.287** **0.056 (trend)** **45**	0.161 0.268 49	0.104 0.475 49	0.061 0.678 49
LVC response to PASAT	0.179 0.218 49	**0.246** **0.103 (trend)** **45**	**0.237** **0.101 (trend)** **49**	0.159 0.274 49	0.121 0.406 50
Forearm blood flow response to PASAT	-0.113 0.435 50	0.035 0.816 46	0.081 0.577 50	0.148 0.305 50	0.198 0.168 50
FVC response to PASAT	-0.124 0.392 50	0.002 0.991 46	0.104 0.473 50	0.160 0.267 50	**0.250** **0.080 (trend)** **50**

Spearman's correlation. Values expressed are Spearman's rho, P value and N. * Significance P≤0.05.

Appendix 6 Scatter plots demonstrating significant associations between inflammation and autonomic function



Scatter plots demonstrating significant associations between ANS function and inflammatory markers including: hs-CRP and resting HR (Panel A); TNF- α and HR response to CPT (Panel B); and IL-10 and HR response to CPT (Panel C), HR response to PASAT (Panel D), forearm blood flow response to CPT (Panel E) and FVC response to PASAT (Panel F).

Appendix 7 Correlations between QT, QTc, HR, age, CRP, ESR and cytokines

	HR *	Age	CRP	ESR	IL-6	TNF-α	IL-1α	IL-1β	IL-10
QT	**-0.807**	0.038	-0.126	**-0.205**	-0.057	0.099	0.113	0.132	0.108
	<0.001	0.694	0.185	**0.030**	0.552	0.299	0.235	0.165	0.257
	112	112	112	**112**	112	112	112	112	112
QT_{BAZ}	**0.517**	**0.293**	**0.202**	0.134	**0.222**	**0.199**	0.158	**0.210**	**0.333**
	<0.001	**0.002**	**0.033**	0.158	**0.016**	**0.036**	0.097	**0.026**	**<0.001**
	112	**112**	**112**	112	**112**	**112**	112	**112**	**112**
QT_{FHS}	-0.041	**0.244**	0.088	-0.010	0.129	0.180	0.180	**0.220**	**0.294**
	0.668	**0.009**	0.356	0.917	0.175	0.057	0.058	**0.020**	**0.002**
	112	**112**	112	112	112	112	112	**112**	**112**
HR			**0.251**	**0.248**	0.175	0.034	-0.031	0.004	0.109
			0.008	**0.008**	0.064	0.724	0.744	0.744	0.251
			112	**112**	112	112	112	112	112
CRP				**0.568**	0.185	0.021	-0.004	0.088	0.0083
				<0.001	0.0051	0.830	0.965	0.356	0.387
				112	112	112	112	112	112
ESR					**0.323**	0.153	0.144	**0.196***	0.121
					0.001	0.106	0.129	**0.038**	0.203
					112	112	112	**112**	112

IL-6	**0.300**	**0.497**	**0.511**	**0.205**
	0.001	**<0.001**	**<0.001**	**0.030**
	112	**112**	**112**	**112**
TNF-α		**0.784**	**0.767**	**0.775**
		<0.001	**<0.001**	**<0.001**
		112	**112**	**112**
IL-1α			**0.917**	**0.853**
			<0.001	**<0.001**
			112	**112**
IL-1β				**0.902**
				<0.001
				112

Values reported are correlation coefficient (Spearman's rank rho or * Pearson product r), P Value and N. Significance = P<0.05, P<0.01, P<0.001 shown in bold.

CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, HR = heart rate, IL=interleukin, QT_{BAZ} = QT corrected using Bazett's formula, QT_{FHS} = QT corrected using Framingham Heart Study formula, TNF=tumour necrosis factor.

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