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Fatigue and inflammation
A psychoneuroimmunological approach o chronic fatigue

Russell, Alice Elizabeth

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**FATIGUE AND INFLAMMATION:
A PSYCHONEUROIMMUNOLOGICAL APPROACH
TO CHRONIC FATIGUE**

By

Alice E. Russell

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degree of Doctor of Philosophy at King's College London

Department of Psychological Medicine

Institute of Psychiatry, Psychology and Neuroscience

King's College London

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Abstract

Chronic Fatigue Syndrome (CFS) is characterised by severe fatigue, endured for at least six months, together with symptoms including impaired cognitive function, sleep disturbance, and musculoskeletal pain. The pathogenesis is still unknown, resulting in a lack of treatment options and the stigmatization of patients. Both psychological and biological factors have been implicated in the development of CFS. To date, evidence has come largely from cross-sectional studies, and there have been a paucity of longitudinal studies.

The aim of this study was to explore interferon-alpha (IFN- α) induced persistent fatigue as a proxy model of CFS. IFN- α is an immunotherapy for chronic Hepatitis C Virus (HCV) infection. It induces a range of side effects including fatigue, which in some patients persists post-treatment. This model allows for the identification of risk factors and monitoring of biological and behavioural changes from the perspective of the trigger, to determine factors relevant to the persistence of fatigue after the original stimulus is no longer present. Fifty-five patients undergoing IFN- α treatment for chronic HCV were assessed at baseline, during treatment, and six-months post-treatment. Clinical, inflammatory and cortisol measures were obtained. Fifty-four CFS patients and 57 healthy volunteers completed the same measures at a one-off assessment, which were compared with post-treatment measures from HCV persistent and resolved fatigue patients.

IFN- α induced persistent fatigue was associated with an exaggerated response to IFN- α , with increased fatigue, depressive symptoms, and perceived stress, a greater decline in health status, and higher inflammation. This higher symptomatology during treatment put these patients at a disadvantage for their subsequent recovery. Neither IFN- α induced persistent fatigue nor CFS was associated with continued peripheral inflammation, emphasising the importance of the response to the initial trigger. Future studies are needed to elucidate the mechanisms behind the exaggerated response, and the 'conversion' to chronic illness in the absence of peripheral immune activation.

Publications related to this thesis

Hepgul, N., Pariante, C. M., Baraldi, S., Borsini, A., Bufalino, C., **Russell, A.**, ... Hotopf, M. (2016). Depression and anxiety in patients receiving interferon-alpha: The role of illness perceptions. *Journal of Health Psychology*, 1-10. DOI: 10.1177/1359105316658967.

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Baumeister, D., **Russell, A.**, Pariante, C. M., & Mondelli, V. (2014). Inflammatory biomarker profiles of mental disorders and their relation to clinical, social and lifestyle factors. *Social Psychiatry and Psychiatric Epidemiology*, 49(6), 841–9. doi:10.1007/s00127-014-0887-z

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Abbreviations

Abbreviation	Meaning
3-HK	3-Hydroxykynurenine
3-HK/Kyn	Ratio of 3-Hydroxykynurenine to Kynurenine
ACTH	Adrenocorticotrophic hormone
ALT	Alanine Aminotransferase
ANOVA	Analysis of variance
AUCg	Area Under the Curve with respect to the ground
AUCi	Area Under the Curve with respect to the increase
AUDIT	Alcohol Use Disorders Identification Test
BBB	Blood Brain Barrier
BCAA	Branched Chain Amino Acids
BH4	Tetrahydrobiopterin
BLE	Brief Life Events (questionnaire)
CAA	Competing Amino Acids
CAR	Cortisol Awakening Response
CBSQ	Cognitive and Behavioural responses to Symptoms (questionnaire)
CDC	Centers for Disease Control and prevention
CECA-Q	Childhood Experience of Care and Abuse Questionnaire
CF	Chronic Fatigue
CFQ	Chalder Fatigue Questionnaire
CFS	Chronic Fatigue Syndrome
CHT	Childhood trauma
CNS	Central Nervous System
CRH	Corticotrophin Releasing Hormone
DAA	Direct Acting Antiviral
DIOS	Dubbo Infection Outcomes Study (cohort)
EBV	Epstein-Barr Virus
FU	Follow-up (six-months post-treatment)
HADS-A	Hospital Anxiety and Depression Scale – Anxiety subscale
HCC	Hepatocellular
HCV	Hepatitis C Virus
HPA	Hypothalamic-Pituitary-Adrenal (axis)
hsCRP	High-sensitivity C-Reactive Protein
IDO	Indoleamine 2,3 dioxygenase
IDS	Inventory of Depressive Symptomatology
IFN- α	Interferon-alpha
IFN- γ	Interferon-gamma
IL-	Interleukin-
ILES	Intrusive Life Events Schedule
IM	Infectious Mononucleosis
IPQ	Illness Perceptions Questionnaire

Abbreviation	Meaning
JAK	Janus-activated kinase
kPA	Kilopascal pressure unit
KYN	Kynurenine
Kyn acid / KYN-A	Kynurenic acid
KYN/TRP	Ratio of Kynurenine to Tryptophan
LNAA	Large Neutral Amino Acids
ME	Myalgic Encephomyelitis
MINI	Mini International Neuropsychiatric Interview
MRS	Magnetic Resonance Spectroscopy
NMDA	N-methyl-D-aspartate (receptor)
Peg	Pegylated
PET	Positron Emission Tomography
PF	Persistent Fatigue
PHE	Public Health England
PIC	Picolinic Acid
PIFS	Post-infective Fatigue Syndrome
PNI	Psychoneuroimmunology
PR	Pegylated-IFN- α /Ribavirin (combination therapy)
PSS	Perceived Stress Scale
PVFS	Post-viral Fatigue Syndrome
PWID	People Who Inject Drugs
Quin Acid / QUIN	Quinolinic acid
Quinald Acid	Quinaldic acid
RF	Resolved Fatigue
RNA	Ribonucleic Acid
RVR	Rapid Virological Response (to IFN- α treatment)
SEID	Systemic Exertion Intolerance Disease
SEM	Standard Error of the Mean
SF-36	Short-Form 36 (Medical Outcomes Survey)
sIL-6R	Soluble Interleukin-6 Receptor
Sim	Simeprevir
SNP	Single Nucleotide Polymorphism
SSRI	Selective Serotonin Reuptake Inhibitors
STAT	Signal Transducers and Activators of Transcription (STAT)
sTNF-R2	Soluble Tumour Necrosis Factor Receptor 2
SVR	Sustained Virological Response (to IFN- α treatment)
Tel	Telaprevir
TGF- β	Transforming Growth Factor-beta
TNF- α	Tumour Necrosis Factor-alpha
TRP	Tryptophan
TW	Treatment week
VEGF	Vascular Endothelial Growth Factor
XAN	Xanthurenic acid

1 Introduction

Here I will first introduce Chronic Fatigue Syndrome (CFS), summarising the symptoms on which the diagnosis is based, characteristics of the condition, and the burden it places on society and patients themselves. I will also give a brief synopsis of current debates surrounding the condition, relating to nomenclature and aetiology. Both biological and psychological factors have been implicated in the pathogenesis of CFS; I will review research concerning the immune and neuroendocrine systems, as well as some psychological factors thought to be involved. I will briefly introduce studies of Post-infective Fatigue Syndrome (PIFS), and their contribution to the field. Such studies have allowed for the identification of risk factors, and the study of longitudinal changes in those who recover from infectious diseases versus those who continue to experience persistent symptoms. The insights gained from these studies have inspired this thesis, and I will introduce here a novel, proxy-model of CFS based on Interferon-alpha (IFN- α) induced persistent fatigue.

IFN- α is an immunotherapy for the treatment of Chronic Hepatitis C Virus infection (HCV). It is effective for treating the virus, but induces a wide range of debilitating side effects, including fatigue. While the majority of patients recover soon after treatment, around 40% of patients continue to experience persistent fatigue post-treatment. I will provide further information about HCV, the treatments prescribed and the effects that IFN- α administration has on patients. I will then summarise research related to the predictors of IFN- α induced fatigue, as well as some of the mechanisms explored for IFN- α induced acute fatigue, which may provide insights into those underlying the persistence of fatigue also. Finally, I will summarise the aims and hypotheses for this thesis.

1.1 What is Chronic Fatigue Syndrome?

Chronic Fatigue Syndrome (CFS) is a debilitating illness, characterised predominantly by severe mental and physical fatigue not relieved by rest. Endured for at least six months, such fatigue is accompanied by a host of other symptoms, including cognitive impairment, flu-like symptoms, musculoskeletal pain, and sleep disturbances (see Figure 1.1) (Fukuda et al., 1994).

A recent report by the National Institutes of Health (NIH) in the United States concluded that accurate estimates of the number of individuals affected by the illness have been hampered by the lack of a universally accepted case definition (Green et al., 2015). However, a recent report from the charity the ME Association estimated that 2-4 per 1000 adults are affected, translating to around 150,000-250,000 UK residents (Shepherd & Chaudhuri, 2016). It occurs more often in women, with a ratio of around 2:1 female to males. Cases of CFS have been reported worldwide across ethnicities (Jason, 1999), though both U.S and U.K studies have highlighted an under-representation of ethnic minorities and those on low incomes in tertiary care versus primary care and community samples (Dinos et al., 2009; Euba et al., 1996; Jason et al., 2003).

The mean economic burden of CFS patients recruited through primary care services alone in the UK has been estimated to be £1406 per patient for a three-month period (McCrone et al., 2003). Concerning the level of disability caused, a recent study found that patients with CFS report a greater impact on wellbeing and functioning than reported in other chronic health conditions, including cancer (Nacul et al., 2011).

The Centers for Disease Control and Prevention (CDC) (1994)

Diagnostic Criteria:

- *The individual has severe chronic fatigue for 6 or more consecutive months that is not due to on-going exertion or other medical conditions associated with fatigue (these other conditions need to be ruled out by a doctor after diagnostic tests have been conducted)*
- *The fatigue significantly interfered with daily activities or work*
- *The individual concurrently has four or more of the following symptoms:*
 - *Post exertional malaise lasting more than 24 hours*
 - *Unrefreshing sleep*
 - *Significant impairment of short-term memory or concentration*
 - *Muscle pain*
 - *Pain in the joints without swelling or redness*
 - *Headaches of a new type, pattern or severity*
 - *Tender lymph nodes in the neck or armpit*
 - *A sore throat that is frequent or recurring*

These symptoms persisted or recurred during 6 or more consecutive months of illness and they cannot have first appeared before the fatigue

Figure 1.1 The CDC Diagnostic Criteria for CFS (Fukuda et al., 1994)

Since CFS is still medically unexplained, the diagnosis is one of exclusion, with patients required to have an extensive range of tests to rule out other conditions which may explain their symptoms before a diagnosis is made. Based solely on symptoms, there are at least five sets of diagnostic criteria. The so-called Fukuda (CDC) criteria described above are still the most used for research, while the Oxford criteria are often used in clinical settings in the UK (Sharpe et al., 1991).

As with other chronic health conditions, the degree of symptoms, specific symptom profiles, and the degree of fluctuation of symptoms varies among individuals. There is a campaign by patients and others to rename the condition, to better reflect the nature of the illness. Indeed, this was considered in the recent U.S. Institute of Medicine report *“Beyond Myalgic Encephalomyelitis/Chronic Fatigue Syndrome – Redefining an Illness”* (2015). The report suggested instead a name of “Systemic Exertion Intolerance Disease” or “SEID”. The term Chronic Fatigue Syndrome is generally preferred by the medical community owing to the lack of medical explanation about cause. However, patients who feel that the name undermines the seriousness and medical nature of their condition often reject it. It is argued that the alternative, Myalgic Encephalomyelitis, or “M.E.”, does not accurately reflect the known pathology (Holgate et al., 2011). However, this too is a subject of great debate and recently an effort was made to better characterise M.E. based on more up to date evidence, through the development of international consensus criteria (Carruthers et al., 2011). Some researchers have argued that M.E. and CFS overlap, but may be slightly different conditions, according to symptoms and/or original criteria (Jason et al., 2013; Maes, Twisk, & Johnson, 2012). Since at the present time the new name (SEID) has not been universally

adopted, for the purpose of this thesis, I will use the term “Chronic Fatigue Syndrome” or the acronym “CFS” throughout.

With regard to prognosis, a meta-analysis found a median “full recovery” rate of 7%, and 39.5% for improvement, though the authors note the heterogeneity across studies (Cairns & Hotopf, 2005). Patients with symptoms at the milder end of the spectrum may have symptoms that, while profoundly affecting their ability to lead a normal life, do not prevent them from working full-time. At the other end of the spectrum are those defined as ‘severely affected’, who experience periods, sometimes incredibly long, of being house- or even bed-bound, and may report other atypical symptoms such as double vision, blackouts, atypical seizures and loss of speech/swallowing (see Shepherd & Chaudhuri, 2016). In any case, the illness is most often a fluctuating one that leaves patients unable to set short- or often long-term goals.

1.2 The pathogenesis of CFS

Despite the known burden for both patients and wider society, and a growing body of research in the last 25 years, the pathogenesis is still unknown. This not only presents a problem for understanding and treating the illness, but also contributes towards the stigmatization of patients unable to fully explain their symptoms. Peers and employers are bad enough; worse still are anecdotes of doctors who remain unwilling to recognise the condition (Holgate et al., 2011). In the UK, the National Institute for Health and Care Excellence (NICE) guidelines for the management of CFS in the NHS were last updated almost a decade ago in 2007; a review in 2014 found that a lack of significant developments meant no changes would be made until at least 2017. The main

treatments recommended are based on cognitive-behavioural and physiotherapy techniques targeting coping with and management of the illness, and some factors thought to perpetuate symptoms. Clinical trials of pharmacological interventions have taken place, and others are on-going; however, the guidelines state that aside from generally prescribed medications for sleep, mood and pain, there is insufficient evidence for any particular pharmacological intervention aimed at treating CFS (National Institute for Health and Clinical Excellence, 2007).

Though the aetiology is unclear, theories implicating both psychological and biological processes have been proposed, with a differing emphasis on each, described by Maes and colleagues as '*biopsychosocial*' versus '*bio(psychosocial)*' (Harvey & Wessely, 2009; Maes & Twisk, 2010). This too is a source of great debate among patients, researchers, and healthcare providers, though underlying both efforts is an emphasis on the link between, rather than the dichotomy of, mind and body, which still persists and is often misunderstood. The overarching criteria have undoubtedly been key in advancing the field of CFS research. However, noting the likely multiple causes, prominent scientists in the field have highlighted the need for larger studies which first establish sub-phenotypes of the condition, before the pursuit of associated pathways and finally relevant therapeutics which may target them (Holgate et al., 2011). An increasing effort is being put into the phenotyping of patients using clinical and genomic approaches (Collin et al., 2016; Vollmer-Conna et al., 2006; Zhang et al., 2010). These strategies aim to benefit research, and to move towards a more personalised approach to treatment to improve outcomes for patients.

1.3 The immune system and CFS

1.3.1 Introduction

There is much evidence connecting CFS with the immune system, and of some degree of continued immune dysfunction. Some of the key findings in this regard come from evidence showing that CFS often follows an initial infective trigger (Hickie et al., 2006). For example, a strong link has been observed with infectious mononucleosis (IM), also known as glandular fever, often caused by the Epstein-Barr Virus (EBV; (White et al., 2001). An increasing body of research has pointed to the role of polymorphisms in genes relevant to the immune response, including functional polymorphisms shown to result in the increased production of cytokines in response to a particular trigger. Moreover, a more intense initial response has been linked to the triggering of related pathways involved in nitrosative and oxidative stress processes, and in turn, and also independently, more prolonged symptoms (Morris et al., 2016). However, further work is needed to fully elucidate how an acute infective trigger results in such a chronic, disabling condition in some, but not all patients (Holgate et al., 2011). Finally, the overlap between CFS symptoms and those observed in the context of inflammatory conditions, as well as so-called 'sickness behaviours' in response to infection and the experimental or therapeutic induction of inflammation, also point towards a role for the immune system. To date, a great deal of the evidence has come from cross-sectional case-control studies, or a few shorter longitudinal studies measuring inflammatory or related markers in CFS patients at different stages. Studies have been also been conducted which prospectively monitor new cases of viral or bacterial infection, to examine characteristics and changes which may identify those at greater risk of developing post-infective CFS.

Here I will summarise research findings related to inflammatory markers measured in CFS patients. I will also highlight the relevance of 'sickness behaviours' that occur in response to injury or infection; the overlap between the behaviours and CFS symptoms adds to the evidence for immune dysfunction in CFS. I will then introduce key cohort studies of Post-infective Fatigue Syndrome (PIFS). Such studies allow for the tracking of new cases of infectious disease to the development of PIFS, a syndrome overlapping with CFS. Lastly, I will summarise findings concerning the related kynurenine pathway. Activated following increases in inflammation, this pathway has been implicated in the manifestation of symptoms associated with CFS. Taken together, this evidence forms the basis of the core aim of this thesis, to explore an alternative, proxy-model of Chronic Fatigue Syndrome.

1.3.2 Elevated inflammatory markers in CFS

Evidence for the role of the immune system in CFS can be found in studies revealing raised inflammatory markers, most notably cytokines. For example, cytokines such as interleukin (IL)-6, IL-1 (alpha/beta) and tumour necrosis factor (TNF)- α have been shown to be elevated in these patients, both in serum and also cerebrospinal fluid (Maes, Twisk, & Ringel, 2012; Silverman et al., 2010). An acute phase reactant originating in the liver, high-sensitivity C-reactive protein (hsCRP) has also been shown to be elevated in CFS patients (Raison et al., 2009; Spence et al., 2008). Notably, Raison and colleagues found the marker to be raised in both chronic and mild fatigue, suggesting an association with fatigue as a symptom as opposed being specific to CFS.

Most recently, differences in cytokine patterns have been found between patients according to illness characteristics. For example, levels of IL-1 β were lower in those with severe illness, but other markers including IL-6, IL-7, IL-8 and IFN- γ were increased in the severely affected versus those with a moderate illness and healthy volunteers. In this case the authors determined severity based on measures of functioning, predominantly related to mobility and occupational function, and measures were obtained from serum samples. Ethnicity was not mentioned, but the sample was Australian (Hardcastle et al., 2015). Another group found no association between cytokine levels and symptom severity when measured as a continuous variable, suggesting that the method applied to measure disease severity is important in determining biological phenotypes. In addition, measures were obtained from plasma, and the sample was ethnically diverse, and from sites across America (Hornig et al., 2015).

Working on data collected from two large multi-centre cohort studies, the authors of the American study did however find that CFS patients had distinct plasma immune signatures, but only if cases were of relatively recent onset; patients who had been ill for less than three years had evidence of activation of pro- and anti-inflammatory cytokines, whereas those with a longer duration of illness did not. The same study observed that some markers were actually lower in those with a longer duration of illness versus healthy controls, including IFN- γ , IL-1 β , IL-4, IL-6, IL-8 and IL-17A (Hornig et al., 2015). Additional work is planned to examine differences in longitudinal changes in inflammatory markers between these categories of illness duration in the same cohorts.

Another recent study, which also explored differences in cytokine patterns according to the duration of illness also found an effect on cytokine levels, this time based on a cut-off of two years. Early onset patients had higher IL-1 α , replicating the earlier finding, while IL-6 and IL-8 were higher in those who had been ill for more than 2 years, in contrast to Hornig and colleagues' reports (Russell et al., 2016). While both in plasma, each group used different assays. In addition, though this was an American sample, there was no mention of the ethnicity of participants. The difference in cut-off used may also be of relevance to the difference in findings. Based on this line of work, in addition to case-control comparisons of CFS patients and healthy controls I will also explore differences according to the duration of illness.

Patients with CFS have also been shown to have increases in levels of auto-antibodies and levels of type 2 cytokine-producing cells, and suppression of mitogen-induced lymphocyte proliferation and natural killer (NK) cell activity, among other immune alterations (see Silverman et al., 2010). Additionally, research has demonstrated some immune related-genes to be differentially modulated in a CFS group (Steinau et al., 2004), and increased gene expression in pathways linked to clinical and immune features of CFS, such as those linked to cytokines and their receptors (Fang et al., 2006; Kerr et al., 2008). As mentioned briefly above, some studies have also explored rates functional genetic polymorphisms (single nucleotide polymorphisms, or SNPs) associated with differential production of cytokines in patients versus healthy volunteers, a selection of which are presented in Table 1.1 (see page 36).

It should be noted, however, that despite the ever-growing body of evidence, a meta-analysis of findings published up to 2003 found no evidence for immune dysfunction (Lyall et al., 2003), and negative findings have been published since. A recent meta-analysis of circulating cytokines in CFS found that of the 77 measured in serum or plasma, only transforming growth-factor beta (TGF- β) was consistently raised, though the authors noted the lack of information about some cytokines, and highlighted heterogeneity across studies in the markers measured (Blundell et al., 2015). Indeed, inflammatory states may be induced by a host of lifestyle factors, or undiagnosed physical or mental health conditions which are not covered by some such studies (see Raison et al., 2009). This may also be attributable to the heterogeneity seen among CFS patients with respect to the clinical features and causes of their condition. The more recent studies highlight the need to explore differences in patient characteristics in order to better identify patterns of immune dysfunction, and also changes in cytokine levels over time. To this end, the current drive to share data and recruit patients into large cohort studies should yield some further useful results.

The majority of research to date has been cross-sectional; further longitudinal studies that measure changes in a wider range of cytokines across time in individuals are essential for understanding whether differences in patterns of different cytokines may contribute to the manifestation and persistence of symptoms. I will do so in this thesis.

1.3.3 Post-infective Fatigue Syndrome (PIFS)

Also encompassing post-viral fatigue (PVFS), this diagnosis may be given where patients do not meet criteria for CFS, or patients with CFS may be labelled as such when a clear path can be identified from the infective trigger (Shepherd & Chaudhuri, 2016). Cohort studies of new cases have provided a basis on which to monitor fatigue and identify characteristics associated with increased risk of more severe fatigue, or a subsequent PIFS/CFS diagnosis. UK studies have focussed largely on infectious mononucleosis, also known as glandular fever (e.g. see Candy et al., 2002).

A large-scale prospective cohort study has been conducted in Australia, named the 'Dubbo Infection Outcomes Study', referred to hereafter as the DIOS cohort. The team aimed to capture all cases of Ross River virus (RRV) and the Epstein Barr virus (EBV), the cause of glandular fever, as well as the bacterial infection Q fever, monitoring patients during the acute sickness phase, and identifying a sub-group who subsequently met the criteria for CFS. Resources for the study have allowed for the publication of several studies of the cohort, encompassing various measures of the acute response, risk factors associated with a greater acute response, and independently, a later diagnosis of PIFS, as well as biological and clinical measures at follow-up versus patients who promptly recovered. These studies will be referred to throughout this thesis. Some findings, concerning functional genetic polymorphisms associated with the acute response to the trigger, are also summarised overleaf in Table 1.1.

Table 1.1 Selection of research about functional genetic polymorphisms related to cytokine production in CFS/PIFS

<i>Gene SNP/Loc</i>	<i>Genotype/allele (association)</i>	<i>Finding</i>	<i>Reference</i>
IL6 -174 (rs1800795)	G (Higher levels)	CFS: rates no higher in patients vs. controls	<i>Carlo-Stella et al. 2006</i>
		PIFS (DIOS): no association with higher fatigue in response to infection; greater mood disturbance (additive effect if +IL-10 -592 A (lower levels); trend towards association with higher pain	<i>Piraino et al. 2012</i>
IFN-γ +874 (rs2430561)	AA (Lower levels)	CFS: rates lower in patients vs. controls	<i>Carlo-Stella et al. 2006</i>
	T (Higher levels)	PIFS (DIOS): best predictor of fatigue severity during acute sickness	<i>Piraino et al. 2012</i>
IL-10 -592 (rs1800872)	C (Higher levels)	PIFS (DIOS): less likely to experience neurocognitive effects or mood disturbances during acute sickness	<i>Piraino et al. 2012</i>
TNF-α -308 (rs1800629)	A (Higher levels)	CFS: rates no higher in patients vs. controls	<i>Carlo-Stella et al. 2006</i>
TNF-α -857 (rs1799724)	T (Higher levels)	CFS: rates higher in patients vs. controls	<i>Carlo-Stella et al. 2006</i>

1.3.4 Cytokine induced sickness behaviours

Valuable insights have also been gained from studies of the impact of inflammation on behaviour. Pro-inflammatory cytokines themselves are known to mediate the so-called 'sickness behaviours'. In response to acute injury or infection, symptoms including fatigue, malaise and hyperalgesia are induced as part of an adaptive, coordinated effort to conserve energy, encourage recovery and enable the resolution of inflammation as required to deal with the insult.

This process has been extensively studied since it was established in the late 1980s (Dantzer & Kelley, 2007; Hart, 1988). Studies have demonstrated the behavioural effects of cytokine administration in both pre-clinical and clinical studies. For example in mice, chronic systemic administration of IL-2 was found to impair spatial working memory and reduce exploration, while in rats, spinal injections were found to increase pain sensitivity (Cata et al., 2008; Lacosta et al., 1999). In clinical practice, chronic cytokine exposure from cytokine immunotherapy for cancer and viral hepatitis often results in severe fatigue, flu-like symptoms, muscle-ache and cognitive impairment, as is observed in CFS (Capuron et al., 2002; Maddock et al., 2005). Conversely, medications that block pro-inflammatory cytokines have been shown to alleviate fatigue and pain in patients with Rheumatoid Arthritis and Psoriasis (Strand & Singh, 2007; Tying et al., 2006). A recent narrative review highlighted how key sickness behaviours – anhedonia and anorexia – do not feature in CFS unless part of a comorbid depression. They add that the range of complex triggers, including inflammatory and autoimmune triggers, contrast to this acute, well-defined evolutionary response (Morris et al., 2013). Nonetheless, such studies demonstrate the link between inflammation and CFS-like symptoms.

1.3.5 The kynurenine pathway and CFS

Another route by which inflammation may elicit symptoms observed in CFS is through dysregulation of the kynurenine pathway. This metabolic pathway starts from tryptophan (TRP), the amino acid precursor to serotonin. Increased inflammation upregulates the expression of the enzyme indoleamine 2,3 dioxygenase (IDO) that breaks TRP down into kynurenine, which can be further metabolised into kynurenic acid, 3-hydroxykynurenine and quinolinic acid (QUIN), in addition to other metabolites (see Figure 1.2).

The increased production of potentially neurotoxic metabolites following IDO activation has been implicated in the experience of pain and other physio-somatic symptoms as observed in CFS. For example, QUIN has an excitotoxic effect on the glutamate N-methyl-D-aspartate (NMDA) receptor, the stimulation of which may cause hyperalgesia and central sensitisation, or an increased sensitivity to pain (Anderson et al., 2014; Romano et al., 2015). Of relevance, drugs that block the NMDA receptor have shown beneficial effects for the treatment of pain associated with central sensitisation, as has been seen in CFS and the associated condition fibromyalgia (Nijs et al., 2011).

The pathway has been extensively studied in depression and other neuropsychiatric diseases, as well as other medical conditions where immune activation and inflammation are key features, where increased ratios of kynurenine to tryptophan have been found (Leonard & Maes, 2012; Morris, Carvalho, et al., 2015).

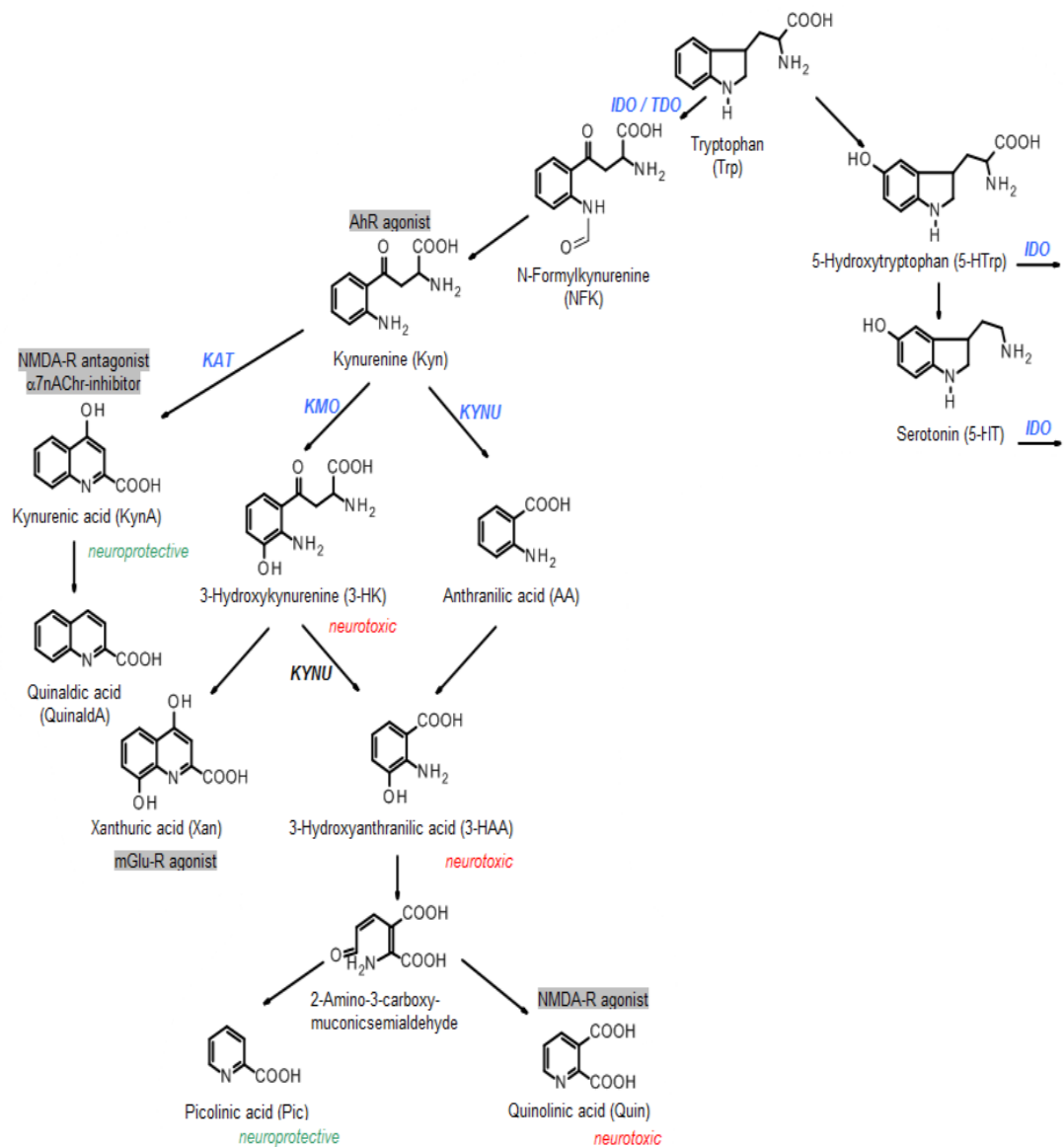


Figure 1.2 The kynurenine pathway

(Modified and used with permission from (Schütze, 2014))

Continuing from the perspective of symptoms, some findings of interest have come from other related or overlapping conditions. A preclinical study demonstrated a reduction in post-exercise fatigue by blocking TRP access to the CNS, through administration of branched-chain amino acids, which compete with tryptophan for access via the same transporter (the large neutral amino acid transporter-1, or LAT-1). The same study observed cognitive impairments following administration of TRP and quinolinic acid, as well as, perhaps surprisingly, kynurenic acid, which is typically thought of as neuroprotective (Yamamoto et al., 2012). Conversely, in a study of patients with somatization, overlapping with CFS, levels of tryptophan were lower, and the ratio of kynurenine to TRP (KYN/TRP) was higher versus healthy controls. In addition, they found lower levels of kynurenic acid, which as highlighted previously is considered protective against the neurotoxic effects of this pathway (Maes et al., 2011).

Further evidence of dysfunction of the kynurenine pathway comes from a study of Epstein-Barr virus (EBV). While the infection is usually self-limiting, some patients will develop chronic active infection, associated with persistent symptoms including fatigue. One study explored the relevance of TRP metabolism to the persistence of symptoms. The authors observed a pattern of aggravated TRP degradation in patients with more severe symptoms. Higher fever was significantly associated with a higher Kyn/TRP ratio and lower TRP, while there were trends towards associations between a higher KYN/TRP ratio and severe tiredness and night sweats (Bellmann-Weiler et al., 2008). This suggests that inflammation induced activation of IDO may be relevant for the persistence of symptoms, including fatigue, following an immune trigger.

In CFS specifically there have been few studies conducted. The majority of research has concerned TRP and the availability of TRP in the brain, explored from the perspective of increased serotonin. One method to assess likely availability of TRP in the brain is the calculation of the ratio of TRP to levels of the five competing amino acids. Studies in both serum and plasma have shown CFS patients to have higher levels of free TRP, and a higher ratio of free TRP to levels of brain-chain amino acids and large-neutral amino acids, indicating increased availability of TRP in the brain (Badawy et al., 2005; Georgiades et al., 2003). However, another study in plasma found the converse, with lower levels of free TRP in plasma, and lower levels of the ratio of TRP to neutral amino acids. They did not find a difference in total TRP (Vassalo et al., 2001). One such study suggested that there may be subgroups of CFS patients with serotonergic dysfunction, while in others results are normal (Badawy et al., 2005). In order to contribute towards this emerging picture, I will examine TRP levels, as well as a range of metabolites involved in the kynurenine pathway that, to the best of my knowledge have not yet been so extensively studied in this population, or from the perspective of persistent fatigue following an immune trigger.

1.4 The neuroendocrine system and CFS: HPA-axis dysfunction

Another biological system that interacts with the immune system is the neuroendocrine system. The most widely investigated biological factor in the study of the aetiology of functional somatic disorders such as CFS is Hypothalamic-Pituitary-Adrenal axis dysfunction (see Tak et al., 2011). In normal conditions, corticotrophin releasing hormone (CRH) is released from the paraventricular nucleus of the hypothalamus. This in turn leads to the release of adrenocorticotrophic hormone (ACTH) in the pituitary, inducing discharge of cortisol from the adrenal cortex, which exerts the negative feedback mechanism controlling its production by regulating the synthesis of CRF. Evidence for dysfunction of the HPA axis can be seen in studies examining cortisol in urine, blood and saliva, as well as endocrine challenge studies.

Studies have shown patients with CFS to have a flattening of cortisol slope during the day, decreased cortisol levels in plasma and saliva, and decreased urinary free cortisol secretion (see Silverman et al., 2010). While results have tended to be mixed, a meta-analysis found that across 38 studies, CFS patients were shown to have significant basal hypocortisolism when compared with healthy volunteers (Tak et al., 2011). Further support for the role of HPA dysfunction in CFS comes from the therapeutic use of cortisol in these patients. Low-dose cortisol replacement therapy produced promising results in alleviating fatigue and associated symptoms in patients with CFS (Cleare et al., 1999; McKenzie et al., 1998). However, the side effect profile, short-term effects after cessation and limited efficacy among the wider CFS population were noted as the reasons why this was not pursued (Cleare, 2004).

As is the problem identified in a wide range of CFS research, mixed results are likely attributable to heterogeneity within the sample, with patients presenting with different clinical features and apparent causes (see Silverman et al., 2010), as well as the wide range of factors known to influence HPA-axis activity. HPA dysfunction may be associated with a number of factors, including impaired sleep, alcohol use, smoking, use of certain medications, obesity, stress, physical inactivity and co-morbid depressive disorder (see Tak et al., 2011). For example, experience of early life stress has been found to stratify patients in this regard, with those who reported traumatic experiences during childhood having hypocortisolism, but those without exhibiting normal levels (Heim et al., 2009). Interestingly, this paper too had originally found a significant increase in the group overall, prior to stratification, as compared to controls. Therefore, there may be moderating factors that account for some of the association between the illness and HPA dysfunction observed. Periods of inactivity, as well as the chronic stress associated with the pattern of symptoms and suffering from an unexplained illness may also contribute to this state (Cleare, 2004; see Silverman et al.). Indeed, a prospective study of factors predictive of the persistence of fatigue following glandular fever found no link with any measures of cortisol, during what would be the earlier phase of illness (Candy et al., 2003).

Despite the number of existing studies, examination of cortisol levels as they relate to post-infective fatigue, or indeed chronic fatigue syndrome, is a vital part of the psychoneuroimmunological picture, and will be included in my thesis.

1.5 Psychological factors and other clinical factors and CFS

Extensive research has been done too to identify psychological risk factors for the syndrome. One such risk factor is experience of childhood trauma (Heim et al., 2006, 2009), which has also been shown by our group to be independently associated with inflammation (Danese et al., 2007). In addition, a study using data from the 1958 cohort (an epidemiological study of individuals born that year) identified a significant association between a history of physical parental abuse, with both self-report CFS/ME, as well as “CFS-like” illness, determined according to operational criteria (Clark et al., 2011). A recent review by our group found rates of CFS and Fibromyalgia to be 2-3 fold higher in those exposed to childhood stressors (Borsini et al., 2013). Furthermore, as mentioned above, in one study hypocortisolism was associated with CFS only in those with a history of early life stress (Heim et al., 2009). Stress throughout life may act as a trigger for CFS, with patients reporting considerably more life events than healthy controls (Hatcher & House, 2003; Salit, 1997), though stressors experienced in the last year did not contribute to the development of CF in another study (White et al., 2001).

A personal history of a psychiatric disorder has also been found to be a risk factor for CFS, with increased levels of psychopathological symptoms preceding the symptoms of fatigue (Clark et al., 2011; Harvey et al., 2008). On the issue of comorbidity, rates across CFS vary, though one community study of unexplained fatigue, using data from the household survey of psychiatric morbidity, found high rates of psychiatric co-morbidity in patients reporting fatigue. Moreover, the authors reported that the disability associated with fatigue was largely explained by psychiatric morbidity (Skapinakis et al., 2000). Fatigue is also a symptom of depression, and studies have found mixed results

with regards to the presence of depression in patients who have been properly diagnosed with CFS. A study in a London CFS specialist service of 68 patients, found that 21 out of 31 patients who had received such a diagnosis had no evidence of psychiatric illness past or present; on the other hand, 13 of the remaining 37 did, highlighting the risk of a misrepresentation in the literature where patients are not properly characterised (Deale & Wessely, 2000).

Nonetheless, it is important to recognise the risk of depression in CFS. There are two explanations related to the condition itself, which are not mutually exclusive. Firstly, depression may follow as a consequence of living with a chronic health condition. Second, as is seen in other inflammatory conditions, high rates of co-morbid depression are likely due to underlying shared pathways relating to dysfunction of the central nervous system (Holgate et al., 2011). However, while pathways may be shared, evidence shows the two conditions as separate illnesses, which require specialist treatment protocols (Rimes et al., 2014). Additional evidence for this comes from the effectiveness of specialist CBT for CFS, regardless of co-morbid psychiatric disorder, and the inefficacy trials of fluoxetine for CFS (Prins et al., 2006).

Also important are illness and treatment perceptions, which have been shown to be associated with the onset of chronic fatigue following infectious diseases. For example, a study by Candy and colleagues found perceptions about the expected illness duration and long-term impact to be predictive of poorer outcomes up to six-months after onset of Infectious Mononucleosis (IM) (Candy et al., 2003). A later, larger study of predictors of CFS following IM found that negative illness perceptions - including believing the condition to be serious and distressing, that it would last a long time, and was uncontrollable - predicted a subsequent diagnosis. Of the cognitive and behavioural responses to symptoms

examined, 'all or nothing' behaviour, defined as bursts of activity with a need to rest afterwards, also predicted a subsequent diagnosis, and in fact was the strongest predictor (Moss-Morris et al., 2011). Moreover, in a study comparing patients already diagnosed with CFS with those suffering from rheumatoid arthritis - a more clearly defined condition with overlapping symptoms - CFS patients held more negative illness perceptions. Physical disability was comparable across the two groups. However, CFS patients reported greater problems with social functioning and limitations to everyday activities as a result of their symptoms (Moss-Morris & Chalder, 2003).

Illness or symptom attributions, related to the factors which patients or individuals feel have caused their condition or made it worse, may also be relevant. There are three overarching categories: somatic (i.e. a physical abnormality), psychological (e.g. emotional upset) and normalising (external events, e.g. a change in environment or behaviour) (Moss-Morris, 2005). A study of PVFS found that tendency to attribute symptoms to somatic factors was the strongest risk factor for subsequent chronic fatigue (Cope et al., 1994). CFS patients have been shown to be less likely to make psychological attributions, and to make similar physical attributions to Multiple Sclerosis patients (Dendy et al., 2001). A community study of fatigued individuals found that those attributing their fatigue to social factors were more 'protected' than those making biological attributions from later fatigue and disability (Chalder et al., 1996). Findings concerning the effect on outcome following treatment for CFS have been mixed. One study in primary care found that physical illness attributions predicted a worse outcome (Chalder et al., 2003). Studies of tertiary care services, however, found no link (Deale et al., 1998; Kempke et al., 2010).

Another concept that has been examined in CFS patients is 'willingness'. In this case, 'willingness' can be defined as the appreciation that avoidance and control of symptoms are not always possible. A lack of 'willingness' indicates a lack of acceptance of fatigue, which has been linked to increased disability. However, it has also been shown to be modifiable following cognitive behavioural therapy interventions (Brooks et al., 2011)

In this thesis, I will explore the relevance of illness perceptions and cognitive and behavioural responses to fatigue to the persistence of fatigue following another immune trigger not yet studied in this context. Specifically, in patients who have been treated with IFN- α , to be described in the next section. I will also compare attributions made, and levels of acceptance of fatigue in the IFN- α treated patients, healthy volunteers and patients with CFS.

1.6 IFN- α induced persistent fatigue: a proxy model of CFS

1.6.1 Introduction

To shed further light on the pathogenesis of CFS, it is important to better understand how an infective or inflammatory trigger may lead to the pattern of symptoms seen in this illness. To this end, valuable information has been gained from studies where the context has enabled researchers to prospectively follow individuals from the perspective of the trigger. These studies have allowed for the identification of behavioural and biological factors relevant to non-recovery, or the persistence of symptoms. So far, I have highlighted insights gained from studies of the experimental induction of inflammation, as well as from post-infective fatigue cohorts. However, effects of the experimental studies are short-lived, and post-infective cohorts have recruited recent onset cases, some of whom would have been ill for some weeks before enrolment. Thus, the opportunity to obtain true 'baseline' measurements has been missed.

While cross-sectional studies have provided a great deal of information also, speculation about the cause or contributory factors are restricted largely to patient report, and cohort studies which track individuals before and after the trigger to CFS diagnosis are costly and resource intensive, and thus few and far between. This highlights the importance of the use of proxy-models to further our understanding. The model that I will present and explore in this thesis is persistent fatigue induced by Interferon-alpha (IFN- α) treatment.

Produced naturally as part of our immune response, the therapeutic administration of IFN- α represents an immune trigger, causing the body to act as though an infection is present, even in the absence of infection.

Depression induced by IFN- α has been extensively studied as an inflammatory model of depression, and patterns of acute fatigue have been reported in such studies (Felger et al., 2012; Majer et al., 2008).

