WHY ARE PATIENTS WITH ANCA-ASSOCIATED VASCULITIS FATIGUED?

by

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A thesis submitted to the
University of Birmingham
for the degree of
DOCTOR OF MEDICINE

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January 2016

ABSTRACT

Objective: To assess the severity and predictors of fatigue in ANCA-associated vasculitis (AAV), and the contribution of peripheral and central mechanisms.

Methods: Fatigue, anxiety/depression, sleep quality and pain were measured in 152 patients with AAV, 68 patients with CKD, and 71 healthy controls. Muscle mass, strength and endurance, cardio-respiratory fitness, perception of exertion, high-sensitivity C-reactive protein (hsCRP), and dehydroepiandrosterone (DHEA) were measured in 48 patients with AAV and 41 healthy controls.

Results: Fatigue in AAV was more severe than in CKD ($p=0.013$) or controls ($p<0.001$), and correlated with anxiety/depression, sleep quality and pain (all $p<0.001$). There was no difference in muscle mass ($p=0.979$) or strength ($p=0.315$) between AAV and controls, but muscle endurance time was shorter in AAV ($p=0.006$), with greater muscle reserve ($p=0.038$) indicating central activation failure. Perception of exertion ($p=0.006$) and cardio-respiratory fitness ($p=0.029$) were worse in AAV than controls. Only perception of exertion independently predicted AAV fatigue ($p=0.01$). Sleep disturbance predicted altered perception of exertion ($p=0.017$). hsCRP was higher ($p=0.011$) and DHEAS levels were lower ($p<0.001$) in AAV than controls, but neither predicted fatigue.

Conclusion: Fatigue in AAV is more severe than in CKD or health, is due to central mechanisms, and may be amenable to intervention.

DEDICATION

To the memory of my father, Sean McClean.

ACKNOWLEDGMENTS

The work presented in this thesis would not have been possible without the contributions of many people. First and foremost, I would like to thank my supervisors, Prof Lorraine Harper and Dr Jos Bosch, without whose support, guidance and wisdom this project could never have been completed. A special thank you also goes to Prof David Jones, who gave huge amounts of personal support in addition to his invaluable scientific advice.

I am grateful to Dr Matthew Morgan for his advice on statistics and scientific writing, and to Dr Neil Basu at the University of Aberdeen, particularly for his collaboration, help and advice with the questionnaire study. Thanks also go to Dr Richard Borrows, Winnie Chan, Dr Angela Taylor, Prof Wiebke Arlt, Dr Alison Whitelegg, Prof Mark Drayson, Dr Peter Nightingale and everyone else at the University of Birmingham who made vital contributions to the work in this thesis.

Thank you to Theresa Brady, Golaleh Didarzadeh, Richard Martin, and all the other nurses at the Wellcome Trust CRF, for their help with running the study.

This work could not have been done without the selfless participation of everyone who volunteered to take part in this study, or the support of John and Susan Mills, and everyone else at Vasculitis UK.

Lastly, but by no means least, I would like to thank my wife Sinead for her love, support and encouragement through all of the ups and downs, my children Ruairi, Niamh and Eoghan for never failing to bring a smile to my face, and my mum Anne for her love and support too.

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ABBREVIATIONS

AAV	ANCA-Associated Vasculitis
ACTH	Adrenocorticotropic Hormone
ADLs	Activities of Daily Living
ANCA	Anti-Neutrophil Cytoplasmic Antibodies
AS	Ankylosing Spondylitis
ATP	Adenosine Triphosphate
BBB	Blood-Brain Barrier
BMI	Body Mass Index
BVAS	Birmingham Vasculitis Activity Score
CAF	Central Activation Failure
c-ANCA	Cytoplasmic Anti-Neutrophil Cytoplasmic Antibodies
CBT	Cognitive Behavioural Therapy
CFS	Chronic Fatigue Syndrome
CHCC	Chapel Hill Consensus Conference
CKD	Chronic Kidney Disease
CNS	Central Nervous System
CRF	Cancer-Related Fatigue
cPR3	Complimentary Proteinase 3
CRH	Corticotrophin-Releasing Hormone
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid

CSS	Churg-Strauss Syndrome
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
DEXA	Dual Energy X-ray Absorptiometry
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone Sulphate
DNA	Deoxyribonucleic Acid
EAV	Experimental Autoimmune Vasculitis
EGPA	Eosinophilic Granulomatosis with Polyangiitis
ELISA	Enzyme-Linked Immunosorbent Assay
ENT	Ear, Nose and Throat
ES	Electrical Stimulation
FBC	Full Blood Count
FSS	Fatigue Severity Scale
FMS	Fibromyalgia Syndrome
GET	Graded Exercise Therapy
GPA	Granulomatosis with Polyangiitis
HADS	Hospital Anxiety and Depression Scale
HR	Heart Rate
HRQOL	Health-Related Quality of Life
hsCRP	High-Sensitivity C-reactive Protein
IFN-γ	Interferon Gamma
Ig	Immunoglobulin (e.g. IgG = Immunoglobulin G)
IL	Interleukin (e.g. IL-8 = Interleukin 8)

IQR	Interquartile Range
IV	Intravenous
KDIGO	Kidney Disease: Improving Global Outcomes
MCS	Mental Component Score
MFI-20	Multidimensional Fatigue Inventory
MHC	Major Histocompatibility Complex
MOS	Medical Outcomes Study
MPA	Microscopic Polyangiitis
MPO	Myeloperoxidase
mRNA	Messenger Ribonucleic Acid
MS	Multiple Sclerosis
MVC	Maximal Voluntary Contraction
NCCN	National Comprehensive Cancer Network
p-ANCA	Perinuclear Anti-Neutrophil Cytoplasmic Antibodies
PASAT	Paced Auditory Serial Addition Test
PBC	Primary Biliary Cirrhosis
PCS	Physical Component Score
PD	Parkinson’s Disease
PFS	Revised Piper Fatigue Scale
PR3	Proteinase 3
PROM	Patient-Reported Outcome Measure
PSQI	Pittsburgh Sleep Quality Index
PSS	Primary Sjögren's Syndrome

RA	Rheumatoid Arthritis
RCT	Randomized Controlled Trial
RPGN	Rapidly Progressive Glomerulonephritis
RPE	Rating of Perceived Exertion
SF-36	Short-Form (36) Health Survey
SLE	Systemic Lupus Erythematosus
SSRI	Selective Serotonin Reuptake Inhibitor
TMC	Theoretical Maximal Contraction
TNF-α	Tumour Necrosis Factor Alpha
U&E	Urea and Electrolytes
VCAM-1	Vascular Cell Adhesion Molecule 1
VDI	Vasculitis Damage Index
VMA	Voluntary Muscle Activation
$\dot{V}O_2max$	Maximum rate of oxygen uptake

1 INTRODUCTION AND LITERATURE REVIEW

1.1 ANCA-Associated Vasculitis

1.1.1 The Classification of Vasculitis

Vasculitis comprises a group of diseases which are all characterised by necrotising inflammation of blood vessel walls, with the endothelium as the primary target. However, the diseases included under this umbrella term vary widely in immunopathogenic mechanisms, clinical presentation and pathology. In 1994, the 'Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis' (CHCC) (1) noted that different names were being used to describe the same disease, and that the same name was being used for different diseases. They attempted to standardise the diagnostic terms used to classify vasculitis; their conclusions still form the basis of how vasculitis is described today, and their definitions were revised in 2012 and published the following year (2). They divided the vasculitides according to the smallest blood vessels affected into large, medium and small vessel vasculitis, and noted the clinical utility of subdividing the small vessel vasculitides into those associated with the presence of anti-neutrophil cytoplasmic antibody (ANCA), and those not. The different types of ANCA-associated vasculitis (AAV) which they described were: Wegener's granulomatosis, which is now called 'granulomatosis with polyangiitis' (GPA); microscopic polyangiitis (MPA); and Churg-Strauss syndrome, which today is called 'eosinophilic granulomatosis with polyangiitis' (EGPA). In 2007 the European Medicines Agency developed an algorithm designed to help in the classification of the different types of AAV, and although they used different definitions which they felt were more specific, they

kept the same names used by CHCC (3). A further refinement of the classification which includes ANCA specificity (described below) has been proposed more recently (4).

1.1.2 What are ANCA?

ANCA are IgG autoantibodies directed against specific lysosomal constituents of neutrophils, first identified as recently as 1982 in a group of patients with segmental necrotising glomerulonephritis (5). The investigators discovered what is now known as c-ANCA, that is, ANCA that results in diffuse cytoplasmic staining of ethanol-fixed neutrophils on indirect immunofluorescence. In 1988, Falk and Jennette identified that there were two different patterns of staining: in addition to c-ANCA, they described ANCA showing an artefactual perinuclear staining pattern now referred to as p-ANCA. Using the technique of enzyme-linked immunosorbent assay (ELISA), they discovered that p-ANCA is usually directed against the antigen myeloperoxidase (MPO) (6). We now also know that c-ANCA is normally directed against proteinase 3 (PR3).

MPO is a 150kDa chloride peroxidase, which in health is used by the neutrophil to kill phagocytosed bacteria; during the 'respiratory burst', it catalyses the formation of hypochlorous acid from hydrogen peroxide and chloride ions. PR3 is a 29kDa serine protease with a host of functions, including modulation of inflammatory mediators (for example, the cleavage of IL-8) (7). MPO and PR3 are both lysosomal enzymes, and are most abundant in the cytoplasmic primary granules of neutrophils and monocytes. Despite being present in the cytoplasm, MPO is not normally found on the cell membranes of resting neutrophils, but rather only on the membranes of neutrophils that have been 'primed', as will be discussed later. On the other hand, PR3 membrane expression is bimodal on resting neutrophils – i.e., resting neutrophils may be divided into subsets with either low or high membrane expression

even in healthy subjects. PR3 membrane expression patterns are stable for an individual, and twin studies suggest the level of membrane expression is genetically determined (8).

The association between ANCA and AAV is strong, with antibodies against PR3 found in 66% of patients with GPA, and antibodies against MPO being found in 58% of patients with MPA in one large study, where the specificity related to disease controls was 87% for anti-PR3 and 91% for anti-MPO (9). However, around 10% of people with AAV will have no detectable ANCA in their sera by either indirect immunofluorescence or ELISA. Also, ANCA has been found in the sera of patients with other autoimmune diseases, inflammatory bowel disease, and infections such as tuberculosis, although in those diseases the ANCA is not usually directed against MPO or PR3. The positive and negative predictive values of ANCA results are very dependent on the clinical presentation, and must be interpreted as such by clinicians (10).

1.1.3 Is AAV an Autoimmune Disease?

Research done during the last two decades has provided fairly compelling evidence that AAV is an autoimmune disease, and that ANCA is pathogenic. The strongest direct evidence is the case report of the baby born of a mother with active AAV, who developed a pulmonary-renal syndrome 48 hours after delivery (11). The baby was found to have comparable levels of anti-MPO ANCA to its mother, presumably due to transplacental transfer, and recovered following treatment with steroids and plasma exchange. The other evidence may be divided into circumstantial evidence, evidence from in-vitro experiments, and that from animal models.

1.1.3.1 Circumstantial Evidence

There is an association between ANCA positivity and disease activity in humans – often a rise in anti-PR3 or anti-MPO ANCA titre may herald a flare in the disease. However, it must

be pointed out that some sufferers will demonstrate consistently high ANCA levels without clinically active disease, and conversely that some flares will occur without a rise in titre, and meta-analyses suggest that ANCA measurements during remission are of limited value in guiding treatment decisions (12). Supportive evidence also comes from the MEPEX trial (13), which showed that patients with severe AAV had a lower risk of being dialysis-dependent at 12 months if they were treated with plasma exchange instead of methylprednisolone - plasma exchange is a treatment that would be expected to remove circulating IgG from the blood, although of course it could have other effects such as altering cytokine levels.

1.1.3.2 In-Vitro Evidence

The first in-vitro evidence for the pathogenicity of ANCA was the demonstration that ANCA could induce respiratory burst and degranulation (14). ANCA activation of neutrophils can cause release of a number cytokines and chemokines, including IL-1, IL-8, and TNF-α (15), damage to endothelial cells (16), and accelerated and dysregulated neutrophil apoptosis in-vitro (17).

1.1.3.3 Animal Models

The first convincing animal work was carried out on mice by Xiao and colleagues in 2002 (18). They immunised MPO knockout mice with mouse MPO, inducing production of anti-MPO antibodies. They then injected splenocytes from those mice into a mouse model known as Rag2 (-/-) which lacks functioning T- and B-lymphocytes. This introduced anti-MPO T- and B-lymphocytes, and caused a dose-dependent anti-MPO IgG ANCA production in the Rag-2 (-/-) mice. All mice that received splenocytes developed mild to moderate glomerular immune deposits, and those that received the highest doses developed a severe necrotizing and crescentic glomerulonephritis, as well as haemorrhagic pulmonary capillaritis and some granuloma formation.

They then tested the ability of ANCA alone to produce such effects, by injecting purified anti-MPO IgG ANCA into Rag2 (-/-) and wild-type mice. They found that both strains developed focal necrotising and crescentic glomerulonephritis without immune complex deposition. They concluded that anti-MPO IgG ANCA could produce a pauci-immune necrotising crescentic glomerulonephritis, both in the setting of absent lymphocytes, and in an intact immune system.

A rat model of anti-MPO ANCA has been developed by Little and colleagues (19). They injected human MPO into Wistar/Kyoto (WKY) rats, which then produced anti-MPO antibodies that cross-reacted against rat neutrophils. The rats developed experimental autoimmune vasculitis (EAV), comprising pauci-immune crescentic glomerulonephritis and lung haemorrhage. They also used a technique known as intravital microscopy to demonstrate in-vivo that the ANCA conferred enhanced adhesion of neutrophils to the endothelial wall, as well as transmigration across the endothelium. Experiments by Nolan and colleagues on a mouse model also demonstrated evidence of enhanced leukocyte–endothelial cell interactions in the presence of anti-MPO IgG (20).

1.1.4 Why do Patients Lose Tolerance to MPO and PR3?

Existing evidence suggests this may be due to a combination of genetic and environmental factors.

1.1.1.1 Genetic Factors

A number of genetic factors are associated with an increased incidence of AAV. As mentioned previously, a variable proportion of human neutrophils express high levels of PR3 on their surface membrane in the resting state. This has been shown to have a genetic basis, and an association has been demonstrated between higher levels of surface expression and the risk of AAV (21).

A genome-wide association study in the UK and Northern Europe recently found major histocompatibility complex (MHC) and non-MHC associations with AAV, and also found genetic distinctions between GPA and MPA (22).

Studies by Kamesh and colleagues showed evidence that CTLA-4, a susceptibility locus for a number of common autoimmune diseases, may be involved in the development of AAV (23). Other pointers to genetic predisposition include the increased expression of C3F and C4A3 complement gene polymorphisms in patients with AAV (24), and the markedly increased frequency of α1 antitrypsin defects in patients with GPA. Such defects confer a worse prognosis (25), which is perhaps unsurprising given that α1 antitrypsin normally inhibits PR3.

1.1.4.1 Environmental Factors

The most common environmental factor linked with the development of AAV is exposure to silica, which may cause production of ANCA through its potent stimulation of T- and B-lymphocytes. Silica exposure can also cause release of PR3 and MPO, and accelerated apoptosis. It is theorised that disordered apoptosis, whether due to silica exposure or otherwise, could result in cross-presentation of self-antigens by dendritic cells (reviewed in (26)).

Infection is also commonly suggested as a reason for loss of self-tolerance in AAV. There is some evidence that chronic nasal carriage of Staphylococcus aureus increases relapse rates in GPA (27), and that long-term co-trimoxazole therapy reduces relapse rates (28). One theory which might explain this link with infection was put forth by Pendergraft (29), who observed that many of their patients had antibodies not only to PR3, but also to a protein they termed ‘complimentary PR3’ (cPR3), a peptide translated from the anti-sense strand of the DNA encoding PR3. They immunised mice with cPR3, and found that antibodies were produced

not only to cPR3, but also to PR3. Both Staphylococcus aureus and Entamoeba histolytica have been found to express proteins mimicking cPR3, thereby providing a potential stimulus for anti-PR3 ANCA production.

Many drugs can cause the development of ANCA, most classically propylthiouracil. The clinical manifestations of drug-induced AAV are similar to those of idiopathic AAV, but with significant differences. For example, the ANCA found usually demonstrates multi-antigenicity, and the diagnosis is made by excluding other medical conditions and linking the temporal onset of disease to use of the offending drug. Treatment also differs: removal of the drug is most important, rather than immunosuppression (reviewed in (30)).

1.1.5 Pathogenesis of AAV

Current evidence suggests that there are four basic steps in the immunopathogenesis of AAV:

1. Both the neutrophil and the endothelium must be 'primed' in order for the process to begin;
2. The primed neutrophil adheres to the endothelium;
3. ANCA interacts with the adhered neutrophil, leading to neutrophil activation;
4. Neutrophil activation results in damage to the endothelium.

This is an over-simplification of the situation as it is in-vivo, but it provides us with a framework for looking at the process, as illustrated in **Figure** 1.1.

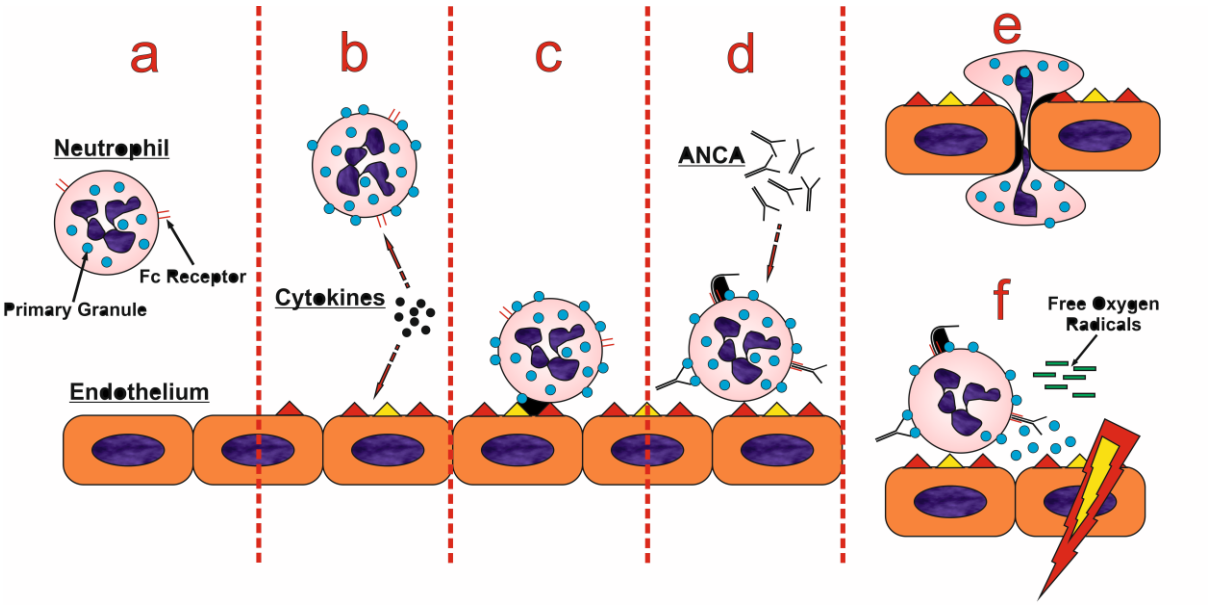


Figure 1.1: The interaction of cytokines, neutrophils, ANCA and endothelium.
(a) In the resting state of a neutrophil, most PR3 and MPO is found in the primary granules, and resting endothelium does not support neutrophil adhesion. (b) An inflammatory stimulus, such as infection, causes the release of pro-inflammatory cytokines, including TNF-α. These induce neutrophils to display PR3 and MPO on their surface, and also cause the expression of P- and E-selectins on the endothelium. (c) Selectin on the endothelium engages with the ligands on the neutrophil cell surface, allowing the neutrophil to roll on the endothelial surface. (d) Once the neutrophil is rolling, ANCA acts on PR3/MPO on the cell surface, activating the neutrophil. (e) ANCA brings about a change in neutrophil surface molecules, allowing it to adhere firmly and transmigrate through into surrounding tissues. (f) The activated neutrophils undergo degranulation and respiratory burst, resulting in endothelial injury.

1.1.1.1 Neutrophil Priming

As described previously, there is normally no MPO on the surface of neutrophils, and the surface expression of PR3 varies considerably between individuals (8, 31). In order therefore for effective interaction to occur between ANCA and antigen, the neutrophils must be 'primed' in such a way that they are induced to carry PR3 or MPO on their cell surface. It is believed that this is brought about by the action of cytokines, particularly tumour necrosis factor-α (TNF-α) interleukin-1(IL-1), and interleukin-8 (IL-8) (31). In-vitro and animal experiments suggest that TNF-α is central to neutrophil priming, and in some animal models it has been shown that anti-TNF-α therapy will arrest glomerular crescent formation and pulmonary haemorrhage (32).

1.1.5.1 Activation of the Endothelium, and Neutrophil Adhesion

There is in-vitro evidence that adherence to the endothelium is a prerequisite for neutrophil activation (33). However, without stimulation by cytokines, glomerular endothelium does not support neutrophil adhesion because it does not normally express VCAM-1, P- or E- selectin on its surface. Once expression of these molecules have been induced by cytokines such as IL-1, IL-4, TNF-α and IFN-γ (34), the neutrophil begins to roll on the surface of the endothelium. A second signal, such as ANCA, causes a conformational change in β2-integrin on the neutrophil surface. This allows the neutrophil to stop rolling and to adhere firmly to, and even transmigrate through, the endothelium.

1.1.5.2 ANCA activation of the Neutrophil

Once neutrophils have been primed by cytokines, they are 'activated' by binding with ANCA, then undergo several changes, including the increased expression and altered conformation of β2-integrins. This has been demonstrated in-vitro, where neutrophils flowing over an endothelial monolayer are induced by incubation with ANCA to stop rolling and adhere firmly to the monolayer, resulting in a ten-fold increase in neutrophil transmigration (35); intravital microscopy has also demonstrated this phenomenon in-vivo (19). ANCA also induces polymerisation of the actin cytoskeleton within the neutrophil, making the cell more rigid. This could lead to sequestration within capillary beds, explaining why AAV tends to mainly affect regions with major capillary beds, such as the kidneys and lungs (36).

Activated neutrophils secrete further cytokines including TNF-α and IL-1, the chemokine interleukin-8, monocyte chemoattractant protein 1 and leukotrine-B4 (15, 37). These substances act as chemoattractants to other neutrophils, monocytes and lymphocytes, resulting in perpetuation of unregulated inflammation and damage (38). Additionally, apoptosis is dysfunctional in neutrophils that have been activated by ANCA, with a delay in the expression of apoptotic surface molecules required for effective phagocytosis by

macrophages. This delays their removal from the tissues, resulting in secondary necrosis, the release of damaging intracellular contents, and yet more spiralling inflammation (26).

1.1.5.3 Damage to the Endothelium Results in the Classical Histopathology of AAV

When primed neutrophils are incubated together in-vitro with ANCA and endothelial cells, endothelial damage can be demonstrated (16, 39). This occurs by two main mechanisms: once neutrophils are firmly adhered to endothelium and activated by ANCA they undergo 'respiratory burst' to produce superoxide and other reactive oxygen species, and they also degranulate to release MPO, PR3, and the other proteolytic constituents of their primary granules into the microenvironment (14, 40). Once internalised by endothelial cells, PR3 can induce apoptosis (programmed cell death) (41); likewise, MPO may be internalised and then damage the endothelial cells by intracellular generation of active oxygen species (42).

As the process spirals out of control, detachment of the endothelium from its basement membrane occurs; the exposed basement membrane initiates thrombosis in the capillary lumen, and when that occurs in the kidney it results in segmental necrosis of the glomerulus. If the segmental necrosis causes vessel rupture, bleeding into Bowman's space causes epithelial cells and monocytes to form a characteristic crescentic scar, leading to the classical histopathology seen on renal biopsy of 'pauci-immune, focal and segmental, necrotising and crescentic glomerulonephritis'. If on the other hand, such vessel rupture occurs in the lungs, it may cause pulmonary haemorrhage.

1.1.6 The Complement Cascade in AAV

Traditionally it has been thought that the complement system is not involved in AAV, for two reasons: because the histology is 'pauci-immune', and because serum complement levels are normal despite active disease. However, both of these arguments are flawed.

Firstly, IgG is also rarely found on biopsy in AAV, even though it is central to the whole disease process. Also, several pathology studies in AAV have shown a degree of immune complex deposition, both in skin biopsies (43) and also in early renal biopsies (44). C3, and to a lesser extent C1q have both been shown. Secondly, hypocomplementaemia is a very insensitive way of measuring complement consumption; anti-GBM disease, for example, features extensive complement deposition but normal serum complement levels.

Positive evidence of complement involvement in AAV has been found in both in-vitro and in-vivo experiments. In-vitro experiments have suggested that the oxygen radicals, MPO and various proteases released by ANCA-activated neutrophils are all capable of activating the complement system. Mouse studies have shown that the alternative pathway of the complement system appears to be critical to the model of anti-MPO ANCA-induced crescentic glomerulonephritis. Finally, in humans it has been found that there is skewed expression of certain gene polymorphisms: increased C3F expression was found among people who were PR3-ANCA positive, and increased expression of C4A3 was found in AAV as a whole, although the clinical relevance is not clear (reviewed in (45)).

1.1.7 What is the Role of Lymphocytes?

The role of B-lymphocytes and plasma cells in AAV is obviously critical, since there is now convincing evidence that the ANCA which they produce is central to the disease immunopathogenesis. This has been illustrated in recent years by the success of rituximab in controlling resistant disease. Rituximab is a chimeric antibody directed against the CD20 antigen on B cells, and it has been shown to induce remission in disease which is refractory to corticosteroid and cyclophosphamide therapy (46).

Culton and colleagues (47) have demonstrated that the surface expression of CD19 in AAV is 20% lower than on the B-cells of healthy controls. This antigen is involved in signal

transduction, and the authors propose that the lower CD19 expression results in lower levels of signal transduction, perhaps allowing autoreactive B-cells to avoid being selected out.

T-lymphocytes probably have a significant role to play as well. Since ANCA are high-affinity, class-switched antibodies, it is likely that CD4+ T-cell help is required. Further evidence of their involvement comes from the high numbers of T cells found in renal biopsy specimens; they are a major feature of the interstitial infiltrate, and their numbers correlate with the number of crescentic glomeruli and the serum creatinine level (48).

There are many changes within the circulating T-cell populations in AAV. Often up to 20% are found to be activated, even during remission (49). Lymphopenia and markedly low numbers of CD4+ T-cells have been found even in untreated patients, suggesting that it is secondary to the disease itself rather than to therapy (50). There is skewing towards effector memory cells, which could be a reason for the common relapsing remitting course, and could be a target for new therapies (reviewed in (51).

1.1.8 Monocytes in AAV

In the same way that neutrophils do, monocytes from the peripheral blood of patients with AAV express MPO and PR3 on their surface during periods of active disease. In-vitro, ANCA has been shown to activate monocytes, causing the release of interleukin-8 (52), a potent attractor for neutrophils, and monocyte chemoattractant protein-1 (53). It even causes monocytes to release reactive oxygen species (54).

As discussed previously, macrophages are responsible for removing apoptotic neutrophils, but the process of neutrophil apoptosis is disturbed by ANCA activation. When these activated neutrophils are eventually ingested, the ingesting macrophages display enhanced phagocytotic activity and TNF-α release (55).

1.1.9 Epidemiology

Granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and Eosinophilic granulomatosis with polyangiitis (EGPA) are all uncommon diseases; the incidence and prevalence of each disease in the UK is shown in Table 1.1.

	GPA	MPA	EGPA
Incidence	10.2	5.8	4.2
Prevalence	109	28	38

Table 1.1: Incidence and prevalence of AAV in the UK, expressed per million population.
Data from ref (56)

AAV can affect any age group, but peak onset is between 65 and 74 years. Unlike many other auto-immune diseases there is a slight male preponderance. Across the Western Hemisphere it is a disease which affects Caucasians much more commonly: for example, a recent study in France reported that the prevalence was twice as high in those individuals who were of European compared to non-European descent (57). Within Europe, GPA seems to be more common in colder Northern European countries than in the Mediterranean countries, whereas MPA seems to have the opposite trend; in Japan up to 90% of AAV seems to be MPO-positive and to show clinical characteristics in keeping with MPA (56).

1.1.10 Clinical Presentation

The clinical presentation of AAV can be extremely variable, and a high index of suspicion must be maintained so as not to miss the diagnosis. Many symptoms such as fever, malaise, arthralgia, myalgia, anorexia and weight loss are common in all types of AAV, sometimes described as a preceding ‘flu-like illness’, and a purpuric skin rash is a common clinical sign. Each disease does have a characteristic pattern of organ involvement, as detailed below, but these classical presentations are not always seen.

1.1.1.1 Microscopic Polyangiitis

The kidneys are the organ most commonly involved in MPA (90% of cases) with evidence of necrotising crescentic glomerulonephritis. Manifestations can range in severity from a rapidly progressive glomerulonephritis (RPGN) which may result in complete renal failure, to microscopic haematuria and proteinuria at the other end of the spectrum. The vessels of the lower respiratory tract are involved in around 50% of cases, resulting in shortness of breath, cough and haemoptysis. Unlike the other forms of AAV, granuloma formation does not occur, and upper respiratory tract involvement is rare (58).

1.1.1.2 Granulomatosis with Polyangiitis

In addition to necrotising vasculitis (as seen in MPA), GPA is characterised by the presence of necrotising granulomatous lesions of the upper and lower respiratory tract. Granulomas are microscopic collections of macrophages, which in the lung may form large cavitating masses. The classical triad of organ involvement seen in GPA is upper respiratory tract (90% of cases), lower respiratory tract (90%), and kidneys (80%). Ear nose and throat (ENT) symptoms are often prominent, and GPA may even be ENT-limited. Although MPA may involve the same organs, the presence of granulomas is required for some clinical manifestations such as saddle nose deformity, which therefore do not occur in MPA.

1.1.10.1 Eosinophilic Granulomatosis with Polyangiitis

EGPA is also characterised by both necrotising vasculitis and necrotising granulomas, but there are a number of differences between it and GPA. It is associated with eosinophils in the blood and in areas of inflammation, and clinical features of asthma may also be seen. Although the neurological system may be involved in any form of AAV, it is most often seen in EGPA (70% of cases).

1.1.11 Prognosis and Relapse

Without immunosuppressive treatment AAV has a very poor prognosis of around 7% survival at two years (59), but with modern treatments survival is good at 80% at 5 years (60). A recent Dutch study showed that two-year survival was only 61.8% for the period 1979-1989 and 59.2% for the period 1990-2000, but had improved to 84.8% between 2001 and 2009 (61); the authors postulated this was due to increased awareness, earlier diagnosis, and improvement in treatment strategies. Factors which are known to correlate with poorer outcomes in AAV are older age (62), pulmonary haemorrhage, renal impairment at presentation, and above all, dialysis-dependant renal failure (63). patients with AAV with active urinary sediment but normal function have more than twice the risk of death than those with no renal involvement, and those with impaired renal function at diagnosis have more than five times the risk of death (64). For patients who have renal involvement, the renal biopsy is also of significance: patients who have predominantly sclerotic glomeruli on biopsy have the worst prognosis in terms of renal recovery (65), and by extension would be expected to have worst survival.

AAV remains an incurable disease, and treatment advances have turned it from a rapidly fatal disease into one with a chronic relapsing-remitting course. Around 40-50% of patients will relapse during the first five years (58), and with each relapse there is a risk of accruing irreversible damage to vital organs such as the kidneys and lungs, so close monitoring is essential. Hogan et al (66) identified anti-PR3 seropositivity (hazard ratio 1.87 [confidence interval (CI) 1.11 to 3.14]), lung involvement (hazard ratio 1.71 [CI 1.04 to 2.81]), and upper respiratory tract involvement (hazard ratio 1.73 [CI 1.04 to 2.88]) as carrying increased risk of relapse; patients with all three of these risk factors had a 3.7-fold increased risk of relapse (CI 1.4 to 9.7). Walsh et al (67) looked at 535 patients enrolled across four RCTs, and again identified anti-PR3 antibodies as a risk factor for relapse (subhazard ratio (sHR) 1.62 [CI

1.39–1.89]), but in their cohort did not find a significant association with lung or ENT involvement, instead finding that cardiovascular involvement carried an increased risk of relapse (sHR 1.59 [CI 1.07–2.37]). Between 18% and 40% of patients with GPA will relapse in the first two years after diagnosis, compared with only 8% at two years for MPA (68), which is unsurprising given the strong association between GPA and PR3 positivity.

1.1.12 Treatment

Treatment of AAV is divided into three phases: aggressive initial treatment with the aim of halting active inflammation ('induction of remission'), followed by less toxic treatment in the longer term with the aim of preventing inflammation from recurring ('maintenance of remission'), and finally treatment of any subsequent relapses.

1.1.1.1 Induction Therapy

Modern induction therapy for AAV usually consists of corticosteroids and cyclophosphamide. The precise dosage schedules used vary somewhat from unit to unit, but are often based on regimens used in the large multi-centre randomised control trials (RCTs) which have provided the evidence-base for modern strategies.

Corticosteroids achieve immunosuppression through many mechanisms, including lymphopenia and monocytopenia, as well as suppression of cytokine production (including IL-1, IL-2 and TNF-α). Although they cause a neutrophilia, they impair neutrophil chemotaxis and adhesion. The main corticosteroid used is prednisolone, at an initial dose of 1mg/kg which is tapered over time. Corticosteroid therapy is also commonly delivered as intravenous pulses of methylprednisolone, at doses varying from 500mg to 1g for three consecutive days at the start of therapy. There is little direct evidence for the use of methylprednisolone, as the only trial it was tested in (MEPEX) showed it to be less efficacious than plasma exchange in induction of remission (69); however it does have a

more rapid immunosuppressive effect than oral prednisolone, and is therefore a common part of induction therapy. Corticosteroids have many side-effects, both short- and long-term, including infection risk, hypertension, diabetes, hyperlipidaemia, peptic ulcer disease, osteoporosis, weight gain and psychiatric disturbances.

Cyclophosphamide is a nitrogen mustard alkylating agent which interferes with DNA replication within cells by forming intrastrand and interstrand DNA crosslinks. Its effects are dose dependent, and it must be given at high doses in order to cause the immunosuppressive effects employed in the treatment of autoimmune diseases. Cyclophosphamide provides a significant benefit when added to corticosteroid therapy, improving remission rate from 56% to 85% and reducing the risk of relapse three-fold (70). There is still some debate about the best regimen for cyclophosphamide delivery; the CYCLOPS trial (71, 72) compared daily oral with pulse IV regimens, and found that the pulse IV regimen allowed administration of half the cumulative cyclophosphamide dose of the daily oral regimen, but long term follow-up showed that the cost of this was almost twice as many relapses. However despite the higher relapse rate, survival and renal function were no different between the two groups at the end of the study, and this coupled with the significantly lower cyclophosphamide exposure is a convincing argument in favour of a pulse IV regimen. Cyclophosphamide is toxic, associated with potentially life-threatening side-effects, including leukopenia (associated with infection and death (73)), increased cancer risk (particularly transitional cell carcinoma of the bladder and acute myeloid leukaemia), haemorrhagic cystitis, and temporary or permanent sterility. There is an increasing body of evidence that the cumulative cyclophosphamide dose is important in determining toxicity risk (74).

Plasma exchange is a treatment in which a proportion of the patient's plasma is removed by either plasma filtration or centrifugation, and the same volume is then replaced with another fluid, usually 5% human albumin solution. It is sometimes used in AAV to remove ANCA

from the circulation, but compared to other therapies there is relatively little evidence to support its role in this disease. The main evidence base for its use in AAV comes from the MEPEX trial, in which patients underwent seven plasma exchanges within fourteen days, on each occasion exchanging a volume of 60ml per kg of body weight(69). The trial showed that when patients with AAV presented with severe acute kidney injury (creatinine >500μmol/l), the addition of plasma exchange to cyclophosphamide and prednisolone resulted in a higher rate of renal recovery than did the addition of IV methylprednisolone. Side-effects of plasma exchange include transfusion reactions, complications related to the central venous catheters which are required to deliver the treatment, sepsis, bleeding, and electrolyte abnormalities.

Rituximab is a chimeric monoclonal antibody directed against the CD20 antigen on B cells. Because of the convincing evidence that ANCA are pathogenic, there has been great interest in intravenous rituximab both as an induction agent and as a maintenance agent. Two RCTs recently reported on the use of rituximab for induction. The RITUXIVAS trial (75) compared a regimen of rituximab combined with only two doses of IV pulse cyclophosphamide to a conventional ten dose regimen of IV pulse cyclophosphamide and found that rates of remission and adverse events were similar in both groups. RAVE (76) compared rituximab to oral cyclophosphamide, and again found no significant difference in rates of remission, adverse events, or rates of relapse. However due to the relatively unknown long-term efficacy and safety of rituximab, as well as its comparative cost, cyclophosphamide remains first line therapy. The Kidney Disease: Improving Global Outcomes (KDIGO) international guidelines currently recommend that rituximab should be used in conjunction with steroids as an alternative induction treatment for those patients “without severe disease or in whom cyclophosphamide is contraindicated” (77). They also recommend the use of rituximab in ANCA-positive glomerulonephritis resistant to induction therapy with cyclophosphamide and corticosteroids.

1.1.12.1 Maintenance Therapy

It is conventional to move to a maintenance regime following disease remission. Such regimens typically include a much lower dose of prednisolone (e.g. 5mg/day) and either azathioprine or mycophenolate.

Azathioprine is a purine analogue which works by blocking purine synthesis, thereby inhibiting DNA synthesis and the proliferation of cells in the body, particularly affecting lymphocytes (other cells are less affected due to salvage pathways which lymphocytes lack). Azathioprine has the best evidence of the available remission agents, with RCT evidence that introducing azathioprine after only three to six months of cyclophosphamide therapy resulted in similar relapse rates after eighteen months as when cyclophosphamide was continued for twelve months (78).

Mycophenolate also inhibits purine synthesis, and it has therefore been suggested as an alternative maintenance agent. However there is RCT evidence that relapse rates with mycophenolate are significantly higher than with azathioprine. It is therefore only recommended as maintenance therapy in those patients who are allergic to or intolerant of azathioprine (77).

Methotrexate is sometimes used for remission in patients without renal involvement. An RCT appeared to show no difference in efficacy between methotrexate and azathioprine (79). It must be used with caution in renal impairment, but is recommended by some authors for ENT-limited disease (80)

Trimethoprim-sulfamethoxazole is a combination sulfonamide antibiotic used in the treatment of a variety of bacterial infections. Interest in the use of this antibiotic in the treatment of AAV arose from the association between chronic nasal carriage of Staphylococcus aureus and increased relapse rates in GPA (27). Long-term use of this drug

can reduce the rate of upper respiratory tract relapses in GPA, although it has not been shown to reduce relapse rates in other organ systems (28).

As yet, no large scale RCTs have looked at the use of rituximab as a long-term maintenance agent, but there is significant interest in using the drug in this way, particularly for patients with refractory disease in whom the immunosuppressive burden is highest. An English study of 73 patients showed that routine re-treatment with rituximab at fixed intervals reduced the rates of relapse in such patients from 75% to 22% at 24 months, as wells as reducing the percentage of patients requiring other immunosuppressive drugs such as azathioprine and allowing corticosteroid withdrawal in 40% (81). Similarly encouraging results were reported by American retrospective studies (82, 83). The ongoing RITAZAREM trial is examining whether maintenance rituximab is more effective than azathioprine for the prevention of relapse after rituximab induction for relapsing AAV, as well as relapse rates following withdrawal of these therapies (84).

1.1.12.2 Treatment of Relapses

Relapse is defined as the occurrence of increased disease activity after a period of partial or complete remission. Treatment depends on the severity of the relapse: a severe relapse that threatens life or an organ should be treated according to the same guidelines as for induction therapy, whereas a milder relapse may be treated by restarting or increasing immunosuppressive therapy with agents other than cyclophosphamide (77).

1.2 What is Fatigue?

Fatigue is a common symptom in the healthy general population (85-88), as well as in a host of physical (89-96) and psychological (97-99) illnesses, but despite this there remains no universally accepted definition of exactly what fatigue is. A common medical definition of

fatigue is “that state, following a period of mental or bodily activity, characterised by a lessened capacity for work and reduced efficiency of accomplishment, usually accompanied by a feeling of weariness, sleepiness or irritability” (100), but although this is a reasonable definition of fatigue in normal healthy life, it does not match the experiences of patients in a number of ways that will be explored in this chapter. Efforts by researchers to arrive at a more satisfactory definition of fatigue have been hampered by three main issues: 1) fatigue is a very subjective experience, and descriptions sometimes vary greatly between individuals; 2) it has proven very difficult to separate fatigue reliably from other every-day symptoms such as drowsiness, tiredness or weariness; 3) the impact of fatigue has only truly been recognised in the last two decades, and compared to many other symptoms fatigue research is still in its relative infancy.

In 2008, Mills and Young (101) developed what they hoped would be a unifying definition of fatigue. They interviewed a UK cohort of multiple sclerosis (MS) patients, then administered a questionnaire survey based on the qualitative data gained. They arrived at a definition of fatigue as a “reversible, motor and cognitive impairment with reduced motivation and desire to rest, either appearing spontaneously or brought on by mental or physical activity, humidity, acute infection and food ingestion. It is relieved by daytime sleep or rest without sleep. It can occur at any time but is usually worse in the afternoon.” Although much more comprehensive, this definition is still contradicted in parts by qualitative studies in other diseases.

Hewlett et al conducted qualitative research looking at the nature and impact of fatigue in rheumatoid arthritis (RA) (102); their findings were similar to other studies in RA (103, 104) and fibromyalgia syndrome (FMS) (105) and might be applicable to many of the long-term conditions associated with fatigue. Their patients reported that the fatigue associated with their RA was fundamentally different from the fatigue they had experienced in health in a

number of ways: it was much more severe and overwhelming; there was often no obvious physical or mental trigger, making it feel 'unearned', unfair and unpredictable to patients; it was only partly or not at all relieved by rest. Two main descriptions of the nature of fatigue in RA emerged: firstly that it was akin to carrying a weight around with you, so that everyday tasks became very much harder than they had previously been, and secondly that some people experienced it as sudden waves of extreme exhaustion that left them unable to carry on, termed 'wipe-out' by Hewlett. The reported duration of fatigue episodes in those patients varied from minutes to days, and for some it was constant. It had significant mental and emotional components, with a reported loss of 'mental energy', motivation and concentration, and a tendency to make some people feel very tearful. The study found that fatigue had an impact on every sphere of life, with effects on work, leisure and activities of daily living (ADLs), as well as a profound effect on inter-personal relationships. Patients felt uncertain about how best to manage their fatigue, employing a whole range of coping techniques with varying levels of success, and invariably felt that their fatigue was either ignored or dismissed by health-care professionals.

Another qualitative study in 2003 instead looked at how healthy working adults describe fatigue (88). They interviewed 40 healthy adults between the ages of 30 and 60 about their experiences of fatigue, and defined fatigue in healthy adults as "an acute, subjective, sometimes overwhelming, but temporary state (with physical, emotional mental and behavioural manifestations) caused by stress and overwork in one's life roles, which disrupts activity and alerts the person to take restorative measures such as rest, sleep, or reordering of activities and goals". Whilst some parts of this definition are not dissimilar to the definitions of Mills (101) and Hewlett (106), the acute and temporary nature, the direct relation to physical or mental stressors, and the restorative power of rest and sleep all confirm the assertion of Hewlett's patients that fatigue in health and fatigue in illness are quite different

entities. Previous reports have suggested that the prevalence of chronic fatigue lasting more than six months is as high as 1-3% among healthy adults in the community (107), seemingly contradicting the notion that fatigue in healthy people is transient, but other studies suggest that these contradictory findings might be explained by undiagnosed illnesses such as Chronic Fatigue Syndrome (CFS - see Section 1.7) or depression. One such study found evidence of undiagnosed hypothyroidism, psychiatric illness, and CFS (108). In another, 18.3% of primary care patients described significant fatigue for six months or longer, but a strong correlation was found between chronic fatigue and psychological symptoms (86).

1.3 How is Fatigue Measured?

Quantifying fatigue is difficult because the symptom is subjective and heterogeneous. Some physical and physiological aspects of fatigue may be measured: for example if we measure how long a person is able to continue a physical task such as lifting a known weight this could be seen as an objective measure of their fatigability, but this would only capture one aspect of fatigue and in isolation would be of very limited use. Common sense suggests that subjective fatigue, regardless of cause, would lead inevitably to a reduction in objectively measurable activity, so that pedometers and other similar devices could be used to obtain an objective surrogate measure of fatigue, but although a good correlation was found between such measurements and subjective fatigue for patients with CFS, this was not the case for patients with MS (109).

In view of these difficulties, the only way to reproducibly and reliably measure the subjective experience of fatigue is through the use of self-rating questionnaires. As the importance of fatigue has been increasingly recognised in the last few decades, so the number of fatigue tools has proliferated – there are now more than thirty different scales designed specifically to

measure fatigue, and many more such as the Medical Outcomes Study Short Form-36 (SF-36) which include fatigue as one dimension of a broader health outcome (110). One study of fatigue instruments identified 252 different ways in which fatigue had been measured, of which 150 had only ever been used once (111)! Although in theory all of these scales assess the same symptom, in reality the questions asked and therefore the information derived can vary considerably based on the scale author's own concept of fatigue. When researchers are deciding which scale to use, they must consider which tool is best suited to answer the questions they want answered in the target population they wish to study and within the practical constraints of their study.

The first question to consider is whether to use a unidimensional scale or a multidimensional scale. Unidimensional scales are designed to sum up all the aspects of fatigue into a single summary score; they are usually short and easy to administer, and equally easy to score and interpret with minimal training. At its most basic a unidimensional scale might only consist of a single visual analogue scale, but the best known and most widely cited unidimensional fatigue scale is the Fatigue Severity Scale (FSS). This is a tool which was first developed for patients with chronic medical illnesses including MS and systemic lupus erythematosus (SLE), and consists of nine items each scored on a 7-point Likert scale (where 1 = 'strongly disagree' and 7 = 'strongly agree'), with statements including "I am easily fatigued" and "Fatigue is among my most disabling symptoms". It has been used across a wide range of illnesses including MS, Parkinson's disease (PD), CFS, cancer and sleep disorders, and has been shown to have good internal consistency (Cronbach's α=0.88 - i.e., there is good correlation between different items on the scale), good test-retest reliability (0.84), and high sensitivity to changes in symptoms with time (112).

Multidimensional scales are generally longer and more time-consuming to complete, as well as being more complex to score and interpret. However, the advantage of multidimensional

scales is that they provide more detailed quantitative and qualitative data, thereby allowing a more meaningful assessment of the nature of fatigue in the disease being studied, and also potentially allowing researchers to gain insights into the mechanisms underlying the different aspects or 'dimensions' of fatigue. A good example of a multidimensional fatigue scale is the Multidimensional Fatigue Inventory (MFI-20). This tool consists of 5 subscales, each purporting to represent a different dimension of fatigue, the last two of which are sometimes considered to be outcomes of fatigue: General Fatigue, Physical Fatigue, Mental Fatigue, Reduced Activity, and Reduced Motivation. All five subscales have been shown to have good internal consistency across a variety of illness and in health, as well as good test-retest reliability results (113). It was developed in cancer patients, but has since been used across a multitude of illnesses including AAV (114, 115), cancer (116-122), CFS (123-127), CKD (128-131), Primary Sjögren's syndrome (132, 133), Ankylosing Spondylitis (AS) (134, 135), RA (132, 135, 136), inflammatory bowel disease (137-139), MS (139-142) as well as in health (143, 144). There were initial concerns regarding the ability of the scale to distinguish between healthy people and those with illnesses (113), but the scale was further developed following the initial study, and other studies seem to have allayed those initial fears (145, 146).

Once a decision has been made to use either a unidimensional or multidimensional scale, the next step is to decide which scale most closely matches the aspects of fatigue the study is interested in measuring. The names of the scales can often be misleading: for example the Fatigue Severity Scale is not actually a measure of the severity of fatigue per se, but rather a measure of the impact of fatigue, with eight of the nine statements describing the effect of fatigue on motivation, social life etc. Finally, it is important to check whether the scale of interest has been used before and preferably validated in the disease group to be studied, although this might not be the case for any scales if studying a rare disease.

1.4 Classifying the Determinants of Fatigue

When considering fatigue, as for any symptom, it is helpful to create models which categorise or group the possible causes, helping researchers to more easily understand the symptom and create hypotheses which may then be tested. Leading on from the qualitative research discussed earlier, Hewlett's group developed one such conceptual model of fatigue in RA (106), separating the causes of fatigue into three interacting groups:

- *Disease factors*, which Hewlett proposed for RA might include deconditioning, muscle atrophy, joint damage, anaemia, medications, pain, poor sleep, and abnormal cortisol responses due to ongoing inflammation (147);
- *Cognitive and behavioural factors*. This aspect of the model is based on a recognised pre-existing model (148). Such factors might include illness beliefs such as low self-efficacy, feelings such as anxiety and depression, and behaviours such as over- and under-activity.
- *Personal life issues*, including role responsibilities, standard of housing, a lack of social support, and comorbid illnesses.

Hewlett proposed that any of these factors might predispose to fatigue, precipitate fatigue episodes, or help perpetuate fatigue which had been caused by other factors, and that the relative importance of different factors varied from one individual to another. This model could easily be applied to AAV or to any other disease or fatigued state; the 'disease factors' might differ depending on the illness being looked at, for example in cancer they might include the administration of chemotherapy (149), but the same over-riding themes apply.

Another useful way to consider the determinants of fatigue is by classifying the possible causes into 'peripheral fatigue' and 'central fatigue', although it is important to note that peripheral and central fatigue may coexist within the same individual. 'Peripheral fatigue'

describes fatigue which is either due to: a) cardio-respiratory problems such as deconditioning, which results in normal tasks being carried out at a higher percentage of the maximum possible heart rate (150); or b) neuromuscular problems outside of the central nervous system such as muscle atrophy, impaired muscular contractility, or impaired neurotransmission in peripheral nerves. A loss of muscle mass due to protein breakdown is common in many acute and chronic illnesses (151, 152), particularly when inflammation is a major component, and because this would result in a reduction in strength and endurance capacity it is likely to be a relatively common cause of peripheral fatigue in long-term illnesses (153). Central fatigue describes abnormalities in perception, motivation or central motor activation, which are probably caused by alterations in neurotransmitter pathways within the brain. It has been proposed previously that central fatigue is more important than peripheral fatigue in patients with chronic illnesses, "although the absolute contribution of peripheral and central fatigue… may vary significantly between different diseases" (154).

1.4.1 Peripheral Fatigue in Health and Disease

In young healthy people, peripheral fatigue is most significant during high intensity exercise such as running up a flight of stairs. This is because energy utilization in the form of ATP breakdown is happening at a faster rate than the rate at which new ATP can be generated by oxidative metabolism or glycolysis (155). Even in the fittest athlete the blood supply and the ability of the muscle to take up and utilise the oxygen in the blood are too slow to meet the demands of a high-intensity activity.

In disease states, metabolic abnormalities within skeletal muscle could result in peripheral fatigue through acceleration of the same processes described in health, but such abnormalities are very rare and are therefore not a common cause of peripheral fatigue (156). Peripheral fatigue is often assumed to be significant only in those diseases (primarily rheumatological,

such as RA) which are characterised by either muscle atrophy, where increased demand is placed on the remaining muscle, or by joint deformities, where there is alteration of the angle of torsion of the muscles acting on the joint. However the contribution of peripheral fatigue in other diseases might actually be more important than commonly thought, because most of the diseases associated with fatigue become more common with increasing age, and as a result a significant number of patients might also be affected by sarcopenia. Sarcopenia is the loss of muscle mass, strength and endurance associated with ageing that occurs through mechanisms including reduced protein synthesis, mitochondrial dysfunction, malnutrition and altered levels of anabolic hormones including dehydroepiandrosterone (DHEA – see Section 1.6.2) (157). It is estimated that around 5% of muscle mass is lost per decade from the fourth decade onwards (158, 159), and although loss of muscle mass occurs in all individuals to an extent, it is accelerated by inactivity, poor nutrition and chronic illness (157). The prevalence of sarcopenia (as defined by muscle mass of more than two standard deviations below the sex-specific young-normal mean) has been reported as up to 15% amongst over 65 year-olds in the community (160), and numerous studies have shown that these changes are associated with reduction in functional measurements such as gait speed and stair-climbing speed (reviewed in (157)).

1.4.1.1 Skeletal Muscle Contraction and Fatigue

The three major muscle types are cardiac, smooth, and skeletal muscle; this section will discuss the basic structure and function of skeletal muscle, with regard to its role in peripheral fatigue in health and disease.

Skeletal muscle is found in muscles which are attached to the skeleton, are under voluntary control, and are used to provide movement to the body; a typical example is the biceps muscle, which is used to flex the arm around the elbow joint. Skeletal muscle cells, commonly referred to as muscle fibres, may range from 10 to 80 micrometres in diameter and

often extend for the entire length of whichever muscle they form part of. Each individual fibre is generally innervated by only a single efferent nerve known as a motor neuron, which runs from its origin in the spinal cord to an interface at the surface of the muscle fibre known as the neuromuscular junction. Each motor neuron innervates anywhere between a few and almost two thousand skeletal muscle fibres (161), all of which will therefore be stimulated together. Collectively a motor neuron and all of the muscle fibres that it innervates are known as a 'motor unit'; groups of motor units often work together to control the overall function of a single muscle, and the greater the number of activated motor units the greater the force of the overall muscle contraction (162).

Contraction of a muscle fibre is initiated when an action potential travels along the length of the motor neuron to the neuromuscular junction, causing the release of the neurotransmitter 'acetylcholine'. Acetylcholine diffuses across the neuromuscular junction and binds to receptors on the muscle fibre cell membrane, causing ion channels in the cell membrane to open; this allows an influx of sodium ions, depolarising the muscle fibre membrane and starting a cascade of events which culminate in the contraction of that fibre (162).

There are a variety of skeletal muscle fibre types, and they may be classified in a number of different ways based on either histochemical staining or function. Although in reality muscle fibres may not fall as neatly into discrete subtypes as was previously believed (161), when discussing fatigue it is still very useful to classify fibres by function, into 'fast-twitch' and 'slow-twitch' fibres:

- Fast-twitch fibres are about twice as large in diameter compared with slow-twitch fibres. They contain a high density of glycogen, and display high activity levels for the enzymes involved in rapid energy release through anaerobic pathways (the 'phosphagen' and 'glycogen-lactic acid' metabolic systems). These features allow

fast-twitch fibres to provide short bursts of explosive force, but only for eight to ten seconds at maximal power and up to two minutes at sub-maximal power.

- Slow-twitch fibres, in contrast, have features which make them more suitable for prolonged endurance at lower intensity. They have a higher concentration of mitochondria and myoglobin, as well as a higher capillary density, and the enzymes involved in the aerobic generation of energy from glucose, amino acids and fatty acids are much more active than in fast-twitch fibres. Though not capable of the same explosive power as fast-twitch fibres, the effective use of aerobic metabolism allows these fibres to sustain low-intensity exercise for a few hours or more in trained athletes.

Individual muscles are usually a mix of both of these types of fibre, although the proportions are known to vary from muscle-to-muscle and from person-to-person. All of the fibres in any individual motor unit will usually be of the same type (162), and so whole motor units are often described as being fast- or slow-twitch.

Although there are known to be exceptions to the rule, according to 'Henneman's size principle', we understand that when the body carries out a movement there is normally an orderly recruitment of motor units from smallest to largest potential force; in other words, the least powerful units will be recruited first, usually comprising slow-twitch muscle fibres, and the more powerful units of fast-twitch fibres will only be recruited when greater force is required (163). When healthy subjects become fatigued quickly during high-intensity exercise such as running a 100m race, this is largely due to the aforementioned limitations of anaerobic energy production within the more powerful fast-twitch motor units. However, as will be discussed later, people with chronic disease often complain of fatigue after very low-level exertion, or even in the absence of overt physical exertion. This might suggest that there is something different about their muscles, for example that there has been a shift in the ratio

of slow-twitch to fast-twitch fibres, or a reduction in the density of mitochondria within the slow-twitch fibres. Alternatively, perhaps they are experiencing central rather than peripheral fatigue.

1.4.2 Central Fatigue in Health – Interoception and the Central Governor Theory

During less intensive exercise (e.g. when running at around 5 m/sec), the generation of ATP can keep up with the energy demands of the muscles, and in theory a subject could continue exercising until their body's supply of carbohydrate was exhausted. This would normally take between one and three hours, depending on whether the subject had eaten a large carbohydrate meal recently and increased his or her muscle glycogen stores. If the speed were further reduced to running at about 2-3 m/sec, the metabolism of fat could also provide sufficient energy and, again in theory, exercise could continue for tens of hours since there are large quantities of fat in the body (164-166). However although elite athletes can do this, the average person finds it difficult to exercise for more than about 30-40 minutes at 60-70% maximum HR. Why should this be? There is little evidence of peripheral muscle failure or that cardiac output has reached a limit in such instances, so the conclusion is that there has been some failure of motivation, desire, or 'drive' of the subjects to continue to exercise; this loss of central drive to use muscles to their full potential is sometimes referred to as 'central activation failure' (CAF) (167, 168), and is one manifestation of what was referred to earlier as 'central fatigue'.

The mechanisms within the brain that lead to exercise-induced central fatigue have been the subject of research for the last 30 years without a definitive answer. One thing we know is that central fatigue during prolonged exercise is sensitive to high body temperature (169) and that exercise can be prolonged by 'precooling' (170). It has been suggested that when body temperature during exercise begins to rise to dangerously high levels (~40^{o}C) there is a

protective reflex that comes into play which reduces the level of exercise and hence prevents a further rise in body temperature. This type of evidence has given rise to the 'central governor' theory of fatigue ((171), critiqued in (172)) which says that exercise intensity and duration is regulated subconsciously to maintain homeostasis. According to this theory the brain has a subconscious image of 'self' which defines the safe limits of activity, and it monitors multiple physiological processes and regulates exercise intensity to keep the body working within safe limits – for example by preventing a dangerously high temperature. In these circumstances the subject's feelings of fatigue are their conscious perception of the subconscious workings of the brain pathways involved in these protective reflexes. Body temperature may be one factor that the brain monitors but there are many other consequences of physical activity: increased heart rate, ventilation, high forces in muscles, tendons and in joints as well as the stimulation of chemo- and mechanical receptors in muscle. There will also be changes in blood metabolite and hormone levels and circulating cytokines.

This subconscious monitoring of the internal physiological state of the body is known as 'interoception' (173), and the sensitivity of these pathways may vary between people and with the state of training; in one individual a very sensitive pathway may shut down exercise very rapidly with a large safety margin, whereas other people may operate closer to the limits. Thus an untrained person may be very sensitive to dyspnoea, body temperature or pains in his or her muscles when running, but with training their interoception becomes less sensitive and there is increased tolerance of these sensations (174).

1.4.3 Central Fatigue in Ill Health

What is it that prevents patients with chronic illnesses from exercising to a similar extent as healthy people? Patients with orthopaedic, cardiac or respiratory disease have obvious 'peripheral' reasons to stop exercising prematurely, but in the absence of such problems most

patients should have enough muscle glycogen to support low intensity exercise for at least an hour or so. Quite possibly if the reward was high enough, or the danger threatening enough, they could do it, but it would be a constant mental battle to keep going despite an overwhelming urge to stop, and afterwards they would feel completely exhausted for hours or even days. If we consider this in terms of interoception and the Central Governor theory, then chronic illness seems to cause interoception to become much more sensitive, so that afferent signals from working muscles or joints that in health would be unexceptional now become unpleasant and inhibiting, and the brain's subconscious image of self becomes altered to a much lower level. In this way, the central fatigue associated with chronic illness is remarkably similar to what is known in the setting of acute illness as 'sickness behaviour' (175).

1.5 Sickness Behaviour

Everyone who has had a significant viral or bacterial infection has experienced sickness behaviour at first hand: when sick, we all exhibit traits of fatigue, anorexia, disinterest in surroundings and activities, and depressed and irritable mood. Although I have introduced it in the context of physical exercise, it also encompass other aspects including mental activity, social interaction, depression and anxiety, in much the same way as the chronic fatigue associated with long term illness was described by the qualitative studies outlined earlier in this chapter (Section1.2).

Sickness behaviour is clearly an evolutionarily advantageous behaviour pattern: by reducing activity and interaction it protects a wounded animal from danger at times of physical vulnerability, and once an acute illness has resolved the person or animal would then be expected to resume normal behaviour. But what are the biological mechanisms underlying

sickness behaviour, and why in chronic illnesses such as AAV or RA do these patterns of behaviour seem to persist even after a severe acute flare has settled? There is circumstantial, experimental and clinical evidence to suggest that sickness behaviour in acute illness and chronic fatigue in the setting of long-term illness are both caused by actions of the immune system on the brain – and in particular the actions of pro-inflammatory cytokines.

1.5.1 Circumstantial Evidence

During the early stages of an acute illness such as a bacterial or viral infection, the body's immune response is coordinated through a massive release of pro-inflammatory cytokines, and this is also the time when fatigue and the other symptoms of sickness behaviour are at their worst. When infections and other brief illnesses resolve, cytokine synthesis settles down to very low baseline levels, and with this settling of the inflammatory response there is a corresponding return to normal behaviour patterns. In contrast, it is known that in many long-term illnesses such as RA (176, 177), SLE (176, 178), MS (179), chronic liver disease (180) and AAV (our own data, unpublished) cytokine levels never return to the very low levels seen in health, and all of these diseases are characterised by fatigue which is chronic rather than acute in nature. Some studies have shown direct correlation between the concentration of pro-inflammatory cytokines and disease activity (176, 178), but as will be discussed later, the evidence of a direct correlation between cytokine concentrations and the severity of fatigue itself is tenuous.

1.5.2 Experimental / Clinical Evidence

Injection of IL-1β or TNF-α into rats and mice has been shown to directly induce not only fever but also sickness behaviour, and further experiments suggested that the resultant sickness behaviour was mediated primarily by central actions of IL-1β on receptors within the CNS (181-184). Similarly, injection of IL-6 in humans induced anorexia and fatigue

(185) (both hallmarks of sickness behaviour), and when Interferon has been used therapeutically one of the major side-effects was fatigue (186). Injection of IL-6 was further shown in mice to induce increased concentrations of serotonin within the brain (187), and with this in mind it is interesting that a study of patients with CFS found evidence suggestive of the up-regulation of hypothalamic serotonin receptors (188). There is even some evidence that fluoxetine (a selective serotonin reuptake inhibitor, SSRI) can treat fatigue in patients with fibromyalgia (189); however this finding was not replicated in CFS (190) and the beneficial effect could possibly be explained by improved depression rather than any direct effect on central fatigue pathways.

1.5.3 How Could Systemic Inflammation Influence the Brain?

Despite all the evidence discussed, until the last decade it was difficult to conceive of any mechanisms by which peripheral inflammation could exert influence on the brain to cause sickness behaviour. The brain was considered to be an 'immune-privileged' organ because, in the absence of an intrinsic CNS injury or illness, inflammatory cells and cytokines are unable to cross the blood-brain barrier (BBB) (191). However we now know that not only does the immune-protection offered by the BBB vary with age and brain region (192), but also that the meninges and parts of the brain parenchyma actually contain their own immune cells such as macrophages (known in the brain parenchyma as microglial cells) and dendritic cells (193). Researchers have now identified four ways in which peripheral inflammation can be monitored by the brain, which probably act in parallel to result in the changes we see in sickness behaviour:

- *Synthesis and release of cytokines within the CNS.* Observational work on patients with RA demonstrated that concentrations of IL-1β within the cerebrospinal fluid (CSF) of patients were markedly elevated compared with serum concentrations in

those same patients, and also compared to the CSF cytokine concentrations of healthy controls, and that there was a good correlation between CSF IL-1β concentration and subjective fatigue ($r=0.55$, $p<0.05$). Follow-on work in mice with 'serum-transferred arthritis' showed that IL-1β mRNA and TNF mRNA levels within the spinal cord were increased at the peak of peripheral arthritic disease activity (194). This work along with other studies demonstrates that cytokines can be synthesised within the CNS itself (195-197).

It has subsequently been shown that at the BBB there are IL-1 receptors on perivascular macrophages and the endothelial cells of brain venules (198-200); when stimulated by IL-1 these cells synthesise and release prostaglandin E2 into the CSF (reviewed in (201)), and this prostaglandin then acts as a stimulant for cytokine production by microglial cells within the brain parenchyma (194).

- *Direct transport of cytokines across the BBB*. Transport mechanisms have been identified for IL-1β, IL-6, and TNF-α (202). It has been suggested that these receptors have too low a capacity to be a significant mechanism in the acute phase of illness (201), but they can transport quantities of cytokines that are comparable to the passage of therapeutic doses of morphine across the BBB (202), and could be an important mechanism in long-term illnesses where there is low-level grumbling inflammation.
- *Passage of cytokines via the circumventricular organs*. Circumventricular organs are structures in the brain that are characterized by their extensive vasculature and lack of a normal blood brain barrier (BBB) (203). The lack of a BBB allows cytokines to enter the brain by volume diffusion (204) at these points.

- *Signalling to the brain through the activation of peripheral nerves*. Sensory neurones of the vagus nerve express receptors for IL-1β and are thus activated by IV injection of IL-1β (205). Following on from this, it has been found that sickness behaviour secondary to intraperitoneal or visceral inflammation can be blocked or reduced by resection of the vagus nerve without compromising the peripheral immune response (206-208). Cytokines are similarly capable of activating the trigeminal nerve during oro-lingual infections (209). It is believed that these afferent nerve signals may sensitise target brain structures for the production and action of cytokines (210).

The pathways which subsequently translate these cytokine and afferent nerve signals within the brain into the observed aspects of sickness behaviour remain elusive (193), and are the focus of ongoing research.

1.6 Hormones Implicated in Central Fatigue

Cytokines are not the only chemicals in the body which have been implicated in central mechanisms of fatigue; a number of hormones have also been the subject of investigation, and perhaps the two hormones of most interest are corticotrophin-releasing hormone and dehydroepiandrosterone.

1.6.1 Corticotrophin-Releasing Hormone (CRH)

CRH is a peptide hormone secreted by the paraventricular nucleus of the hypothalamus in response to stress, and with its effector the pituitary-adrenal axis and the sympathetic and autonomic nervous systems it is one of the central pillars of the body's stress response system (211). The best-known function of CRH is to stimulate release of ACTH by the anterior lobe of the pituitary gland, which in turn stimulates production of other hormones in the pituitary gland including cortisol and dehydroepiandrosterone. However this is not its only role: CRH-

containing nerve fibres extend from the hypothalamus into brainstem autonomic nuclei and many other parts of the brain, and CRH has been localised within a number of these areas including limbic and autonomic structures; it is therefore thought that CRH is involved in not only the hormonal responses to stress, but also the behavioural and autonomic responses (212, 213).

There is evidence of abnormal synthesis and release of CRH in some of the human diseases in which chronic fatigue is a prominent symptom. The most direct evidence comes from studies in CFS, which have repeatedly shown abnormalities of the hypothalamic-pituitary-adrenal axis, and in particular reduced CRH secretion by the hypothalamus (214, 215). Indirect evidence of reduced CRH synthesis and / or release has also been shown in SLE (216), RA (217), MS (218) and primary biliary cirrhosis (PBC) (154). Experimental evidence in rats suggests that the effect of chronic stress on CRH production and release might depend on what the stressor is: whereas various physical and psychological stressors have all been shown to induce an up-regulation of CRH in the hypothalamus (219-221), animal models of immune-mediated arthritis (222), SLE (223), MS (224) and cholestatic liver disease (225) all suggest that if that stressor is inflammation then there is instead a reduction in CRH synthesis and secretion. In reviewing this evidence, Swain (154) suggested that fatigue in these chronic inflammatory diseases may be due in part to defective CRH release.

1.6.2 Dehydroepiandrosterone (DHEA)

In humans DHEA is synthesised from cholesterol primarily in the adrenal glands, with smaller quantities produced in the gonads and in the brain. Most DHEA in the blood is not found in its free form, but rather as the sulphate ester dehydroepiandrosterone sulphate (DHEAS), with serum levels of DHEAS around 300 times greater than free DHEA; conversion between the two forms is reversible, and occurs in the adrenals, liver, small

intestine and many other peripheral tissues. DHEAS is often measured in preference to DHEA not only because it is more abundant, but also because DHEA concentrations ebb and flow in a diurnal variation, whereas DHEAS concentrations do not. It is known that endogenous DHEA production increases in response to exercise in humans (226, 227) and calorie restriction in primates (228). DHEA levels decline slowly with age – a process which, coupled with the relative glucocorticoid excess that often occurs in older people, is referred to as 'adrenopause' (229).

The main function of DHEA is probably as a precursor to androgens and oestrogens, but over the last couple of decades it has been discovered not to be inert, but rather to have roles in obesity, aging, stress and immune responses amongst other effects (230). There is evidence for example in older individuals (229, 231, 232) that adrenopause is important in many of the changes associated with aging, including cognitive impairment, ischaemic heart disease, immunodeficiency, malignancies and osteoporosis. It is extremely difficult to prove to what extent these effects are caused by DHEA itself or whether they are entirely effects of its metabolites, but for example DHEA is known to be a full agonist in its own right of the ERβ oestrogen receptor, and because the normal physiological concentrations of DHEA in the human body are fairly high it probably acts as an important endogenous oestrogen (233). It also has a low affinity to the androgen receptor, but this is so much weaker than testosterone as to be insignificant (234). It does not bind to the glucocorticoid or mineralocorticoid receptors, and in fact it has an anti-glucocorticoid action, partly though effects on the enzyme 11β-HSD1 which is essential for the synthesis of glucocorticoids such as cortisol (230).

Studies in many patient populations including CFS, SLE, MS and PSS (235-242) have shown that DHEA levels are often reduced. This is unsurprising, since chronic inflammation results in reduction of DHEA and DHEAS levels, with IL-6 and TNF playing an important role (243). Compounding the effects of inflammation, treatment of such illnesses usually includes

the use of corticosteroids, which have also been shown to suppress DHEA and DHEAS levels as a result of the suppression of pituitary ACTH secretion (244). Some studies have shown that low DHEA levels are associated with fatigue (235, 240), and low levels of androgen steroids (synthesised from DHEA) have also been associated with fatigue in AAV (114), but other studies have not shown a correlation between lower DHEA levels and greater fatigue (236, 238). Similarly, although some interventional studies of DHEA replacement have shown improvements in wellbeing, psychological symptoms and fatigue (245-247), others have been negative (237, 241, 248, 249).

1.7 Chronic Fatigue Syndrome (CFS)

CFS is an acquired illness which is primarily characterised by severe, debilitating fatigue which is aggravated by physical or mental exertion and is not relieved by rest (250) – in other words, fatigue which is very much the same as that associated with sickness behaviour, and with AAV and many other chronic illnesses. What is fundamentally different between CFS and sickness behaviour or the fatigue of chronic illness is that in CFS there is no known specific cause, and there is no diagnostic test; indeed the name was chosen in 1988 specifically because it included no statement of aetiology (251). The diagnosis is instead made on the basis of exclusion of other illnesses, subjective clinical assessment and patient self-report (252). The fatigue found in CFS is often extremely severe, with a profound impact on function in personal, social and professional life and resultant economic consequences for sufferers and society (253, 254).

Because there is no diagnostic test, clinicians and researchers must diagnose CFS on the basis of ‘case definitions’, in other words “an approved criterion of disease specific signs and symptoms, which is used to determine the presence of a disease or health complaint” (255).

Unfortunately there has not been agreement on how best to diagnose the disease, resulting in there being at least six commonly used case definitions (250). This is not merely an inconvenience for clinicians and researchers - it has been estimated that the prevalence of CFS in the adult population can vary between 0.007% and 2.8% depending on which case definition is used (256). The 1994 US Centres for Disease Control and Prevention (CDC) Case Definition (257), which is the most common case definition used, lists the following diagnostic criteria:

- The fatigue must have been persistent or relapsing for at least six months;
- There should be significant reductions in previous levels of occupational, educational, social and personal activities;
- The fatigue cannot be explained by medical or psychiatric conditions;
- It must be accompanied by at least 4 of 8 self-reported symptoms: sore throat; tender lymph nodes; muscle pain; arthralgia without joint swelling or redness; new headaches; unrefreshing sleep; post-exertional malaise lasting more than 24 hours; cognitive dysfunction (impaired short-term memory or concentration). These symptoms must also have been present for >6 months and must not have predated the fatigue.

CFS affects all ages including children, and although it affects people of both genders, and across all ethnicities and socioeconomic strata, it has been shown that it is twice as common in women, and that healthcare workers have a higher prevalence than those in any other occupation (258). The cause of CFS is unknown; suggested causes include neuroendocrine abnormalities (giving rise to the alternative name of ‘myalgic encephalomyelitis’ or ME which is preferred by many patient groups), Epstein Bar Virus infection (post-viral fatigue syndrome), immunological abnormalities (chronic fatigue immune dysfunction syndrome),

genetic predisposition and psychological trauma, but no one of these factors can be attributed to more than a small percentage of all the cases of CFS. Various models have been proposed to explain CFS, but as with sickness behaviour and the chronic fatigue associated with illnesses such as RA or MS, the best explanation is probably that of an altered representation of self and an increase in the sensitivity of interoception. According to this theory an increased sensitivity to afferent pain signals would be expected and this has been described in a limited number of studies.

Because the cause of CFS is unknown, the treatments used are the same as those used in the fatigue of chronic illness where there is no identifiable reversible factor, as described in Section 1.11. The prognosis of the illness is not good: a systematic review of 14 studies showed that the median full recovery rate was only 5% (range 0-31%), and the median proportion of patients who improved during follow-up was 39.5% (range 8-63%). Good prognostic factors were less severe fatigue at baseline, a sense of control over symptoms, and not attributing the illness to a physical cause; return to work was only measured in three studies, and ranged from 8-30% (259).

1.8 Fatigue in Autoimmune Disease

Fatigue is a common symptom of virtually all known autoimmune diseases, and because some of these other autoimmune diseases are significantly more common than AAV, there is a correspondingly larger body of existing fatigue literature. For example RA has a prevalence in the UK of around 0.8% (260), and up to 80% of patients with RA describe fatigue (261). Repeated studies have shown that fatigue is at least as prevalent and severe a symptom of RA as is pain (262, 263) and that most patients consider fatigue to be both more important and more difficult to deal with (136, 264, 265). This has led to the RA research community

showing increasing interest in fatigue over the last decade, with an international consensus in 2006 that it should be measured in all subsequent clinical trials (266). Studies have shown it to be no less of a problem in other autoimmune diseases – for example the prevalence of fatigue is 90% in SLE (267), 68% in primary Sjögren's syndrome (PSS) (268) and 78% in MS (269).

1.8.1 Fatigue, Disease Activity and Inflammation

Despite numerous studies across different diseases, controversy remains over whether disease activity is a predictor of fatigue in autoimmune disease. One early study found that in a cohort of 59 SLE patients with severe fatigue there was a significant correlation between fatigue and the physician's clinical rating of disease activity (270), another study found that although disease activity, depression and helplessness all correlated with each other and with fatigue, only disease activity was a predictor of future fatigue three months later (271), and a number of other studies in SLE have also shown evidence of significant correlation between fatigue and disease activity (272-275). However, most of the studies in SLE which have shown a correlation with disease activity utilised a disease activity tool known as Systemic Lupus Activity Measure, which actually includes perceptions such as fatigue as component scores. Studies in SLE which have used other instruments to assess activity (276), and most studies in RA, have found no significant correlation between fatigue and disease activity (262, 277), and nor did a study of 88 patients with primary biliary cirrhosis (PBC, an autoimmune liver disease) (278). There is similarly conflicting evidence on whether fatigue worsens with disease duration: whilst some studies have shown higher levels of fatigue amongst those who have had RA for a longer time (279, 280), others have found greater fatigue amongst those patients with a more recent diagnosis (281), and there was no association found between disease duration and fatigue in PBC (278).

As described in Section 1.5, pro-inflammatory cytokines probably have an important role in inducing sickness behaviour, and therefore could be at least partly responsible for central mechanisms of fatigue. There is also a great deal of evidence for a link between inflammation and depression (reviewed in (282)). It is puzzling therefore that although some clinical studies have shown a correlation between higher circulating cytokine levels and greater fatigue (283), many have not (133, 284, 285), but there are a number of very plausible explanations. Firstly, perhaps the wrong fluid is being sampled: clinical studies of fatigue have almost invariably measured serum cytokine levels, but a study by Lampa et al in RA patients showed that CSF concentrations of IL-1 were much higher than those in the serum, perhaps reflecting local production within the CNS (194); if this is true then only CSF levels would be expected to correlate with fatigue, as indeed they did in that study. Alternatively it could be a technical issue, as cytokines are relatively difficult to store and test without degradation of samples. It could be an issue of timing: cytokines concentrations appear to have a circadian rhythm, perhaps explaining why many symptoms of inflammatory diseases have a rhythmical pattern of severity; the 'perfect' time for sampling probably does not exist, as it has been shown that the peak concentrations of different cytokines occur at different times in the 24hr cycle, and that the cycle is altered in RA depending on severity of disease, but undoubtedly all study participants should have samples taken at approximately the same time of day (147). Finally, most studies that have attempted to look at the relationship are relatively small, and since there are so many other factors influencing fatigue levels (particularly the psychosocial influences discussed below) it may be that the influence of cytokines is not strong enough to show statistical significance in small studies.

1.8.2 The Interplay between Psychiatric Disease, Psychosocial Factors and Fatigue

In primary care fatigue is strongly correlated with depression (286), and this finding has been replicated in autoimmune disease. Higher levels of fatigue in RA are predicted by 'trait anxiety' (287) (a tendency to respond with anxiety in anticipation of threatening situations), current depressive or anxiety symptoms (277) and also by a lifetime history of mood disorder (288). Longitudinal fluctuations in mood appear to affect fatigue also: patients with RA reported higher levels of fatigue during the same annual assessments at which they also reported lower mood, a finding most marked in those individuals with less aggregate depressive symptoms (288); and patients with SLE who reported the greatest increases in anxiety and depression over the first nine months of a study subsequently reported greater fatigue at the fifteen month time-point (289). There appears to be a similarly strong correlation between fatigue and depression in PBC (278). An early study by Krupp (290) in patients with MS found no association between fatigue and depression, but this was a small study and such findings are in the minority.

Several studies appear to demonstrate a link between pain and fatigue in inflammatory disease. For example, on days when patients with RA reported increased pain they also reported increased fatigue (291), and the association is replicated in other RA studies (277) as well as studies of juvenile rheumatic disease (292, 293). Pollard et al found that pain and depression were the two characteristics that most strongly correlated with fatigue in RA (261).

Sleep also seems to be an important predictor of fatigue. Patients with RA who reported poor sleep in one study also had higher levels of pain and fatigue (294), and in a study of 1488 patients with rheumatic disease (a mixture of patients with RA, osteoarthritis and FMS were studied), 90% of fatigue was explained by pain, sleep disturbance and depression (262). A number of SLE studies have shown that patients with poor sleep have higher levels of fatigue

(273, 295-297), and in PBC, sleep and depression were the two strongest predictors of fatigue (278).

Other psychosocial factors may also be important determinants of fatigue. In RA, for example, those patients who perceived themselves to have less help at home experienced greater fatigue (287), as did those with a higher level of functional disability (287, 298). There is a suggestion that the coping mechanisms used to deal with fatigue might be important, with an association found between avoidant coping and greater fatigue (299).

1.9 Fatigue in Cancer

Cancer-related fatigue (CRF) is almost universal among cancer patients (300), and has the same wide-ranging effects on all aspects of patients' lives that are seen in autoimmune disease; it is also similarly disproportionate to the patient's level of exertion and is not relieved by rest or sleep (301). Most commonly fatigue in cancer is associated with radiotherapy and chemotherapy treatments; whilst a positive aspect of this is that patients may find it more predictable, there is some evidence that the onset of fatigue outside of therapy courses can cause fear of disease progression for patients (302). Fatigue often begins before the diagnosis of cancer is made (303), and may continue for a considerable amount of time beyond the end of treatment (304, 305). The MFI-20 has shown that fatigue continues to affect patients after the start of treatment across all five dimensions (306, 307). Although it has been suggested that research into CRF lags behind research into cancer-related pain, nausea and vomiting (308, 309), it has still received more attention than fatigue has done in most other diseases due to the sheer number of people who suffer from cancer and CRF.

1.9.1 Prevalence and Impact

The prevalence of CRF is influenced by patient characteristics, primary malignancy, and the type and intensity of treatment, but has been variably reported in 60-99% of cancer patients who undergo treatment with chemotherapy, radiotherapy or both (310-314). Indeed it is so prevalent and severe that CRF is now a distinct diagnosis in the International Classification of Diseases 10th Revision (ICD-10). The prevalence and impact of CRF during and after chemotherapy was assessed in a large telephone interview study of 379 patients (315), 62% of whom had breast cancer. When patients were asked to identify the side-effect which had affected them most during chemotherapy, 18% reported fatigue (second only to nausea, 34%), and fatigue was the symptom with the greatest impact during the period after completion of treatment (25%). 76% reported that they had suffered from fatigue on at least a few days each month during their most recent chemotherapy, with 30% experiencing fatigue every day. Patient characteristics associated with fatigue included female gender and age >55years, as well as depression and pain ($p<0.05$ for all). Patients reported reduced physical activity across a variety of measures, and also reported the need for an average of 2.8 additional hours of sleep per day; 88% stated that they had to alter their daily routine due to fatigue, and on average they reported only being able to complete 55% of the activities they would normally have performed. It also had a profound psychosocial impact, with effects on motivation, mood and cognition. Of the 177 patients who had been employed prior to cancer diagnosis, 75% changed their employment status as a direct result of fatigue, with 28% leaving work altogether.

1.9.2 Aetiology

Many of the determinants of fatigue in cancer are poorly understood, but a review of the causes by Wagner and Cella (91) listed them under the following construct:

1. Direct effects of cancer and tumour burden;
2. Treatment side-effects – chemotherapy, radiotherapy, surgery and medication;
3. Psychosocial factors – coping with chronic illness, anxiety and depression;
4. Exacerbating comorbid symptoms – chronic pain, sleep disturbances, deconditioning;
5. Comorbid medical conditions – Anaemia, malnutrition, thyroid dysfunction, infection.

The first point on this list is obviously unique to cancer, but otherwise the list is practically identical to the causes of fatigue in autoimmune disease (e.g. Hewlett's construct in RA (106)). Reviews of CRF have implicated cytokines, serotonin and the hypothalamic-pituitary axis in the pathogenesis of the symptom (309), and a study by Bower et al suggested that CRF may be associated with a T-cell mediated inflammatory process (316), all of which are theories which have been discussed in this chapter in the context of autoimmune disease.

In summary the prevalence, impact and proposed causes of CRF are all remarkably similar to those in auto-immune disease, suggesting that fatigue in chronic disease may have much more to do with the biological and psychosocial processes that are common to all long-term illnesses than anything which is unique to a particular disease.

1.10 Fatigue in AAV

Compared to diseases such as cancer, RA and CFS, there has been little fatigue research conducted in AAV, perhaps because the monitoring of patients with AAV has traditionally been focused on what doctors thought was important rather than patients' priorities. The tools used to assess the disease, such as the Birmingham Vasculitis Activity Score (BVAS) (317, 318) and the Vasculitis Disease Index (VDI) (319) were designed without any patient input, and their purpose is to help doctors to know if there is any active inflammation, or any damage to specific target organs, rather than how the patient is feeling subjectively. This

might be due to the relatively recent improvements in patient mortality – issues of quality of life for patients with AAV have only come to the fore in the last few decades in parallel with improving survival.

In the late 1990s awareness began to grow of the huge impact AAV was having on health-related quality of life (HRQOL), as the disease became a chronic relapsing-remitting disease for many patients rather than a fatal one. In 1998, the first study to attempt to use patient-reported outcome measures (PROMs) in AAV was a questionnaire study of 60 patients by Hoffman et al (320) which investigated the patient-perceived effects of GPA on health, function, income and relationships. 80% of the patients surveyed said that their activities of daily living were compromised, and the effect on employment and income was also huge: 57% of patients had to take sick-leave for more than six consecutive months, 51% had to reduce their work hours, 31% had to completely leave work on disability benefits, and the average income one year after diagnosis had dropped by 26%.

That study stimulated interest in PROMs in AAV, and three significant studies were all reported in 2002. One of those studies had a similar focus to Hoffman's study, this time administering a questionnaire to 60 patients with GPA who were under the age of 40 at diagnosis (321). The researchers found that 27% of those who were employed at time of diagnosis had left work and gone onto work disability benefit within 39 months, and that the risk of this was three-fold greater for women than for men. Those who were unemployed suffered a considerable reduction in HRQOL compared with those who were employed, independent of disease factors. Another questionnaire study of 701 GPA patients also reported significant effects of the disease on disability and employment, although they found that the impact was less in patients who were diagnosed in the 1990's than those diagnosed in previous decades (322). A questionnaire study by Boomsma et al looked at 79 patients with

GPA and 114 patients with SLE, and found that both diseases had profound effects on levels of disability, employment, mood and personal relationships (323).

Although those earlier studies helped to increase awareness of the socioeconomic, psychological and inter-personal effects of AAV, the first study to comprehensively address patients' subjective experiences and priorities in AAV was only published as recently as 2010. Herlyn et al (324) conducted a questionnaire study of 264 patients with AAV across three countries (USA, Germany and the UK) which, in addition to questions about demographics and disease activity, also asked the patients to rate to what extent 40 different symptoms related to AAV had impacted upon their lives. Of the symptoms that more than 50% of patients reported having experienced, the five most severe were: 'Tiredness/Fatigue' (reported by 95% of patients, mean rating 3.5 / 5); 'Loss of Energy' (reported by 93%, mean rating 3.4); Weight gain (76%, mean 3.1); Joint pain (83%, mean 2.9); Sinusitis (70%, mean 3.0); severe organ manifestations such as seizures, CKD and oxygen dependency were all rated lower by patients. In a free text section where patients were asked to list the five most important aspects of the disease in their daily life, fatigue/energy loss (75%), pain (31%) and musculoskeletal symptoms (24%) were listed most frequently. Herlyn's study was the first to illustrate this startling difference between what health care professionals thought was important in AAV and the priorities of patients. It brought the problem of fatigue in AAV to the fore as a major cause of reduced HRQOL, highlighted the fact that none of the disease-monitoring tools currently being used clinically or in AAV research included fatigue in their scoring schemes, and gave a compelling argument for the inclusion of measures of PROMs in all future studies in AAV.

The underlying cause of fatigue in patients with AAV is not understood. The inflammatory nature of the disease might be critical, because even during disease remission patients with AAV have evidence of inflammation with elevated circulating pro-inflammatory cytokines

such as IL-1, IL-6 and TNF-α (our unpublished data and (325)); however it has already been shown that data in other autoimmune diseases has not shown a consistent link between either disease activity or inflammation and fatigue. Another possible cause could be end-organ damage – for example, it is known that many patients with AAV have renal involvement and are left with degrees of renal impairment varying from mild CKD to dialysis-dependence (326). Previous studies have shown that the prevalence of fatigue in haemodialysis patients is around 70%, and that the correlates of fatigue in CKD are virtually the same as those in cancer, including: comorbidity, raised body mass index (BMI), inflammation, lack of physical exercise, poor mental health, pain and sleep problems (92, 93). These possible causes can be most easily explored by dividing them again into causes of peripheral fatigue and causes of central fatigue.

1.10.1 Potential Causes of Peripheral Fatigue in AAV

In a previous study by our group patients with AAV cited muscle fatigue rather than breathlessness as a reason for exercise termination, and peripheral muscle weakness was significantly correlated with reduced exercise tolerance (327). No studies have previously systematically examined whether peripheral muscular problems contribute to fatigue in AAV, but there are a number of reasons why this might be the case:

1. *Loss of Muscle Mass or Contractility*: As with other chronic inflammatory illnesses, we might expect significant muscle atrophy in AAV, leading to fatigue due to muscular weakness as described previously (153, 328, 329). Reduced muscle strength may also be due to impaired peripheral nerve conductivity due to inflammation-induced epineural endothelial damage (330, 331). Alternatively, pro-inflammatory cytokines may affect muscle contractility by reducing the resting membrane potential of muscle and nerve (332, 333). In elderly patients, serum TNF-α concentration was

associated with reduced muscle endurance (334), although it might be argued that this finding could be due to a central mechanism. In addition to the direct effects of inflammation on muscle, corticosteroids and cyclophosphamide, the mainstay of treatment in AAV, are known to reduce myofibrillar mass and may also alter aerobic metabolism via reductions in mitochondria (335-337). Corticosteroids may induce the development of the metabolic syndrome, including insulin resistance. Insulin resistance results in impaired amino acid uptake into the muscle fibre, reduced protein synthesis, and increased protein degradation resulting in a progressive loss of muscle mass (338, 339).

2. *Blood supply and oxygen delivery to the muscles*: Microangiopathy resulting in reduced oxygen delivery to muscle cells has been suggested as an important cause of lowered exercise capacity and increased fatigue (340) in SLE, and similar pathological changes could occur in AAV. In health, various studies have shown a 40-50% increase in the number of perfused muscle capillaries, and up to a 100% increase in capillary blood volume, in response to physiological increases in insulin, and even larger increases are seen during exercise (341, 342). TNF-α, has been shown to block insulin-mediated microvascular recruitment (341, 343). This blunting of the haemodynamic effects of insulin and exercise by TNF-α may reduce glucose and oxygen transport to muscles, possibly leading to premature fatigue (344).
3. *Uraemia:* CKD in many patients with AAV may cause selective muscle weakness, as uraemia affects fast twitch fibres more than slow twitch (345).
4. *Deconditioning of Skeletal Muscle:* The effects of reduced muscle strength are often compounded by "deconditioning", which lowers muscle mitochondrial content, resulting in enhanced glycogen breakdown, glycolysis and acidosis, stimulating muscle afferents leading to an exaggerated cardiovascular response to exercise (346).

5. *Reduced Cardiac* Output: Maximal sustained muscle power output is partly determined by cardiac output, which in turn is determined by stroke volume. Stroke volume decreases with age, inactivity, or inflammation (all of which are features of AAV), as a result of which normal activities are carried out at a higher percentage of maximum aerobic capacity than would otherwise be the case, and at a higher heart rate (347). Additionally, as explained above, the increased metabolic demand of deconditioned muscles with a low oxidative capacity results in an exaggerated stimulation of the muscle heart reflex (346, 348). Collectively these mechanisms can cause normal physical activities to be more readily experienced as exhausting.
6. *Ischaemic Heart Disease*: Systemic inflammation is an independent risk factor for ischaemic heart disease (349, 350).

1.10.2 Central Fatigue in AAV

The literature reviewed in the previous sections suggests that fatigue in many chronic illnesses might be primarily central in origin, and there is some evidence that this might also be the case in AAV.

One Danish study of 68 GPA patients in 2010 found no association between HRQOL and any measures of disease extent or severity, although it did not look at whether fatigue or any other psycho-social factors might have explained the reduced quality of life in that cohort (351). A questionnaire study by Basu et al, also in 2010 (352), attempted to identify which factors in AAV contributed to reduced HRQOL: patients were twice as likely as healthy controls to suffer from mild/moderate fatigue (OR 2.0; 95% CI 1.1-3.8) or severe fatigue (OR 2.5; 95% CI 1.4- 4.5), and fatigue appeared to be a major determinant of HRQOL. As in the aforementioned Danish study, fatigue did not correlate with disease activity or damage. A US study of 55 patients with GPA found no association between fatigue and disease duration,

damage or activity, or with steroid use; rather, significant correlations were found between fatigue and FMS, sleep disorder and depression (353). Another British questionnaire study of 51 patients with AAV found that patients who experienced moderate-to-severe pain had significantly reduced HRQOL as well as significantly greater fatigue, depression and sleep disturbance than those who reported little or no pain. The authors hypothesised that pain was a major determinant of HRQOL, but acknowledged that longitudinal work would help to establish causal pathways (354).

No studies thus far have attempted to identify the mechanisms linking these psychosocial factors with fatigue, and no studies have attempted to study the treatment of fatigue in AAV either.

1.11 Treatment of Fatigue

Studies have shown that patients believe health professionals dismiss their fatigue (102), forming a significant barrier to treatment. Numerous treatment strategies exist however, and although the evidence of efficacy is variable, the widely held view among patients and physicians that fatigue is unmanageable is probably not justified. The first step in managing chronic fatigue in any patient must be to look for easily correctable causes such as anaemia, hypothyroidism, electrolyte imbalances, or culprit medications, as highlighted by the National Comprehensive Cancer Network (NCCN) guidelines for cancer-related fatigue (300); the second step, which will be the focus of this section, is the pharmacological and non-pharmacological management of fatigue that either occurs in the absence of, or persists following the treatment of such correctable causes.

1.11.1 Pharmacological Treatments

A number of pharmacological treatments might help treat fatigue in patients with AAV, particularly for individuals who have a readily reversible cause of fatigue. For example, treating anaemia (which might be due to an associated CKD) with erythropoiesis-stimulating agents would be expected to help, as might treating a co-incidental co-morbidity such as hypothyroidism, or withdrawing drugs known to cause fatigue such as benzodiazepines or beta-blockers. Depression was shown earlier to be an important predictor of fatigue, and antidepressants have shown some promise in reducing fatigue in FMS (355); therefore depression and anxiety should be actively looked for and treated in any patients with chronic fatigue regardless of which long term condition they have.

In RA, several studies have shown improvements in HRQOL (of which fatigue is a component) with the administration of drugs that are designed to reduce inflammation, including the biological agent infliximab (an anti-TNF-α antibody that was previously used to treat AAV but has not been shown to be effective). The largest RCT looking at the use of infliximab in RA was ATTRACT, in which 428 patients received infliximab or placebo in addition to methotrexate (356). In ATTRACT, all groups achieved statistically significant and clinically meaningful improvements in HRQOL as measured by the physical component score (PCS) of the SF-36 quality of life questionnaire, but whereas the 'methotrexate plus placebo' group achieved a median score improvement of only 2.8 at two years, the 'infliximab plus methotrexate' groups achieved an overall improvement of 6.4 ($p=0.011$ for the lowest dose, and $p<0.001$ for higher doses). A smaller RCT also showed that infliximab improved HRQOL more than methylprednisolone in patients with active RA (357). It should be noted that although the PCS of the SF-36 does include a measure of fatigue ('vitality'), both of these trials found greater improvements in some of the other subscales which contribute to the PCS, particularly 'bodily pain', and that observational work by Pollard et al

found that most of the improvement in fatigue scores with anti-TNF therapy could be explained by improvements in pain (261). However, since pain has been shown to predict fatigue as already discussed, these RCT findings remain fairly encouraging.

Some promising results have also been demonstrated for rituximab in RA: in REFLEX, an RCT that recruited 520 patients with active RA, patients treated with rituximab plus methotrexate achieved statistically significant and clinically meaningful improvements in both the PCS and mental component score (MCS) of the SF-36 compared to those treated with methotrexate plus placebo (358).

1.11.2 Non-Pharmacological Treatments

There have been a large number of studies looking at the non-pharmacological management of fatigue in various illnesses, and whilst the studies have mainly been small and of variable quality, they have provided some evidence of benefit from exercise and / or psychological therapies. The role of these therapies remains very controversial for many patient groups, particularly CFS patient groups, who have highlighted negative reports from some of their members (359). There is an assumption that the use of such therapies implies that the pathogenesis of the symptom is entirely psychological, a view which many patients find unpalatable (360). Nonetheless, therapies such as graded exercise therapy and cognitive behavioural therapy are probably the best evidence-based therapies available to treat fatigue at the moment. Some beneficial effects have been reported in small studies which looked at homeopathy, osteopathy and massage therapy, but none of these studies were of good quality (reviewed in (361)).

1.11.2.1 Graded Exercise Therapy (GET)

Graded exercise programmes have been assessed across a number of illnesses associated with fatigue with mixed but mainly positive results. One of the problems in comparing the results

of GET studies is the heterogeneity of the programmes being tested: they are of varying intensity and duration; they are variably aerobic-only, strength-only, or a mixture of both; perhaps most importantly they may be exercise only, or exercise combined with a non-exercise component such as cognitive behavioural therapy. The exercise component of any such programmes might be expected to work through a number of mechanisms:

- There is some evidence that aerobic and strength training may improve depression (362, 363), and as previously discussed there is evidence that depression is a strong predictor of fatigue;
- Exercise has been shown to help sleep complaints (364, 365), another known predictor of fatigue;
- Improvements in cardio-respiratory fitness would be expected to improve maximal aerobic capacity, thereby reducing the percentage of maximal aerobic capacity at which normal ADLs are carried out.

In a study of 93 patients with SLE, one RCT compared a twelve week programme of GET with relaxation sessions to no intervention, and found a significant improvement in fatigue in the exercise group (as measured by the Vitality subscale of the SF-36 and the Chalder Fatigue Scale) which was maintained at follow-up after three months but only in those individuals who continued to exercise (366). Dalgas et al (142) enrolled 38 patients with MS into either a twelve week progressive resistance training group or a control group, and measured fatigue (with both the FSS and the MFI-20), mood and quality of life (with the MOS SF-36). At baseline all these parameters were comparable between the two groups, but by the end of 12 weeks the intervention groups reported statistically significant improvements in fatigue (on the FSS score and the General Fatigue scale of the MFI-20, but not the other MFI-20 dimensions), mood and quality of life (on the SF-36 Physical but not Mental Component

Score) when compared to the control group, and all of these improvements were maintained after a further 12 week follow-up period. A larger American RCT included 347 patients with varying types of arthritic illnesses, 25% of whom had RA, and found a statistically significant improvement in fatigue at both completion of the eight week programme, and at six months follow-up (367).

A recent large RCT in patients with CFS challenged the view of patients' organisations that GET and cognitive behavioural therapy (CBT – see Section 1.11.2.3 below) can be harmful by randomising 641 patients to one of four groups: specialist medical care (SMC) alone; SMC plus GET; SMC plus CBT; or SMC plus adaptive pacing therapy. After one year of follow-up they found that mean fatigue scores and mean physical function scores were significantly better in the groups who received GET or CBT compared with SMC alone, but that adaptive pacing therapy (favoured by some patients' groups) conferred no benefit over just SMC, and that there was no difference in the incidence of serious adverse reactions (359). A prospective RCT of fluoxetine therapy with and without GET, also in patients with CFS, found that fluoxetine on its own only helped depression, but exercise significantly improved health perception and fatigue at 28 weeks (190). Two other RCTs that looked at GET in CFS also found a statistically significant improvement in fatigue and other measures (368), as did six separate studies of cancer patients (369-374)

On the other hand, an RCT which recruited patients with both SLE and RA found less impressive results with a 'minimally supervised' home exercise programme: although there were improvements in fatigue in both exercise groups, especially in the RA patients, the results did not reach statistical significance (375). A Cochrane review of RCTs of GET in FMS was also fairly disappointing; although some studies showed evidence of improvements in fatigue from exercise (376-378), those were trials of exercise combined with education,

and studies without education showed no benefit. They concluded that "it is not known whether exercise training… improves other symptoms such as fatigue."

It is unclear why these studies have yielded such variable results, but common themes amongst the studies which have yielded the most promising improvements in fatigue seem to be that the exercise program was well supervised rather than self-directed or 'minimally supervised', that the GET was of lower intensity or aerobic in nature, and also that combining an element of education seems to be beneficial.

1.11.2.2 Taught Self-Management Strategies

Qualitative studies have found that in the general absence of clinician-led treatment for their fatigue, patients have developed varying self-management strategies. Hewlett (379) described some such strategies:

- Behavioural – resting for periods, pacing overall activity level so as to maximise productivity without provoking exhaustion, planning ahead and adjusting other activities accordingly to 'conserve energy' for crucial tasks, using appliances which minimise energy expenditure;
- Cognitive – distraction, re-normalising life;
- Social – Seeking emotional and practical support.

Occasionally such strategies can be maladaptive – for example in Hewlett's own study (102) patients reported that they were sacrificing social activities in order to conserve energy for mundane tasks which they perceived to be more essential, undoubtedly reducing their quality of life in the process. Counselling from health professionals is important to help patients to use self-management strategies effectively, and a careful balance must be struck – although strategies such as pacing are frequently reported as helpful by patients (360), it has been shown that excessive rest worsens fatigue in cancer (380), and as previously discussed a large

RCT of patients with CFS found no benefit from adaptive pacing therapy (359). Nonetheless many patients claim benefit from such strategies, and they are recommended in the NCCN guidelines for the management of CRF (300). This recommendation is based on evidence from studies such as that by Barsevick et al, which showed that cancer patients undergoing chemotherapy or radiotherapy experienced significantly less fatigue if they were randomised to an energy conservation and activity management intervention than to a control group that was similar in terms of time and attention (381).

Evidence supporting the efficacy of taught self-management strategies can be found in a large RCT in UK primary care which studied 544 patients with a diagnosis of arthritis (37% RA, 52% OA, 11% 'other'), comparing a treatment group that was enrolled into the Arthritis Self-Management Programme (ASMP) to a control group that was placed on a waiting list for the same programme (382). The ASMP is a community-based programme delivered by lay people who have arthritis themselves, which aims to enhance perceived ability to control various aspects of the disease through four main strategies: 'skills mastery', which involves participants learning and practicing new behaviours such as cognitive symptom management techniques (e.g. distraction, visualisation, and 'contracting', a technique based on setting realistic goals to be achieved in the coming week); 'modelling', where the course leaders act as positive role models; persuasive communication; reinterpretation of physiological symptoms, which helps participants to distinguish disease symptoms from similar symptoms that can arise for example from exercise. In addition to significant improvements in perception of control and health behaviours, the treatment group achieved a significant reduction in fatigue (measured by the VAS, $p=0.02$), depression and anxiety (measured by the Hospital Anxiety and Depression Scale (HADS), <0.0005 and 0.014 respectively) at four months, and not only were these differences even more pronounced at a twelve month

follow-up, but visits to general practitioners regarding arthritis were also significantly reduced ($p<0.0005$).