To the best of my knowledge, this is the first time it has been explored as a model of CFS, though from patient reports it is known that a number of patients report CFS-like symptoms post-treatment (Hepatitis C Trust, 2010; Hopwood, 2013). Since as in CFS the original inflammatory stimulus is no longer present, this makes it a suitable study that can be conducted within a short, well-defined timeframe. In addition, patients may be recruited ahead of treatment initiation, allowing for baseline measurements to be captured. Furthermore, studies have shown that acute sickness behaviours, and associated changes in inflammatory markers are consistent across different infectious diseases. This provides further evidence that such behaviours relate to a generalised immune response on the part of the host (Vollmer-Conna et al., 2004). Indeed, CFS, or post-infective/viral fatigue syndromes (PIFS/PVFS) may occur following different conditions, but manifest in the same way (Shepherd & Chaudhuri, 2016). This means that we may use such studies to further understand the relevance of different factors in the development of CF or CFS, and are not restricted by the infectious trigger.

1.6.2 Hepatitis C Virus infection

Hepatitis C Virus (HCV) infection was first identified in 1989. Initially an acute illness lasting around six-months, an estimated 75% of patients will develop Chronic HCV, associated with progressive fibrosis of the liver, risk of cirrhosis and hepatocellular carcinoma (HCC) (Dore et al., 2014). Such is the current burden of the disease, coupled with the promise of new treatments (see below), the World Health Organisation have published a Global Health Sector Strategy on Viral Hepatitis (2016-2021), with a view to eliminating it as a major public health threat by 2030. Hepatitis C is estimated to cause 48% of the 1.4 million deaths annually worldwide attributed to acute infection, and hepatitis-related liver cancer (HCC) and cirrhosis (World Health Organisation, 2016). An estimated 214,000 individuals are living with Chronic HCV in the UK (Public Health England, 2016). There are six strains of the virus, genotypes 1-6, with further subtypes. In the UK, reflective of global trends, the most common are 1 and 3, each representing around 45% of cases (Messina et al., 2015). Historically, one of the most common routes to transmission was exposure to contaminated blood, or blood products, which of course had not been screened for the disease. However, following routine blood testing the risk of contracting HCV via this route reduced markedly (Donahue et al., 1992). One of the greatest risks now is to those using drugs intravenously, known in the medical field as 'People who inject drugs' (PWID) (Alter, 2007). Transmission is also still known to occur as a result of inadequate healthcare practices including sharing and improper sterilisation of equipment (Alter, 2007; Ver Hoeve et al., 2013), with a report estimating that un-safe injections in healthcare settings account for 24% of HCV infections (World Health Organisation, 2009).

1.6.3 The treatment of Hepatitis C infection

The rapidly changing therapeutic landscape in Chronic HCV necessitates a brief summary of the relevant changes since the initiation of my study. Historically, IFN- α , and later pegylated-IFN- α , has been the standard treatment. However, more recently there has been greater recognition of the growing burden of the disease, and great promise shown in clinical trials (Dore, 2012). This has resulted in an increasing number of drugs being awarded fast-track status by the U.S Food and Drug Administration (FDA), enabling them to be brought to market more quickly. Such was the rate of change, that the two original protease inhibitors to be introduced in 2012, Boceprevir and Telaprevir, fairly quickly became redundant. As it became apparent that more effective, better-tolerated IFN- α free regimens would soon be available, NHS England held back funding for IFN- α based regimens. However, the cost of the newer medications has prohibited their use in many countries (Dore et al., 2014; Iyengar et al., 2016). Despite the biggest investment in any single condition in 2016, even with recent NICE approvals, treatment numbers remain capped by NHS England; as a result of such high costs, they are limiting treatment unless urgent treatment is indicated by disease progression and severity. A system has been put into place whereby treatment across 'operational delivery networks' are now managed by central hubs which each have their own quota. In an apparently unprecedented move, NICE gave NHS England permission to delay the prescription of drugs they had already approved. In addition, a scheduled update to the NICE treatment guidelines expected in September 2016 was postponed until further notice, due to the continued changes and lack of stability in the field. Furthermore, the latest approvals do not cover all genotypes (Ryder, 2015). Therefore, IFN- α side effects remain a current concern.

1.6.4 IFN- α for Hepatitis C infection

IFN- α is a type 1 interferon and cytokine released as part of our natural innate immune response, playing an important role in the response to pathogens. It attaches to receptors on the cell surface that signal via Janus-activated kinase (JAK) and signal transducers and activators of transcription (STAT) proteins, which in turn leads to the induction of interferon-stimulated genes. Such genes inhibit the translation of the viral protein, and destabilise the viral messenger RNA (Hoofnagle & Seeff, 2006). It also triggers the production of other cytokines involved in the immune response, such as IL-6 (Raison, Borisov, et al., 2010).

The antiviral and immunomodulatory properties led to the development of a synthetic equivalent, the structure of which was later modified through the process of pegylation to improve half-life (Hoofnagle, 1999). Combination therapy for HCV consists of weekly subcutaneous injections of pegylated-IFN- α , referred to throughout this thesis as IFN- α , together with oral Ribavirin taken twice daily. Since some are more difficult to treat, treatment duration is at least in part determined by genotype, with genotypes 2 and 3 usually treated for 24 weeks, and 1, 4 and 5 for 48 weeks. Viral clearance is achieved in around 50% of those with genotype 1, and 80% of those with genotypes 2 or 3 (Hadziyiannis et al., 2004).

From 2012-2015, Genotype 1 patients were also likely to have been treated with one of two first-generation protease inhibitors, Boceprevir or Telaprevir. The addition of these drugs, known as 'triple therapy', increases clearance rates to around 65% (Pearlman, 2012). In 2015, Simeprevir was approved in the UK. It is a 'second generation' protease inhibitor according to its specificity, with response rates of 80-81% (Jacobson et al., 2014; Manns et al., 2014). Another

form of triple therapy, the once-daily oral drug is approved for use with peg-IFN- α and ribavirin for genotypes 1 and 4. Unlike combination therapy, where treatment discontinuation is flexible according to virus clearance (and side-effects), those on 'triple therapy' are subject to stricter protocols; if the viral load is not significantly reduced at certain milestones, treatment is stopped, to avoid additional cost, unnecessary side effects and treatment resistance. See Figure 1.3 for treatment schedules.

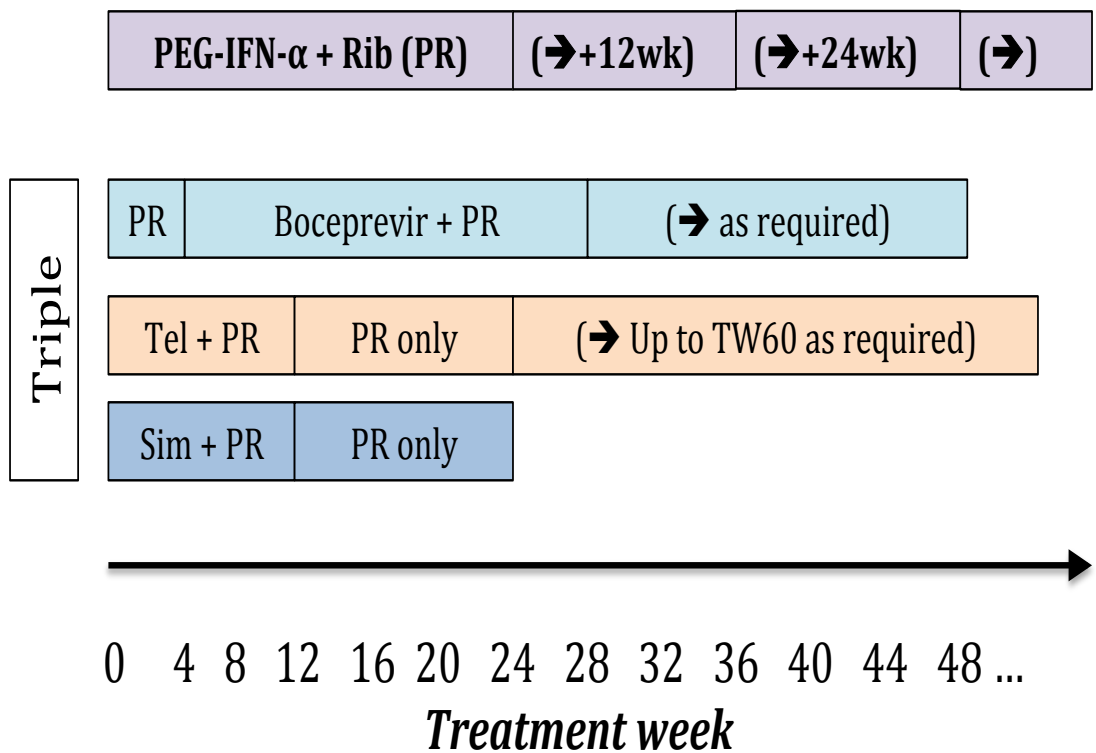


Figure 1.3 Treatment schedules for combination and triple therapies

1.6.5 IFN- α induced side effects

Following the initial dose of IFN- α , the majority of patients will experience cytokine-induced sickness behaviours, including myalgia, malaise, anorexia, fatigue, and mild cognitive impairment, in addition to other flu-like symptoms. Following more chronic administration, the flu-like symptoms including fever, coughs and sore-throats will subside, though the aforementioned symptoms of fatigue, malaise and cognitive symptoms will persist, with additional mood disturbance usually occurring later, after around eight to twelve weeks (Capuron & Miller, 2004).

1.6.6 Predictors of IFN- α induced acute fatigue

Few studies have been able to identify clear predictors of IFN- α induced acute fatigue, during treatment. Since depression is more severe an adverse event, and more likely to be associated with treatment discontinuation, to date research has focussed more heavily on predictors of depression development. Some studies have examined fatigue as part of the umbrella of depressive symptoms, which while useful, poses some problems, since studies have often found distinct patterns in mechanisms underlying IFN- α induced depression versus fatigue (Bull et al., 2009; Felger et al., 2012). One such study found that those HCV IFN- α treated patients who experienced a greater increase in “somatic” symptoms of depression early on in treatment (including loss of appetite, fatigue, irritability) had lower baseline levels of serotonin, and higher levels of the cytokine TNF- α (Loftis et al., 2013).

For those studies measuring fatigue specifically, unlike what we see for risk of IFN- α induced depression, one study found no effect of a past history of depression on IFN- α induced fatigue levels (Majer et al., 2008). Another American study mentioned previously, of genotype 1 patients, found that female versus males, and Caucasian versus African American patients experienced greater problems with fatigue during treatment. Though not a predictor, increased fatigue was also reported by those who would go on to achieve a sustained virological response (Sarkar et al., 2012). A prospective study of early changes in response to the first four weeks of IFN- α treatment for HCV found no association between the initial, immediate increases in cytokines and subsequent fatigue (Dowell et al., 2016). The authors did, however, identify baseline fatigue as a predictor of acute fatigue during IFN- α treatment, which the authors suggested could also be linked to response bias, or the tendency to over/under report symptoms (Dowell et al., 2016). Another study found higher baseline fatigue in individuals with the CG or GG genotypes of a IL-6 polymorphism (rs1800795), where the 'G' allele is associated with higher levels of the cytokine (Udina et al., 2013). A review of Hepatitis C related fatigue found little or no evidence for an association with most markers of disease severity, including viral load (Wessely & Pariente, 2002). However, it has been linked to the stage of disease, for example those with cirrhosis of the liver may be more prone to fatigue (Sarkar et al., 2012). Higher fatigue has also been reported by women with HCV versus their male counterparts, in younger participants, and perhaps surprisingly, those with lower levels of Alanine Aminotransferase (ALT), the presence of which is associated with liver injury or disease (Sarkar et al., 2012).

1.6.7 IFN- α induced persistent fatigue

Where longer-term outcomes of IFN- α have been considered, research has tended to overlook the persistence of side effects in favour of the study of clinical outcomes (Aronsohn & Reau, 2009). Indeed, some patients report having felt misled or mismanaged regarding the long-term effects of treatment (Hopwood, 2013). A report by the Hepatitis C trust found that around 60% of patients continued to experience fatigue six-months post-treatment, with around 30% still experiencing fatigue 12-months post-treatment, in addition to a range of CFS-like symptoms (see Table 1.2) (Hepatitis C Trust, 2010).

One study of genotype 1 patients focussing on patterns of fatigue pre-, during and post-IFN- α observed improved rates of fatigue post-treatment versus baseline; at 12 week follow-up, 36% of responders and 42% of non-responders were still reporting fatigue, with lower overall fatigue scores (Sarkar et al., 2012). Another study reported decreases in fatigue post-treatment, to levels similar to baseline, though on this occasion the authors measured severity as opposed to incidence (Huckans et al., 2015). A study of health related quality of life used a measure of 'vitality' (energy/fatigue), comparing HCV IFN- α treated patients against US population norms. They examined 'sustained response', whereby the virus is not detected in the blood six-months post-treatment ('Sustained Virological Response; 'SVR'). They found that in 'sustained responders' pre-treatment vitality scores had been significantly lower, indicating greater problems, than the population, but by six-months post-treatment levels had improved so that they were no longer different. They also found significant associations with improvements in measures of HCV disease severity including viral load and hepatic inflammation (McHutchison et al., 2001).

Table 1.2 The similarities between IFN- α induced persistent side effects and CFS symptoms

CFS Symptoms	IFN- α induced side effects
Severe fatigue	Fatigue (<i>lack of energy, sleepiness, run</i>
Post-exertional malaise	<i>down</i>)
Muscle pain / multi-joint pain	Joint/muscle aches (<i>myalgia</i>)
Cognitive impairment (<i>memory, concentration, word finding</i>)	'Brain fog' (<i>confusion, memory loss, sudden blankness</i>)
Unrefreshing sleep	Unrefreshing sleep
Headaches	Flu-like symptoms
Sore throat	
Tender cervical or axillary lymph nodes	

1.6.8 Predictors of IFN- α induced persistent fatigue

Very few studies have identified predictors of persistent fatigue following IFN- α treatment. Studies conducted have found higher levels of fatigue in those who did not achieve a sustained virological response (SVR), versus those who had cleared the virus (Huckans et al., 2015; Sarkar et al., 2012). The earlier study found that there was no difference in presence or severity of fatigue according to whether the virus had returned during treatment ('viral breakthrough') or during the follow-up period ('relapse'). Depression was also associated with fatigue, with increasing significance over the course of treatment and beyond (r_s = 0.53 baseline; r_s = 0.66 TW24; r_s = 0.73 six-month post-treatment) (Sarkar et al., 2012).

Lastly, illness perceptions, highlighted earlier as a risk factor for CFS, or chronic fatigue following an infection, have been found to be predictive of the subsequent severity of symptoms of depression and anxiety. In a study of the same sample of patients as to be studied here in this thesis, scores were associated with pre-treatment perceptions of a strong illness identity, greater consequences associated with HCV, and a strong emotional response to the illness (Hepgul et al., 2016). However, neither the association with acute nor persistent fatigue has yet been studied in this population, a gap in the literature that I will address in this thesis. In addition, risk factors for both IFN- α induced acute- and persistent-fatigue have not yet been well defined, and further characterisation of patients most at risk will also form a part of this work.

1.6.9 Mechanisms of IFN- α induced fatigue

1.6.9.1 Introduction

The evidence for the role of the immune and neuroendocrine systems in CFS has been discussed elsewhere. Here I will focus on providing a summary of some of the evidence that has sought to explain the mechanisms underlying IFN- α induced fatigue. As suggested previously, the neuro-immune basis for fatigue has not received as much attention as depression in the context of the administration of IFN- α , nor in connection with inflammation more generally. Where mechanisms have been explored, they have related to acute fatigue experienced during treatment, and not the persistence of fatigue later. A recent review by Dantzer and colleagues highlighted the different effects peripheral inflammation, such as that induced by IFN- α treatment, has on neuro-immune pathways, which may lead to the experience of fatigue in different conditions (Dantzer et al., 2014). Cytokines released in the periphery, as part of this response, cause the production and release of inflammatory mediators, such as prostaglandins and cytokines. They are released by different immune or immune-related cells in the central nervous system, and in turn impact on cell function. They also act on the brain through their effects on the bioavailability of amino acid precursors to neurotransmitters, including serotonin and dopamine. First, straddling the gap between “predictor” and “mechanism”, I will summarise some of the research relating to functional genetic polymorphisms in relevant genes. Then, I will summarise some of the research concerning the effect of IFN- α on dopaminergic, glutamatergic and serotonergic function, as well as TRP and the kynurenine pathway.

1.6.9.2 Genetic polymorphisms

A few functional genetic polymorphisms have been explored related to altered cytokine production, though notably less than have been explored in the context of IFN- α induced depression (see Table 1.3). Where a link has been made, it has typically been with low energy, as opposed to higher fatigue (Lotrich et al., 2010, 2011). Also of note is the connection between the IL28B polymorphism (CC genotype) and a higher pre-treatment ratio of kynurenine to tryptophan, which may contribute to the increased somatic symptoms during treatment that are also associated with this genotype (Lotrich et al., 2011; Zoller et al., 2015).

Findings concerning serotonin are not presented in the table, though studies of a polymorphism in the promoter region of the serotonin transporter gene, '5-HTTLPR', have found the 'LL' genotype, associated with higher transcription, to be protective against IFN- α induced depression, and linked to a later onset of symptoms relative to baseline. There was no significant association with IFN- α induced fatigue, suggesting that serotonin may be less important for the experience of IFN- α induced fatigue (Bull et al., 2009; Udina et al., 2013).

Genetic polymorphisms are not examined in this thesis, as such studies typically require larger participant numbers. However, I will examine baseline levels and changes in a range of cytokines in response to IFN- α , including IL-6, IFN- γ and TNF- α . These studies provide useful context regarding a possible mechanism underlying an elevated inflammatory response to IFN- α , which may result in more severe fatigue, and the persistence of fatigue post-treatment. Moreover, they highlight both overlapping and distinct measures of interest for cytokine-mediated mood disturbances and fatigue.

Table 1.3 Selection of evidence relating to genetic polymorphisms associated with altered cytokine production, and their relationship to IFN- α induced fatigue

<i>Gene/Loc</i>	<i>Genotype/allele (Association if known)</i>	<i>Finding</i>	<i>Reference</i>
IL6 -174 (rs1800795)	CC (Lower levels)	Protective against IFN- α depression, but not fatigue	<i>Bull et al. 2009</i>
		Sig. lower changes in IFN- α depression and anxiety scores; lower changes in fatigue scores (n.s.)	<i>Udina et al. 2013</i>
		Later onset of sig. changes in fatigue relative to baseline	
IFN-γ +874 (rs2430561)	T (Higher levels)	Associated with IFN- α induced depression (fatigue not examined)	<i>Oxenkrug et al. 2011</i>
TNF-α -308 (rs1800629)	A (Higher levels)	Greater problems with energy (BDI composite measure)	<i>Lotrich et al. 2010</i>
IL-28B (rs1297860)	C	Greater problems with energy and somatic symptoms but not fatigue (BDI item) or MDD development; also associated with improved virus clearance	<i>Lotrich et al. 2011</i>
	CC	Higher Kyn/TRP ratio pre-IFN- α therapy	<i>Zoller et al. 2015</i>

1.6.9.3 Cytokine effects on neurotransmitters

IFN- α induced changes in neurotransmitters are one mechanism by which behavioural changes occur. To further elucidate the role of such changes in IFN- α induced fatigue, the most recent evidence has come from neuroimaging studies. Such studies have demonstrated cytokine effects on brain regions mediating the effects of inflammation on behaviour. A recent observational study of IFN- α treated HCV patients used structural magnetic resonance imaging to further explore the link between the basal ganglia and IFN- α induced fatigue. The authors found that acute changes to microstructure of the striatum, part of the basal ganglia, occurring within hours of the initial injection, predicted the development of acute IFN- α induced fatigue at treatment week 4 (Dowell et al., 2016). They proposed that the rapid induction of this effect, coupled with relatively low levels of the other cytokines, points towards a direct action of IFN- α on the brain. However, the specific mechanism by which IFN- α reaches the brain is still unclear, with theories focussing on a leaky blood-brain barrier in the context of immune activation, or activation of cells spanning the BBB which result in IFN- α production in the brain (Dowell et al., 2016). A Positron Emission Tomography (PET) imaging study in cancer patients found an association between lower energy and higher glucose metabolism in the basal ganglia during IFN- α treatment (Capuron et al., 2007).

Altered basal ganglia function is implicated in psychomotor slowing, which has been associated with elevated IL-6 in major depressive disorder (MDD) (Goldsmith et al., 2016). Furthermore, IFN- α induced decreases in psychomotor speed are associated with IFN- α induced fatigue and depressive symptoms (Majer et al., 2008). More specifically, IFN- α has been shown to induce changes in dopamine function via changes in the basal ganglia, linked in an acute

challenge to the evolutionary sickness response, but to disabling fatigue in the context of chronic inflammation (Felger & Miller, 2012). Of interest, decreased activation of the basal ganglia has also been seen in CFS (Miller et al., 2014).

Further evidence comes from the study of tyrosine metabolism, the primary precursor of dopamine. A recent study found that IFN- α is associated with decreased conversion of phenylalanine (Phen) to tyrosine (Tyr); higher fatigue symptoms during IFN- α treatment were associated with a lower Phen/Tyr ratio, and lower dopamine levels in the CSF (Felger et al., 2013). Furthermore, the activation of guanosine-triphosphate cyclohydrolase-1 (GTP-CH1) by peripheral pro-inflammatory cytokines leads to a reduction in tetrahydrobiopterin ('BH4'), which is essential for the conversion of tyrosine to dopamine (Dantzer et al., 2014).

Elevated levels of glutamate have also been linked with fatigue more generally, thought to occur via cytokine induced reductions in the uptake of glutamate by astrocytes, and limits to the expression of glutamate transporters (Morris, Berk, et al., 2015). Moreover, quinolinic acid has also been shown to be relevant for IFN- α induced neuropsychiatric symptoms acting as an agonist of the glutamatergic NMDA receptor (Müller & Schwarz, 2007). A study of HCV patients utilising Magnetic Resonance Spectroscopy (MRS) observed an increase in glutamate following IFN- α ; while overall fatigue did increase during treatment, only reduced motivation was correlated with the elevated glutamate levels (Haroon et al., 2014). A second MRS study found only elevated glutamine, and no link with fatigue, though only overall fatigue was measured. Furthermore, there was no change in fatigue levels in response to treatment in their sample (Taylor et al., 2014).

Finally, further to the work described above concerning polymorphisms related to serotonergic function, while research has suggested some link with serotonin, findings concerning IFN- α induced fatigue specifically have been mixed. As suggested previously, there has been stronger evidence for the link to IFN- α induced depression. Indeed, use of Selective Serotonin Reuptake Inhibitors (SSRIs) such as Paroxetine, while effective for depression, were not found to be effective in preventing IFN- α induced fatigue (Capuron et al., 2002).

1.6.9.4 The kynurenine pathway and IFN- α

An alternative mechanism linked to TRP, already described in the context of CFS, concerns the kynurenine pathway. Though IFN- γ is thought to be the most potent activator, IFN- α is also capable of activating IDO, resulting in the catabolism of TRP as described earlier. So too do TNF- α and IL-6, which are also elevated following the therapeutic administration of IFN- α (Morris, Berk, et al., 2015). In IFN- α specifically, the pathway has typically been examined in the context of IFN- α induced depression, with few studies of IFN- α induced fatigue. One such study examined IFN- α induced decreases in energy, and found that it was associated with increases in the ratio of kynurenine to kynurenic acid (KYN/KYN-A) (Wichers et al., 2005). Outside of IFN- α , however, a study of rats found elevated levels of KYN-A and TRP in the brains of fatigued, sleep-disordered animals (Yamashita & Yamamoto, 2014). Of note, increased KYN-A has also been linked with a decrease in dopamine, which in turn is associated with fatigue; for example, one study observed a decrease in dopamine following the administration of KYN-A in rats (Wu et al., 2007). Also, as previously described, KYN is further metabolised into quinolinic acid (QUIN), which is a strong agonist of the glutamatergic NMDA receptor. To date, research into the

kynurenine pathway has focussed on depression. In this thesis, I will explore a possible role of the pathway, and increases in IDO activation, in IFN- α induced acute and persistent fatigue. In addition, new techniques mean I will explore a wider range of metabolites than have previously been reported in IFN- α studies.

1.6.9.5 HPA axis

Cytokine effects on neuroendocrine function are also of relevance; IFN- α itself activates the HPA axis, and administration of the cytokine is associated with inhibition of the axis (Dafny & Yang, 2005). One study observed that IFN- α treatment was associated with a flattening of the diurnal ACTH and cortisol patterns, as well as lower evening levels of the hormones. Moreover, the changes in cortisol, though not ACTH, were associated with increases in depressive and fatigue symptoms; there were associations with reduced activity, reduced motivation, physical and mental fatigue, but not with general fatigue. However, they were not associated with concurrent measurements of pro-inflammatory cytokines and their receptors (Raison, Borisov, et al., 2010). A study of IFN- α treated cancer patients found an association with higher initial, acute changes in ACTH and cortisol and IFN- α induced depression. However, there was no association with neurovegetative symptoms, including fatigue. Moreover, the effect related only to the acute response, with no differences evident following chronic administration (Capuron et al., 2003). To the best of my knowledge, HPA axis function has not yet been studied as it relates to the persistence of symptoms post-IFN- α treatment, as will be examined here.

1.7 Aims and hypotheses of the study

The overarching aim of the study, to which each individual aim below contributes, is to investigate IFN- α induced persistent fatigue as a proxy-model of Chronic Fatigue Syndrome. I will do so from a psychoneuroimmunological perspective, exploring immunological, neuroendocrine and psychological factors that may be involved in the development of persistent fatigue following an immune trigger. Most patients will experience some degree of side effects in response to this immune challenge, though the majority will recover after the cessation of treatment, once IFN- α and any other adjunctive medications are no longer having a direct effect on the body. However, other patients report persistent side effects, including fatigue and other CFS-like symptoms, even up to six or twelve months post-treatment. This is remarkably similar to what is seen in CFS induced by an immune/infective trigger. This thesis will aim to understand which characteristics, and clinical, behavioural and biological factors are most relevant to the experience of chronic fatigue long after the original immune stimulus is no longer present.

Aim 1. To examine the effect of IFN- α on the whole HCV cohort

To provide context, it is first important to examine the effect of IFN- α on the whole sample. IFN- α is widely known to be associated with a range of debilitating side effects including fatigue and other neuropsychiatric symptoms. Furthermore, it has effects on multiple biological systems. I will measure baseline levels, and track changes in:

- ***Clinical symptoms:*** *fatigue, depression, anxiety, stress, sleep*
- ***Health status:*** *including measures of general and mental health, as well as functioning*
- ***Biological measures:*** *cytokines and the kynurenine pathway*

Based on previous work, I hypothesise that IFN- α treatment will result in increases in levels of fatigue, depression, anxiety, stress and sleep. In particular, levels of fatigue will increase by TW4, while increases in depression and anxiety will occur later, around TW12. Measures of health status will reveal a marked decline in functioning across treatment. There will be increases in cytokines, reflecting the increase in the inflammatory response induced by IFN- α . Levels of TRP will decrease, while levels of KYN, or the KYN/TRP ratio will increase.

Aim 2. To identify factors associated with the severity of fatigue

To further investigate IFN- α induced fatigue specifically I will first examine the severity of fatigue. Work to date has focussed on the presence or severity of acute fatigue experienced during IFN- α treatment, though few studies have identified clear predictors of such fatigue. One predictor that has been identified is baseline fatigue. Few studies have examined post-treatment fatigue as a problem in itself, instead focussing on its relationship to treatment response. In the field of CFS, the severity of the acute illness has been found to be a key predictor of subsequent diagnosis of CFS following an infectious illness. I will therefore assess the relationship with severity of fatigue at three key time points: (i) baseline (treatment week 0, TW0), (ii) acute fatigue (the initial response to IFN- α , at treatment week 4, TW4), and (iii) six-months post-treatment.

I will explore an association with the aforementioned clinical and biological markers and health status, as well as socio-demographic characteristics and virus and treatment characteristics, experience of psychosocial stress (recent events; lifetime intrusive events; childhood trauma) and illness perceptions. I hypothesise that baseline fatigue severity will predict both acute fatigue, and fatigue post-treatment. I expect that more severe acute fatigue will be associated with more negative illness perceptions, but post-treatment fatigue will not. Finally, I also expect post-treatment fatigue to be associated with the clinical response to IFN- α , with those who have not cleared the virus experiencing more severe fatigue post-treatment.

Aim 3. To identify risk factors for IFN- α induced persistent fatigue, and examine the effects of IFN- α in persistent versus resolved fatigue

I will then examine the persistence of IFN- α induced fatigue. I will stratify patients according to whether fatigue persists post-treatment (Persistent Fatigue, 'PF') or resolves (Resolved Fatigue, 'RF'), based on the change from baseline. First, I will identify possible risk factors by exploring differences in the socio-demographic and virus and treatment characteristics, as well as prior experience of stress and trauma, and illness perceptions as described above. I will also examine baseline biological markers. Then, I will examine baseline measures, and monitor changes in clinical symptoms, measures of health status, and biological markers where longitudinal data is available (cytokines, kynurenine pathway), to explore differences in patterns, and the degree of change in response to IFN- α treatment. Through the identification of risk factors, and differential effects of the trigger (IFN- α), I aim to better understand which factors may contribute towards chronic illness in some, while the majority of individuals recover normally.

Based on the limited IFN- α data available, and findings concerning CFS/PIFS I hypothesise that patients in whom fatigue persists post-treatment will experience a more exaggerated response to IFN- α treatment, with (i) increased clinical symptoms (ii) a greater decline in health status, including functioning and (iii) elevated inflammatory markers, blunted cortisol, and increased IDO activity. I expect that HCV PF patients will have a more protracted recovery from IFN- α than HCV RF patients. Finally I hypothesise that recent experience of psychosocial stress; a history of trauma, and IFN- α treatment failure will be associated with an increased likelihood of persistent fatigue post-treatment.

Aim 4. A cross-sectional comparison with CFS patients and controls

This comparison will provide additional information for IFN- α induced persistent fatigue as a potential proxy-model of CFS. I will compare the measurements obtained from the IFN- α treated HCV cohort at the follow-up visit, six-months post-treatment, with a one-off assessment of CFS patients and Healthy Controls. HCV patients will again be stratified into HCV RF and PF groups. In doing so, I aim to better understand the similarities and differences between the groups - primarily the HCV PF and CFS groups - on all of the relevant elements examined: socio-demographic characteristics, experience of psychosocial stress, clinical symptoms, health status and biological markers. In addition, since the duration of illness has been found to be important for inflammatory markers in CFS, I will explore a link between each biological marker in turn, and the duration of illness. Finally I will examine psychological responses to fatigue, as well as provide some descriptive information concerning attributions of fatigue made by each group.

Based on comparisons between CFS and other chronic health conditions I hypothesise that CFS patients will report greater symptoms, and greater physical disability than all other groups. Patients in the HCV PF group will in turn report greater clinical symptoms and associated functional decline than HCV RF patients and controls. I also hypothesise that CFS patients and HCV PF patients may have similar levels of inflammation, higher than HCV RF patients and controls, though any differences may be relatively small versus acute inflammation. Finally, I hypothesise that patients with CFS will have blunted cortisol versus both HCV groups and healthy volunteers, though HCV PF patients will have lower cortisol measures than HCV RF patients.

2 Methods

2.1 My contribution

This study was conducted as part of a wider, MRC funded study 'Persistent Fatigue Induced by Interferon-alpha: A new Immunological Model for Chronic Fatigue Syndrome'. The study design, protocol, and much of the set-up had been organised by my senior colleagues and the previous study coordinator. On joining the study team in November 2012, I took on partial responsibility for subsequent decisions made regarding the measures used, in collaboration with my senior colleagues on the project. I had sole responsibility for the preparation and submission of subsequent amendments to our ethical approval, and the inclusion and setting up of new sites. I coordinated and assisted with the data collection and initial processing of blood samples (separation of serum/plasma), with support from my colleagues Miss Zuzanna Zajkowska, Miss Alessandra Borsini, Mr David Baumeister and two placement students who I had trained, each of whom spent varying lengths of time working on the project. I was the main contact person for the study, and liaised with all medical professionals involved. I alone coordinated recruitment from the Chronic Fatigue Syndrome clinics. Miss Zajkowska coordinated the healthy control recruitment, with assistance from me as required. Dr Naghmeh Nikkheslat measured Cytokines. Natalie Moll and colleagues in Professor Markus Schwarz's group measured levels of tryptophan and the kynurenine pathway metabolites at the Institute for Laboratory Medicine, Munich University Hospital. Dr Patricia Zunszain, then a senior laboratory coordinator, and later Dr Naghmeh Nikkheslat measured all cortisol samples. All provided raw values for me to work with. I entered data along with Dr Hepgul and Miss Zajkowska, with support from placement students. I alone conducted the statistical analyses contributing to this thesis.

2.2 Prospective HCV (IFN- α) cohort study

2.2.1 Study Design

A prospective cohort design was used to investigate the effects of IFN- α on a range of clinical, health status, and biological measures. Patients were assessed at baseline, also abbreviated to treatment week 0 (TW0), monthly for the first three months, at TW4, 8, and 12, then three-monthly thereafter until the end of their treatment, at TW24, 36, or 48. They were then followed up, and seen again six-months post-treatment.

2.2.2 Participants selection

Patients were recruited from the liver outpatient services at six London hospitals: King's College Hospital, Guy's and St. Thomas' Hospitals, St. George's Hospital, Queen Mary's Hospital and the Royal London Hospital. Eligible patients were adults with chronic hepatitis C virus (HCV) infection who were due to commence antiviral therapy with IFN- α and ribavirin, with or without an additional direct-acting antiviral (Boceprevir, Telaprevir or Simeprevir). Patients were initiated on treatment for 24-72 weeks. This comprised weekly subcutaneous IFN- α injections (1.5 μ g per kg of body weight) and daily ribavirin tablets (800 to 1400 mg orally per day in 2 divided doses). A figure depicting the timetable for triple therapy regimens can be found on page 54. Patients on Boceprevir took 2400mg orally per day in 3 divided doses. They had a four-week 'lead in' of IFN- α and ribavirin before the third drug was added for 24 or more weeks. Patients on Telaprevir took 2250mg orally per day in 2 divided doses in combination with IFN- α and ribavirin for 12 weeks, followed by an additional 12 or more weeks of IFN- α and ribavirin only. Patients on Simeprevir took one 150mg tablet orally per day. Patients had 12 weeks of all three drugs,

plus 12 weeks of IFN- α and ribavirin. Exclusion criteria included age below 18 years, any autoimmune disorder, any cause for liver disease other than HCV, inadequate English language and co-infection with HIV or Hepatitis B. Written informed consent was obtained from all participants after they had received verbal information about the study from a trained researcher, and had read the participant information sheet and had the opportunity to ask questions. The main study was approved by the London Dulwich Research Ethics Committee (REC ref: 12/LO/1368), connected to an earlier study of depression in the same subjects (10/H0808/30). All patients were recruited from September 2010 to April 2015 with the last follow-up data collected in April 2016.

A total of 106 participants were recruited into the study; however it was not possible to include 51 patients, leaving a final sample of 55 (see Figure 2.1). Of this 51, 31 patients did not attend the follow-up visit for an assessment of post-treatment fatigue. A small proportion of patients withdrew, or had insufficient data due to early termination of treatment. Reasons for exclusion were (i) insufficient English language, which had not been apparent when consenting ($n = 1$), (ii) physical health diagnoses received during the course of the study (lung cancer; congestive heart failure; scleroderma; decompensation of the liver; haemochromatosis) and (iv) relapse of prior alcohol dependence. Because of the system for referral, via clinical nurse specialists, it was not possible to collate information on non-eligible cases. Anecdotally, very few patients who were approached declined to participate. Two patients included finished treatment early at 9 and 16 weeks. Both achieved a Sustained Virological Response (SVR) indicating virus clearance six-months post-treatment.

For HCV patients, limited information was available on comorbid conditions, which were not recorded after the clinical nurse specialists had deemed patients eligible. As discussed further in patient characteristics in the results section, ten patients were taking opioids as part of drug rehabilitation programs. Among the other medications taken: two patients were taking steroid inhalers for asthma (beclomethasone); six were taking medication for gastro oesophageal reflux (lansoprazole; omeprazole; ranitidine); seven were taking antihypertensives (irbesartan, losartan, lisinopril, enalapril, amlodipine, prazosin, indapamide); one patient was on a statin (simvastatin). Two patients were taking occasional benzodiazepines for sleep (nitrazepam, lorazepam). One patient was taking occasional diclofenac for pain relief. Four patients were on antidepressants (sertraline, mirtazapine, citalopram), discussed further in the results section. One patient was started on thyroxine during the course of the study. Antidepressants were initiated in a further three patients (SSRIs).

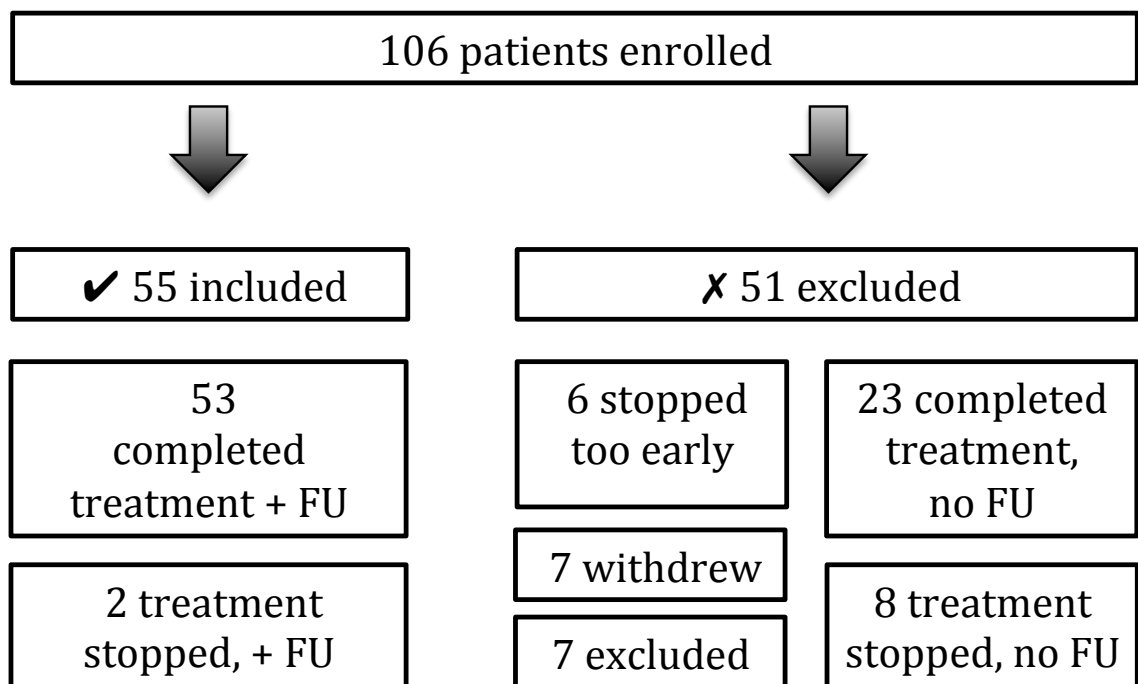


Figure 2.1 Flowchart for HCV recruitment

2.2.3 Clinical data collection at baseline

Baseline measures were obtained to establish baseline function prior to the initiation of IFN- α therapy, and to examine risk factors and/or predictors of IFN- α induced persistent fatigue. Where possible, measures were obtained prior to the first injection. In some cases, due to restrictions in the clinics, measures were obtained immediately afterwards. Baseline fatigue levels were also used to calculate a delta score together with the fatigue score at follow-up six-months post-treatment, to determine whether fatigue had persisted ($FU > BSL$) or resolved ($FU \leq BSL$) post-treatment. A schedule of the measures completed at each visit can be found in Appendix A. Copies of all questionnaires completed can be found in the appendices and are described in section 2.5.

2.2.4 Clinical data collection at follow-up assessments

To monitor changes during treatment, levels of fatigue, depression and anxiety, sleep quality and general health status were measured at all visits. Other measures were completed less frequently. (See Appendix A). Since the end of treatment was different across participants, an 'end of treatment' variable was also created for each relevant measurement. In most cases, this was TW24, 36 or 48. For three individuals it was TW8, TW16 and TW72. At six-months post-treatment, measures were repeated to assess the persistence of side effects, levels of perceived stress, current general health status, cognitive and behavioural responses to symptoms, acceptance of fatigue and attribution of fatigue. At TW24, the end of treatment visit if different, and at six-month post-treatment, measures on alcohol use, socio-demographic characteristics and recent stressful life events were repeated to account for any changes

2.3 Cross-sectional study

2.3.1 Study Design

A cross-sectional study design was then used to compare data collected six-months post-treatment in the prospective cohort study, of HCV patients with persistent fatigue six-months post-treatment (HCV PF), and patients in whom fatigue had resolved (HCV RF), with data from a one-off assessment of Chronic Fatigue Syndrome patients and healthy volunteers, on the full range of biological and behavioural measures available.

2.3.2 Participants selection

2.3.2.1 Chronic Fatigue Syndrome (CFS) patients

Patients were recruited from the CFS specialist clinics at the South London and Maudsley (SLaM) and the Royal Free NHS Foundation Trusts. Eligible patients were adults who had received a diagnosis of CFS according to the Oxford criteria (Sharpe et al., 1991) from a trained clinician at either service. The Oxford criteria state that fatigue must be the main symptom, and must have been present for at least six months. It should be present at least 50% of the time, and be of definite onset (not lifelong). It must be severe, disabling and affect physical and mental functioning. Patients should be excluded if they have a medical condition associated with chronic fatigue, or a diagnosis of schizophrenia, manic depression, substance abuse or an eating disorder. However, other psychiatric disorders, including depression and anxiety, are generally not excluded. Both CFS services ensured other possible diagnoses had been ruled out according to the NICE guidelines (National Institute for Health and Clinical Excellence, 2007).

Exclusion criteria for this study were age below 18 years, any autoimmune disorder, inadequate English language or use of medication known to affect the biological measures examined in this study which they were unable to abstain from taking for the sake of their health. Patients were not included if the case was described as 'atypical' or 'idiopathic' CFS/Chronic Fatigue. Written informed consent was obtained from all participants after they had received verbal information about the study from a trained researcher, and had read the participant information sheet and had the opportunity to ask questions. The same ethical approval applied. Recruitment was from March 2013 to February 2015.

A total of 59 participants were recruited into the study, however it was not possible to include 5 patients (corticosteroid inhalers for asthma; use of antibiotics; insulin dependent diabetes), resulting in a final sample of 54 CFS patients. It was not possible to monitor rates of patients not recruited but for reference the SLAM service would see on average 3 new adult referrals each week, with as many as 8 seen on some of the busier weeks. Rates of patients seen at the Royal Free service, first added as a site in June 2014, were lower as there was only one assessor who was part-time. Though there was interest in the study, many patients declined, as they did not feel able to manage the journey or commit to planning a visit while unwell. Since the service is a specialist service covering a wide area, often patients lived too far away. Efforts were made to accommodate patients through the provision of taxis and flexible visit times, as well as encouraging patients that cancellations were expected given the nature of their condition, measures which were sometimes, but not always, effective.