1.11.2.3 Cognitive Behavioural Therapy

Cognitive behavioural therapy (CBT) is the collective term for therapies that address patients' thoughts and beliefs about their symptoms (cognition) as well as how they cope with those symptoms (behaviour) through the setting and achievement of individualised goals. For example if the aim was to help a patient to cope with anxiety, CBT might include sessions to identify maladaptive thinking patterns that lead to increased stress and anxiety, as well as any dysfunctional coping strategies the patient might use, and then setting a 'homework' goal of practicing newly taught techniques by talking to a stranger before the next session. Typical CBT programs consist of anywhere between six and eighteen face-to-face sessions of around an hour each with a trained therapist, with a gap of 1–3 weeks between sessions. This initial programme might be followed by some booster sessions, for instance after one month and three months, although this is not always the case. Sometimes other techniques are combined with CBT – for example a study by Greco et al in SLE patients used 6 sessions of CBT which were re-enforced by auditory electromyographic biofeedback from the trapezius area as an indicator of muscle relaxation (383).

CBT for fatigue reduction has probably been used most in CFS, where it has achieved both significant improvements in fatigue (359, 384-386) and also some more disappointing results (387-389), but it has also been used increasingly in other illnesses such as autoimmune disease (390). Given et al conducted an RCT of 237 patients with a variety of different solid organ and haematological cancers, and found that a 20 week CBT program produced a significant improvement in fatigue levels among patients who had moderate or severe fatigue at baseline. An RCT of 64 patients with RA of less than 8 years duration, all of whom achieved high scores on a psychosocial risk profile, compared treatment with or without

tailor-made CBT treatment in addition to standard medical therapy. The study showed that those in the CBT group achieved significantly lower levels of fatigue and depression post-treatment which were maintained at six months follow-up (391). A study of SLE patients found that although CBT did have a marginal effect on levels of fatigue, this effect was no greater than two control groups who either underwent a 6-session 'symptom-monitoring support programme' where a therapist's role was to listen empathetically but avoid giving advice or teaching any new skills, or else only had 'usual care' but completed follow-up evaluations in the same manner as the other groups (383). It should be noted, however, that fatigue was not one of the primary symptoms studied, and this may have been reflected in the CBT provided. A study of CBT combined with education and exercise in patients with FMS demonstrated an improvement in fatigue at four months (change in Fibromyalgia Impact Questionnaire fatigue score of -0.61 vs +0.09, p=0.02), but this effect was lost at 8 months.

1.11.2.4 Expressive Writing

Although CBT is the main psychological intervention which has been studied in fatigue, it is not the only one to have shown some benefit. Expressive writing has shown some promise in the treatment of fatigue, as it has also in the treatment of depression. In a study by Broderick et al, 92 patients with FMS were randomised to either 'written emotional disclosure' (ED), 'neutral' (purely factual) writing, or usual care. ED describes a technique where subjects are asked to speak or write about a traumatic event not only factually but also with deep emotional expression and cognitive reflection, and the technique has been described in physical illnesses as long ago as 1997 (392), when it was shown to result in better psychological and physical functioning in RA; since then it has been tested in a variety of illnesses including asthma (393), prostate cancer (394), renal cell carcinoma (395) and breast cancer (396). Broderick's study found that the ED group had improvements in fatigue, pain and psychological well-being at four months compared to the control groups (change in SF-

36 vitality score of -13.7 for ED vs -3.7 for controls, p=0.05), although this was lost by 10 months follow-up. Another study in patients with RA or SLE found that two different types of expressive writing both improved fatigue levels compared to factual writing at three month follow-up (397).

1.11.2.5 Mindfulness

'Mindfulness-based interventions (MBIs)' are being increasingly proposed as a way of helping those with chronic physical or psychiatric illness to cope with pain and other symptoms (398), including fatigue (399), anxiety and depression (400-402), sleep disturbance (403) and stress (404). Mindfulness is a form of meditation which was historically associated with Buddhism, but many of its modern applications are not inherently spiritual or religious. Most therapeutic programmes are based on 'mindfulness-based stress reduction' (MBSR), which was developed in Massachusetts (USA) by Jon Kabat-Zinn, who defines mindfulness as 'the effort to intentionally pay attention, non-judgmentally, to present-moment experience and sustain this attention over time' (405). MBSR is an eight-week group workshop which teaches a number of techniques including: mindfulness meditation; 'body scanning', where the subject is to lie on their back and focus their attention fully on one part of the body at a time, moving from the toes up to the head; and gentle yoga (406). Another programme based on many of the same principles is 'mindfulness-based cognitive therapy' (MBCT), which combines elements of MBSR with CBT. It was initially developed to prevent relapses of depression, and encourages participants to 'change their relationship to thoughts and feelings… to discover that these are fleeting events in the mind and the body that they can choose to engage with – or not' (400). To date there have been no studies of the use of MBIs in patients with AAV, but the literature available for cancer, rheumatoid arthritis and a great many other chronic illnesses suggest it may have a potential role in relieving fatigue and other psychological symptoms.

In the first study of MBIs in cancer, 90 patients with a variety of different cancers were randomised to either MBSR or 'waiting list' (i.e., a control group which received no psychological intervention), and found that the intervention group benefitted from significant improvements in a variety of psychological symptoms including anxiety and depression ($p<0.001$). However the results for fatigue were mixed: they used the POMS questionnaire which includes subscales for both 'vigour' and 'fatigue', and although there was a significant improvement in the 'vigour' subscale ($p<0.05$), the improvement in the 'fatigue' subscale did not reach significance (p value not supplied) (407). Another study by the same group looked at sleep disturbance and cancer-related fatigue in 63 patients; again the participants had a variety of cancer types and stages, and were randomised to MBSR or waiting list, but this cohort of patients experienced a significant improvement in sleep quality ($p<0.001$) as well as much more marked improvements in fatigue as measured by the POMS fatigue ($p<0.001$) and vigour ($p=0.016$) subscales (408). The benefits of MBIs for sleep and fatigue were further supported by the findings of a smaller qualitative study (409). A small study of 24 patients with breast cancer in Iran found that a MBSR programme significantly reduced fatigue compared to no intervention ($p<0.001$) (410), but a similar study in the UK found a less impressive improvement in fatigue severity which was not statistically significant ($p=0.62$) (411).

Rimes and Wingrove investigated whether mindfulness would be of use in patients with CFS who had failed to respond to CBT. They conducted a small pilot study in England comparing MBCT to 'waiting list' in patients who were still excessively fatigued despite CBT, and found that the MBCT group reported significantly lower levels of fatigue after therapy than the control group ($p=0.014$), and that this improvement was sustained at six months (399). It is worth pointing out that this study did not directly compare the two therapy types, so

although the findings strongly suggest a possible role for MBCT in patients who have failed to benefit from CBT, it cannot be concluded that MBCT was superior.

Many studies have shown that MBIs are beneficial for patients with chronic pain (406), and therefore one disease of particular interest is fibromyalgia. Numerous studies have shown MBSR to be a useful intervention in fibromyalgia, with improvements in common symptoms including pain, fatigue and depression (412-414). However it has not shown additional benefit compared with other psychological interventions, and has yet to be compared with CBT (406).

In RA, an observational questionnaire study of 201 patients found that greater mindfulness, as assessed by the Freiburg Mindfulness Inventory-short-form, was associated with lower levels of psychological distress at baseline and at twelve-months follow-up (415). Zangi et al conducted an RCT of 73 patients with inflammatory joint diseases including RA and AS, comparing their own MBI to a control group that received routine care plus access to a CD with mindfulness-based home exercises. They found that the formal group intervention resulted in significantly greater improvements in psychological distress, fatigue and overall wellbeing (416). Another RCT of 63 patients with RA again randomised patients to MBSR or 'waiting list'; although there were no significant differences between groups after the eight-week course, follow-up after a further four-month maintenance programme showed improved psychological distress ($p=0.04$) and well-being ($p=0.03$) (417).

1.12 Conclusion / Rationale for this Study

Based on clinical observations and the evidence presented in this chapter, there is good reason to believe that a high proportion of patients with AAV who are treated and stable nevertheless complain of excessive fatigue. Furthermore, anecdotal evidence suggests that

this fatigue has a major effect on their quality of life. However, fatigue is a complex multidimensional phenomenon and, to date, there have been no studies of the nature of the problem in AAV, whether, for instance, it is more the physical or the cognitive and psychological aspects of fatigue that affect the patients. Likewise the extent to which the symptoms of fatigue degrade the quality of life to the patients has not been quantified.

It is unlikely that any successful treatment for fatigue in AAV can be devised without understanding more about the underlying mechanisms, and there are many possibilities. Through mechanisms outlined previously, there might be atrophy or weakness of skeletal muscle, meaning that the patient would be making a greater effort to achieve the same outcome during the activities of daily living. Alternatively, or in addition, patients might have lower levels of cardio-respiratory fitness, meaning that the activities of daily living are carried out at a higher percentage of maximum aerobic capacity. These changes might be described as "peripheral" causes of fatigue, but inflammatory disorders give rise to sickness behaviour which in some patients can lead to depression. Although fatigue and depression are not the same condition, there are close associations between the two and it is possible that the long lasting fatigue could be due to persistent systemic inflammation or to some central adaptation as a result of the stress of the disease.

The aim of the work presented in this thesis was to obtain a better understanding of the fatigue experienced by patients with AAV, and to identify possible causes or mechanisms that may then be amenable to pharmacological, nutritional or lifestyle interventions.

To these ends, the objectives of this study were to:

1. Determine the prevalence, nature and severity of fatigue in patients with AAV in comparison with patients with CKD and healthy controls, and to assess the impact of fatigue on their quality of life.

2. Determine whether fatigue in AAV is associated with depression, anxiety or other psycho-social variables.
3. Measure the strength and fatiguability of skeletal muscle in patients with AAV compared with matched healthy controls.
4. Determine the cardiorespiratory fitness of patients compared with matched controls.
5. Investigate whether any deficiencies observed in skeletal muscle function or cardiorespiratory fitness might be contributing to subjective fatigue.
6. Determine the extent of any central mechanisms of fatigue, including altered perception of exertion and reduced central motor drive.
7. Determine whether there is any reduction in global cognitive function in patients with AAV, and whether any reduction is associated with fatigue.
8. Determine whether patients with AAV who are in sustained remission have measurably increased levels of systemic inflammation compared with healthy controls, and whether any inflammation is related to their symptoms of fatigue.

Objectives 1 & 2 were assessed by questionnaire, objectives 3 - 7 were achieved with laboratory-based studies, and blood samples were taken for objective 8.

2 MATERIALS AND METHODS

2.1 Study Structure

The study was conducted as two cross-sectional sub-studies which ran in parallel: a large questionnaire study which examined the prevalence, nature, severity, impact and predictors of fatigue in AAV compared to disease and healthy controls, and a laboratory-based study which examined the mechanisms resulting in fatigue. Throughout the original ethical and R&D applications, as well as in the information and consent forms, the questionnaire study was referred to as 'Part 1' of the study, and the mechanistic study as 'Part 2'.

2.2 Questionnaire Study

2.2.1 Subjects

Three groups of participants were recruited to the questionnaire study:

- **Patients with AAV**. This group comprised adult patients meeting the European Medicines Agency (EMA) vasculitis classification algorithm (3) for AAV, all of whom were in sustained remission. The majority of patients recruited to this group were regular attenders at the University Hospitals Birmingham NHS Foundation Trust (UHBNFT) vasculitis clinic; patients were pre-screened to ensure that they met the inclusion criteria and did not meet any of the exclusion criteria (as detailed below), and all patients who were eligible were then approached at their next scheduled outpatient visit. Additionally, there were pro-active approaches by some patients who did not attend clinic at UHBNFT, but who had heard about the study through various

patient support groups (Vasculitis UK, previously known as The Stuart Strange Vasculitis Trust, and Vasculitis Support Group West Midlands (VSGWM)); these patients were screened prior to recruitment using the same inclusion and exclusion criteria as local patients, using information supplied by the patient.

- **Patients with CKD.** This group was utilised as a disease control group, because CKD is a common consequence of AAV and is itself independently associated with fatigue. Patients were only recruited to this group if their CKD was not secondary to a systemic inflammatory disease, and were pre-screened and recruited consecutively from the UHBNFT CKD clinics.
- **Healthy controls**. The majority of healthy controls were recruited through a 'buddy system', whereby each recruited AAV patient was asked to identify a partner, relative or friend who was of a similar age. Additional healthy volunteers were recruited using the following strategies:
    - Outreach research clinics using the 'Health Research Bus' of the Wellcome Trust Clinical Research Facility (Birmingham). The bus spent time parked in prominent Birmingham city centre locations, and recruited members of the public who had no link to UHBNFT outpatient clinics;
    - Recruitment of healthy volunteers from the Staff at the University of Birmingham (UoB) and UHBNFT.

2.2.2 Sample Size and Statistical Analysis

Over the course of the year during which the study was designed, there had been around 170-180 patients attending the vasculitis clinic at UHBNFT. We therefore aimed to recruit 150 patients with AAV into the questionnaire study, and to match this by recruiting 150

participants into each of the CKD and healthy control groups. *A priori* power calculations were not carried out during the design of this part of the study.

In fact, although 152 participants were eventually recruited to the AAV group, only 68 were recruited to the CKD group and 71 to the Healthy Control group; when it is also taken into account that some of the questionnaires were not fully completed (see Table 3.1), there was enough data to generate a General Fatigue score for 145 patients with AAV, 67 patients with CKD, and 66 Healthy Controls. *Post-hoc* power calculations were carried out and showed that, taking a conventional alpha of 0.05 into account, this study had 61.8% power to detect an effect size of 0.4 between the AAV and CKD groups, 61.3% power to detect an effect size of 0.4 between the AAV and Healthy Control groups, and 46.1% power to detect an effect size of 0.4 between the CKD and Healthy Control groups in analyses of variance.

Statistical analysis was carried out using IBM SPSS Statistics 21.0. Categorical data was summarised with percentages, and quantitative data was summarised with mean and standard error (SEM), or with median and interquartile range (IQR) according to normality of distribution. Comparisons of results between the groups were assessed using $\chi 2$ tests for categorical data and Mann-Whitney tests for quantitative data, and Spearman's rank order correlation was used to assess the strength of associations between variables. Unless stated all correlations include both patients with AAV and healthy controls. Multivariable analysis was carried out using linear regression, having first confirmed that the distribution of the standardised residuals was normal.

2.2.3 Inclusion and Exclusion Criteria

2.2.3.1 Inclusion Criteria

- **Patients with AAV**: Adult patients with biopsy-proven disease, meeting the European Medicines Agency (EMA) vasculitis classification algorithm (3) for AAV. All recruited patients were in sustained remission (Birmingham Vasculitis Activity Score =0 for >6 months) at time of recruitment. Those patients with a recent diagnosis were required to have been diagnosed at least 9 months prior to entry into the study, and to have had an uncomplicated treatment course with remission within three months of diagnosis.
- **Patients with CKD**: Adult patients with a diagnosis of CKD due to any cause other than systemic inflammatory disease, and with disease no more advanced than CKD stage IV (i.e., estimated glomerular filtration rate ≥15ml/min/1.73m^2).
- **Healthy Controls**: People not known to suffer from AAV, CKD or from any other major organ disease on screening. Minor illnesses such as osteoporosis, hypertension or hyperlipidaemia were permissible, provided that there was no known damage to any major organ, and that the illness had not been previously associated with fatigue in the literature.
- For all three groups, written informed consent was a pre-requisite for inclusion.
- Age ≥ 16 years. There was no upper age limit for inclusion.

2.2.3.2 Exclusion Criteria

- Symptoms, signs or investigation results in keeping with sepsis within the six weeks prior to enrolment.
- Any known active malignancy, or still on active treatment for previous malignancy.

- Patients with AAV were excluded if they had received high dose steroids (>30mg prednisolone daily) within the previous 6 months, as this was interpreted to represent significant clinical suspicion of a relapse.
- Patients with CKD and healthy controls were excluded if there was any evidence to suggest the presence of a systemic inflammatory disease.

2.2.4 Data Collection and Baseline Bloods

A data sheet was completed for all subjects who entered the study, comprising the following information which was gained from interview with the participant, as well as their medical notes (where available):

- Gender, date of birth, height and weight;
- Presence or absence of comorbidities including CKD, SLE, RA, other inflammatory arthritis, asthma/COPD, bronchiectasis, inflammatory bowel disease (Crohn's disease or ulcerative colitis, irritable bowel syndrome, thyroid disease;
- List of medications;
- For the patients with AAV– diagnosis, date of diagnosis, organ involvement, relapses, and pharmacological treatments given.

Baseline blood samples were taken on entry to the study and tested for full blood count (FBC), urea & electrolytes (U&E), albumin and C-reactive protein (CRP).

2.2.5 Questionnaire

The questionnaire was divided into ten 'sections', each of which gathered a different sub-set of information. 'Section 1' of the questionnaire collected the following information:

- Age, gender, ethnicity, marital status, education and employment status;
- Smoking history;

- Caffeine and alcohol intake;
- Habitual physical activity – participants were asked how many hours per week they had spent engaging in various physical activities such as walking, swimming and gardening during the previous year.

The other sections of the questionnaire included a number of pre-existing and widely used symptom-rating tools, as follows:

2.2.5.1 Section 2 - The Short Form (36) Health Survey

The Short Form (36) Health Survey, more commonly known as the 'SF-36', remains the most commonly used and well validated tool for the assessment of HRQOL. It was constructed following the Medical Outcomes Study (MOS) (418), a two-year American study in which 22,462 patients with chronic illnesses including diabetes, hypertension, ischaemic heart disease and depression were studied to determine whether variations in patient outcomes were explained by differences in system of care, and to develop more practical tools for the routine monitoring of patient related outcomes in medical practice. From the forty physical and mental health concepts included in the original MOS, the eight which were seen as most important were subsequently selected for inclusion in the SF-36 (419).

The SF-36 was designed to be as brief as possible without sacrificing measurement precision, and to be self-administered. It includes 36 statements which the respondent is asked to score using a Likert scale (420); these 36 items contribute towards eight subscale scores, each of which assesses a different domain of HRQOL (421). Raw scores are transformed into a 1-100 scale for each domain, where higher scores indicate better HRQOL:

- Physical functioning – 10 items on the SF-36 contribute towards this variable. Low scores indicate great limitation in all physical activities due to health problems;

- Role-Physical – Four items contribute towards this measure of problems with work or other daily activities as a result of physical health. Lower scores indicate more significant problems;
- Bodily Pain – Two items contribute to this score, where low scores indicate severe and very limiting pain;
- General Health – Five items contribute to this measure of the patient's own perception of his or her health. Low scores indicate that the individual perceives their health to be poor and likely to get worse;
- Vitality – Four items contribute towards this measure which is often considered to be the inverse of fatigue. Low scores indicate that the individual feels tired and 'worn out' all of the time;
- Social Functioning – Two items contribute to this measure of the ability of the individual to engage in normal social activities. Low scores indicate that the individual encounters extreme and frequent interference with normal activities due to either physical or emotional problems;
- Role-Emotional – Three items contribute to this scale. Similarly to the Role-Physical scale, this measures the impact of emotional problems on work and other daily activities, where lower scores indicate greater impact;
- Mental Health – Five items contribute to this score, which is a measure of how nervous or depressed the individual is. Lower scores indicate poor mental health.

In addition to the 35 items which contribute to the eight primary scales listed above, there is also one item used to derive an outcome known as Reported Health Transition.

A recent enhancement of the scale has been the development of summary scores, whereby pooling of the eight primary scale scores allows researchers to calculate a Physical Component Score (PCS) and a Mental Component Score (MCS) (422). The SF-36 is the

mostly widely used HRQOL measure in AAV, including in UK populations, and as such is well validated for our study population (354, 423).

2.2.5.2 Section 4 - The Multi-Dimensional Fatigue Inventory (MFI-20)

As described in Chapter 1, the MFI-20 is one of the most commonly used multi-dimensional fatigue scales (110), and is well validated across healthy (113, 143, 144) and disease populations (116-142, 146) including AAV (114, 115). It allows assessment not only of the severity of fatigue, but also of the nature and impact of the symptom. Participants are asked to rate the truth of twenty statements using a Likert scale, and the responses generate five scores, each representing a different 'dimension' or aspect of fatigue: 'General Fatigue', 'Physical Fatigue', 'Mental Fatigue', 'Reduced Activity' and 'Reduced Motivation'; Reduced activity and Reduced Motivation are sometimes regarded as measures of the impact of fatigue. The score for each item ranges from 1-3, and therefore the score for each dimension ranges from 4 (no fatigue) to 20 (maximal fatigue). There is no overall or total score, and the author's recommendation is that the 'General Fatigue' score should be used in place of a summary score when comparing between studies (113, 118).

There is no 'upper limit of normal' reported for the MFI-20, i.e. there is no accepted cut-off above which a person would be considered to suffer from fatigue and below which they would not. It was decided, therefore, that we would define patients with AAV and CKD as having 'severe fatigue' if their MFI-20 'General Fatigue' scores were above the 95% confidence limits of our matched healthy controls.

2.2.5.3 Section 5 - The Revised Piper Fatigue Scale

Part of the Revised Piper Fatigue Scale (PFS) was included in order to assess the direct impact of fatigue on role impairment (424). Questions 2-6 of the Behavioural/Severity subscale were used, supplemented by an additional question relating to the impact of fatigue

on activities of daily living (ADLs); it was not necessary to include the whole scale since the other sections did not add to the information already provided by the MFI-20.

2.2.5.4 Section 6 - The Hospital Anxiety and Depression Scale (HADS)

The HADS is a short but comprehensive instrument designed to identify people suffering from anxiety and / or depression (425). The HADS was chosen above other such measures because its authors purposely excluded somatic symptoms, thereby enabling it to more accurately define psychological illness in people who are also suffering from physical illness (426, 427), and also because it has previously been shown to be acceptable and discriminative in AAV (354).

The scale consists of 14 items which are scored from 0-3 on a Likert scale to generate two summary scores: an 'Anxiety Score' ranging from 0 (no anxiety) to 21 (maximal anxiety), and a 'Depression Score' which also ranges from 0 to 21 (425). In addition to analysing the raw scores, it is common to categorise the scores into 'Normal' (scores of 0-7), 'Borderline Abnormal' (scores of 8-10), and 'Abnormal' (11-21); there is good evidence that such score cut-offs are useful for predicting psychiatric morbidity (427).

2.2.5.5 Section 9 - The Pittsburgh Sleep Quality Index (PSQI)

The PSQI (428) is a self-rated questionnaire which assesses sleep quality and disturbance over the previous month. The questionnaire consists of nineteen items, each scored on a Likert scale from 0-3; these nineteen scores are used to derive seven component scores (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction), and the seven component scores are further summed to yield a global PSQI score ranging from 0 (best possible sleep quality) to 21 (worst possible sleep quality). The tool has been used across a

wide spectrum of illnesses as well as in healthy populations to assess sleep quality and disturbance, and has been appropriately validated as such (429).

2.2.5.6 Section 11 - Brief COPE

As discussed in Chapter 1, associations have been seen between the severity and impact of fatigue and the coping mechanisms employed by patients. The 'Brief COPE' is a 30 item scale which assesses fourteen dimensions of generalised coping relevant to chronic diseases (430). These dimensions comprise: dysfunctional coping (denial, self-distraction, self-blame, substance use, venting, and behavioural disengagement); emotion-focused coping (acceptance, use of emotional support, humour, positive reframing, and religion); and problem-focused coping (instrumental support, planning, and active coping) (431). Each item is scored on a four point ordinal scale ranging from 1 'I don't do this at all' to 4 'I have been doing this a lot'.

2.2.5.7 Questionnaire Sections Not Used In This Study

Four additional sections of the questionnaire were not used in this study, but were included as part of a collaborative study. These were:

- Section 3 - The Chalder Fatigue Scale (CFS)
- Section 7 - The Sino-Nasal Outcome Test-25 (SNOT-25)
- Section 8 - The EQ-5D-3L
- Section 10 - Assessment of Chronic Widespread Pain

2.3 Mechanistic Study

2.3.1 Subjects

Initially it was planned that patients from all three groups would also be enrolled into the mechanistic study. However, initial results of the questionnaire study indicated that there were unlikely to be major differences between the fatigue experienced by patients with AAV and that experienced by patients with CKD. In view of this, and in order to optimise the use of available testing time, the CKD disease control group was not included in the mechanistic study. All participants from the AAV and Healthy Control groups who completed the questionnaire study were screened for eligibility for the mechanistic study, and if they fulfilled inclusion criteria and were not excluded by the additional exclusion criteria (see below) they were given the relevant information leaflets to take away and read for a minimum of 24 hours, following which they were contacted and offered the opportunity to participate.

2.3.2 Sample Size

The aim for the mechanistic study was to recruit 50 participants to each of the AAV and healthy groups. This sample size was determined on the basis of power analyses (G-power, v3.01), imputing moderate to large effect sizes (r=0.40 - 0.60). The projected effect size was based on prior research using comparable methods (327), on clinical relevance (i.e., weak associations between fatigue markers and clinical characteristics are unlikely to inform effective interventions), and on the basis of the existing literature on fatigue in other inflammatory conditions (94, 432). On the basis of published data (324), it was anticipated that approximately 80% of patients with AAV would report fatigue compared to 20% in the healthy population. With the intended sample size of 50 participants per group, and taking a

conventional α of 0.05 into account, an effect size of 0.40 would give 97% power to detect an effect between groups in analyses of variance and 97% power to detect an effect between groups in multiple regression analyses.

2.3.3 Inclusion and Exclusion Criteria

Because all of the participants in the mechanistic study comprised a sub-population of those in the questionnaire study, all of the same inclusion and exclusion criteria applied. However in view of the nature of the tests included in the mechanistic study, the following additional exclusion criteria were applied:

- Any proven or suspected acute coronary syndrome within the last six months, defined by the American College of Cardiology and American Heart Association (433) as either:
    - Unstable angina – angina commencing when the patient is at rest, or of new-onset (less than 2 months), or of increasing intensity, duration, and/or frequency;
    - Non-ST Elevation Myocardial Infarction;
    - ST Elevation Myocardial Infarction.

  This exclusion was necessary to reduce the risk of adverse events, particularly during exercise testing.
- Known moderate or severe aortic stenosis (AS), as defined by the American College of Cardiology (valvular cross-sectional area of less than 1.5cm^2, mean trans-valvular gradient greater than 25 mm Hg, or jet velocity greater than 3.0m/s) (434). This exclusion was necessary both because of the risk of adverse events during exercise, and also because of the association between significant AS and fatigue. Screening for AS was done by clinical assessment and by examination of the medical notes at the

time of recruitment; routine pre-study echocardiograms were not deemed necessary because in the absence of symptoms and signs it is unlikely that a patient would have AS in the moderate or severe categories.

- Pregnancy, which would have been a contra-indication for the radiation involved in DEXA-scanning.
- Severe musculoskeletal problems or levels of general debility that were deemed likely to impede participation in any of the investigations.
- Severe deafness, which would have made participation in the Paced Auditory Serial Attention Test impossible.
- Stage V CKD, whether on dialysis or pre-dialysis, because this would have been a major confounding factor in interpreting the results of many of the tests. This was felt to be particularly important once the decision was made no longer to recruit patients with CKD to the mechanistic study.

2.3.4 Physiological Investigations

Subjects underwent a series of tests of muscular and cardiovascular function. All of the tests conducted were volitional, so that each allowed assessment of both peripheral and central mechanisms.

2.3.4.1 Muscle Strength and Voluntary Activation

Muscle function was assessed by the participants performing a series of maximal isometric voluntary contractions (MVC) of the quadriceps on their dominant side, utilising the twitch interpolation technique (168, 435) to assess the extent of voluntary activation and theoretical maximal strength.

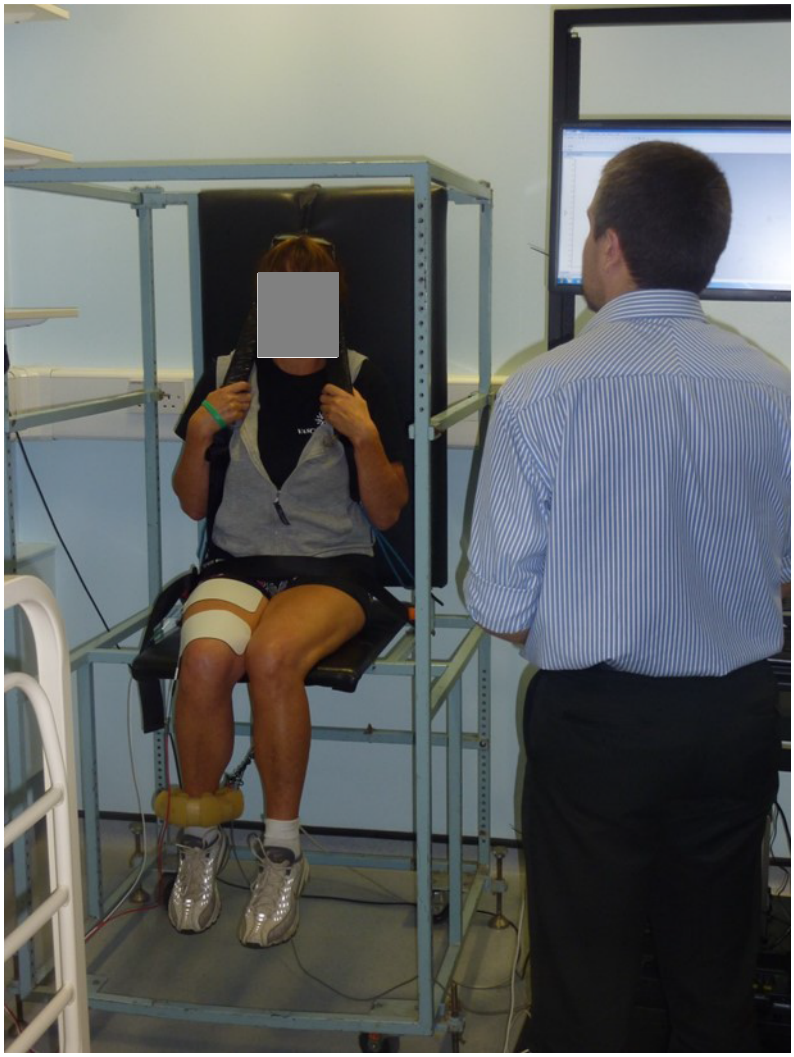


Figure 2.1: Participant in position for muscle testing.
Participant has adopted the position described in the text, the self-adhesive carbon-rubber electrodes have been applied to the dominant leg quadriceps muscle, and the strain gauge attached to the ipsilateral ankle.

Participants sat upright, firmly strapped into an isometric testing chair (436) with hip and knee at 90° flexion. Self-adhesive carbon-rubber electrodes (American IMEX, USA, 4x7 inches) were affixed to the skin over the mid and proximal regions of the quadriceps, and a strain gauge attached to the ankle (see Figure 2.1). The force was recorded digitally by a Micro1401-3 data acquisition unit (Cambridge Electronic Design (CED), UK).

In preparation for testing, the maximum tolerated electrical stimulation (ES) was then determined at rest using square wave doublet pulses, each of 50μs duration with a 10-ms

inter-stimulus gap, delivered via the electrodes by an electrical stimulator (168) (Digitimer DS7AH High Voltage Constant Current Electrical Stimulator, UK) and the force response measured. The amplitude of the stimulus was increased in 50mA increments (starting at 150mA) until either no additional force was generated or the subject's tolerance was reached. This final current, the 'maximal intensity doublet', was used for electrical stimulation during subsequent testing.

A minimum of 3 MVCs were performed (up to a maximum of five if required for consistency), separated by one-minute rest periods. The electrical stimulation was delivered first at rest, and then super-imposed on the MVC three seconds after the start of the contraction. Participants could see the force record and received strong verbal encouragement to make a maximal effort. Spike2 software (CED, UK) was used for data analysis.

As illustrated in Figure 2.2, four measurements were made from the force records:

1. The force generated by electrical stimulation at rest (Ti);
2. The greatest voluntary force exerted before the superimposed stimulus (F0, designated the MVC force);
3. The voluntary force measured immediately prior to the super-imposed stimulation (F1);
4. The additional force, i.e. the force above F1, generated by the superimposed electrical stimulus (Ts).

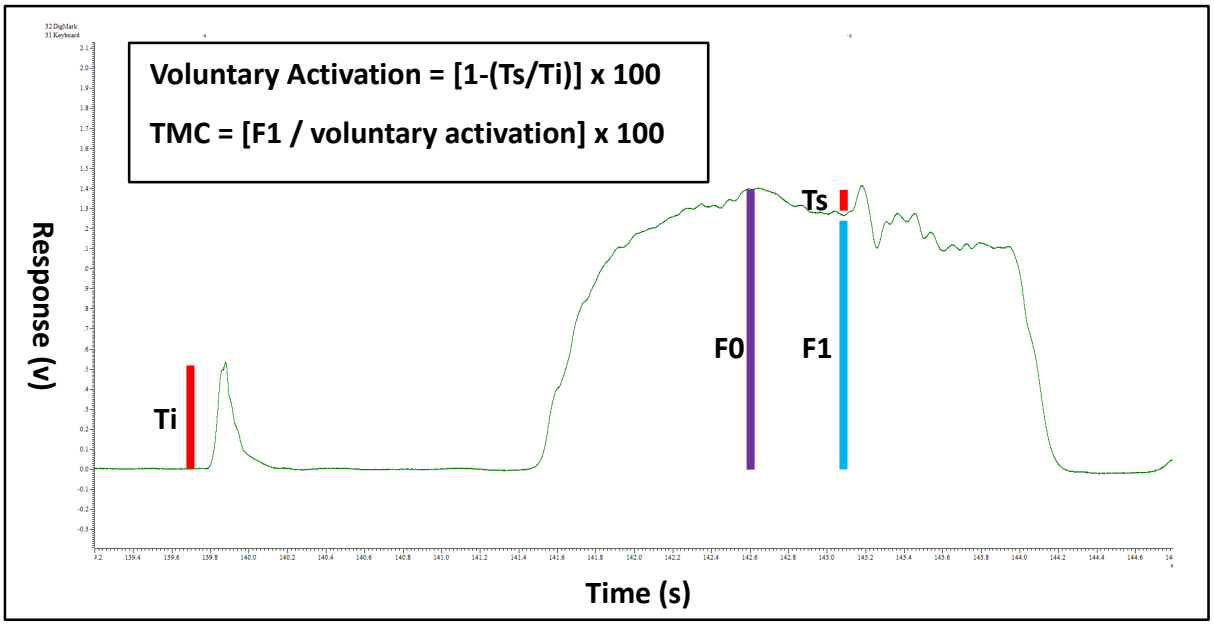


Figure 2.2: A typical MVC with twitch interpolation.
Ti = response from a resting electrical stimulation; Ts = response from electrical stimulation super-imposed on a MVC; F0 = maximal voluntary contraction force; F1 = voluntary power just prior to superimposed stimulation.

These direct measurements were then used to calculate the two primary outcome variables:

1. 'Voluntary Muscle Activation'. This is a measure of how fully the individual activates their muscle of their own volition, derived from the ratio of response to stimulation super-imposed on the voluntary contraction to that in the resting condition, expressed as a percentage:

   Voluntary Muscle Activation = [1-(Ts/Ti)] x 100

2. 'Theoretical Maximal Contraction (TMC)', derived from the percentage voluntary muscle activation and the measured voluntary strength.

   Theoretical Maximal Contraction = [F1 / voluntary muscle activation] x 100.

2.3.4.2 Muscle Endurance

A large variety of different techniques have been used to assess peripheral muscle fatigue or endurance, but they may be roughly divided into those studies which have examined a sustained sub-maximal contraction (437), those which have used repeated maximal strength contractions (438), and those which have used a combination of both (140, 439). As described in Chapter 1, the fatigue of chronic disease is often reported at rest or after extremely low levels of physical exertion, and it is not effectively relieved by a period of rest. It therefore seems unlikely that fatigue in AAV is related to the use of high-power fast-twitch motor units, and so for this study it was decided to investigate prolonged *sub-maximal* exertion in an attempt to more closely replicate real-life scenarios.

Participants were asked to maintain a single sustained quadriceps contraction at 50% of their MVC force for as long as possible, using the same limb tested for strength and voluntary activation. Participants matched the force trace to a '50% target' on the computer screen and received strong verbal encouragement to continue. The end point was either volitional fatigue or when the force fell more than 5% below the target for three seconds. The muscles were electrically stimulated as described above every 10s during the contraction to monitor the level of voluntary activation. The outcome measures for this test were:

1. Endurance Time - the time for which the participants were able to maintain a 50% MVC;
2. Muscular Reserve - The ratio of the response to the last superimposed ES before task failure compared with the response to the resting stimulation. This ratio indicates the proportion of muscle that is able to contract in addition to that being used to achieve the 50% target. If, at the point of task failure the superimposed stimulus evoked no additional force the muscle reserve would also be zero and the fatigue would be considered of entirely peripheral origin. If the superimposed stimulus was a quarter

of the resting doublet then the muscle reserve would be 25%, indicating a substantial failure of central activation. The greater the muscular reserve at the point of task failure, the greater the extent of central fatigue.

2.3.4.3 Body Composition

Body Composition was measured by dual energy x-ray absorptiometry (DEXA; Hologic Discovery A QDR Bone Densitometer, Massachusetts, USA) (440, 441). Bone, lean and fat mass were calculated for the whole body and for each limb.

2.3.4.4 Incremental Submaximal Exercise Test

Participants undertook a 10 minute incremental exercise protocol on a cycle ergometer (Lode, Groningen, the Netherlands), beginning with a 1 minute warm-up at a workload of 10W, followed by 3 minute periods at 25W, 50W and 75W. Oxygen uptake ($\dot{V}O_2$) was recorded continuously with an on-line gas analysis system (MOXUS, AEI Technologies, USA). Heart rate was monitored continuously with a Polar T31 Coded Transmitter (see Figure 2.3).

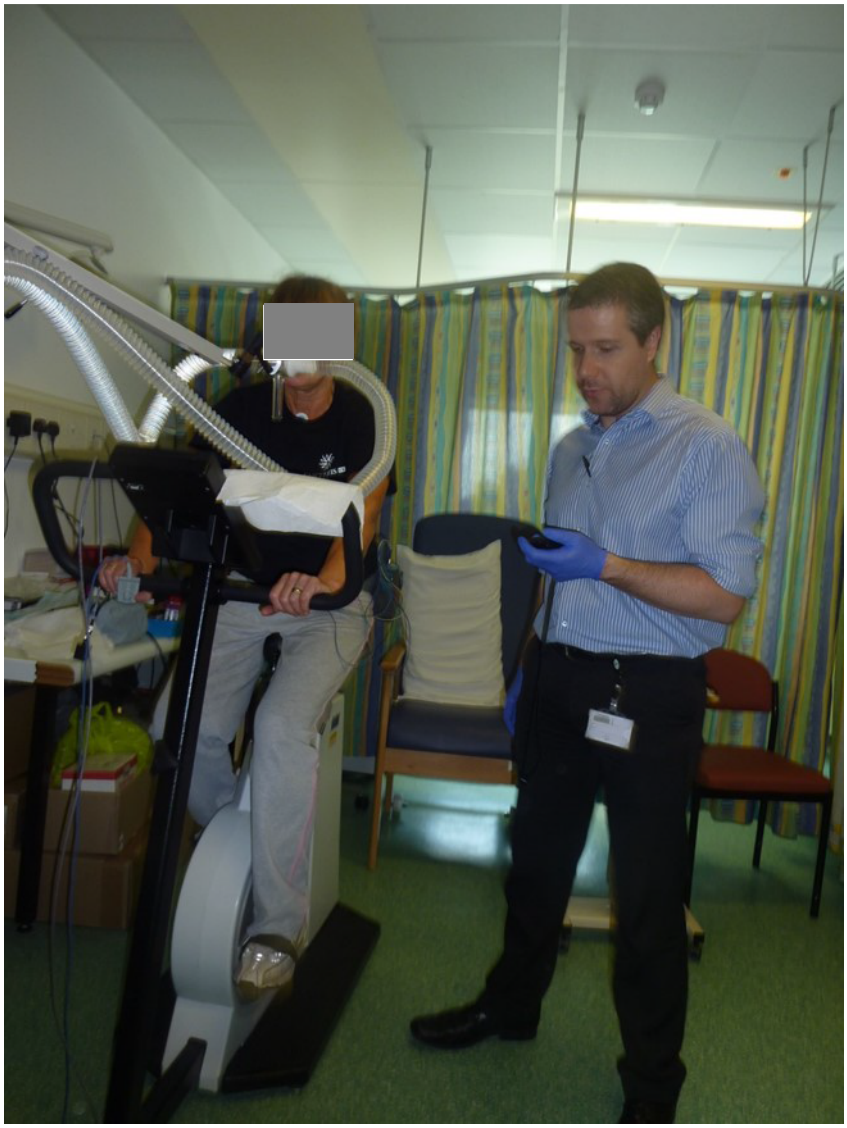


Figure 2.3: Participant performing a submaximal exercise test.
Participants were asked to rate their perception of exertion (RPE) during the last 30 seconds of each exercise stage, i.e. just before the transition to the next work load, using the Borg 6-20 RPE scale (442, 443).

The outcome measures for this test were:

- Cardio-respiratory fitness, which was assessed using two variables:
    - The Oxygen Pulse (O2 Pulse). This was determined as the slope of the linear regression of $\dot{V}O_2$ against heart rate using the data points at the end of each exercise period (444). To allow for differences in body size of the

participants, the O2 Pulse was divided by lean body mass since, in healthy control subjects, O2 Pulse varies in a linear fashion with lean body mass;

- Estimated maximum oxygen uptake (estimated $\dot{V}O_2max$). This was also estimated by extrapolating oxygen uptake as a function of heart rate, assuming it to be linear (445), to an estimated maximum heart rate calculated as [205.8 – (0.685x age)].

- Perception of exertion. This was also assessed in two ways:
  - The RPE at the end of the highest workload completed by the participant.
  - RPE_{index}. The Borg RPE scale was constructed based on the physiology and psychology of young healthy individuals, whose resting and maximum heart rates were assumed to be approximately 60bpm and 200bpm respectively. Many studies have shown that in such individuals there is a strong correlation (0.8-0.9) between heart rate and Borg RPE ratings at a ratio of ten to one - e.g. exertion sufficient to cause a heart rate of 170bpm would be rated as 17 on the RPE scale (446). However this relationship is not precise, and is influenced by many variables including age. Because the average age of the participants in this study was expected to be 60-70 years, many would be expected to have a maximum heart rate significantly below 200bpm, but a rating of ’20’ on the Borg RPE scale would still be expected to equate to an individual’s maximum heart rate in the absence of abnormally high central fatigue. Therefore, if the relationship between HR and RPE remains linear across all age groups, the following equation should always be true:

$$\frac{HR\ at\ Exhaustion\ or\ End\ of\ Exercise}{Age\ predicted\ \max HR} = \frac{RPE\ at\ Exhaustion\ or\ End\ of\ Exercise}{Maximum\ RPE\ (i.e.\ 20)}$$

Rearranging these variables:

$$RPE_{index} = \frac{RPE\ at\ Exhaustion\ or\ End\ of\ Exercise\ \times\ Age\ predicted\ max\ HR}{HR\ at\ Exhaustion\ or\ End\ of\ Exercise\ \times\ 20}$$

2.3.5 Cognitive Performance

A common complaint of individuals reporting chronic fatigue is the perceived inability to adequately perform cognitive tasks. In order to investigate whether this perceived problem was objectively measurable, subjects in the mechanistic study undertook a prolonged cognitive task which primarily tests attention, concentration, short-term recall, and executive function (Paced Auditory Serial Addition Test; PASAT). The test was performed at 4 speeds, using inter-digit intervals of 2.4s, 2.0s, 1.6s, and 1.2s and the number of correct responses was recorded.

2.3.6 Laboratory analysis

Serum samples were collected from participants at four time-points: a fasting morning sample was taken at rest between 9am and 10am, and non-fasting samples were taken at the end of the exercise, and at 1 hour and 2 hours post-exercise. These samples were allowed to sit undisturbed for between 30 minutes and one hour to allow the blood to clot, and the clot was then removed by centrifuging at 5,000rpm for ten minutes in a refrigerated centrifuge. The resultant serum was then apportioned into 500µl aliquots and stored at -80^0C for batch processing after all samples had been collected.

2.3.6.1 Cytokine Analysis

Samples from each of the four time-points were subsequently used to measure serum interleukin (IL)-1b, IL-1RA, IL-6, IL-8, IL-10 and TNF-α by multiplex luminex assay

according to the manufacturer's instructions (Bio-Rad, UK). This process was carried out by colleagues in the Clinical Immunology Service at the University of Birmingham. Precise instructions for duplicating the process they followed are available from bio-rad at:

http://www.bio-rad.com/webroot/web/pdf/lsr/literature/10044282.pdf

2.3.6.2 Hormone Analysis

Serum concentrations of cortisol, DHEA and DHEAS were also measured (in the fasting baseline samples only) using liquid chromatography/tandem mass spectrometry (447, 448). This technique is superior to radioimmunoassay because it avoids antibody cross-reactivity and allows the simultaneous measurement of multiple steroids with a high degree of accuracy (449). The analysis was carried out by colleagues in the Centre for Endocrinology, Diabetes and Metabolism (CEDAM) at the University of Birmingham, where methods and assays have been validated as described by Honour (450) so that the sensitivity of the system to each steroid, the accuracy, reproducibility, and extraction efficiency are known.

The first step in the process was the extraction of steroids from the serum samples, a process which is different for cortisol/DHEA compared with that for DHEAS:

- For cortisol/DHEA, 200µL of serum was pipetted into a silanized glass thin layer chromatography tube, and 20µL of internal standard solution was added. The tube was vortexed, 1ml of MTBE is added, and the tube vortexed again. After freezing for >30 minutes, the top layer was pipetted into a 96 well plate then evaporated to dryness under nitrogen at 55ºC. The sample was then reconstituted in 125µL of 50/50 methanol/water and centrifuged at 3000rpm for 10 minutes. The sample was then stored at -20ºC until analysis.
- To prepare for DHEA analysis, the above sample was then evaporated to dryness under nitrogen, and 100µL of hydroxylamine in pyridine (0.16g/8mL of pyridine) was

added. The plate was vortexed for 10 minutes, then centrifuged at 3000rpm for 10 minutes, heated for 1.5hrs at 60°C, then centrifuged at 3000rpm for another 10 minutes. The sample was evaporated to dryness under nitrogen, reconstituted in 125µL of 50/50 Methanol/water, then centrifuged at 3000rpm for 10min. The sample was then stored at -20°C until analysis.

- For DHEAS, 20µL of serum was pipetted into a low volume 96 well plate, followed by 20µL of internal standard, 20µL of zinc sulphate solution (0.1mmol/L) and 100µL of acetonitrile. The plate was placed for 10 minutes on a plate shaker, and then centrifuged at 3000rpm for a further 10 minutes. 100µL of supernatant was transferred into another plate, evaporated to dryness under nitrogen at 55°C, reconstituted in 125µL of 50/50 methanol/water, then stored at -20°C until analysis.

The samples then underwent liquid chromatography/tandem mass spectrometry in an automated process using a Waters Xevo (triple quadrupole) mass spectrometer.

2.3.7 Statistical Analysis

As with the questionnaire study, all analysis was carried out using IBM SPSS Statistics 21.0. Categorical data was summarised with percentages, and quantitative data was summarised with mean and standard error (SEM), or with median and interquartile range (IQR) according to normality of distribution. Comparisons of results between the groups were assessed using χ^2 tests for categorical data and Mann-Whitney tests for quantitative data, and Spearman's rank order correlation was used to assess the strength of associations between variables. Unless stated all correlations include both patients with AAV and healthy controls. Multivariable analysis was carried out using linear regression, having first confirmed that the distribution of the standardised residuals was normal.

3 RESULTS CHAPTER 1: WHICH PATIENT CHARACTERISTICS AND SYMPTOMS ARE PREDICTORS OF FATIGUE?

3.1 Introduction

One hundred and fifty two patients with AAV, sixty eight patients with CKD and seventy one healthy controls completed a questionnaire which consisted of a 'Participant Characteristics' section and several symptom-rating tools as outlined in Chapter 2. The questionnaire was designed with two purposes: firstly to assess the differences in severity and nature of fatigue and other symptoms between the three groups, and secondly to determine which characteristics and symptoms within the patient group were associated with greater or lesser severity of fatigue.

Based on the experience of the investigating team it was predicted that the questionnaire would take around half an hour to complete, and in order to minimise inconvenience to the participants it was completed at home unsupervised.

Data collection was almost 100% for simple sections where only a single response by the participant was required to generate data – for example only four participants did not give valid data on marital status (completion rate was 98.6% for all participants, 98.0% for patients with AAV, 100% for CKD patients, and 98.6% for healthy controls). For more complex sections the completion rates were lower, e.g. only 82.8% of participants provided enough responses for us to be sure about whether they participated in sport (84.9% of patients with AAV, 80.9% of participants with CKD, and 80.3% of healthy controls), but

encouragingly the completion rates were very similar across the three groups and this is therefore unlikely to have significantly influenced results.

The completion rates for some of the important summary scores are shown in Table 3.1. The lowest completion rates obtained were for the summary scores which require the greatest number of valid responses in order to be calculated: for example, calculation of the Physical Component Score (PCS) of the MOS SF-36 requires valid responses to all 36 items on the scale, whereas calculation of General Fatigue on the MFI-20 requires only four valid responses.

	All Participants	AAV	CKD	Healthy
General Fatigue	95.5	95.4	98.5	93.0
Physical Fatigue	95.2	95.4	97.1	93.0
Mental Fatigue	95.9	96.1	98.5	93.0
Reduced Activity	94.5	95.4	95.6	91.5
Reduced Motivation	95.9	96.1	98.5	93.0
Anxiety	96.2	96.1	100	93.0
Depression	96.6	96.1	100	94.4
Physical Component Score (PCS)	77.7	80.3	70.6	78.9
Mental Component Score (MCS)	77.7	80.3	70.6	78.9
Global PSQI Score	86.9	86.8	85.3	88.7

Table 3.1: Completion rates for the symptom-rating scales in the questionnaire.
Percentage of participants giving enough valid answers to generate summary scores for the main symptom-rating tools included in the questionnaire. Results are expressed as percentage of all participants in the study and of all participants in each group. General Fatigue, Physical Fatigue, Mental Fatigue, Reduced Activity and Reduced Motivation – Multi-dimensional Fatigue Inventory (MFI-20); Anxiety and Depression – Hospital Anxiety and Depression Scale (HADS); PCS and MCS – MOS SF-36; Global PSQI Score – Pittsburgh Sleep Quality Index.

3.2 Participant Characteristics

As shown in Table 3.2, there were no significant differences between any of the groups for age, gender or ethnicity, and nor was there any difference in the proportion of subjects across the groups who had a living partner.

	AAV	CKD	Healthy	Significance
n	152	68	71	-
Age (yrs)	63.1 (49.8-71.5)	64.4 (47.8-75.2)	61.9 (49.8-67.0)	0.355
Male Gender	73 (48.0%)	39 (57.4%)	35 (49.3%)	0.429
White Ethnicity	141 (92.8%)	61 (89.7%)	66 (93.0%)	0.530
Living Partner	102 (67.5%)	48 (70.6%)	56 (78.9%)	0.221
Age Left Education (yrs)	16 (15-18)	16 (15-18)	18 (16-22)	**<0.001**
Obtained a University Degree	18 (12.1%)	8 (11.8%)	27 (39.7%)	**<0.001**
Currently Working/Studying	51 (34.2%)	27 (39.7%)	33 (48.5%)	0.133
Current Smoker	14 (9.3%)	10 (14.7%)	3 (4.3%)	0.109
Caffeine Intake (g/Wk)	1.8 (0.9-2.5)	1.6 (0.9-2.4)	1.8 (1.2-2.4)	0.592
Alcohol Intake (Units/Wk)	1.5 (0-9.0)	1.8 (0-9.8)	4.8 (0-16.4)	**0.035**
Involved in Exercise or Sport	62 (48.1%)	21 (38.2%)	40 (70.2%)	**0.002**
Hb (g/l)	132 (125-140)	127.5 (117-138)	139 (131-149)	**<0.001**
eGFR (ml/min/1.73m^2)	61.0 (37.0-75.5)	38.8 (27.0-56.0)	79.0 (71.0-90.0)	**<0.001**
Disease duration (months)	68 (29-120)	-	-	-
VDI	4 (3-6)	-	-	-

Table 3.2: Characteristics of the study participants by group.
'Male Gender', 'White Ethnicity', 'Living Partner', 'Obtained a University Degree', 'Currently Working/Studying', 'Current Smoker' and 'Involved in Exercise or Sport' are expressed as number of participants and as percentage of valid responses; other results expressed as median with IQR. eGFR = estimated GFR, calculated using Modification of Diet in Renal Disease (MDRD) formula; VDI = Vasculitis Damage Index. Significant differences across the three groups are highlighted in bold.

Table 3.3 shows the most important disease-specific characteristics of the AAV group. As shown, the vast majority (86.2%) of participants had a documented diagnosis of either GPA or MPA; 7.2% had a biopsy-proven diagnosis of AAV but no precise phenotypic diagnosis given by the clinical team. 88 participants had renal involvement, of who 17 remained dialysis-dependent, and 65 had lung involvement. Table 3.4 (on the next page) details the demographic characteristics of the patients with AAV who had a documented diagnosis of either GPA or MPA.

	Number and Percentage of Participants
GPA	89 (58.6%)
MPA	42 (27.6%)
eGPA	6 (3.9%)
Renal-Limited AAV	4 (2.6%)
Other or Uncertain Diagnosis	11 (7.2%)
Renal Involvement	88 (57.9%)
Dialysis-Dependent	17 (11.2%)
Lung Involvement	65 (42.8%)
ENT Involvement	70 (46.1%)
Joint Involvement	37 (24.3%)
Peripheral Nerve Involvement	28 (18.4%)
Previous Exposure to Cyclophosphamide	108 (71.1%)
Currently on Prednisolone	131 (86.2%) (Median dose 5mg, IQR 2.5-5)

Table 3.3: Disease-specific characteristics of the AAV group
Results are expressed as absolute number, with percentage of all patients with AAV in brackets. 'Other or Uncertain Diagnosis' includes three participants who were felt to have drug-induced AAV, and the rest had a documented diagnosis of AAV but without a specific phenotypic diagnosis.

	GPA	MPA	Significance
n	89	42	-
Age (yrs)	58.1 (46.5-67.7)	68.9 (61.2-76.2)	**<0.001**
Male Gender	46 (51.7%)	14 (33.3%)	**0.049**
White Ethnicity	84 (94.4%)	39 (92.9%)	0.734
Living Partner	62 (70.5%)	29 (69%)	0.870
Age Left Education (yrs)	16 (15-18)	16 (15-17)	0.240
Obtained a University Degree	10 (11.4%)	3 (7.5%)	0.502
Currently Working/Studying	33 (37.9%)	11 (26.8%)	0.217
Current Smoker	10 (11.4%)	1 (2.4%)	0.085
Caffeine Intake (g/Wk)	1.8 (0.7-2.1)	1.8 (1.2-2.8)	0.124
Alcohol Intake (Units/Wk)	1.5 (0-9.0)	1.5 (0-5.0)	0.289
Involved in Exercise or Sport	42 (52.5%)	11 (36.7%)	0.139
Hb (g/l)	135 (126-142)	127 (119-134)	**0.008**
eGFR (ml/min/1.73m^2)	63.0 (47.5-75.5)	41.5 (26.0-69.0)	**0.004**
Disease duration (months)	76 (38-126)	74.5 (24.5-115)	0.527
VDI	4 (3-7)	5 (3-6)	0.997

Table 3.4: Characteristics of the patient group with AAV by phenotypic diagnosis.
'Male Gender', 'White Ethnicity', 'Living Partner', 'Obtained a University Degree', 'Currently Working/Studying', 'Current Smoker' and 'Involved in Exercise or Sport' are expressed as number of participants and as percentage of *valid* responses; other results expressed as median with IQR. eGFR = estimated GFR, calculated using Modification of Diet in Renal Disease (MDRD) formula; VDI = Vasculitis Damage Index. Significant differences are highlighted in bold.

As shown, patients with GPA were significantly younger and more likely to be male than those with MPA. They also had significantly better Hb and eGFR, but any other differences were not significant – in particular they were not significantly more likely to be involved in exercise.

3.2.1 Education and Employment

Better education, which has previously been associated with lower levels of fatigue (451), was assessed in our questionnaire by the participants' age of leaving the education system and by the percentage of participants who had attained formal qualifications. There was no difference in school-leaving age between the AAV and CKD disease groups ($p=0.225$), but mean school-leaving age in the healthy group was significantly higher ($p<0.001$) than in either disease group. Likewise, there was no difference in the proportion of patients with university degrees between the two disease groups ($p=0.571$), but healthy controls were significantly more likely to have obtained a university degree ($p<0.001$) compared with either of the other groups. Disease duration could not have accounted for this difference, as most patients were not affected by the disease in childhood or early adulthood, and the difference is probably largely due to the recruitment of hospital and university employees into the healthy control group. Studies have also previously found that educational attainment has an influence on enrolment in research (452).

Although there was no overall significant association between study group and the likelihood of currently being in employment or education (as shown in Table 3.2), direct comparison between the AAV and healthy groups showed that patients with AAV were less likely to be currently working or studying ($p=0.045$); this could be important, as there have been links in previous studies between employment status and fatigue (85, 453).

3.2.2 Lifestyle

Smoking status was asked for because it has been associated with fatigue previously (454, 455), perhaps due to carbon monoxide hampering the delivery of oxygen to muscle mitochondria (456). Overall in this study there was no significant difference in smoking

status between the groups, although sub-analysis showed that those in the CKD group were significantly more likely to be current smokers than those in the healthy group (p=0.034).

Questionnaire participants were asked to estimate their intake of both caffeine and alcohol. There is evidence that antagonism of adenosine receptors in the brain by caffeine can reduce fatigue, particularly in the setting of exercise (457), and it is widely used in Western society as a way of reducing tiredness. It exhibits a U-shaped dose-response curve, with generally positive effects on attention and concentration at lower doses but decreases in performance and higher levels of anxiety with higher dose, and there is evidence of greater effects in fatigued than non-fatigued individuals (458). Participants were asked how many cups of coffee and tea they consumed in a typical week, with weekly caffeine intake calculated by assuming the average caffeine content of a cup of coffee or tea to be 100mg and 50mg respectively; as shown in Table 3.2 no differences in intake were seen between the groups. Self-reported alcohol intake in units per week was also calculated, with the alcohol content of drinks assumed to be 1.5 units for a small glass of wine (125ml), 1 unit for a measure of fortified wine (50ml), 2.5 units for a pint of beer or cider, and 1 unit for a 25ml measure of spirits or a 50ml measure of liqueur. A significant association was found between participant group and alcohol intake, with the healthy control group admitting to around three times the alcohol consumption of the CKD and AAV groups (p=0.01 for AAV vs healthy group, p=0.077 for CKD vs healthy group). The explanation for this might be that the patients had been told by their doctors that they must not drink excessive amounts of alcohol due to the medications they were on, or that the patients themselves perceived that to be the case – for example those on prednisolone might have been concerned about the increased risk of gastritis or peptic ulcers caused by the combination of alcohol and oral steroids. They might even have thought that simply having AAV or CKD means they should drink less alcohol. However the effects of alcohol are in many respects similar to those of fatigue, particularly

with regards to the reduction they cause in attention and concentration (459), so perhaps patients were avoiding excessive intake so as not to make their fatigue symptoms worse.

Activity levels were much lower than had been anticipated when the questionnaire was designed, so responses to questions 21-28 in Section 1 were dichotomised to 'not involved' (participant answered 'Never') or 'involved' (participant gave any of the other possible answers). The responses to involvement in jogging or running, swimming, cycling, and exercise or dance classes (and 'other' if appropriate) were then analysed together as 'involvement in exercise or sport'; as shown, the CKD group were found to be the most sedentary and the healthy control group were the most active ($p=0.005$ for AAV vs healthy and $p=0.001$ for CKD vs healthy). It may be that patients were more sedentary than healthy controls because the severity of their baseline fatigue made them feel disinclined to engage in sport and exercise. Alternatively, perhaps they were actively avoiding exercise because their previous experience told them that they could avoid developing significant fatigue if they did so. An alternative explanation might be the variability of knowledge about and attitudes towards fatigue amongst physicians and other health care workers. In clinic, physicians are frequently asked by fatigued patients for advice on how best to manage the symptom, and it would not be surprising if some had been advising their patients that they should conserve energy by not exerting themselves excessively.

3.2.3 Biological Factors

Anaemia and renal impairment are causes of fatigue, and in the present study Hb and eGFR in the AAV group were both significantly lower than in the healthy group ($p=0.001$ for Hb and $p<0.001$ for eGFR) but significantly higher than in the CKD group ($p=0.026$ for Hb and $p<0.001$ for eGFR).

3.3 The Severity of Fatigue in AAV

The severity of subjective fatigue symptoms were assessed using the Multi-Dimensional Fatigue Inventory (MFI-20). Responses from the three groups are illustrated in Figure 3.1, with median scores and interquartile ranges shown for the five different dimensions of fatigue. Scores across all dimensions of fatigue were significantly higher in both of the disease groups compared with healthy controls ($p<0.001$ for all comparisons, except $p=0.011$ for CKD vs healthy in Mental Fatigue), indicating that both disease groups experience much more subjective general, physical and mental fatigue, as well as greater reductions in self-reported activity and motivation. There is no 'overall' or 'total' score for fatigue in the MFI-20, and it has been previously suggested that the General Fatigue score should be used when a single score is desired to compare groups (113, 118); the General Fatigue scores of the AAV group (median=15, IQR=12-17) were significantly greater than those of the CKD group (median=13, IQR=10-16, $p=0.013$), although differences between the scores of the two disease groups were not significant for the other four dimensions of fatigue.

There is no cut-off for the presence or absence of fatigue in the MFI-20, so in order to assess the prevalence of severe fatigue in the AAV and CKD groups patients were dichotomised into 'fatigued' and non-fatigued' according to their General Fatigue score, using the 95th centile of the healthy control group as the threshold of excessive or severe fatigue. Using the resultant threshold score of ≥16, the prevalence of severe fatigue was 42.8% in the AAV group and just 29.9% in the CKD group, although this difference was not statistically significant ($p=0.073$).

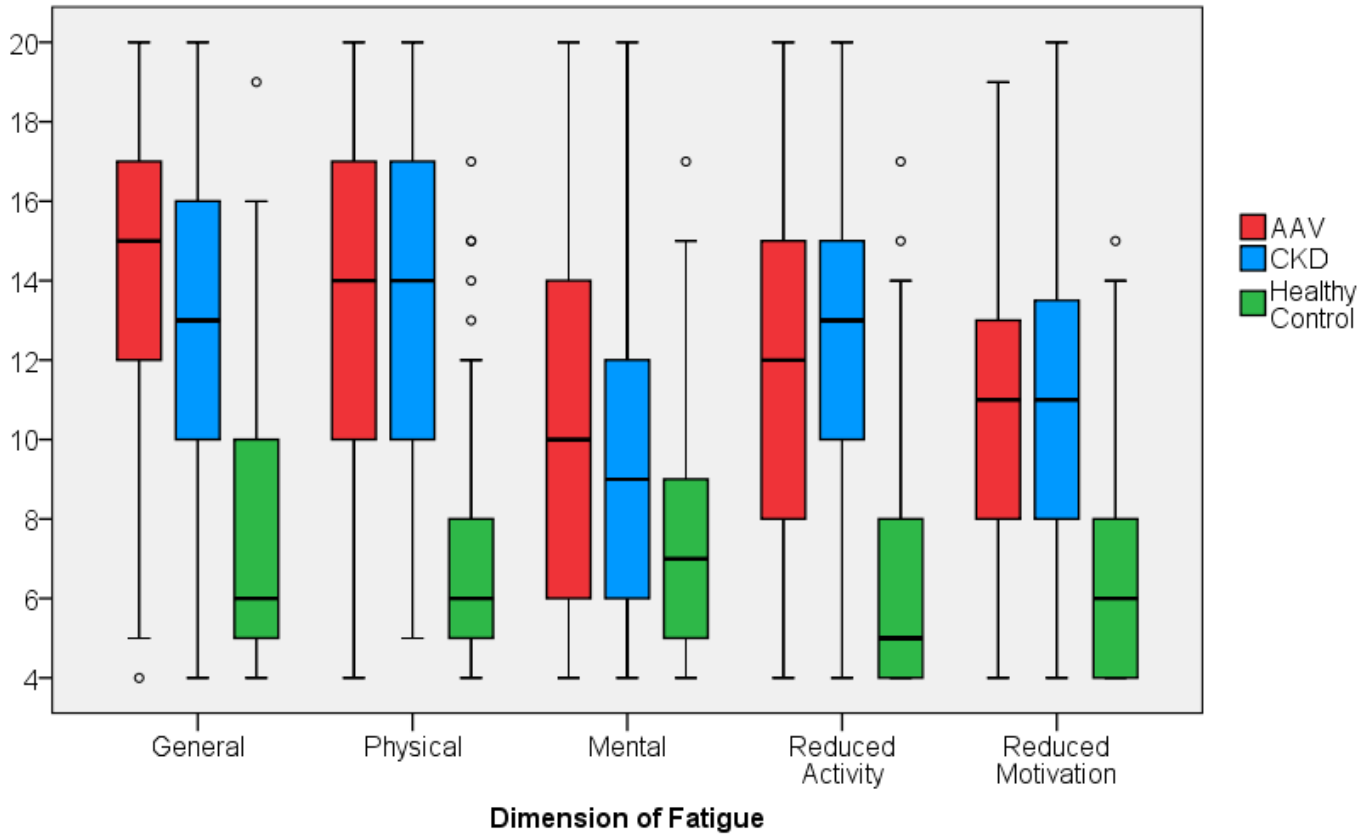


Figure 3.1: Median values and IQR for the 5 dimensions of fatigue on the MFI-20.
Bars indicate median, boxes indicate the interquartile range, whiskers indicate the range, and dots represent outliers (values greater than 1.5 interquartile ranges away from the 25th or 75th percentiles).

3.3.1 Were Patients With MPA More Fatigued Than Patients with GPA?

As described previously, those patients with AAV in the study who had a phenotypic diagnosis of GPA had significantly higher Hb and eGFR than those with a diagnosis of MPA, and they were also younger. In view of these differences, it seemed possible that the MPA subgroup might be much more fatigued than the GPA subgroup, but as the results in Table 3.5 show, the only difference between the groups was that patients with MPA reported slightly worse motivation. Because there is so little difference in fatigue or self-reported activity levels (see Table 3.4) between the two groups, the patients with AAV were analysed as a single group in the remainder of this thesis.

	GPA	MPA	Significance
General Fatigue	15 (12-18)	14 (12-17)	0.581
Physical Fatigue	14 (10-17)	14.5 (10-17)	0.427
Mental Fatigue	10 (5-14)	10 (6-13)	0.859
Reduced Activity	11.5 (8-14)	12 (8.5-15.5)	0.283
Reduced Motivation	10 (7-12)	12 (8-14)	**0.020**

Table 3.5: Differences in fatigue between patients with GPA and MPA.
Median values and IQR are shown for the 5 dimensions of fatigue on the MFI-20. Comparisons are shown between patients with with GPA and those with MPA, with significant differences highlighted in bold.

3.4 The Qualitative Nature of Fatigue in AAV

Because the MFI-20 splits fatigue symptoms into five different dimensions, it may also be used to inform about the quality or nature of the fatigue experienced by respondents. Sample statements taken from the questionnaire which help to illustrate the differences between the five dimensions are: “I feel tired” (General Fatigue), “Physically I feel only able to do a little” (Physical Fatigue), “It takes a lot of effort to concentrate on things” (Mental Fatigue),

"I think I do very little in a day" (Reduced Activity) and "I dread having to do things" (Reduced Motivation). As shown in Figure 3.1, symptoms of Physical Fatigue were much more severe for both the AAV and the CKD groups than were symptoms of mental fatigue, and the self-reported impact of fatigue on activity was greater than the impact on motivation; this suggests that the fatigue experienced in both AAV and CKD has a very physical quality.

Becoming fatigued on occasion is a normal part of life for healthy people, and the multidimensionality of the MFI-20 allows the nature of this fatigue to be examined. In comparison to the AAV and CKD groups, the healthy control group reported Mental Fatigue symptoms that were slightly more severe than their physical fatigue symptoms, and a marginally greater impact of fatigue on motivation than on activity, which suggests that there might be something different about the fatigue experienced in AAV and CKD compared with that experienced by healthy people in every-day life. This is in keeping with previous research in RA (102, 379) where patients have described the fatigue related to their disease as being fundamentally different from the fatigue they experienced in health before the onset of their disease. However although in that research patients described sudden episodes of physical 'wipeout', the authors highlighted differences in the predictability and chronicity of fatigue in RA rather than a more physical nature to the symptoms *per se*.

3.5 Health-Related Quality Of Life (HRQOL)

Table 3.6 shows the median scores for the 8 sub-scales and two summary scores of the MOS SF-36 for each of the three groups. It should be remembered that, in contrast to all of the other symptom scales used in this study, higher scores on the SF-36 indicate *better* condition – for example, a higher score on the PF subscale indicates better physical functioning.

	AAV	CKD	AAV vs CKD	Healthy	AAV vs Healthy
Physical functioning (PF)	55 (32.5-80)	60 (40-90)	0.401	95 (85-100)	**<0.001**
Role Physical (RP)	25 (0-75)	62.5 (0-100)	**0.030**	100 (100-100)	**<0.001**
Bodily Pain (BP)	62 (41-84)	62 (41-74)	0.350	84 (72-100)	**<0.001**
General Health (GH)	42 (21-62)	45 (25-67)	0.379	82 (72-92)	**<0.001**
Vitality (VT)	45 (30-55)	50 (30-65)	0.357	80 (62.5-85)	**<0.001**
Social Functioning (SF)	75 (50-100)	87.5 (62.5-100)	0.454	100 (100-100)	**<0.001**
Role Emotional (RE)	100 (0-100)	100 (0-100)	0.278	100 (100-100)	**<0.001**
Mental Health (MH)	68 (52-84)	74 (62-88)	0.077	84 (76-92)	**<0.001**
Physical Component Score (PCS)	38.7 (26.9-48.4)	39.0 (30.3-50.9)	0.280	54.7 (49.8-56.7)	**<0.001**
Mental Component Score (MCS)	50.2 (39.4-56.9)	52.5 (40.4-58.7)	0.538	56.2 (52.7-58.4)	**<0.001**

Table 3.6: Health-related Quality of Life by SF-36 domains.
Median values and IQR are shown for the 8 domains and the two summary scores of the SF-36. Comparisons are shown between the AAV group and the other two groups, with significant differences highlighted in bold.

Across all eight domains of the SF-36 and both of the summary scores (PCS and MCS), both of the disease groups reported much poorer HRQOL than the healthy group ($p<0.001$ for all comparisons). Comparing the two disease groups, the only significant difference was that the AAV group reported greater role limitations due to physical health (RP) ($p=0.03$) (see also section 3.6).

3.5.1 Did Fatigue Significantly Influence Health-Related Quality Of Life?

Table 3.7 shows the correlations found between the five domains of the MFI-20 and the 8 primary domains and two summary scores of the SF-36 when participants from all groups were included in the analysis; all of the correlations shown were significant at the level of $p<0.001$ and, as indicated, the majority were very strong correlations of $r_s>0.5$. When considering the patients with AAV only, analysis showed that all correlations remained significant at the level of <0.001 except for those between Mental Fatigue and PF ($r_s=-0.173$, $p=0.051$), Mental Fatigue and RP ($r_s=-0.231$, $p=0.007$), Mental Fatigue and PCS ($r_s=-0.143$, $p=0.122$), and Reduced Activity and MCS ($r_s=-0.264$, $p=0.004$). It is unsurprising that Mental Fatigue correlated less strongly with Physical Functioning and the Physical Component Score of the SF-36 than with the mental aspects of HRQOL, and as a whole these results suggest that fatigue is a major cause of reduced HRQOL in AAV, supporting reports in the existing literature (324). Importantly, they also show a very strong association between fatigue and both pain (measured by the Bodily Pain domain of the SF-36) and psychological health (measured by the Mental Health domain) in our patients with AAV. This may indicate that bodily pain is one of the causes of increased fatigue in AAV, although it could also be argued that severe fatigue might itself increase the perception of pain. The relationship between fatigue and mental health will be explored further in Section 3.9.1.

SF-36	MFI-20				
	General Fatigue	**Physical Fatigue**	**Mental Fatigue**	**Reduced Activity**	**Reduced Motivation**
Physical functioning (PF)	**-0.672**	**-0.791**	-0.314	**-0.695**	**-0.582**
Role Physical (RP)	**-0.644**	**-0.686**	-0.376	**-0.639**	**-0.543**
Bodily Pain (BP)	-0.495	**-0.568**	-0.305	-0.468	-0.407
General Health (GH)	**-0.753**	**-0.835**	-0.418	**-0.664**	**-0.624**
Vitality (VT)	**-0.857**	**-0.777**	-0.489	**-0.696**	**-0.655**
Social Functioning (SF)	**-0.643**	**-0.650**	-0.496	**-0.587**	**-0.594**
Role Emotional (RE)	**-0.510**	-0.463	-0.479	-0.463	-0.457
Mental Health (MH)	**-0.554**	-0.452	**-0.585**	-0.398	-0.476
Physical Component Score (PCS)	**-0.681**	**-0.804**	-0.315	**-0.696**	**-0.547**
Mental Component Score (MCS)	**-0.568**	-0.415	**-0.574**	-0.382	-0.493

Table 3.7: Correlations between fatigue (MFI-20) and health-related quality of life (SF36).
Correlations (r_s) are shown between the five domains of the MFI-20 and the 8 primary domains and two summary scores of the SF-36. Correlations of >0.5 are considered particularly significant and are highlighted in bold. All correlations were significant at the level of <0.001.

3.6 Fatigue and Role Impairment

The SF-36 results (section 3.5) revealed that both of the disease groups experienced significant role impairments due to their physical health (SF-36 RP), and also that these role impairments correlated strongly with severity of fatigue across all domains of the MFI-20. However the questions in the SF-36 questionnaire do not allow investigators to separate whether these role impairments are due to fatigue or other physical problems or symptoms. The direct effect of fatigue on life roles was therefore explored further in a modified version of the Piper Fatigue Scale, the results of which are shown in Table 3.8. Both disease groups experienced severe role impairments as a direct result of chronic fatigue across all aspects of their working, family and social life which were highly significant when compared to the healthy group. Although the AAV group reported more severe impairment of all roles than did the CKD group, none of the comparisons were statistically significant.

	AAV	CKD	AAV vs CKD	Healthy	AAV vs Healthy
Work or School	4 (1.5-7)	2 (1-6)	0.241	1 (1-2)	**<0.001**
Socialising with Friends or Family	3 (1-7)	2 (1-5)	0.345	1 (1-1)	**<0.001**
Sex Life	5 (1-9)	4 (1-8)	0.259	1 (1-2)	**<0.001**
Enjoyable Activities	6 (2-8)	3 (1-8)	0.081	1 (1-2)	**<0.001**
Activities of Daily Living (ADLs)	4 (2-7)	2 (1-6)	0.051	1 (1-2)	**<0.001**

Table 3.8: Impact of fatigue on professional, family and social life.
Results shown are for the modified Piper Fatigue scale, where scores range from 0 ('No impact') to 10 ('Greatest possible impact'). Median scores (with IQR) are shown, and statistically significant results are highlighted in bold.

3.7 Which Participant Characteristics Influenced Fatigue?

	General Fatigue		Physical Fatigue		Mental Fatigue		Reduced Activity		Reduced Motivation	
	r_s	p	r_s	p	r_s	p	r_s	p	r_s	p
Age (yrs)	-0.003	0.959	**0.197**	**0.001**	**-0.162**	**0.007**	**0.277**	**<0.001**	**0.172**	**0.004**
Female Gender	**0.193**	**0.001**	0.096	0.113	0.029	0.634	0.017	0.778	0.005	0.933
Ethnicity	0.074	0.216	0.020	0.745	0.042	0.486	0.029	0.634	0.006	0.918
No Living Partner	**0.161**	**0.007**	**0.164**	**0.006**	**0.121**	**0.044**	**0.136**	**0.024**	**0.191**	**0.001**
Age Left Education	**-0.200**	**0.001**	**-0.292**	**<0.001**	-0.48	0.423	**-0.284**	**<0.001**	**-0.259**	**<0.001**
Obtained University Degree	**-0.207**	**0.001**	**-0.235**	**<0.001**	-0.055	0.365	**-0.194**	**0.001**	**-0.185**	**0.002**
Unemploy-ed	**0.181**	**0.003**	**0.304**	**<0.001**	0.028	0.638	**0.350**	**<0.001**	**0.195**	**0.001**
Current Smoker	0.091	0.129	**0.146**	**0.015**	0.063	0.296	**0.143**	**0.018**	**0.162**	**0.007**
Caffeine Intake	-0.041	0.499	-0.33	0.588	-0.094	0.120	-0.041	0.498	-0.071	0.241
Alcohol Intake	**-0.247**	**<0.001**	**-0.291**	**<0.001**	-0.089	0.141	**-0.278**	**<0.001**	**-0.240**	**<0.001**
Exercise or Sport	**-0.255**	**<0.001**	**-0.361**	**<0.001**	-0.045	0.494	**-0.345**	**<0.001**	**-0.210**	**0.001**
Hb (g/l)	**-0.291**	**<0.001**	**-0.264**	**<0.001**	-0.078	0.211	**-0.253**	**<0.001**	**-0.161**	**0.010**
eGFR	**-0.240**	**<0.001**	**-0.355**	**<0.001**	-0.038	0.556	**-0.428**	**<0.001**	**-0.244**	**<0.001**
Disease duration	-0.004	0.964	0.044	0.618	-0.084	0.338	-0.004	0.965	-0.020	0.820
VDI	0.141	0.162	**0.362**	**<0.001**	0.035	0.732	**0.258**	**0.010**	0.180	0.073

Table 3.9: Correlations between participant characteristics and MFI-20 scores.
Study participants from all three groups are included in these correlations. Significant correlations ($p<0.05$) are highlighted in bold.

Table 3.9 shows the univariate correlations that were found between the five dimensions of fatigue on the MFI-20 and each of the participant characteristics when the participants from all three study groups were analysed together. Since the healthy group had much lower levels of fatigue in all five dimensions than the two disease groups, there was a concern that differences in the characteristics between the healthy group and the disease group could lead

to misleading results, so univariate correlations were also performed using only the participants in the AAV group, as detailed in each subsection below.

3.7.1 Participant Demographics

When participants from all three groups were analysed together, positive correlations were found between participant age and Physical Fatigue, Reduced Activity and Reduced Motivation, in keeping with existing literature (451) but a negative correlation was found between age and Mental Fatigue, and the results were very similar whenever only AAV participants were included in the analysis (r_s=0.163, p=0.05 for Physical Fatigue; r_s=0.252, p=0.002 for Reduced Activity; r_s=0.161, p=0.052 for Reduced Motivation; r_s=-0.273, p=0.001 for Mental Fatigue). The positive correlation with Physical Fatigue and Reduced Motivation might be explained by the natural reduction in physical reserve (particularly cardiovascular) seen with increasing age, and one explanation for the negative correlation with mental fatigue might be that younger participants were more aware of their mental fatigue because they were more likely to be in employment or education (correlation between age and unemployment for the patients with AAV was r_s=0.563, p<0.001), although the lack of any correlation between unemployment and mental fatigue (see section 3.7.3) perhaps makes this explanation less likely.

In this study female gender was associated with greater General Fatigue, and when patients with AAV were analysed separately not only was this still the case but there was also a positive correlation between female gender and Physical Fatigue (r_s=0.174, p=0.036); however no correlations existed between gender and the other dimensions of fatigue. Studies exploring gender differences in fatigue severity have previously given conflicting results (451, 460) and it is not yet clear how important an influence gender is on fatigue levels. No correlation was seen between ethnicity and fatigue, although it was unlikely such a

correlation would be found in view of the very low percentage of participants who were not Caucasian in this study.

3.7.2 Marital Status

Not having a living spouse or partner correlated positively with higher scores across all dimensions of fatigue, which is unsurprising since for most people their partner is their main source of psychological support. Since there was no significant difference in marital status between the groups, it was surprising that this association was not seen when the AAV group was analysed in isolation. This might be explained by the smaller number of participants in the analysis, or perhaps other predictors of fatigue were so strong for these patients that marital status became less important.

3.7.3 Education and Employment

Increasing educational attainment was associated with a lower severity of fatigue, in keeping with previous literature (451); this was not simply a result of the better educational attainment in the healthy group (discussed in Chapter 3.2.1) because age at the time of leaving education was also negatively correlated with MFI-20 scores within the AAV group (r_s=-0.225, p=0.007 for Physical Fatigue; r_s=-0.188, p=0.025 for Reduced Motivation). Unemployment was associated with higher scores in all dimensions of fatigue except for Mental Fatigue as shown, and this was also true in sub-analysis of the AAV group: r_s=0.160, p=0.056 for General Fatigue; r_s=0.329, p<0.001 for Physical Fatigue; r_s=0.369, p<0.001 for Reduced Activity; r_s=0.234, p=0.005 for Reduced Motivation. This finding was in keeping with previous studies which have found that chronic fatigue is strongly associated with occupational disability and unemployment (461).

3.7.4 Lifestyle

When all groups were analysed together, being a current smoker correlated positively with higher levels of Physical Fatigue, Reduced Activity, and Reduced Motivation. This finding is in keeping with previous literature (454-456), but despite there being no difference in smoking prevalence between the groups these correlations were not found in sub-analysis of only the AAV group. The proposed link between smoking and fatigue is that CO reduces oxygen delivery to peripheral muscles, so this finding might suggest that muscular fatigue is not an important contributor to the overall experience of fatigue in AAV. An alternative explanation might be that smoking does increase fatigue in AAV, but that other influences are so much stronger as to render the effect of smoking insignificant in a relatively small sample of patients.

No association was found in this study between caffeine intake and fatigue severity when all participants were analysed together, but when the AAV group was analysed separately there was a negative correlation between mental fatigue and caffeine intake (r_s=-0.169, p=0.042), perhaps suggesting that for some patients with AAV, caffeine was a helpful way of reducing mental fatigue. There was a negative correlation between alcohol intake and fatigue (other than mental fatigue), and this was not simply a result of the much lower alcohol intake reported by patients with AAV in this study because the negative correlation was even stronger when the AAV group was analysed separately (r_s=-0.257, p=0.002 for General Fatigue; r_s=-0.302, p<0.001 for Physical Fatigue; r_s=-0.289, p<0.001 for Reduced Activity; r_s=-0.270, p=0.001 for Reduced Motivation). As mentioned previously the most likely explanation for these fairly strong correlations might be that more fatigued patients were inclined to avoid the further sedative effects of alcohol.

As would be expected, there were strong negative correlations (as shown in Table 3.9) between involvement in sport or exercise and greater severity in all of the dimensions of

fatigue other than mental fatigue. When the AAV group were analysed separately, strong negative correlations were still found between involvement in sport and both Physical Fatigue (r_s=-0.339, p<0.001) and Reduced Activity (r_s=-0.226, p=0.011). This part of the questionnaire was problematic for two reasons: the level of completion was relatively low, with 17% of participants not completing the section and, as discussed previously, the selection of possible answers anticipated a much higher level of participation in exercise than was found in the participants. In view of these problems, the results should be interpreted with some caution.

3.7.5 Biological Factors

Lower levels of haemoglobin (Hb) and poorer renal function, both recognised independent causes of fatigue, were associated with higher levels of fatigue in this cohort as shown. Sub-analysis of the AAV group showed similarly strong negative correlations between Hb and General Fatigue (r_s=-0.286, p=0.002), Physical Fatigue (r_s=-0.216, p=0.012), Reduced Activity (r_s=-0.268, p=0.002) and Reduced Motivation (r_s=-0.178, p=0.039); the association with eGFR was weak in this group, with only Reduced Activity reaching significance (r_s=-0.270, p=0.002). It seems improbable that reduced renal function would not cause fatigue in AAV, and it is probable that we only found no correlation because of the relatively well preserved renal function in the patients with AAV in this study. Disease duration did not significantly correlate with fatigue in our patients with AAV, although it should be noted that all patients in this study had been in remission for at least six months. The degree of accumulated disease-related damage (as assessed by the VDI) was strongly associated with greater physical fatigue and reduced activity.

3.8 Did Recruitment of Healthy Controls From the University and Hospital Affect The Results?

Out of the seventy-one healthy controls, thirteen (18%) were recruited from among the staff of UoB and UHBNFT. A sensitivity analysis is therefore important to assess whether the recruitment of these particular healthy controls had any important effect on the results.

	AAV	CKD	Healthy	Significance
n	152	68	58	-
Age (yrs)	63.1 (49.8-71.5)	64.4 (47.8-75.2)	62.6 (51.6-68.7)	0.568
Male Gender	73 (48.0%)	39 (57.4%)	30 (51.7%)	0.439
White Ethnicity	141 (92.8%)	61 (89.7%)	57 (98.3%)	0.149
Living Partner	102 (67.5%)	48 (70.6%)	47 (81.0%)	0.156
Age Left Education (yrs)	16 (15-18)	16 (15-18)	18 (15-21)	**0.001**
Obtained a University Degree	18 (12.1%)	8 (11.8%)	20 (35.1%)	**<0.001**
Currently Working/Studying	51 (34.2%)	27 (39.7%)	23 (40.4%)	0.613
Current Smoker	14 (9.3%)	10 (14.7%)	2 (3.5%)	0.102
Caffeine Intake (g/Wk)	1.8 (0.9-2.5)	1.6 (0.9-2.4)	1.8 (1.0-2.3)	0.666
Alcohol Intake (Units/Wk)	1.5 (0-9.0)	1.8 (0-9.8)	4.0 (0-16.5)	0.081*
Involved in Exercise or Sport	62 (48.1%)	21 (38.2%)	31 (66.0%)	**0.018**
Hb (g/l)	132 (125-140)	127.5 (117-138)	140 (130-151)	**<0.001**
eGFR (ml/min/1.73m^2)	61.0 (37.0-75.5)	38.8 (27.0-56.0)	77.0 (70.0-90.0)	**<0.001**

Table 3.10: Characteristics of the study participants by group, excluding UoB/UHBNFT employees. 'Male Gender', 'White Ethnicity', 'Living Partner', 'Obtained a University Degree', 'Currently Working/Studying', 'Current Smoker' and 'Involved in Exercise or Sport' are expressed as number of participants and as percentage of valid responses; other results expressed as median with IQR. eGFR = estimated GFR, calculated using Modification of Diet in Renal Disease (MDRD) formula; VDI = Vasculitis Damage Index. Significant differences across the three groups are highlighted in bold.

As shown in Table 3.10, excluding the employees of UoB and UHB from the analysis makes reassuringly little difference to the group characteristics. For example, although it results in the healthy control group having a slightly lower level of educational attainment and a lower level of participation in sport and exercise, these differences are much less than might be anticipated, and do not at all change the statistical significance of the differences between the groups. The only exception to this is with regards to alcohol intake: the healthy group have a lower level of alcohol intake in this new analysis, and the change is enough to mean that the difference in alcohol intake between the groups is no longer statistically significant. The reason that the recruitment of UoB and UHBNFT employees seems to have made little difference to the group characteristics is probably because care was taken to keep the group as representative as possible: all sections of the work-force were approached, so although doctors and professors were recruited, so were nurses, secretaries etc. Also, it is important to note that they comprised less than 20% of the total healthy control group.

Although the recruitment strategy did not greatly alter the participant characteristics of the healthy control group, it is still important to examine whether it changed the level of subjective fatigue reported. Table 3.11 shows a direct comparison between the median fatigue levels reported by the healthy control group when the hospital and university employees are included or excluded. The inclusion of the employees does not seem to have made any meaningful difference to the fatigue levels in any domain of the MFI-20, and since their inclusion has altered neither the characteristics nor the fatigue levels of the group, it seems entirely appropriate to include them in the remainder of the analysis.

	General Fatigue	Physical Fatigue	Mental Fatigue	Reduced Activity	Reduced Motivation
UoB/UHBNFT Employees Included	6 (5-10)	6 (5-8)	7 (5-9)	5 (4-8)	6 (4-8)
UoB/UHBNFT Employees Excluded	6 (5-9)	6 (5-8)	6 (5-8.5)	5 (4-8)	6 (4-8.5)
Significance	0.740	0.802	0.860	0.948	0.907

Table 3.11: Comparison of the subjective fatigue reported by the healthy control group depending on whether the data of university and hospital employees are included or excluded.
Results shown are for the five dimensions of the MFI-20; median scores (with IQR) are shown.

3.9 Mental Health

As discussed in Section 3.5, the participants' responses to the SF-36 suggested that both the AAV and the CKD disease groups were suffering from much poorer mental health than the healthy control group, and that their mental health correlated strongly with the severity of their fatigue. These findings were further explored with analysis of the participants' responses to the Hospital Anxiety and Depression Scale (HADS), which has three important advantages over the Mental Health domain of the SF-36: it was specifically designed for use with patients, and as such does not ask about symptoms which might be due to physical rather than psychological illness (426, 427); it separates psychological symptoms into those attributable to depression and those attributable to anxiety, and scores in the 'abnormal' range have been shown to be predictive of psychiatric disease (427); it has been previously validated in AAV (354).

As shown in Figure 3.2, the AAV group reported significantly more severe anxiety and depression than did the healthy group ($p<0.001$ for both comparisons), but there were no significant differences compared with the CKD group ($p=0.342$ for anxiety and $p=0.694$ for depression). Figure 3.3 illustrates the median total anxiety and depression scores for each of

the study groups categorised into ‘Normal’ (scores of 0-7), ‘Borderline’ (8-10) and ‘Abnormal’ (11-21, highly predictive of psychiatric disease). Significantly more patients with AAV than healthy controls had ‘Abnormal’ scores for anxiety (p=0.001) and depression (p=0.003); although more patients with AAV than patients with CKD had ‘Abnormal’ scores for anxiety and more patients with CKD had ‘Abnormal’ scores for depression, these differences did not reach statistical significance. In both of the disease groups, more than 25% of participants reported HADS scores predictive of clinical anxiety, depression, or both.

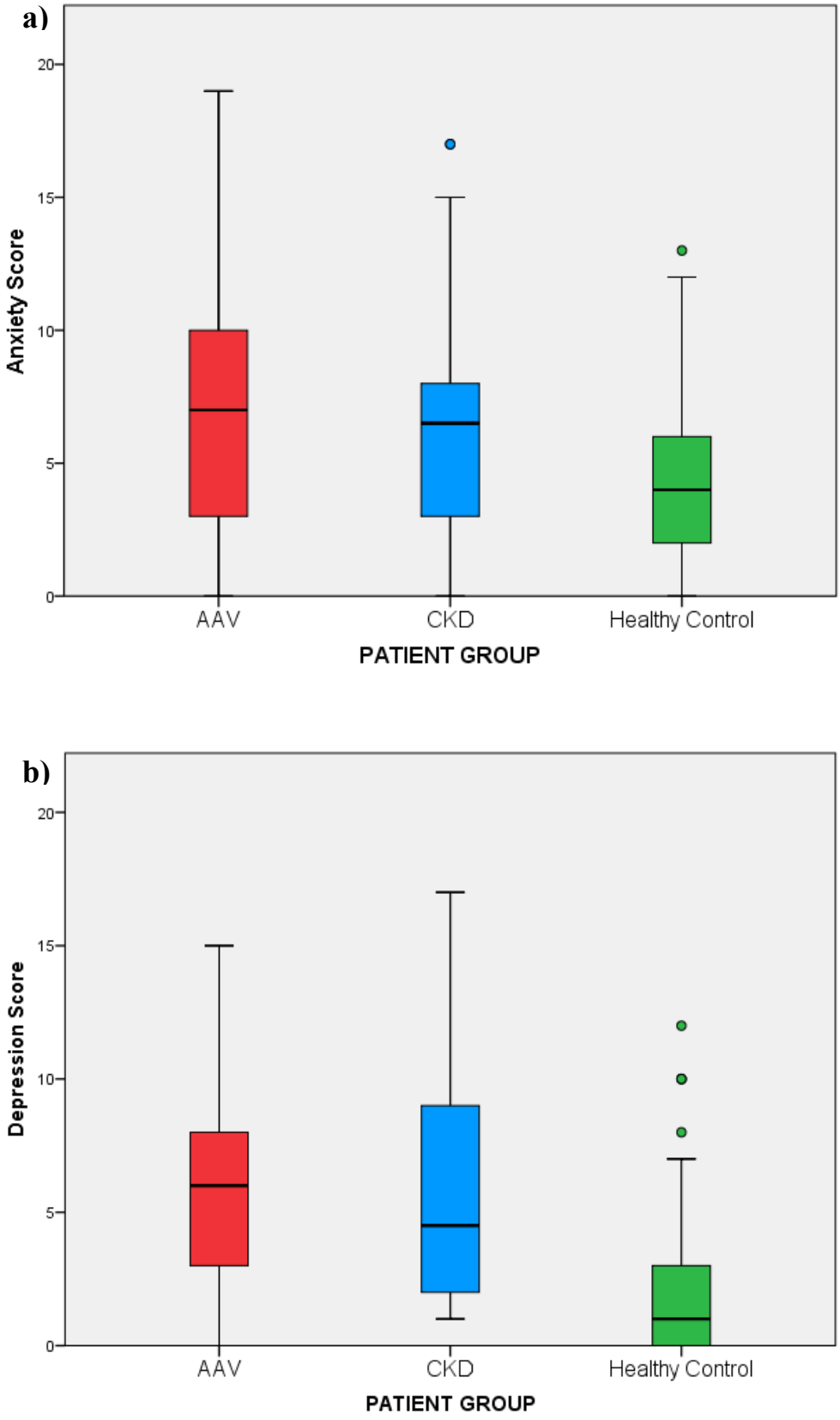


Figure 3.2: Anxiety and depression scores from the Hospital Anxiety and Depression Scale (HADS). Figure a) illustrates total anxiety score, and figure b) illustrates total depression score. Bars indicate median, boxes indicate the interquartile range and whiskers indicate the range.

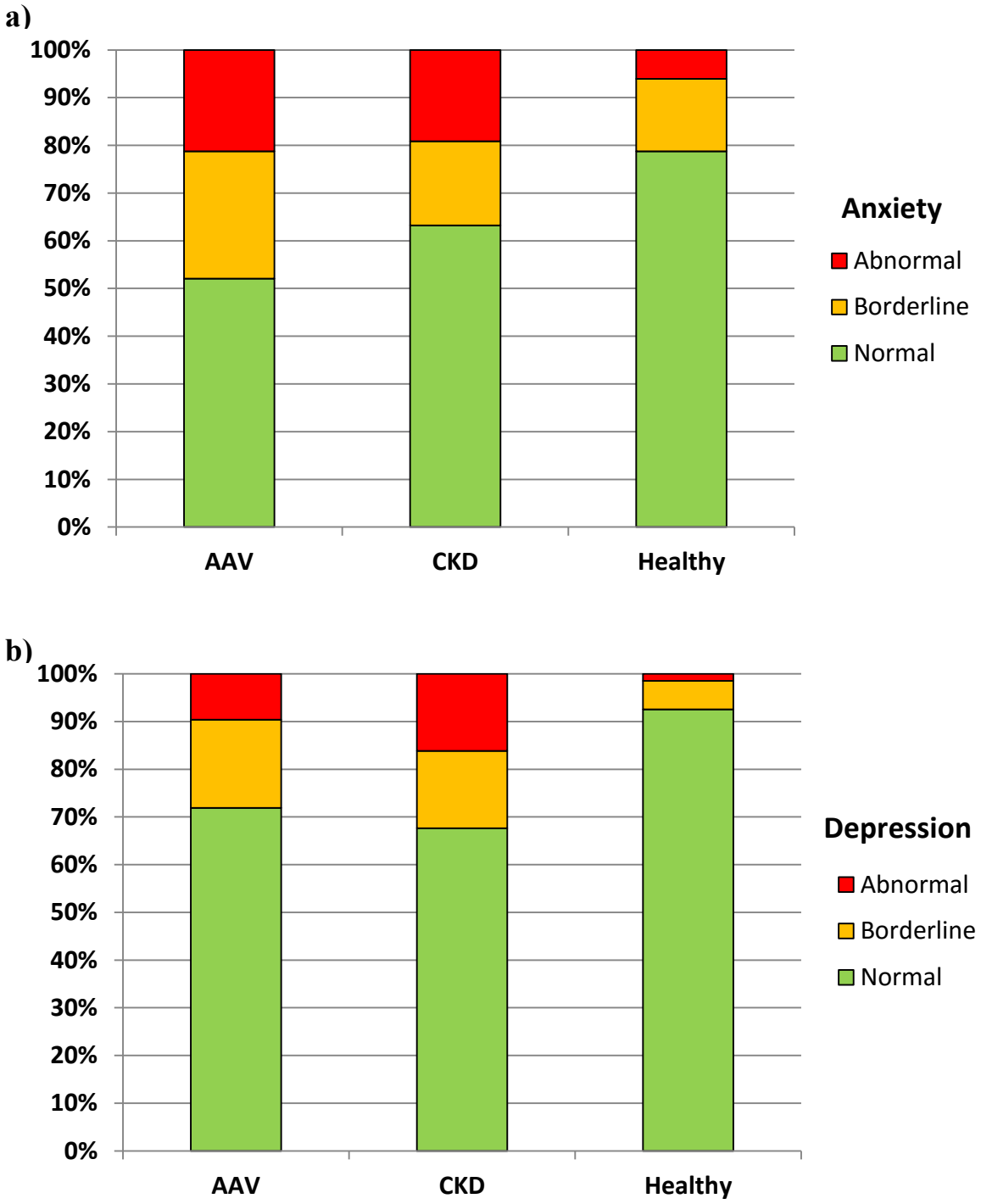


Figure 3.3: Percentage of participants with HADS scores suggestive of psychiatric morbidity.
Figure a) illustrates the percentage of subjects in each group who reported anxiety scores on the HADS which were predictive of clinical anxiety, and figure b) illustrates the percentage with depression scores predictive of clinical depression.

3.9.1 The Relationship Between Fatigue and Mental Health

Table 3.12 shows the correlations which were found between fatigue and mental health for all participants as well as for AAV only. All correlations were highly significant as shown in the table, with depression in particular showing very strong positive correlations with all five dimensions of fatigue on the MFI-20.

	All Participants		**Patients with AAV**	
	Anxiety	**Depression**	**Anxiety**	**Depression**
General Fatigue	**0.559**	**0.701**	0.458	**0.560**
Physical Fatigue	0.435	**0.703**	0.390	**0.642**
Mental Fatigue	**0.576**	**0.586**	**0.556**	**0.511**
Reduced Activity	0.298	**0.689**	0.192 (p=0.022)	**0.550**
Reduced Motivation	0.427	**0.718**	0.398	**0.638**

Table 3.12: Correlations between fatigue and mental health.
Correlations (r_s) are shown between the five domains of the MFI-20 and total anxiety and depression scores of the HADS. Correlations of >0.5 were considered particularly significant and are highlighted in bold. All correlations were significant at the level of <0.001, except for where indicated.

These very strong correlations between psychological morbidity and all aspects of fatigue suggest that central mechanisms are likely to be making a large contribution to the overall fatigue in AAV, although it is impossible in this cross-sectional study to prove whether depression and anxiety were contributing to the participants' fatigue or vice-versa, or indeed whether a third variable was causing both symptoms.

3.10 Sleep Quality

Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI), with results shown in Table 3.13. The AAV group reported sleep that was significantly worse than the healthy controls across all domains except for sleep duration, i.e. the quality of sleep was found to be reduced rather than the duration. Patients took longer to get to sleep, experienced more frequent disturbances, used medications to help them sleep more often, and reported more daytime dysfunction as a result of poor sleep. None of the differences between the AAV and CKD groups reached statistical significance.

	AAV	CKD	AAV vs CKD	Healthy	AAV vs Healthy
Subjective Sleep Quality	1 (1-2)	1 (1-2)	0.338	1 (0-1)	**<0.001**
Sleep Latency	1 (0-2)	1 (0-2)	0.677	0 (0-1)	**<0.001**
Sleep Duration	0 (0-1)	0 (0-1)	0.493	0 (0-1)	0.067
Sleep Efficiency	1 (0-2)	1 (0-2)	0.198	0 (0-1)	**<0.001**
Sleep Disturbances	1 (1-2)	1 (1-2)	0.286	1 (1-1)	**<0.001**
Sleep Medication	0 (0-0)	0 (0-0)	0.413	0 (0-0)	**0.008**
Daytime Dysfunction	1 (1-1)	1 (1-1)	0.843	0 (0-1)	**<0.001**
Global PSQI Score	7 (4-11)	7 (3-9)	0.272	3 (2-5)	**<0.001**

Table 3.13: Sleep quality as assessed by the Pittsburgh Sleep Quality Index.
Median values and IQR are shown for the 7 domains and the Global PSQI Score. Comparisons are shown between the AAV group and the other two groups, with significant differences highlighted in bold.

The correlations between Global PSQI score (i.e. overall sleep quality) and fatigue, psychological morbidity and pain are shown in Table 3.14. As shown, poor sleep quality correlated with all dimensions of fatigue as well as anxiety, depression and pain at a significance level of <0.001, regardless of whether all participants were analysed together or the patients with AAV were analysed separately. Whilst it is impossible to attribute definite

cause on the basis of the data from this study, there is clearly a close and complex relationship between these symptoms which warrants further investigation.

	Global PSQI Score All Participants	Global PSQI Score Patients with AAV Only
General Fatigue	**0.602**	0.493
Physical Fatigue	0.470	0.370
Mental Fatigue	0.429	0.396
Reduced Activity	0.397	0.308
Reduced Motivation	0.432	0.367
Anxiety	**0.579**	**0.539**
Depression	0.488	0.451
Bodily Pain	-0.444	-0.395

Table 3.14: Correlations between sleep quality and fatigue, anxiety and depression.
Correlations (r_s) are shown between Global PSQI score and the five domains of the MFI-20, total anxiety and depression scores of the HADS, and the Bodily Pain domain of the SF-36. Correlations of >0.5 were considered particularly significant and are highlighted in bold. All correlations were significant at the level of <0.001.

3.11 Coping Strategies

Coping strategies were assessed using the Brief COPE questionnaire. As shown in Table 3.15, the use of most coping strategies was the same across the groups, although the AAV group reported:

- More use of 'Active Coping' (defined as "taking action, exerting efforts, to remove or circumvent the stressor") and less 'Substance Use' than the CKD group;
- More use of 'Denial' ("attempting to reject the reality of the stressor") and 'Behavioural Disengagement' ("giving up, or withdrawing effort from, the attempt to attain the goal with which the stressor is interfering"), but less use of 'Active Coping' than the healthy group.

In other words, the AAV group reported slightly less use of strategies which might be regarded as maladaptive compared to the CKD group, but more use than the healthy control group.