According to the rates of depression among eligible patients, and the pace of recruitment it was not possible to exclude co-morbid depression. A summary of characteristics relating to the CFS sample specifically can be found below; for medications, the numbers represent total patients reporting use of each medication, and overlap should be considered (see Table 2.1). Additional medications taken included co-codamol and ibuprofen, and low dose benzodiazepines as required (diazepam $n = 1$; clonazepam $n = 1$), though patients were encouraged to avoid use of these medications 48 hours before the appointment where it would not cause unnecessary suffering. Eleven patients were taking receiving hormonal contraceptive medication: 1 patient had a contraceptive coil; one had a sub-dermal implant; and a further 9 were taking oral contraceptives (Combined $n = 4$; progesterone only $n = 4$; information not available $n = 1$). One additional patient had a copper coil. Two patients were taking Hormone Replacement Therapy (Climaval, prog only; Climagest, combined).

Apart from depression (see Table 2.1), common comorbid conditions included fibromyalgia ($n = 6$), headache, including migraine ($n = 4$) and Irritable Bowel Syndrome (IBS; $n = 3$). Two patients were taking anti-hypertensives (Ramipril; Irbestartan). Since current depression as assessed by the MINI (see section 2.5.2) or a formal diagnosis of depression were exclusions for both the HCV cohort for most of the study, and healthy volunteers throughout, any analysis of rates of depression in the four groups are descriptive only, and should not be taken as indicative of higher rates of depression in the CFS group. Analyses of levels of depressive symptoms using the Inventory of Depressive Symptomatology (IDS) measure should also be considered with this in mind.

Table 2.1 CFS specific sample characteristics

	CFS patients (<i>n</i> = 54*)
Duration of symptoms	
Months, self-report	83.6±12.2
<3 years	20 (39%)
≥3 years	31(61%)
Depression status***	
Neither	21 (39%)
Not Depressed, <i>taking AD*</i>	15 (28%)
Depressed, <i>taking AD*</i>	10 (19%)
Depressed, <i>no AD</i>	7 (13%)
Information not available	1 (2%)
Medication (see also text)	
Citalopram	8
Fluoxetine	5
Sertraline	5
Amitriptyline (<i>depression/sleep</i>)	4
Venlafaxine	1
Nortriptyline (<i>sleep</i>)	1
Propranolol	2^
Trazodone	2
Bupropion (<i>smoking cessation</i>)	1
Lofepramine (<i>migraine</i>)	1
Pregabalin	6
Gabapentin	2
Naproxen	2
Tramadol	2

Note - * information not available in *n* = 3 patients ** some patients had been prescribed antidepressants primarily for their effects on pain and sleep *** % rounded up/down ^ in both cases, used in addition to citalopram

2.3.2.2 Healthy control participants

Healthy controls were recruited from a number of sources: advertisements placed on the website 'Gumtree' (www.gumtree.co.uk); a circular email sent out to all staff and students of King's College London; leaflets placed in local community settings and through word of mouth. Volunteers were invited to attend a one-off visit after passing an initial telephone screening confirming that they had no significant health conditions, were not taking any regular medication in the last three-months except for contraception, had not experienced a recent or recurrent episodes of mental illness, and had no past or present substance abuse or dependency. A total of 64 participants were recruited into the study, however it was not possible to include 7 patients, resulting in a final sample of 57 healthy control participants. Reasons for exclusion included information obtained at the visit which was not disclosed during screening (suicidal ideation; disclosure of Hepatitis B; excessive alcohol consumption query dependence; use of regular antibiotics and/or steroids for recurrent mouth ulcers); suspected current drug use identified through refusal to respond to a drug questionnaire and behavioural clues; excessive fatigue with an unknown cause and refusal to respond to a large proportion of questionnaires.

Four healthy control participants were in receipt of hormonal contraceptives: progesterone only oral, implant and coil ($n = 1$ each), and a combined hormone patch ($n = 1$). Three participants reported occasional use of paracetamol and ibuprofen for headaches, and diazepam for sleep, but participants were asked to refrain from using these medications 48 hours before the study visit. One patient was using hydrocortisone (1%) for a small patch of eczema on her face.

2.3.3 Clinical data collection

Data from measures obtained at the follow-up visit six-months post IFN- α treatment, and additional information regarding risk factors obtained at the baseline visit were used for HCV patients, as described earlier. CFS patients completed a one-off assessment incorporating all measures. Healthy control participants completed a one-off assessment incorporating all measures except for the Cognitive and Behavioural Responses to Symptoms Questionnaire, and the attribution question concerning the nature of their symptoms (see Appendix S) as these measures were patient/illness specific measures.

2.4 Data analysis

All data were analysed with IBM SPSS statistical software version 22. Where data is presented graphically, unless stated, the mean score is presented with error bars representing the standard error of the mean (SEM). The significance value for all tests was set at $\alpha = 0.05$. Throughout the thesis, significant test results are in **bold** font, and trends are underlined. For all relevant analyses, associations between two continuous variables were explored using Pearson's product moment correlations (Pearson's r ; r) where data for both variables was normally distributed, or Spearman's rank correlation co-efficient (Spearman's Rho; r_s) where either was not. For cross-sectional comparisons of two groups, differences in continuous variables were analysed using independent samples t-tests. Where equal variance could not be assumed, as assessed by the Levene's test ($p < 0.05$) the results reported are those where the degrees of freedom have been adjusted using the Welch-Satterthwaite method. For all categorical variables, chi-squared (χ^2) tests were used.

For the effect of IFN- α in the whole sample, paired t-tests were used to examine changes in variables between time points. To explore whether there were differences in the response to IFN- α treatment in persistent fatigue (PF) versus resolved fatigue (RF), for clinical measures and measures of health status, independent t-tests were conducted on both the scores at each time point, as well as the change in the level (delta score; Δ) relative to baseline functioning. This allowed for the examination of all data collected in all individuals, since some data points were missing.

To examine changes over the same period in cytokine levels, cost considerations meant that only the samples of those patients who had been seen at TW0, 4, 24 and FU (six-months post-treatment) were analysed. In this case a series of repeated measures analyses of variance (ANOVAs) were conducted, and where appropriate, pairwise comparisons corrected for multiple comparisons using the Bonferroni correction are reported. Where Mauchly's test indicated that the assumption of sphericity was violated ($p < 0.05$), results reported are those with the Greenhouse-Geisser [G-G] correction to the degrees of freedom applied. Attempts to address the positive skew through transformation of the data were not successful; where a group difference was found in the analysis of the raw data, additional post-hoc independent t-tests and Mann-Whitney U tests were conducted, and the results from the two tests compared.

For kynurenine pathway metabolites, data collected at TW0, TW8 and TW24 was considered, and again a series of repeated measures ANOVAs were conducted. There was insufficient data from the follow-up visit to include in the repeated measures analysis, however this data was evaluated in the cross-sectional comparison described in the next section (2.2). It was possible to address the positive skew of the kynurenine data using logarithmic transformation (Log_{10}), and an ANOVA was repeated on transformed data, for comparison. Where used, both analyses are reported. Finally, since in the case of the kynurenine pathway a larger data set was available, using further t-tests, cross-sectional comparisons of levels in the two groups at each time point as well as of delta scores relative to baseline were also conducted.

For the cross-sectional comparisons of the four groups, for continuous variables a series of one-way ANOVAs were performed. Where there was a difference between groups, post-hoc comparisons were conducted with Tukey's Honest Significant Difference (HSD) tests. Where the Levene's test result indicated that equal variance could not be assumed, the Welch's ANOVA was conducted instead, and post-hoc comparisons were made with the Games-Howell post-hoc tests, a non-parametric post-hoc test which accounts for unequal variance. For the cytokine analysis, as reported previously attempts to address the positive skew of the data were not successful. If group differences were observed, the same strategy of running additional post-hoc tests was employed. For kynurenine pathway data, ANOVAs were again repeated on data transformed using the same logarithmic method, and where used both sets of analyses are reported. Additional correlational analyses and independent samples t-tests were conducted to examine the association between biological measures and the duration of illness in CFS patients.

2.5 Questionnaire Data

This section includes all questionnaires administered for both the prospective cohort and cross-sectional studies. As described above, the questionnaires shown below were administered according to the schedule shown in Appendix A for HCV patients, and at a one-off assessment of CFS patients and controls.

2.5.1 Socio-demographic Data (SDS)

Socio-demographic data relating to age, gender, self-rated ethnicity, level of education reached, current relationship status and current employment status were collected at baseline or the one-off assessment using a modified version of the MRC socio-demographic schedule (Mallett et al., 2002). (See Appendix B). The sample size and low numbers necessitated the collapsing of some of the categories. As such, I created some dichotomised variables: White British/Irish versus other ethnicity, university degree versus other level of education, living with or married to partner versus other categories for relationship status, and unemployed versus other categories of work status.

2.5.2 Depression (Mini International Neuropsychiatric Interview; MINI)

The MINI was administered at baseline or the one-off assessment, to diagnose a current depressive episode or a previous history of MDD. The MINI was then repeated to assess for current depression where a score of ≥ 7 was recorded for either of the Hospital Anxiety and Depression Scale (HADS) subscales: depression or anxiety. A positive diagnosis of major depressive disorder on the MINI was then used to determine whether a patient had developed IFN- α induced depression during treatment. The MINI is a structured diagnostic

interview for psychiatric disorders according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) and the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10). With an administration time of approximately 15 minutes, the MINI was designed to meet the need for a short yet accurate, structured psychiatric interview for use in multi-centre clinical trials and epidemiology studies, and to be used as a first step in outcome tracking in non-research clinical settings (Sheehan et al., 1998). The full interview includes diagnoses of 19 disorders. For the purpose of this study I used only the section relating to major depression. (See Appendix C). All researchers were trained in administering the MINI with the use of training videos. The training videos show actors simulating symptoms of depression that are consistent with the information assessed by the MINI.

2.5.3 Family history of psychiatric illness (FIGS)

A family history of mental illness was obtained using a modified version of the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992). (See Appendix D). Patients considered any first-degree relatives aged 18 or above at the time of the episode, and reported “yes” or “no” to whether their family members had had a particular experience of mental illness as described by the questionnaire, also noting their relationship to the participant. A dichotomised variable was used ‘yes/no’ according to whether patients had responded ‘yes’ to one or more questions.

2.5.4 Drug use (Cannabis Experience Questionnaire)

To summarise past and present drug use, a modified version of the Cannabis Experience Questionnaire was used (Section 2 of CEQ) (Barkus et al., 2006). (See Appendix E). It is an overview of the individual's lifetime pattern of substance use, including questions on age at first use and frequency of use of substances including cannabis, amphetamines and opioids. Most of the HCV patients had used drugs in their lifetime, and as such I chose to focus on reporting and comparisons of the use of (i) opioids, (ii) alcohol and (iii) smoking. For alcohol and smoking, I coded use as: past use (0), current use (1) or never used (2). For opioid use in the HCV analysis only, I created two variables. First, I considered current use of opioids as part of drug rehabilitation programs: (0) past use of opioids for recreational purposes (1) past use + current use for rehab and (2) never used. Second, I considered past recreational use of opioids specifically: never used versus past recreational use, including those currently on opioids as part of rehabilitation programs. For the cross-sectional comparison, since no other groups contained individuals on rehab programs, I examined the past recreational versus never variable only.

2.5.5 Alcohol Use (AUDIT)

The Alcohol Use Disorders Identification Test (AUDIT; (Saunders et al., 1993) was used to capture additional information on alcohol use. It was repeated in HCV patients. (See Appendices A and F). Higher scores indicate greater hazardous/harmful use of alcohol. In the original paper, a score of ≥ 8 characterised most patients (92%) with a formal diagnosis of hazardous or harmful use.

2.5.6 Recent Life Events (Brief Life Events scale)

The Brief Life Events (BLE) scale was administered to assess recent stressful events (Brugha & Cragg, 1990). (See Appendix G). This is a self-report questionnaire examining the incidence of different categories of negative life events over the previous 6 months. It assesses life stressors involving moderate or long-term threats such as illness or injury, the death of a close friend or relative, unemployment, financial loss and loss of important relationships. A dichotomised variable was created with 0 if no life events were experienced in the previous 6 months, and 1 if one or more type of life event was experienced in the previous 6 months prior to baseline. Recent experience of stressful events was assessed at baseline, TW24 and end of treatment if different, and six-months post-treatment in HCV patients, and at the one-off assessment of CFS patients and healthy controls.

2.5.7 Intrusive Life Events (schedule; ILES)

The Intrusive Life Events schedule (ILES) was used to examine lifetime experience of intrusive (traumatic) life events (Bebbington et al., 2004). Participants were asked to report whether they had ever experienced each event on the list. (See Appendix H). Dichotomised variables were created for each event to determine whether each individual had experienced that event in their lifetime, yes or no. The variable analysed was then the dichotomised variable created according to whether participants reported yes to one or more events.

2.5.8 Childhood Experiences of Care and Abuse (CECA-Q)

A modified version of the Childhood Experiences of Care and Abuse Questionnaire (CECA-Q) was used to collect information about childhood trauma. (See Appendix H). The CECA-Q is a self-report measure designed to elicit information concerning childhood experiences before the age of 17, including information about parental loss, separation from parents for 6 months or more, physical and sexual abuse (Bifulco et al., 2005). Cut-off points were used to dichotomise responses on physical and sexual abuse variables. Using the cut-off points published by Bifulco et al., physical abuse was defined as repeated exposure to physical violence, from either the main mother or main father figure before the age of 17 years. In order to be considered 'severe', these incidents had to meet at least two of the following criteria; (a) being hit with a belt/stick or being punched or kicked; (b) resulting in an injury, including broken limbs, black eyes or bruising; (c) the perpetrator was considered to be out of control. Mild forms of punishment such as being smacked or hit with a slipper were excluded. A participant was considered as having experienced sexual abuse if at least one of the screening questions for sexual abuse had been answered 'yes' ("When you were a child or teenager, did you ever have any unwanted sexual experiences?" "Did anyone force you or persuade you to have sexual intercourse against your wishes before age 17?", "Can you think of any upsetting sexual experiences before age 17 with a related adult or someone in authority, e.g., a teacher?").

A composite variable was created by adding together presence of one of the four dichotomised variables considered (loss of parents, separation from parents for 6 months or more, severe physical abuse and presence of sexual abuse); the score of this variable ranged from 0 (absence of any childhood

trauma) to 4 (presence of all four types of childhood trauma investigated). This variable was then further dichotomised as 0 if no childhood trauma was experienced and 1 if one or more type of childhood trauma was experienced.

2.5.9 Illness Perceptions (IPQ-R)

The revised Illness Perceptions Questionnaire (IPQ-R) is made up of eight subscales, which measure the components of illness representation specified in Leventhal's self-regulatory model of illness. The five original overarching dimensions assess identity (the symptoms the patient associates with the illness), cause (personal ideas about aetiology), timeline (the perceived duration of the illness), consequences (expected effects and outcome), and cure control (beliefs about potential for cure and control of the illness) (Weinman et al., 1996). The additional subscales introduced in the revised questionnaire (IPQ-R) being used here assess illness coherence and emotional representations of the illness (Moss-Morris et al., 2002). (See Appendix J). Higher scores indicate a strong illness identity, stronger beliefs that the illness is chronic, that it is cyclic in nature, that it has serious consequences, that control or cure is possible (treatment or personal), that they have a good understanding or comprehension of their illness, and that they have strong emotional representations of their illness.

2.5.10 Fatigue (Chalder Fatigue Questionnaire)

The CFQ has been widely used to measure the severity of fatigue and consists of 11 questions measuring fatigue-related symptoms over the previous month, compared to usual. (See Appendix K). The CFQ contains 7 items that address physical fatigue and 4 items that address mental fatigue. Items are scored on a 0-3 scale – 0 = less; 1 = no more than; 2 = more, 3 = much more than usual - giving total scores ranging from 0-33 with higher scores indicating more fatigue (Chalder et al., 1993). Scores of ≤ 18 are considered to be within normal range for fatigue, and on this basis I refer to fatigue “caseness” as a CFQ score of >18 (Cella & Chalder, 2010).

2.5.11 Depressive symptoms (Inventory of Depressive Symptomatology)

The IDS is a 30-item questionnaire asking subjects to rate how they have felt over the past seven days, assessing the frequency, duration and/or severity of a range of depressive symptoms (Rush et al., 1996). . (See Appendix L). The scale assesses the 9 symptom domains required for a DSM-IV diagnosis of a major depressive episode. It includes items to assess melancholic and atypical symptom features, as well as associated symptoms such as fatigue, pain and anxiety. The IDS allows for the detection of milder levels of depression, and items are scored on a 0-3 scale. Respondents are instructed to answer either question 11 or 12 (decreased appetite or increased appetite), and 13 or 14 (weight loss or weight gain). As such, the total score range is 0-84 with higher scores indicating greater symptom severity. The authors have suggested the following severity indications: <12 , normal; 13-23, mild; 24-36, moderate; 37-46, moderate-severe and >47 , severe.

2.5.12 Anxiety symptoms (Hospital Anxiety and Depression Scale)

The HADS is a 14-item self-report questionnaire designed to screen for the presence and severity of depression and anxiety symptoms in medical patients over the past week (Zigmond & Snaith, 1983). This questionnaire comprises a 7-item depression subscale and a 7-item anxiety subscale. Preferring to use the aforementioned IDS according to the range of depressive symptoms measured, for the purpose of this study I focused only on the anxiety subscale in my analyses (items highlighted in Appendix M). However, as indicated above, the full questionnaire was completed as part of the aims of the wider study within which this work was collected. The depression subscale was also used, alongside the anxiety subscale, to determine whether or not the MINI should be completed. Items on the HADS are scored on a 0-3 scale, and each subscale of 7 questions is summed to give a total score range of 0-21. Scores in the range of 0-7 are considered normal; 8-10, mild, 11-14, moderate, and 15- 21, severe. Therefore, where 'above-normal' scores were indicated, the MINI was completed to assess for a major depressive episode (see section 2.5.2).

2.5.13 Perceived Stress (Scale; PSS)

The 10-item Perceived Stress Scale (PSS) measures the degree to which situations in one's life are appraised as stressful (Cohen & Williamson, 1988). In this study, the 10-item version was used. Each item asks the subject to rate how often they have experienced each scenario over the last month (e.g. difficulties piling up so high that you could not overcome them). (See Appendix N). It uses a 5-point scale (0=never to 4=very often). Four items are positive scenarios and are reverse-scored. The sum score is then used (range 0-40).

2.5.14 Sleep quality (Jenkins Sleep Scale)

The Jenkins Sleep Scale is a brief scale evaluating the frequency of a series of common categories of sleep disturbance experienced by the responder in the last month (Jenkins et al., 1988). (See Appendix O) Participants respond to four items on falling asleep, waking several times in the night, trouble staying asleep, and unrefreshing sleep. Participants indicate on how many days in the last month they have experienced each problem on a five-point scale: not at all (= a score of 0), 1-3 (=1), 4-7 (=2), 8-14 (=3), 15-21 (=4) or 22-31 days (=5). A sum score is then calculated.

2.5.15 Health Status (Medical Outcomes Survey, Short-Form 36; SF-36)

The SF-36 is a self-report measure of general health and has been extensively used to assess health status in patients with mood and anxiety disorders. (See Appendix P). (Ware et al., 1993). It assesses both physical and emotional wellbeing based on how an individual has functioned over the previous 4 weeks. The SF-36 assesses 8 primary dimensions: physical functioning, physical role limitation, bodily pain, social functioning, mental health, emotional role limitation, vitality (energy versus fatigue) and general health. The items are scored either as “yes/no” or on 3, 5 or 6-point scales. The 8 subscales have score ranges of 0-100, where higher scores indicate better health status.

2.5.16 (Lack of) acceptance

The first measure related to psychological responses to fatigue is the 'acceptance of fatigue' questionnaire, a modified version of the Chronic Pain Acceptance Questionnaire (CPAQ) for use in CFS patients (Brooks et al., 2011; McCracken et al., 2004). The CPAQ is divided into two factors: pain willingness, and activity engagement. The modified version (see Appendix Q) only covers 'fatigue willingness' and includes 9 items that are measured on a scale 0-6 according to how often the statement given is 'true' of the respondent, where 0 = never true, and 6 = always true. 'Willingness' relates to the recognition that avoidance and control of symptoms are often not possible, which translates to a reduced need to attempt to avoid or control fatigue. Conversely, a higher score indicates a lack of acceptance.

2.5.17 Cognitive and Behavioural Responses to Symptoms (CBSQ)

The second measure is the Cognitive and Behavioural Responses to Symptoms Questionnaire (CBSQ) (Skerrett & Moss-Morris, 2006). (See Appendix R). There are five cognitive subscales: fear avoidance, embarrassment avoidance, catastrophising about symptoms, beliefs that symptoms signal damage to the body (damage beliefs) and symptom focus. The two behavioural subscales measure resting and avoidance of activity, and all-or-nothing behaviour. All items are scored 0-4; either corresponding to the degree with which the respondent agrees with the statement - 'strongly disagree' to 'strongly agree' – or to the frequency with which they adopt behaviours to manage their symptoms - 'never' to 'all the time'. A sum score is then calculated for each subscale from the related items. Only the patient groups completed this measure.

2.5.18 Attribution of fatigue

Two sets of questions were used to assess the attribution of fatigue. The first concerns the attribution of fatigue to a range of factors, including treatment, psychosocial stress, and infective items. (See Appendix S). This is a modified version of a set of questions used in the study of CFS and PVFS, updated to include 'current treatment' to accommodate IFN- α (Chalder et al., 1996; Sharpe et al., 1992). Participants were asked to decide whether each variable was 'not a factor', 'might be a factor' or was 'definitely a factor' in having caused their fatigue or made it worse. For the purpose of this study, the proportion of each group attributing fatigue to each factor is reported descriptively only. These questions were asked of all individuals as part of the cross-sectional study only, where patients had identified experiencing at least one problem in the Chalder Fatigue Questionnaire (CFQ) more than usual, or much more than usual (see Appendix K). The second question concerns the nature of symptoms, and the degree to which the symptoms are physical or psychological in nature, as used in an earlier study of CFS (Powell et al., 1990). This is a single item and participants are asked to choose one response from the five available options, which range from symptoms 'are physical' to 'are psychological' in nature. Again, data obtained was used descriptively only. Since this item is symptom focussed, only the patient groups completed this measure.

2.6 Laboratory methods

2.6.1 Cytokines

Cytokines were measured in serum collected at baseline (TW0), TW4, TW24 and at follow-up six-months post-treatment in the HCV IFN- α cohort, or at the one-off assessment of CFS patients and healthy volunteers. Blood samples were collected using 6ml BD vacutainer plastic tubes (silica clot activator) and left to clot for at least 30 minutes at room temperature. Following this period, the blood was centrifuged at 1850g for 10 minutes at room temperature, after which the serum was separated and stored in aliquots at -80°C for analysis later. All candidate cytokines were measured in the serum samples using Meso Scale Discovery (MSD) V-PLEX sandwich immunoassays according to the manufacturer's instructions. MSD Pro-inflammatory Panel 1 (human) kits were used for the measurement of IFN- γ , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNF- α , and a custom Cytokine Panel 1 (human) kit was used for the measurement of IL-7, IL-17A and VEGF.

Each plate is pre-coated with capture antibodies. Patient samples are added to individual wells, together with a solution containing detection antibodies conjugated with electrochemiluminescent labels ("sulfo-tag", an MSD trademark). Each analyte in the sample binds with the capture antibodies on the plate, then, in turn, the "sulfo-tag" labelled detection antibodies bind to their respective analyte, thus creating the "sandwich". A buffer is then added which allows for reading of the electrochemiluminescent labels, before the plate is inserted into the MSD instrument, where a voltage is applied to the electrodes within the plate, causing light to be emitted. The intensity of the light emitted provides a quantitative measure of the level of each cytokine in the sample.

First, the lyophilised calibrator mix was reconstituted. Eight standards (or 'calibrators') were created by performing a series of four-fold dilution steps, with a final 'zero-calibrator' or blank consisting of undiluted diluent. Patient samples, and where appropriate, controls, were diluted two-fold. Standards, diluted sample or controls were added to each well. All samples were measured in duplicate. Plates were incubated at room temperature for 2 hours, on a horizontal orbital microplate shaker at 700rpm, and then washed three times with wash buffer, before the detection antibody solution was added to each well. After an additional incubation at room temperature for 2 hours, and a further washing step, read buffer was added to each well. Finally plates were inserted into the instrument for reading.

With few exceptions, all samples from the same patient were analysed together in the same plate. Each pair of plates (pro-inflammatory panel / customised cytokine panel) were analysed together so that the full range of analytes were measured in each patient sample in one session. High and low controls were used to assess variance between plates, where indicated by different lot numbers. The inter-assay coefficient of variations were <10%. Cytokines were selected according to the aims of this study, and an overlapping project of depression development in the HCV cohort. Due to cost it was not possible to analyse all markers in all participants (see results section). Some cytokines could not be measured: of the 206 samples analysed for IL-4 levels, 74 samples (36%) recorded a result of "not detected", and a further 69 samples (33%) recorded a result that was lower than the validated lower limit of detection (69% in total). Similarly, for IL-1 β levels, 167 samples (81%) recorded a result of "not detected", and so it was not possible to analyse either of these markers.

2.6.2 Kynurenine pathway

Levels of tryptophan and the kynurenine pathway metabolites kynurenic acid, quinaldic acid, 3-hydroxykynurenine (3-HK), xanthurenic acid, picolinic acid and quinolinic acid were measured in plasma collected at baseline, TW8, TW24, and end of treatment if different, as well as six-months post-treatment in HCV patients. In CFS patients and healthy controls it was collected at the one-off assessment. Ratios of kynurenine to tryptophan and 3-HK to kynurenine were then calculated by dividing one by the other and multiplying the value by 100. Levels of 5-hydroxy-L-tryptophan and 5-hydroxyindole-3-acetic acid were also measured but were not detectable. Measures of anthranilic acid and 3-hydroxyanthranilic acid (3-HAA) had a high coefficient of variation and were not analysed. Raw values for kynurenine levels in some patients were above the calibration limit, and the decision was made not to include this data in the analyses, with the kynurenine/tryptophan ratio instead representing increases in kynurenine.

Blood samples were collected using 9ml VACUETTE® plasma separation, sodium heparin tubes. On arrival at the laboratory the samples were centrifuged at 500g for 10 minutes at room temperature, and then plasma was separated and stored in aliquots at -80°C for analysis later. After thawing, analysis of the metabolites in plasma was conducted using liquid chromatography with tandem mass spectrometry (LC-MS/MS). The chromatographic system was composed of a Waters Acquity UPLC separations module connected to a Xevo TQ MS triple-quadrupole mass spectrometer, equipped with a Z-spray ESI ion source (Waters Corp., Milford, MA, U.S.). Separation was carried out using a Kinetex XB-C18, 2.6 µm, 2.1 x 150 mm column (Phenomenex, Torrance, California, U.S.). Reagents for protein precipitation, derivatisation, and chromatography

were purchased from Sigma-Aldrich (St. Louis, MO, U.S.) and Biosolve (Valkenswaard, NL). Tryptophan, 3-hydroxykynurenine (3-HK), kynurenic acid, xanthurenic acid, picolinic acid, quinaldic acid and quinolinic acid were purchased from Sigma-Aldrich (St. Louis, MO, U.S.). Standards and controls were established by adding defined volumes of the stock solutions to a human plasma obtained from a blood bank. Quality control samples were generated to obtain low and high amounts of the analytes.

A total sample volume of 300 µl plasma was used. Analytes were extracted from samples and calibrators/controls by adding 50 µl of 2.0 M urea and 50 µl of internal standard solution containing KYNA-D5, PIC-D4 and TRP-D5 as internal standards. Two precipitation steps by subsequently adding methanol/ethanol and acetonitrile were carried out. The supernatant was separated into two portions, which were evaporated separately. One of these portions was directly reconstituted in mobile phase, while the other portion was used for derivatisation by addition of HCl/Butanol. After evaporation to dryness, this portion was reconstituted in mobile phase, too. 7.5 µl of the reconstituted samples/calibrators/controls were loaded onto the LC-MS/MS system. 3-HK, quinolinic acid and picolinic acid were analysed from the derivatized sample, while all other analytes were determined from the underivatized sample. A gradient method with a total duration of 7.5 min was used for chromatographic separation. Mobile phase A was composed of 0.1% formic acid and 0.01% HFBA in water; mobile phase B was methanol. Flow rate was set at 0.25 ml/min, column temperature was set at 30.0 °C. Retention times for the analytes were between 3.1 and 6.0 min.

The Xevo TQ MS was operating in atmospheric pressure electrospray ionization in positive mode (ESI+). Ion source settings were: capillary voltage, 1.00 kV;

desolvation temperature 650°C; source temperature, 150°C; nitrogen was used as desolvation gas with a API gas flow rate of 1200 l/h; argon was used as collision gas at a flow rate of 0.15 ml/min. The analytes and internal standards were detected using multi reaction monitoring (MRM) technique. System operation, data acquisition and data processing were controlled using MassLynx V4.1 software (Waters, Milford, USA). Natalie Moll in the research group of Professor Markus Schwarz, University of Munich, Germany conducted these analyses.

2.6.3 Salivary cortisol

Saliva samples were collected to measure salivary cortisol, at baseline and six-months post-treatment in the HCV cohort, and in a one-off assessment of CFS patients and healthy controls using a salivette device (Sarstedt, Leicester, UK) in which saliva is absorbed. Subjects were instructed to collect saliva samples by chewing on the pad, described in materials as cotton wool but consisting of a synthetic pad. Samples were collected at 6 time points in a single day; immediately after awakening (0 minutes), +15, 30 and 60 minutes after awakening, and at 12pm and 8pm. Subjects were instructed to wake up before 10 am, and to have the sample available to them beside their bed to take immediately. They were advised not to have breakfast or brush their teeth during the first hour of awakening, or in the 30 minutes before taking the samples at 12pm and 8pm. This is in order to avoid falsely high cortisol values due to plasma exudates from minor bleeding in the oral cavity, or from meal-stimulated rises in cortisol. During collection, subjects were instructed not to touch the samples with their hands. "Information sheets" were provided to guide participants through the process, including all necessary instructions, and also

obtain real time information on the times samples were collected, any accidental consumption of food or drink in the prohibited periods, and notes on any difficult or tense situations (see Appendix U). Samples were kept in the refrigerator immediately after samples were taken to provide some protection from bacterial growth, left there overnight and then collected at the visit appointment or sent back to the laboratory in the morning using the return padded envelope provided.

On arrival at the laboratory samples were frozen at -20°C . After thawing, saliva samples were centrifuged at 3000rpm for 15 minutes at room temperature, which resulted in a clear supernatant of low viscosity. Determination of cortisol levels was achieved using the High Sensitivity Salivary Cortisol ELISA KIT from Salimetrics, following the recommended procedure. Briefly, 25 μl of saliva and standards were assayed in duplicates, by incubation on a microtitre plate coated with monoclonal antibodies against cortisol. Cortisol linked to horseradish peroxidase was then added, to compete with cortisol in the standards and unknowns for the antibody binding sites. After incubation, unbound components were washed away and bound cortisol peroxidase measured by reaction of the peroxidase enzyme on the substrate tetramethylbenzidine. The amount of cortisol peroxidase detected, as measured by the intensity of colour developed, is inversely proportional to the amount of cortisol present. Optical density was read at 450 nm with correction at 620 nm, using a PHERAstar 'FS' (BMG Labtech, Aylesbury, UK). Values of cortisol were calculated using SoftMax Pro 4.8 software, following a 4-parameter fit. The inter-assay co-efficient of variations were $<10\%$. Dr. Patricia Zunszain and later Dr Naghmeh Nikkheslat, both in my research group, conducted all of the cortisol analysis.

To investigate the cortisol response to awakening, I calculated the Area Under the Curve of the with respect to the increase (AUCi), the recommended calculation for this measurement (see Figure 2.2) (Pruessner et al., 2003; Stalder et al., 2016). This study included measures of cortisol levels after awakening, considering the changes in cortisol levels from baseline (0 minutes) to 15, 30, and 60 minutes after awakening. Time differences between time points were standardised in the analyses. Any sample where there had been poor compliance with the protocol in general, or at these sample times specifically was excluded from the analysis.

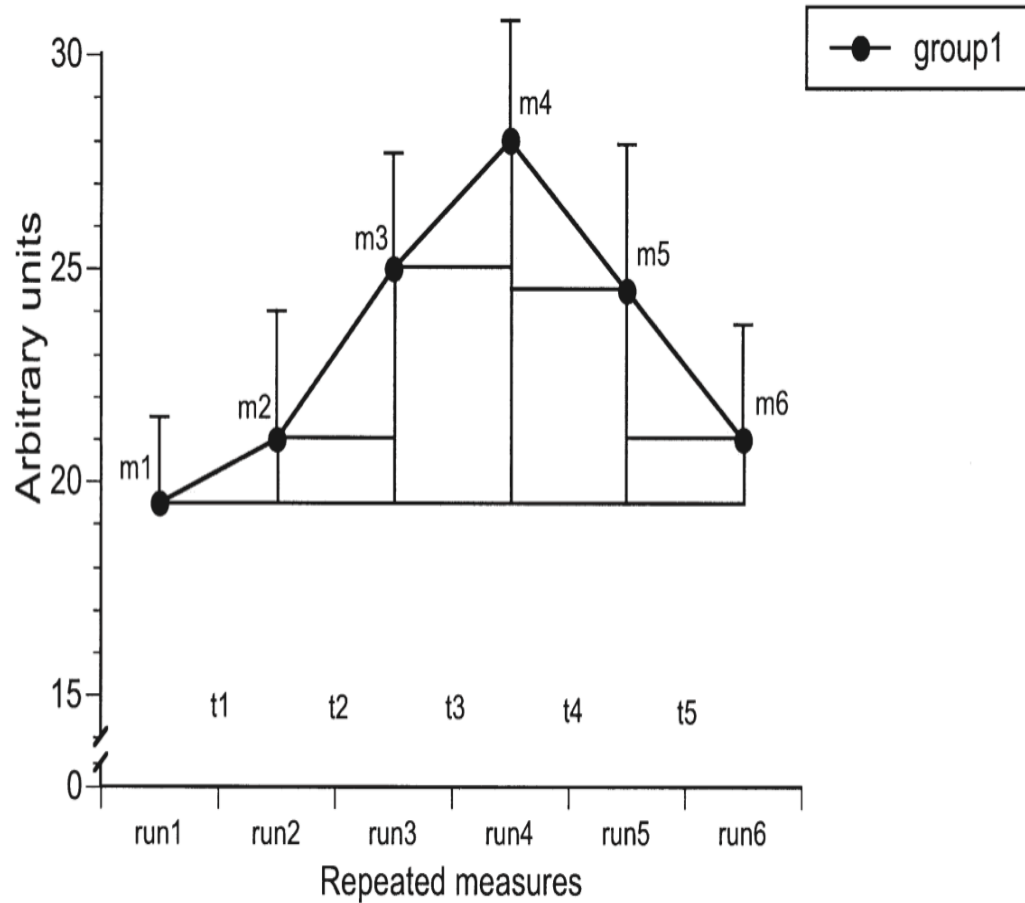


Fig. 2. Time course of group 1 over the six measurements; the triangles and rectangles illustrate the composition of the area under the curve with respect to the increase (AUC_i). m_1 to m_6 denote the single measurements, and t_1 to t_5 denote the time interval between the measurements. Note that although in this example, the time interval between the measurements is identical for all observations, individual time intervals can vary depending on the study.

Figure 2.2 Area Under the Curve with respect to the increase (AUC_i)

(Taken from Pruessner et al. 2003)

To investigate the cortisol levels during the day, I calculated the Area Under the Curve with respect to the ground (AUCg) of cortisol levels at 0 minutes after awakening, at 12pm and at 8pm. Another example using six time points, versus our three, can be seen in Figure 2.3. Both formulas for the calculation of the AUCs were derived from the trapezoid formula (Pruessner et al., 2003). Time difference between these points was calculated per patient and included in the formula, to reflect the different awakening times. Again, poor compliance with the protocol in general, or at the relevant sampling points resulted in exclusion from the analyses. On some occasions, participants had complied with the protocol at some sampling points, but not others. In this case they may have been included for one of the two analyses conducted at each time point. Participant numbers for each analysis are given separately in the results section.

For the analysis of cortisol levels, additional HCV patient samples had been collected during IFN- α treatment. However, patient engagement with this part of the study protocol was poor. Of those patients who did complete samples, poor compliance with the protocol meant that the number of samples that could be included was low. In addition, very few patients completed multiple measures, making any interpretation of changes in response to IFN- α difficult. Therefore, I examined cortisol in cross-sectional analyses only, using samples obtained at baseline and six-months post-treatment for HCV patients, and from the one-off assessment by CFS patients and healthy controls. I examined baseline cortisol as a predictor of IFN- α induced persistent fatigue, and the six-months post-treatment measure as part of the cross-sectional study only.

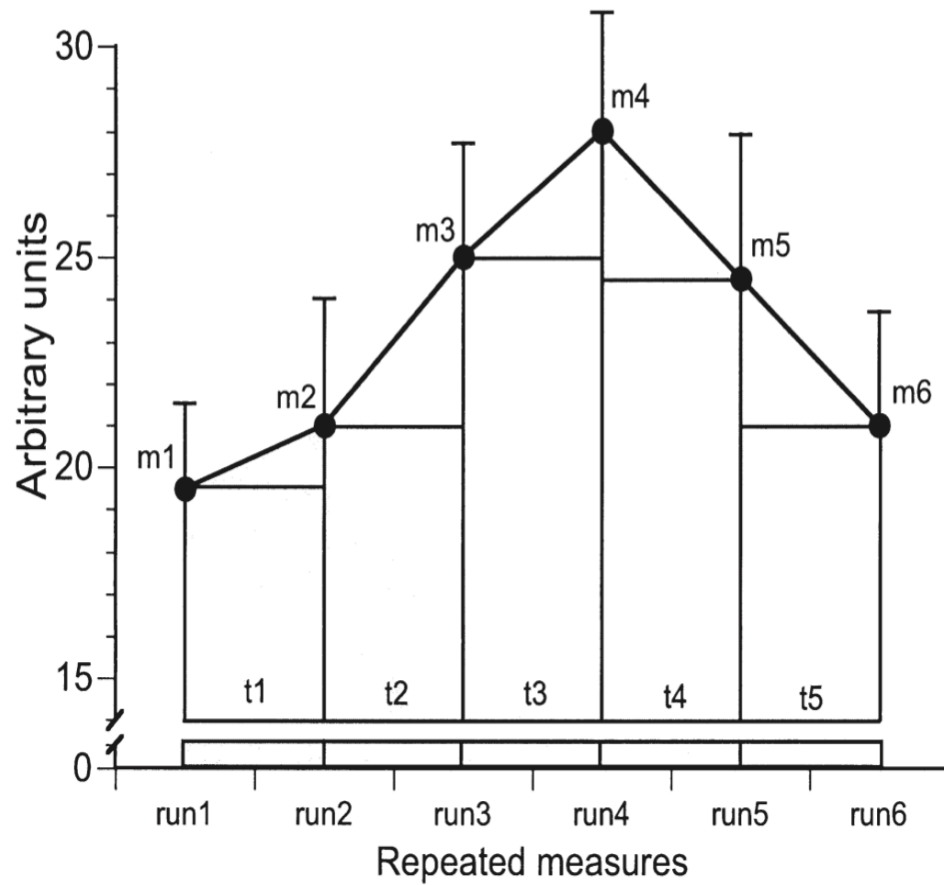


Fig. 1. Time course of an artificial dataset with six measurements; the triangles and rectangles illustrate the composition of the area under the curve with respect to ground (AUC_G). m_1 to m_6 denote the single measurements, and t_1 to t_5 denote the time interval between the measurements. Note that although in this example, the time interval between the measurements is identical for all observations, individual time intervals can vary depending on the study.

Figure 2.3 Area Under the Curve with respect to the ground (AUC_G)

(Taken from Pruessner et al. 2003)

2.6.4 Time from sampling to analysis

Because the study was conducted in stages, it was necessary to explore differences in the time from sampling to analysis between the groups. Cortisol was analysed regularly in batches within six-months of receipt, after storage at -20°C (Stalder et al., 2016). Therefore only days in storage to analysis of cytokines and the kynurenine pathway measures were considered. For HCV patients, cytokine measurements were conducted with all samples collected from one patient analysed at one time, with very few exceptions. I examined the number of days in storage before analysis to see if there was a difference between the two groups (RF vs. PF). I conducted t-tests for two variables: days in storage to analysis of baseline samples, and follow-up (six-months post-treatment) samples. There were no differences between groups in days in storage to analysis of baseline (RF vs. PF, Mean \pm SEM days: 1291 ± 71 vs. 1406 ± 134 ; $t(47) = -0.83$, $p = 0.41$), or follow-up samples (902 ± 69 vs. 974 ± 127 ; $t(45) = -0.55$, $p = 0.59$).

The analysis was then repeated for the cross-sectional comparison. Data regarding the number of days in storage before analysis of cytokines was normally distributed in each of the HCV groups (HCV RF: $p = 0.66$; HCV PF: $p = 0.11$), who were recruited over a longer period, but not in the CFS ($p = 0.001$) or healthy control groups ($p = 0.007$), which were positively skewed. Inspection of the boxplots showed that there were no outliers in any of the four groups. The assumption of the homogeneity of variances was violated ($p < 0.001$), as assessed by the Levene's test, and so the Welch's ANOVA was used, which confirmed a significant difference between the four groups (Welch's $F(3,47.42) = 25.99$, $p < 0.001$). Mean statistics showed HCV samples to have been stored for the longest (Mean \pm SEM days; RF: 913 ± 67 ; PF: 995 ± 120), then CFS

samples (542 ± 41) and healthy controls (397 ± 19). Games-Howell post-hoc tests showed no difference between HCV groups ($p = 0.93$), but a longer duration in storage in HCV RF versus CFS and healthy control groups ($p < 0.001$), and HCV PF versus CFS ($p = 0.010$) and healthy control groups ($p < 0.001$). CFS samples were also stored for significantly longer than healthy control samples ($p = 0.010$). This should be considered in the interpretation of results.

Measurements of tryptophan and the kynurenine pathway metabolites were conducted at two time points, in two sets of samples. For HCV samples, where possible all samples from one patient were analysed together. However, at the point at which measurements were obtained at time one, analysis of samples after TW24 ('end' if not TW24; six-months post-treatment, or 'follow-up') was not completed. Therefore, all follow-up samples were analysed at time two, in addition to end of treatment samples where this was not TW24 (i.e. TW8, 16, 36 or 48). For the analysis presented in this thesis, since they were collected separately for a large proportion of patients, follow-up samples were only evaluated in a cross-sectional comparison.

I also examined the number of days in storage before analysis for the kynurenine pathway measurements. As described above, samples were in general analysed together for each HCV patient, however in this case there were some exceptions for 'end of treatment' and follow-up samples. Therefore, t-tests were conducted for three variables: days in storage to analysis of baseline samples, end of treatment samples, and follow-up samples. There were no differences between groups in the number of days in storage to analysis of baseline (RF vs. PF, Mean \pm SEM days: 925 ± 207 vs. 943 ± 245 ; $t(42) = -0.23$, $p = 0.82$), end of treatment (775 ± 36 vs. 714 ± 76 ; $t(39) = 0.82$, $p = 0.42$) or follow-up samples (943 ± 70 vs. 1046 ± 122 , $t(41) = -0.78$, $p = 0.44$). Secondly,

the proportion of patients whose samples were analysed at each time was checked, to see if the distribution of samples analysed at each time was different. There was no difference in the proportion of baseline samples analysed at time 1 (RF vs. PF; Time 1: 57% vs. 67%; $\chi^2 (1) = 0.40, p = 0.53$) or end of treatment samples (Time 1: 54% vs. 60%; $\chi^2 (1) = 0.15, p = 0.70$).

All kynurenine pathway measurements for the cross-sectional comparison (CFS; healthy controls; HCV RF and PF six-months post-treatment) were measured together at time 2. Data regarding the number of days in storage before analysis was normally distributed in each of the HCV groups (HCV RF: $p = 0.122$; HCV PF: $p = 0.143$), recruited over a longer period, but not in the CFS ($p = 0.002$) or healthy control groups ($p = 0.005$), which were positively skewed. Boxplots showed that there were no outliers in any of the four groups. The assumption of the homogeneity of variances was violated ($p < 0.001$). Welch's ANOVA confirmed a significant difference between the four groups (Welch's $F (3,39.68) = 27.72, p < 0.001$). HCV samples had been stored for the longest (Mean \pm SEM days; RF: 968 ± 67 ; PF: 1046 ± 122), then CFS samples (581 ± 41) and healthy controls (430 ± 19). Games-Howell post-hoc tests showed no difference between HCV groups ($p = 0.942$). There was a longer duration in storage in HCV RF versus CFS and healthy control groups ($p < 0.001$), and HCV PF versus CFS ($p = 0.012$) and healthy control groups ($p = 0.001$). CFS samples were also stored for significantly longer than healthy control samples ($p = 0.007$). This should also be considered in the interpretation of results.

3 Results

In this section, I will first present results from the Prospective Cohort study, followed by the Cross-Sectional study (see the overview overleaf, Figure 3.1). To begin, I will outline the characteristics of the whole IFN- α treated HCV cohort. Then, I will examine the effect of IFN- α treatment in the whole sample, on the clinical measures (fatigue, depression, anxiety, perceived stress), measures of health status (overall health and functioning) and biological variables examined (cytokines and kynurenine pathway).

Next, I will further explore IFN- α induced fatigue from two perspectives. First, I will look at the association between each of these variables, as well as cortisol, and the severity of fatigue at three key time points: (i) baseline, (ii) acute fatigue (TW4) and (iii) six-months post-treatment (follow-up). Then I will examine each of these variables and their relationship to the persistence of fatigue. Based on the change in individuals' fatigue scores I will stratify patients into two groups: Persistent Fatigue (PF) and Resolved Fatigue (RF). Fatigue will be considered persistent where levels at follow-up are worse than reported at baseline, or resolved if levels have returned to baseline levels or improved. I will examine baseline characteristics, clinical measures and health status, as well as biological markers for possible risk factors for the later persistence of fatigue. I will also compare the effect of IFN- α treatment longitudinally in each group.

Finally, for the cross-sectional study, I will compare measures obtained in four groups (healthy controls, HCV RF, HCV PF and CFS patients), for each of the areas in turn. I will also add psychological responses to fatigue and fatigue attributions, as well as consider some CFS-specific characteristics.

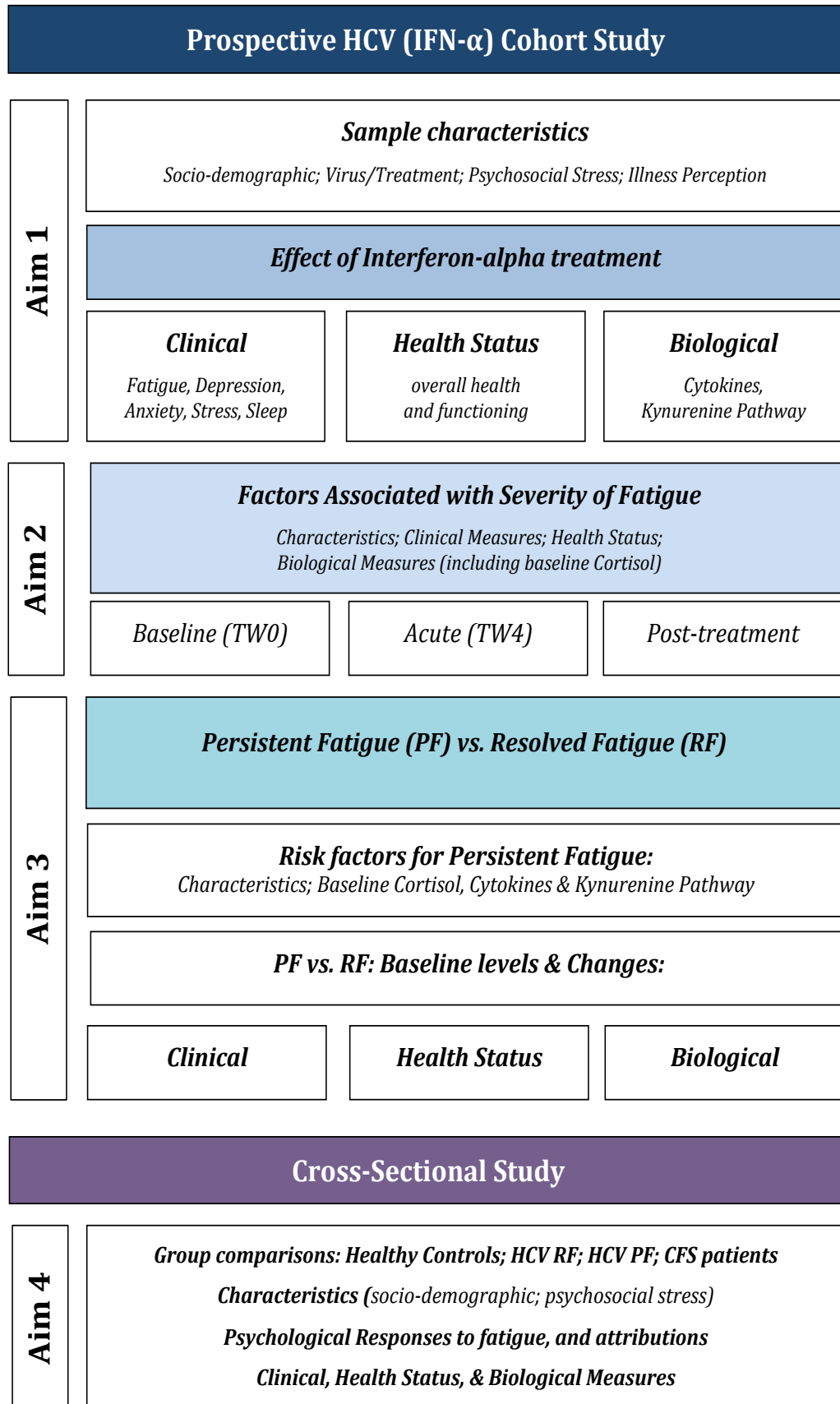


Figure 3.1 Summary of analyses conducted, to achieve each aim

3.1 Prospective Cohort Study

Fifty-five patients were included in the study. Participants received at least 9 weeks of an IFN- α based treatment regimen, and were seen at a follow-up visit, six-months post-treatment.

3.1.1 Effects of IFN- α treatment in the whole HCV cohort

I first characterised the whole IFN- α treated HCV cohort, and then I examined baseline scores, and monitored changes in clinical symptoms and self-report health status in response to IFN- α treatment. Finally, I examined baseline levels, and monitored changes in levels of cytokines and kynurenine pathway metabolites.

3.1.1.1 Socio-demographic characteristics

The age range of the sample was 18-68, with a mean age of 44.6. The sample was predominantly Male (80%), and White British/Irish (50.9%). At the baseline assessment, 5 patients (9.1%) reported experiencing problems with fatigue such that they could be considered 'cases' (CFQ score >18). One patient met the MINI criteria for a current depressive episode, and was treated with an antidepressant (sertraline). A further 3 patients were on antidepressant treatment (mirtazapine; sertraline; citalopram), of which one had been initiated prophylactically. Twenty patients (36.4%) reported a history of depression, and 17 (30.9%) reported a first-degree family history of mental illness. Ten (18.2%) reported current medical use of opioids (drug replacement; pain relief) (see Table 3.1).

3.1.1.2 Virus and treatment characteristics

These characteristics are summarised in Table 3.2. Most patients had HCV genotype 3 (63.6%), with a mean pre-treatment viral load (the number of viral particles per ml of blood presented in millions) of 2.79 ± 0.46 . As a measure of damage to the liver, data from the pre-treatment FibroScan was also obtained. This is a non-invasive procedure that measures liver stiffness, where a score of less than 7 means no or minimal fibrosis; 8-9 of moderate fibrosis; 9-14 of severe fibrosis, and 14.6 kilopascals (kPa) or more indicates cirrhosis or advanced fibrosis. The sample had a mean score of 8.51 ± 0.85 , indicating moderate fibrosis, though 3 patients were cirrhotic. Patients were predominantly treated with combination therapy comprising IFN- α and Ribavirin (85.5%). The remaining eight patients were also treated with an additional direct-acting antiviral (DAA).

Table 3.1 Socio-demographic characteristics, whole HCV cohort

	Patients (n = 55)
Age (years) Mean±SEM	44.6±1.60
Gender Male	44 (80%)
Ethnicity White British/Irish	28 (50.9%)
Education Level Degree	18 (32.7%)
Employment status Unemployed	19 (34.5%)
Relationship status Married/living with someone	22 (40%)
Fatigue 'case' at baseline (CFQ score >18)	5 (9.1%)
History of Depression	20 (36.4%)
Family history of mental illness (first degree)	17 (30.9%)
Drug use Current opioid use (medical; drug replacement) <i>Methadone; buprenorphine; suboxone; morphine</i>	10 (18.2%)
History of opioid abuse	24 (43.6%)
Current smoking	22 (40%)

Table 3.2 Virus and treatment characteristics, whole HCV cohort

	Patients (n = 55)
HCV genotype	
1	10 (18.2%)
2	9 (16.4%)
3	35 (63.6%)
4	1 (1.8%)
HCV viral load, millions	
Mean±SEM	2.8±0.5
Liver stiffness, kPa	
Mean±SEM	8.5±0.9
Treatment type	
IFN-α + ribavirin	47 (85.5%)
Triple (as above, plus DAA)	8 (14.5%)
<i>Telaprevir</i>	5
<i>Simeprevir</i>	2
<i>Boceprevir</i>	1
Treatment duration, weeks	
<24	2 (3.6%)
24	35 (63.6%)
36	4 (7.3%)
48	14 (25.5%)

3.1.1.3 Experience of psychosocial stress

As evaluated using the Brief Life Events scale, around half of all patients (25; 45.5%) reported having experienced at least one stressful life event in six-months before the initiation of treatment. Using the Intrusive Life Events Schedule, 37 patients (67.3%) reported having experienced at least one intrusive life event in their lifetime. As evaluated using the CECA questionnaire, around half of all patients (27; 49.1%) reported having experienced some form of childhood trauma, with the most common being separation from either parent for six months or more, up to age 17) (See Table 3.3).

3.1.1.4 Illness Perceptions

Out of the possible 14 symptoms evaluated (see Appendix I), patients in the sample did not attribute many symptoms experienced since diagnosis to their condition, with a mean identity subscale score, and therefore number of symptoms, of 2.59. Similarly, they did not have a strong perception of their illness as cyclical in nature (mean score 9.49 / 20). They had positive beliefs about their personal understanding of their condition, scoring 19.24 out of a possible 20 on illness coherence. Patients also had positive beliefs about their own personal ability to control their illness (mean score 23.20 / 30), as well as treatment (22.06 / 25). More moderate beliefs were held regarding the seriousness of the consequences of the illness (19.36 / 30) and their emotional representations of it (17.20 / 30). There was no clear picture regarding beliefs about the chronic versus acute nature of the condition (15.58 / 30) (see Table 3.4).

Table 3.3 Experience of psychosocial stress in the whole HCV cohort

Type of psychosocial stress (<i>Measure</i>)	Patients (<i>n</i> = *52-55)
Recent stressful life events (<i>BLE</i>)	
Any last six months, yes	25 (45.5%)
Lifetime intrusive life events* (<i>ILES</i>)	
Any lifetime, yes	37 (67.3%)
<i>Serious injury or assault</i>	26 (47.3%)
<i>Bullying</i>	16 (29.1%)
<i>Homeless</i>	14 (25.5%)
<i>Violence in the home ever</i>	13 (23.6%)
<i>Sexual abuse</i>	7 (12.7%)
<i>Running away from home</i>	11 (20.0%)
<i>Expelled from school</i>	8 (14.5%)
<i>Time in a children's institution</i>	6 (10.9%)
<i>Violence at work ever</i>	5 (9.1%)
<i>Taken into local authority care</i>	5 (9.1%)
Childhood trauma (<i>CECA-Q</i>)	
Any, yes	27 (49.1%)
Forms of trauma, yes	
<i>Separation from parent</i>	16 (29.1%)
<i>Loss of parent</i>	9 (16.4%)
<i>Physical abuse</i>	9 (16.4%)
<i>Sexual abuse</i>	6 (10.9%)

Table 3.4 Illness Perceptions characteristics in the whole HCV cohort

IPQ Dimension	Patients (n = 49)
	<i>Mean±SEM</i>
Identity	2.6±0.5
Timeline (acute vs. chronic)	15.6±0.8
Consequences	19.4±1.0
Personal control	23.2±0.5
Treatment control	22.1±1.2
Illness coherence	19.2±0.6
Timeline (cyclical)	9.5±0.6
Emotional representations	17.2±0.9

3.1.1.5 Clinical changes in response to IFN- α treatment

Also as part of the first aim of my thesis, I examined baseline levels, and monitored changes in fatigue, depression and anxiety in the whole sample, during the first 24 weeks of treatment (see Figure 3.2). Changes in sleep quality were examined in a smaller sample ($n = 20$). Patients experienced the biggest increases in depressive and fatigue symptoms in the first four weeks of treatment. Whereas depressive symptoms worsened during treatment, fatigue scores remained stable. More modest increases were seen in anxiety symptoms; though symptoms did increase over the treatment course, there were no statistically significant increases relative to baseline levels. Sleep quality worsened during the first four weeks of treatment, but varied over the treatment course and perceived levels of stress experienced increased steadily over the same period (data not shown).

3.1.1.6 Health status changes in response to IFN- α treatment

I also examined baseline levels, and monitored changes in the general, physical and mental health of patients as a result of IFN- α treatment, as well as their level of functioning, as represented by the 8 dimensions of the SF-36 Medical Outcomes Survey. For this measure, higher scores represent higher functioning, with a maximum score of 100. Patients experienced a decline in functioning and wellbeing across all dimensions. Patients were most notably affected in relation to functioning (physical functioning; social functioning) and limitations caused by physical health (physical role limitation) and emotional problems (emotional role limitation), as well as the measure of energy/fatigue ('vitality') (see Figures 3.2-3.5).

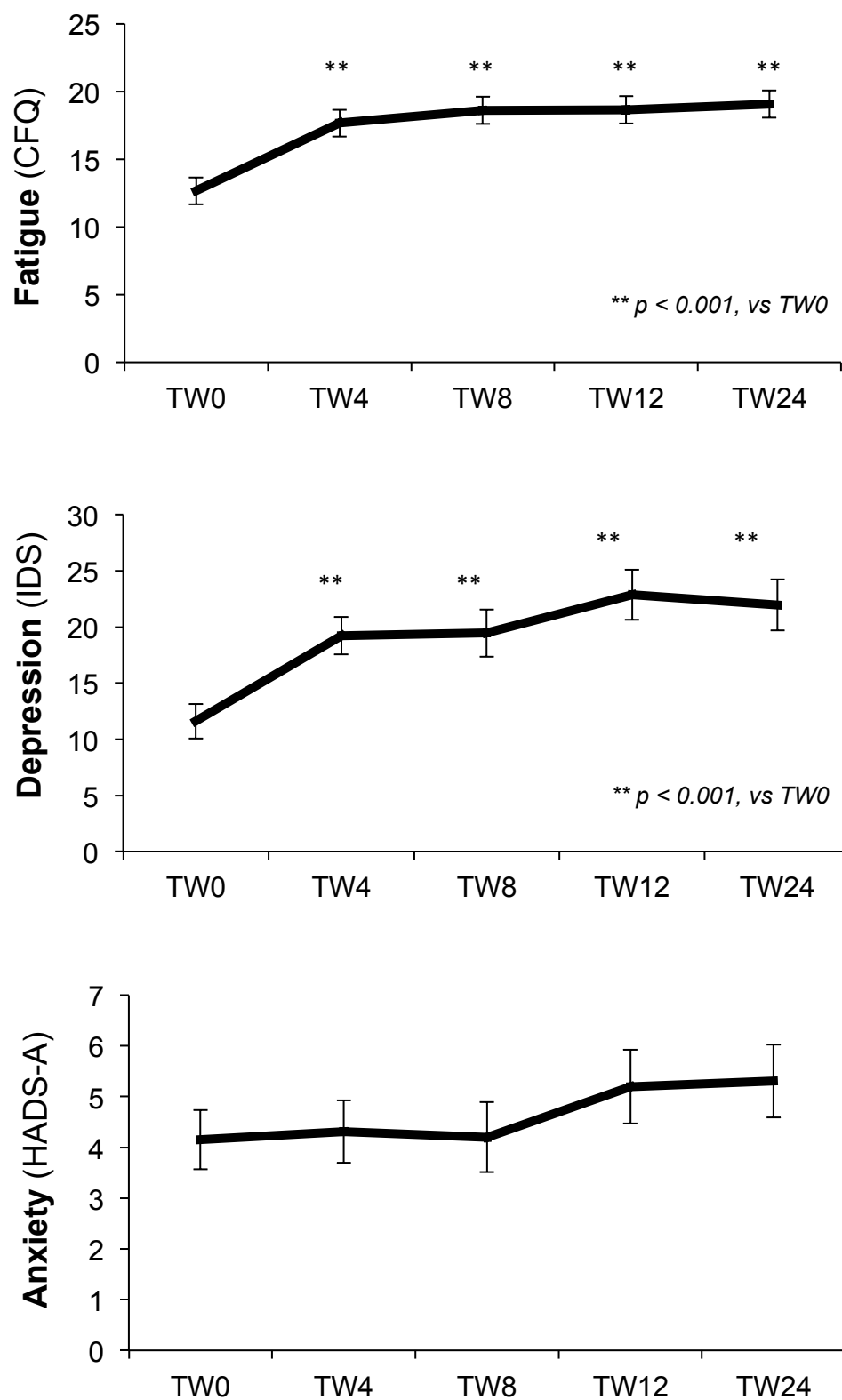


Figure 3.2 Baseline levels, and changes in (i) Fatigue, (ii) Depression and (iii) Anxiety in response to IFN- α in the whole HCV cohort

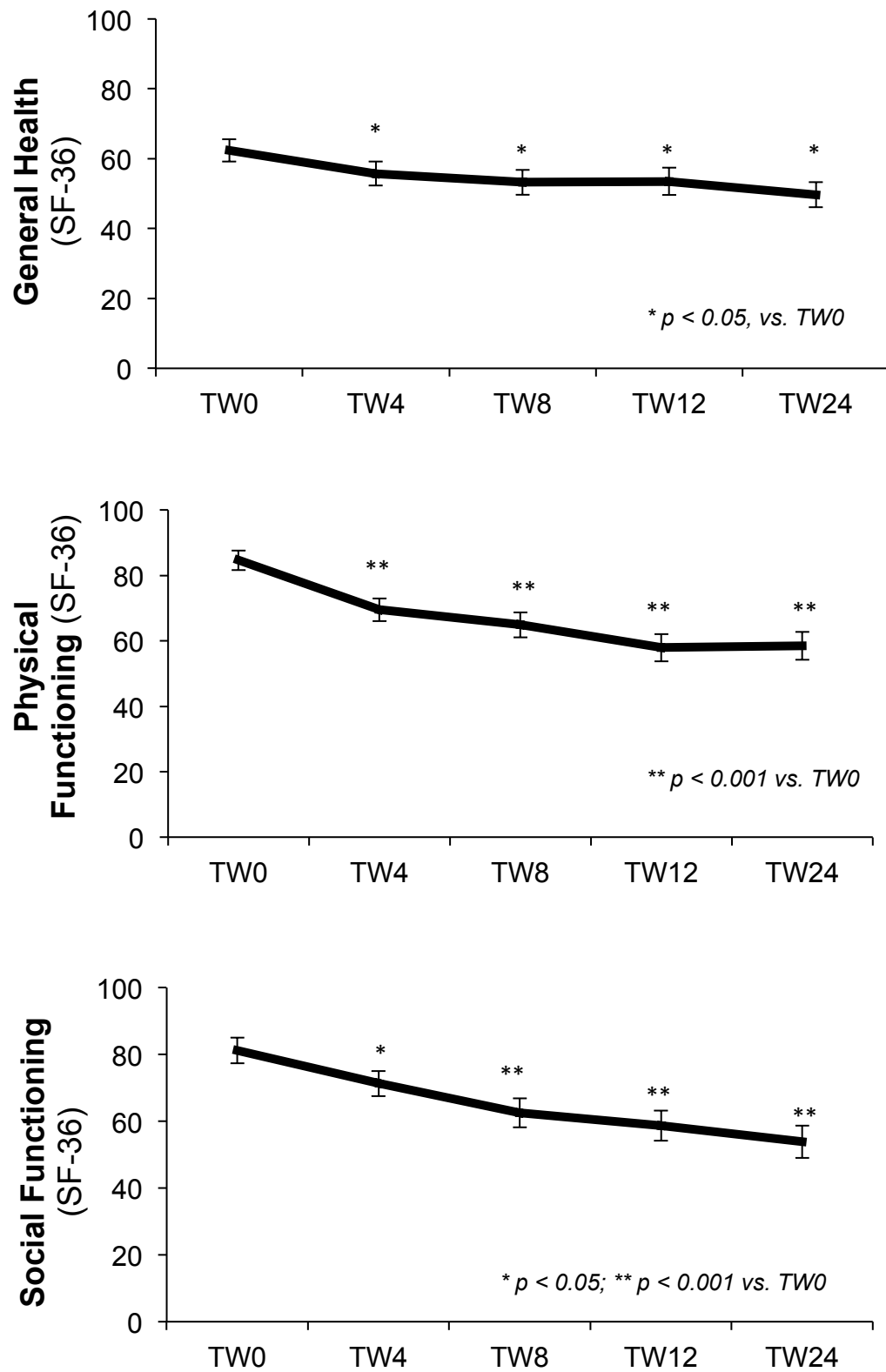


Figure 3.3 Baseline levels, and changes in ratings of (i) General Health, (ii) Physical Functioning and (iii) Social Functioning in response to IFN- α in the whole HCV cohort

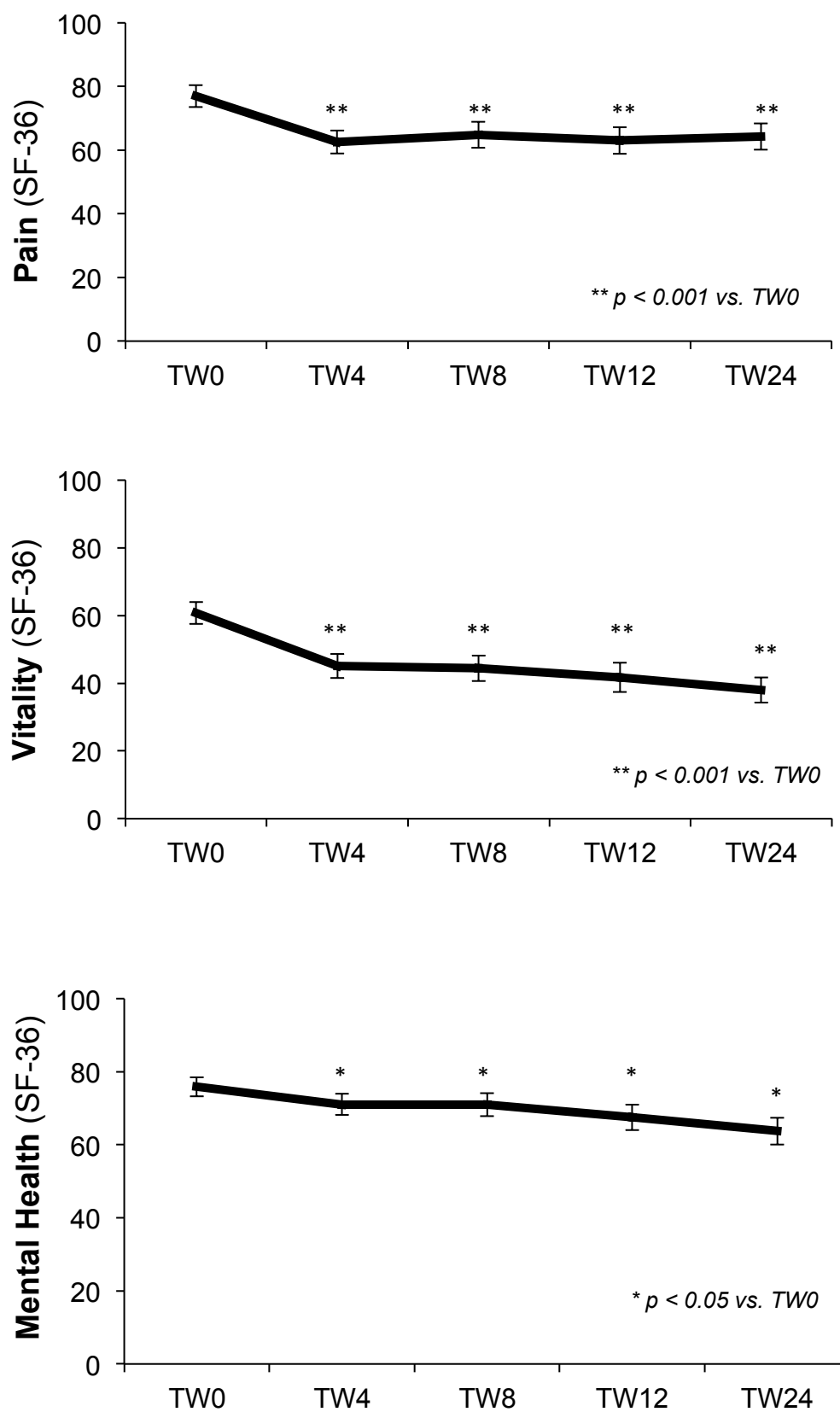


Figure 3.4 Baseline levels, and changes in (i) Pain, (ii) Vitality, and (iii) Mental Health, in response to IFN- α in whole HCV cohort

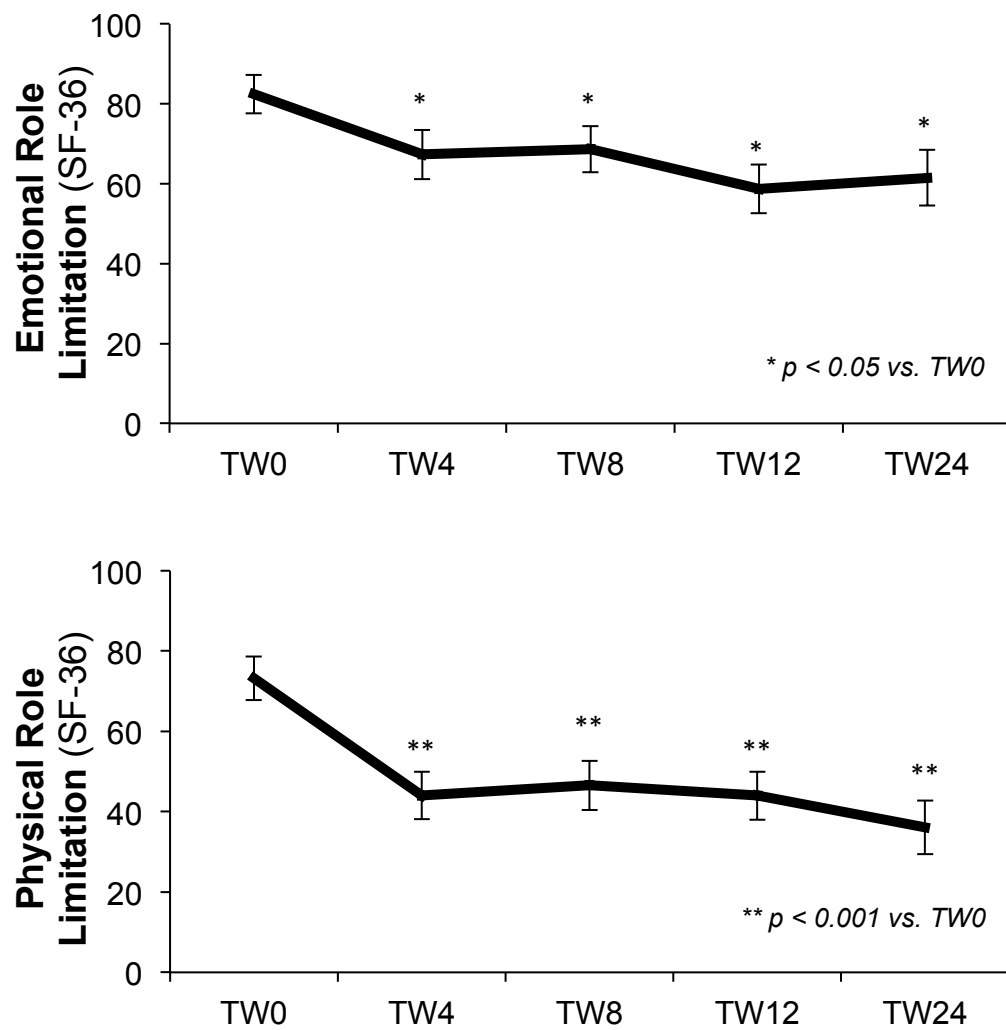


Figure 3.5 Baseline levels, and changes in limitations to roles due to (i) Emotional problems and (ii) Physical Health, in response to IFN- α in whole HCV cohort

3.1.1.7 Biological changes in response to IFN- α treatment

As the last part of the first aim of my thesis, I examined baseline levels, and monitored changes in cytokines and kynurenine pathway metabolites in response to IFN- α treatment in the whole HCV cohort, in the first six-months of treatment (24-weeks). I also explored a link between the analytes measured and treatment response. Absolute levels of all measures are in Appendix V.

Cytokines

Data for cytokine levels was available in 49 of 55 patients. Levels were measured at baseline, TW4 and TW24. Levels of Interferon-gamma (IFN- γ), IL-8 and IL-10 did not change significantly in response to IFN- α treatment (data not shown). Where significant changes were observed, data is presented in Figure 3.7 - Figure 3.9. Levels of IL-2 decreased initially, then increased following more chronic administration. Levels of IL-6 and IL-17A increased significantly by TW4, but did not change from TW4-24; a similar trend was found in levels of IL-7. There was a trend towards a decrease in levels IL-12p70 in the first four weeks of treatment, with no significant change between treatment weeks (TW)-4 and 24. Levels of Tumour Necrosis Factor-alpha (TNF- α) rose steadily throughout treatment. There were also trends towards decreases in levels of Vascular Endothelial Growth Factor (VEGF) across the six-months.

I then explored a link with the clinically relevant measures of treatment response: rapid virological response, or 'RVR' (HCV not detectable at TW4), and sustained, or 'SVR' (not detectable six-months post-treatment). At TW4, data was available in 27 patients who had achieved an RVR, and 19 patients who had not. Cytokine levels at TW4 were the same across patients, regardless

of whether patients had cleared the virus or not. Next, I examined cytokine levels at the follow-up visit six-months post-treatment and whether patients achieved RVR or not. Data was available in 20 patients who had not, and 27 who had. Cytokine levels at follow-up were not associated with RVR at TW4. Finally, I examined cytokine levels at follow-up and SVR. Data was available in 6 patients who had not, and 41 patients who had cleared the virus. Again, there was no association between cytokine levels at follow-up and SVR. In conclusion, I found no link between cytokine levels and clinically relevant measures of treatment response.

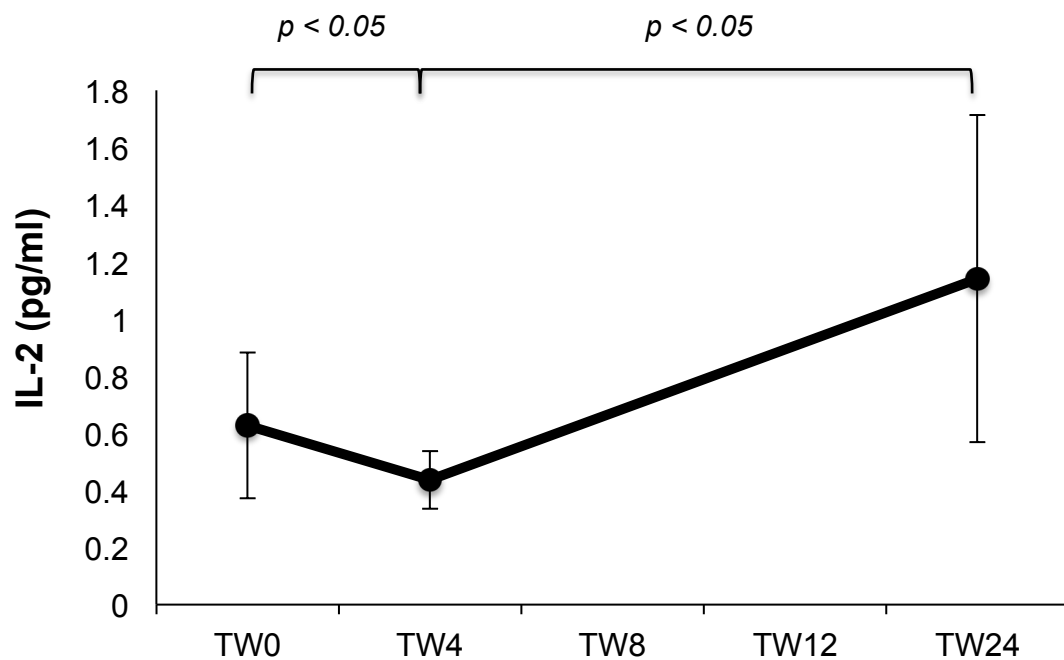


Figure 3.6 Baseline levels, and changes in IL-2 in response to IFN- α in the whole HCV cohort

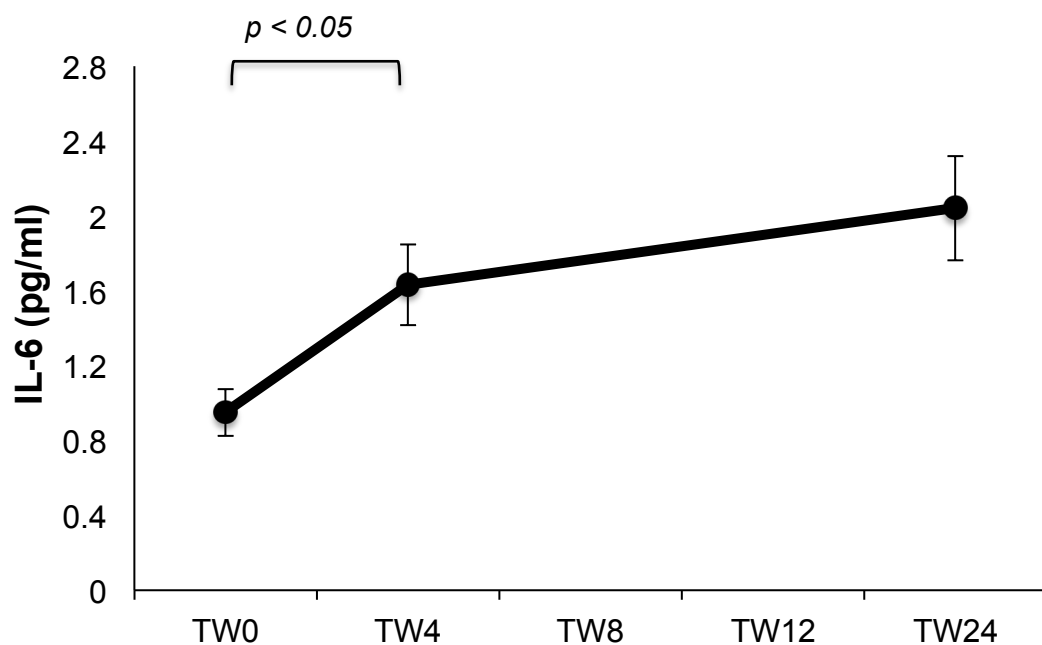


Figure 3.7 Baseline levels, and changes in IL-6 in response to IFN- α in the whole HCV cohort

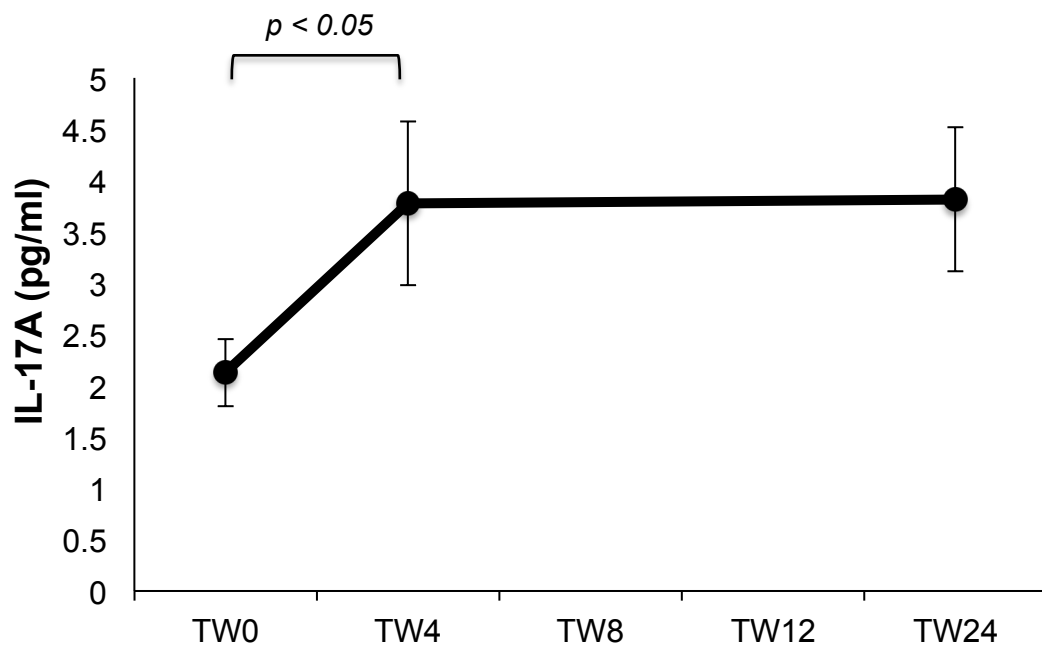


Figure 3.8 Baseline levels, and changes in IL-17A in response to IFN- α in the whole HCV cohort

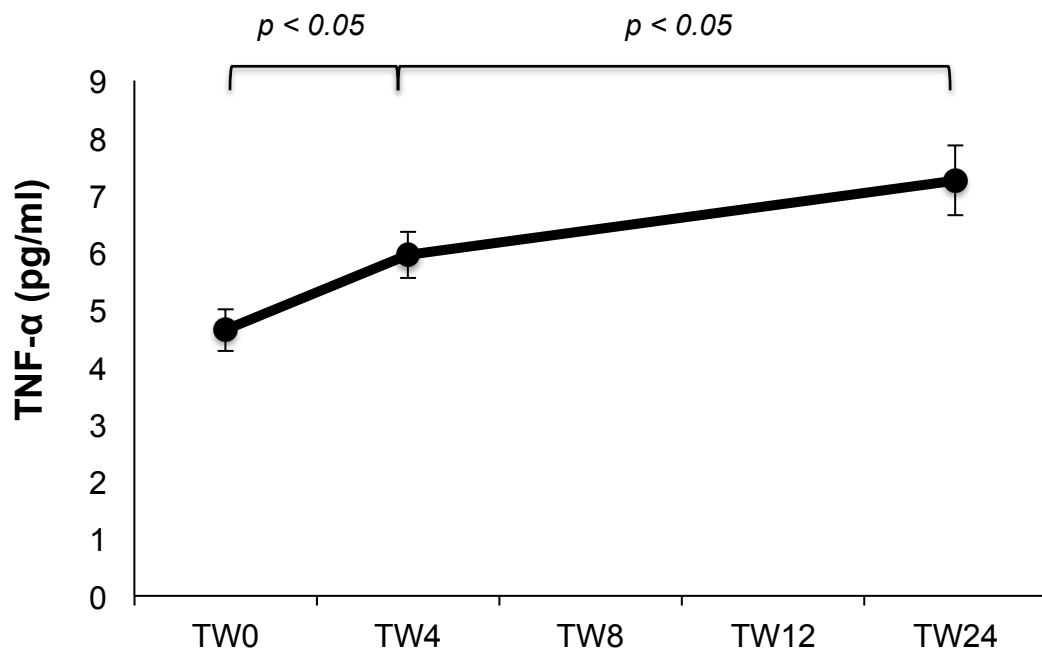


Figure 3.9 Baseline levels, and changes in TNF- α in response to IFN- α in the whole HCV cohort

Kynurenine pathway

Metabolites in the kynurenine pathway were measured at baseline (TW0), TW8 and TW24. Data was available in up to 49 of 55 patients. There was a significant decrease in tryptophan from baseline to TW24, with the greatest increase occurring in the first eight weeks of treatment (see Figure 3.10). This was in line with the increases in the ratio of kynurenine to tryptophan over the same timeframe (Figure 3.11). Kynurenine may be metabolised into kynurenic acid or 3-hydroxykynurenine (3-HK). Levels of kynurenic acid did change in response to treatment, as measured in the change from TW0 to TW24. There was also a trend towards a decrease from TW8 to TW24 (Figure 3.12). Levels of quinaldic acid decreased significantly throughout treatment, with the greatest change occurring from TW0 to TW8 (Figure 3.13). There was no significant change in the ratio of 3-HK to Kynurenine, however levels of 3-HK increased by TW24, relative to baseline levels (Figure 3.14). It may be further metabolised into xanthurenic acid, picolinic acid or quinolinic acid. There was no change in levels of xanthurenic acid or quinolinic acid in response to IFN- α . However, there was a trend towards a decrease in picolinic acid by TW24 relative to baseline, with a trend towards a decrease also from TW0 to TW8 (data not shown).