The correlations between coping strategies and General Fatigue, Physical Fatigue and Reduced Activity for the patients with AAV were very weak, and the only coping strategy which correlated with severity of fatigue across all dimensions was Behavioural Disengagement, the use of which was associated with increased severity of all dimensions of fatigue (r_s=0.225, p=0.007 for General Fatigue; r_s=0.365, p<0.001 for Physical Fatigue; r_s=0.299, p<0.001 for Mental Fatigue; r_s=0.302, p<0.001 for Reduced Activity; r_s=0.462, p<0.001 for Reduced Motivation). Coping strategies were more predictive of Mental Fatigue and Reduced Motivation, as shown in Table 3.16, suggesting that teaching adaptive coping strategies might help reduce at least these aspects of fatigue.

	AAV	CKD	AAV vs CKD	Healthy	AAV vs Healthy
Active Coping	5 (4-7)	5 (3-7)	**0.040**	6 (5-8)	**0.036**
Planning	6 (4-7)	5 (3-7)	0.232	6 (4-8)	0.081
Positive Reframing	5 (4-7)	4 (3-6)	0.240	5 (4-7)	0.142
Acceptance	6 (5-8)	6 (4-8)	0.880	6 (5-8)	0.623
Humour	4 (3-6)	4 (3-6)	0.808	4 (2.5-6)	0.690
Religion	2 (2-4)	2 (2-3.5)	0.316	2 (2-4)	0.079
Using Emotional Support	4 (3-6)	4 (2-5)	0.103	4 (3-5)	0.605
Using Instrumental Support	4 (3-5)	4 (2-6)	0.858	4 (3-6)	0.946
Self-Distraction	5 (3.5-6)	4 (3-5.5)	0.173	4 (3-6)	0.259
Denial	2 (2-3)	2 (2-3)	0.553	2 (2-2)	**0.001**
Venting	3 (2-5)	3 (2-5)	0.103	4 (2.5-4)	0.832
Substance Use	2 (2-2)	2 (2-4)	**0.020**	2 (2-3)	0.234
Behavioural Disengagement	2 (2-4)	2 (2-3)	0.202	2 (2-2)	**0.009**
Self-Blame	4 (2-5)	3 (2-5)	0.875	4 (3-5)	0.073

Table 3.15: Use of different coping strategies as assessed by the Brief COPE.
Median values and IQR are shown for the 14 domains. Comparisons are shown between the AAV group and the other two groups, with significant differences highlighted in bold.

	Mental Fatigue		Reduced Motivation	
	r_s	p	r_s	p
Active Coping	-0.099	0.242	-0.208	**0.013**
Planning	-0.005	0.955	-0.205	**0.013**
Positive Reframing	-0.025	0.767	-0.233	**0.005**
Acceptance	0.006	0.945	-0.055	0.508
Humour	0.026	0.757	0.012	0.888
Religion	-0.113	0.175	0.039	0.639
Using Emotional Support	0.141	0.092	0.069	0.411
Using Instrumental Support	0.049	0.563	0.052	0.534
Self-Distraction	0.213	**0.010**	0.027	0.746
Denial	0.235	**0.005**	0.143	0.089
Venting	0.235	**0.005**	0.189	**0.023**
Substance Use	0.140	0.093	0.047	0.571
Behavioural Disengagement	0.299	**<0.001**	0.462	**<0.001**
Self-Blame	0.328	**<0.001**	0.187	**0.024**

Table 3.16: Use of coping strategies and severity of fatigue for patients with AAV.
Correlations (r_s) are shown between the fourteen coping strategies and Mental Fatigue and Reduced Motivation scores for the AAV group. Statistically significant results ($p<0.05$) are highlighted in bold.

3.12 Which Factors are Independent Predictors of Fatigue?

To identify which of the variables that were identified as most strongly associated with fatigue in univariate analysis were important independent predictors of each of the dimensions of fatigue, these variables (age, gender, age leaving education, employment status, haemoglobin, anxiety, depression, sleep disturbance, pain) were then submitted to logistic regression models using a backward stepwise selection technique. In these models only the patients with AAV were included, and it was confirmed that the standardised residuals from these regressions were normally distributed. Results were as follows:

- For General Fatigue, Depression Score (standardised beta coefficient=0.429, $p<0.001$), Global PSQI Score (standardised beta coefficient=0.275, p=0.001) and haemoglobin (standardised beta coefficient=-0.200, p=0.007) were the significant independent predictors; the R^2 for the model was 0.438, and the adjusted R^2 was 0.423.
- For Physical Fatigue, Depression Score (standardised beta coefficient=0.512, $p<0.001$), Bodily Pain (standardised beta coefficient=-0.183, p=0.023) and employment status (standardised beta coefficient=0.150, p=0.042) were the significant independent predictors; the R^2 for the model was 0.496, and the adjusted R^2 was 0.477.
- For Mental Fatigue, Anxiety Score (standardised beta coefficient=0.350, $p<0.001$), age (standardised beta coefficient=-0.322, $p<0.001$), and Depression Score (standardised beta coefficient=0.285, p=0.002) were the significant independent predictors; the R^2 for the model was 0.447, and the adjusted R^2 was 0.432.
- For Reduced Activity, Depression Score (standardised beta coefficient=0.568, $p<0.001$), employment status (standardised beta coefficient=0.203, p=0.008) and Global PSQI Score (standardised beta coefficient=-0.178, p=0.044) were the

significant independent predictors; the R^2 for the model was 0.416, and the adjusted R^2 was 0.395.

- For Reduced Motivation, Depression Score (standardised beta coefficient=0.565, $p<0.001$) and Bodily Pain (standardised beta coefficient=-0.190, $p=0.013$) were the significant independent predictors; the R^2 for the model was 0.456, and the adjusted R^2 was 0.448.

3.13 Summary of Findings

One hundred and fifty two patients with AAV, sixty eight patients with CKD and seventy one healthy controls participated in the questionnaire study. The study confirmed that patients with AAV suffer from severe fatigue, with MFI-20 General Fatigue scores that were significantly higher than those found in both the CKD and the healthy control groups, and results also suggested that the nature of fatigue experienced by patients with either AAV or CKD might be quite different from that experienced day-to-day by healthy people. A number of patient characteristics, biological factors and other symptoms correlated with fatigue in univariate analysis, and stepwise linear regression suggested that the most important independent predictors of fatigue symptoms in AAV were depression, sleep quality, haemoglobin, pain, and employment status.

The severity and nature of fatigue were assessed using the MFI-20. Results confirmed that patients with AAV were suffering from severe fatigue, with General Fatigue scores that were significantly worse than the scores of the CKD and healthy groups in this study, and were comparable to those reported elsewhere in cancer, RA, and other chronic illnesses in which fatigue is a prominent feature (116, 119, 122, 128, 132, 136, 462). Qualitative analysis of the pattern of symptoms across the five dimensions of fatigue suggested that in AAV and CKD physical symptoms, such as physically only being able to do a little, were much more severe than mental symptoms. This contrasts with the healthy group in whom fatigue was a less physical experience. This is in keeping with research in RA, which also suggested that fatigue in disease states is fundamentally different from the fatigue of healthy people (102, 379). These findings might suggest that the overall experience of fatigue in chronic disease states such as AAV and CKD is more driven by muscular or cardiovascular fatigue than it is in health. On the other hand the perception or reporting of physical fatigue does not necessarily mean that there are physiological deficits in the cardiovascular or muscular systems. The

mechanisms underlying this severe physical fatigue will be examined in detail in the mechanistic study (see Chapters 4 and 5).

Health-related quality of life as measured by the SF-36 was significantly worse in the AAV and CKD groups than in the healthy control group, and all domains of HRQOL correlated very strongly with fatigue, supporting the existing evidence that fatigue is a major cause of reduced HRQOL in AAV. Although the SF-36 does not ask directly whether problems are caused by fatigue, a modified version of the Piper fatigue scale showed in this study that fatigue was a direct cause of severe role impairments in all aspects of professional, family and social life.

In univariate analysis, a number of patient characteristics were shown to be associated with fatigue in AAV including age, which was positively correlated with physical fatigue but negatively correlated with mental fatigue. The positive correlation between age and Physical Fatigue is to be expected, because as people age their physical reserve generally declines due to a number of physiological factors including a lower maximum heart rate (451). The negative correlation between age and Mental Fatigue is more difficult to explain, but since in this study age was negatively correlated with being employed or in education, perhaps older participants were less likely to be carrying out the sorts of tasks which would exacerbate mental fatigue. Female gender was also positively correlated with General Fatigue and Physical Fatigue in patients with AAV, although reasons for this are not apparent and the existing literature on the impact of gender on fatigue is conflicting (451, 460). Educational attainment was negatively correlated with fatigue, in keeping with existing literature (451); potential reasons for this might be that education enables people to have a better understanding of their disease and symptoms, giving them a better sense of control, or perhaps this finding relates instead to subsequent employment, with university graduates less likely to find employment which requires manual labour that might exacerbate symptoms of

physical fatigue. Fatigue was positively correlated with unemployment; there is evidence in the literature to suggest that severe chronic fatigue causes people to have to give up their jobs (461), and whilst this is perhaps the likeliest explanation for the findings of this study, it could also be argued that unemployment might itself lead to inactivity, depression and fatigue.

In this study both the AAV and CKD groups reported symptoms in keeping with much higher levels of anxiety and depression than the healthy control group as measured by the HADS, with over 25% of patients reporting symptoms that suggested clinically significant anxiety, depression, or both. The disease groups also reported significantly worse sleep quality as assessed by the PSQI, and higher levels of bodily pain as assessed by the SF-36 BP domain than did the healthy control group. These symptoms were shown to correlate strongly with fatigue and with each other, suggesting that there was a complex relationship between fatigue, pain, sleep quality and psychiatric disease. It could be argued that pain, poor sleep quality and psychological symptoms might all have been contributing towards the severe fatigue experienced by patients with AAV, but in a cross-sectional observational study such as this cause and effect can never be substantiated; indeed fatigue, psychological symptoms or poor quality sleep might lower an individual's pain threshold (463, 464), and severe depression is a well-established cause of poor quality sleep (465). Data in this study from the Brief COPE questionnaire showed that patients with AAV were also more likely to use maladaptive coping strategies than the healthy group were, particularly the methods of behavioural disengagement and denial.

To try to unravel which characteristics and symptoms were most predictive of fatigue, linear regression analysis was carried out. This suggested that depression was by far the strongest predictor of fatigue in our patients with AAV, although the best predictors varied with the dimension of fatigue tested for, with pain, employment status, anxiety and poor sleep quality

also shown to be important independent predictors of fatigue in this study. These findings suggest a number of symptoms for which targeted interventions might also bring about improvements in fatigue for patients with AAV. These potential interventions will be discussed further in Chapter Six.

4 RESULTS CHAPTER 2: HOW DO BODY COMPOSITION, MUSCLE FUNCTION, INFLAMMATION AND ENDOGENOUS HORMONE SUPPRESSION RELATE TO FATIGUE IN AAV?

4.1 Introduction

Forty eight of the patients with AAV and 41 of the healthy controls who had participated in the questionnaire study also participated in a mechanistic study, undergoing a series of physiological and laboratory tests as described in Chapter 2. This chapter will explore the results of the body composition and muscle function tests, as well as how these results relate to the symptoms reported in the questionnaire, and with measurements of inflammation and endogenous hormones previously implicated in fatigue.

4.2 Participant Characteristics and Self-Reported Symptoms

As can be seen in Table 4.1, both groups in the mechanistic study were highly representative of the larger populations from which they were recruited. The median age of both groups was slightly lower in the mechanistic study (58.4 years [IQR=47.2-65.0] vs 63.1 years [IQR=49.8-71.5] for the larger AAV group, 58.2 years [IQR=49.0-62.5] vs 61.9 years [IQR=49.8-67.0] for the larger healthy group); although there was no maximum age for entry to the mechanistic study per se, this was undoubtedly a consequence of the stricter exclusion criteria and the nature of the tests involved, but the difference was small. The two study groups in this part of the study remained well matched. In virtually all indices measured the same differences existed between the groups in this study as were observed in the

questionnaire study described in the previous chapter, but there was a noticeable difference with regards to involvement in sport and exercise; not only was this activity much more prevalent for both groups in the mechanistic study (70% vs 48.1% for the larger AAV group, 83.3% vs 70.2% for the larger healthy group), but there was no longer a significant difference between the disease and control groups. This was almost certainly another consequence of the nature of the recruitment of subjects for the tests involved, with those patients with AAV who reported no involvement in sport and exercise in their questionnaire being either less able or less inclined to participate in the mechanistic study.

There was also a clear difference in educational level for both groups between studies, perhaps because those with an academic background were more inclined to volunteer for more time- and effort-intensive research: for the AAV group the proportion who had a university degree was only 12.1% in the larger questionnaire study but 21.3% in the mechanistic study, and for the healthy group these proportions were 39.7% and 50% respectively.

The severity and nature of the fatigue experienced by both groups was virtually identical to that reported by the larger groups in the questionnaire study, as illustrated by their MFI-20 scores (see Figure 4.1). Scores across the other symptom-rating scales were also very similar to those seen in the questionnaire study, with Table 4.2 showing a comparison of the summary scores for the SF-36, HADS and PSQI, as well as those domains of the Brief COPE where significant differences between health and illness were found in the questionnaire study. The only SF-36 domain in which scores varied appreciably from those seen in the questionnaire study was Physical Functioning, where the AAV group in the mechanistic study scored 75 compared with 55 for the larger questionnaire study cohort, consistent with the variance in reported involvement in sport and exercise.

	AAV	Healthy	Significance
n	48	41	-
Age (yrs)	58.4 (47.2-65.0)	58.2 (49.0-62.5)	0.610
Male Gender	26 (54.2%)	23 (56.1%)	1.0
White Ethnicity	48 (100%)	40 (98%)	0.513
Living Partner	32 (66.7%)	32 (78.0%)	0.170
Age Left Education (yrs)	16 (15-18)	20 (16-23)	**<0.001**
Obtained a University Degree	10 (21.3%)	19 (50%)	**0.005**
Currently Working/Studying	25 (53.2%)	22 (57.9%)	0.416
Current Smoker	2 (4.2%)	1 (2.4%)	0.560
Caffeine Intake (g/Wk)	1.8 (0.9-2.5)	1.8 (1.2-2.1)	0.922
Alcohol Intake (Units/Wk)	4.3 (0.0-11.0)	9.5 (2.5-17.5)	**0.016**
Involved in Exercise or Sport	28 (70%)	30 (83.3%)	0.137
Hb (g/l)	135 (124-146)	140 (131-148)	0.195
eGFR (ml/min/1.73m^2)	65.5 (50.0-78.0)	81.5 (74.0-90.0)	**<0.001**
Disease duration (months)	64 (22-98)	-	-
VDI	4 (2-5)	-	-

Table 4.1: Characteristics of the study participants by group.
Gender, ethnicity, 'Living Partner', 'Obtained a University Degree', 'Currently Working/Studying', smoking status and 'Involved in Exercise or Sport' are expressed as number of participants and as percentage of the total number of respondents who provided the required information; other results are expressed as median with IQR. VDI=Vasculitis Damage Index; eGFR=Estimated GFR, calculated using Modification of Diet in Renal Disease (MDRD) formula.

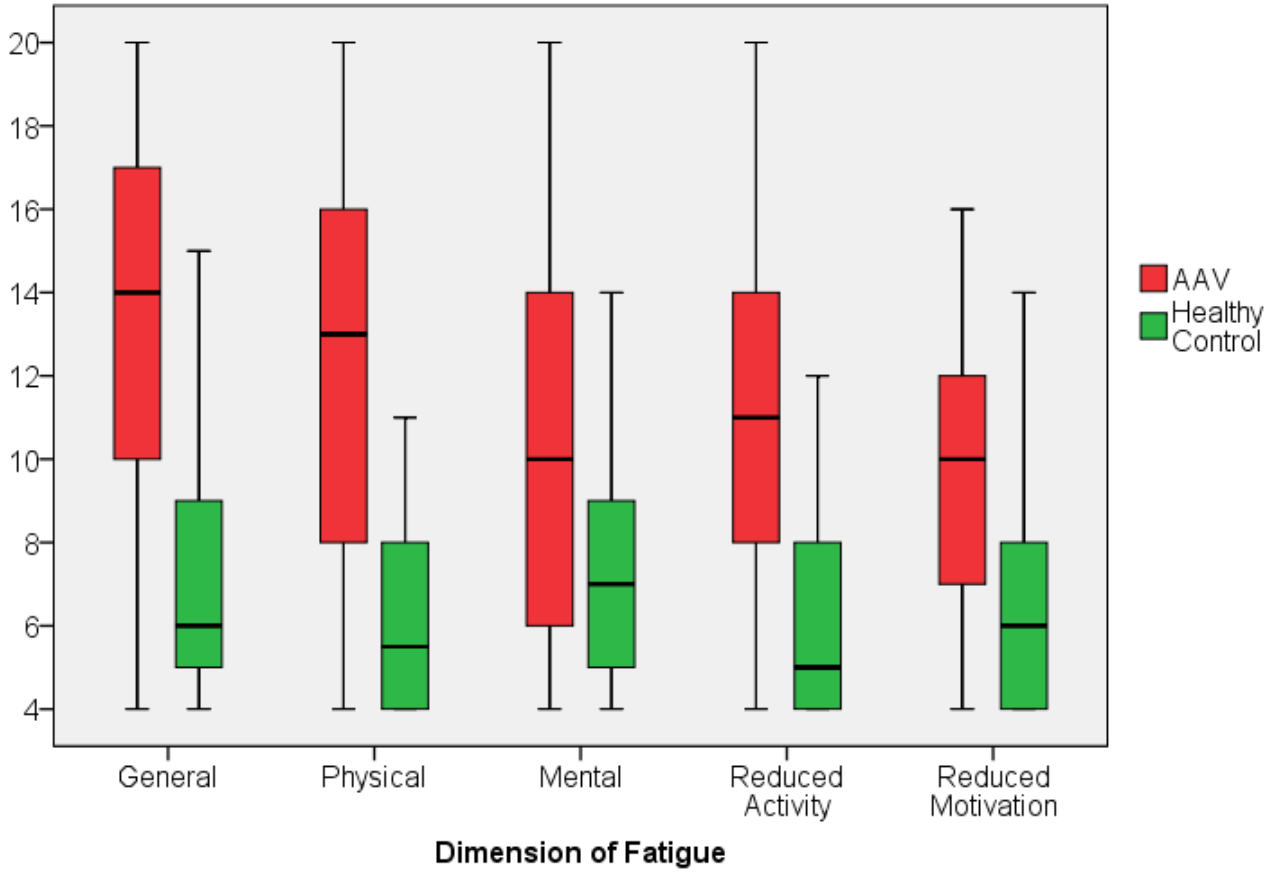


Figure 4.1: Self-reported fatigue using the Multi–Dimensional Fatigue Inventory (MFI-20).
Bars indicate median, boxes indicate the interquartile range and whiskers indicate the range.

	Questionnaire Study			Mechanistic Study		
	AAV	**Healthy**	**p**	**AAV**	**Healthy**	**p**
Physical Component Score (PCS)	38.7 (26.9-48.4)	54.7 (49.8-56.7)	**<0.001**	42.5 (32.4-50.3)	55.3 (53.0-57.5)	**<0.001**
Mental Component Score (MCS)	50.2 (39.4-56.9)	56.2 (52.7-58.4)	**<0.001**	48.4 (39.2-55.0)	56.3 (54.4-58.4)	**<0.001**
Anxiety	7 (3-10)	4 (2-6)	**<0.001**	6.5 (3-9)	3 (1-6)	**0.005**
Depression	6 (3-8)	1 (0-3)	**<0.001**	5 (2-8)	1 (0-2)	**<0.001**
Global PSQI Score	7 (4-11)	3 (2-5)	**<0.001**	6.5 (4-9.5)	4 (2-5)	**<0.001**
Active Coping	5 (4-7)	6 (5-8)	**0.036**	6 (4-7)	6 (5-8)	0.267
Denial	2 (2-3)	2 (2-2)	**0.001**	2 (2-3)	2 (2-2)	**0.035**
Behavioural Disengagement	2 (2-4)	2 (2-2)	**0.009**	2 (2-3)	2 (2-2)	0.284

Table 4.2: HRQOL, psychological symptoms, sleep quality and coping mechanisms.
HRQOL measured by the physical and mental summary scores of the SF-36, anxiety and depression measured by the HADS, sleep quality measured by the PSQI global score, and three of the component scores from the Brief COPE, comparing scores from those in the questionnaire study to the sub-populations in the mechanistic study. Scores are expressed as median with IQR.

4.3 Obesity and Fatigue

In this study, two assessments of obesity were made: body mass index (BMI) was calculated from direct measurement of height and weight (BMI=weight (kg)/height^2 (m^2)), and patients underwent DEXA scanning which supplied detailed body composition data (Table 4.3).

	AAV	Healthy	p
Body Mass Index (kg/m^2)	28.1 (24.8-32.0)	24.8 (23.6-27.9)	**0.019**
Percentage Body Fat	32.5 (25.6-40.5)	26.5 (20.6-32.6)	**0.007**

Table 4.3: BMI and percentage body fat in patients and healthy controls.
BMI calculated as weight/height2, percentage body fat measured by DEXA. All results expressed as median with IQR.

Obesity, assessed by BMI, was found to be positively correlated with the General Fatigue, Physical Fatigue, and Reduced Activity domains of the MFI-20 in patients with AAV, as shown in Table 4.4, but not with mental fatigue or motivation. Stronger correlations were seen with the direct measurement of percentage body fat on DEXA, which may be due to the influence on BMI of other factors such as muscle mass. There was no significant correlation between obesity and fatigue in the healthy controls, perhaps as a result of the generally low levels of fatigue experienced by this group. In keeping with the association between obesity and physical but not mental fatigue, it was also found to be associated with the physical component score of HRQOL on the SF-36, but not the mental component score. Interestingly, despite the strong correlation between both physical and mental fatigue and psychological symptoms, no correlation was seen between obesity and either anxiety or depression on the HADS. Obesity correlated strongly with poor sleep quality on the PSQI, in keeping with existing literature (466-468).

	Correlation with BMI	p	Correlation with % Body Fat	p
General Fatigue	0.333	**0.026**	0.405	**0.006**
Physical Fatigue	0.318	**0.038**	0.390	**0.010**
Mental Fatigue	0.032	0.835	0.177	0.250
Reduced Activity	0.252	0.099	0.398	**0.007**
Reduced Motivation	0.188	0.216	0.264	0.079
PCS	-0.200	0.210	-0.328	**0.036**
MCS	-0.104	0.518	-0.104	0.519
Anxiety	0.040	0.796	0.207	0.178
Depression	0.191	0.214	0.270	0.076
Global PSQI Score	0.337	**0.029**	0.371	**0.016**

Table 4.4: Correlation between obesity and fatigue for patients with AAV.
Spearman's rank correlations shown, with statistically significant correlations ($p<0.05$) highlighted in bold.

4.4 Muscle Mass

Lean body mass, measured by DEXA, was used as an approximation of muscle mass. Table 4.5 presents the lean body mass data split by group for the whole body and for the participants' dominant lower limb (i.e. the limb subsequently used for muscle function testing). No differences were found between the groups, and neither measurement correlated with any domain of fatigue in the AAV group, and there was therefore no evidence to suggest that fatigue in AAV is due in any part to muscle atrophy.

	AAV (N=48)	Healthy (N=41)	Significance
Total Lean Body Mass (kg)	52.4 (42.2-61.9)	51.4 (45.0-59.8)	p=0.979
Dominant Lower Limb Muscle Mass (kg)	8.3 (6.7-9.9)	8.8 (7.1-9.8)	p=0.483

Table 4.5: Muscle mass in patients and healthy controls.
Lean body and leg mass determined by DEXA, expressed as median and IQR.

4.5 Muscle Strength and Voluntary Activation

Muscle function was next assessed with a series of isometric maximal voluntary contractions (MVC) of the dominant side quadriceps muscle, with twitch interpolation to assess the extent of voluntary activation and theoretical maximal strength. This is a widely used and well validated technique in sports science, but to ensure investigator technique was satisfactory and that the equipment was functioning correctly, validation work was performed on two test subjects.

4.5.1 Results for Test Subjects

Two healthy volunteers each underwent testing on two separate occasions within a two week period. For reference, Figure 4.2 shows an example tracing and demonstrates how 'Voluntary Activation' (VA) and 'Theoretical Maximal Contraction' (TMC) were derived from the measured data. Note that since the protocol was to administer the super-imposed stimulation three seconds after the start of the contraction, TMC was calculated using VA and the voluntary contraction strength immediately prior to that superimposed stimulation (F1), which was not necessarily the same as the MVC.

Table 4.6 shows the mean data obtained for the two test subjects. It should be noted that the test subjects used were not typical of the study subjects (both were post-graduate students in their 30's), and it was a very small sample. Nonetheless, there were no problems with equipment, and it was considered to provide sufficient evidence of reliable equipment and adequate investigator technique.

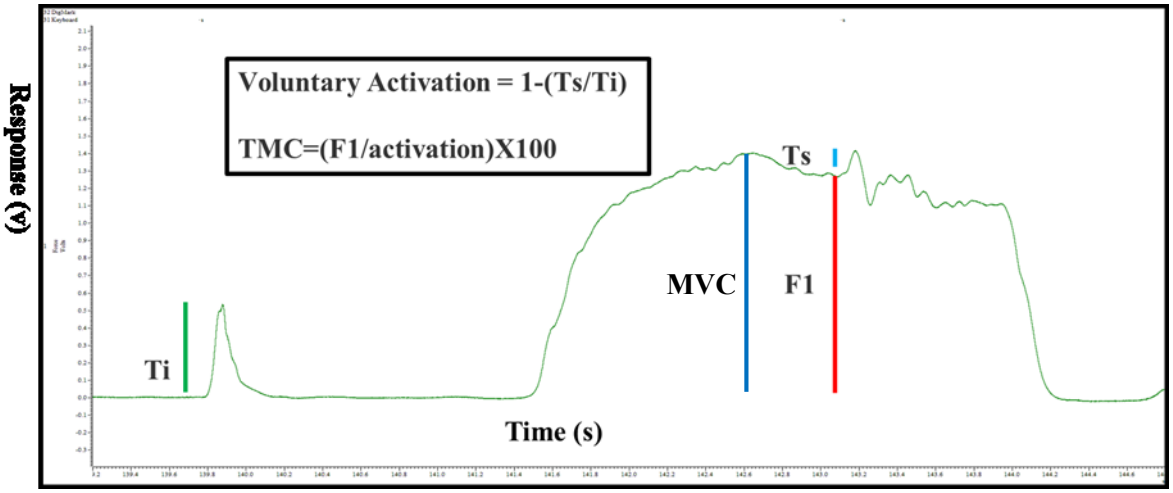


Figure 4.2: A typical MVC with twitch interpolation.
Ti = response from a resting electrical stimulation; Ts = response from electrical stimulation super-imposed on a MVC; MVC = maximal voluntary contraction force; F1 = voluntary power just prior to superimposed stimulation.

		Voluntary Activation (VA)	MVC (N)	TMC (N)
Test Subject A	**Test 1**	93%	509.5	534.7
	Test 2	90%	517.5	547.2
Test Subject B	**Test 1**	91%	393.3	413.6
	Test 2	95%	390.9	402.3

Table 4.6: Muscle function results of test subjects.
Results given are the mean values obtained from 3-5 MVCs, as described in Chapter 2.

4.5.2 Results for AAV and Healthy Control Groups

No adverse events occurred, and all participants were able to complete the test satisfactorily. Table 4.7 shows the data obtained for both groups during the study.

	AAV (N=48)	Healthy (N=41)	Significance
Maximal Voluntary Contraction (MVC) (N)	275.2 (210.7-336.6)	322.4 (240.2-385.4)	**p=0.050**
Voluntary Muscle Activation (%)	68.5 (58.2-81.1)	76.2 (67.5-86.5)	p=.074
Theoretical Maximal Contraction (TMC) (N)	347.4 (277.1-441.3)	395.3 (314.6-479.8)	p=0.315

Table 4.7: Muscle strength and activation in patients and controls.
Results expressed as medians with IQR.

There was a significant difference in median MVC between the two groups, suggesting an apparent deficiency in muscle strength in the AAV group despite there being no difference in muscle mass. However there was also a trend towards the patients with AAV failing to voluntarily activate their muscles to the same extent as the healthy controls, and because of this there was no difference found in the TMC between groups, i.e. there was no difference in actual strength once the difference in voluntary activation was accounted for.

It is notable that in this study voluntary muscle activation in the healthy control group was lower than would have been anticipated, and very much lower than for the two test subjects used in the pilot experiments. This is difficult to explain, as there was considerable verbal encouragement given to all participants, and they appeared to be well motivated during the experiments. It could possibly be that the participants were reluctant to contract fully because they knew that electrical stimulations were going to follow, however this was not apparent during the experiments. Moreover when a participant is purposely 'holding back' when performing an MVC, the shape of the resulting response trace usually takes on a bizarre shape (for example it might show a steep drop-off in force after the very first moments of the

contraction as the stimulation approaches) but this also was not the case very often. In fact, all of the tracings were screened as the results were calculated, and any obviously unusual or suspicious tracings were excluded from analysis. It also seems unlikely to be a problem with either the equipment or the operator technique, since the test subjects and some of the subjects in the main study showed excellent voluntary contraction using the same equipment, set-up and operator. Perhaps it was a function of the difference in age between the two test subjects and the healthy controls in the study, since the test subjects were both around thirty years younger than the median age of the healthy controls. Reports in the literature are conflicting, and although some studies have shown no difference in voluntary muscle activation across different age groups (469), others have shown that muscle activation is either reduced or much more variable in older age (470, 471). Amongst the healthy controls in this study, there was actually a weak positive correlation between age and VMA (r_s=0.157, p=0.326).

4.5.2.1 Were There Any Differences Between Males and Females For Muscle Strength?

Because there is usually a difference in muscle strength between males and females it is important to look at whether splitting the groups by sex changes the results. As can be seen in Table 4.8, there were slight differences seen when the sexes were analysed separately: the difference in MCV between groups was more marked for males, and the difference in VMA was greater in females. However, none of these differences reached statistical significance, and it can only be speculated whether with greater numbers of participants these differences might have reached significance or might in fact have evened out. Most importantly perhaps, there was no difference in TMC between groups for either males or females, confirming again that there was no difference in actual muscle strength between patients with AAV and healthy controls.

A)	AAV (N=26)	Healthy (N=23)	Significance
Maximal Voluntary Contraction (MVC) (N)	322.6 (255.8-382.9)	369.8 (328.6-405.6)	0.061
Voluntary Muscle Activation (%)	71.0 (55.4-81.1)	74.2 (67.2-84.7)	0.459
Theoretical Maximal Contraction (TMC) (N)	421.3 (343.9-620)	460.9 (420.0-543.1)	0.459

B)	AAV (N=22)	Healthy (N=18)	Significance
Maximal Voluntary Contraction (MVC) (N)	215.4 (188.5-277.4)	238.9 (200.4-309.3)	0.338
Voluntary Muscle Activation (%)	65.7 (60.8-80.7)	81.0 (70.5-89.9)	0.075
Theoretical Maximal Contraction (TMC) (N)	288.2 (251.4-346.3)	306.4 (261.2-341.8)	0.476

Table 4.8: Muscle strength and activation in patients and controls, divided by sex into A) Males and B) Females.
Results expressed as medians with IQR.

4.5.2.2 Did Muscle Strength or Voluntary Activation Correlate With Fatigue?

When only the patients with AAV were analysed, there was no correlation between muscle strength or voluntary activation and any of the dimensions of fatigue (Table 4.9), further suggesting that muscle strength is not an important contributor to fatigue in AAV.

	Correlation with MVC	p	Correlation with VA	p	Correlation with TMC	p
General Fatigue	-0.123	0.411	-0.253	0.086	-0.006	0.965
Physical Fatigue	-0.208	0.169	-0.142	0.352	-0.144	0.344
Mental Fatigue	-0.098	0.515	-0.182	0.227	-0.072	0.635
Reduced Activity	-0.287	0.054	-0.160	0.288	-0.215	0.152
Reduced Motivation	-0.009	0.952	-0.035	0.813	0.019	0.901
PCS	0.118	0.450	-0.087	0.587	0.175	0.262
MCS	-0.010	0.950	0.147	0.346	-0.001	0.997
Anxiety	-0.086	0.571	-0.096	0.525	-0.121	0.424
Depression	-0.090	0.551	0.034	0.821	-0.153	0.310
Global PSQI Score	0.005	0.972	0.025	0.871	-0.059	0.705

Table 4.9: Correlations between muscle function and symptoms for patients with AAV.
No correlations were statistically significant.

4.6 Muscle Fatigability

Having examined muscle strength and central motor drive in short bursts of maximal voluntary contraction over only a few seconds, muscular fatigability was tested next, by asking participants to maintain an isometric quadriceps muscle contraction for as long as possible at a force equal to 50% of their own MVC (as measured in the previous test). As described in Chapter 2, superimposed electrical stimulation was applied to the quadriceps muscle at 10-second intervals throughout the test. The results are shown in Table 4.10.

	AAV (N=48)	Healthy (N=41)	Significance
Endurance Time (S)	70.0 (50.2-89.5)	91.2 (71.2-122.4)	**p=0.006**
Muscular Reserve at Point of Fatigue (%)	31.6 (21.0-42.1)	25.0 (17.1-32.3)	**p=0.038**

Table 4.10: Muscle fatigability for patients with AAV and healthy controls.
Median and IQR shown for time to volitional fatigue when maintaining a 50% MVC, and the muscular reserve at the point of that volitional fatigue for patients and controls.

Although there was some overlap between the groups, on average the patients with AAV were only able to maintain a 50% MVC for around 75% of the time achieved by the healthy controls. This crude measurement is evidence of reduced endurance in the patients with AAV, but to distinguish whether task failure was due to peripheral muscular fatigue or to a failure of voluntary motor activation (i.e. central fatigue), the response to superimposed electrical stimulation must be examined.

Figure 4.3 illustrates the response to electrical stimulation for both groups at the beginning of the test and at the point of volitional fatigue. Since the task was to maintain a contraction at 50% of MVC, at the beginning of the test there should be 50% muscular reserve, and this was approximately the case for both groups. As a participant's muscle becomes increasingly

fatigued during a sustained contraction, there should be a compensatory increase in voluntary muscle activation as the nervous system attempts to maintain a constant force. In theory this might result in there being no measurable response at all to a superimposed electrical stimulus at the point of volitional fatigue, since at that point there should already be maximal intrinsic stimulation of the muscle. In fact, as shown in Figure 4.3, both groups in this study still had a considerable response to electrical stimulus at the point where they felt unable to continue with the test – evidence of failure of voluntary muscle activation. The difference between the 'muscular reserve' of the two groups at this point was significant however, demonstrating evidence of increased central fatigue in the AAV group.

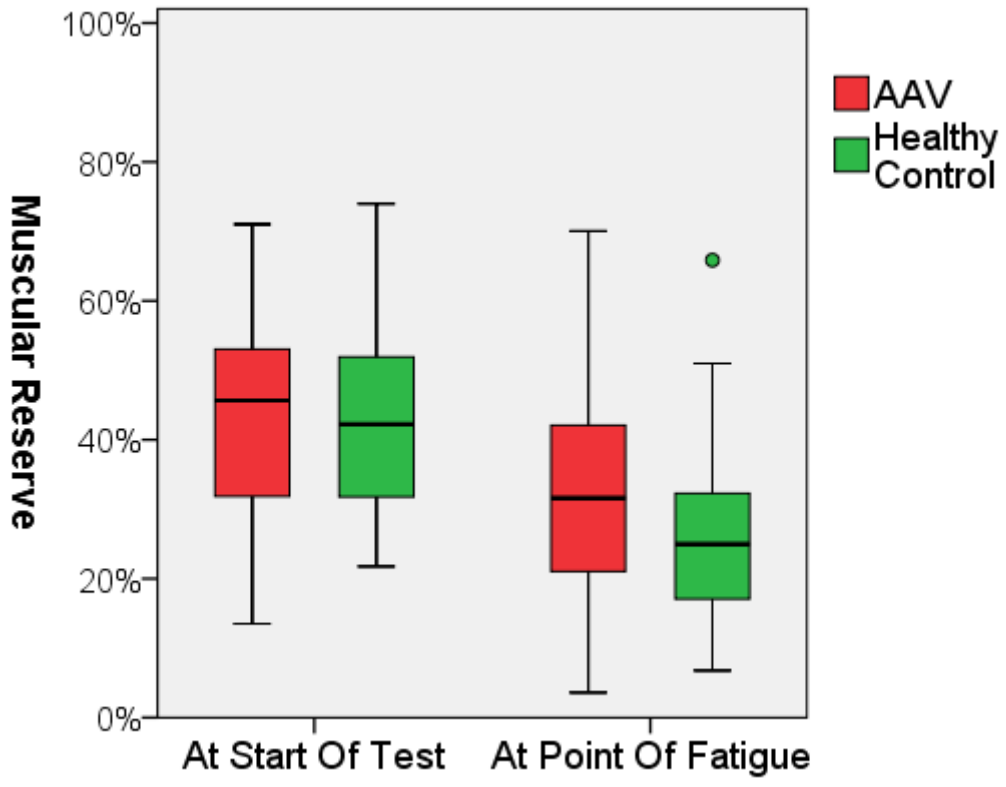


Figure 4.3: Muscular reserve at the beginning and end of the fatigue test for patients and controls.
'Muscular Reserve' is the response to superimposed electrical twitch at that time-point as a percentage of the response to a resting twitch. Bars indicate median, boxes indicate the interquartile range, whiskers indicate the range, and the dot indicates an outlier.

When analysing all study participants together, strong correlations were seen between endurance time and General Fatigue ($r=-0.385$, $p<0.001$), Physical Fatigue ($r=-0.310$, $p=0.004$), Reduced Activity ($r=-0.303$, $p=0.005$) and Reduced Motivation ($r=-0.338$, $p=0.002$), and also between muscular reserve at point of volitional fatigue and General Fatigue ($r=0.241$, $p=0.026$). However, when the patients with AAV were analysed alone these correlations were lost, suggesting that either the study was underpowered to find such correlations, or that the apparent correlations were simply a reflection of the differences between the two groups.

4.7 Inflammation

4.7.1 hsCRP

In this cohort of patients with AAV the serum concentration of hsCRP was within the normal range, but was still significantly higher than in the healthy controls, suggesting ongoing subclinical inflammation (Table 4.11). Although hsCRP concentration did correlate with General Fatigue, Physical Fatigue and Reduced Activity across all of the participants in the mechanistic study, correlations with these indices did not reach statistical significance when the patients with AAV were analysed separately (Table 4.12). Correlations for hsCRP with indices of quality of life, anxiety and depression, and sleep quality also failed to reach statistical significance when the patient group was analysed separately.

	Patients with AAV	**Healthy Controls**	**p**
hsCRP (mg/ml)	1.9 (1.15-3.65)	0.92 (0.49-2.14)	**0.011**
IL-1b (ng/ml)	3.5 (1.5-5.3)	1.5 (1.1-2.7)	**0.007**
IL-1RA (ng/ml)	187.9 (80.5-266.6)	70.0 (48.1-146.9)	**0.004**
IL-6 (ng/ml)	13.6 (6.3-20.0)	5.3 (4.2-8.9)	**<0.001**
IL-8 (ng/ml)	27.7 (9.9-39.2)	8.5 (6.2-19.1)	**<0.001**
IL-10 (ng/ml)	11.4 (7.4-14.6)	6.9 (4.5-10.2)	**0.002**
TNFα (ng/ml)	61.0 (33.4-74.1)	31.9 (25.8-45.7)	**0.001**

Table 4.11: Comparison between groups for hsCRP and a panel of cytokines.
Median results shown, with interquartile range. Correlation between serum concentrations and MFI-20 general scores are also shown.

	hsCRP – All Participants	p	hsCRP – Patients with AAV Only	p
General Fatigue	0.234	**0.031**	0.169	0.257
Physical Fatigue	0.287	**0.008**	0.264	0.080
Reduced Activity	0.229	**0.036**	0.264	0.077
PCS	-0.358	**0.001**	-0.113	0.469
MCS	-0.190	0.100	-0.114	0.466
Anxiety	0.103	0.351	-0.048	0.751
Depression	0.194	0.077	0.162	0.281
Global PSQI Score	0.234	**0.036**	0.269	0.078

Table 4.12: Correlation of hsCRP with fatigue, HRQOL, anxiety, depression and sleep quality.
General Fatigue, Physical Fatigue and Reduced Activity from MFI-20; PCS and MCS summary scores from SF-36; Anxiety and Depression subscores from HADS. Median results shown with IQR.

4.7.2 Cytokines

The measured cytokine concentrations (Table 4.11) were lower than might be anticipated in patients with AAV, but this is less surprising when it is considered that the patients in this study had all been immunosuppressed for a prolonged period of time, and that the majority of the patients were still taking prednisolone regularly at the time they were tested. Indeed, all patients with AAV who participated in the study had been in sustained disease remission for a minimum of six months.

Although none of the patients had any clinical evidence of disease activity, all of the serum cytokines were present in significantly higher concentrations in patients' serum than in the healthy controls' serum, again suggesting ongoing sub-clinical inflammation (Table 4.11). Despite the significant differences in serum cytokine concentrations between groups however, none of those cytokine concentrations correlated significantly with subjective

fatigue, quality of life, psychological symptoms, or sleep quality when the AAV group was analysed separately (data not shown).

As discussed in Chapter 4.3, patients were found to be more obese than healthy controls, and measures of adiposity were significantly correlated with fatigue. Obesity has previously been linked with inflammation, and in this cohort of patients with AAV, hsCRP correlated with BMI ($r=0.368$, $p=0.012$), but not with percentage body fat as measured by DEXA. There was no correlation between serum cytokine levels and adiposity.

Neither hsCRP nor any of the cytokines correlated with measures of central motor drive (voluntary muscle activation during MVC, or muscular reserve at end of 50% MVC).

4.8 Endogenous Hormones

Serum DHEA and DHEAS concentrations at 9am were significantly reduced in patients with AAV compared with healthy controls, which was expected given that most patients were receiving low dose prednisolone (median dose 5mg, IQR 0-5mg). The suppression of cortisol levels in patients with AAV did not reach statistical significance. Current prednisolone dose correlated very strongly with hormone levels as shown (Table 4.13).

	Patients with AAV	Healthy Controls	p	Prednisolone Dose Correlation	p
DHEA (nmol/L)	2.4 (1.7-5.2)	6.9 (4.2-13.5)	**<0.001**	-0.544	**<0.001**
DHEAS (mmol/L)	1 (0.3-2.2)	4.7 (2.7-5.8)	**<0.001**	-0.552	**<0.001**
Cortisol (mmol/l)	161.3 (101.0-228.0)	184.0 (142.8-256.1)	0.058	-0.487	**0.001**

Table 4.13: DHEA, DHEAS and cortisol levels, and correlation with prednisolone dose.
Median results are shown, with interquartile range.

Within the AAV group, there was a correlation between DHEAS and Physical Fatigue (r=-0.294, p=0.05), but not with the other dimensions of fatigue on the MFI-20, and there were no significant correlations between DHEA or cortisol and subjective fatigue. None of the measured hormones correlated significantly with anxiety or depression scores on the HADS, or with sleep quality (Global PSQI Score). Likewise, serum hormone levels did not correlate with either voluntary muscle activation or with muscular reserve (the measures of central motor drive) when only patients with AAV were analysed. Current prednisolone dose did not correlate with any of the symptoms measured in the questionnaire, and nor did it correlate with the measures of central motor drive.

Despite hormone levels correlating quite poorly with the above indices, for the patients with AAV there were significant correlations between DHEA/DHEAS levels and HRQOL measured by the SF-36, as shown in Table 4.14. This suggests that DHEA/DHEAS

suppression causes problems which have a deleterious effect on HRQOL but are not well measured by the tools used in this study.

	Correlation with DHEA	p	Correlation with DHEAS	p
Physical functioning (PF)	0.379	**0.011**	0.569	**<0.001**
Role Physical (RP)	0.210	0.166	0.111	0.465
Bodily Pain (BP)	0.344	**0.018**	0.341	**0.019**
General Health (GH)	0.157	0.291	0.374	**0.010**
Vitality (VT)	0.137	0.357	0.209	0.159
Social Functioning (SF)	0.195	0.189	0.268	0.068
Role Emotional (RE)	0.193	0.200	0.100	0.510
Mental Health (MH)	-0.027	0.856	-0.310	0.835
Physical Component Score (PCS)	0.339	**0.026**	0.464	**0.002**
Mental Component Score (MCS)	0.068	0.667	0.095	0.545

Table 4.14: Correlations between DHEA/DHEAS and HRQOL for patients with AAV.
Statistically significant correlations are highlighted in bold.

4.9 Summary of Results

In the last chapter it was shown that fatigue in AAV was severe, and that patients experienced greater physical symptoms than mental symptoms of fatigue. It was found that fatigue had important associations with quality of life and role impairments in patients, and that it was predicted by psychological symptoms, pain, and quality of sleep. This chapter attempted to explore some of the possible mechanisms underpinning physical fatigue in AAV, and found evidence of links with body adiposity and central motor drive, but no evidence linking fatigue with muscle mass or strength.

Patients in this study had significantly higher body adiposity than healthy controls, whether measured directly by DEXA or indirectly by BMI. In the patient group, body adiposity was significantly correlated with General Fatigue, Physical Fatigue and Reduced Activity on the MFI-20, and with the Physical Component Score of the SF-36. A link between obesity and fatigue is well reported in the literature (439, 472-474), and although this study did not find any correlation in the healthy control group, this may be a result of the relatively small sample size and the low levels of fatigue reported by those participants.

There are a number of plausible mechanisms linking adiposity and fatigue in patients with AAV, and in this study there is evidence supporting at least three. Fatigue would be expected to result in a more sedentary lifestyle and over time lead to increased body adiposity, and this is somewhat supported by the lower levels of exercise reported by the larger group in our questionnaire study. Obesity is strongly linked to poor sleep quality through mechanisms including obstructive sleep apnoea, which in turn is linked to fatigue in the literature, and in patients with AAV in this study it was found that Global PSQI score, measures of body adiposity, and the physical dimensions of fatigue in the MFI-20 all correlated with each other. Finally, obesity is also linked to inflammation as part of the metabolic syndrome, and this

study found a correlation in patients with AAV between BMI and hsCRP, although it did not find any statistically significant correlation between serum inflammatory markers and fatigue.

Prolonged steroid use is often associated with muscle atrophy, and muscle contractility could also be reduced in patients with AAV through mechanisms including endothelial damage to the epineurial blood vessels (475-477) and reduction of the resting membrane potential of muscle and nerve by pro-inflammatory cytokines (478, 479). However this study did not find any evidence of reduced muscle mass in patients with AAV compared to healthy controls with DEXA, and during maximal voluntary contractions there was also no difference found in muscle strength, once reduced voluntary muscle activation was accounted for. However, despite having no muscle mass or strength deficit, patients with AAV were less able than controls to sustain an isometric contraction at 50% of their MVC, and superimposed electrical stimulation demonstrated that at the point of volitional fatigue patients with AAV had a significantly greater reserve of muscle force. This suggests a reduction in central motor drive in patients with AAV, as does the trend towards reduced voluntary muscle activation that was observed during MVC testing (although this did not reach statistical significance).

Although inflammation, using hsCRP as a surrogate marker, was associated with fatigue across all participants in this study, no association was demonstrated between hsCRP and fatigue or measures of central motor drive in the patients with AAV. Likewise, there was no correlation found with the concentrations of inflammatory serum cytokines measured in this study. However it is important to note that all patients in this study were in sustained remission, with BVAS scores of 0 and hsCRP concentrations within the normal range, and it may be that inflammation at the onset of disease or during flares is a more important contributor to fatigue. Inflammation during such phases of the disease may cause central changes which result in fatigue, as has been suggested in RA (194), and it may be that for unknown reasons these central changes persist following disease remission when the level of

inflammation reduces. It has however also been shown in some studies that serum and CNS concentrations of cytokines do not correlate, so perhaps the correct fluid compartment was not sampled to demonstrate the true effects of inflammation on fatigue.

Both inflammation and prednisolone use have been associated with suppressed levels of DHEA and DHEAS, which have themselves been associated with fatigue, although with contradictory findings (232, 238, 480, 481). Low levels of DHEAS have been associated with fatigue in patients with MS (482) where there is also evidence of failure of central motor drive such as we propose in our patients (483). Suppression of DHEA and DHEAS in this study correlated significantly with current prednisolone dose, but did not associate with fatigue or central motor drive in patients. It did however correlate strongly with some measures of HRQOL, and it seems that DHEA and DHEAS suppression are important in ways which are not well captured by the tools used in this study.

The isometric muscle contractions considered in this Chapter are valuable objective measures of muscle function but prolonged isometric contractions are a relatively rare activity in daily life and may not, therefore, provide a true reflection of the problems facing patients. Neither do isometric contractions test the respiratory nor cardiovascular systems deficits which can severely restrict aerobic exercise capability. The following Chapter addresses this issue.

5 RESULTS CHAPTER 3: HOW DO CARDIO-RESPIRATORY FITNESS, PERCEPTION OF EXERTION, AND MENTAL FUNCTION RELATE TO FATIGUE IN AAV?

5.1 Introduction

The work described in the last chapter explored the relationship between fatigue and body composition, isometric muscle function, and central motor drive in patients with AAV. It was found that patients with AAV had higher body fat than healthy controls, and that adiposity correlated positively with the General and Physical dimensions of fatigue, as well as reduced activity. No difference was found in peripheral muscle mass or contractile strength between patients and healthy controls, but patients showed evidence of a failure of central motor drive, a central mechanism of fatigue. Although patients had significantly higher peripheral blood markers of inflammation and lower levels of the hormones DHEA and DHEAS than the healthy control group, no correlation was found between those levels and either subjective fatigue or markers of central motor drive.

The data presented in this chapter are of the submaximal exercise test, which examined both cardio-respiratory fitness and perception of exertion, and the Paced Auditory Serial Addition Test (PASAT), which was used as a measure of global cognitive function. As previously described, the mechanistic study included forty eight of the patients with AAV and 41 of the healthy controls who had participated in the questionnaire study; further details of the inclusion and exclusion criteria as well as the test methods can be found in Chapter 2.

5.2 Pilot Work – What Is The Optimum Method of Assessing Cardio-Respiratory Fitness In Patients with AAV?

In sports science literature, the most common measure of cardio-respiratory fitness is '$\dot{V}O_2$max', which is defined as an individual's maximum possible rate of oxygen consumption during exercise. It is measured by finding the point during an incremental exercise test where oxygen consumption reaches a steady state or 'plateau', after which any further increases in workload are no longer accompanied by an increase in $\dot{V}O_2$. It is important to note that if a participant stops exercising before that point is reached then the final measurement is simply a 'peak $\dot{V}O_2$', although such measurements are sometimes misrepresented as $\dot{V}O_2$max. Not only would it be unsafe to push patients with AAV to such physical limits, but if there were any element of central fatigue then patients would be expected to stop before their true $\dot{V}O_2$max was reached anyway. For the mechanistic study it was therefore planned instead to make an *estimate* of the participants' $\dot{V}O_2$max using the data obtained from a *submaximal* exercise protocol.