I then explored a link with the clinically relevant measures of treatment response, the Rapid ('RVR') and Sustained Virological Response 'SVR'. At TW8, the first available data after the RVR result, levels were measured in up to 26 patients who had achieved an RVR, and 20 patients who had not. Levels of all metabolites were the same in both groups. Exploring an association with RVR and metabolite levels post-treatment, levels of quinolinic acid were higher at FU in those who had not achieved an RVR (Mean \pm SEM ng/ml; 58.3 \pm 50.3; t

(41) = 2.73, $p = 0.009$), but there were no other associations. Finally, an association between SVR and follow-up levels was explored. Up to 38 patients had achieved an SVR who also had levels measured, and 5 had not. Levels of Kynurenic Acid were significantly higher in those who had achieved an SVR (9.7 ± 6.7 ; $t(40) = -2.11$, $p = 0.042$). There was also a trend towards higher Xanthurenic acid in the same direction (4.5 ± 2.3 ; $t(40) = -1.87$, $p = 0.07$). In summary, though there were some findings that may be worthy of further exploration, there was no consistent evidence of an association between the kynurenine pathway and clinically relevant measures of treatment response.

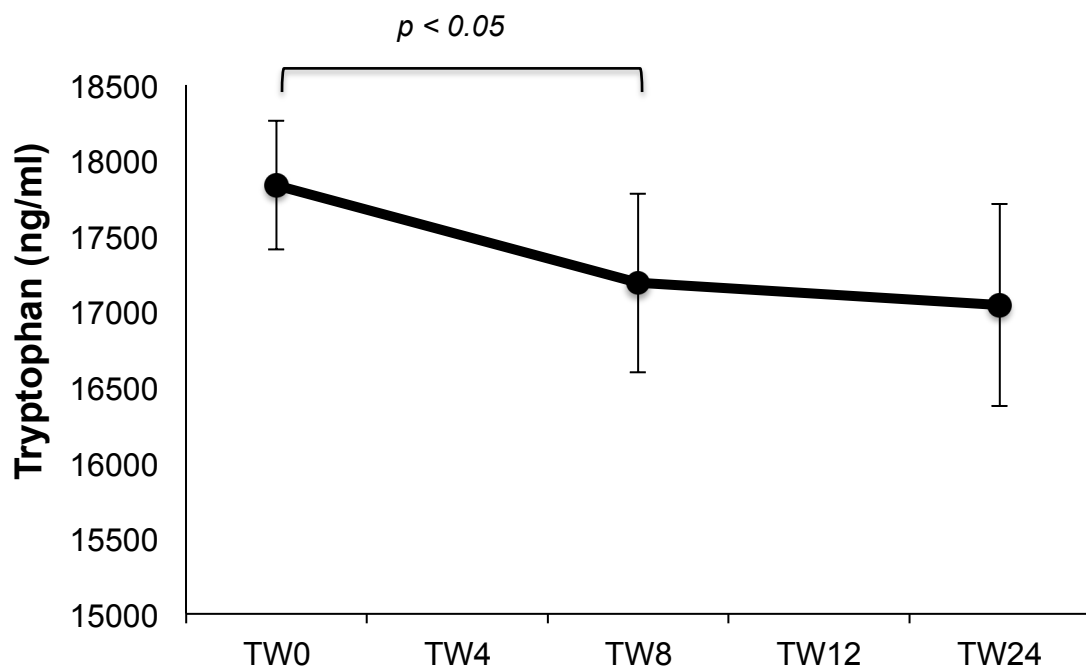


Figure 3.10 Baseline levels, and changes in tryptophan in response to IFN- α in the whole HCV cohort

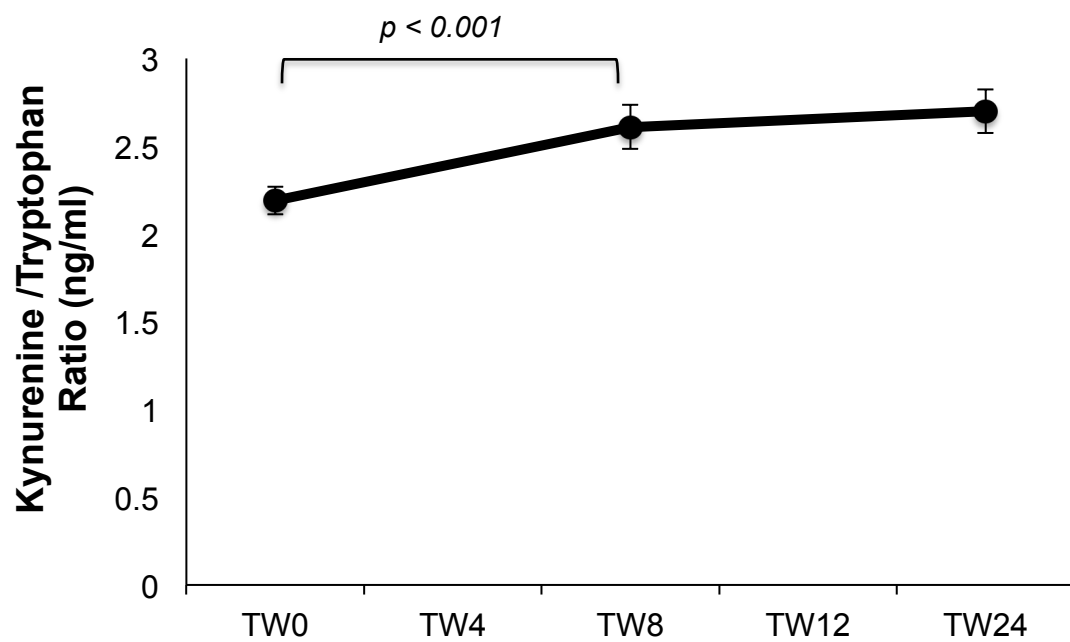


Figure 3.11 Baseline levels, and changes in KYN/TRP ratio in response to IFN- α in the whole HCV cohort

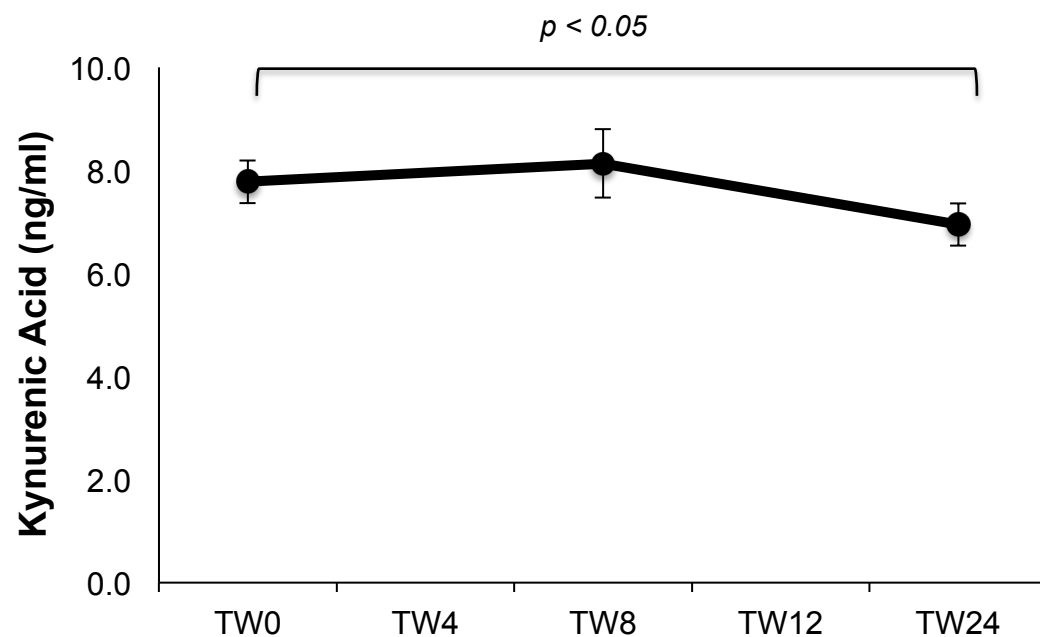


Figure 3.12 Baseline levels, and changes in kynurenic acid in response to IFN- α in the whole HCV cohort

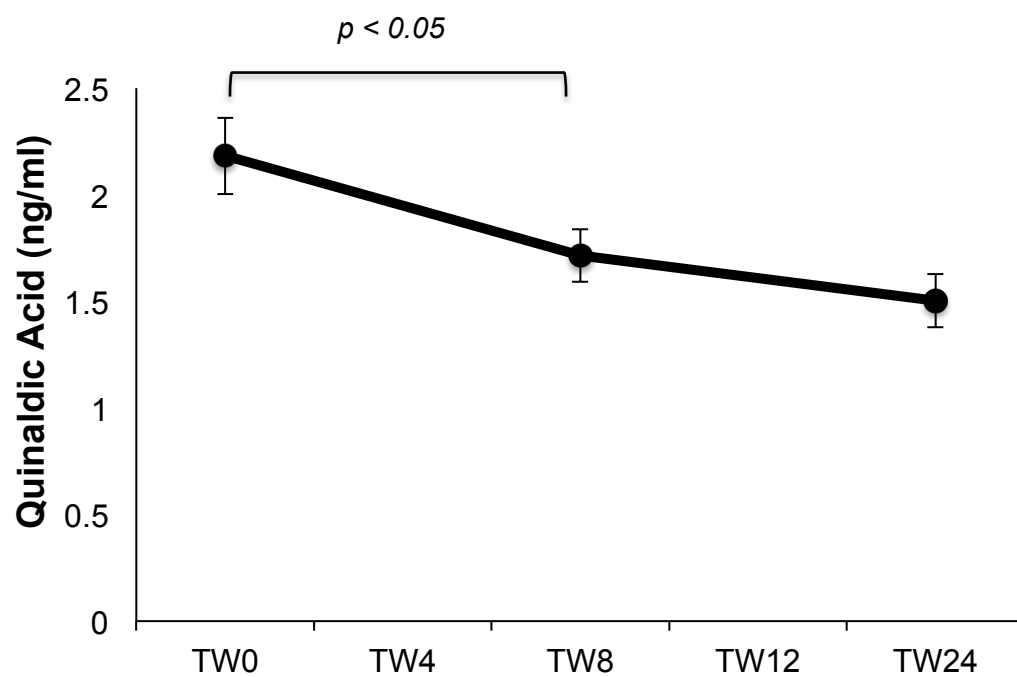


Figure 3.13 Baseline levels, and changes in quinaldic acid in response to IFN- α in the whole HCV cohort

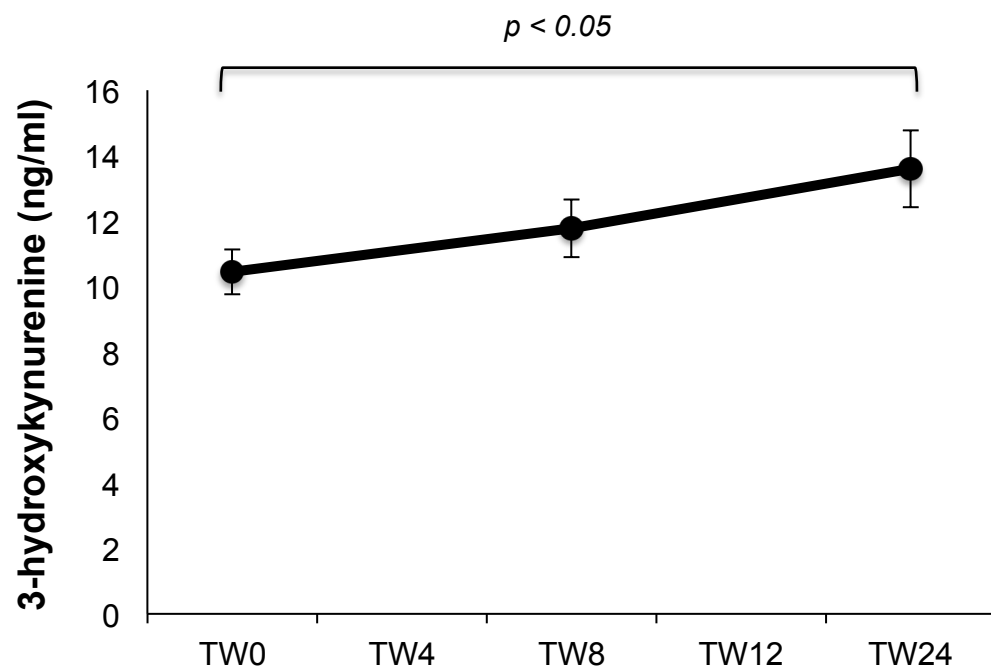


Figure 3.14 Baseline levels, and changes in 3-HK in response to IFN- α in the whole HCV cohort

3.1.1.8 Effect of IFN- α treatment in the whole HCV cohort: a summary

The figure overleaf, Figure 3.15 summarises findings in relation to the first aim of my thesis. The aim was to examine the effect of IFN- α in the whole HCV cohort. I did so by obtaining baseline measurements in the whole sample, and monitoring subsequent changes in response to IFN- α treatment in the first six-months of treatment. Patients saw an increase in side effects relating to fatigue, mood and perceived stress, as well as a decline in wellbeing and overall health and functioning. There were changes in some, but not all of the biological markers examined. For biological markers specifically, where there was an effect of IFN- α , I have highlighted below the overall direction of change, as well as the change between each measurement. In the case of cytokines, this was from baseline (TW0) to TW4, and TW4 to 24. For kynurenine pathway measures, this was TW0 to 8, and TW8 to 24. I did not find an effect on sleep in my sample.

Prospective HCV (IFN- α) Cohort Study

Aim 1	Effect of Interferon-alpha treatment		
	Clinical	Health Status	Biological*
	Fatigue \uparrow Depression \uparrow Anxiety \uparrow Stress \uparrow X Sleep	General Health \downarrow Physical Functioning \downarrow Social Functioning \downarrow Pain \uparrow Energy \downarrow Mental Health \downarrow Limitations to daily activities \uparrow	IL-2 = \downarrow \nearrow IL-6, IL-7 \uparrow \uparrow \rightarrow IL-17A \uparrow \uparrow \rightarrow TNF- α \uparrow \uparrow \uparrow VEGF \downarrow \searrow \searrow Trp \downarrow \downarrow \rightarrow Kyn/Trp \uparrow \uparrow \rightarrow KA \downarrow \rightarrow \searrow Quinald Acid \downarrow \downarrow \searrow 3-HK \uparrow \rightarrow \rightarrow
Key: \uparrow / \downarrow =increase/decrease overall during treatment; \searrow / \nearrow trend towards; \rightarrow no diff *Bio: \uparrow / \downarrow =Overall change TW0-24 + \downarrow \nearrow =early change (TW0-4/8) & later (TW4/8-24)			

(Note – small n for sleep measurements earlier in treatment should be noted)

Figure 3.15 Summary of results for Aim 1: the effect of IFN- α in the whole HCV cohort

3.1.2 Factors associated with the severity of fatigue

To address aim two of this thesis, to understand which characteristics, clinical and biological measures may be associated with severity of fatigue, I conducted a series of analyses looking at the association between severity of fatigue and clinical measures, health status and biological measures. I examined severity of fatigue at three key time points: baseline (or treatment week zero; TW0), treatment week 4 (TW4), representing acute fatigue and the initial response to IFN- α treatment, and six-months post-treatment, or follow-up (FU). A summary of the variables found to be associated with fatigue for each set of analyses can be found on page 170. Absolute levels of each biological marker at each treatment time point can be found in Appendix V at the back of this thesis.

3.1.2.1 Severity of baseline fatigue

Analyses were conducted to examine the characteristics of those HCV patients who experienced more problems with fatigue before treatment began, which has been found to be a significant predictor of IFN- α induced fatigue. As many patients were reporting lower scores at this point, data was not normally distributed ($p < 0.001$), as assessed by the Shapiro-Wilk test. Visual examination of the boxplot showed that there were two outliers \pm three times the interquartile range. Spearman's Rho Correlations (r_s) were therefore used to examine the relationship with other continuous variables. Independent t-tests were used to explore the relationship with dichotomised variables. Where equal variance could not be assumed, as assessed by the Levene's test ($p < 0.05$), the t-test result reported was that where the degrees of freedom had been adjusted according to the Welch-Satterthwaite method.

Socio-demographic characteristics

Data is presented in Table 3.5. For some variables, results were adjusted where equal variance could not be assumed. Although the spread of scores were slightly different, scores were negatively skewed in both groups (ethnicity; past depression), for both analyses. There were no significant associations, though some patterns were apparent. There was a weak, negative association with age, with younger participants reporting more problems with fatigue. A similar association was found in non-White British participants. There was a trend towards a positive association with a personal history of depression, with those with a history reporting slightly higher baseline fatigue levels. Though again not significant, higher levels were also reported by those with a first-degree family history of mental illness.

Virus and treatment characteristics

Data is also summarised in Table 3.5. The T-test results were both adjusted to account for unequal variances. For both genotype and treatment type, all groups most often reported scores of '11' (all items 'no more than usual'), however for the 2/3 group and those on combination therapy (IFN- α + ribavirin) there were more patients reporting higher scores. Indeed, baseline fatigue was associated with genotype, with individuals with genotypes 2 and 3 reporting higher baseline levels of fatigue. There was also a trend towards higher baseline fatigue in those with a personal history of depression. There was no indication of a relationship with the available markers of disease severity, baseline HCV RNA (viral load) or the FibroScan (liver stiffness) result.

Table 3.5 Association between socio-demographic and virus/treatment characteristics and baseline fatigue

Characteristics	Fatigue score, TW0	
	<i>Mean±SEM</i>	<i>Test and statistic</i>
Age (years)	-	$r_s = -0.20, p=0.15$
Gender Male/Female	12.7±0.5 vs. 12.5±1.1	$t (53) = -0.21, p=0.83$
Ethnicity White/other	12.0±0.5 vs. 13.2±0.7	$t (49.1) = 1.30, p=0.20$
Education Degree/other	13.1±0.8 vs. 12.4±0.6	$t (53) = -0.69, p=0.50$
Employment Unemployed/other	12.9±0.5 vs. 12.2±0.9	$t (53) = -0.82, p=0.42$
Relationship Married or living with/other	13.0±0.7 vs. 12.4±0.4	$t (53) = -0.60, p=0.55$
History of Depression yes/no	13.9±1.1 vs. 12.0±0.6	$t (25.2) = -1.71, p=0.099$
Family History First degree – yes/no	13.7±1.0 vs. 12.0±0.5	$t (48) = -1.63, p=0.11$
HCV genotype, 1/4 vs. 2/3	11.2±0.6 vs. 13.0±0.6	$t (32.3) = 2.36, p=0.025$
HCV viral load, <i>Millions</i>	-	$r_s = -0.06, p=0.69$
Liver stiffness, <i>kPa (n = 48)</i>	-	$r_s = -0.006, p=0.97$
Treatment type <i>IFN-α + ribavirin–y/n</i>	12.8±0.5 vs. 11.8±0.5	$t (27.5) = 1.46, p=0.16$

Experience of psychosocial stress

Experience of any stressful life event in the six-months prior to the initiation of treatment, lifetime experience of any intrusive life event, and experience of any childhood trauma were examined for any association with baseline fatigue (see Table 3.6). The result for recent life events was adjusted, as equal variance could not be assumed; histograms of scores showed again that the majority of patients in both groups reported a score of '11', though the 'yes' group had more high scores. Indeed, levels of fatigue were significantly higher in those who had experienced at least one stressful life event in the last six months. Though not significant, mean baseline fatigue levels were slightly higher in those patients who had experienced one or more 'intrusive' life event in their lifetime. No association was observed with childhood trauma.

Illness Perceptions

Next, an association was explored with patients' illness perceptions, recorded at the baseline visit. Data is shown in Table 3.7. There was a significant, moderate correlation with 'emotional representations', with those with greater emotional representations of their HCV having higher levels of fatigue. There was also a trend towards an association with the 'Identity' dimension score, indicating that those who attributed more symptoms experienced to their HCV had slightly more severe baseline fatigue. The other illness perceptions were less important for baseline fatigue; there were weak correlations between 'consequences' and fatigue, with those who felt their HCV had greater consequences having slightly higher fatigue, and with personal control, with those reporting feeling less personal control of their illness having greater fatigue symptoms.

Table 3.6 Association between experience of psychosocial stress and baseline fatigue

Risk Factor	Fatigue score, TW0	
	<i>(Mean±SEM)</i>	<i>Test and statistic</i>
Recent stressful events		
Pre-treatment, Yes/No	14.0±0.4 vs. 11.5±0.4	<i>t</i> (37.67) = -2.79, <i>p</i> = 0.008
Lifetime intrusive events		
Yes/No	13.0±0.6 vs. 11.9±0.7	<i>t</i> (53) = -1.07, <i>p</i> = 0.29
Childhood trauma		
Yes/No	12.3±0.6 vs. 13.0±0.7	<i>t</i> (53) = 0.76, <i>p</i> = 0.70

Table 3.7 Association between illness perceptions and baseline fatigue

IPQ Dimension	Fatigue score, TW0
Identity	<i>r_s</i> = 0.27, <i>p</i> = 0.059
Timeline (acute vs chronic)	<i>r_s</i> = 0.05, <i>p</i> = 0.75
Consequences	<i>r_s</i> = 0.23, <i>p</i> = 0.12
Personal control	<i>r_s</i> = -0.20, <i>p</i> = 0.17
Treatment control	<i>r_s</i> = -0.05, <i>p</i> = 0.75
Illness coherence	<i>r_s</i> = -0.05, <i>p</i> = 0.71
Timeline (cyclical)	<i>r_s</i> = 0.17, <i>p</i> = 0.25
Emotional representations	<i>r_s</i> = 0.42, <i>p</i> = 0.002

Baseline clinical symptoms

Data for the association between baseline fatigue and clinical symptoms is presented in Table 3.8. Scores for baseline depression, anxiety, and stress were available in all 55 patients. Scores for sleep quality, a measure introduced later in the study, were only available in 18 patients. There were significant associations between baseline fatigue and baseline symptoms of depression, anxiety, and perceived stress in the month preceding treatment. There was no association with quality of sleep, though the sample size should be noted.

Baseline health status

To explore the association between poorer baseline health status and baseline fatigue, correlation analyses were also run with each baseline dimension subscale score of the SF-36 and the fatigue score as measured at the same time point (see Table 3.9). As described previously, lower SF-36 subscale scores indicate worse functioning. Interestingly, there was no relationship between baseline fatigue and the patients' rating of their general health. There was a trend towards associations with physical functioning and the degree to which patients' felt limited in carrying out their day-to-day activities by their physical health, suggesting that any issues with fatigue were not impacting too greatly on physical functioning. On the other hand, there were significant, moderate associations with measures of mood: mental health, and the degree to which 'emotional problems' were limiting their day-to-day activities. Baseline fatigue was also moderately related to poorer perceived social functioning and higher ratings of pain. Finally, there was a moderate association with scores of 'vitality', representing a balance of energy/fatigue.

Table 3.8 Association between baseline clinical symptoms and baseline fatigue

<i>Baseline clinical symptom (measure)</i>	<i>Fatigue score, TW0</i>
Depression (IDS)	$r_s = 0.44, p = 0.001$
Anxiety (HADS-A)	$r_s = 0.32, p = 0.017$
Perceived Stress (PSS)	$r_s = 0.39, p = 0.004$
Sleep quality (Jenkins)	$r_s = 0.26, p = 0.30$

Table 3.9 Association between baseline health status and baseline fatigue

<i>SF-36 Dimension</i>	<i>Fatigue score, TW0</i>
Emotional Role Limitation	$r_s = -0.37, p = 0.006$
General Health	$r_s = -0.14, p = 0.33$
Mental Health	$r_s = -0.44, p = 0.001$
Pain	$r_s = -0.31, p = 0.022$
Physical Role Limitation	$r_s = -0.25, p = 0.062$
Physical Functioning	$r_s = -0.24, p = 0.083$
Social Functioning	$r_s = -0.32, p = 0.018$
Vitality	$r_s = -0.43, p = 0.001$

Baseline Biological Measures

I examined baseline cytokine, kynurenine pathway metabolite and cortisol levels and their association with baseline fatigue. The range of cytokines and kynurenine pathway metabolites examined, as well as participant numbers and results are presented in Table 3.10. Absolute levels can be found in Appendix V. For cytokine levels, significant, positive associations were found with 8 out of the 11 markers measured. The strongest associations were with levels of TNF- α , IL-7, IFN- γ , and IL-2. This indicates that higher levels of cytokines were associated with greater problems with fatigue pre-treatment.

Baseline fatigue was not associated with levels of the kynurenine pathway metabolites. Interestingly though, the direction of the correlations were almost all negative, linking lower levels with higher symptoms. There was, however, a trend towards a positive association with levels of 3-HK, with higher levels associated with more severe fatigue.

I examined two measures of cortisol, also shown in Table 3.10. The first measure is the Cortisol Awakening Response, or 'CAR', calculated using the Area Under the Curve with respect to the increase ('AUCi') from the level at awakening. This calculation is made including levels from samples taken within the first hour after awakening. The second measure represents diurnal cortisol output, as calculated using the AUC with respect to the 'ground' (zero; AUCg). This calculation includes levels obtained from sampling at awakening, 12 noon, and 8pm. There was no link between salivary cortisol levels and the severity of baseline fatigue.

Table 3.10 Association between baseline biological measures and baseline fatigue

Biological Marker	Fatigue score, TW0
<u>Cytokines</u> (<i>n</i> = 49 ^{*#})	
IL-10	$r_s = 0.15, p = 0.29$
IL-13	$r_s = -0.06, p = 0.66$
IFN- γ^*	$r_s = 0.58, p < 0.001$
IL-2	$r_s = 0.52, p < 0.001$
IL-6	$r_s = 0.30, p = 0.03$
IL-7	$r_s = 0.62, p < 0.001$
IL-8	$r_s = 0.33, p = 0.02$
IL-12p70 [#]	$r_s = 0.37, p = 0.048$
IL-17A	$r_s = 0.38, p = 0.006$
TNF- α	$r_s = 0.64, p < 0.001$
VEGF [*]	$r_s = -0.15, p = 0.37$
<u>Kynurenine pathway</u> (<i>n</i> = 49)	
Tryptophan	$r_s = -0.17, p = 0.25$
Kyn/Trp ratio	$r_s = 0.006, p = 0.97$
Kynurenic Acid	$r_s = -0.05, p = 0.74$
Quinaldic Acid	$r_s = -0.21, p = 0.14$
3-HK/Kyn ratio	$r_s = -0.16, p = 0.26$
3-HK	$r_s = 0.40, p = 0.052$
Xanthurenic Acid	$r_s = -0.04, p = 0.79$
Picolinic Acid	$r_s = -0.15, p = 0.32$
Quinolinic Acid	$r_s = 0.19, p = 0.19$
<u>Cortisol</u> (<i>n</i> = 25; 28)	
Awakening Response (CAR AUC _i)	$r_s = 0.01, p = 0.95$
Diurnal (DAY AUC _g)	$r_s = -0.08, p = 0.69$

**n* = 39; [#] *n* = 29

(For absolute levels see Appendix V)

3.1.2.2 Severity of fatigue at TW4: IFN- α induced acute fatigue

Patients experience the greatest increase in fatigue in the first four weeks of IFN- α , early on in treatment. First I explored associations with baseline variables, as potential predictors. Then I analysed cytokine levels at TW4 and their association with fatigue scores, to understand if concurrent inflammation may be associated with IFN- α induced acute fatigue. Data on the severity of fatigue at TW4 was available in 54 patients, and was normally distributed ($p = 0.80$). There were no outliers. Pearson's correlations (r) were performed where both data sets were normally distributed, or Spearman's Rho correlations (r_s) if they were not. Independent samples t-tests were used to explore differences in fatigue in groups, with results adjusted where variances were not equal.

Socio-demographic characteristics

First, an association with socio-demographic characteristics was explored (see Table 3.11). Interestingly, there was only a trend towards a positive association with baseline fatigue 'caseness' (a Chalder Fatigue Scale score of >18). Though not significant, there were also slightly higher scores reported by those with a personal history of depression, and those with a family history.

Virus and treatment characteristics

This data is also presented in Table 3.11. For genotype, the test result was adjusted since equal variance could not be assumed between the two subtype groups. Histograms of the scores in each group showed that fatigue scores were normally distributed in those with genotype 2 or 3, but slightly positively skewed in those with 3 or 4. Though not significant, there were slightly higher fatigue scores in those on combination therapy (IFN- α and ribavirin).

Table 3.11 Association between socio-demographic and virus/treatment characteristics and the severity of fatigue at TW4

Characteristics	Fatigue score, TW4	
	<i>Mean±SEM</i>	<i>Test and statistic</i>
Age (years)	-	$r = -0.11, p=0.44$
Gender Male/Female	18.2±1.8 vs. 17.5±0.7	$t (52) = 0.39, p=0.70$
Ethnicity White/other	18.2±0.8 vs. 17.1±1.1	$t (52) = -0.82, p=0.42$
Education Degree/other	18.7±1.3 vs. 17.2±0.8	$t (52) = -0.99, p=0.33$
Employment Unemployed/other	18.0±0.9 vs. 17.0±1.1	$t (52) = -0.66, p=0.51$
Relationship Married or living with/other	18.3±1.2 vs. 17.2±0.8	$t (52) = -0.81, p=0.42$
Baseline fatigue ‘case’ CFQ>18 – yes/no	21.2±1.2 vs. 17.3±0.7	$t (52) = -1.68, p=0.099$
History of Depression Yes/no	18.8±1.0 vs. 17.0±0.9	$t (52) = -1.23, p=0.20$
Family History First degree – yes/no	18.8±1.1 vs. 17.1±1.0	$t (47) = -1.07, p=0.29$
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HCV genotype, 1/4 vs. 2/3	17.9±2.2 vs. 17.6±0.7	$t (10.99) = -0.14, p=0.86$
HCV viral load, millions	-	$r_s = -0.10, p=0.48$
Liver stiffness, kPa (n = 47)	-	$r_s = 0.06, p=0.68$
Treatment type IFN- α + ribavirin – y/n	18.0±0.7 vs. 15.6±2.5	$t (52) = 1.17, p=0.25$
Treatment duration ≤24 / >24	17.5±0.9 vs. 18.0±1.1	$t (52) = -0.34, p=0.73$
Rapid Virological Response Yes/No	17.8±1.0 vs. 17.4±0.9	$t (52) = -0.29, p=0.78$

Experience of psychosocial stress

Experience of any stressful life event in the six-months prior to the initiation of treatment, lifetime experience of any intrusive life event, and experience of any childhood trauma were each examined as possible predictors of more severe fatigue after the first four weeks of IFN- α treatment. Though variance was not equal in each of the childhood trauma groups (yes/no), scores were normally distributed in both groups. Interestingly, the mean scores suggest that those who reported having experienced one or more stressful events in the lead up to treatment had a higher fatigue score at TW4 than those who had not (see Table 3.12). However, this association did not reach statistical significance.

Illness Perceptions

Next, an association was explored with patients' illness perceptions, as recorded before the initiation of treatment, at the baseline visit. Data is shown in Table 3.13. There was a strong link between patients' perceptions and acute IFN- α induced fatigue. Fatigue scores were significantly, positively correlated with the 'Identity' and 'Timeline (cyclical)' dimensions, and strongly so with 'emotional representations'. This indicates that those who attributed a greater number of symptoms experienced to their HCV, versus any other external cause, experienced greater difficulties with fatigue at TW4. So too did those who believed their illness to be more cyclical in nature, and those with stronger emotional representations of their illness. A significant negative relationship was observed with scores for personal control and illness coherence, with those reporting less confidence in their personal control over and understanding of their illness experiencing greater fatigue. No significant relationship was found with the consequences dimension, or with the treatment control dimension.

Table 3.12 Association between experience of psychosocial stress and the severity of fatigue at TW4

Risk Factor	Fatigue score, TW4	
	<i>(Mean±SEM)</i>	<i>Test and statistic</i>
Recent stressful events		
Yes/No	18.6±0.83 vs. 16.9±1.0	$t(52) = -1.3, p = 0.21$
Lifetime intrusive events		
Yes/No	17.6±0.76 vs. 17.8±1.4	$t(52) = 0.19, p = 0.85$
Childhood trauma		
Yes/No	17.9±0.70 vs. 17.5±1.2	$t(43.7) = -0.28, p = 0.78$

Table 3.13 Association between baseline illness perceptions and the severity of fatigue at TW4

IPQ Dimension	Fatigue score, TW4	
Identity	-	$r_s = 0.40, p = 0.006$
Timeline (acute vs chronic)	$r = 0.27, p = 0.065$	-
Consequences	-	$r_s = 0.29, p = 0.042$
Personal control	$r = -0.33, p = 0.021$	-
Treatment control	-	$r_s = -0.19, p = 0.20$
Illness coherence	-	$r_s = -0.40, p = 0.004$
Timeline (cyclical)	-	$r_s = 0.30, p = 0.038$
Emotional representations	$r = 0.58, p < 0.001$	-

Baseline clinical symptoms

An association between baseline clinical symptoms and the severity of fatigue was also explored (see Table 3.14). Scores for baseline fatigue, depression, anxiety, and perceived stress were available in all 54 patients. Scores for sleep quality, a measure introduced later in the study, were only available in 18. There were significant, positive associations with baseline symptoms of depression and anxiety, and levels of perceived stress. No correlation was found with baseline sleep quality. Interestingly, and indeed surprisingly, there was no significant association between baseline fatigue and fatigue severity at TW4.

Baseline health status

To explore poorer baseline health status as a risk factor for greater problems with fatigue in response to treatment, correlation analyses were run with each baseline dimension subscale score of the SF-36 and the fatigue score at TW4 (see Table 3.15). Lower scores indicate worse functioning. Unlike fatigue on the CFQ, another measure of energy/fatigue, the 'vitality' subscale score, was moderately, negatively correlated with subsequent fatigue scores at TW4. This may be attributable to the fact that the measure is of the balance between energy and fatigue over the same period (past month), as opposed to fatigue as a problem. It should also be noted that this data was normally distributed, and therefore might provide a greater insight into the relationship between baseline fatigue and fatigue at TW4. Moderate correlations were also found with baseline ratings of general health, mental health, pain and social functioning. There was a trend towards a negative association with the 'emotional role limitation' score. The 'physical role limitation' score was not associated with fatigue at TW4, though there was a trend towards an association with 'physical functioning'.

Table 3.14 Association between baseline clinical symptoms and fatigue at TW4

<i>Baseline clinical symptoms (measure)</i>	<i>Fatigue score, TW4</i>
Fatigue (CFQ)	$r_s = 0.19, p = 0.16$
Depression (IDS)	$r_s = 0.45, p = 0.001$
Anxiety (HADS-A)	$r_s = 0.48, p = 0.001$
Perceived Stress (PSS)	$r_s = 0.44, p = 0.001$
Sleep quality (Jenkins)	$r_s = 0.07, p = 0.79$

Table 3.15 Association between baseline health status and fatigue at TW4

<i>SF-36 Dimension</i>	<i>Fatigue score, TW4</i>
Emotional Role Limitation	$r_s = -0.24, p = 0.074$
General Health	$r_s = -0.45, p = 0.001$
Mental Health	$r_s = -0.47, p < 0.001$
Pain	$r_s = -0.36, p = 0.017$
Physical Role Limitation	$r_s = -0.22, p = 0.108$
Physical Functioning	$r_s = -0.23, p = 0.096$
Social Functioning	$r_s = -0.59, p < 0.001$
Vitality	$r = -0.44, p = 0.001$

Baseline biological measures

Next, I examined baseline biological measures as predictors of fatigue severity in response to acute IFN- α treatment. Data is presented in Table 3.16. Again, absolute levels can be found in Appendix V. Since by TW4 fatigue scores were normally distributed, if data for the biological measure was normally distributed Pearson's correlation coefficient was used. In this case, this was only true of levels of tryptophan (TRP), and the ratio of kynurenine to tryptophan (KYN/TRP). Otherwise, Spearman's Rho correlations were performed and reported below. Though the relationship between baseline cytokines levels and acute fatigue was not as strong as had been seen at baseline, interestingly I did find a moderate association between more severe acute fatigue and higher baseline levels of IL-10 and TNF- α . There was also a trend towards a link with higher baseline IL-6. With regards to the kynurenine pathway, again the direction of results were largely negative, indicating that lower levels were associated with higher fatigue symptoms. Of the metabolites examined, lower baseline kynurenic and xanthurenic acid were significantly associated with greater fatigue at TW4. For cortisol, there was a trend towards higher fatigue levels at TW4 in those who had a higher Cortisol Awakening Response ('CAR') at baseline. The reverse pattern was found with diurnal levels, with those who had lower baseline cortisol across the day having more problems with fatigue at TW4.

Table 3.16 Association between baseline biological measures and fatigue at TW4

<i>Biological Marker</i>	<i>Fatigue score, TW4</i>
<i>Cytokines</i> (<i>n</i> = 48* #)	
IL-10	$r_s = 0.34, p = 0.02$
IL-13	$r_s = -0.18, p = 0.23$
IFN- γ^*	$r_s = 0.20, p = 0.22$
IL-2	$r_s = 0.08, p = 0.60$
IL-6	<u>$r_s = 0.28, p = 0.05$</u>
IL-7	$r_s = 0.07, p = 0.65$
IL-8	$r_s = -0.05, p = 0.73$
IL-12p70 [#]	$r_s = 0.05, p = 0.79$
IL-17A	$r_s = 0.12, p = 0.41$
TNF- α	$r_s = 0.35, p = 0.02$
VEGF*	$r_s = -0.06, p = 0.72$
<i>Kynurenine pathway</i> (<i>n</i> = 48)	
Tryptophan	$r = 0.13, p = 0.38$
Kyn/Trp ratio	$r = -0.15, p = 0.31$
Kynurenic Acid	$r_s = -0.41, p = 0.004$
Quinaldic Acid	$r_s = -0.24, p = 0.10$
3-HK/Kyn ratio	$r_s = 0.10, p = 0.48$
3-HK	$r_s = 0.007, p = 0.96$
Xanthurenic Acid	$r_s = -0.31, p = 0.03$
Picolinic Acid	$r_s = -0.08, p = 0.60$
Quinolinic Acid	$r_s = -0.16, p = 0.29$
<i>Cortisol</i> (<i>n</i> = 24; 27)	
Awakening Response (CAR AUC _i)	<u>$r = 0.40, p = 0.052$</u>
Diurnal (DAY AUC _g)	$r_s = -0.39, p = 0.045$

* *n* = 38; # *n* = 29;

(For absolute levels see Appendix V)

Cytokine levels at TW4

Finally for this measure of acute fatigue, I explored a link with cytokine levels measured at the same time point. Results are presented in Table 3.17. Absolute levels can be seen in Appendix V. Interestingly, fatigue scores at TW4 were not associated with concurrent measures of peripheral inflammation in these patients. There was a trend towards greater fatigue in those with higher levels of IL-2, though there were no significant associations across the 11 analytes.

Table 3.17 Association between cytokine levels and severity of fatigue at TW4

Cytokine, TW4 (<i>n</i> = 46^{*#})	Fatigue score, TW4
IL-10	$r_s = 0.24, p = 0.11$
IL-13	$r_s = -0.03, p = 0.83$
IFN- γ^*	$r_s = 0.15, p = 0.39$
IL-2	<u>$r_s = 0.26, p = 0.08$</u>
IL-6	$r_s = 0.10, p = 0.49$
IL-7	$r_s = 0.19, p = 0.19$
IL-8	$r_s = -0.09, p = 0.57$
IL-12p70 [#]	$r_s = -0.06, p = 0.78$
IL-17A	$r_s = 0.14, p = 0.36$
TNF- α	$r_s = 0.17, p = 0.26$
VEGF [*]	$r_s = 0.11, p = 0.54$

^{*} *n* = 36 [#] *n* = 28

(For absolute levels see Appendix V)

3.1.2.3 Severity of fatigue post-treatment

First, I explored possible predictors of more severe fatigue six-months post-treatment by analysing each baseline variable together with the fatigue score at the follow-up visit ('FU'). Because of the particular interest in the severity of fatigue post-treatment, I then concluded by exploring a link with the measures collected at the same visit. As many patients were again reporting lower scores at this point, data was again non-normal ($p = 0.002$), as assessed by the Shapiro-Wilk test. There were no outliers \pm three times the interquartile range. Spearman's Rho Correlations (r_s) were used to examine the relationship with other continuous variables. Independent samples t-tests were used to explore the relationship with dichotomised variables. Where equal variance could not be assumed, results reported are again those with the welch-satterthwaite adjustment applied.

Socio-demographic characteristics

Data relating to socio-demographic characteristics and fatigue severity is presented in Table 3.18. Equal variance could not be assumed between those with and without a personal history of depression: for the 'no' group, there was a slight positive skew around scores of '11', while in the 'yes' group scores were more normally distributed. Those who developed IFN- α induced depression during treatment were more likely to experience problems with fatigue at FU, six-months post-treatment. Though not significant, individuals with a history of depression, and those who did not complete a university education also had higher levels of fatigue. Interestingly, there was no significant association with baseline fatigue 'caseness', though mean fatigue scores were slightly higher in those who had met the criteria. The low number of 'cases' ($n = 5$) should of course be noted.

Virus and treatment characteristics

These characteristics are also summarised in Table 3.18. Individuals on triple therapy regimens, and those on treatment for longer than 24 weeks had slightly higher fatigue levels at FU. Though this association did not reach statistical significance, this implies that longer exposure to the trigger may be associated with more severe fatigue post-treatment. Interestingly, whether or not an individual had attained a 'Sustained Virological Response' six-months post-treatment – the virus was still not detectable - was not linked with the severity of fatigue. However, there was a trend towards a link to whether they had attained a 'Rapid Virological Response', with those who did not attain this result, and therefore 'fighting the virus' for longer, experiencing more severe fatigue post-treatment.

Table 3.18 Association between socio-demographic and virus/treatment characteristics and the severity of fatigue at follow-up

Characteristics	Fatigue score, FU	
	<i>Mean±SEM</i>	<i>Test and statistic</i>
Age (years)	-	$r_s = -0.008, p=0.96$
Gender Male/Female	13.0±0.6 vs. 11.7±1.4	$t (53) = -0.84, p=0.41$
Ethnicity White/other	13.2±0.8 vs. 12.2±0.9	$t (53) = -0.84, p=0.40$
Education Degree/other	11.6±1.0 vs. 13.2±0.7	$t (54) = 1.32, p=0.19$
Employment Unemployed/other	12.6±0.7 vs. 13.0±1.1	$t (53) = 0.31, p=0.76$
Relationship Married or living with/other	12.6±0.9 vs. 12.8±0.8	$t (53) = 0.23, p=0.82$
Baseline fatigue ‘case’ CFQ>18 – yes/no	14.2±3.3 vs. 12.6±0.6	$t (53) = -0.80, p=0.43$
History of Depression , y/n	14.1±1.2 vs. 11.9±0.6	$t (29.8) = -1.67, p=0.11$
Family History First degree – yes/no	13.0±1.2 vs. 12.8±0.7	$t (48) = -0.09, p=0.93$
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HCV genotype , 1/4 vs. 2/3	12.0±1.1 vs. 12.9±0.7	$t (53) = 0.60, p=0.55$
HCV viral load , millions	-	$r_s = -0.05, p=0.71$
Liver stiffness , kPa ($n = 48$)	-	$r_s = 0.009, p=0.95$
Treatment type IFN- α + ribavirin, y/n	11.1±0.8 vs. 13.0±0.7	$t (53) = 1.12, p=0.27$
Treatment duration ≤24 / >24	12.1±0.7 vs. 14.0±1.1	$t (53) = -1.56, p=0.13$
IFN-α depression , y/n	14.6±1.0 vs. 11.6±0.7	$t (52) = -2.58, p=0.013$
Response (clinical) RVR (TW4) – yes/no	11.8±0.7 vs. 14.1±0.9	$t (53) = 1.98, p=0.053$
SVR (FU) – yes/no	12.8±0.7 vs. 12.0±1.0	$t (53) = -0.46, p=0.65$

Experience of psychosocial stress

I then explored an association between severity of fatigue at follow-up (FU) and experience of psychosocial stress. Experience of any stressful life event in the six-months prior to the initiation of treatment, during treatment, or in the six-months post-treatment follow-up period was examined. So too was lifetime experience of any intrusive life event, and experience of any childhood trauma. Data on events during treatment was available in 31 patients (yes/no; 13/18), as it was not measured until later in the study. There was some difference in the variances between groups; individuals who reported no events during treatment were more likely to report a score of '11', whereas data in the 'yes' group was more normally distributed. Scores in those who did or did not experience intrusive life events were similarly focussed around a score of '11', though in those who did report at least one event, there were more high scores. With regards to results, experience of a stressful event pre-treatment was not linked to the severity of fatigue at FU, six-months post-treatment. However, there was a trend towards higher levels of fatigue in those who had experienced one or more stressful events during treatment. In addition, the data suggest higher fatigue scores in those who reported experiencing at least one stressful event in the six-months post-treatment, though this was not significant. I did not find the expected association with childhood trauma, though there was a trend towards higher fatigue scores in those who had experienced one or more intrusive life events in their lifetime (see Table 3.19).

Illness Perceptions

Next, an association was explored with patients' illness perceptions, as recorded before the initiation of treatment, at the baseline visit. Data is shown in Table 3.20. Unlike the strong association with IFN- α induced acute fatigue, there were no significant correlations between any of the illness perceptions dimensions and fatigue severity at follow-up. However, there was a trend towards a positive association with 'consequences' and 'emotional representations'. This indicates that to some extent, those who perceived their HCV as having greater consequences, and those who had more emotional representations of their illness had more severe fatigue at follow-up. Interestingly, the link with emotional representations appears to be consistent, having also been important for baseline fatigue, as well as acute IFN- α induced fatigue.

Table 3.19 Association between experience of psychosocial stress and the severity of fatigue at follow-up

Risk Factor	Fatigue score, FU	
	<i>(Mean±SEM)</i>	<i>Test and statistic</i>
Recent stressful events		
Pre-treatment, Yes/No	12.7±0.9 vs. 12.7±0.7	$t(53) = -0.01, p = 0.99$
During treatment, Yes/No	13.4±1.1 vs. 11.0±0.8	$t(28.0) = -1.83, p = 0.078$
During follow-up, Yes/No	12.9±0.7 vs. 11.3±0.9	$t(48) = -1.47, p = 0.15$
Lifetime intrusive events		
Yes/No	13.3±0.8 vs. 11.4±0.6	$t(52.1) = -1.85, p = 0.070$
Childhood trauma		
Yes/No	13.2±0.8 vs. 12.2±0.9	$t(53) = -0.86, p = 0.39$

Table 3.20 Association between Illness Perceptions characteristics and the severity of fatigue at follow-up

IPQ Dimension	Fatigue score, FU
Identity	$r_s = 0.12, p = 0.41$
Timeline (acute vs. chronic)	$r_s = 0.16, p = 0.26$
Consequences	$r_s = 0.24, p = 0.092$
Personal control	$r_s = -0.08, p = 0.56$
Treatment control	$r_s = -0.09, p = 0.54$
Illness coherence	$r_s = -0.005, p = 0.973$
Timeline (cyclical)	$r_s = 0.01, p = 0.95$
Emotional representations	$r_s = 0.25, p = 0.081$

Baseline clinical symptoms

An association between baseline clinical symptoms and the severity of fatigue was also explored. The data is presented in Table 3.21. Scores for baseline fatigue, depression, anxiety, and stress were available in all 55 patients. Scores for sleep quality, a measure introduced later in the study, were only available in 18 patients. There were no significant associations between fatigue at FU and baseline symptomatology. Interestingly, as has also been shown in relation to baseline fatigue 'caseness', there was only a trend towards an association between fatigue at follow-up, and levels reported at baseline.

Baseline health status

To explore poorer baseline health status as a risk factor for greater problems with fatigue post-treatment, correlation analyses were also run with each baseline dimension subscale score of the SF-36 and the fatigue score at follow-up (see Table 3.22). As described previously, lower SF-36 subscale scores indicate worse functioning. For fatigue at FU, baseline health status was less important than had been apparent for acute fatigue. There was a weak, negative correlation with patients' self reported ratings of their mental health, with those with more mental health related issues at baseline experiencing more problems with fatigue at FU. In line with this, there was a significant negative association with the baseline 'emotional role limitation' subscale score, indicating that those patients who felt more limited in their everyday lives by 'emotional problems' prior to treatment, were more prone to experiencing more fatigue post-treatment.

Table 3.21 Association between baseline clinical symptoms and fatigue at follow-up

Baseline clinical symptoms (measure)	Fatigue score, FU
Fatigue (CFQ)	$r_s = 0.25, p = 0.062$
Depression (IDS)	$r_s = 0.17, p = 0.21$
Anxiety (HADS-A)	$r_s = 0.13, p = 0.35$
Perceived Stress (PSS)	$r_s = 0.20, p = 0.15$
Sleep quality (Jenkins)	$r_s = 0.08, p = 0.74$

Table 3.22 Association between baseline health status and fatigue at follow-up

SF-36 Dimension	Fatigue score, FU
Emotional Role Limitation	$r_s = -0.33, p = 0.015$
General Health	$r_s = -0.16, p = 0.25$
Mental Health	$r_s = -0.24, p = 0.074$
Pain	$r_s = -0.22, p = 0.11$
Physical Role Limitation	$r_s = -0.14, p = 0.32$
Physical Functioning	$r_s = -0.21, p = 0.13$
Social Functioning	$r_s = -0.11, p = 0.43$
Vitality	$r_s = -0.13, p = 0.34$

Baseline biological measures

I then explored baseline biological measures as predictors of more severe fatigue post-treatment (see Table 3.23). Absolute levels can be seen in Appendix V. I found a moderate association between more severe fatigue at FU, and lower baseline levels of the cytokine IL-13, and higher levels of IL-17A. There was also a trend towards lower baseline xanthurenic acid and higher post-treatment fatigue. There was no significant association with baseline HPA axis function, as assessed via salivary cortisol measurements. However, there was a trend towards a negative association with the cortisol awakening response, with those with a lower baseline Cortisol Awakening Response ('CAR') reporting more problems with fatigue post-treatment.

Table 3.23 Association between baseline clinical measures and fatigue at follow-up

<i>Biological Marker, TW0</i>	<i>Fatigue score, FU</i>
<i>Cytokines</i> (<i>n</i> = 49* #)	
IL-10	$r_s = 0.03, p = 0.83$
IL-13	$r_s = -0.31, p = 0.029$
IFN- γ^*	$r_s = 0.08, p = 0.61$
IL-2	$r_s = 0.05, p = 0.75$
IL-6	$r_s = 0.18, p = 0.23$
IL-7	$r_s = 0.01, p = 0.96$
IL-8	$r_s = 0.09, p = 0.55$
IL-12p70 [#]	$r_s = -0.11, p = 0.57$
IL-17A	$r_s = 0.29, p = 0.042$
TNF- α	$r_s = 0.04, p = 0.80$
VEGF*	$r_s = 0.12, p = 0.46$
<i>Kynurenine pathway</i> (<i>n</i> = 42)	
Tryptophan	$r_s = -0.07, p = 0.64$
Kyn/Trp ratio	$r_s = -0.12, p = 0.40$
Kynurenic Acid	$r_s = -0.15, p = 0.31$
Quinaldic Acid	$r_s = -0.06, p = 0.70$
3-HK/Kyn ratio	$r_s = 0.02, p = 0.88$
3-HK	$r_s = 0.02, p = 0.88$
Xanthurenic Acid	<u>$r_s = -0.25, p = 0.09$</u>
Picolinic Acid	$r_s = -0.07, p = 0.63$
Quinolinic Acid	$r_s = -0.06, p = 0.68$
<i>Cortisol</i> (<i>n</i> = 25; 28)	
Awakening Response (CAR AUC _i)	<u>$r_s = -0.37, p = 0.07$</u>
Diurnal (DAY AUC _g)	$r_s = -0.04, p = 0.83$

* *n* = 39; # *n* = 29

(For absolute levels see Appendix V)

Follow-up clinical symptoms

At the follow-up visit six-months post-treatment, all clinical measurements were available in 55 patients, apart from sleep quality, which was measured in 40 patients. There was a strong, positive association between fatigue and concomitant depressive symptoms at this time point, indicating that the patients who reported the most problems with fatigue were also experiencing more problems with low mood and other associated symptoms. Analysis was also run looking at the difference in scores between those who fulfilled the MINI criteria for a current depressive episode and those who did not, but it is not reported as only 2 individuals met the criteria at that time. There were significant, moderate correlations linking higher perceived stress and more problems with sleep with higher fatigue. Higher anxiety was also associated with more severe fatigue (see Table 3.24).

Follow-up health status

When measured at follow-up, some of the strongest associations with fatigue related to physical problems, with patients who reported poorer physical functioning, and more limitations to their every day activities as a result of their physical health also reporting more problems with fatigue (see Table 3.25). Poor social functioning also continued to be associated with more severe fatigue, as did measures of pain and mental health (including limitations to every day activities as a result of emotional problems). At follow-up, the link with 'vitality' scores was stronger than had been found previously. However, the association with ratings of general health was still weak, again suggesting that patients in this sample did not factor in their experience of fatigue in their assessment of their overall health.

Table 3.24 Association between clinical symptoms and fatigue at follow-up

<i>Clinical symptoms, FU (measure)</i>	<i>Fatigue score, FU</i>
Depression (IDS)	$r_s = 0.58, p < 0.001$
Anxiety (HADS-A)	$r_s = 0.27, p = 0.046$
Perceived Stress (PSS)	$r_s = 0.30, p = 0.025$
Sleep quality (Jenkins)	$r_s = 0.38, p = 0.015$

Table 3.25 Association between health status and fatigue at follow-up

<i>SF-36 Dimension, FU</i>	<i>Fatigue score, FU</i>
Emotional Role Limitation	$r_s = -0.45, p = 0.001$
General Health	$r_s = -0.17, p = 0.20$
Mental Health	$r_s = -0.33, p = 0.014$
Pain	$r_s = -0.39, p = 0.004$
Physical Role Limitation	$r_s = -0.51, p < 0.001$
Physical Functioning	$r_s = -0.45, p = 0.001$
Social Functioning	$r_s = -0.47, p < 0.001$
Vitality	$r_s = -0.51, p < 0.001$

Follow-up biological measures

Finally, I explored a link between the different biological markers measured at follow-up, and the severity of fatigue reported at the same visit (see Table 3.26). Absolute levels can be seen in Appendix V. For cytokine measurements, there was a trend towards higher IFN- γ levels in those with more severe fatigue, and a significant association with higher IL-6. There was no link between fatigue at follow-up and the kynurenine pathway. Interestingly there was a significant negative association with the cortisol awakening response, with those with a lower cortisol response to awakening reporting more problems with fatigue. However, there was no significant association with diurnal cortisol output.

Table 3.26 Association between biological measures and fatigue at follow-up

<i>Biological Marker, FU</i>	<i>Fatigue score, FU</i>
<u>Cytokines</u> (<i>n</i> = 47* #)	
IL-10	$r_s = 0.19, p = 0.21$
IL-13	$r_s = -0.14, p = 0.37$
IFN- γ^*	$r_s = 0.31, p = 0.07$
IL-2	$r_s = 0.08, p = 0.60$
IL-6	$r_s = 0.30, p = 0.04$
IL-7	$r_s = 0.08, p = 0.60$
IL-8	$r_s = 0.07, p = 0.63$
IL-12p70 [#]	$r_s = -0.11, p = 0.57$
IL-17A	$r_s = 0.17, p = 0.25$
TNF- α	$r_s = 0.10, p = 0.51$
VEGF*	$r_s = 0.02, p = 0.92$
<u>Kynurenine pathway</u> (<i>n</i> = 49)	
Tryptophan	$r_s = -0.10, p = 0.54$
Kyn/Trp ratio	$r_s = -0.09, p = 0.58$
Kynurenic Acid	$r_s = -0.21, p = 0.17$
Quinaldic Acid	$r_s = -0.06, p = 0.73$
3-HK/Kyn ratio	$r_s = 0.09, p = 0.56$
3-HK	$r_s = -0.18, p = 0.25$
Xanthurenic Acid	$r_s = -0.19, p = 0.23$
Picolinic Acid	$r_s = 0.26, p = 0.10$
Quinolinic Acid	$r_s = 0.01, p = 0.97$
<u>Cortisol</u> (<i>n</i> = 25; 28)	
Awakening Response (CAR AUC _i)	$r_s = -0.49, p = 0.025$
Diurnal (DAY AUC _g)	$r_s = 0.31, p = 0.16$

* *n* = 37; # *n* = 27

(For absolute levels see Appendix V)

3.1.2.4 Severity of fatigue: a summary

A summary of findings from all three sets of analyses, relating to baseline, acute and post-treatment fatigue, and addressing aim two of this thesis, can be found overleaf (Figure 3.16). For clarity, only statistically significant findings are reported, though it is also indicated where there were no significant associations within a category of measures. Those variables measured at baseline, whether associated with baseline fatigue, or examined as predictors of acute and fatigue post-treatment, appear at the beginning of the table. For acute and post-treatment fatigue, measures obtained at the same time point (TW4, six-months post-treatment respectively) were also examined, and these appear at the bottom of the table.

Prospective HCV (IFN- α) Cohort Study

Aim 2	Factors Associated with Severity of Fatigue <i>Characteristics; Clinical Measures; Health Status; Biological Measures (including baseline Cortisol)</i>		
	Baseline (TW0)	Acute (TW4)	Post-treatment (FU)
	HCV Genotype 2/3 ↑	✗ baseline characteristics	IFN- α depression ↑ Virus cleared by TW4 ↓
	Recent stressful event ↑	✗ measures of stress	Stressful event during IFN- α ↑
	Emotional representations ↑	Negative Baseline Illness Perceptions ↑↑	baseline Illness Perceptions ✗
	Depressive Sx ↑ Anxious ↑ Perceived Stress ↑	TW0 Depressive Sx ↑ Anxious ↑ Perceived Stress ↑	baseline clinical symptoms ✗
	Health Status ↓↓	Baseline Health Status ↓↓	Limitations to role due to emotional probs ↑
	↑ Pro-inflammatory cytokines IFN- γ ; IL-2; IL-6; IL-7; IL-8; IL-12p70; -17A; TNF- α	Baseline IL-10, TNF- α ↑ Baseline Kyn acid ↓ Baseline Xan acid ↓ Baseline Diurnal ↓	IL-13 ↓; IL-17A ↑
		TW4 IL-2 ↑	Current Clinical Sx: Dep, Anx, Stress ↑ Sleep ↓ Current Health Status ↓ Current IL-6 ↑ Current CAR ↓
Key: ↑=higher fatigue/increased levels associated with higher fatigue; ↓=lower fatigue/lower levels/functioning associated with higher fatigue; ✗=no association			

Figure 3.16 Summary of results for Aim 2: factors associated with the severity of fatigue

3.1.3 IFN- α induced persistent fatigue

Addressing the third aim of my thesis, to further understand the persistence of fatigue specifically, I then conducted additional analyses based on changes in fatigue levels in individuals. This is different from the approach used in section 3.1.2 where I used the absolute levels of fatigue to explore the factors relevant to fatigue severity. The approach presented here fits more closely with the proposed basis for IFN- α induced persistent fatigue as a proxy-model. It does so by focussing on the degree of change occurring in response to the trigger, and the resolution or not of these symptoms to baseline functioning after the stimulus was no longer present, independent of baseline fatigue severity. I explored differences in various measures in those in whom fatigue persisted post-treatment, versus those who subsequently recovered. Patients were stratified according to delta Chalder Fatigue Questionnaire scores, deducting the baseline score from that reported at follow-up, six-months post-treatment (CFQ Total FU – CFQ Total TW0). Negative scores, indicating an improvement in fatigue, and scores of 0, indicating a return to baseline fatigue levels, were labelled 'Resolved Fatigue' ('RF'; $n = 37$; 67.3%). Positive scores, indicating a worsening of fatigue, were labelled 'Persistent Fatigue' ('PF'; $n = 18$; 32.7%). First, I examined possible risk factors for IFN- α induced persistent fatigue by looking at the characteristics of each group, and also comparing baseline biological variables (cortisol, cytokines, kynurenine pathway metabolites). Then I compared baseline measures, and changes in clinical and biological measures in response to IFN- α treatment in the two groups, to explore possible differences in the effect of IFN- α on these patients, that may contribute towards IFN- α induced persistent fatigue.

3.1.3.1 Risk factors for IFN- α induced persistent fatigue

As in the whole sample, I examined socio-demographic characteristics, as well as those relating to treatment and the virus, baseline and history of psychopathology, baseline health status, experience of recent and historic psychosocial stress and illness perceptions. I then examined baseline levels of cytokines and kynurenine pathway metabolites, and baseline functioning of the HPA-axis through salivary cortisol levels.

Socio-demographic characteristics

There were few significant differences in the socio-demographic characteristics of each group. Unexpectedly, the only significant result related to a lower number of patients on opioids as part of rehabilitation programs in the PF group (versus never/past recreational use). Otherwise, there were some interesting patterns apparent (see Table 3.27). There were a higher proportion of males in the PF group. There was no significant difference in ethnicity, though no Asian participants reported a worsening of baseline levels of fatigue, and there were a higher proportion of White British/Irish individuals in the PF group. A slightly higher proportion of patients in the PF group reported a history of major depression, and separately, developed IFN- α induced depression. With respect to drug use, a slightly higher proportion of patients in the PF group had a history of opioid use (including current drug rehab users, versus never used), and/or were current smokers, though this was not significant. There was also a trend towards higher current alcohol use in the PF group. Otherwise, groups did not differ in terms of age, employment, relationship status or family history of mental illness. As had been found for the severity of fatigue post-treatment, clinically relevant baseline fatigue ('caseness') did not predict persistent fatigue.

Table 3.27 Socio-demographic characteristics in the HCV PF vs. RF groups

	RF (n = 37)	PF (n = 18)	Test and statistic
Age (years)			
Mean±SEM	43.7±2.0	46.4±2.8	t (53) = -0.79, p=0.43
Gender			
Male	28 (75.7%)	16 (88.9%)	X ² (1) = 1.32, p=0.25
Ethnicity			X ² (3) = 5.18, p=0.16
White (British/Irish)	16 (43.2%)	12 (66.7%)	
Asian	7 (18.9%)	0	
Black	1 (2.7%)	1 (5.6%)	
Other	13 (35.1%)	5 (27.8%)	
Education Level			
Degree	14 (37.8%)	4 (22.2%)	X ² (1) = 1.34, p=0.25
Employment status			
Unemployed	12 (32.4%)	7 (38.9%)	X ² (1) = 0.22, p=0.64
Relationship status			
Married/ Living with someone	15 (40.5%)	7 (38.9%)	X ² (1) = 0.01, p=0.91
Fatigue 'case' (TW0 CFQ score >18)	4 (10.8%)	1 (5.6%)	X ² (1) = 0.41, p=0.53
History of Depression	12 (32.4%)	8 (44.4%)	X ² (1) = 0.76, p=0.39
Family history	11 (33.3%)	6 (35.3%)	X ² (1) = 0.02, p=0.89
IFN-α induced Depression*	12 (32.4%)	9 (50.0%)	X ² (2) = 1.91, p=0.39
Drug use			
Current opioid use (medical)**	9 (25.0%)	1 (6.3%)	X² (2) = 7.03, p=0.030
History of opioid abuse	15 (41.7%)	9 (56.3%)	X ² (1) = 0.95, p=0.33
Current smoking**	13 (36.1%)	9 (52.9%)	X ² (2) = 1.42, p=0.49
Current alcohol**	15 (41.7%)	11 (64.7%)	X ² (2) = 5.51, p=0.064

* Vs. no/baseline depression; ** vs. past/never; some missing

Virus and treatment characteristics

There were no significant differences between groups in any virus or treatment characteristics, though again some interesting patterns were suggested (see Table 3.28). There was a slightly higher rate of HCV genotype 3 in the RF versus PF group, though overall there was no significant difference in the distribution of genotypes in each group. Building on previous research highlighting the lack of an association with disease severity and fatigue, patients had similar baseline virus levels, and almost the same level of liver damage, as measured by the FibroScan, was observed in each group. However, as had been associated with the severity of fatigue post-treatment earlier, there was a trend towards an association with a longer duration of treatment, and longer exposure to the trigger. Specifically, more of the RF group had 24 weeks or less treatment (~75%), while in the PF group it was a 50/50 split. This, in addition to being relevant for the duration of the exposure to the exogenous trigger, may also be considered as a proxy for more difficult to treat presentations. I found no difference between groups in the treatments prescribed, suggesting that while improving clinical efficacy, the newer drugs don't necessarily protect against IFN- α side effects. No differences were observed in clinically relevant measures of treatment response: 'Rapid Virological Response' (RVR), whereby the virus is undetected after four weeks of treatment, or 'Sustained Virological Response' whereby the virus is undetected six-months post-treatment, the measure on which the success of treatment is determined. Lastly, appointments were based on hospital appointments in existing clinics, meaning we were unable to standardise the time of appointments. However, whether responses and blood samples were collected in the morning or afternoon was recorded, and no difference was found between the two groups.

Table 3.28 Treatment and virus characteristics in the HCV PF vs. RF groups

	RF (n = 32*-37)	PF (n = 16*-18)	Test and statistic
HCV genotype			$\chi^2 (3) = 3.64, p=0.30$
1	6 (16.2%)	4 (22.2%)	
2	4 (10.8%)	5 (27.8%)	
3	26 (70.3%)	9 (50.0%)	
4	1 (2.7%)	0 (0.0%)	
HCV viral load, millions			
Mean±SEM	2.7±0.6	3.0±0.8	$t (53) = -0.34, p=0.43$
Liver stiffness, kPa*			
Mean±SEM	8.6±1.1	8.4±1.3	$t (46) = 0.09, p=0.93$
Treatment duration, wks			$\chi^2 (1) = 3.6, p=0.057$
≤24	28 (75.7%)	9 (50%)	
>24	9 (24.3%)	9 (50%)	
Treatment type			$\chi^2 (1) = 0.25, p=0.61$
IFN-α + ribavirin	31 (83.8%)	16 (88.9%)	
Triple therapy	6 (16.2%)	2 (11.1%)	
Telaprevir	4	1	
Simeprevir	1	1	
Boceprevir	1	0	
RVR			
Yes	24 (64.9%)	9 (50.0%)	$\chi^2 (1) = 1.12, p=0.29$
SVR			
Yes	31 (83.8%)	17 (94.4%)	$\chi^2 (1) = 1.24, p=0.27$
Blood time taken			
AM	17 (45.9%)	10 (55.6%)	$\chi^2 (1) = 0.48, p=0.50$

Experience of psychosocial stress

With regards to recent psychosocial stress, a similar proportion of patients in each group reported having experienced at least one stressful life event in the six-months before the initiation of treatment. Taking into account a lifetime history of experience of more intrusive life events, there was a trend towards a higher proportion of the PF group having experienced at least one of the events listed in their lifetime (87.5% vs. 63.9%, $p = 0.08$). Looking at childhood experiences of care and abuse, later development of persistent fatigue was not associated with a history of early-life stress, with around 50% of both groups reporting at least one form of trauma (loss of parent, separation from parent, physical and/or sexual abuse). Data from these measures are shown in Table 3.29. Furthermore, there were no differences in the experience of specific types of trauma when considered individually, across the lifetime and childhood measures (data not shown).

Illness perceptions

The patients 'illness perceptions', as measured at baseline and relating to HCV and treatment ahead, were also examined as a possible risk factor for the subsequent development of persistent fatigue. As had been shown in relation to the severity of fatigue post-treatment, baseline illness perceptions did not predict the persistence of fatigue post-treatment (data shown in Table 3.30, page 178). Interestingly though, this included 'emotional representations', which had been consistently associated with the severity of fatigue throughout, albeit more weakly post-treatment (see pages 140, 148, 160).

Table 3.29 Experience of psychosocial stress in the HCV PF vs. RF groups

	RF (n = 36*-7)	PF (n =16*-18)	Test and statistic
Recent stressful life events			
Any last six months, yes	18 (48.6%)	7 (38.9%)	$X^2 (1) = 0.47, p = 0.50$
Lifetime intrusive life events*			
Any lifetime, yes	23 (63.9%)	14 (87.5%)	$X^2 (1) = 3.01, p = 0.08$
<i>Serious injury or assault</i>	16 (44.4%)	10 (62.5%)	
<i>Bullying</i>	9 (25.0%)	7 (43.8%)	
<i>Homeless</i>	8 (22.2%)	6 (37.5%)	
<i>Violence in the home ever</i>	8 (22.2%)	5 (38.5%)	
<i>Sexual abuse</i>	4 (11.1%)	3 (18.8%)	
<i>Running away from home</i>	7 (19.4%)	4 (25.0%)	
<i>Expelled from school</i>	4 (11.1%)	4 (25.0%)	
<i>Time in a children's institution</i>	4 (11.1%)	2 (12.5%)	
<i>Violence at work ever</i>	3 (8.3%)	2 (12.5%)	
<i>Taken into local authority care</i>	4 (11.1%)	1 (6.3%)	
Childhood trauma			
Any, yes	18 (48.6%)	9 (50.0%)	$X^2 (1) = 0.01, p = 0.93$
Forms of trauma, yes			
<i>Separation from parent</i>	10 (27.0%)	6 (33.3%)	
<i>Loss of parent</i>	7 (18.9%)	2 (11.1%)	
<i>Physical abuse</i>	7 (18.9%)	2 (11.1%)	
<i>Sexual abuse</i>	2 (5.4%)	4 (22.2%)	

Table 3.30 Illness perceptions in the HCV PF vs. RF groups

IPQ Dimension	RF	PF	Test and statistic
	(<i>n</i> = 32)	(<i>n</i> = 18)	
	Mean±SEM		
Identity	2.5±0.7	2.8±0.8	<i>t</i> (47) = -0.27, <i>p</i> = 0.79
Timeline (acute vs chronic)	15.0±1.0	16.6±1.4	<i>t</i> (48) = -0.92, <i>p</i> = 0.36
Consequences	18.5±1.5	20.8±1.0	<i>t</i> (48) = -1.07, <i>p</i> = 0.29
Personal control	23.2±0.6	23.3±0.6	<i>t</i> (47) = -1.22, <i>p</i> = 0.90
Treatment control	23.0±1.9	20.4±0.8	<i>t</i> (48) = 1.01, <i>p</i> = 0.32
Illness coherence	19.6±0.8	18.6±0.8	<i>t</i> (48) = 0.85, <i>p</i> = 0.40
Timeline (cyclical)	9.8±0.7	8.9±1.0	<i>t</i> (47) = 0.79, <i>p</i> = 0.44
Emotional representations	16.9±1.1	17.8±1.4	<i>t</i> (48) = -0.49, <i>p</i> = 0.62

Baseline biological measures

Data is presented in Table 3.31. First, I compared baseline cytokine levels in the Persistent versus Resolved Fatigue groups. There were no statistically significant differences in baseline levels of any of the cytokines measured. However, interestingly, examination of the mean values did show that levels of IL-6 were slightly higher in those who would later experience persistent fatigue post-treatment (PF vs. RF, pg/ml; 1.2 ± 0.3 vs. 0.8 ± 0.1 , $p = 0.14$), as were levels of IFN- γ (8.0 ± 1.0 vs. 11.8 ± 3.6 , $p = 0.17$). There were no differences in any of the kynurenine pathway metabolites measured, and in fact, some levels (quinaldic, kynurenic and picolinic acid) were almost identical in both groups.

Next, I examined salivary cortisol levels. I compared the baseline cortisol response to awakening ('CAR') and the diurnal cortisol output of patients in the PF/RF groups. Data for salivary cortisol are presented in Table 3.32 (page 181), and depicted in Figure 3.17 and Figure 3.18. Data was available in 12 PF patients. In the RF group, data was available for the CAR in 13 patients, and on diurnal cortisol in 16 patients. Patients were excluded from each analysis separately where the completed diary indicated poor compliance with the protocol at the relevant sampling points. Examination of the mean levels showed that patients who would later develop Persistent Fatigue (PF) had a lower cortisol response to awakening (27.7 ± 73.2 vs. 131.5 ± 62.5) and diurnal cortisol output (2685.3 ± 367.1 vs. 3733.0 ± 594.3), though the difference did not reach statistical significance. Indeed, in the PF group overall, cortisol levels dipped after awakening, the opposite pattern to what might be expected, of increases in the first 30 minutes.

Table 3.31 Baseline cytokine levels, and levels of the kynurenine pathway metabolites in HCV PF vs. RF groups

<i>Biological Measure</i>	RF (<i>n</i> = 34 [#] -35 [^])	PF (<i>n</i> = 14 [^] -15 ^{*#})	Test and statistic
<i>Cytokines</i>			
IL-10	1.0±0.2	1.6±0.6	<i>t</i> (47) = -0.95, <i>p</i> = 0.35
IL-13	0.5±0.1	0.4±0.2	<i>t</i> (47) = 0.22, <i>p</i> = 0.83
IFN-γ*	8.0±1.0	11.8±3.6	<i>t</i> (37) = -1.39, <i>p</i> = 0.17
IL-2	0.7±0.4	0.4±0.1	<i>t</i> (47) = 0.67, <i>p</i> = 0.51
IL-6	0.8±0.1	1.2±0.3	<i>t</i> (47) = -1.49, <i>p</i> = 0.14
IL-7	16.1±1.8	14.4±1.8	<i>t</i> (47) = 0.56, <i>p</i> = 0.58
IL-8	28.4±8.5	15.0±2.0	<i>t</i> (47) = 1.04, <i>p</i> = 0.30
IL-12p70 [#]	0.3±0.1	0.2±0.1	<i>t</i> (27) = 0.61, <i>p</i> = 0.55
IL-17A	2.0±0.4	2.5±0.7	<i>t</i> (47) = -0.72, <i>p</i> = 0.47
TNF-α	4.4±0.4	5.2±0.8	<i>t</i> (47) = -1.10, <i>p</i> = 0.30
VEGF*	196.3±27.0	215.8±47.8	<i>t</i> (37) = -0.38, <i>p</i> = 0.71
<i>Kynurenine pathway[^]</i>			
Tryptophan	18086.6±509.0	17210.8±771.2	<i>t</i> (47) = 0.93, <i>p</i> = 0.36
Kyn/Trp ratio	2.2±0.1	2.2±0.1	<i>t</i> (47) = -0.37, <i>p</i> = 0.72
Kynurenic Acid	7.8±0.5	7.8±0.6	<i>t</i> (47) = 0.004, <i>p</i> = 0.997
Quinaldic Acid	2.2±0.2	2.2±0.3	<i>t</i> (47) = 0.01, <i>p</i> = 0.99
3-HK/Kyn ratio	2.7±0.2	2.9±0.3	<i>t</i> (47) = -0.60, <i>p</i> = 0.55
3-HK	10.3±0.8	10.8±1.2	<i>t</i> (47) = -0.37, <i>p</i> = 0.71
Xanthurenic Acid	3.5±0.4	2.8±0.3	<i>t</i> (47) = 1.10, <i>p</i> = 0.28
Picolinic Acid	71.3±5.8	73.4±8.7	<i>t</i> (47) = -0.20, <i>p</i> = 0.84
Quinolinic Acid	52.7±4.7	50.4±4.7	<i>t</i> (47) = 0.29, <i>p</i> = 0.78

* *n* = 28/11; [#] *n* = 19/10; [^] *n* = 35/14

Table 3.32 Baseline salivary cortisol measurements in the HCV PF vs. RF groups

<i>Cortisol measure</i>	RF (<i>n</i> = 13*-16)	PF (<i>n</i> = 12)	Test and statistic
CAR*			
<i>AUC_i</i> nmol min/l	131.5±62.5	27.7±73.2	<i>t</i> (23)=1.08, <i>p</i> = 0.29
<i>Levels</i> nmol/L			
Awakening	7.0±1.1	7.2±1.5	
+15mins	9.5±1.2	6.7±1.3	
+ 30mins	9.7±1.5	8.7±1.3	
+ 60mins	9.4±1.3	7.5±1.6	
Diurnal output			
<i>AUC_g</i> nmol h/l	3733.0±594.3	2685.3±367.1	<i>t</i> (26)=1.38, <i>p</i> = 0.18
<i>Levels</i> nmol/L			
Awakening	9.8±2.3	7.2±1.5	
12pm	4.8±0.9	2.9±0.4	
8pm	2.9±0.6	1.9±0.3	

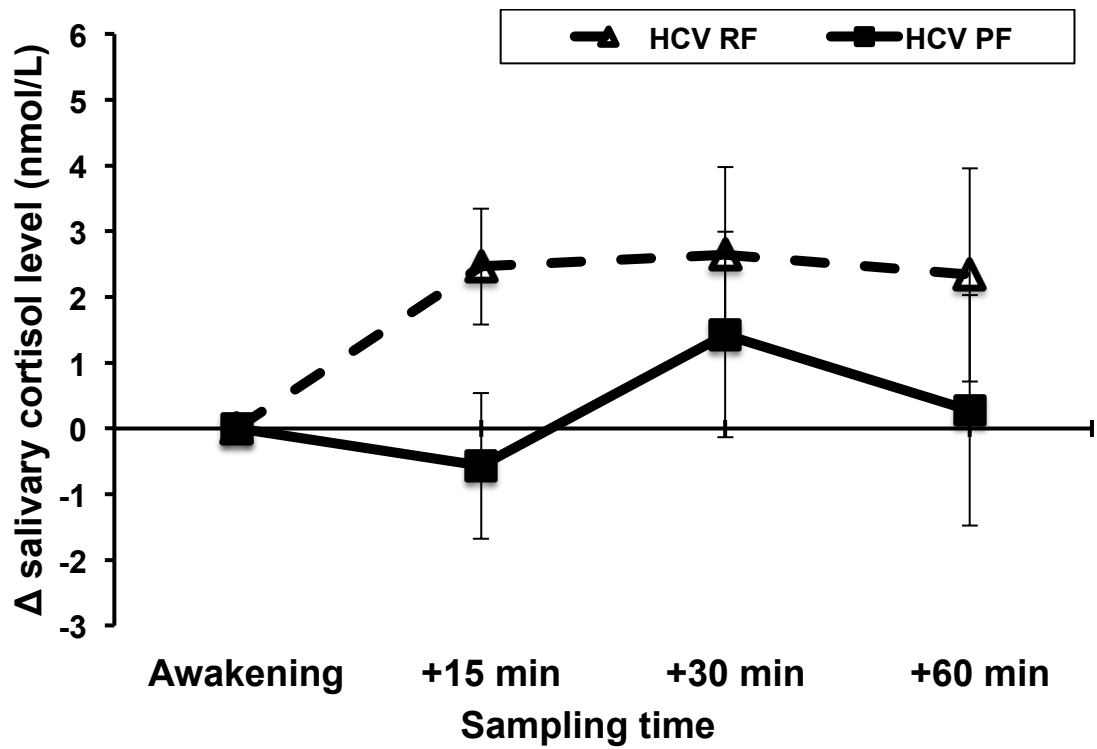


Figure 3.17 Baseline 'Cortisol Awakening Response' in HCV PF vs. RF patients

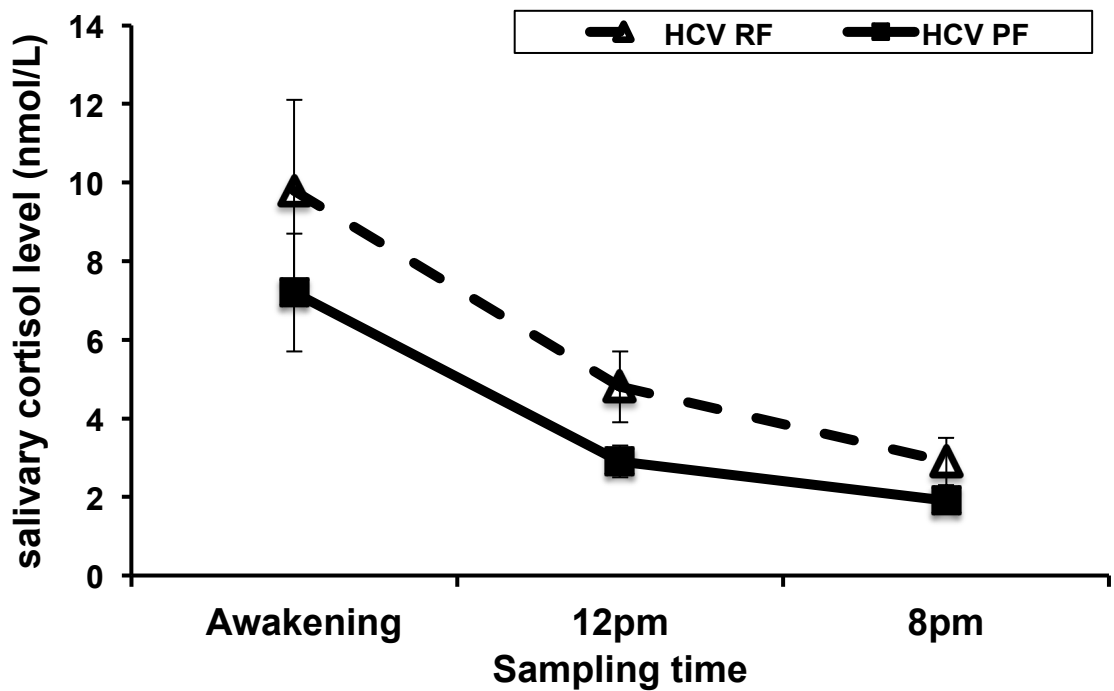


Figure 3.18 Baseline diurnal cortisol levels in HCV PF vs. RF patients

3.1.3.2 Baseline clinical symptoms, and changes in persistent versus resolved Fatigue

I also examined whether patients who would later report persistent fatigue had different patterns of clinical symptoms at baseline and in response to IFN- α , compared with those in whom fatigue resolved. A summary of results can be found in Table 3.38 (see page 198).

Fatigue

Data relating to fatigue scores are presented in Table 3.33, and depicted in Figure 3.19 and Figure 3.20. Contrary to expectations, there was no difference in baseline fatigue levels between the PF and RF groups, indicating that it was not simply that PF patients were already more fatigued before IFN- α treatment. However, levels became higher in the PF group by treatment week (TW) 4. While fatigue levels were elevated in the PF group at the intermediate time points, they were not significantly higher again until TW24, and/or end of treatment, and of course at six-month follow-up. Looking to the delta scores, the PF group had a more exaggerated response to treatment in the first four weeks (TW0 vs. 4), with greater increases following more chronic treatment (TW0 vs. 24; TW0 vs. END) than those in the RF group. There was no difference between groups in the delta between baseline and TW8, and only a trend towards a higher increase in the PF group from baseline to TW12. This reflects the gradual increase in the experience of acute fatigue early on, and the levelling out of symptoms later in the RF group, versus the upward trajectory of the PF group. Again, contrary to expectations, there was no difference between groups in the change over the six-month follow-up period ('end' vs. FU). Mean scores show that the PF group experienced this change from a higher level.