The relationship between oxygen consumption and heart rate is linear during incremental exercise (445), so it was theorised that extrapolating the relationship between heart rate and $\dot{V}O_2$ observed during any individual's submaximal exercise test out to that same individual's predicted maximum heart rate would provide an acceptable estimate of $\dot{V}O_2$max (see Figure 5.1). Pilot tests were carried out to examine the acceptability of this technique by asking two healthy volunteers to each undergo exercise testing on two separate occasions a few weeks apart. On the first occasion the participants undertook exactly the same submaximal exercise protocol that would subsequently be used in the mechanistic study; on the second occasion the same protocol was used initially, but after completion of the final 75W stage the workload was then further increased in 25W and then 10W increments every three minutes until the true $\dot{V}O_2$max was observed.

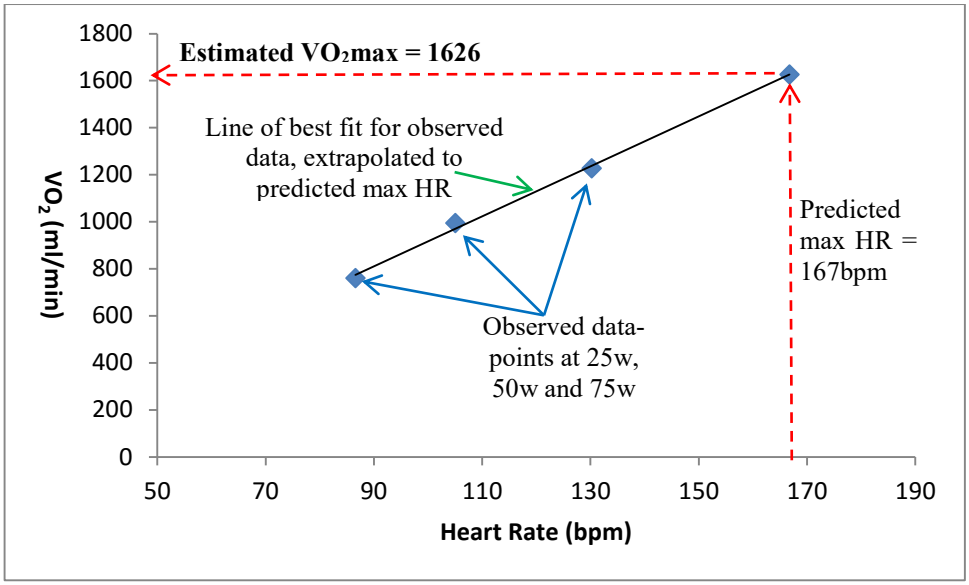


Figure 5.1: Technique for estimation of $\dot{V}O_2$max.
The steps in the estimation of $\dot{V}O_2$max were as follows: 1) Data for heart rate and $\dot{V}O_2$ observed during the last 30 seconds of each stage in the sub-maximal exercise test were plotted; 2) A 'line of best fit' for the observed data was extrapolated out to the estimated maximum heart rate; 3) The corresponding $\dot{V}O_2$ was then taken as the estimated $\dot{V}O_2$max.

The estimated and measured $\dot{V}O_2$max values from the pilot tests are illustrated in Table 5.1. Any difference of less than 10% between estimated and measured $\dot{V}O_2$max was considered tolerable but, as shown, one of the estimated values for subject A was 15% greater than the measured value. A large part of the discrepancy between that individual's estimated and measured $\dot{V}O_2$max was due to the reliance on equations for predicting maximum heart rate, even the best of which are known to be unreliable (484). For Subject A the predicted maximum HR was 161bpm but the actual measured maximum HR was only 148bpm in the max test, whereas for Subject B the predicted and measured maximum HR were close at 176bpm and 174bpm respectively. If the vectors for HR/$\dot{V}O_2$ derived from the Subject A's two submaximal exercise tests had been extrapolated out to their true maximum heart rate rather than the inaccurate predicted value, the two $\dot{V}O_2$max estimates would have differed from the true $\dot{V}O_2$max by just 2.3% and 4.9%.

	Test Subject A	Test Subject B
First estimated $\dot{V}O_2$max	34.5 (115%)	32.5 (92%)
Second Estimated $\dot{V}O_2$max	31.9 (106%)	32.7 (92%)
Measured $\dot{V}O_2$max	30.0	35.5

Table 5.1: Results of the pilot submaximal exercise tests.
Results are shown in ml/min/kg, and 'estimated VO_2max' results are also expressed as a percentage of that participant's measured $\dot{V}O_2$max in brackets.

It was decided that estimated $\dot{V}O_2$max should be retained in the study as a measurement of cardio-respiratory fitness because $\dot{V}$O2max is the value most often quoted in the literature. However, because this method of estimation was shown in the pilot work to be somewhat unreliable, 'O_2 pulse' was added as a second measure of cardiorespiratory fitness which did not rely on estimated maximum HR. O_2 pulse, which is the volume of oxygen consumed during exercise *per heart beat*, is widely regarded to be a good reflection of cardio-respiratory efficiency (485) and can be simply determined as the slope of the linear regression of $\dot{V}O_2$ against heart rate (444).

5.2.1 Correcting for Total Body Weight versus Lean Body Mass

In health, cardiac output varies in a linear fashion with lean body mass (LBM) (486). Generally in the literature, cardio-respiratory variables such as $\dot{V}O_2$max and O_2 pulse are corrected for total body weight rather than LBM, mainly because total body weight is easier to measure. It makes more physiological sense, however, to correct instead for lean body mass (LBM), since the quantity of body fat is highly variable and does not consume significant amounts of oxygen during exercise. As described in the previous chapter the AAV group had a significantly greater percentage body fat than the healthy group, so in this study

it was decided to express measures of cardio-respiratory fitness relative both to total body weight and LBM.

5.3 Incremental Submaximal Exercise Test

As described in Chapter 2, all participants in the mechanistic study undertook a 10 minute submaximal exercise test on a cycle ergometer, with a one minute warm-up at a workload of 10W followed by three sequential three-minute periods at 25W, 50W and 75W. Heart rate and oxygen consumption ($\dot{V}O_2$) were recorded continuously to assess cardiorespiratory fitness, and perception of exertion was assessed with the Borg 6-20 RPE Scale at the end of each exercise period.

There were no adverse events during testing, and the majority of the participants completed all three stages of the test. Eight female patients, one male patient, and one female healthy control stopped the exercise before completing the final stage, citing a combination of dyspnoea and leg pain as their reasons for stopping the test early. The data from four patients with AAV was excluded from the analysis of cardio-respiratory fitness – two of the four because they only managed to complete the first stage of the exercise protocol, and the other two because they were not able to maintain a sufficient cadence during the test to cause a meaningful rise in heart rate.

5.3.1 Cardio-respiratory Fitness

5.3.1.1 Were Patients Less Fit Than Healthy Controls?

Comparing the two groups, there was no significant difference between the resting heart rates or the heart rates at each of the three workloads during the exercise test (Figure 5.2). This

might suggest similar levels of fitness, but is a very crude measurement compared with estimated V̇O2max or O2 pulse.

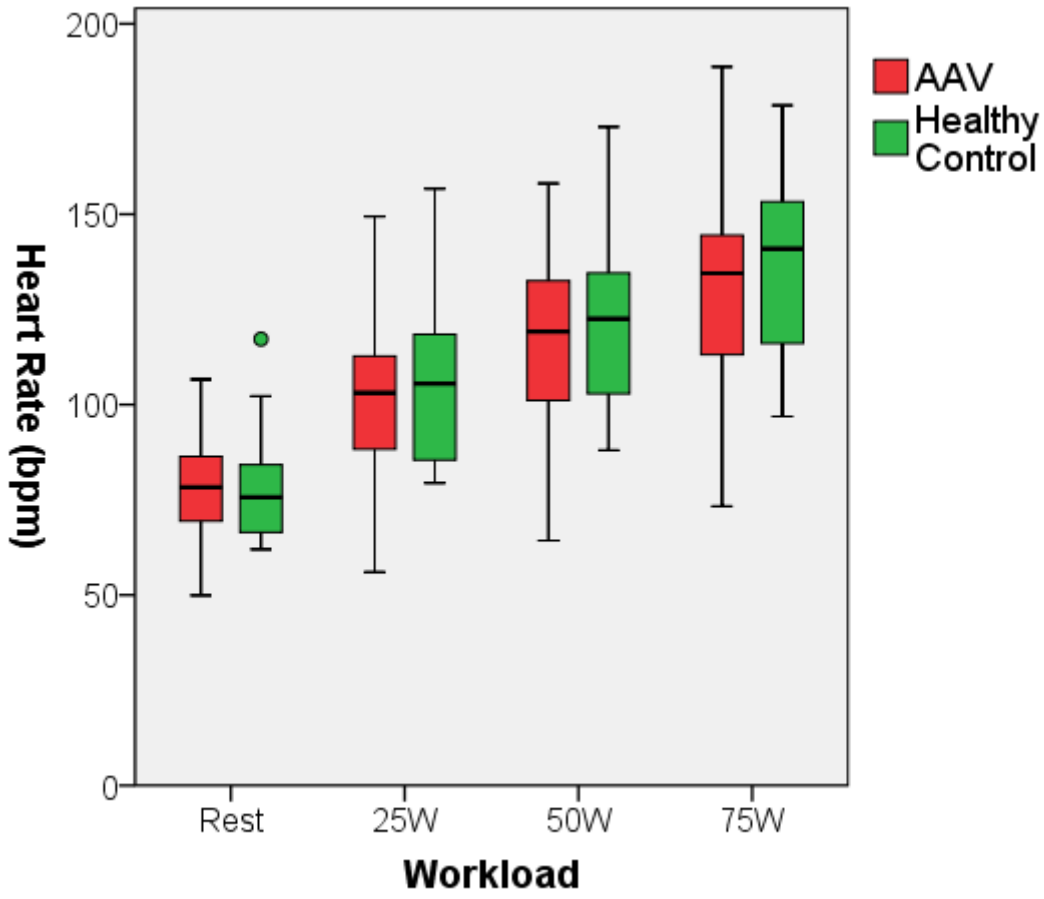


Figure 5.2: Comparison of heart rates at rest and during exercise for patients and controls.
There were no significant differences at rest or at the end of any of the workloads. Bars indicate median, boxes indicate the interquartile range, whiskers indicate the range and dot indicates an outlier..

As shown in Figure 5.3, patients had lower estimated $\dot{V}O_2$max than healthy controls: the median estimated $\dot{V}O_2$max per kg of total body weight of the patient group was 24.8 (IQR=21.6-31.9) compared to 30.5 (IQR=24.5-35.3) for the healthy controls, and the median estimated $\dot{V}O_2$max per kg of LBM was 37.9 in the AAV group (IQR=34.1-49.5) compared to 43.4 (IQR=38.2-50.5) for the healthy controls. The difference between groups was not

statistically significant, although it was closer to significance (p=0.056) when adjustment was made for total body weight than when LBM was adjusted for (p=0.103). As previously discussed fat is not metabolically active during exercise, therefore it is more valid to normalise these results to LBM and to conclude that there was no difference between groups for estimated V̇O2max.

The median O_2 pulse adjusted for total body weight was 0.196 (IQR=0.155-0.280) in the AAV group and 0.259 (0.212-0.331) in the healthy control group, and in contrast to the difference between groups for estimated $\dot{V}O_2$max this difference was highly significant (p=0.007). When O_2 pulse was instead adjusted for LBM, the difference between groups was still significant, but less so: median O_2 pulse for the AAV group was 0.310 (IQR=0.258-0.419) compared with 0.371 (IQR=0.323-0.456) for the healthy group (p=0.029). Therefore, even using the more valid method of normalising the results to LBM, the patients with AAV still had lower O_2 pulse than the healthy controls.

Due to the previously described inaccuracies inherent in predicting participants' maximum heart rate, estimated $\dot{V}O_2$max is a less reliable measurement of cardio-respiratory fitness than O_2 pulse. For this reason it is reasonable to assert that there was indeed a significant difference between the groups, and O_2 pulse will be used in the rest of this chapter when discussing cardio-respiratory fitness.

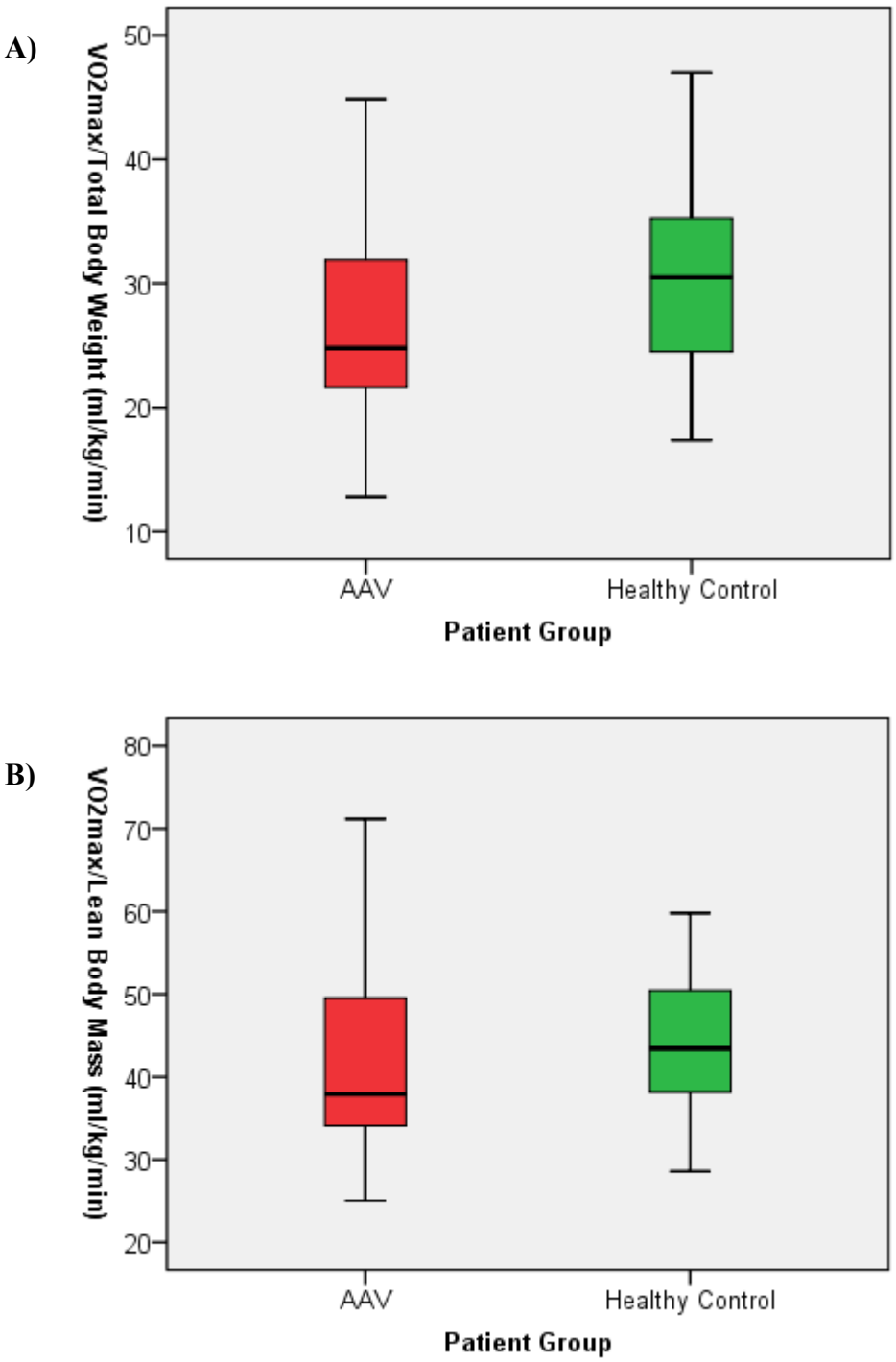


Figure 5.3: Estimated $\dot{V}O_2$max of patients with AAV and healthy controls.
Figure A shows the $\dot{V}O_2$max adjusted for total body weight, and Figure B shows the $\dot{V}O_2$max adjusted for lean body mass for patients with AAV and healthy controls during exercise testing. Bars indicate median, boxes indicate the interquartile range and whiskers represent the highest and lowest values within 1.5 times the interquartile range

5.3.1.2 Was Reduced Cardio-respiratory Fitness a Cause of fatigue in AAV?

As shown in Table 5.2, when O_2 pulse was adjusted for total body weight it correlated with the General Fatigue, Physical Fatigue and Reduced Activity MFI-20 scores of all participants, suggesting that subjective fatigue was related to cardio-respiratory fitness in this study. However, when O_2 pulse was instead more correctly adjusted for LBM it did not correlate with any the MFI-20 dimensions of fatigue. General Fatigue, Physical Fatigue and Reduced Activity scores were shown in the last chapter to correlate strongly with percentage body fat, and it seems likely that this relationship is responsible for the apparent correlation when adjusting O_2 pulse for total rather than lean body weight. Furthermore, when only the patients with AAV are analysed the association between fatigue symptoms and O_2 pulse falls short of statistical significance even when adjusted for total body weight ($p=0.077$ for general fatigue). In summary, although there was a significant difference in cardio-respiratory fitness between groups it does not appear that it was an important cause of subjective fatigue.

	O_2 Pulse Adjusted For Total Body Weight		**O_2 Pulse Adjusted For LBM**	
	Correlation (r_s)	**p**	**Correlation (r_s)**	**p**
General Fatigue	-0.268	**0.016**	-0.154	0.176
Physical Fatigue	-0.239	**0.034**	0.110	0.341
Mental Fatigue	-0.193	0.087	-0.120	0.297
Reduced Activity	-0.220	**0.048**	-0.110	0.337
Reduced Motivation	-0.170	0.128	-0.096	0.402

Table 5.2: Cardio-respiratory fitness and participants' symptoms.
Non-parametric correlations between cardiorespiratory fitness and: the five dimensions of fatigue on the MFI-20; the physical and mental component scores on the SF-36; anxiety and depression scores on the HADS; and overall sleep quality score on the PSQI. Analysis includes patients with AAV and healthy controls.

5.3.2 Perception of Exertion

The perception of exertion for any given workload increases with increasing age, most likely because the maximum heart rate decreases with age, resulting in that workload being carried out at an ever increasing fraction of the maximum aerobic capacity. Similar arguments apply to differences in body size but because the patient and healthy control groups were well matched for age (as well as for gender and ethnicity) it is reasonable to look for differences between groups using the raw RPE in the last 30 seconds before cessation of the exercise test. The perception of exertion was significantly heightened in the patients compared to the control subjects: median final Borg Score was 14 (IQR=13-15.5) for patients with AAV compared with 12 (IQR=11-13) for healthy controls (p=0.006), even though patients and control subjects finished the exercise at very similar relative work rates, as judged by their heart rates as a percentage of their age predicted maxima (Figure 5.4).

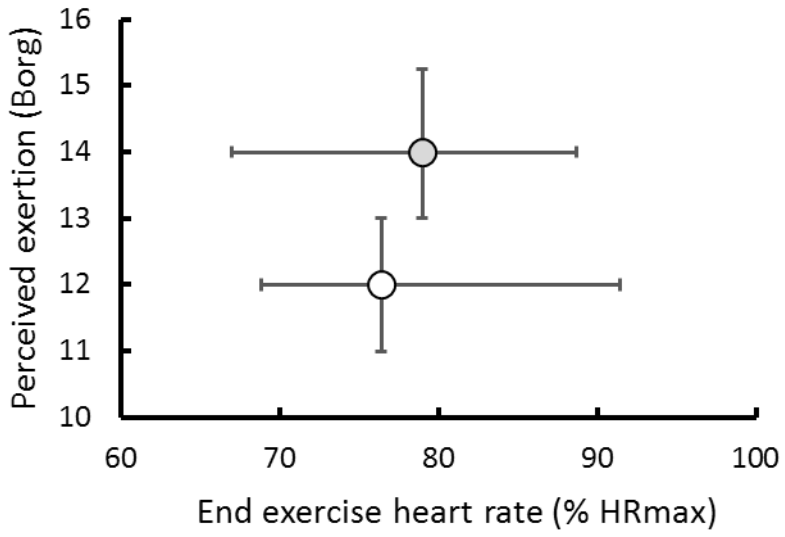


Figure 5.4: Perceived exertion as a function of work rate.
The Borg scale ratings (RPE) at the end of exercise are shown compared to exercise intensity, expressed as actual heart rate as a percentage of estimated maximum heart rate. Closed symbol, patients; open symbol, controls. Data is given as median and IQR.

5.3.2.1 Was Perception Of Exertion An Important Cause Of Subjective Fatigue?

Whilst there were clear differences between the median final RPE scores of the two groups, the most important question is whether there is a correlation between perception of exertion during exercise and the subjective symptoms of fatigue. Since there was a wide range of ages and body sizes within both the patients and healthy control groups, it was necessary to allow for this using the RPE_{index}. The median RPE_{index} was 0.88 (IQR=0.72-1.12) for patients with AAV compared with 0.78 (IQR=0.71-0.85) for healthy controls (p=0.031).

As shown in Table 5.3, perception of exertion correlated strongly with fatigue across all participants, and it also correlated with HRQOL, bodily pain, psychological morbidity and sleep quality. When only the patients with AAV were included in the analysis there were still strong, statistically significant correlations with Physical Fatigue and Reduced Motivation on the MFI-20. There were also reasonably strong correlations with General Fatigue on the MFI-20, Depression and Anxiety scores on the HADS, and Global PSQI score, and although these correlations were not statistically significant this may have been a function of the relatively small participant numbers. Univariate analysis therefore suggests that altered perception of exertion is an important central mechanism of fatigue in AAV, and that it may be related in some way to pain, psychological morbidity and reduced sleep quality.

	RPE_{Index} All Participants		RPE_{Index} Patients with AAV Only	
	r_s	p	r_s	p
General Fatigue	0.391	**<0.001**	0.287	0.051
Physical Fatigue	0.431	**<0.001**	0.337	**0.023**
Mental Fatigue	0.236	**0.031**	0.156	0.302
Reduced Activity	0.319	**0.003**	0.187	0.213
Reduced Motivation	0.451	**<0.001**	0.387	**0.007**
Bodily Pain	-0.256	**0.020**	-0.163	0.274
PCS	-0.358	**0.002**	-0.235	0.130
MCS	-0.267	**0.020**	-0.163	0.296
Anxiety Score	0.292	**0.007**	0.250	0.094
Depression Score	0.404	**<0.001**	0.275	0.064
Global PSQI Score	0.280	**0.011**	0.260	0.089

Table 5.3: Relationship between perception of exertion and participants' symptoms.
Non-parametric correlations between perception of exertion and: the five dimensions of fatigue on the MFI-20; the physical and mental component scores on the SF-36; anxiety and depression scores on the HADS; and overall sleep quality score on the PSQI. Analysis is shown for all participants and also for patients with AAV only.

5.4 Multivariate Analysis – Which Mechanisms Predicted Fatigue in This Study?

As described over the last two chapters, univariate analysis in this study suggested that fatigue in patients with AAV was unrelated to skeletal muscle bulk or strength, or to cardio-respiratory fitness, but was instead predicted, in the statistical sense, by reduced central motor drive and altered perception of exertion. A logistic regression model was constructed using a backward stepwise selection technique to further explore which of these mechanisms were predictive of fatigue in the AAV patient group. The variables entered into the analysis were: muscle mass, voluntary muscle activation (during MVC), theoretical maximal contraction, muscular reserve (at the end of the sustained 50% MVC), O_2 pulse (adjusted for LBM), and RPE_{Index}. Results for the AAV group were as follows:

- For General Fatigue, only RPE_{Index} independently predicted fatigue (standardised beta coefficient=0.396, p=0.010), in a final model which also included voluntary muscle activation (standardised beta coefficient=-0.272, p=0.068). The overall R^2 for the model was 0.208, and the adjusted R^2 was 0.166.
- For Physical Fatigue, only RPE_{Index} independently predicted fatigue (standardised beta coefficient=0.433, p=0.006), in a final model which also included voluntary muscle activation (standardised beta coefficient=-0.418, p=0.070) and muscular reserve (standardised beta coefficient=-0.394, p=0.087). The overall R^2 for the model was 0.246, and the adjusted R^2 was 0.181.
- For Reduced Motivation, only RPE_{Index} independently predicted fatigue (standardised beta coefficient=0.517, p=0.001), in a final model which also included theoretical maximal contraction (standardised beta coefficient=0.247, p=0.092) and O_2 pulse

(standardised beta coefficient=-0.253, p=0.086). The overall R^2 for the model was 0.318, and the adjusted R^2 was 0.263.

- None of the mechanisms studied appeared to significantly predict Mental Fatigue or Reduced Activity in these models.

This multivariate analysis appears to confirm the findings of univariate analysis - that altered perception of exertion is the most important mechanism underlying fatigue in patients with AAV.

5.5 Was Altered Perception of Exertion Related to Suppression of Endogenous Hormones or to Inflammation?

It was shown in the previous chapter that DHEA and DHEAS levels were suppressed in the AAV group, and that this correlated with Physical Fatigue. However, as shown in Table 5.4, although perception of exertion correlated with DHEAS when all participants were analysed together, the correlation was not present when only the patients with AAV were analysed. This apparent correlation in fact was probably due to the large difference in serum concentrations between study groups, and there was no evidence to support a link between hormone suppression and altered perception of exertion.

	RPE_{Index} All Participants		**RPE_{Index} Patients with AAV Only**	
	r_s	**p**	**r_s**	**p**
DHEA	-0.110	0.308	-0.075	0.613
DHEAS	-0.255	**0.019**	-0.155	0.294

Table 5.4: Perception of exertion and endogenous hormone suppression.
Correlations between DHEA/DHEAS and RPE_{Index} when all participants are analysed together and also when patients with AAV are analysed separately. Statistically significant correlations are highlighted in bold.

Inflammation was assessed in all participants by measurement of hs-CRP and a panel of pro-inflammatory cytokines. Although all of these markers were significantly raised in the patients with AAV compared to the healthy controls (as described in the previous chapter), baseline inflammation did not correlate with RPE_{Index} regardless of whether patients with AAV were analysed alone or together with healthy controls.

Because some studies have shown an association between fatigue and the change in serum inflammatory marker concentrations after exercise, the same inflammatory markers that were measured at baseline were also measured after the sub-maximal exercise at three time-points: immediately after cessation of exercise, one hour afterwards, and two hours afterwards. The changes in serum concentration from baseline for each group are shown in Table 5.5. The only change in serum concentration from baseline that was significantly different between groups was that of Il-1RA from baseline to one hour post-exercise; in isolation this would seem to be of highly doubtful clinical significance, and indeed there was no correlation between the change in concentration and any of the dimensions of subjective fatigue or objective measures of central fatigue. In summary, no evidence was found to link inflammation to fatigue either at baseline or post-exercise.

	Change Post-Exercise			Change At 1 Hour			Change At 2 Hours		
	AAV	**Healthy**	**p**	**AAV**	**Healthy**	**p**	**AAV**	**Healthy**	**p**
hs-CRP (mg/ml)	0.08 (0.00-0.21)	0.03 (-0.00-0.10)	0.212	-0.05 (-0.18-0.01)	-0.01 (-0.10-0.02)	0.224	-0.06 (-0.17-0.02)	-0.01 (-0.09-0.02)	0.154
Il-1b (ng/ml)	0.15 (-0.58-0.70)	0.24 (0.00-0.52)	0.477	0.22 (-0.23-0.84)	0.04 (-0.33-0.28)	0.099	-0.17 (-1.45-0.63)	0.08 (0.20-0.66)	0.197
Il-1RA (ng/ml)	7.97 (-8.98-45.67)	16.58 (-7.14-33.95)	0.626	14.9 (-8.95-42.76)	-0.69 (-21.85-13.51)	**0.005**	1.51 (-32.99-30.78)	0.00 (-22.93-19.72)	0.728
Il-6 (ng/ml)	0.71 (-0.86-2.49)	0.89 (0.27-1.83)	0.781	0.11 (-1.47-3.05)	0.43 (-0.87-1.74)	0.645	-0.04 (-2.11-2.75)	0.89 (-0.64-2.03)	0.692
Il-8 (ng/ml)	1.21 (-3.26-7.16)	1.66 (0.13-2.96)	0.781	1.46 (-2.24-9.08)	0.47 (-1.48-1.88)	0.189	-0.66 (-5.38-2.63)	0.36 (-1.58-2.56)	0.626
Il-10 (ng/ml)	1.74 (0.00-3.87)	1.09 (-0.26-1.97)	0.189	1.05 (-0.70-2.52)	-0.43 (-1.80-1.08)	0.052	-0.28 (-1.81-2.00)	0.03 (-1.45-0.75)	0.951
TNF-α (ng/ml)	2.48 (-5.12-8.84)	3.67 (0.00-10.46)	0.869	5.63 (-3.58-14.21)	-0.22 (-5.72-6.64)	0.101	1.22 (-11.37-9.82)	0.23 (-5.47-7.65)	0.905

Table 5.5: Change from baseline of inflammatory marker concentrations.
Changes in concentration are shown from baseline to immediately post-exercise, one hour post-exercise, and two hours post-exercise. Median values are shown, with IQR in brackets.

5.6 Multivariate Analysis – Which Factors Best Predicted Altered Perception Of Exertion in Patients with AAV?

Chapter 3 showed that a number of biological and psychosocial factors appeared to predict fatigue in AAV, including: Depression Score and Anxiety Score on the HADS, Global PSQI Score, Bodily Pain on the SF-36, employment status, and haemoglobin. Multiple regression analysis was carried out, again using a backwards stepwise selection technique, to identify whether any of these factors were predictors of altered perception of exertion in AAV. In this model the only independent predictor of altered perception of exertion in AAV was global PSQI score (standardised beta coefficient=0.372, p=0.017). The overall R^2 for the model was 0.138, and the adjusted R^2 was 0.116.

5.7 Paced Auditory Serial Addition Test (PASAT)

Although patients with AAV in this study reported much more severe physical fatigue than mental fatigue, it is anecdotally very common for patients with AAV to complain of difficulty concentrating. In order to investigate this, the mechanistic study also included the PASAT, which is a test of global cognitive function (see Chapter 2 for details).

As shown in Table 5.6, patients with AAV achieved much lower scores in all four sections of the PASAT, indicating that patients with AAV displayed significant cognitive impairment compared to the healthy controls in this study. As described in Chapter 2, four different inter-digit intervals were used in case either all participants did well at slow speeds because they were too easy, or all did badly at fast speeds because they were too hard, but in this study significant differences were found between the groups across all four speeds.

	Patients with AAV	Healthy Controls	p
Section 1 (60 calculations; 2.4s inter-digit interval)	39 (31-46.5)	46 (38-53)	**0.003**
Section 2 (60 calculations; 2.0s n inter-digit interval)	32.5 (27.5-40.5)	39 (31-47)	**0.001**
Section 3 (60 calculations; 1.6s inter-digit interval)	29 (22.5-36.5)	37 (29-43)	**0.004**
Section 4 (240 calculations, 1.2s inter-digit interval)	18.5 (14-24)	29 (19-33)	**0.001**
Total	120 (93-137.5)	151 (121-177)	**0.001**

Table 5.6: PASAT results for patients with AAV and healthy controls.
The median number of correct answers given (out of a possible 60) for each of the four different sections of the PASAT, and median total scores (out of a possible 240) for each of the groups. IQR in brackets. Differences between groups which reached statistical significance are highlighted in bold.

As expected, cognitive function did correlate with fatigue in both groups of participants, but perhaps unexpectedly, cognition correlated most strongly with Physical Fatigue and Reduced Activity rather than Mental Fatigue (Table 5.7). As shown above, symptoms of Physical Fatigue also correlated with central mechanisms of fatigue such as RPE_{Index}, so one possible explanation might be that whatever had caused the reduced cognition had also affected the centres in the brain responsible for central fatigue, although in this study there was no direct correlation between PASAT scores and voluntary muscle activation, muscular reserve at the end of a 50% MVC, or RPE_{Index}. There is a well-established link between physical activity and cognition (487, 488), so another explanation might be that physical fatigue had led to reduced activity, and this in turn had led to reduced cognition over time - although there was no correlation between disease duration and cognition in the AAV group (r_s=0.172, p=0.259). Where the link between cognitive function and fatigue on the MFI-20 has been reported previously, investigators have not stated which dimension of fatigue correlated best (489).

	All Participants		Patients with AAV	
	r_s	p	r_s	p
General Fatigue	-0.333	**0.002**	-0.211	0.154
Physical Fatigue	-0.370	**0.001**	-0.358	**0.016**
Mental Fatigue	-0.191	0.082	-0.206	0.169
Reduced Activity	-0.364	**0.001**	-0.396	**0.006**
Reduced Motivation	-0.276	**0.011**	-0.212	0.153

Table 5.7: Cognition and fatigue.
Non-parametric correlations are shown between fatigue and total score on the PASAT for all participants and also for patients with AAV separately. Statistically significant correlations are highlighted in bold.

Cognition in this study correlated strongly with educational attainment, whether assessed by age of leaving education (r_s=0.412, p<0.001 for analysis of both groups together) or attainment of a university degree (r_s=0.494, p<0.001 for both groups together), and this is particularly important since the level of educational attainment for participants in this study was much greater in the healthy control group than in the AAV group. To further examine whether or not AAV was independently predictive of reduced cognition, multiple regression analysis was carried out, again using a backwards stepwise selection technique. Both groups were included, and the variables entered into the analysis were 'patient group' (AAV or healthy), age of school-leaving, attainment or not of a university degree, and the five subscales of the MFI-20. In this model the only independent predictors of cognitive level were 'University degree' (standardised beta coefficient=0.461, p<0.001) and Reduced Activity (standardised beta coefficient=-0.384, p<0.001); the overall R^2 for the model was 0.369, and the adjusted R^2 was 0.353. This suggests that in fact educational attainment and activity levels might explain the difference between the two groups in this study, and that the presence or absence of AAV was of little consequence. However, since patients anecdotally

complain of reduced cognition, the factors associated with cognitive level *within the AAV group* were further examined.

5.7.1 Interaction of Biological Factors with Cognition

When only patients with AAV were included in analysis, cognition did not correlate with haemoglobin, renal function, DHEA, DHEAS or CRP. However there were significant correlations with IL-1RA (r_s=-0.411, p=0.004), IL-6 (r_s=-0.457, p<0.001), IL-8 (r_s=-0.306, p=0.034), IL-10 (r_s=-0.363, p=0.011), and TNF-α (r_s=-0.363, p=0.012). The correlation between the PASAT scores of patients with AAV and all of these cytokine concentrations might suggest the possibility of a causal relationship between chronic inflammation and reduced cognitive function; however, in a cross-sectional study such as this it is impossible to prove cause and effect, and in any case it must be remembered that IL-1RA and IL-10 are anti-inflammatory. Vasculitic involvement of the peripheral nerves (as recorded in the patients' records) strongly correlated with reduced cognition (r_s=0.423, p=0.006), but this is difficult to explain and none of the participants in the study had evidence of cerebral vasculitis.

5.7.2 Cognition and Psychosocial Factors

Surprisingly, objective cognition in patients with AAV did not correlate with quality of sleep as measured by the Global PSQI Score (r_s=-0.2, p=0.194). There were strong correlations with Anxiety Score (r_r=-0.501, p<0.001) and Depression Score (r_s=-0.419, p=0.004) on the HADS; although this might suggest that whatever is leading to reduced cognition is also causing psychological symptoms, the relationship might in fact be artifactual, since anxiety could be expected to lead to panic and impaired performance during the PASAT. Bodily Pain (SF-36) correlated strongly with cognition (r_s=0.373, p=0.01), but this might be most easily explained by speculating that reduced cognition could be a predictor of reduced pain

threshold. In view of the relationships observed between cognition and fatigue, anxiety, depression and pain, it is unsurprising that it also correlated with the Social Functioning (r_s=0.405, p=0.005), Role Emotion (r_r=0.326, p=0.027), Mental Health (r_s=0.350, p=0.016) and Mental Component Score (r_s=0.343, p=0.024) measures of HRQOL on the MOS SF-36.

5.8 Summary of Results

In the last chapter evidence was presented that fatigue in patients with AAV was related to reduced central motor drive and also to adiposity, but that it was not related to peripheral muscle mass or strength. In this chapter results showed that fatigue in patients with AAV was related to a heightened perception of exertion, but was not related to cardio-respiratory fitness. Taking these findings together, the evidence suggests that fatigue in AAV is due to central and not peripheral mechanisms. Evidence was also shown of impaired cognition in patients with AAV, which correlated with cytokine concentrations, vasculitis of the peripheral nerves, fatigue, psychological morbidity, pain and HRQOL.

The measure of cardio-respiratory fitness which is most commonly quoted in the literature is $\dot{V}O_2max$, but because it is not safe to measure this directly in patients with AAV, the original study design included a method of estimating $\dot{V}O_2max$ using predicted maximum heart rate. However, pilot work shown in this chapter confirmed that the available equations to predict HR are unreliable, and that the method used in this study to estimate $\dot{V}O_2max$ was therefore unacceptably inaccurate. It was therefore decided to use 'O_2 pulse' as an additional measurement of cardio-respiratory fitness even though it is less commonly used than $\dot{V}O_2max$, since it may be measured directly in a submaximal exercise test.

Submaximal exercise testing confirmed that patients with AAV were less fit than age-matched healthy controls, and when the results were corrected for total body weight and both groups were analysed together cardiorespiratory fitness appeared to correlate with fatigue. However, since adipose tissue makes a negligible contribution to oxygen consumption during exercise, it makes more physiological sense to correct O_2 pulse for LBM, and when this was done there was no correlation with any of the dimensions of fatigue. It was shown in Chapter 4 that fatigue correlated with adiposity in patients with AAV, and this probably skewed the

results when O_2 pulse was corrected using total body weight. In fact, it appears that although cardio-respiratory fitness was reduced in patients with AAV, it was not an important mechanism underpinning their subjective symptoms of fatigue. This is unsurprising when it is considered that patients, unlike healthy people, report that their fatigue is unpredictable and often unrelated to physical exertion.

Perception of exertion was measured using the Borg RPE scale at the end of the three stages of the submaximal exercise test. It was shown that the AAV group had a heightened perception of exertion compared with the healthy group – in other words at the same workload and for the same physiological response, the patients with AAV *felt* like they were working harder. In univariate analysis of the AAV group, perception of exertion correlated strongly with symptoms of fatigue on the MFI-20, reaching statistical significance for symptoms of Physical Fatigue and Reduced Motivation, and in a multivariate analysis of the AAV group RPE_{Index} was the only independent predictor of General Fatigue, Physical Fatigue and Reduced Motivation. These results suggested that perception of exertion was the most important mechanism of fatigue in the patients with AAV in this study. Previous chapters have shown that psychosocial factors including quality of life, anxiety, depression, sleep disturbance and pain were all associated with fatigue, and in this chapter it was shown that these same factors were also associated with perception of exertion. Although the correlations did not reach statistical significance when only the AAV group were analysed this may have been due to the relatively small study size, and the results suggest that these psychosocial factors may result in fatigue through altered perception of exertion in patients with AAV. The link between increased perceived exertion in patients with anxiety and depression has been suggested previously (490), and it may be that patients with psychological symptoms are more likely to catastrophize feelings during physical activity (491). There was no evidence to

support a link between perception of exertion and either hormone suppression or inflammation in patients with AAV.

Anecdotally, patients with AAV often complain to physicians that they are unable to concentrate when performing mental tasks. To investigate this, participants underwent testing with the Paced Auditory Serial Addition Test (PASAT) at four different test speeds. It was demonstrated that patients with AAV in this study had significantly impaired global cognitive function compared to the healthy controls. Cognitive function correlated most strongly with the Physical Fatigue and Reduced Activity dimensions of fatigue on the MFI-20, and perhaps counter-intuitively did not correlate with Mental Fatigue. Perhaps the most likely explanation is that that fatigue had led to reduced physical exertion in these patients, and thereby to reduced cognition over time in keeping with the established literature (487, 488). Indeed, regression analysis showed that the presence of AAV was not independently associated with reduced cognition in this study, and that the only independent predictors of reduced cognition were lower educational attainment and higher scores in the Reduced Activity subscale of the MFI-20. Within the AAV group, correlations were seen between cognition and the concentrations of both pro-inflammatory and anti-inflammatory cytokines. One explanation might be that chronic low level inflammation reduces cognitive level in patients with AAV, but this can only be speculated in a cross-sectional study, particularly when so much of the reduced cognition appears to be explained by education and activity. Anxiety and Depression Scores on the HADS correlated very strongly with PASAT scores in the AAV group, but this is difficult to interpret since anxiety might actually impair a participants' technique in this particular test due to the time pressures involved. Correlations with scores on the MOS SF-36 suggested that reduced cognition was leading to reduced HRQOL in patients with AAV, chiefly through reduced social functioning and role impairment.

6 DISCUSSION

This study has demonstrated that fatigue in AAV is severe, and that it is a principle cause of impaired quality of life in patients with AAV, in keeping with the existing literature (324). This is the first study to demonstrate central mechanisms of fatigue in patients with AAV in stable remission and to show that fatigue in AAV is not due to problems with peripheral muscles or to reduced cardio-respiratory fitness. It has been shown that perception of effort is independently associated with fatigue, and that there is also evidence of reduced central motor drive in patients with AAV. Importantly, the study also showed that previously identified psychosocial factors including quality of life, anxiety and depression and sleep disturbance were all associated with perception of effort and fatigue, suggesting that these psychosocial factors may result in fatigue through heightened perception of exertion and reduced central activation in patients with AAV. DHEAS and inflammation did not independently contribute to fatigue. This is one of the largest studies to date to examine peripheral and central elements associated with fatigue in inflammatory rheumatologic disease in a systematic manner.

6.1 Fatigue in AAV is Severe and Causes Reduced HRQOL

The existing literature suggests that patients with AAV believe fatigue to be their most severe symptom, and that it is the symptom which has the biggest impact in their daily life (324), and that suggestion was supported by the questionnaire data in this study. Patients in the AAV group reported levels of fatigue on the MFI-20 which were greater than that reported by the CKD control group, and comparable to fatigue in other diseases characterised by fatigue, including RA, Sjogren's syndrome and cancer (116, 119, 122, 128, 132, 136, 462). Analysis of the results suggested that patients in both the AAV and CKD groups experienced fatigue

as a very physical phenomenon, and that it was qualitatively different from the fatigue experienced as part of normal every-day life by the healthy control group. This is in keeping with previous research in patients with RA (102, 106, 379), who described the fatigue of RA as 'akin to carrying a weight around with you', and stated that it was fundamentally different from the fatigue they had experienced in every-day life before they developed RA.

In this study, there were very strong correlations for patients with AAV between fatigue measured by the MFI-20 and HRQOL as measured by the MOS SF-36, suggesting that fatigue was indeed a very important cause of the reduced quality of life experienced by patients with AAV. This was further supported by the results of the modified Piper Fatigue Scale, which showed that fatigue was directly responsible for multiple role impairments in working, social and family life, demonstrating the need for a better understanding of the determinants and mechanisms of fatigue in AAV.

6.2 The Mechanisms of Fatigue in AAV Are Central

The very physical nature of fatigue in AAV would suggest that it might be a result of peripheral problems such as skeletal muscle weakness or reduced cardio-respiratory fitness, but the perception of exertion is influenced by emotional and psychological as well as physical factors (446). Physical fatigue can be caused by central mechanisms such as 'central activation failure' (167, 168), and so both peripheral and central mechanisms were examined in the mechanistic part of this study.

There are many reasons why patients with AAV might have significant muscle atrophy or loss of contractility: steroids and cyclophosphamide are mainstays of the treatment of AAV, and can cause muscle atrophy and toxicity (335, 336, 338, 339); pro-inflammatory cytokines can reduce the resting membrane potential of muscle and nerve (475), and have been

implicated in reduced muscle endurance (334); and either the disease itself or complications of the treatment might cause damage to or dysfunction of the epineural endothelium (475-477). However, in this study there was no significant difference between the muscle mass of the AAV group and the healthy control group on DEXA, and there was no correlation between muscle mass and subjective fatigue on the MFI-20. Prednisolone dose was not associated with fatigue, but all of the patients in this study had been taking a dose of ≤7.5mg for longer than 6 months (data not shown), unlike those in the previous study (354).

Muscle strength was tested by a series of brief isometric maximal voluntary contractions (MVCs) of the quadriceps muscle, and the MVCs of the AAV group were significantly weaker than those of the healthy control group. However, the twitch interpolation technique showed that there was evidence of central activation failure in the AAV group, who did not activate their muscles to the same extent as the matched healthy controls, and when this was accounted for there was no difference in muscle strength between the groups. Further evidence was found of central activation failure in the AAV group when participants were asked to maintain a 50% isometric contraction: the endurance time of patients with AAV was around 25% less than the healthy controls, and superimposed electrical stimulation demonstrated that at the point of volitional fatigue patients with AAV had a significantly greater reserve of muscle force.

It may be that interoception is more sensitive in patients with AAV, with afferent signals from the muscles and joints causing the brain to 'shut down' exercise prematurely at a point where the participant would be perfectly able to continue exercise from the perspective of physiological reserve. The findings in this study are similar to those in other diseases associated with increased fatigue, including fibromyalgia and multiple sclerosis (MS), where there is also evidence of central fatigue (140, 437, 492). Patients with fibromyalgia also reach the point of volitional muscle contraction failure more rapidly than healthy controls.

Fibromyalgia is associated with hypersensitisation to many stimuli (493) and it has been suggested that these patients may fail to activate endogenous pain inhibitory mechanisms during muscle contraction (494) resulting in central fatigue. Interestingly, patients with AAV exhibit increased pain scores that correlate with fatigue (90) and have increased rates of fibromyalgia compared with the general population (353). The relationship between neuromuscular central activation failure and fatigue in patients with AAV requires further investigation.

Although they are valuable methods of testing muscle function, repeated or prolonged isometric contractions are not common activities in daily life, and it could therefore be argued that they do not replicate the sorts of activities which lead to fatigue in the lives of patients with AAV. The aerobic exercise capacity of the participants was therefore also assessed using a submaximal exercise test, but although the patients with AAV did have poorer cardio-respiratory fitness than the healthy control group, fitness levels did not correlate with fatigue on the MFI-20. This is perhaps unsurprising since, in contrast to healthy people, patients with AAV and other chronic diseases often complain that their fatigue is unpredictable and frequently occurs without the precipitant of exercise (102).

The perception of exertion during aerobic exercise was significantly heightened in the AAV group compared to the healthy controls, meaning that for the same workload and a similar physiological response the patients with AAV *felt* as if they were working much harder. More importantly, perception of exertion was found to correlate strongly with Physical Fatigue and Reduced Motivation in univariate analysis of the AAV group, and regression analysis showed that perception of fatigue independently predicted fatigue in patients with AAV. This study therefore showed that fatigue in AAV appears to be due to central mechanisms including heightened perception of exertion and central activation failure, and not due to peripheral mechanisms involving skeletal muscle or cardio-respiratory fitness.

6.3 Inflammation and Endogenous Steroid Suppression

Pro-inflammatory cytokines probably have an important role in inducing sickness behaviour (181-183), and injection of pro-inflammatory cytokines into humans has been shown to induce fatigue (185, 186). Animal studies have suggested an important role for cytokines affecting introception centres within the brain (495), which could perhaps explain central activation failure if the same were true in patients with AAV. However, observational studies in autoimmune disease have generally failed to show any association between inflammation and fatigue (133, 284, 285). Likewise, although in this study the baseline concentrations of hsCRP and a panel of pro-inflammatory cytokines were all significantly higher in the AAV group than in the healthy controls, this did not correlate with fatigue, central activation failure or perception of exertion in patients with AAV.

It is possible that the much higher levels of inflammation present when patients have active disease are a more important contributor to fatigue. All patients in the current study were in sustained remission, and although hsCRP was significantly raised compared with the healthy control group, the median concentration remained within the normal range. It may be that inflammation during the initial active stage of the disease causes central changes within the brain leading to fatigue, as has been suggested in rheumatoid arthritis (194), and that these changes then persist following disease remission when the level of inflammation reduces. Perhaps the patients who experience the most severe fatigue during remission are those who had the highest levels of inflammation during that initial stage of disease activity. It could be argued that the very strong correlation found in this study between accumulated disease damage (VDI) and Physical Fatigue supports this theory, since that accumulated damage is the result of active inflammation which has since settled, but this needs further investigation, and a better understanding of the effects of inflammation on the brain in human disease is required. Another possible explanation for the lack of association between inflammation and

fatigue might be that in this study the wrong fluid compartment was sampled, since there is evidence to suggest that cytokine concentrations in the brain do not correlate with those in the blood (194).

Both inflammation and prednisolone use have been associated with suppressed levels of DHEAS which has, itself, been associated with fatigue, although with contradictory results (232, 238). Low levels of DHEAS have been associated with fatigue in patients with MS (240) where there is central activation failure (140). In this study, DHEA and DHEAS were both significantly suppressed in patients with AAV, and the levels correlated strongly with the current prednisolone dose. DHEAS correlated significantly with physical fatigue in the AAV group in univariate analysis, but other correlations between the dimensions of fatigue on the MFI-20 and either DHEA or DHEAS were weak, and in multivariate analysis DHEA and DHEAS did not independently associate with fatigue.

6.4 Anaemia and Renal Function

Previous studies have suggested that anaemia and severity of chronic kidney disease may be related to fatigue, and in the questionnaire study anaemia did correlate quite strongly with fatigue for patients with AAV. eGFR did not correlate with fatigue except in the Reduced Activity dimension on the MFI-20, however it must be noted that the median eGFR in that group was 61 ml/min, a level which would not be expected to cause fatigue.

6.5 Psycho-Social Factors Are of Great Importance

High levels of anxiety and depression have been reported previously in patients with AAV (353), and in this study more than 25% of patients in both the AAV group and the CKD group reported symptoms of anxiety, depression, or both that were predictive of psychiatric

disease. Psychological symptoms, particularly those of depression, correlated very strongly with all dimensions of fatigue in patients with AAV in univariate analysis. Patients in both disease groups also described very poor sleep quality and high levels of chronic pain. Depression, anxiety, pain, poor sleep quality and fatigue all correlated strongly with each other in univariate analysis of the patients with AAV, and it was impossible in this observational study to ascribe any causal relationship between the different variables. In multivariate analysis, depression, anxiety, sleep quality, pain, and employment status were all independent predictors of fatigue in the AAV group. There is clearly a complex interplay between all of these psycho-social factors which warrants further study.

In this study, depression, anxiety, pain and sleep quality all correlated strongly with perception of exertion across all participants. In analysis of only the patients with AAV, correlations with depression, anxiety and sleep quality remained fairly strong, and perhaps were only non-significant because of the modest sample size. The link between increased perception of exertion in patients with anxiety and depression has been suggested previously (496), perhaps because these patients are more likely to catastrophize feelings during physical activity (491). It is interesting that in this study patients with AAV also reported increased use of maladaptive coping strategies ('denial' and 'behavioural disengagement') than did the healthy control group, and that in patients with AAV, there was a strong positive correlation between the use of behavioural disengagement and both Mental Fatigue and Reduced Motivation.

6.6 Which Interventions Might Improve Fatigue in AAV?

This study has identified a number of factors which correlated with fatigue, and whilst it is impossible to identify cause and effect in a cross-sectional study such as this, it seems likely

that interventions which treat those associated factors might also lead to improvements in fatigue.