Table 3.33 Baseline levels, and changes in fatigue in HCV PF vs. RF groups

Fatigue (CFQ)	RF (<i>n</i> = 37)	PF (<i>n</i> = 18)	Test and statistic
<i>Treatment Time point</i>			
TW0	12.8±0.6	12.4±0.9	<i>t</i> (53) = 0.4, <i>p</i> = 0.69
TW4	16.7±0.8	19.5±1.2	<i>t</i> (52) = -1.96, <i>p</i> = 0.056
TW8	17.9±1.0	19.9±1.1	<i>t</i> (50) = -1.28, <i>p</i> = 0.21
TW12	17.6±1.1	20.5±1.3	<i>t</i> (49) = -1.61, <i>p</i> = 0.11
TW24	17.2±1.2	22.5±1.3	<i>t</i> (46) = -2.79, <i>p</i> = 0.008
END (<i>of treatment</i>)	17.9±1.3	22.5±1.5	<i>t</i> (49) = -2.18, <i>p</i> = 0.035
FU	10.6±0.5	17.0±0.8	<i>t</i> (53) = -7.07, <i>p</i> < 0.001
<i>Delta scores</i>			
Δ TW0 vs TW4	4.0±0.8	7.1±1.5	<i>t</i> (52) = -2.05, <i>p</i> = 0.046
Δ TW0 vs TW8	5.1±0.9	7.5±1.4	<i>t</i> (50) = -1.49, <i>p</i> = 0.14
Δ TW0 vs TW12	4.8±1.0	8.1±1.5	<i>t</i> (49) = -1.85, <i>p</i> = 0.071
Δ TW0 vs TW24	4.7±1.1	10.1±1.7	<i>t</i> (46) = -2.81, <i>p</i> = 0.007
Δ TW0 vs FU	-2.2±0.6	4.6±0.9	<i>t</i> (53) = -6.13, <i>p</i> < 0.001
Δ TW0 vs END	5.1±1.1	10.1±1.8	<i>t</i> (49) = -2.50, <i>p</i> = 0.016
Δ END vs FU	-7.3±1.3	-5.3±1.4	<i>t</i> (49) = -0.96, <i>p</i> = 0.34

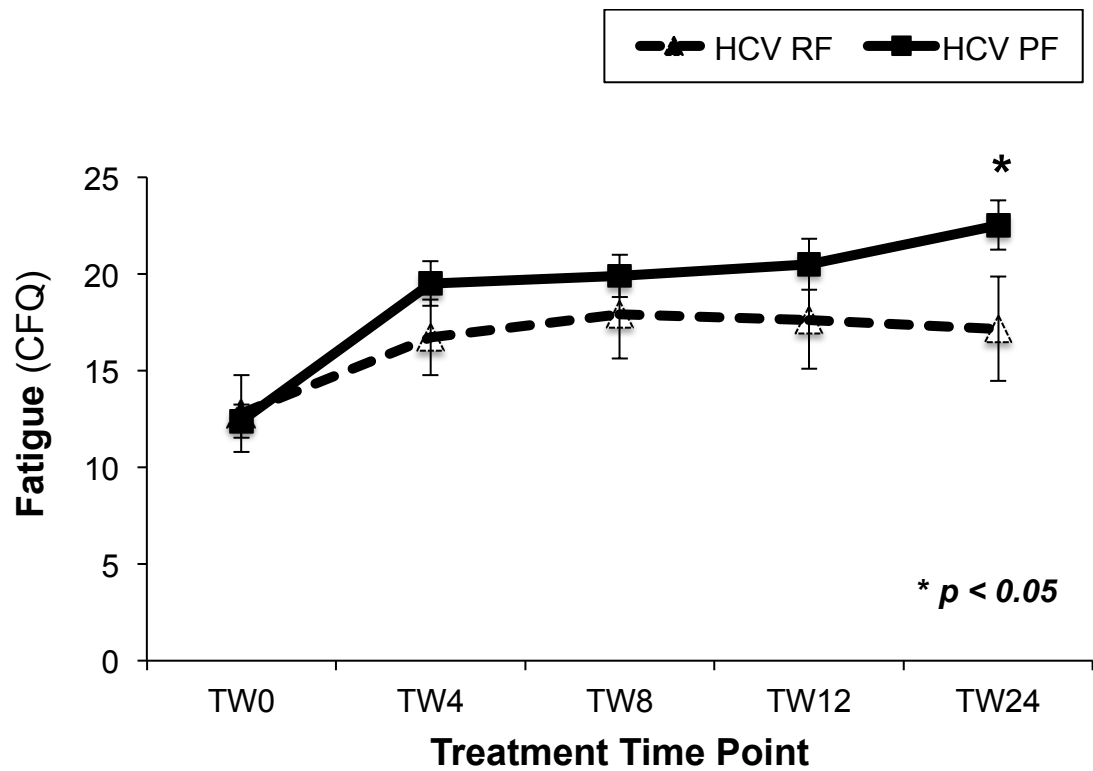


Figure 3.19 Fatigue scores at baseline, and during treatment in HCV PF vs. RF groups

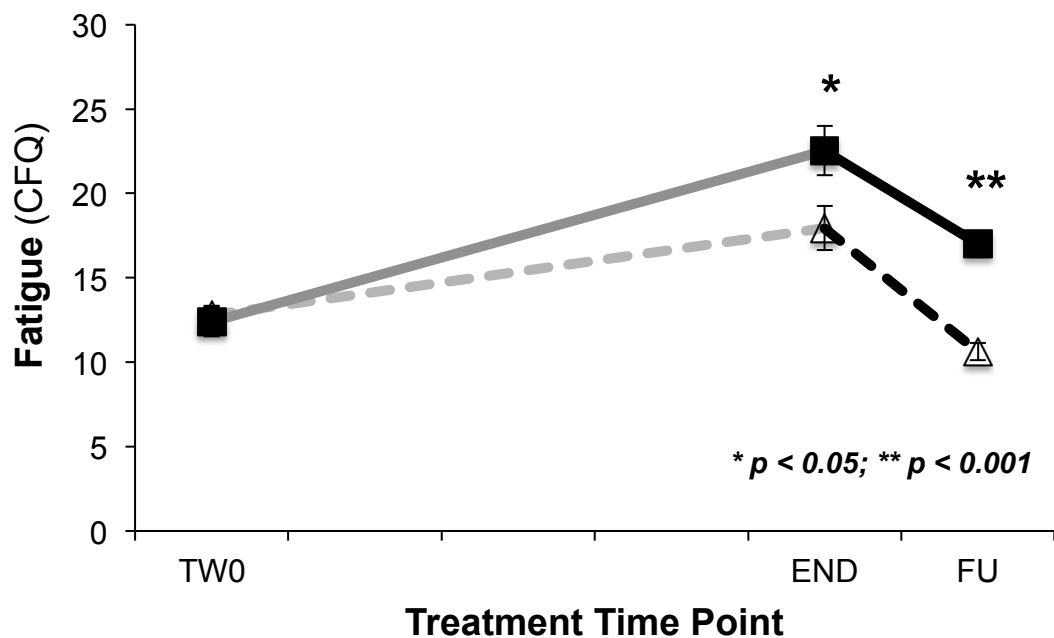


Figure 3.20 Fatigue scores at baseline, and from end of treatment to six-months post-treatment in HCV PF vs. RF groups

Depression

Data relating to depression scores are presented in Table 3.34, and depicted in Figure 3.21 and Figure 3.22. Looking at individual time points, there were no differences between groups until TW8, at which point the PF group had higher levels than did the RF group. An increase in the RF group at TW12 resulted in a difference only at a trend level, but while levels in the RF group became more stable thereafter, in the PF group levels increased. The delta scores reveal that the PF patients experienced much greater increases in depressive scores at each time point from TW8 onwards, relative to baseline depression levels. Similar to fatigue, PF patients were seen to be on an upward trajectory, versus the RF group in whom depressive symptoms levelled out after their peak at TW12. Across the six-month follow-up period post-treatment, again both groups experienced a similar decrease, though in the PF group this was relative to higher levels at the end of treatment. Furthermore, compared to baseline levels of depressive symptoms, the RF group had improved at follow-up while the PF group had worsened, as shown in the delta scores for TW0 vs. FU.

Table 3.34 Baseline levels, and changes in depression scores in HCV PF vs. RF groups

Depression (<i>IDS</i>)	RF (<i>n</i> = 37)	PF (<i>n</i> = 18)	Test and statistic
<i>Treatment Time point</i>			
TW0	12.3±2.0	10.2±2.4	t (52) = 0.63 , <i>p</i> = 0.53
TW4	18.9±2.0	19.9±3.1	t (51) = -0.31, <i>p</i> = 0.76
TW8	16.4±2.3	25.2±4.0	t (50) = -2.03, <i>p</i> = 0.047
TW12	20.1±2.5	28.0±4.1	t (49) = -1.73, <i>p</i> = 0.090
TW24	18.1±2.7	29.0±3.7	t (46) = -0.24 , <i>p</i> = 0.021
END (<i>of treatment</i>)	18.8±2.4	29.1±3.5	t (50) = -2.48, <i>p</i> = 0.017
FU	8.9±1.4	19.4±2.9	t (53) = -3.64, <i>p</i> = 0.001
<i>Delta scores</i>			
Δ TW0 vs TW4	6.8±1.7	9.7±2.5	t (50) = -0.98, <i>p</i> = 0.33
Δ TW0 vs TW8	4.2±2.0	14.9±3.6	t (49) = -2.81, <i>p</i> = 0.007
Δ TW0 vs TW12	8.4±2.0	17.8±3.9	t (48) = -2.35, <i>p</i> = 0.023
Δ TW0 vs TW24	5.8±2.6	18.2±3.3	t (45) = -2.91, <i>p</i> = 0.006
Δ TW0 vs FU	-3.2±1.8	9.2±3.0	t (52) = -3.72, <i>p</i><0.001
Δ TW0 vs END	6.4±2.2	18.9±2.9	t (49) = -3.40, <i>p</i> = 0.001
Δ END vs FU	-10.3±2.0	-9.7±2.6	t (50) = -0.17, <i>p</i> = 0.86

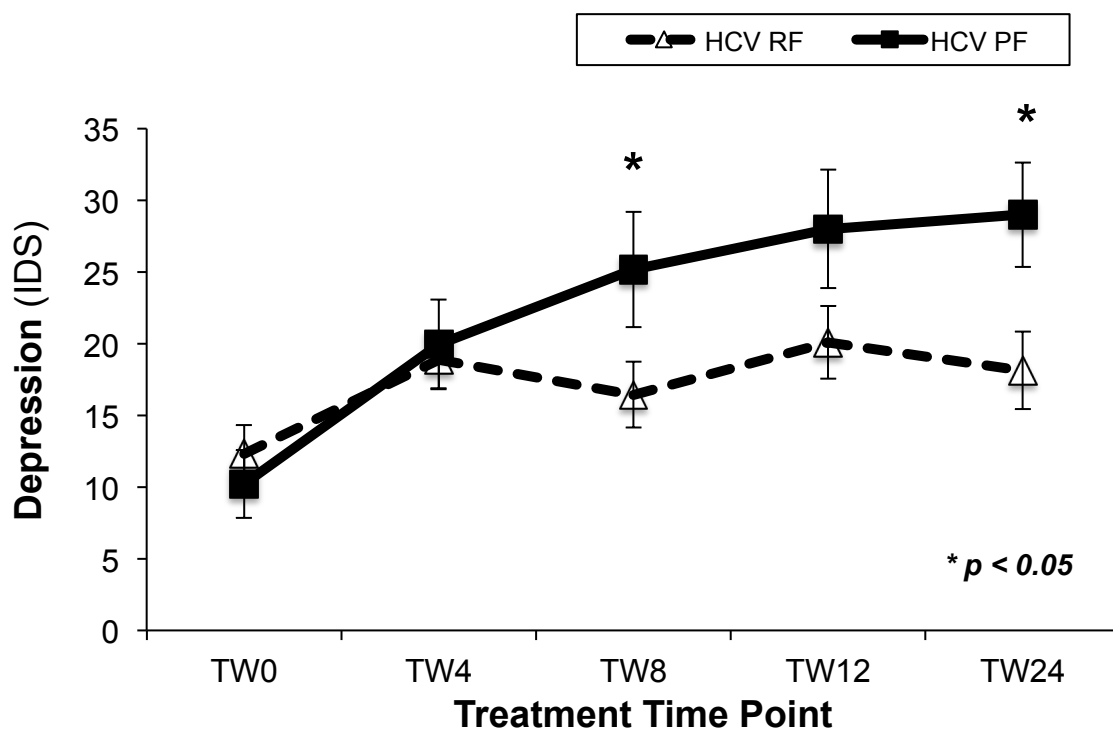


Figure 3.21 Depression scores at baseline, and during treatment in HCV PF vs. RF groups

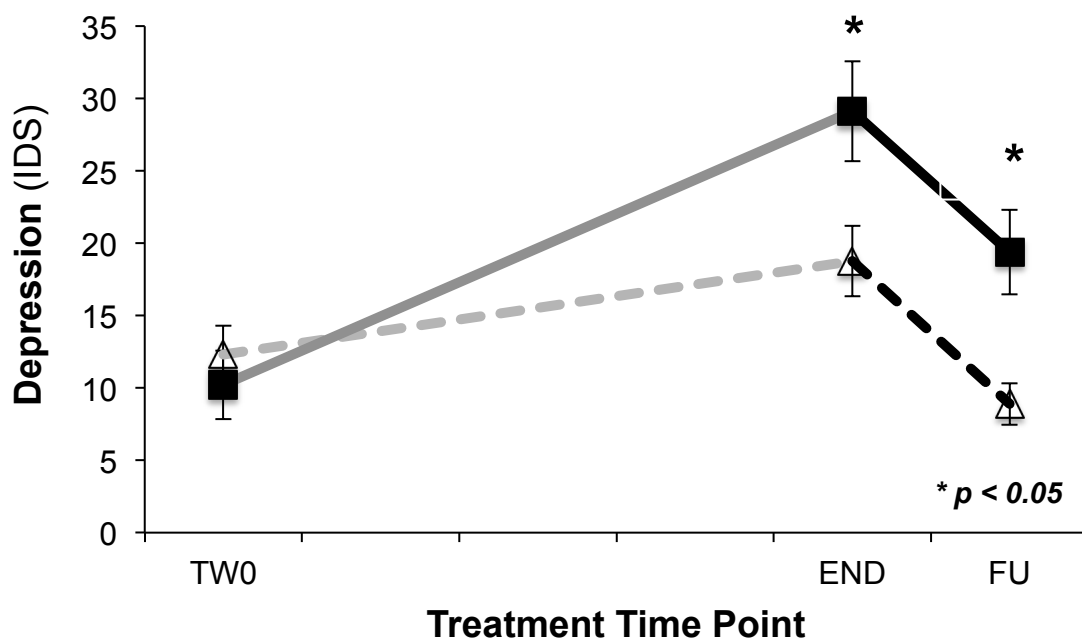


Figure 3.22 Depression scores at baseline, and end of treatment to six-months post-treatment in HCV PF vs. RF groups

Anxiety

Data for anxiety scores are shown in Table 3.35, and depicted in Figure 3.23 and Figure 3.24. The PF group did appear to experience greater anxiety across the treatment course, though changes were small and not statistically significant. However, there was a trend towards increased levels by the end of treatment in the PF versus RF group. The delta scores show a trend towards greater increases at TW8 relative to baseline, and again at TW24 and/or the end of treatment, reflecting again the upward trajectory of neuropsychiatric side effects experienced by the PF group. Across the six-month follow-up, after the cessation of treatment, similar decreases were observed. However, this was again relative to a higher end of treatment score in the PF group.

Table 3.35 Baseline levels, and changes in anxiety scores in HCV PF vs. RF groups

Anxiety (<i>HADS-A</i>)	RF (<i>n</i> = 37)	PF (<i>n</i> = 18)	Test and statistic
<i>Treatment Time point</i>			
TW0	4.2±0.8	3.9±0.9	t (53) = 0.24, <i>p</i> = 0.81
TW4	4.3±0.8	4.3±1.0	t (52) = 0.04, <i>p</i> = 0.97
TW8	3.4±0.8	5.6±1.2	t (49) = -1.54, <i>p</i> = 0.13
TW12	4.6±0.9	6.2±1.2	t (49) = -1.04, <i>p</i> = 0.30
TW24	4.6±0.9	6.7±1.2	t (46) = -1.45, <i>p</i> = 0.15
END (<i>of treatment</i>)	4.2±0.8	6.7±1.2	<u>t (49) = -1.80, <i>p</i> = 0.078</u>
FU	3.7±0.6	4.1±0.7	t (53) = -0.36, <i>p</i> = 0.72
<i>Delta scores</i>			
Δ TW0 vs TW4	0.1±0.8	0.3±1.0	t (52) = -0.21, <i>p</i> = 0.84
Δ TW0 vs TW8	-0.6±0.7	1.7±1.1	<u>t (49) = -1.82, <i>p</i> = 0.074</u>
Δ TW0 vs TW12	0.6±0.9	2.3±1.2	t (49) = -1.15, <i>p</i> = 0.26
Δ TW0 vs TW24	-0.03±0.9	2.5±1.2	<u>t (46) = -1.72, <i>p</i> = 0.093</u>
Δ TW0 vs FU	-0.5±0.2	0.2±0.5	t (53) = -1.43, <i>p</i> = 0.16
Δ TW0 vs END	-0.3±0.8	2.7±1.1	t (49) = -2.29, <i>p</i> = 0.026
Δ END vs FU	-0.3±0.7	-2.6±0.8	t (49) = 2.03, <i>p</i> = 0.048

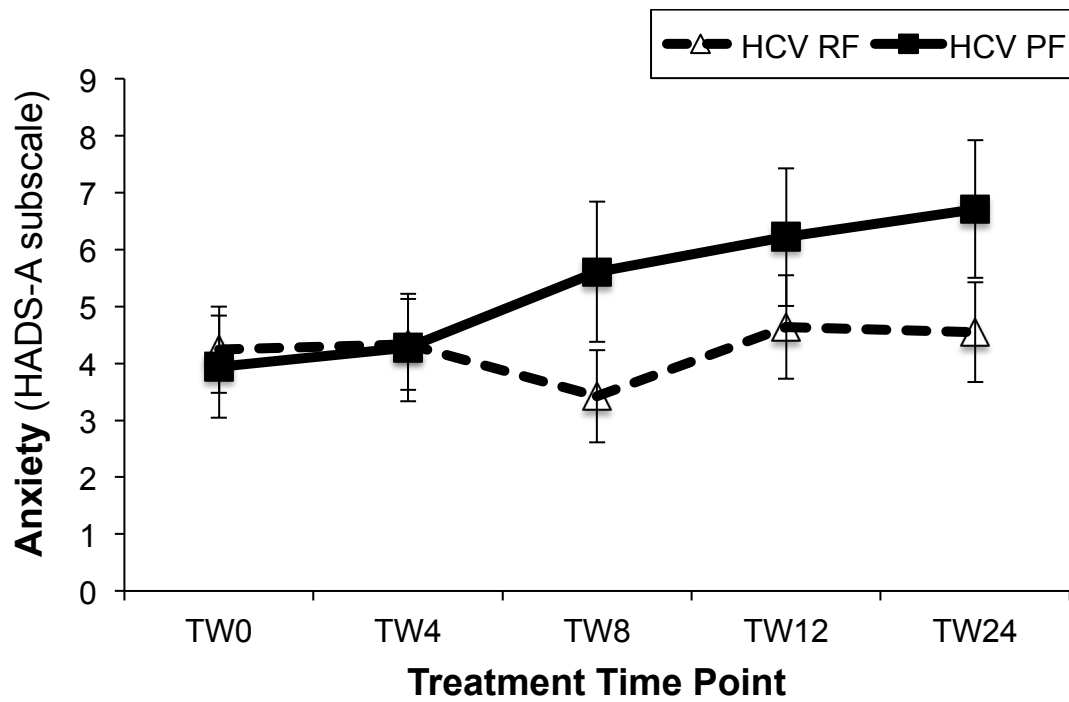


Figure 3.23 Anxiety scores at baseline, and during treatment in HCV PF vs. RF groups

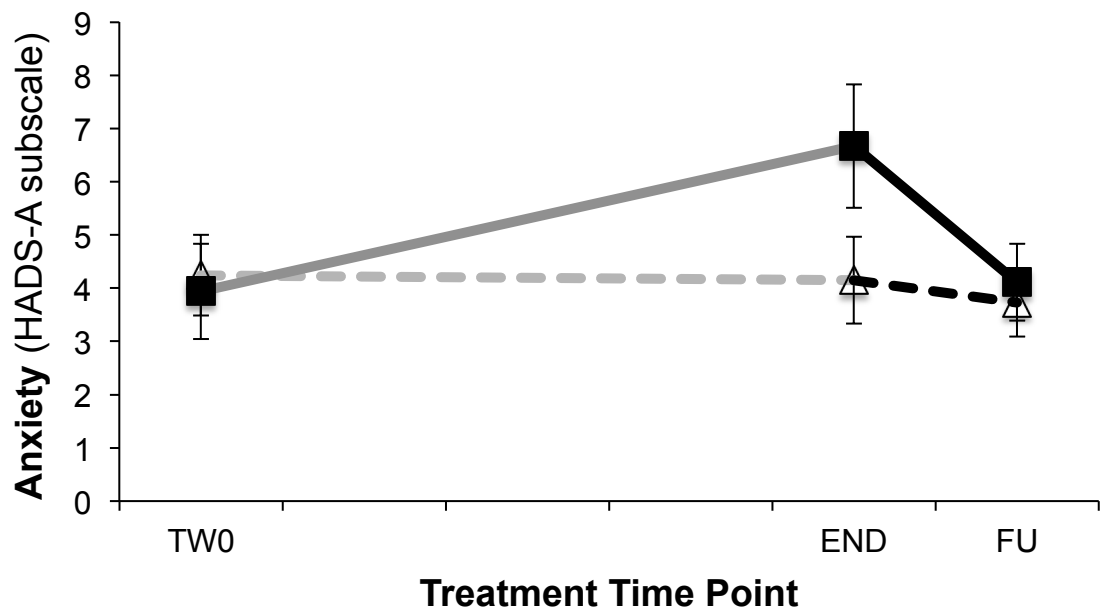


Figure 3.24 Anxiety scores at baseline, and at end of treatment to six-months post-treatment in HCV PF vs. RF groups

Perceived stress

Data for perceived stress levels are presented in Table 3.36 and depicted in Figure 3.25 and Figure 3.26. The result for the comparison of baseline levels was adjusted, since equal variance could not be assumed. While PF data was normally distributed, RF data was not, with a slight positive skew towards lower scores. However, there were no differences between the PF and RF groups in levels of perceived stress at this point, or during the first eight weeks. By TW8, patients in the PF group reported higher levels of perceived stress. There was no difference at TW12, when RF patients experienced an increase, while in PF levels decreased. At TW24 and at follow-up, and to some extent the 'end of treatment' time point, patients in the PF group again experienced higher levels of perceived stress. These results are reflected also in the delta scores. Over the six-month period post-treatment, similar decreases in perceived stress were shown in both groups, though in the PF group this was relative to a higher end of treatment level. In addition, as was seen in depressive symptoms, patients who were persistently fatigued were more stressed at follow-up than they had been pre-treatment, whereas in the RF group stress levels had improved.

Table 3.36 Baseline levels, and changes in Perceived Stress in HCV PF vs. RF groups

Perceived Stress (<i>PSS</i>)	RF (<i>n</i> = 37)	PF (<i>n</i> = 18)	Test and statistic
<i>Treatment time point</i>			
TW0	10.4±1.2	10.2±1.3	<i>t</i> (42.55) = .070, <i>p</i> = 0.9
TW4	10.9±1.3	13.7±1.9	<i>t</i> (51) = -1.26, <i>p</i> = 0.21
TW8	10.8±1.4	17.0±2.3	<i>t</i> (50) = -2.46, <i>p</i> = 0.017
TW12	13.1±1.5	15.6±2.5	<i>t</i> (49) = -0.90, <i>p</i> = 0.37
TW24	12.2±1.7	18.4±2.3	<i>t</i> (46) = -2.14, <i>p</i> = 0.037
END (<i>of treatment</i>)	12.8±1.7	17.9±2.3	<i>t</i> (49) = -1.83, <i>p</i> = 0.074
FU	8.8±1.2	13.8±1.9	<i>t</i> (52) = -2.35, <i>p</i> = 0.023
<i>Delta scores</i>			
Δ TW0 vs TW4	0.8±1.2	3.2±1.6	<i>t</i> (50) = -.120, <i>p</i> = 0.23
Δ TW0 vs TW8	0.9±1.1	6.3±2.1	<i>t</i> (49) = -2.52, <i>p</i> = 0.015
Δ TW0 vs TW12	2.7±1.2	4.5±2.1	<i>t</i> (48) = -0.81, <i>p</i> = 0.42
Δ TW0 vs TW24	1.5±1.7	7.2±2.0	<i>t</i> (45) = -2.10, <i>p</i> = 0.042
Δ TW0 vs FU	-1.6±1.4	3.0±1.1	<i>t</i> (51) = -2.13, <i>p</i> = 0.038
Δ TW0 vs END	2.0±1.4	7.4±1.7	<i>t</i> (48) = -2.27, <i>p</i> = 0.028
Δ END vs FU	-4.3±1.5	-3.9±1.3	<i>t</i> (48) = -0.20, <i>p</i> = 0.84

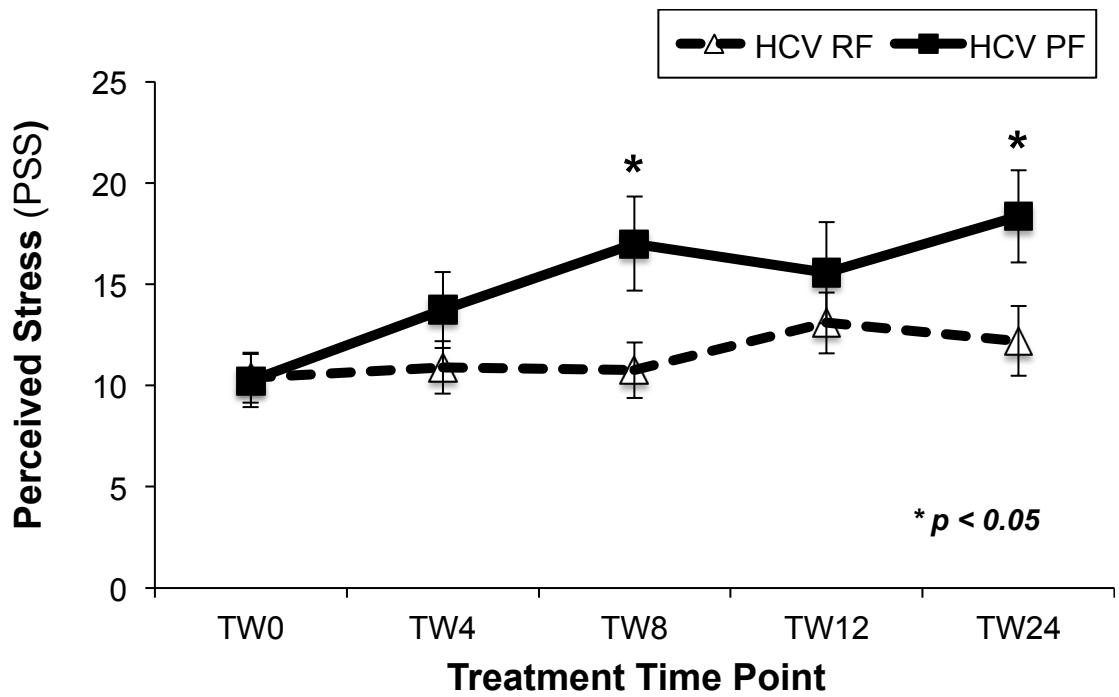


Figure 3.25 Perceived Stress scores at baseline, and during treatment in HCV PF vs. RF groups

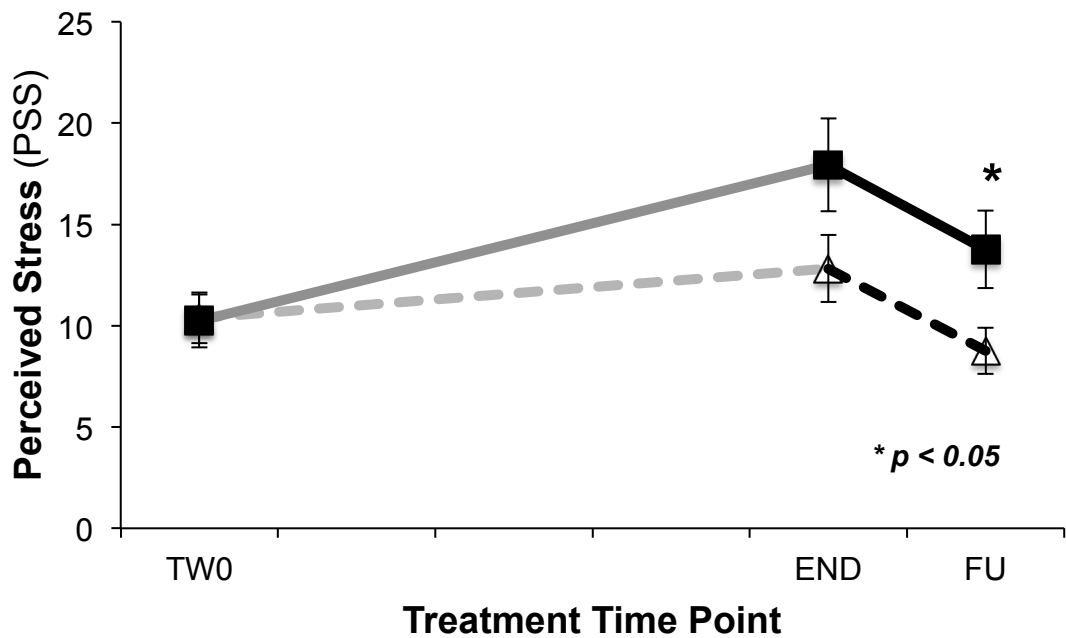


Figure 3.26 Perceived Stress scores at baseline, and end of treatment to six-months post-treatment in HCV PF vs. RF groups

Sleep Quality

Data on sleep quality are presented in Table 3.37, and depicted in Figure 3.27 and Figure 3.28. Higher scores indicate more frequent experience of sleep problems. Sleep quality was measured in a smaller sample, which should be noted in the interpretation of the results. Test results for the comparison of scores at TW4 and TW8 were adjusted since equal variances could not be assumed. Data in both groups was normally distributed at both time points, as assessed by the Shapiro-Wilk test. However, in the RF group, there were slightly more individuals at either end of the spectrum (no problems/more frequent problems), whereas patients in the PF group tended to report more frequent problems. However, the small number of patients mean this is difficult to interpret. Reports of sleep quality showed that results were similar across groups throughout treatment, though by TW24 sleep quality was worse in the PF group compared to the RF group, and this continued to six-month follow-up. Interestingly, again this is a similar pattern to that observed for fatigue, depression and anxiety, albeit with a later onset. Comparison of the delta scores in each group relative to baseline revealed no significant differences in the changes in sleep quality over time.

Table 3.37 Baseline levels, and changes in sleep scores in HCV PF vs. RF groups

Sleep (<i>Jenkins</i>)	RF (<i>n</i> = 13-27*)	PF (<i>n</i> = 5-13*)	Test and statistic
<i>Treatment Time point</i>			
TW0	6.8±2.1	7.4±2.2	t(16) = -0.17, <i>p</i> = 0.87
TW4	8.3±1.7	8.6±1.3	t(15.65) = -0.15, <i>p</i> = 0.89
TW8	7.3±1.9	7.8±1.4	t(14.58) = -0.24, <i>p</i> = 0.82
TW12	8.9±2.0	10.4±2.1	t(16) = -0.45, <i>p</i> = 0.66
TW24	6.0±1.1	10.6±2.3	<u>t(16) = -2.06, <i>p</i> = 0.056</u>
END (<i>of treatment</i>)	8.3±1.2	11.7±2.2	t(22) = -1.36, <i>p</i> = 0.19
FU*	3.6±0.8	7.5±1.6	t(38) = -2.46, <i>p</i> = 0.018
<i>Delta scores</i>			
Δ TW0 vs TW4	2.2±2.2	1.2±2.6	t(16) = 0.25, <i>p</i> = 0.81
Δ TW0 vs TW8	0.7±2.5	0.4±1.8	t(14) = 0.08, <i>p</i> = 0.94
Δ TW0 vs TW12	1.1±2.9	3.0±1.4	t(14) = -0.42, <i>p</i> = 0.68
Δ TW0 vs TW24	-0.9±1.9	3.2±2.8	t(12) = -1.30, <i>p</i> = 0.22
Δ TW0 vs FU	-1.9±2.1	-2.2±3.3	t(16) = 0.09, <i>p</i> = 0.93
Δ TW0 vs END	0.6±2.5	3.6±3.2	t(13) = -0.71, <i>p</i> = 0.49
Δ END vs FU	-4.1±1.2	-6.5±2.1	t(19) = 1.03, <i>p</i> = 0.32

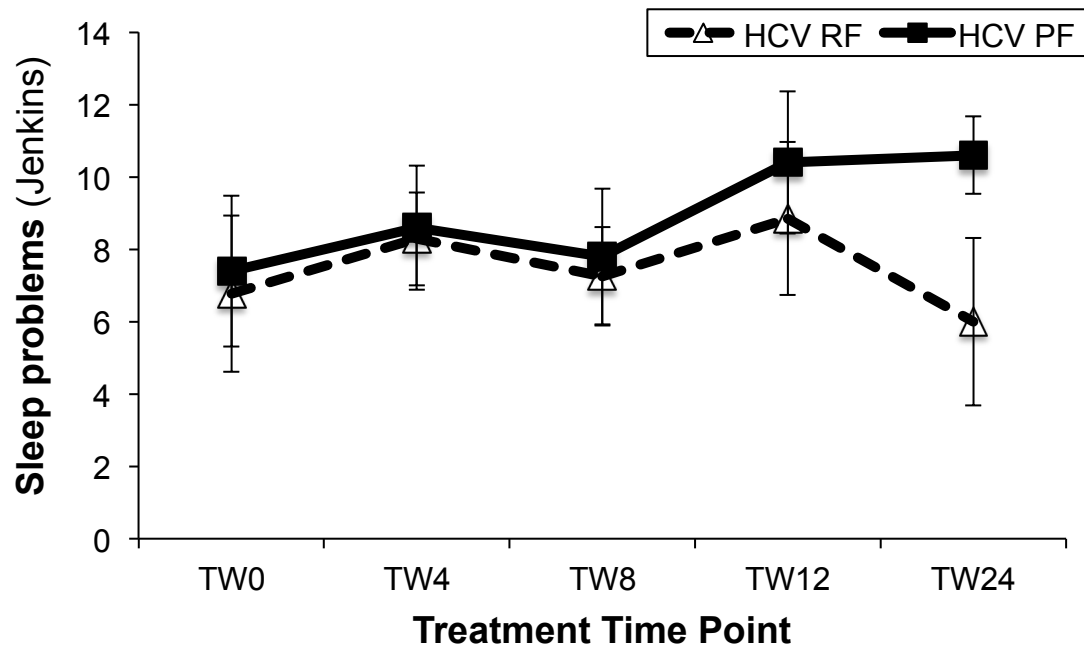


Figure 3.27 Sleep problem scores at baseline, and during treatment in HCV PF vs. RF groups

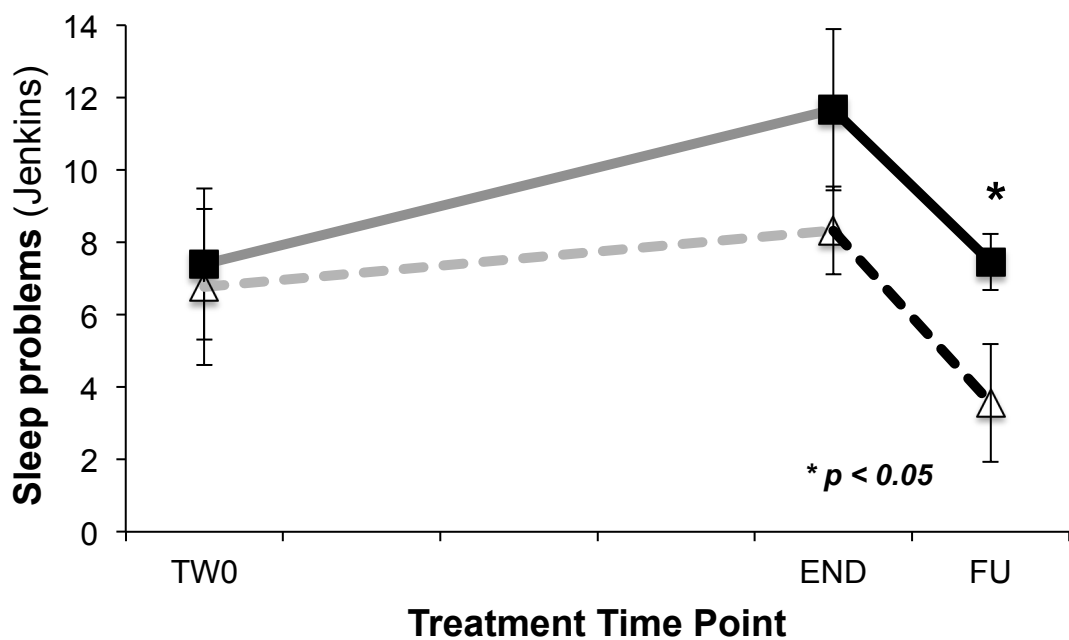


Figure 3.28 Sleep problem scores at baseline, and from end of treatment to six-months post-treatment in HCV PF vs. RF groups

Table 3.38 Summary: clinical symptoms at baseline, and changes during treatment in PF vs. RF groups

	TW0	TW4	TW8	TW12	TW24	END	FU
Fatigue (CFQ)	=	↖	=	=	↑	↑	↑
Depression (IDS)	=	=	↑	↖	↑	↑	=
Anxiety (HADS-A)	=	=	=	=	=	↖	=
Perceived Stress (PSS)	=	=	↑	=	↑	↖	↑
Sleep* (Jenkins)	=	=	=	=	=	↖	↑

	Δ 4	Δ 8	Δ 12	Δ 24	Δ FU	ΔEND	ΔEND vs FU
Fatigue (CFQ)	↑	=	↖	↑	↑	↑	=
Depression (IDS)	=	↑	↑	↑	↑	↑	=
Anxiety (HADS-A)	=	↖	=	↖	=	↑	↓
Perceived Stress (PSS)	=	↑	=	↑	↑	↑	=
Sleep* (Jenkins)	=	=	=	=	=	=	=

Note – (=) – no difference; (↖) – trend towards worse function/greater decline; (↑) – significantly worse function/greater decline; (↗) – trend towards better function/greater improvement; (↓) – significantly better function/greater improvement; * = small *n* earlier during treatment should be noted, see Table 3.37.

3.1.3.3 Baseline health status, and changes in persistent versus resolved Fatigue

I then examined baseline ratings, and changes in response to IFN- α in each of the 8 dimensions of the SF-36 in the Persistent Fatigue (PF) versus Resolved Fatigue (RF) groups. Data from all 8 subscales are presented in Table 3.39, with a summary in Table 3.40 (see page 212). In this case, lower scores indicate lower or poorer levels of functioning, and higher scores (max 100) represent higher or better levels of health.

In relation to **Physical functioning**, patients in both groups reported similar levels of functioning at baseline and during the early weeks of treatment. There was a trend towards lower scores, indicating a worsening of physical functioning, in the PF group at TW12, at the end of treatment and at follow-up, six-months post-treatment. Looking to the delta values, comparing functioning at each time point relative to baseline, the PF group experienced a significantly greater decline during treatment than the RF group. There were similar changes in both groups over the six-month post-treatment period, though patients in the PF group reported a decline in functioning at FU relative to baseline, whereas the RF group reported an improvement. Data is also shown (Mean \pm SEM) in Figure 3.29 and Figure 3.30.

Physical Role Limitation relates to difficulties in performing usual tasks because of their physical health. Results for the comparison of scores at TW24, and 'end of treatment' (TW24/36/38) were adjusted since equal variance could not be assumed. Visual examination of the histograms of scores in each group revealed that, for both measurements, in the RF group there was a split, with more patients either reporting none, or a greater level of disability, versus the PF group where there was a positive skew towards lower scores indicating greater disability. However, there were no significant differences between groups at each time point overall. However, delta scores show a greater decline in PF patients relative to baseline, versus the RF group, particularly at TW24 and/or the end of treatment. Additionally, at follow-up the scores suggest a decline in the PF patients versus an improvement in RF patients, when comparing results to baseline. Data is shown in Figure 3.31 and Figure 3.32.

For limitations to everyday roles or activities attributed to emotional problems – the **Emotional role limitation** subscale score – again there were similar levels reported by both groups. Towards the end of treatment, there was a trend towards poorer functioning in PF versus RF patients at TW24, and a significant difference at the 'end of treatment'. The result for the comparison at follow-up was adjusted since equal variance could not be assumed. Visual examination of the histograms of scores showed that they were negatively skewed in both groups, indicating no disability, though more so in the RF than PF patients. This was reflected in the trend towards poorer functioning in the PF group at follow-up. The only difference in change over time relative to baseline was again at the end of treatment, where the PF group reported a much greater decline than the RF group (-41.2 ± 11.6 vs. -11.5 ± 8.9).

In relation to **Vitality**, measuring a balance of energy/fatigue, some test results were adjusted, as equal variance could not be assumed. At baseline, data in both groups was normally distributed, but with a slight negative skew in the RF group. By TW24, and also for the 'end of treatment' measure, data was positively skewed in both groups, though slightly more so in the RF patients. For the delta score analysis from TW0 to 'end', data was normally distributed in both groups, but slightly negatively skewed in the PF group. Again, for this measure, the most notable differences were at TW24 and/or the end of treatment, and of course at follow-up. In addition, when looking at the delta scores relative to baseline, PF patients reported a greater decline across all treatment time points than did the RF group. The improvement in function over the post-treatment follow-up period was the same, though from a much lower (worse) level in the PF group. By the follow-up visit, the RF patients reported an overall improvement relative to baseline, whereas the PF patients reported persistent problems, as would be expected based on the stratification. Data is shown in Figure 3.34 and Figure 3.35.

Concerning overall **mental health**, again some results were adjusted where equal variance could not be assumed. At both TW8 and follow-up, in the RF group, scores were negatively skewed reflecting better health, while in the PF group scores were normally distributed. While a decline in mental health was reported by the PF group over the treatment course, differences between groups were not significant, with only a trend towards the PF group being worse off by TW24 and/or end of treatment, continuing to the six-month follow-up assessment. Looking to the delta scores, the PF group experienced a greater deterioration from TW8 relative to baseline, and TW24. Differences at

intermediate time points were reduced due to a greater deterioration in mental health reported by the RF group. There were no differences in improvements seen in the follow-up period. However, comparing the change from baseline to follow-up, again the RF group improved while the PF group was still in decline.

For the measure of **Social Functioning**, a lower level of functioning was reported in the PF group versus RF group at TW8, and then again at TW24 and/or end of treatment, and at follow-up. The test result for the comparison at follow-up was adjusted, as equal variance could not be assumed. Data was negatively skewed in both groups, though to a greater extent in the RF group. The RF group experienced a 'peak' decline at TW12, bringing them closer to the PF group, though overall the PF group experienced a greater decline in functioning across each time point, relative to baseline. There was a trend towards a greater recovery of social functioning in the PF group than the RF group in the follow-up period, but again relative to a greater decline during the treatment period. However, overall the PF group still reported a slight decline in social functioning at follow-up relative to baseline, compared to a an improvement in the RF group. Data is also shown in Figure 3.35 and Figure 3.36.

In relation to the reporting of **pain**, a continued downward trajectory for the PF group again resulted in-group differences appearing only at the end of treatment, with a longer recovery and differences also at follow-up. Results for changes during treatment, from TW0 to TW8, and from TW0 – TW24 were adjusted, as equal variance could not be assumed. For both sets of delta

scores, data was normally distributed in both groups, though slightly negatively skewed in the RF groups. As is the theme, the RF group experienced improvements in pain versus the decline observed in the PF group.

Finally, for the measure of **general health**, both groups reported similar levels of perceived overall health at baseline. Again due to the downward trajectory, only towards the end of treatment did PF patients begin to rate their overall general health as worse than the RF group. The result at the end of treatment was adjusted since equal variance could not be assumed, but data was normally distributed in both groups, with a slightly more negative skew in the RF group towards better ratings of overall general health. The measure of general health at six-months follow-up did not reveal significant differences. Only at the end of treatment (TW24 and/or end) was there a trend towards a greater decline in general health in the PF versus RF groups. No difference between groups was observed at follow-up relative to baseline.