Patient factors such as poor sleep, anxiety and depression, increased perception of effort, and reduced central activation may be amenable to treatment with psychological therapies such as cognitive behavioural therapy. Although in this study reduced cardio-pulmonary fitness did not correlate with fatigue, patients with AAV were nonetheless significantly less fit than healthy controls, and it remains reasonable to think that improved fitness levels might bring about some improvement in fatigue. Indeed, even if that were not the case, there would undoubtedly be other benefits such as reduced cardiovascular morbidity and mortality. A similar combination of factors is present in chronic fatigue syndrome, and a recent large study suggests there may be improvements in these patients with cognitive behavioural therapy or exercise (359) or perhaps a combination of both.

In patients with AAV, depression and anxiety were the symptoms which correlated most strongly of all with fatigue in both univariate and multivariate analysis, and these symptoms should be actively sought out and treated, particularly in those patients who complain of severe fatigue. In addition to CBT, pharmacological treatment with antidepressants might be expected to help, and this approach has achieved some success in fibromyalgia (355).

Pain also correlated strongly with fatigue in this study, and pharmacological treatments might again have an important role to play in treating this. An important caveat, however, is that opioids and many other analgesics may actually cause fatigue in some patients, so the risk and benefit of pharmacological treatment should be balanced on an individual basis, and patients observed closely for worsening of fatigue. Depending on the nature of the pain, non-pharmacological treatments such as TENS (transcutaneous electrical nerve stimulation) might

be more appropriate. Indeed a recent randomised controlled study in fibromyalgia suggested that TENS might have benefits for pain, fatigue and hyperalgesia (497).

'Sleep hygiene' is the term used for a number of behaviours and practices which may help people with poor sleep quality, including: avoidance of daytime napping; avoidance of stimulants such as caffeine close to bedtime; vigorous exercise in the morning, or relaxing exercise such as yoga before bed; adequate exposure to natural light during the day. Sleep hygiene has been associated with improvements in pain, fatigue and sleep quality in fibromyalgia (498). Treatment of biological factors is important too. This study showed an association between anaemia and fatigue in patients with AAV, and anaemia should be investigated and treated as appropriate.

6.7 Strengths and Weaknesses of the Study

This is the first study to systematically examine both peripheral and central mechanisms of fatigue in AAV, and is one of the largest to do so in any rheumatological disease. It achieved good recruitment of patients with AAV, and the groups were well matched for age, gender and ethnicity for both the questionnaire and the mechanistic studies. The AAV group was a good representation of patients with AAV as a whole, particularly in the questionnaire study, and it therefore seems reasonable to extrapolate the findings to the larger population of patients with AAV. In combining the questionnaire and mechanistic elements, it enabled the complex aetiology of fatigue to be examined more completely than in any other study to date. The experiments in the mechanistic study were well designed, and no patients withdrew from the study or suffered any adverse events as a result of the study.

Recruitment to the questionnaire study was good for the AAV group but disappointing for the CKD and Healthy Control groups, and this led to the study being underpowered; this must be

considered when interpreting the results. Recruitment to the healthy control group was also problematic in the mechanistic study. The 'buddy system' of recruitment was less effective than anticipated, and this led to a disproportionate number of professionals in this group, recruited from the university or hospital. For future clinical studies conducted locally, it would be useful to keep a list of well-matched healthy controls that are happy to participate in clinical research.

Whilst very comprehensive, the questionnaire was long, and this probably impacted on completion rates. The 'demographics' section of the questionnaire was written specifically for this study, and with hind-sight a number of improvements could have been made if it had been piloted prior to use in the main study. In particular:

- The section which asked about habitual exercise was not well designed for this age-group, and was not sensitive enough to distinguish between the low levels of activity reported by the participants, since the 'least active' option which could be selected for each activity was '1-4 hours per week'.
- The sections which asked about alcohol and caffeine intake were left blank in a number of cases, and this might have been avoided if 'tick-box' options had been provided rather than space left for written answers.
- The questionnaire included some sections which proved to be of no benefit; for example the EQ-5D-3L provided no additional information over the MOS SF-36.

A more robust method of assessing habitual exercise might have been to distribute pedometers instead of administering that particular questionnaire section, or to have given pedometers to a random sample of the participants in order to test the reliability of the information provided in the questionnaire. Pedometers are not expensive, so their use would be recommended in similar studies in the future.

The mechanistic study was comprehensively designed, and the experiments as a whole worked very well. Some of the results were unexpected however – in particular the voluntary activation of skeletal muscle by the healthy group was much lower than expected in the muscle strength experiment, a finding which cannot be easily explained. The pain of the superimposed electrical stimulus might have affected some participants' performance, and perhaps their voluntary muscle activation was therefore not as complete as it would be in a 'real-life' situation. Pain due to electrical stimulation might also have affected the results of the muscle fatigue/endurance test, as some participants might have stopped the test early because they were not keen to endure further electrical stimuli rather than because of true central fatigue. However, although some study participants undoubtedly found the electrical stimulation painful, without using the 'interpolated twitch' technique it would have been difficult to detect central mechanisms of fatigue in these tests.

6.8 Summary

Although of limited size, this study is the largest to date to have investigated the complex aetiology of fatigue in autoimmune rheumatologic disease. The results showed that central mechanisms including 'central activation failure' and 'heightened perception of exertion' may be the most important mechanisms underpinning fatigue in AAV, but that muscle mass, muscle strength and cardio-pulmonary fitness did not predict fatigue in this group. Increased perception of effort is likely to make any physical exertion feel more demanding, thus reducing motivation and constituting a barrier to adhering to exercise advice and undertaking physical activity (324, 499). A number of psychosocial factors including depression, anxiety, and reduced sleep quality might lead to fatigue through these central mechanisms, and interventions such as CBT and sleep hygiene might therefore be expected to improve fatigue in patients with this disease. This study adds to our understanding of the complexity of

fatigue in patients with AAV and is similar to findings in chronic diseases where fatigue is common. The role of DHEA and its sulphate ester remains unclear, but inflammation appears not to be important in the aetiology of fatigue in AAV.

7 APPENDIX 1: SAMPLE QUESTIONNAIRE WITH CODING

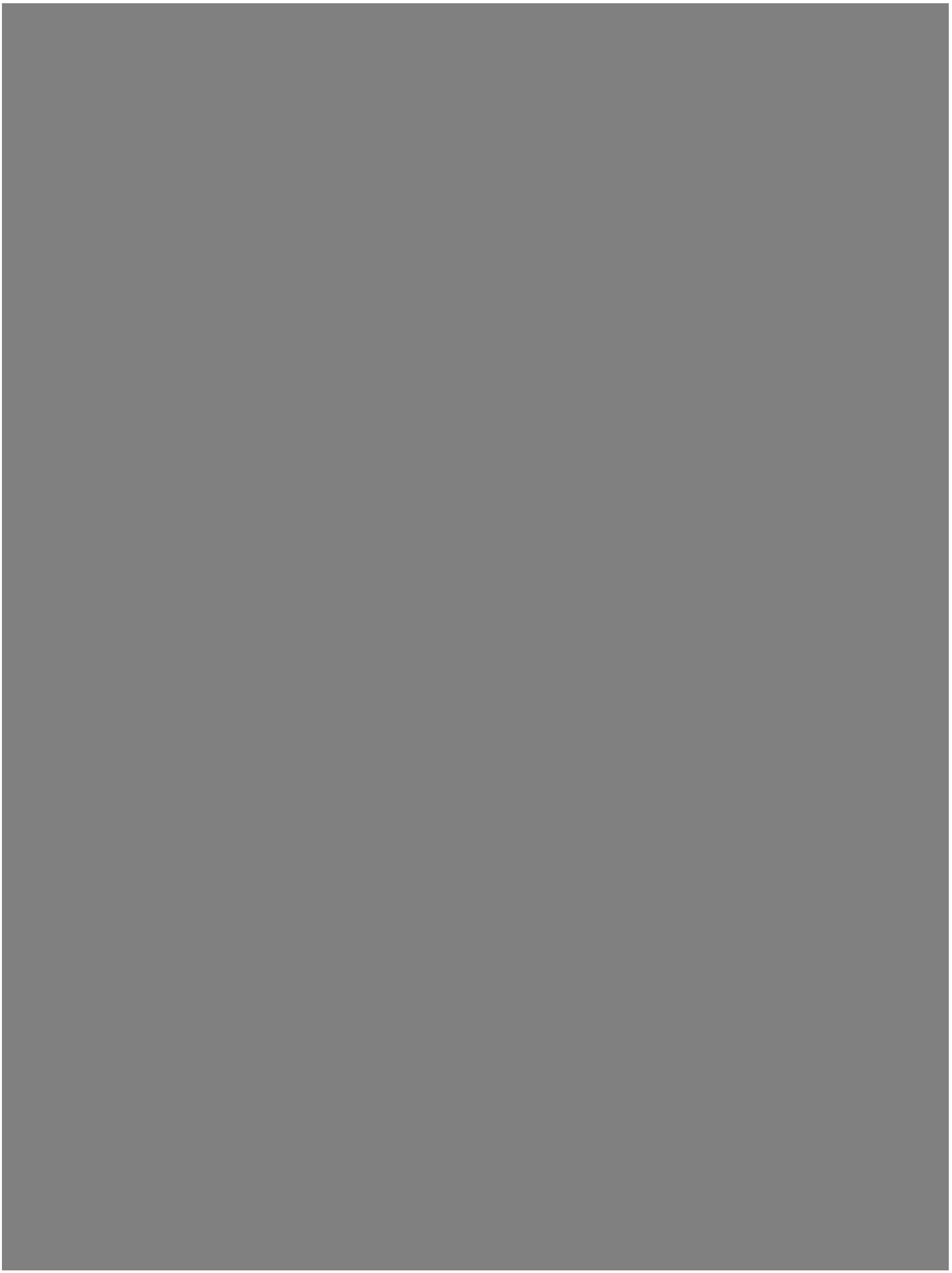
Participant Number:

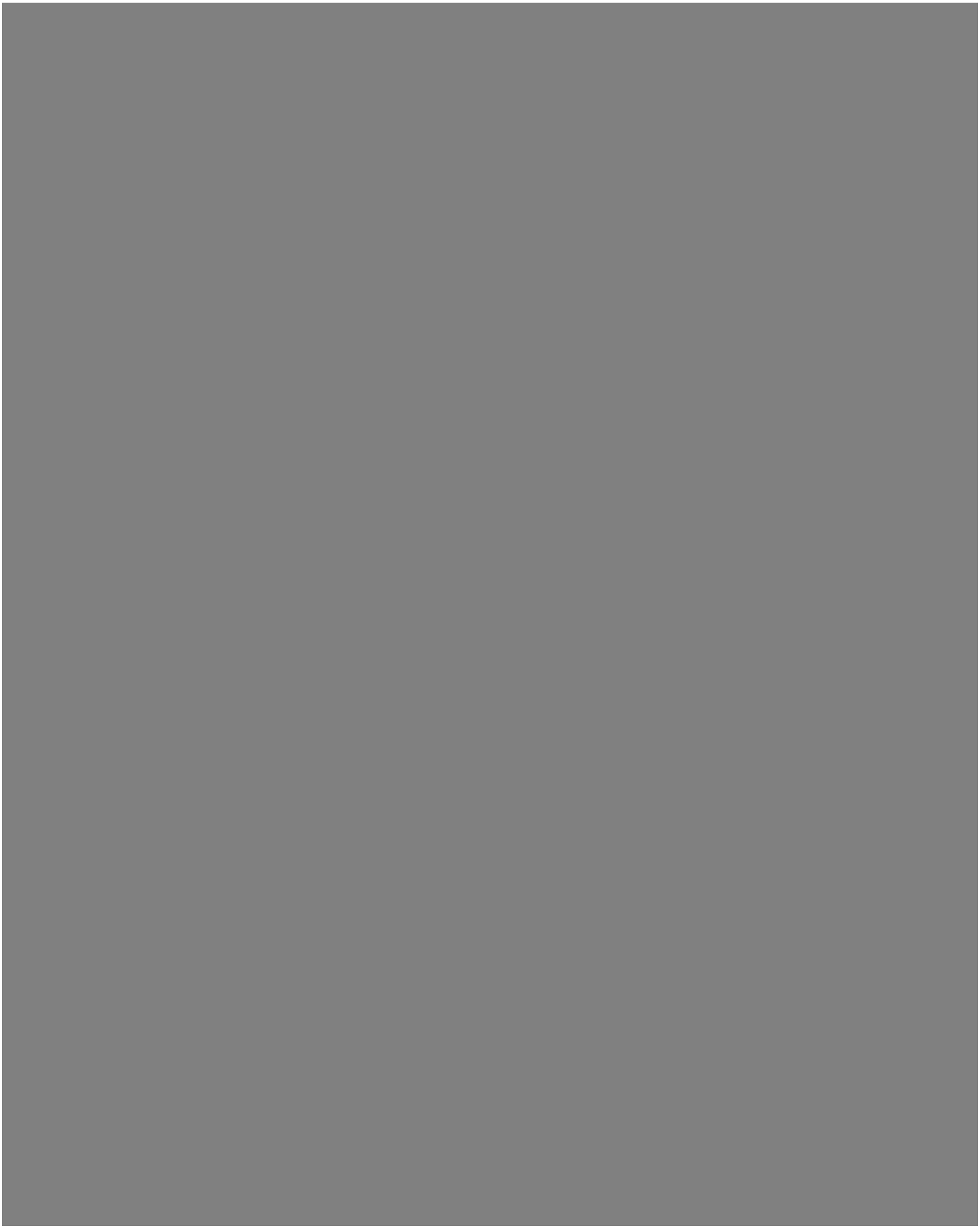
_____

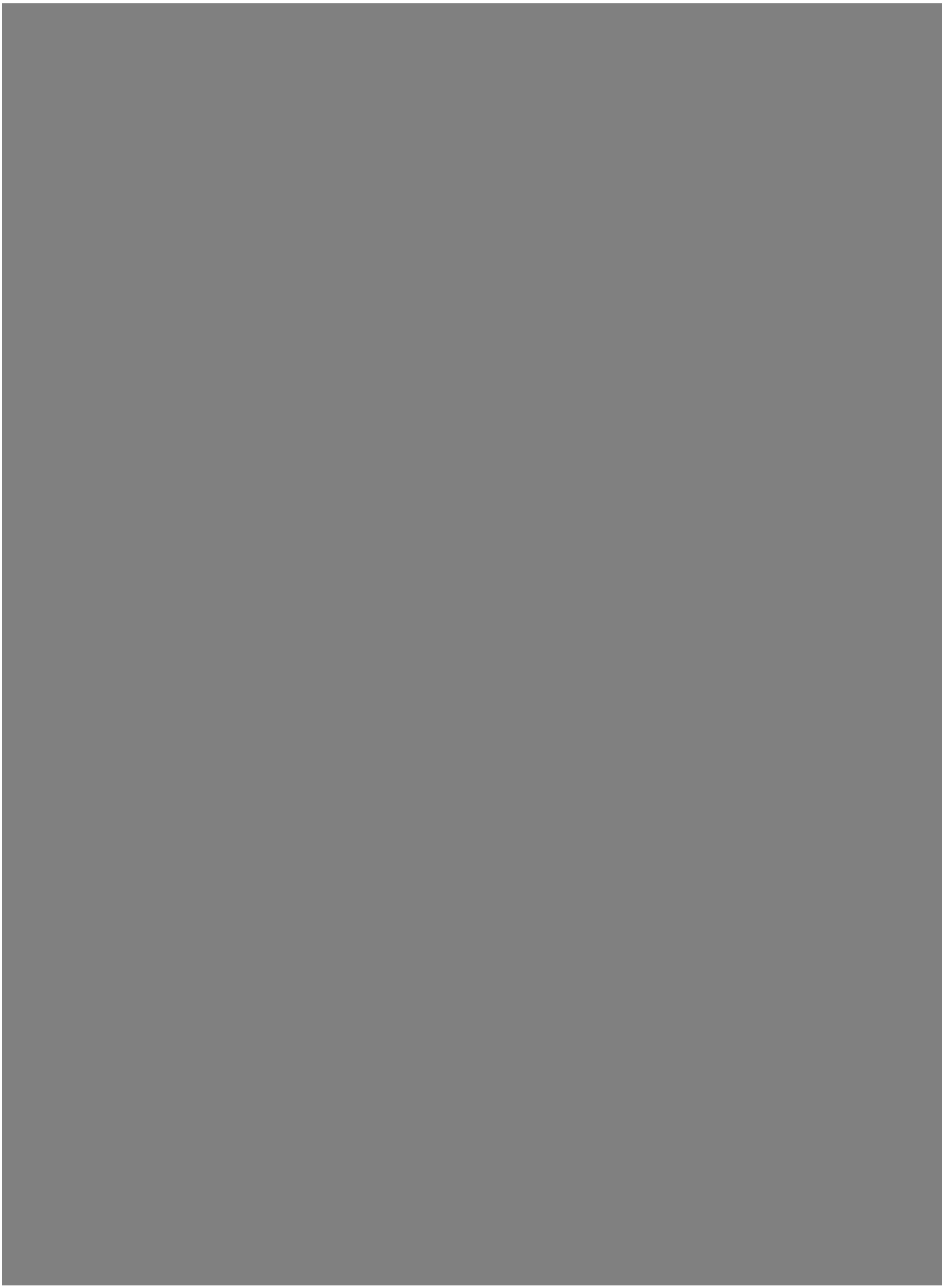
This booklet contains a questionnaire which will tell us how fatigue and illness is affecting your life. This questionnaire will take between 30 and 45 minutes to complete. You may notice that some of the questions sound a bit similar; just try to answer each question independently. There are no right or wrong answers; simply indicate what applies best to you.

Section 1

Firstly, we would like to ask you about yourself in general.

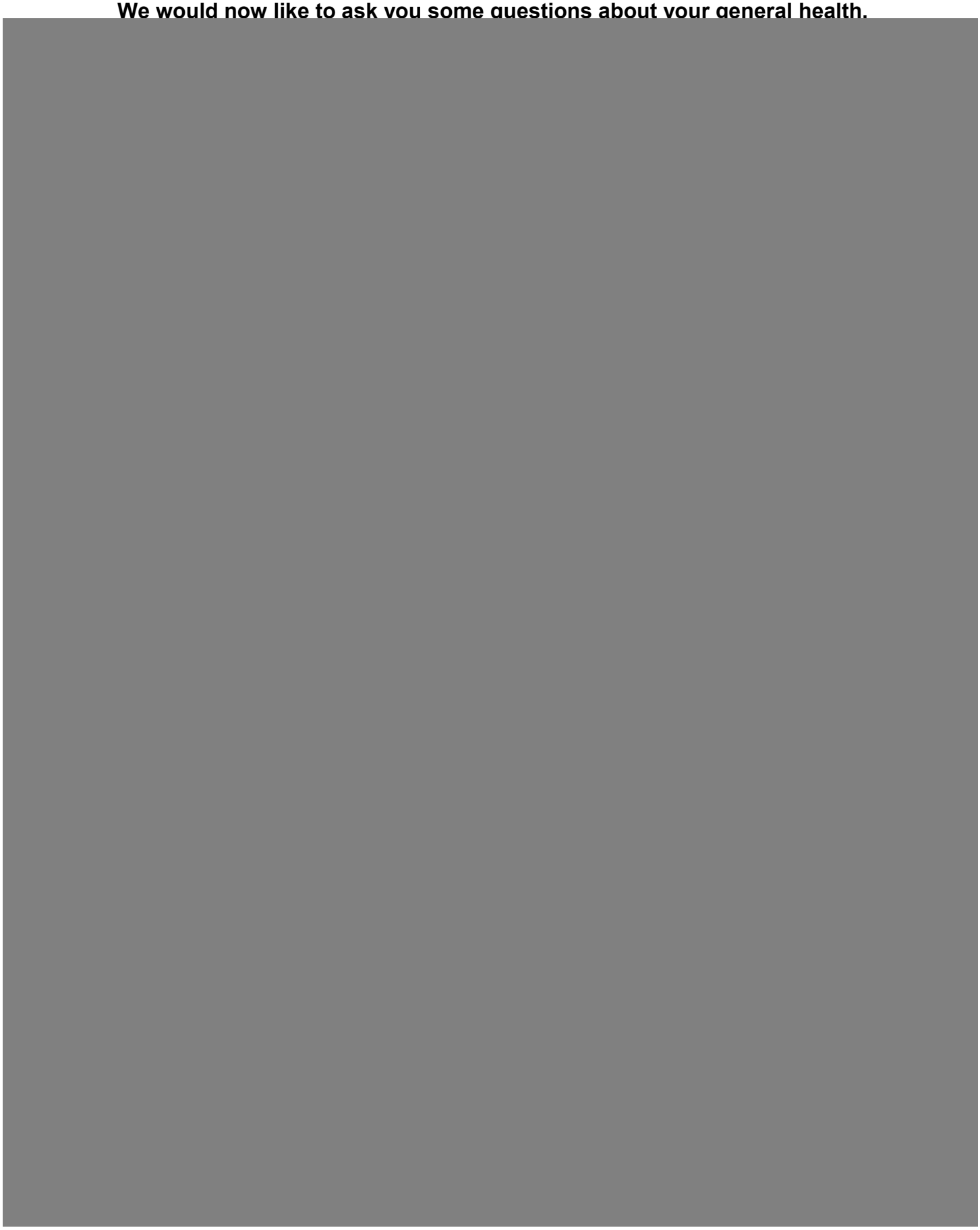


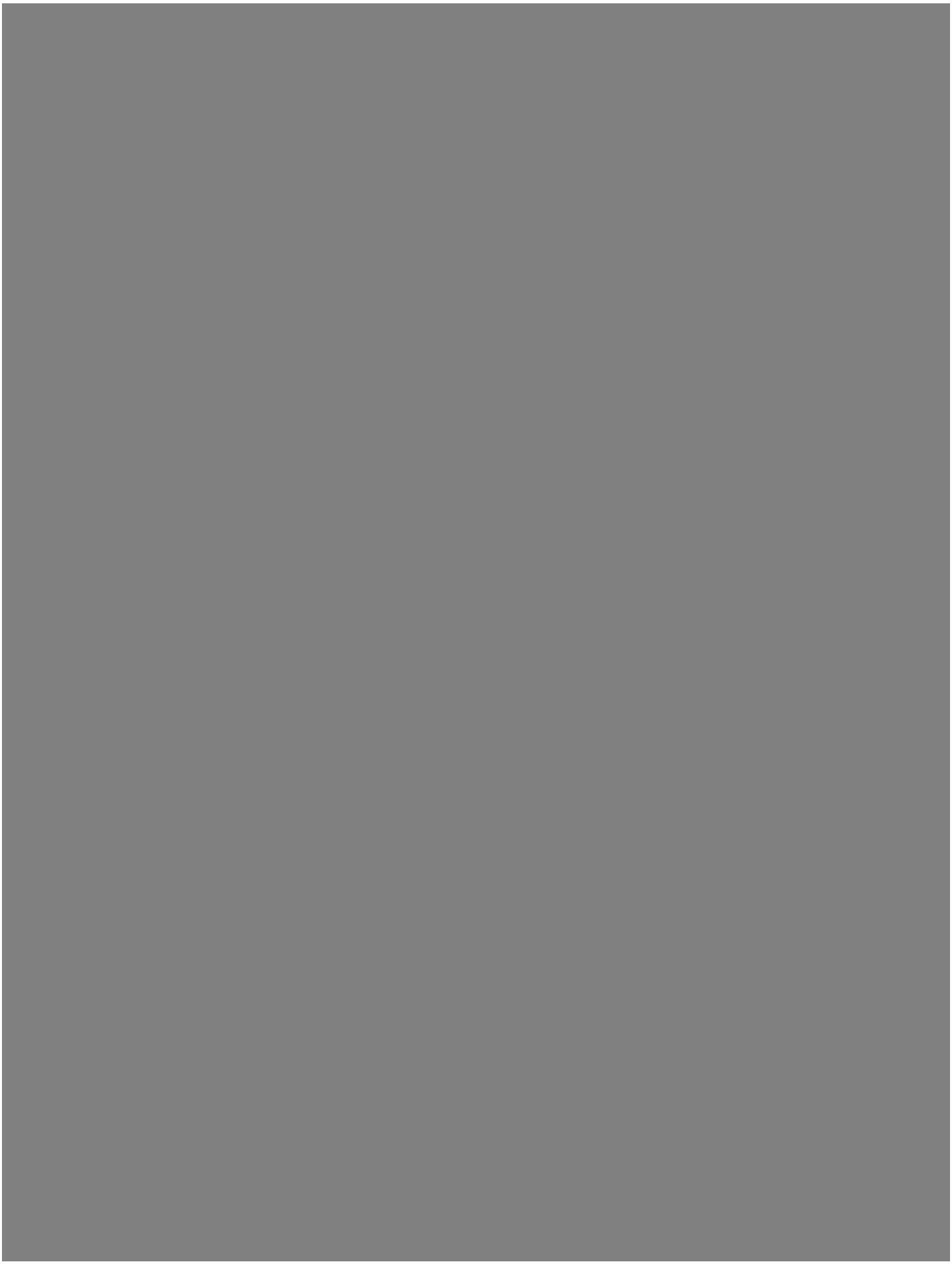




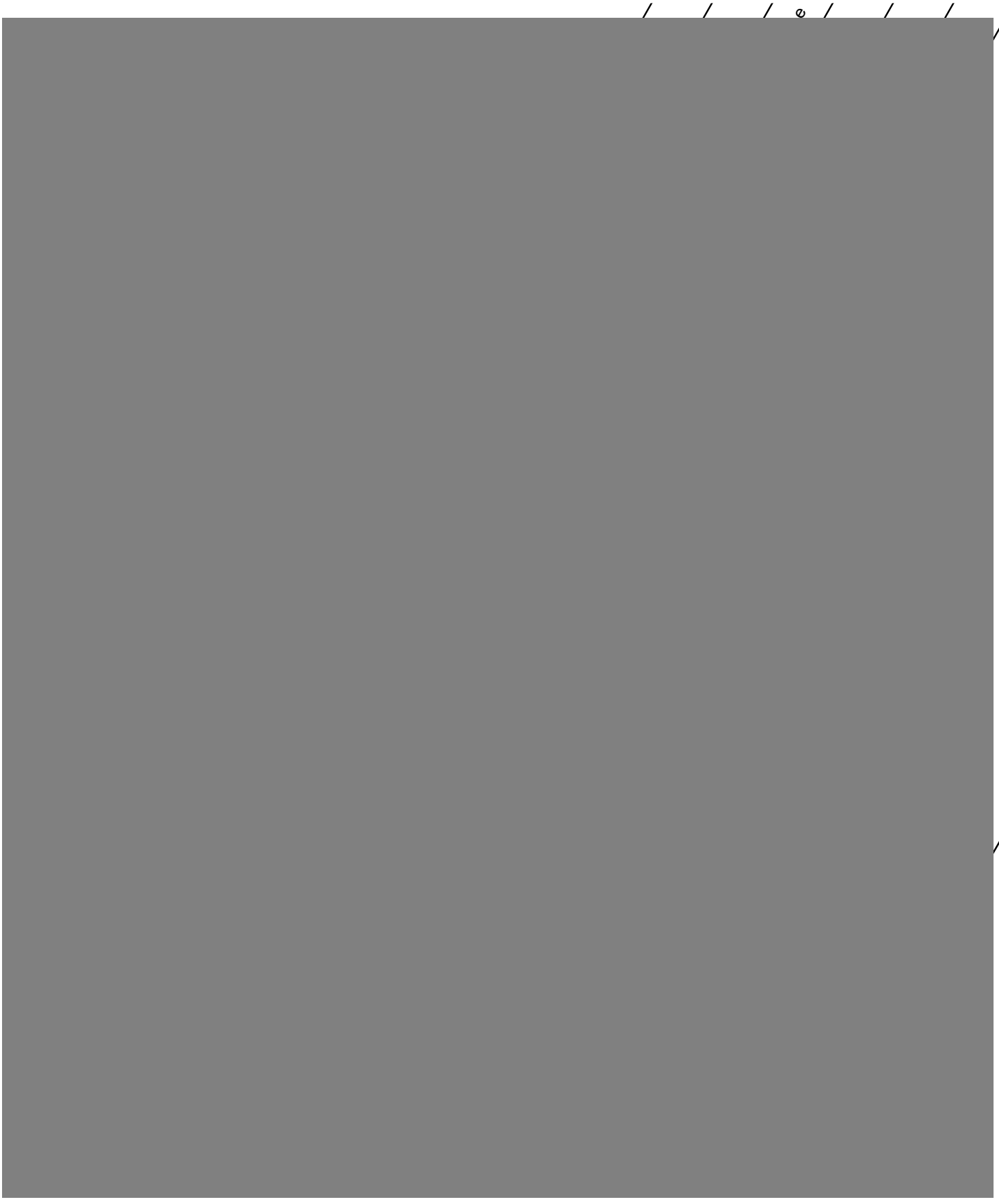
Section 2

We would now like to ask you some questions about your general health.



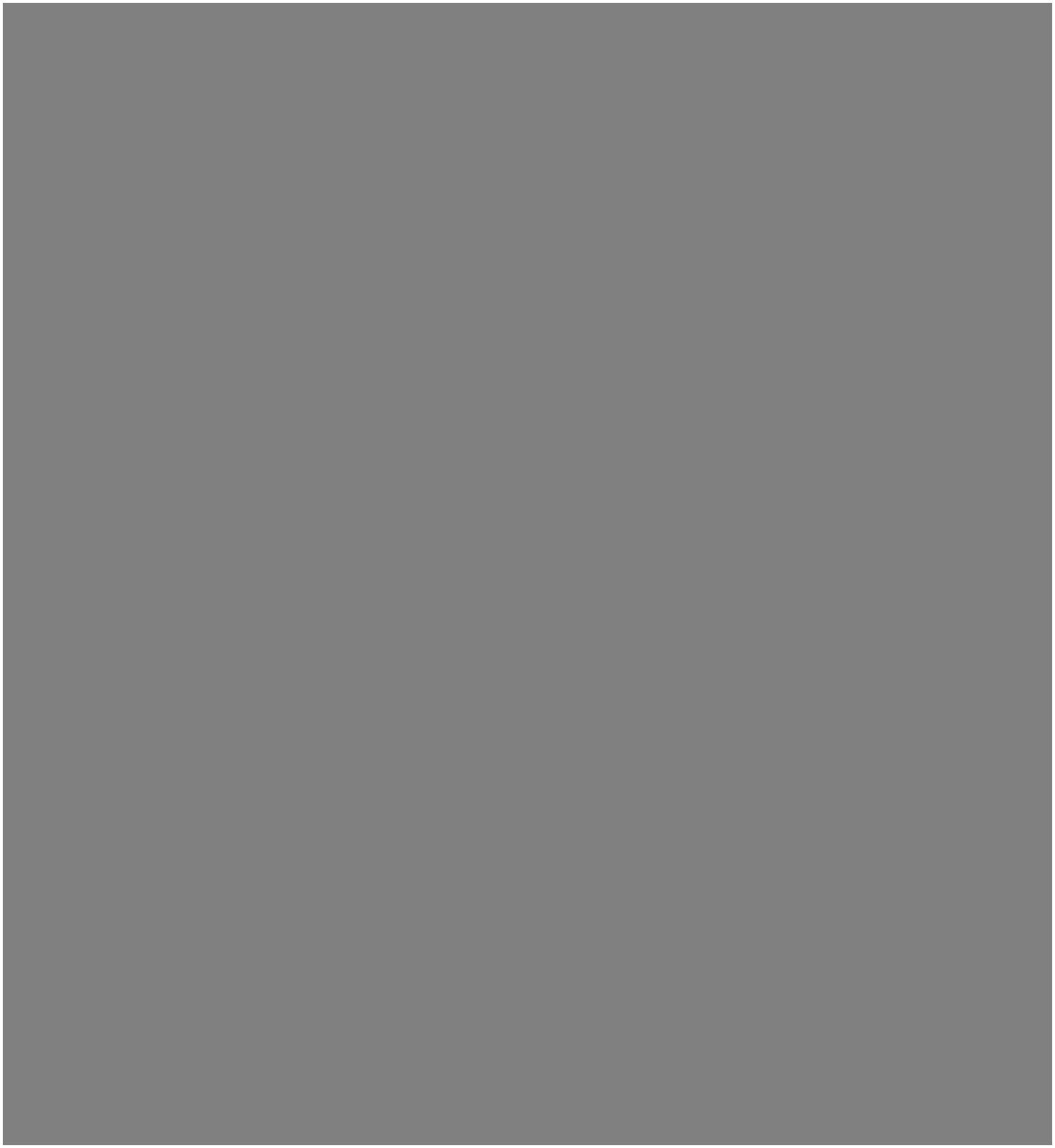


These questions are about how you feel and how things have been with you during the *past 4 weeks*. (For each question, please indicate the one answer that comes closest to the way you have been feeling). How much time during the *past 4 weeks*:



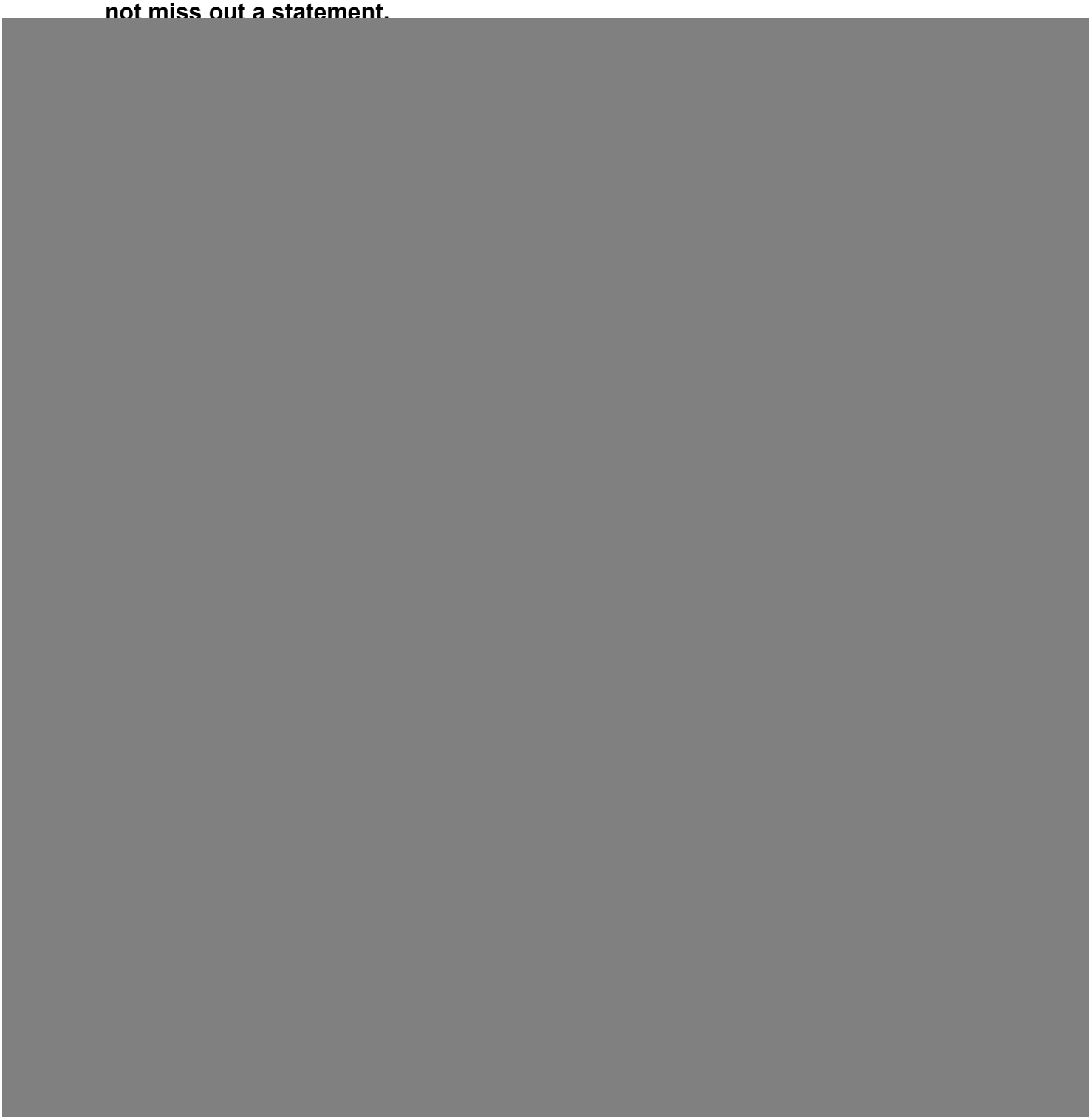
Section 3

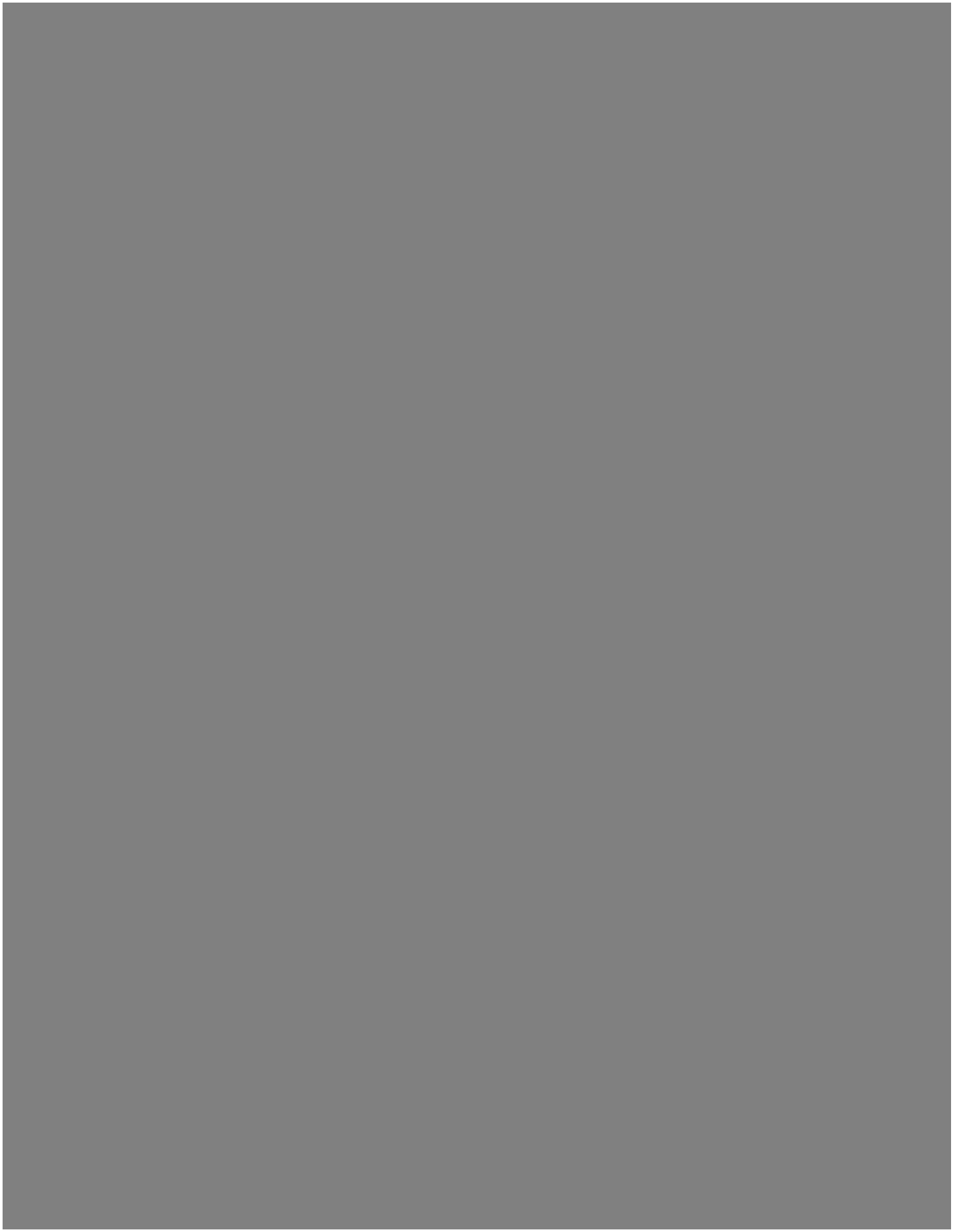
The next section asks about tiredness, fatigue and concentration.



Section 4

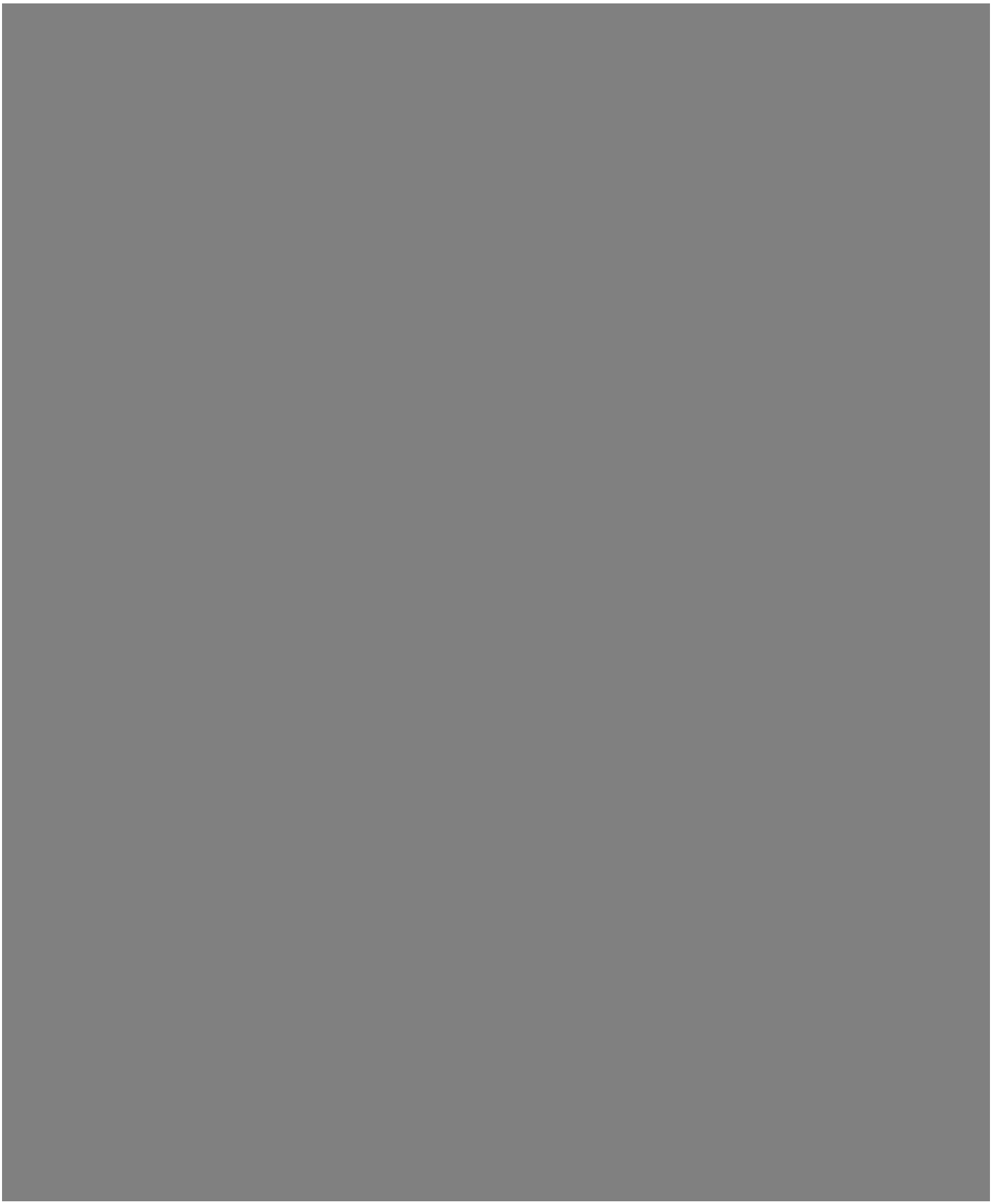
We would like to get an idea of how you have been feeling over the last few days. If you think any of these statements are entirely true, please tick the box for "1" on the extreme left. The more you disagree with the statement, the more you can tick the box in the direction of "no, that is not true". Please do not miss out a statement.

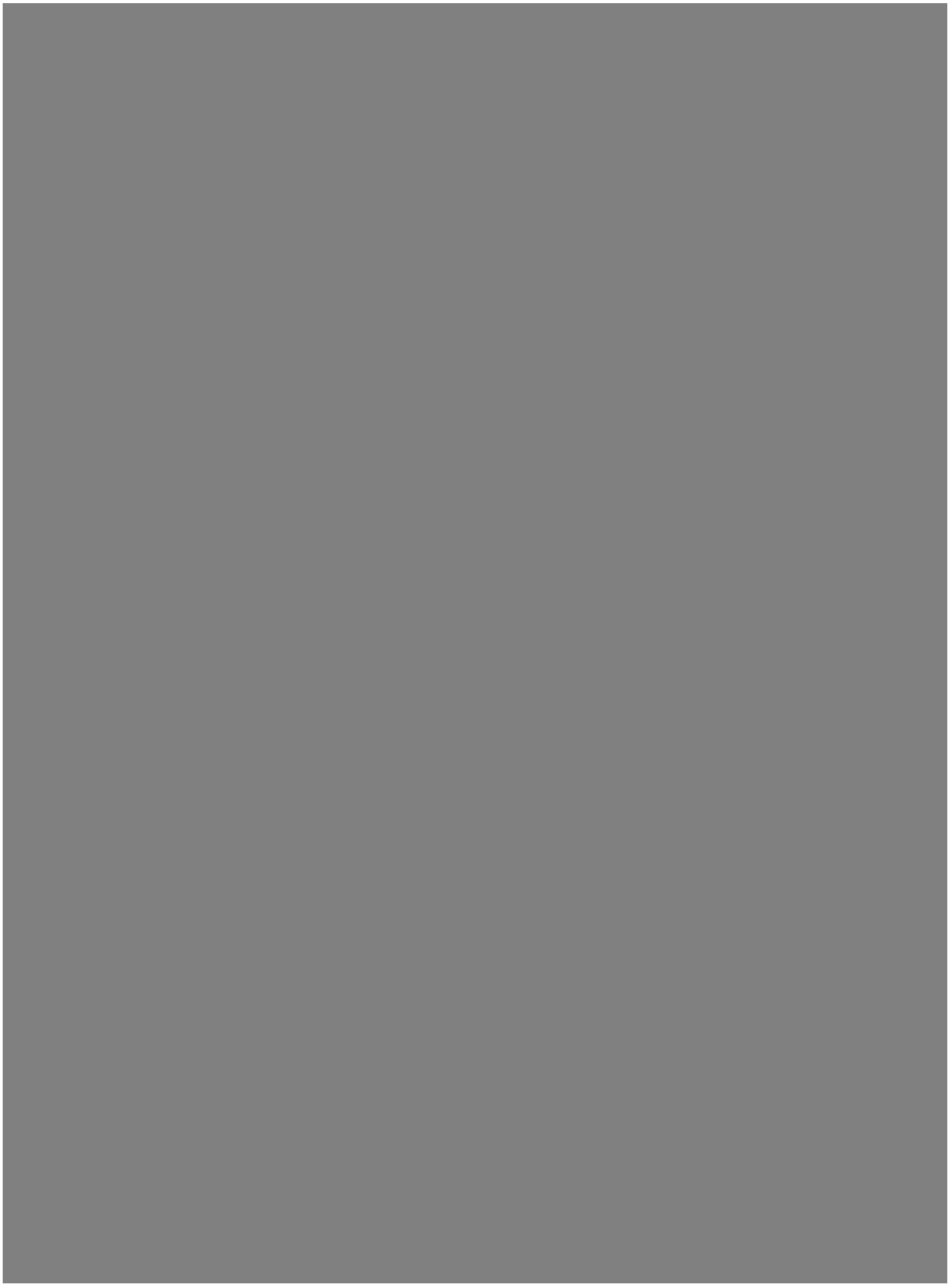




Section 6

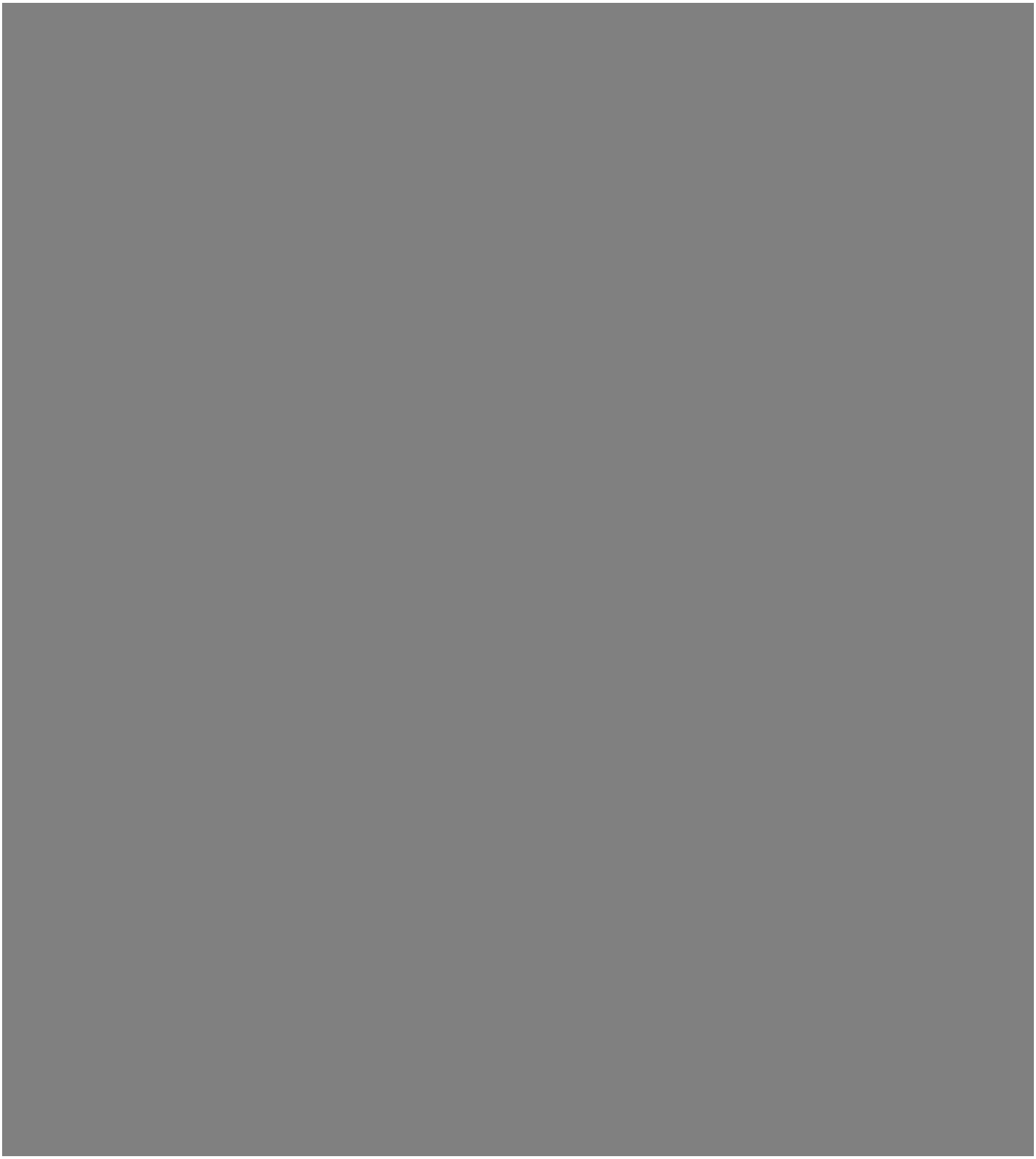
The next questions are designed to help us know how you feel in further detail.

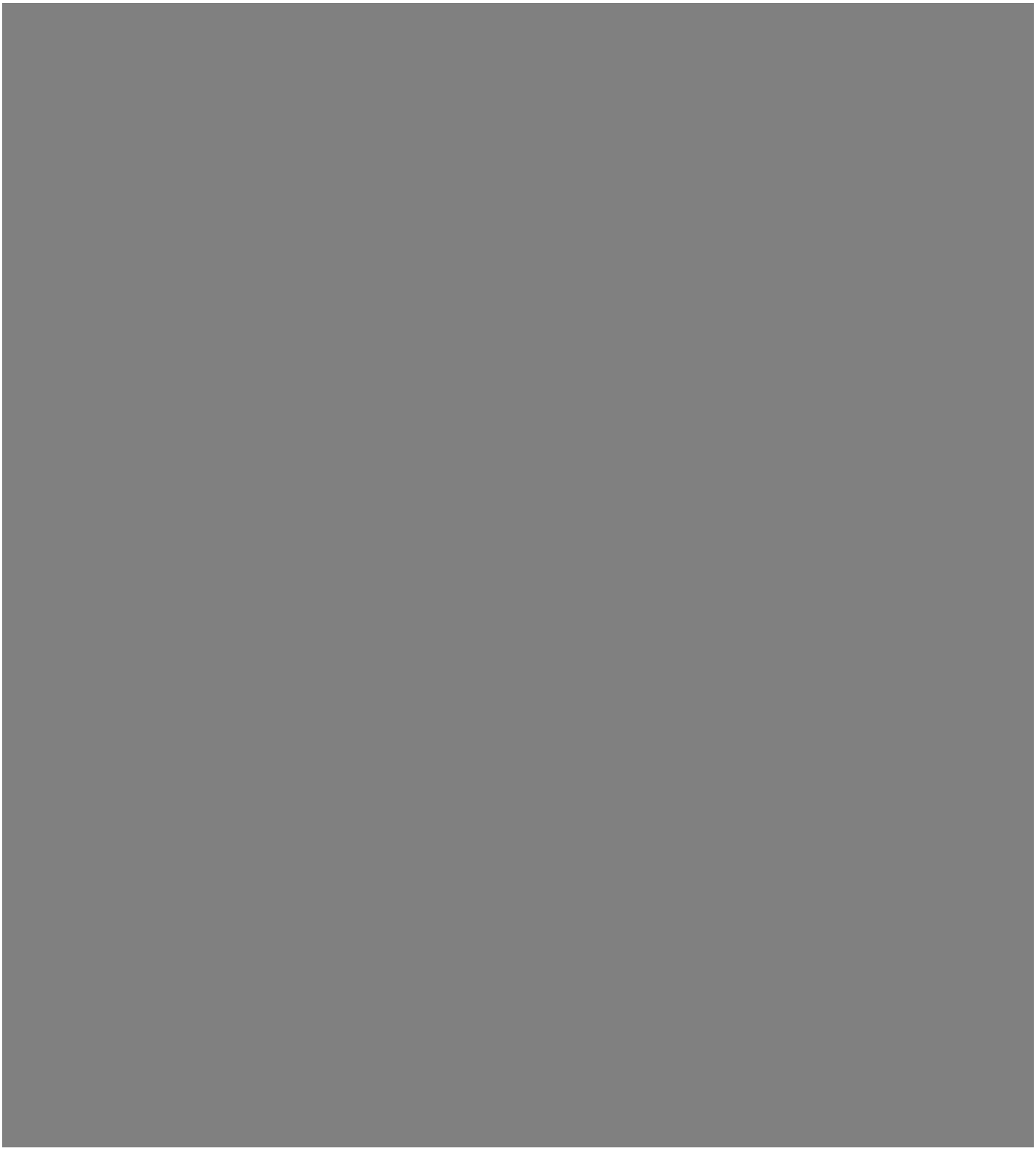




Section 7

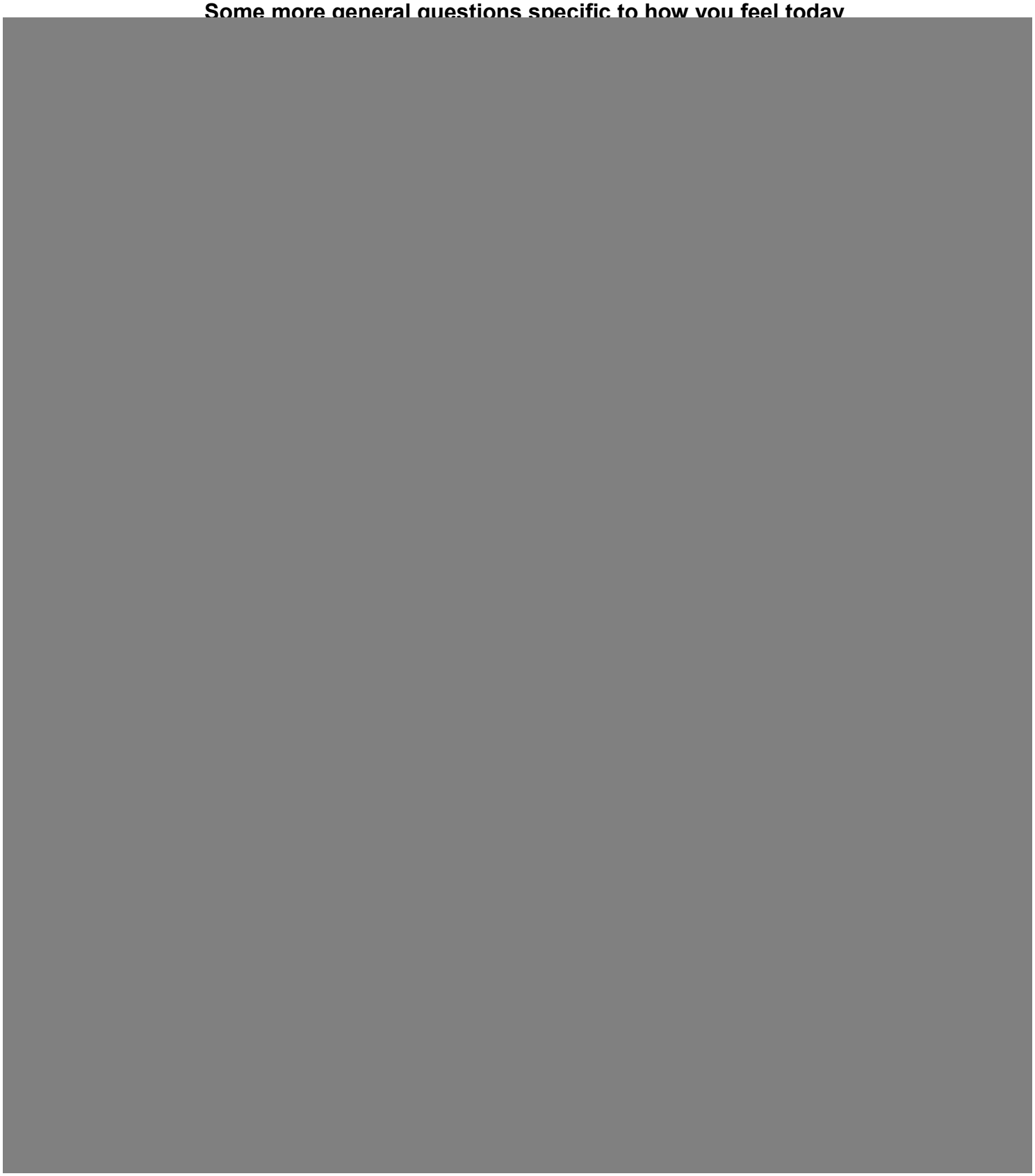
We would now like to ask you about the presence and severity of any ear or nose problems you may have experienced in the last 2 weeks.

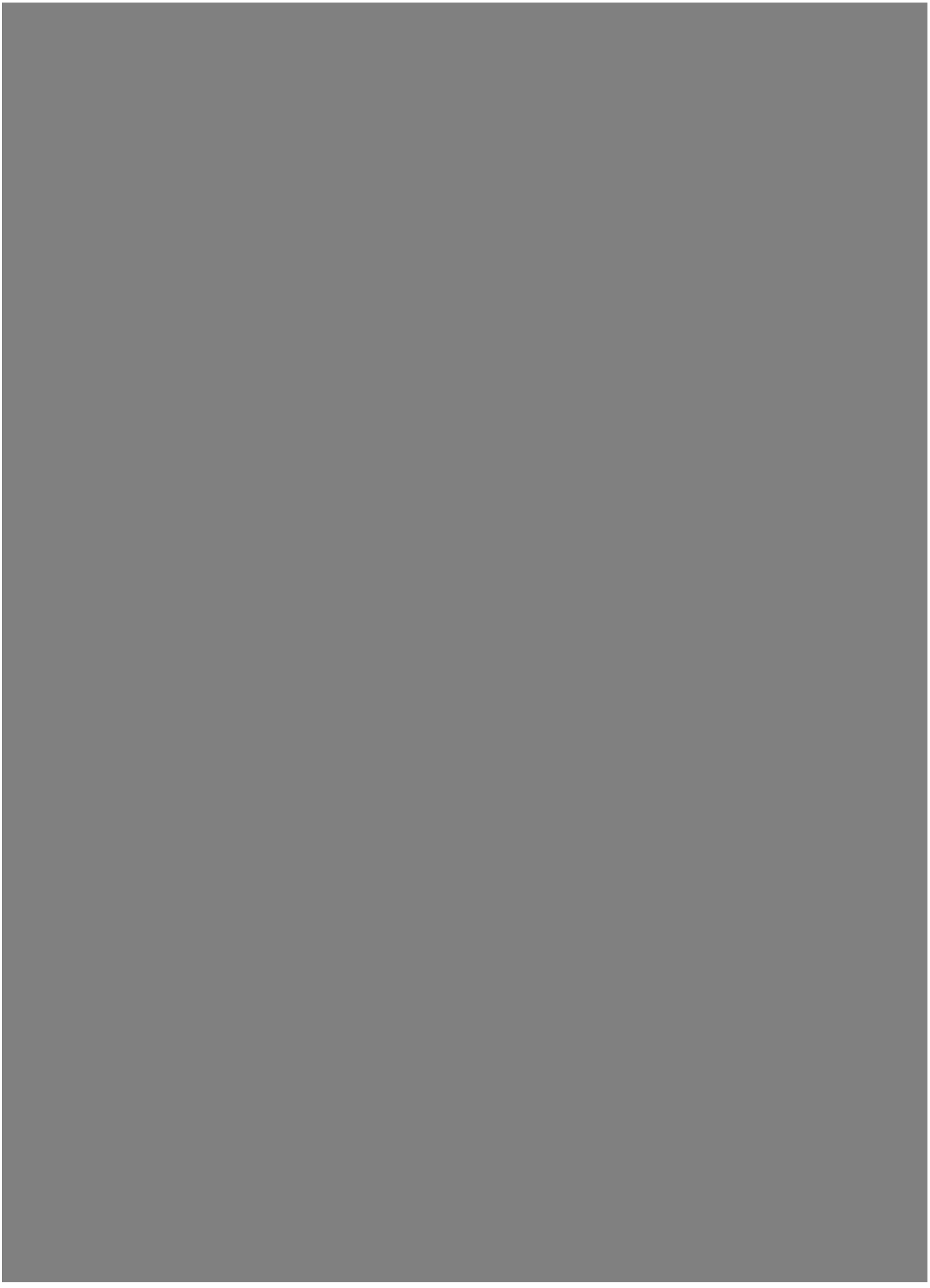




Section 8

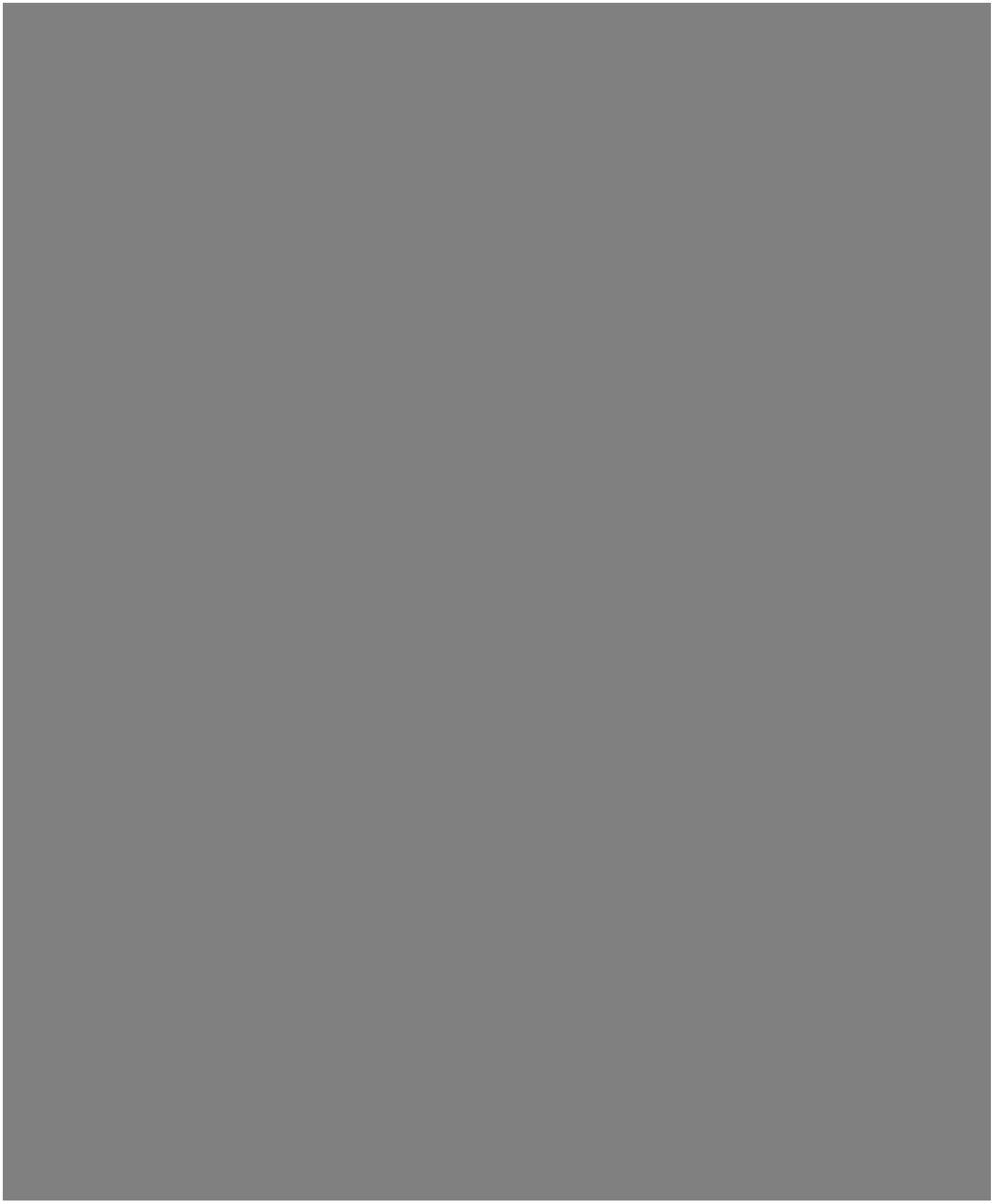
Some more general questions specific to how you feel today

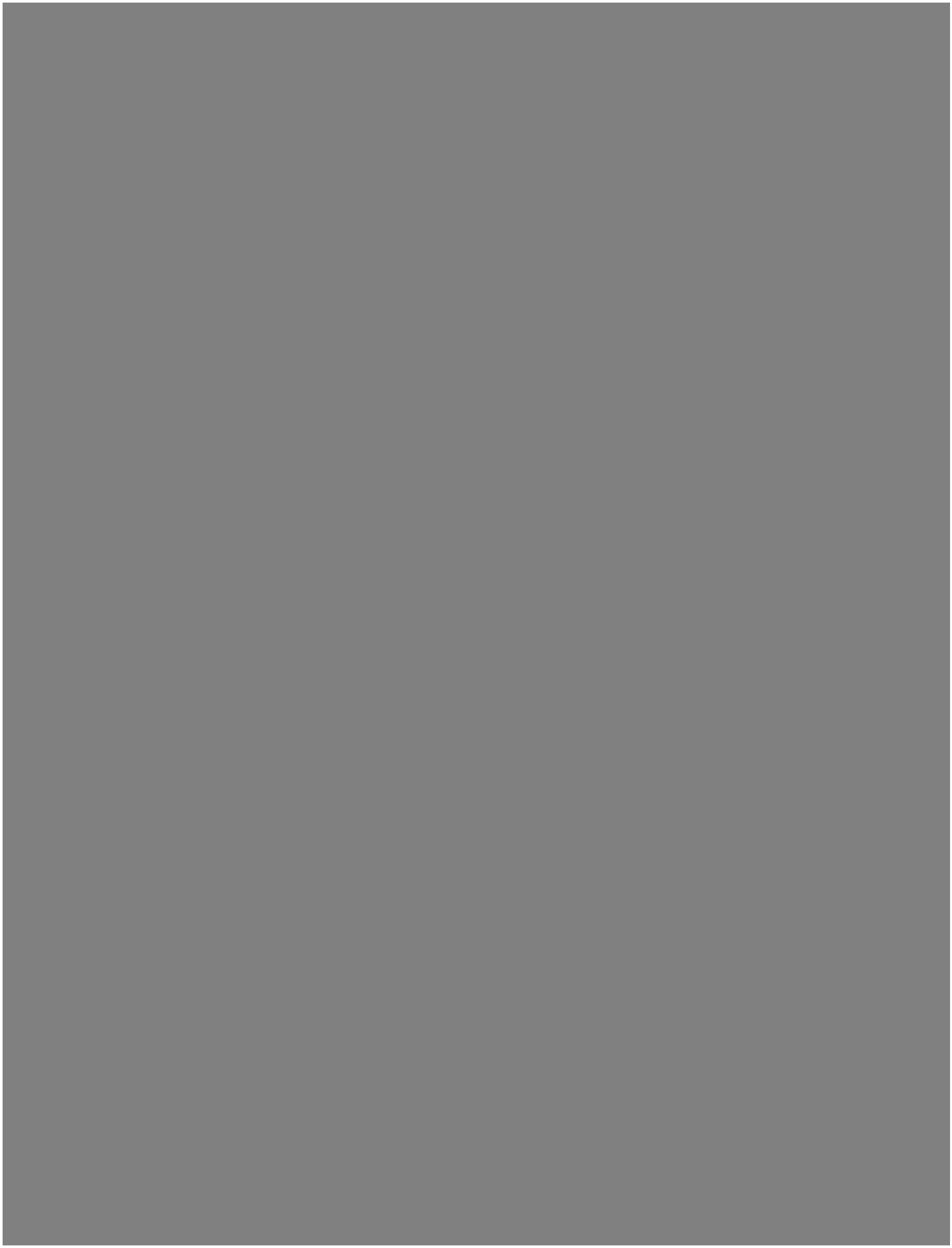


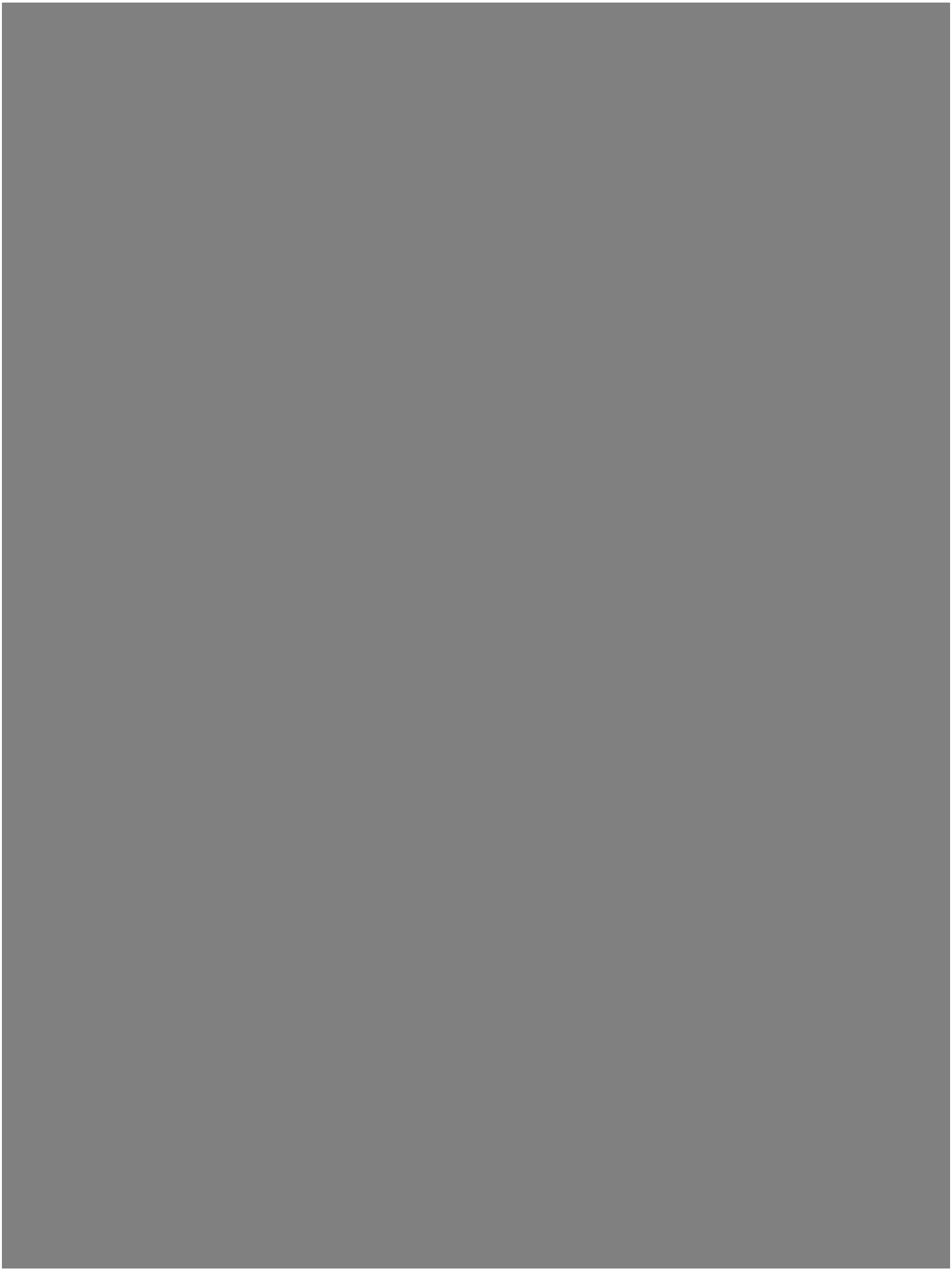


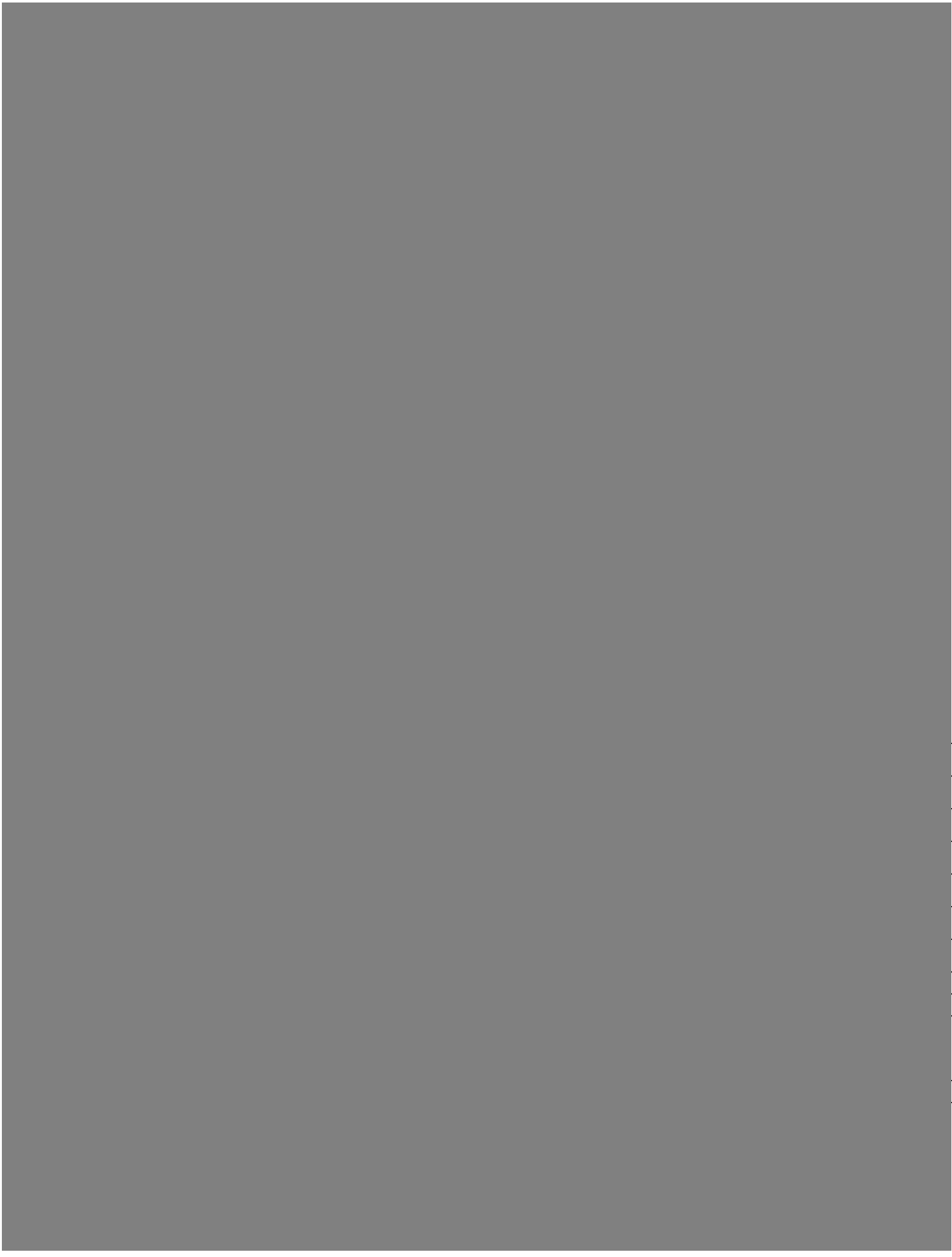
Section 9

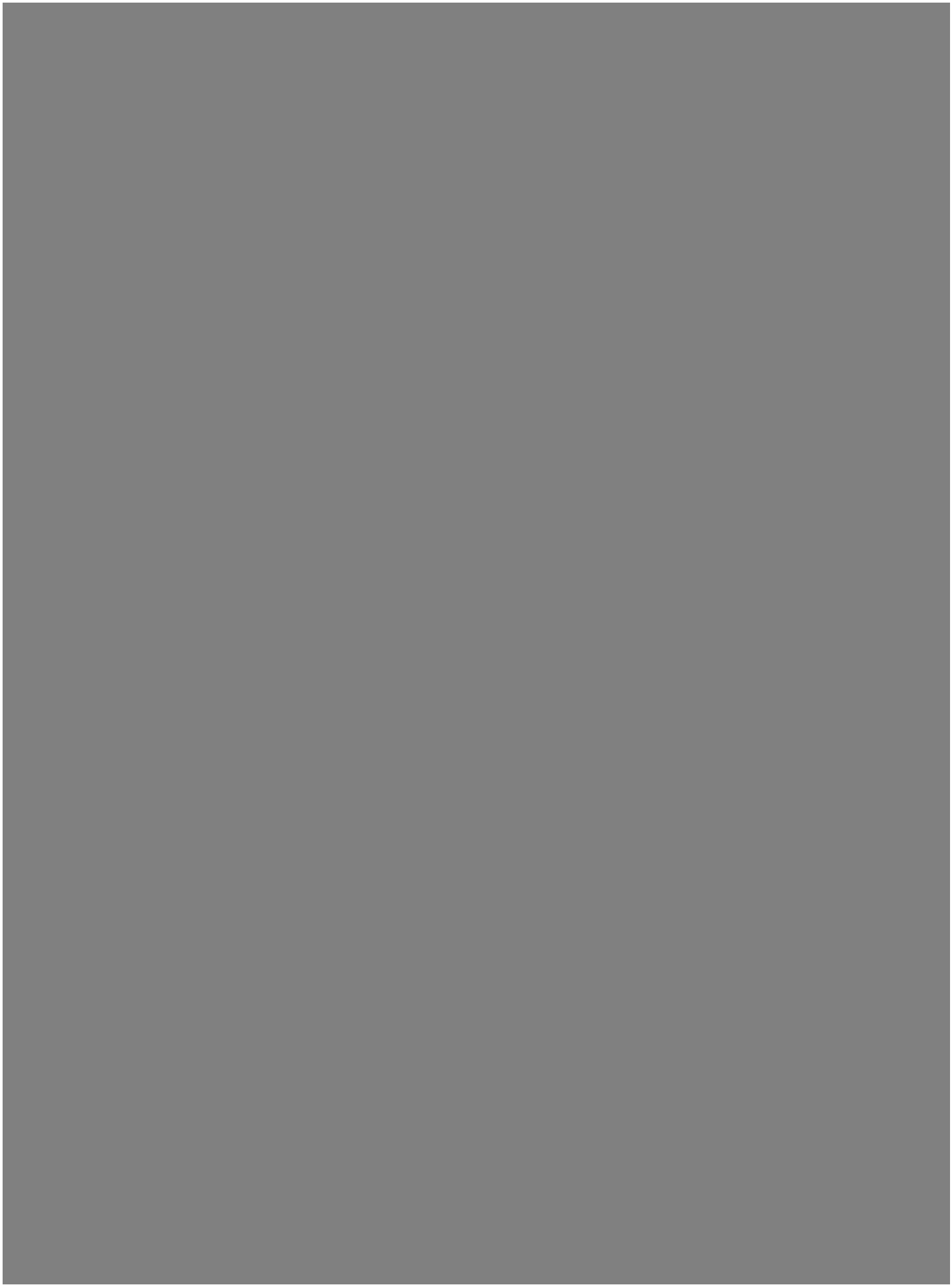
We would now like to ask some questions about sleep.

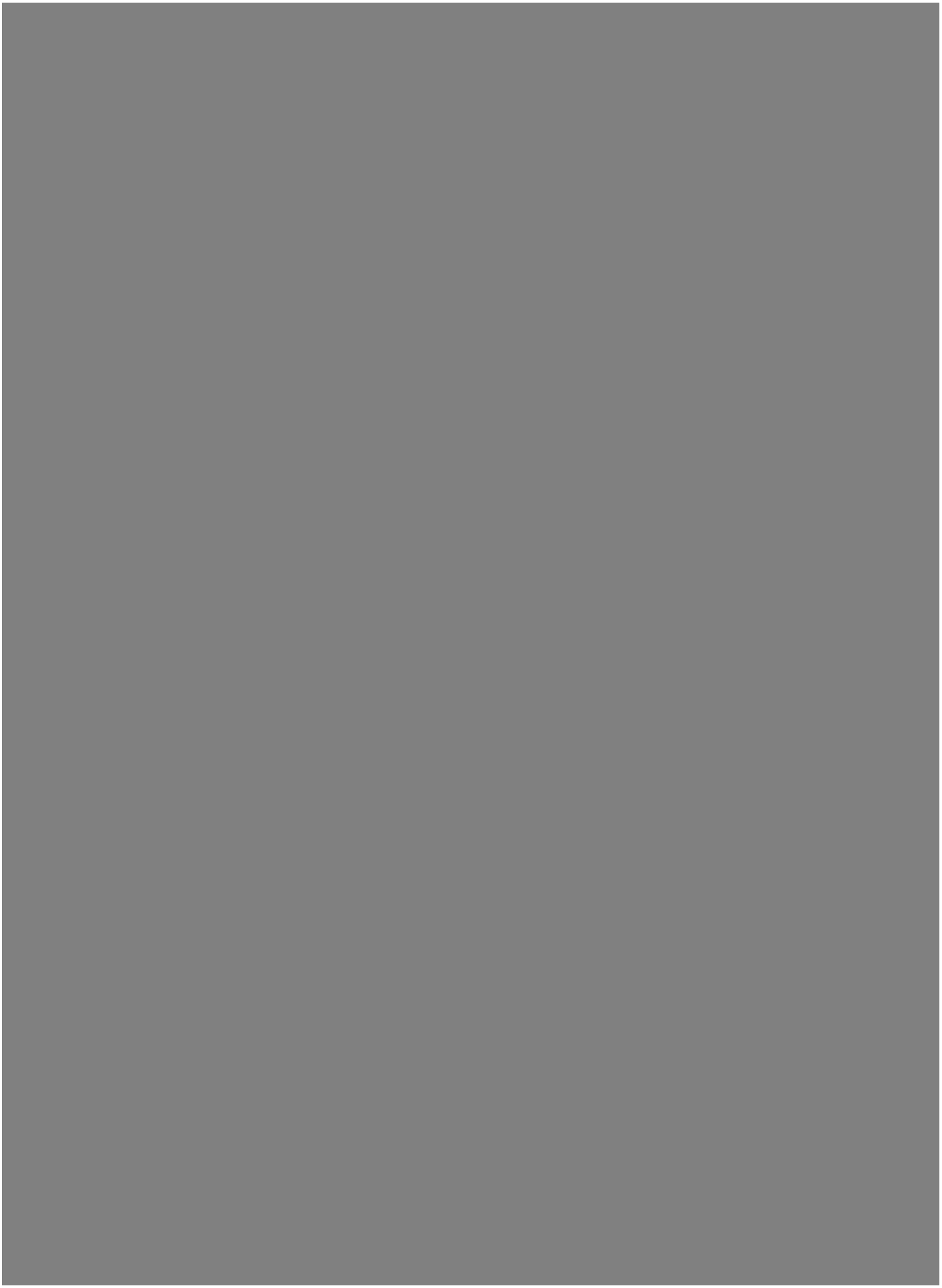












Thank you for taking the time to complete our questionnaire.

This information is very important and will help us to understand chronic fatigue in patients with vasculitis or chronic kidney disease.

When you return your questionnaire, we may contact you to ask if you are interested in helping with a second part of this study, in which we are investigating the causes of fatigue. If you are interested, we will send you an information booklet to help you decide whether or not to participate. You will be free to participate or not participate as you wish, with no impact on your care. Also, if you do decide to participate, you will be free to change your mind again at any time.

FINALLY, IF YOU HAVE A PARTNER, FAMILY MEMBER OR FRIEND OF ABOUT THE SAME AGE, BUT NOT SUFFERING VASCULITIS, CHRONIC KIDNEY DISEASE, OR ANY SERIOUS CHRONIC ILLNESS, WE WOULD BE GRATEFUL IF YOU COULD TALK TO THEM ABOUT THIS STUDY. PLEASE LET THEM READ THE INFORMATION LEAFLET WE HAVE ENCLOSED.
IF THEY ARE INTERESTED IN HELPING US WITH THE STUDY, WE WOULD ASK THEM EITHER TO JOIN YOU AT YOUR NEXT APPOINTMENT, OR TO CONTACT DR ANDREW MCCLEAN AT THE ADDRESS BELOW. (NOTE: ANY INFORMATION PROVIDED BY YOU, YOUR PARTNER, OR FAMILY MEMBER WILL BE KEPT STRICTLY CONFIDENTIAL).

Please put the questionnaire back into the stamped addressed envelope provided, and post it back to us as soon as is convenient.

Dr. Andrew McClean,
Department of Renal Immunobiology,
IBR West Extension,
The Medical School,
University of Birmingham,
Edgbaston,
Birmingham,
B15 2TT.

8 APPENDIX 2: PARTICIPANT INFORMATION LEAFLET FOR QUESTIONNAIRE STUDY (AAV GROUP)

Patient Information Sheet: Investigating Fatigue In People With Vasculitis

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. **One of our team will go through the information sheet with you and answer any questions you have**. This should take about five minutes. Ask us if there is anything that is not clear.

What Is The Study About?

Vasculitis is a group of diseases that may damage various parts of the body by disturbing the immune system and blood vessels. It commonly results in kidney damage known as 'chronic kidney disease'. Vasculitis causes about 10% of all chronic kidney disease. Some research has previously shown that patients with vasculitis feel less energetic than other people, get tired more easily, and cannot do as much physically or mentally. These symptoms, which we describe as 'fatigue' are very common, even when the vasculitis has been brought under control by medication.

Even though fatigue is one of the commonest symptoms in vasculitis, and can have a major impact upon sufferers' lives, we know very little about it. We are carrying out this study to better understand fatigue in vasculitis. We aim to discover how common the symptom is, how it impacts on peoples' lives, and how all of this compares to people with other sorts of chronic kidney disease, and with people who have neither disease. We hope that this study will help us to eventually become better at preventing or treating fatigue.

Why Have I Been Invited?

You have been invited because you have vasculitis.

Who Else Is Taking Part?

We intend to enrol 200 people from the Vasculitis Clinic, 200 people from the 'Chronic Kidney Disease Clinic', and 200 people from the kidney transplant clinic at the Queen Elizabeth Hospital. In addition, we will enrol an equal number of people

who have no vasculitis or CKD, and with your permission we may ask your family and friends whether they would like to help as part of this healthy group.

Do I Have To Take Part?

It is up to you to decide to join the study. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to refuse, without giving a reason. This would not affect the standard of care you receive.

What Will Happen To Me If I Take Part?

If you agree to help us with this study and have signed the consent form, we will ask you to complete a questionnaire booklet. This booklet should take around half an hour to complete. To help us understand the information, we will need your permission to look through your medical notes, and to take a record of your illnesses and medications.

In addition, we will take 5ml of your blood, which we will test for inflammation.

With your permission, we will share your anonymised results with other research groups who are carrying out similar research.

The study will run for around two years in total, during which time we hope to have enrolled the full 800 people. However you will only be asked to fill in the questionnaire on this one occasion.

Afterwards we may ask you whether you wish to be involved in another part of the study. The aim of the other part of the study is to identify what actually causes people with vasculitis to suffer from fatigue. However any involvement in that second part of the study is separate and is again entirely optional.

Payments

There will be no payment made for agreeing to help with this research.

Please note in particular that it will not be possible to reimburse any additional travel or parking expenses.

What Are The Risks And Benefits?

There are no risks involved in this study. Equally, there will be no immediate benefit to you from helping with this study. However, the results will help us to plan further

studies, and we hope that eventually they will help us to be much better at preventing and treating this significant problem.

Will My Taking Part In The Study Be Kept Confidential?

Yes. We will follow ethical and legal practice, and all information about you will be handled in confidence. With your permission we will inform your General Practitioner that you have helped us with this study, but they will have no access to the information you give us.

What Will Happen To The Results Of The Research Study?

It is intended that the results will be published in various scientific journals with an interest in vasculitis, chronic kidney disease, or fatigue. We will also seek to increase public awareness by speaking to patient groups such as the Stuart Strange Trust for Research into Vasculitis, and the West Midlands Vasculitis Group.

In addition, the results will form the basis for future research investigating how we might treat fatigue in vasculitis patients.

Who Is Funding The Research?

The research is being funded by University Hospitals Birmingham Charities. The doctors conducting the research are employees of University Hospital Birmingham NHS Foundation Trust. They will receive no direct payment for including you in this study, and there are no conflicts of interest.

Who has Reviewed this Study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests.

What if I have any Questions?

If you have any queries or concerns please ring [redacted] (Queen Elizabeth Hospital switchboard) and ask to be put through to extension [redacted] (Wellcome Trust Clinical Research Facility). One of the Research Nurses will be able to answer any of your queries and arrange a suitable appointment with Dr Andrew McClean.

9 APPENDIX 3: PARTICIPANT INFORMATION LEAFLET FOR MECHANISTIC STUDY (AAV GROUP)

Participant Information Sheet 2:

Investigating the Causes of Fatigue In People With Vasculitis

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. **One of our team will go through the information sheet with you and answer any questions you have.** This should take about five to ten minutes.

Part 1 tells you the purpose of the study, and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear.

Part 1

What Is The Study About?

Vasculitis is a group of diseases that may damage various parts of the body by disturbing the immune system and blood vessels. It commonly results in kidney damage known as ‘chronic kidney disease’. Vasculitis causes about 10% of all chronic kidney disease. Some research has previously shown that patients with vasculitis feel less energetic than other people, get tired more easily, and cannot do as much physically or mentally. These symptoms, which we describe as ‘fatigue’ are very common, even when the vasculitis has been brought under control by medication.

Even though fatigue is one of the commonest symptoms in vasculitis, and can have a major impact upon sufferers' lives, we still know very little about it. We are carrying out this study to better understand what factors contribute to causing fatigue in vasculitis, as well as how this compares to people with other sorts of chronic kidney disease, and to with people who have neither disease. We hope that this study will help us to eventually become better at preventing or treating fatigue.

Why Have I Been Invited?

You have been invited because you have vasculitis and have already helped us with the first part of this study.

Who Else Is Taking Part?

We intend to enrol 50 people from the vasculitis clinic to this part of the study, 50 people with 'Chronic Kidney Disease', and 50 people with kidney transplants. In addition, we will enrol 50 people who have no kidney disease or vasculitis, and with your permission we may ask your family and friends whether they would like to help as part of this healthy group.

Do I Have To Take Part?

It is up to you to decide to join the study. We will describe the study and go through this information booklet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What Will Happen To Me If I Take Part?

This part of the study will be conducted over two half day sessions, with different investigations occurring on each day. The order in which you undergo these two

different sets of investigations may differ, and will depend on finding you a convenient appointment for each.

Session 1: (approximately 4 hours) This will take place in the Wellcome Trust Clinical Research Facility, at the Queen Elizabeth Hospital. We will ask you not to eat or drink from midnight on the night before this appointment. The tests are as follows:

- Before doing anything else we would like to take a fasting blood sample, and will ask you to complete a very short questionnaire about how you are feeling, which should take no more than around a minute to fill in. Once you have had this blood sample taken, we will provide you with some breakfast, and will give you a short break before doing the next test.

- *Exercise test*: We will ask you to pedal on an exercise bike for nine minutes. The workload will start off very low and every three minutes it will be increased by a small amount. Each minute during the exercise, we will ask you how difficult the exercise feels. During all of this you will have 6 leads similar to ECG leads attached to stickers on your chest and back. These leads will measure your heart rate, as well as measuring the amount of blood your heart is pumping around your body. You will breathe into a rubber mouthpiece similar to a snorkel, which measures the gas in your breath, and will wear a nose plug so that all your breath passes in and out of the mouthpiece. Afterwards you can sit or lie down while you get your breath back and recover. We will take further blood tests immediately after the exercise test and then one and two hours later, and on each of these occasions we will also ask you to fill in the 1 minute questionnaire about how you are feeling.

- We would like to measure your body composition - that is, the amount of muscle, fat and bone in your body using an x-ray test called DEXA scanning. This will expose you to a minimal x-ray radiation. The radiation will be 0.0042 mSv, which is less than a chest x-ray or less than 0.2% of the annual dose from natural background radiation in the UK. In other words, it is less than a day of naturally occurring background radiation in the UK. All risks will be minimised by safe practice, and conducted by a trained NHS radiographer. This test will not involve any blood

tests or injections, and will not involve enclosed spaces that could induce claustrophobia.

Session 2: (approximately 2 hours) This will take place in the Wellcome Research Laboratories in the School of Sport and Exercise Sciences, University of Birmingham. The tests are as follows:

- *Muscle tests*: We will measure the strength of your thigh muscles while you sit in a specially designed chair. First of all we will place two pads onto one of your thighs, and will put a strap around the ankle of the same leg. We will use the pads to apply a small electrical stimulation to the muscles in your thigh. This only lasts for a fraction of a second, and although it can feel a little uncomfortable it is not painful. We will start with a very weak current, and will repeat with a slowly increasing current until the stimulation causes your muscle to contract well.

Next, we will do a test that it allows us to measure your muscle strength and assess the reasons for any weakness. We will ask you to kick your leg out against the strap as strongly as possible for about five seconds, and during your normal muscle contraction we will give another electrical stimulation for a fraction of a second. We will ask you to do this three times in total.

After a few minutes rest, we will perform a test that assesses how easily your muscles become tired. We will ask you to kick out your leg against the strap again, but this time we will ask you to hold the contraction for as long as you are able to. Every 10 seconds we will give a small electrical stimulus.

- *Blood Supply*: To perform the next test, you will lie on a bed, and we will place a device around your forearm which allows us to measure the oxygen levels in your muscles. We will inflate a blood pressure cuff around the same arm, and then ask you to squeeze a hand grip for 1 minute. This will make your forearm muscles feel tired and a little uncomfortable, as if you had done a lot of exercise. As soon as the minute has passed we will ask you to stop squeezing the hand grip, and we will deflate the blood pressure cuff. This will allow your muscles to recover from the exercise, and we will measure the time taken for the oxygen level in the muscles to get back to normal.

• To test your concentration and memory, we will ask you to do a test called the PASAT. This simple test will last for less than half an hour. During it, you will be played a CD through computer speakers. You will hear a series of numbers spoken, and will be asked to add up each pair of numbers. The process will be fully explained to you before we start, and you will also be given some practice before you begin.

Your involvement will end after you have attended each of the half day sessions.

Payments

There will be no payment made for agreeing to help with this research.

Please note in particular that it will not be possible to reimburse any additional travel or parking expenses.

What Will I have To Do?

Apart from attending for the two appointments detailed above, we will ask you to do three other things:

1. On the day before your appointment at the Queen Elizabeth Hospital (described as 'session 1' above) you will have to collect some saliva samples for us. We will give you the labelled bottles beforehand, and one of our Research Nurses will contact you the day before to remind you about the collections. We will ask you to collect five samples through the day: when you waken, and at 10am, noon, 3pm and 9pm. You will then bring these samples along to your appointment, when we will send them for testing.

2. When you are coming up for 'session 1', we will ask you to fast from midnight the night before. Once we have taken the first blood test, we will provide you with breakfast free of charge. When our nurse contacts you to remind you about the

saliva collections, she will also remind you that you will need to fast before the appointment.

3. On both test days, please come wearing loose fitting clothes, preferably shorts and T-shirt.

What Are The Possible Risks of Taking Part?

The assessments described in this section may sound a little technical but you should not worry about this, these assessments are routinely performed in our labs. Our investigators are friendly and experienced and will explain everything to you at the time, answer any questions that you may have and will only proceed when you are happy to do so.

The main potential risk is the exercise bike testing, which could put strain on your heart if you have heart disease. However we have taken a number of steps to minimise any risk to you:

- We will not allow you to undertake the test at all if we know that you have significant heart disease.

- We will only carry on with the test until the point where you ask to stop. We will never attempt to push you beyond your limits.

- The test will always be supervised by NHS staff who are trained in how to help if there are any problems.

- The test will be performed within the Queen Elizabeth Hospital, with full medical facilities onsite for dealing with any possible problems.

Taking the blood tests may result in a small bruise, but this will resolve over a few days. It will be no different from your experience of giving blood samples during your normal clinical care.

The electrical stimulation we will give you during the muscle testing is an unusual sensation but most people find it acceptable and you will be given plenty of time to

get used to it. We will always keep the intensity of the stimulation within the limits you are happy with. The measurement of muscle oxygen content is entirely without sensation.

At the end of the fatigue test you can sit or lie down for as long as you like to recover but we would expect your muscle to be fully recovered within about 10 minutes and you should have no problems or after-effects from the testing.

What Are The Possible Benefits Of Taking Part?

There will be no immediate benefit to you from helping with this study. However, the results will help us to plan further studies, and we hope that eventually they will help us to be much better at preventing and treating this significant problem.

What If There Is A Problem?

Any complaint about the way you have been dealt with during the study, or any possible harm will be addressed. The detailed information about this is given in Part 2.

Will My Taking Part In The Study Be Kept Confidential?

Yes. We will follow ethical and legal practice, and all information about you will be handled in confidence. The details are included in Part 2.

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.

Part 2

What Will Happen If I Don't Want To Carry On With The Study?

Remember that if you are unhappy with the way things are going you can withdraw from the study at any time without having to give an explanation, even if you have signed a consent form. Whether or not you decide to take part in this research, or if you withdraw having started, it will have no effect on the care and treatment you are receiving. If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

What If I Have A Complaint?

If you have any queries or concerns please ring [redacted] (Queen Elizabeth Hospital switchboard) and ask to be put through to extension [redacted] Wellcome Trust Clinical Research Facility). One of the Research Nurses will be able to answer any of your queries and arrange a suitable appointment. If you remain unhappy and wish to complain formally, you can do this through the normal NHS complaints procedure. Details can be obtained from the Patient Advice Liaison Service at the Queen Elizabeth Hospital (Tel: [redacted], or email: [redacted]).

What If I Suffer Harm?

In the event that something does go wrong and you are harmed during the research, and this is due to someone's negligence, then you may have grounds for a legal action against University Hospital Birmingham NHS Foundation Trust. However, you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Will My Taking Part In The Study Be Kept Confidential?

Yes. We will follow standard ethical and legal practice, and all information about you will be handled in confidence. All the data collected will be stored securely in locked filing cabinets, in rooms with locked doors. The results of your investigations will also be kept on computer; the computers will be physically stored behind locked doors, and the data on them will be protected by passwords. All data stored will have your name and address removed so that you cannot be recognised, and only one member of the research team will have the means to link the data to you. The anonymous parts of the data will be stored for three years after the study, so that it can be used to help in further linked studies. However, any such studies will also have to go through detailed assessment by a Research Ethics Committee. Once this period is up, the data will be destroyed securely by shredding it physically and electronically.

With your permission we will inform your General Practitioner that you have helped us with this study, but they will have no access to the information you give us.

What Will Happen To The Samples I Give?

Blood and saliva samples will be taken during this study, as previously described. The blood samples that we take from you will be used for this study, but they will also be stored beyond the end of the study. We may wish to make use of them again for future studies, but we will apply to the relevant authorities for approval if we intend to do this.

The samples will be sent securely to the on-site laboratory for processing. During the short time that they are to be stored, they will be held securely, protected from theft by a locked door. The electronic results of your samples will again have your personal details removed before they are added to the research data, and will be treated in the same manner as all the rest of the data, as described above. Afterwards the samples will be destroyed in accordance with normal NHS standards.

What Will Happen To The Results Of The Research Study?

It is intended that the results will be published in various scientific journals with an interest in vasculitis, chronic kidney disease, or fatigue. We will also seek to increase public awareness by speaking to patient groups.

In addition, the results will form the basis for future research investigating how we might treat fatigue in patients with CKD.

Who Is Funding The Research?

The research is being funded by University Hospital Birmingham Charities. The doctors conducting the research are employees of University Hospital Birmingham NHS Foundation Trust. They will receive no direct payment for including you in this study, and there are no conflicts of interest.

Who Has Reviewed This Study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by Birmingham, East, North and Solihull Research Ethics Committee.

Further Information and Contact Details

If you have any queries or concerns please ring [redacted] (Queen Elizabeth Hospital switchboard) and ask to be put through to extension [redacted] (Wellcome Trust Clinical Research Facility). One of the Research Nurses will be able to answer any of your queries, or arrange a suitable appointment with Dr Andrew McClean (the doctor coordinating the study) if necessary.

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