Table 3.39 Baseline health status, and changes in health status in HCV RF vs. PF groups

SF-36 dimension	RF (<i>n</i> = 37)	PF (<i>n</i> = 18)	Test and statistic
Physical Functioning			
TW0	83.1±4.2	87.8±3.2	<i>t</i> (53) = -0.72, <i>p</i> = 0.47
TW4	73.3±4.1	61.9±6.3	<i>t</i> (52) = 1.56, <i>p</i> = 0.13
TW8	68.2±4.8	58.9±6.2	<i>t</i> (49) = 1.16, <i>p</i> = 0.25
TW12	63.8±4.8	47.5±7.4	<i>t</i> (48) = 1.93, <i>p</i> = 0.06
TW24	62.2±5.4	51.6±6.0	<i>t</i> (43) = 1.21, <i>p</i> = 0.23
END of treatment	65.0±4.8	50.6±7.0	<i>t</i> (47) = 1.73, <i>p</i> = 0.09
FU	88.2±3.6	75.3±5.9	<i>t</i> (53) = 1.96, <i>p</i> = 0.06
Δ TW0 vs TW4	-9.6±3.3	-25.8±6.0	<i>t</i>(52) = 2.59, <i>p</i> = 0.013
Δ TW0 vs TW8	-13.8±4.4	-28.9±6.6	<i>t</i> (49) = 1.95, <i>p</i> = 0.06
Δ TW0 vs TW12	-21.9±3.8	-40.3±7.1	<i>t</i>(48) = 2.51, <i>p</i> = 0.015
Δ TW0 vs TW24	-17.6±5.5	-36.3±6.2	<i>t</i>(43) = 2.14, <i>p</i> = 0.038
Δ TW0 vs FU	5.1±2.5	-12.5±3.8	<i>t</i>(53) = 3.91, <i>p</i> < 0.001
Δ TW0 vs END	-16.1±5.3	-37.6±6.2	<i>t</i>(47) = 2.51, <i>p</i> = 0.016
Δ END vs FU	21.7±5.1	24.1±7.2	<i>t</i> (47) = -0.28, <i>p</i> = 0.78
Physical Role Limitation			
TW0	73.7±6.9	72.2±9.2	<i>t</i> (53) = 0.12, <i>p</i> = 0.90
TW4	49.3±7.5	33.3±9.3	<i>t</i> (52) = 1.28, <i>p</i> = 0.21
TW8	48.5±7.9	43.1±9.9	<i>t</i> (49) = 0.42, <i>p</i> = 0.68
TW12	47.7±7.9	37.5±9.1	<i>t</i> (48) = 0.81, <i>p</i> = 0.42
TW24	45.7±8.6	18.8±9.3	<i>t</i>(37.2) = 2.00, <i>p</i> = 0.04
END of treatment	47.7±7.9	19.1±7.6	<i>t</i>(43.2) = -1.05, <i>p</i> = 0.012
FU	83.1±5.7	66.7±8.8	<i>t</i> (53) = 1.62, <i>p</i> = 0.11
Δ TW0 vs TW4	-23.6±7.1	-38.9±10.9	<i>t</i> (52) = 1.21, <i>p</i> = 0.23
Δ TW0 vs TW8	-22.7±8.0	-29.2±14.0	<i>t</i> (49) = 0.43, <i>p</i> = 0.67
Δ TW0 vs TW12	-28.1±8.5	-34.7±13.1	<i>t</i> (48) = 0.44, <i>p</i> = 0.66
Δ TW0 vs TW24	-24.1±9.4	-51.6±14.7	<i>t</i> (43) = 1.64, <i>p</i> = 0.11
Δ TW0 vs FU	9.5±5.7	-5.6±8.0	<i>t</i> (53) = 1.52, <i>p</i> = 0.13
Δ TW0 vs END	-21.9±8.2	-51.5±11.4	<i>t</i>(47) = 2.11, <i>p</i> = 0.040
Δ END vs FU	32.8±8.4	47.1±10.0	<i>t</i> (47) = -1.05, <i>p</i> = 0.30
<i>Cont. overleaf</i>			

SF-36 dimension	RF (<i>n</i> = 37)	PF (<i>n</i> = 18)	Test and statistic
Emotional Role Limitation			
TW0	82.9±6.0	81.5±8.2	<i>t</i> (53) = 0.14, <i>p</i> = 0.89
TW4	66.7±7.7	68.5±10.3	<i>t</i> (52) = -0.14, <i>p</i> = 0.89
TW8	66.7±7.4	72.2±9.4	<i>t</i> (49) = -0.46, <i>p</i> = 0.65
TW12	58.3±7.8	59.3±9.9	<i>t</i> (48) = -0.07, <i>p</i> = 0.94
TW24	70.1±8.5	45.8±11.3	<i>t</i> (43) = 1.71, <i>p</i> = 0.09
END of treatment	68.8±7.8	39.2±11.5	<i>t</i>(47) = 2.18, <i>p</i> = 0.034
FU	88.3±5.0	68.5±10.3	<i>t</i> (25.5) = 1.95, <i>p</i> = 0.06
Δ TW0 vs TW4	-15.7±7.9	-13.0±11.5	<i>t</i> (52) = -0.20, <i>p</i> = 0.84
Δ TW0 vs TW8	-14.1±6.7	-9.3±10.4	<i>t</i> (49) = -0.41, <i>p</i> = 0.68
Δ TW0 vs TW12	-25.0±8.5	-22.2±10.8	<i>t</i> (48) = -0.20, <i>p</i> = 0.84
Δ TW0 vs TW24	-8.1±9.6	-33.3±11.4	<i>t</i> (43) = 1.64, <i>p</i> = 0.11
Δ TW0 vs FU	5.4±7.1	-13.0±7.7	<i>t</i> (53) = 1.59, <i>p</i> = 0.12
Δ TW0 vs END	-11.5±8.9	-41.2±11.6	<i>t</i> (47) = 1.99, <i>p</i> = 0.052
Δ END vs FU	17.7±8.2	27.5±10.4	<i>t</i> (47) = -0.72, <i>p</i> = 0.48
Vitality			
TW0	59.1±4.4	64.2±4.5	<i>t</i> (45.2) = -0.82, <i>p</i> = 0.42
TW4	47.5±4.5	40.3±5.6	<i>t</i> (52) = 0.96, <i>p</i> = 0.32
TW8	47.9±4.6	38.1±6.4	<i>t</i> (49) = 1.26, <i>p</i> = 0.22
TW12	45.3±5.9	35.6±5.8	<i>t</i> (47) = 1.09, <i>p</i> = 0.28
TW24	44.8±4.9	25.6±4.2	<i>t</i>(41.8) = 2.98, <i>p</i> = 0.005
END of treatment	40.0±4.2	24.1±3.1	<i>t</i>(47.0) = 3.01, <i>p</i> = 0.004
FU	69.7±3.5	53.9±4.3	<i>t</i>(53) = 2.70, <i>p</i> = 0.009
Δ TW0 vs TW4	-12.1±3.5	-23.9±4.2	<i>t</i>(52) = 2.04, <i>p</i> = 0.046
Δ TW0 vs TW8	-11.2±3.7	-26.1±5.5	<i>t</i>(49) = 2.32, <i>p</i> = 0.024
Δ TW0 vs TW12	-14.2±4.4	-28.6±5.4	<i>t</i>(47) = 2.05, <i>p</i> = 0.046
Δ TW0 vs TW24	-12.6±5.2	-35.0±5.3	<i>t</i>(43) = 2.79, <i>p</i> = 0.008
Δ TW0 vs FU	10.7±3.2	-10.3±3.9	<i>t</i>(53) = 3.91, <i>p</i> < 0.001
Δ TW0 vs END	-16.4±4.9	-38.2±4.2	<i>t</i>(45.7) = 3.39, <i>p</i> = 0.001
Δ END vs FU	27.8±4.7	27.7±3.5	<i>t</i> (47) = 0.02, <i>p</i> = 0.98
<i>Cont. overleaf</i>			

SF-36 dimension	RF (<i>n</i> = 37)	PF (<i>n</i> = 18)	Test and statistic
Mental health			
TW0	75.9±3.1	75.8±4.7	<i>t</i> (53) = 0.02, <i>p</i> = 0.98
TW4	73.4±3.4	66.2±5.2	<i>t</i> (52) = 1.19, <i>p</i> = 0.24
TW8	75.0±3.2	63.4±6.5	<i>t</i> (25.1)=1.60, <i>p</i> = 0.12
TW12	70.7±4.0	62.0±6.5	<i>t</i> (47) = 1.21, <i>p</i> = 0.23
TW24	68.8±4.4	54.5±6.2	<i>t</i> (43) = 1.91, <i>p</i> = 0.06
END of treatment	69.7±3.8	56.5±6.1	<i>t</i> (47) = .1.92, <i>p</i> = 0.06
FU	81.7±2.5	71.3±4.9	<i>t</i> (26.2)=1.87, <i>p</i> = 0.07
Δ TW0 vs TW4	-3.1±2.5	-9.6±3.2	<i>t</i> (52) = 1.54, <i>p</i> = 0.13
Δ TW0 vs TW8	-1.8±2.7	-12.3±3.6	<i>t</i>(49) = 2.31, <i>p</i> = 0.025
Δ TW0 vs TW12	-5.2±3.3	-13.8±5.3	<i>t</i> (47) = 1.46, <i>p</i> = 0.15
Δ TW0 vs TW24	-5.1±3.9	-18.5±4.5	<i>t</i>(43) = 2.14, <i>p</i> = 0.038
Δ TW0 vs FU	5.8±3.3	-4.4±3.3	<i>t</i> (53) = 1.95, <i>p</i> = 0.06
Δ TW0 vs END	-4.3±3.9	-17.9±3.9	<i>t</i>(47) = 2.26, <i>p</i> = 0.028
Δ END vs FU	11.1±3.5	13.4±3.1	<i>t</i> (47) = -0.43, <i>p</i> = 0.67
Social functioning			
TW0	79.7±4.9	84.0±6.0	<i>t</i> (53) = -0.53, <i>p</i> = 0.60
TW4	75.0±4.4	63.9±7.1	<i>t</i> (52) = 1.39, <i>p</i> = 0.17
TW8	68.9±4.9	50.7±7.7	<i>t</i>(49) = 2.08, <i>p</i> = 0.043
TW12	63.7±5.7	50.0±7.4	<i>t</i> (47) = 1.46, <i>p</i> = 0.15
TW24	61.6±5.9	39.8±7.5	<i>t</i>(43) = 2.24, <i>p</i> = 0.030
END of treatment	62.5±5.6	36.03±6.24	<i>t</i>(47) = 2.96, <i>p</i> = 0.005
FU	91.2±2.9	79.87±5.26	<i>t</i> (27.98)=1.88, <i>p</i> = 0.07
Δ TW0 vs TW4	-4.2±4.5	-20.1±5.5	<i>t</i>(52) = 2.16, <i>p</i> = 0.035
Δ TW0 vs TW8	-10.2±4.9	-33.3±7.5	<i>t</i>(49) = 2.66, <i>p</i> = 0.010
Δ TW0 vs TW12	-18.6±5.3	-34.0±8.3	<i>t</i> (47) = 1.64, <i>p</i> = 0.11
Δ TW0 vs TW24	-13.4±5.2	-42.2±8.7	<i>t</i>(43) = 3.04, <i>p</i> = 0.004
Δ TW0 vs FU	11.5±3.9	-4.2±6.2	<i>t</i>(53) = 2.23, <i>p</i> = 0.030
Δ TW0 vs END	-14.1±5.0	-47.1±7.2	<i>t</i>(47) = 3.83, <i>p</i> < 0.001
Δ END vs FU	27.3±5.0	42.7±6.2	<i>t</i> (47) = -1.86, <i>p</i> = 0.07
<i>Cont. overleaf</i>			

SF-36 dimension	RF (n = 37)	PF (n = 18)	Test and statistic
Pain			
TW0	78.0±4.3	74.9±6.1	t(53) = 0.42, p = 0.68
TW4	64.2±4.4	59.0±6.4	t(52) = 0.68, p = 0.50
TW8	66.7±5.0	61.5±7.3	t(49) = 0.60, p = 0.56
TW12	66.2±5.1	57.5±7.2	t(47) = 1.01, p = 0.32
TW24	68.5±4.7	56.7±8.0	t(43) = 1.36, p = 0.18
END	68.4±4.6	54.0±7.8	t(47) = 1.71, p = 0.09
FU	82.7±3.7	69.0±5.4	t(53) = 2.09, p = 0.041
Δ TW0 vs TW4	-14.3±3.7	-15.8±5.6	t(52) = 0.23, p = 0.82
Δ TW0 vs TW8	-11.9±2.91	-13.3±7.4	t(22.4) = 0.18, p = 0.86
Δ TW0 vs TW12	-15.0±3.66	-17.4±8.3	t(23.6) = 0.26, p = 0.80
Δ TW0 vs TW24	-8.5±4.4	-17.7±9.5	t(43) = 1.01, p = 0.32
Δ TW0 vs FU	4.7±3.0	-5.8±4.6	<u>t(53) = 1.98, p = 0.05</u>
Δ TW0 vs END	-8.0±4.8	-21.9±9.6	t(47) = 1.45, p = 0.15
Δ END vs FU	13.4±4.9	15.2±8.6	t(47) = -0.19, p = 0.85
General health			
TW0	63.7±3.9	59.7±5.7	t(53) = 0.57, p = 0.57
TW4	57.5±4.3	52.2±5.7	t(52) = 0.73, p = 0.47
TW8	56.1±4.5	48.1±5.8	t(49) = 1.08, p = 0.29
TW12	57.8±5.0	45.8±5.8	t(48) = 1.51, p = 0.14
TW24	54.3±4.6	41.3±5.0	<u>t(43) = 1.80, p = 0.08</u>
END	53.9±4.7	39.4±4.1	t(44.4) = 2.33, p = 0.024
FU	67.2±4.2	55.3±5.2	t(53) = 1.70, p = 0.10
Δ TW0 vs TW4	-5.4±3.4	-7.5±3.6	t(52) = 0.38, p = 0.71
Δ TW0 vs TW8	-7.4±3.8	-11.7±3.9	t(49) = 0.72, p = 0.48
Δ TW0 vs TW12	-5.6±4.4	-13.9±4.8	t(48) = 1.21, p = 0.23
Δ TW0 vs TW24	-4.7±3.9	-16.3±4.6	<u>t(43) = 1.84, p = 0.07</u>
Δ TW0 vs FU	3.6±4.1	-4.4±4.8	t(53) = 1.19, p = 0.24
Δ TW0 vs END	-7.1±3.9	-18.8±5.1	<u>t(46) = 1.79, p = 0.08</u>
Δ END vs FU	10.8±3.8	13.8±3.8	<u>t(46) = -0.51, p = 0.61</u>

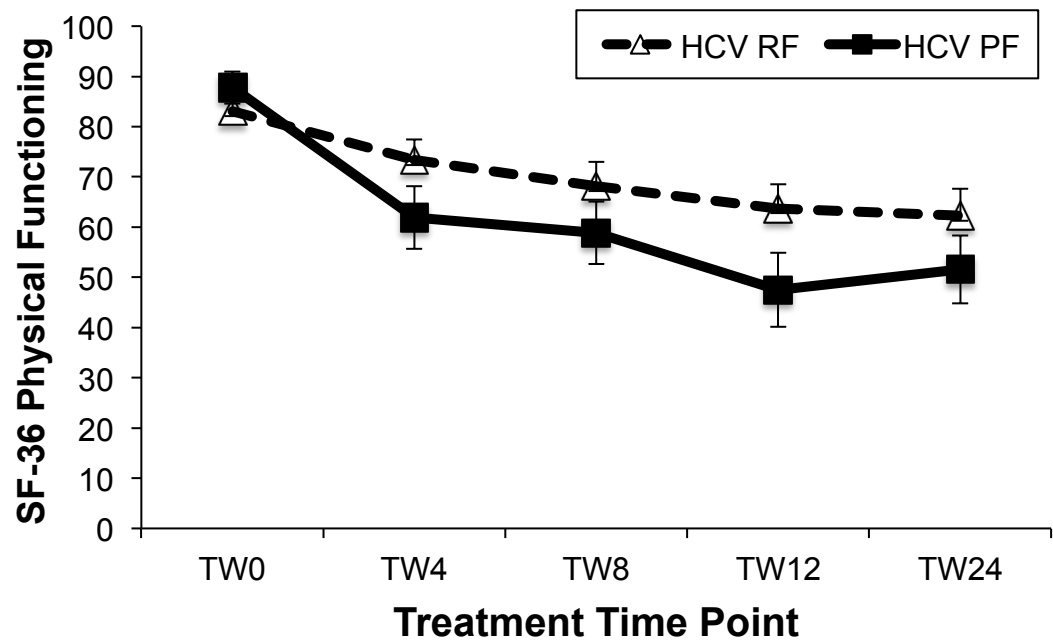


Figure 3.29 SF-36 Physical Functioning scores at baseline, and during treatment in HCV PF vs. RF groups

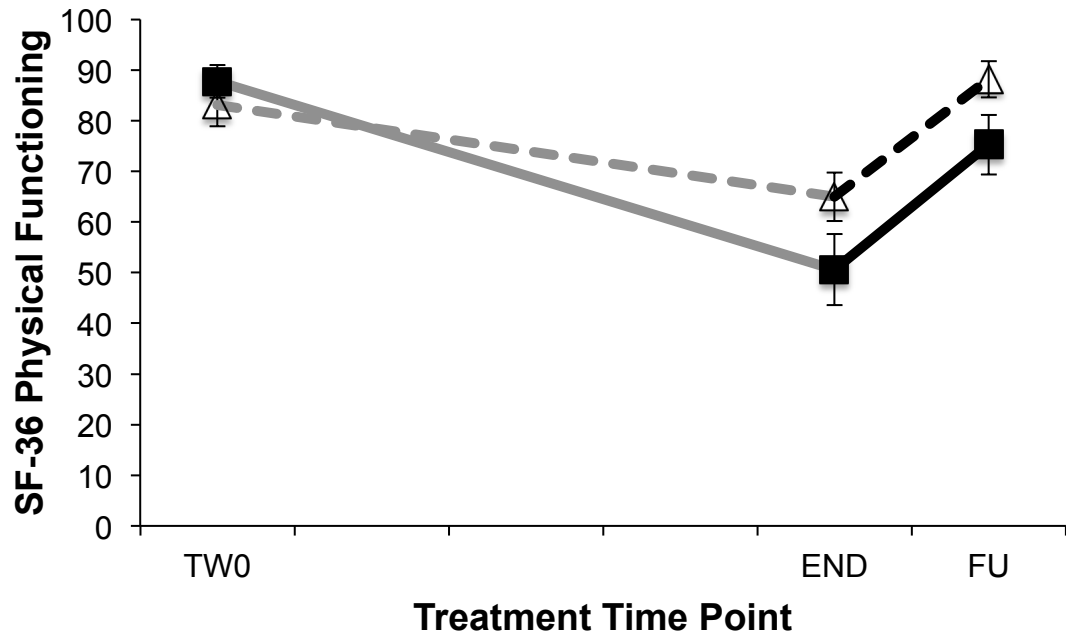


Figure 3.30 SF-36 Physical Functioning scores at baseline, and from end of treatment to six-months post-treatment in HCV PF vs. RF groups

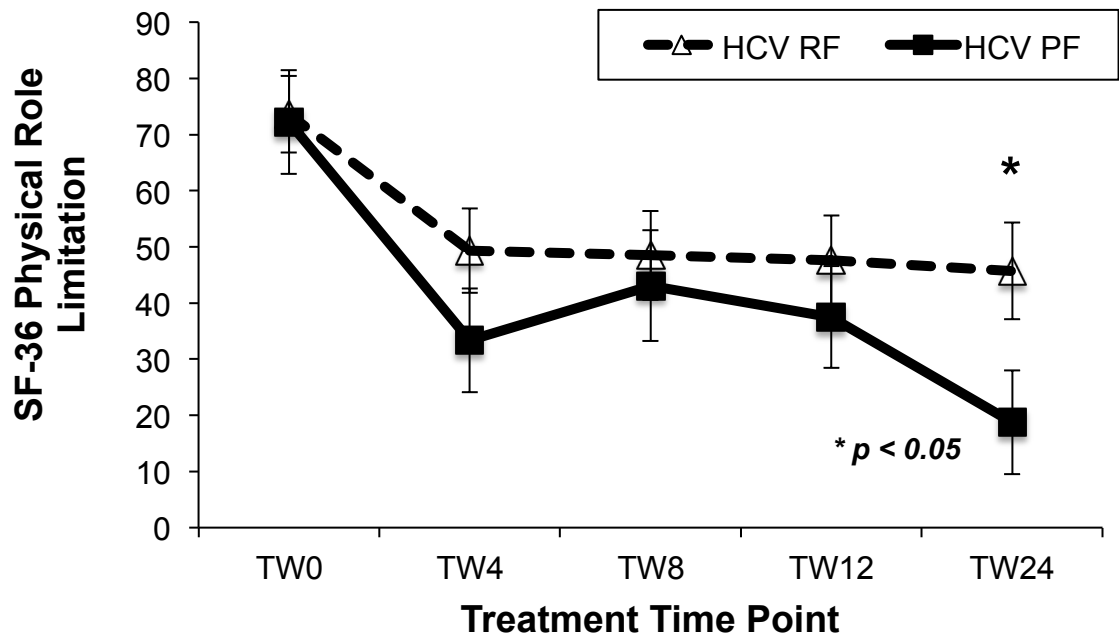


Figure 3.31 SF-36 Physical Role Limitation scores at baseline, and during treatment in HCV PF vs. RF groups

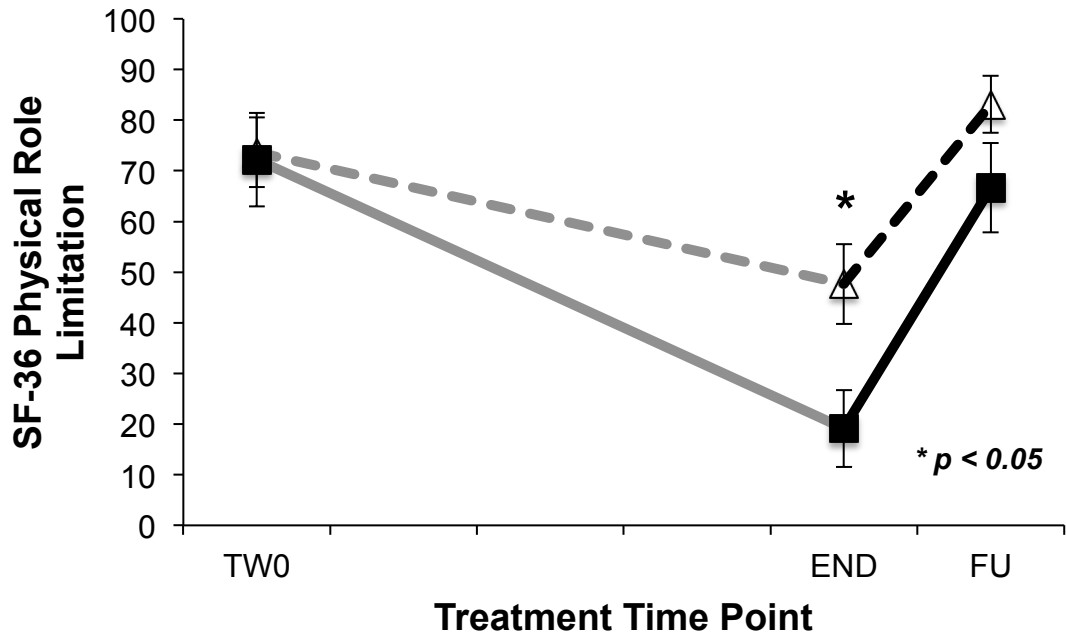


Figure 3.32 SF-36 Physical Role Limitation scores at baseline, and from end of treatment to six-months post-treatment in HCV PF vs. RF groups

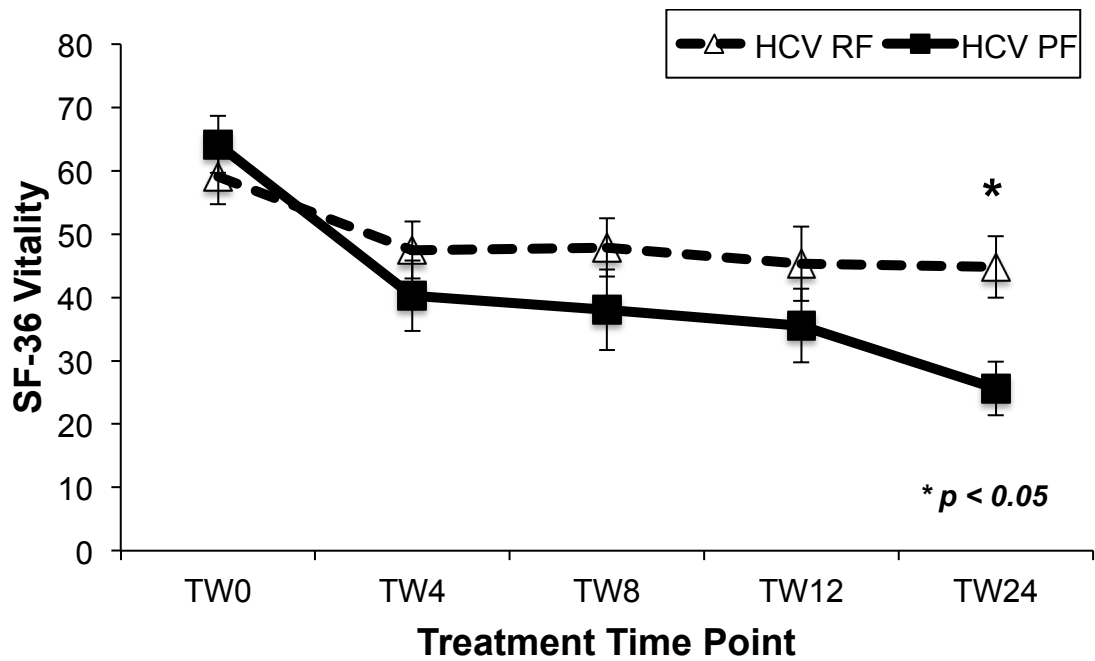


Figure 3.33 SF-36 Vitality scores at baseline, and during treatment in HCV PF vs. RF groups

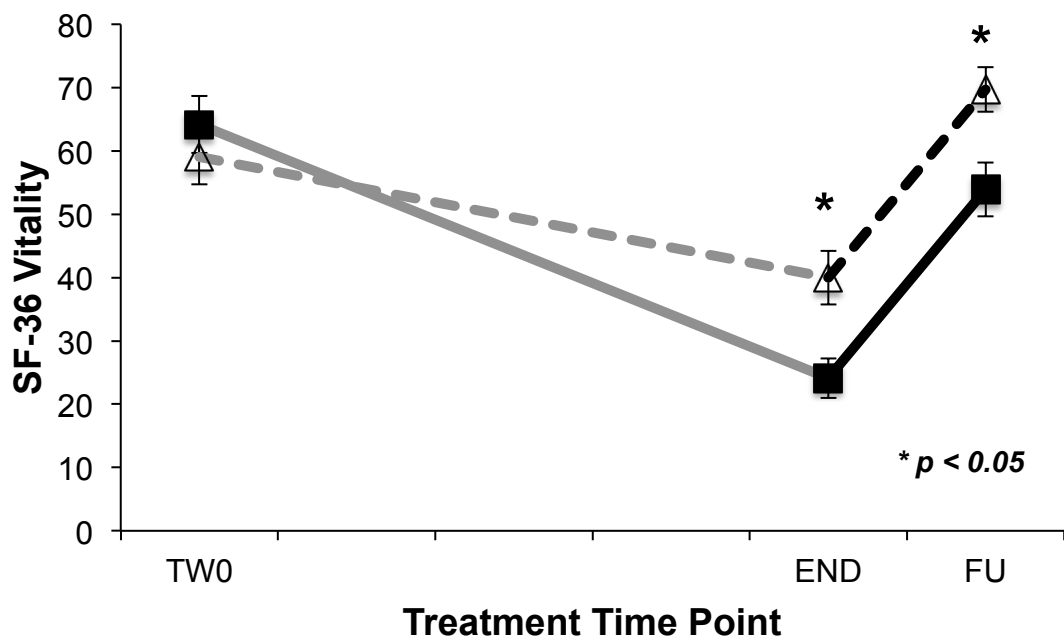


Figure 3.34 SF-36 Vitality scores at baseline, and from end of treatment to six-months post-treatment in HCV PF vs. RF groups

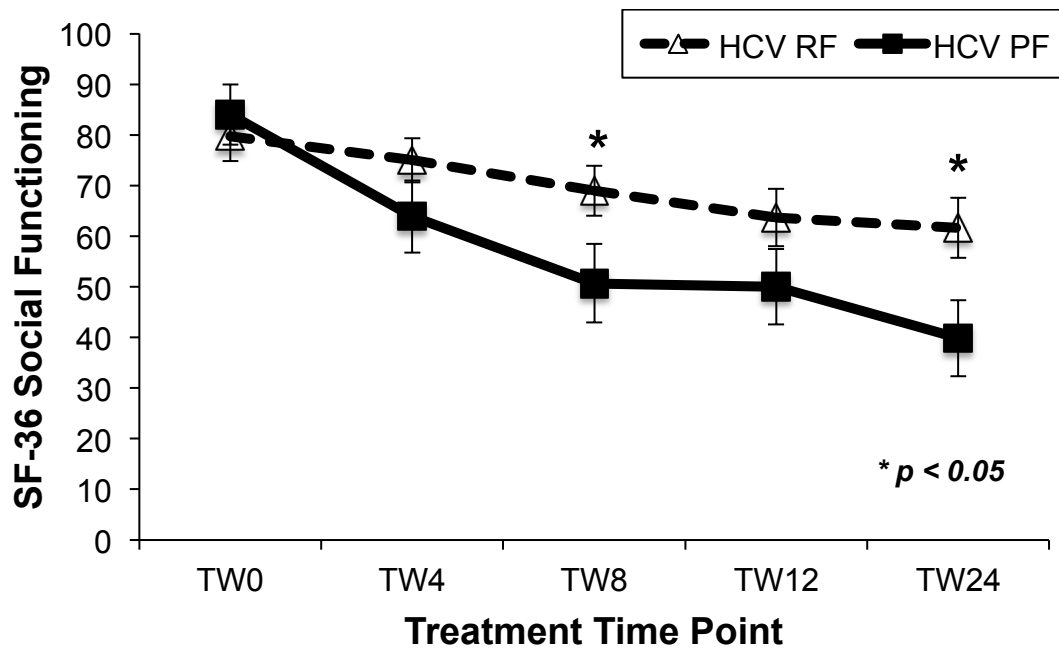


Figure 3.35 SF-36 Social Functioning scores at baseline, and during treatment in HCV PF vs. RF groups

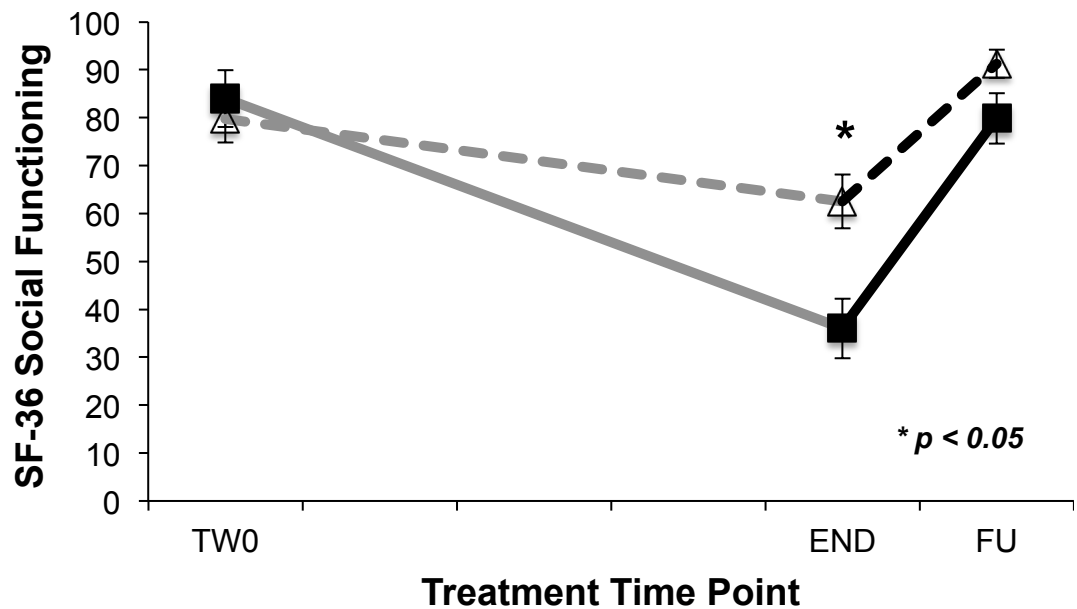


Figure 3.36 SF-36 Social Functioning scores at baseline, and from end of treatment to six-months post-treatment in HCV PF vs. RF groups

Table 3.40 Summary: health status at baseline, and changes during treatment in the PF vs. RF groups

	TW0	TW4	TW8	TW12	TW24	END	FU
Physical Functioning	=	=	=	↘	=	↘	↘
Physical Role Limitation	=	=	=	=	↓	↓	=
Emotional Role Limitation	=	=	=	=	↘	↓	↘
Vitality	=	=	=	=	↓	↓	↓
Mental Health	=	=	=	=	↘	↘	↘
Social Functioning	=	=	↓	=	↓	↓	↘
Pain	=	=	=	=	=	↘	↓
General Health	=	=	=	=	↘	↓	=

	Δ 4	Δ 8	Δ 12	Δ 24	Δ FU	ΔEND	ΔEND vs FU
Physical Functioning	↓	↘	↓	↓	↓	↓	=
Physical Role Limitation	=	=	=	=	=	↓	=
Emotional Role Limitation	=	=	=	=	=	↓	=
Vitality	↓	↓	↓	↓	↓	↓	=
Mental Health	=	↓	=	↓	↘	↓	=
Social Functioning	↓	↓	=	↓	↓	↓	↗
Pain	=	=	=	=	↘	=	=
General Health	=	=	=	↓	=	↘	=

Note – (=) – no difference; (↘) – trend towards worse function/greater decline; (↓) – significantly worse function/greater decline; (↗) – trend towards better function/greater improvement

3.1.3.4 Biological changes in persistent versus resolved fatigue

Next, I compared biological changes in response to IFN- α in the Persistent Fatigue (PF) versus Resolved Fatigue (RF) patients in two measures. First, I explored differences in levels of, and patterns of change in cytokine levels at baseline, during treatment, and at follow-up, six-months post-treatment. I then did the same with levels of metabolites forming a part of the kynurenine pathway at baseline, and during treatment.

Cytokines: longitudinal changes in persistent versus resolved fatigue

The analytes measured were Interferon-gamma (IFN- γ), Interleukin (IL)-10, IL-12p70, IL-13, IL-17A, IL-2, IL-4, IL-6, IL-7, IL-8, Tumour-Necrosis Factor-alpha (TNF- α) and Vascular Endothelial Growth Factor (VEGF). To explore differences in cytokine levels, and patterns of change in response to IFN- α treatment between the RF and PF groups, repeated measures ANOVAs were performed. Levels of the cytokines were often not normally distributed, and for these markers attempts to transform the data were not universally successful in resulting in a normal distribution, meaning it was not possible to compare the analyses with transformed data. Any significant difference found was followed up with a post-hoc comparison of independent t-test and the non-parametric Mann-Whitney U Test. Time points included were baseline (TW0), and treatment weeks (TW)-4, 24, and follow-up, six-months post-treatment (FU). There were 35 participants who had data available for all four time points: 21 in the RF group, and 14 in the PF group. Results for some analytes were not available in all participants: IFN- γ (RF/PF; 18/10); VEGF (18/10) and IL-12p70 (12/9). For sample characteristics for the larger subset of participants ($n = 35$), see Table 3.41 and Table 3.42.

Table 3.41 Socio-demographic characteristics of the Cytokine analysis subsample

	RF (n = 21)	PF (n = 14)	Test and statistic
Age (years)			
Mean±SEM	43.0±2.7	46.1±3.1	t (33) = -0.77, p = 0.45
Gender			
Male	17 (81%)	13 (93%)	X ² (1) = 0.97, p = 0.32
Ethnicity			X ² (3) = 4.66, p = 0.20
White (British/European)	8 (38%)	9 (64%)	
Asian	5 (24%)	0	
Black	1 (5%)	1 (7%)	
Other (incl. mixed)	7 (33%)	4 (29%)	
Education Level			
Degree	5 (24%)	4 (29%)	X ² (1) = 0.10, p = 0.75
Employment status			
Unemployed	12 (57%)	9 (64%)	X ² (1) = 0.18, p = 0.67
Relationship status			
Married/ living with someone	10 (48%)	5 (36%)	X ² (1) = 0.49, p = 0.49
Fatigue 'case' (TW0 CFQ score >18)	2 (10%)	1 (7%)	X ² (1) = 0.06, p = 0.81
History of Depression	5 (24%)	6 (43%)	X ² (1) = 1.41, p = 0.23
Family history	6 (32%)	3 (%)	X ² (1) = 0.28, p = 0.60
IFN-α induced Depression*	5 (24%)	9 (64%)	<u>X² (2) = 5.98, p = 0.050</u>
Drug use			
<i>Opioid</i>			
Current (medical)**	4 (20%)	1 (8%)	X ² (2) = 2.04, p = 0.36
History of abuse***	8 (40%)	6 (50%)	X ² (1) = 0.31, p = 0.58
Current smoking**	5 (24%)	7 (50%)	X ² (3) = 3.06, p = 0.38
Current alcohol**	7 (35%)	9 (69%)	<u>X² (2) = 5.85, p = 0.054</u>

* vs. no/baseline depression; ** vs. past/never; some missing; *** (incl current) vs. never

Table 3.42 Treatment and Virus Characteristics of the Cytokine analysis subsample

	RF (n = 21)	PF (n = 14)	Test and statistic
HCV genotype			$\chi^2 (3) = 4.44, p = 0.22$
1	3 (14%)	4 (29%)	
2	2 (10%)	4 (29%)	
3	15 (71%)	6 (43%)	
4	1 (5%)	0	
HCV viral load, millions			
Mean±SEM	2.22±0.64	3.48±1.02	t (33) = -1.10, p = 0.28
Liver stiffness, kPa*			
Mean±SEM	7.69±2.35	8.28±5.82	t (27) = -0.37, p = 0.71
Treatment duration, wks			$\chi^2 (1) = 1.05, p = 0.31$
24	18 (72%)	6 (55%)	
>24	7 (28%)	5 (45%)	
Treatment type			$\chi^2 (1) = 0.00, p = 1.00$
IFN-α + ribavirin	18 (86%)	12 (86%)	
Triple therapy	3 (14%)	2 (14%)	
RVR			
Yes	12 (57%)	7 (50%)	$\chi^2 (1) = 0.17, p = 0.68$
SVR			
Yes	18 (86%)	13 (93%)	$\chi^2 (1) = 0.42, p = 0.52$
Blood time taken			
AM	10 (48%)	6 (43%)	$\chi^2 (1) = 0.08, p = 0.78$

* Some data missing

Interleukin (IL)-10

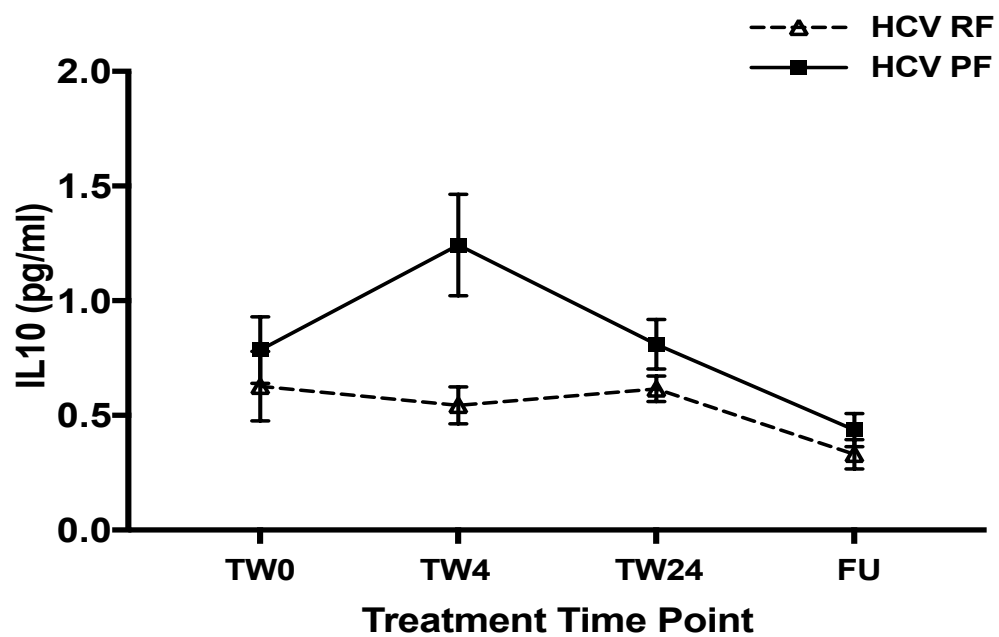
A total of six patients across the two groups were identified as outliers for at least one result of four and thus excluded. With these outliers removed, 17 RF and 12 PF patients were included in the analysis. In the Resolved Fatigue group, data was not normally distributed at TW0, 4 and six-months follow-up, but was at TW24, as assessed by the Levene's test ($p < 0.05$). In the Persistent Fatigue group, data was not normally distributed at TW0 and at follow-up, but was more normally distributed at TW4 and TW24. The assumption of homogeneity of covariance was violated. The assumption of homogeneity of variance was met at treatment time points TW0, 24 and six-month follow-up ($p < 0.05$), but violated at TW4 ($p = 0.005$). The assumption of sphericity was violated ($X^2 (5) = 20.82, p = 0.001$). Results reported are those with the Greenhouse-Geisser correction applied.

There was a trend towards an interaction between the group and time on IL-10 concentration ($F (2.14, 57.86) = 3.09, p = 0.050$, partial $\eta^2 = 0.22, \epsilon = 0.71$). There was a significant main effect of time, showing a difference in mean IL-10 concentration at different time points ($F (2.14, 57.86) = 7.47, p = 0.001$, partial $\eta^2 = 0.22, \epsilon = 0.71$). Post-hoc comparisons revealed statistically significant decreases from TW0 to the follow-up visit (Mean \pm SEM pg/ml; 0.71 ± 0.11 vs. $0.38 \pm 0.05, p = 0.041$), TW4 to follow-up (0.89 ± 0.10 vs. $0.38 \pm 0.05, p = 0.001$) and TW24 to follow-up (0.71 ± 0.06 vs. $0.38 \pm 0.05, p < 0.001$). There was also a main effect of group, indicating a difference in mean IL-10 concentration between the RF/PF groups ($F (1, 27) = 8.25, p = 0.008$, partial $\eta^2 = 0.23$). Post-hoc comparisons of the marginal means showed levels to be higher in the Persistent Fatigue group (0.82 ± 0.08 vs. $0.53 \pm 0.07, p = 0.008$). See Figure 3.37.

Additional post-hoc tests were conducted to further explore the main effect of group. Independent samples t-tests showed that mean IL-10 levels were significantly higher in the Persistent Fatigue group at TW4. There was also a trend towards higher levels at TW24 (data shown in Table 3.43). Non-parametric Mann Whitney U-tests also showed the median IL-10 level to be significantly higher in the PF group at TW4, and then a trend towards increased levels at TW0 and at follow-up. In summary, the effect seems to be driven largely by increased levels of IL-10 at TW4 in the PF group, in line with concurrent increases in fatigue over the same period.

Table 3.43 IL-10: post-hoc parametric and non-parametric comparisons of HCV PF vs. RF groups

Time point	HCV RF	HCV PF	Test and statistic
	Mean±SEM		
TW0	0.63±0.15	0.79±0.15	$t(27)=-0.73, p = 0.47$
TW4	0.55±0.08	1.24±0.22	$t(13.95)=-2.95, p = 0.011$
TW24	0.62±0.06	0.81±0.11	<u>$t(27)=-1.73, p = 0.096$</u>
FU	0.33±0.06	0.44±0.07	$t(27)=-1.10, p = 0.28$
	Median		
TW0	0.45	0.63	<u>$U = 58, z =-1.95, p = 0.051$</u>
TW4	0.43	1.05	$U = 30, z =-3.19, p = 0.001$
TW24	0.57	0.79	$U = 70, z =-1.42, p = 0.16$
FU	0.28	0.34	<u>$U = 59, z =-1.90, p = 0.057$</u>



IL-10 ANOVA: Group*Time: $p = 0.05$; Time: $p < 0.001$; Group: $p = 0.008$

Figure 3.37 Baseline levels, and changes in IL-10 during treatment and at FU in HCV PF vs. RF groups

Interleukin (IL)-13

A total of five individuals who had at least once score outside of the cut-off of three times the interquartile range, four in the Resolved Fatigue group and one in the Persistent Fatigue group, were removed from the analysis of IL-13 levels. Excluding these values, 17 RF and 13 PF patients were included in the analysis. Data was non-normal for all time points in the RF group ($p < 0.05$). In the PF group, data was non-normal at all time points except for TW4 ($p = 0.43$). The assumption of the homogeneity of covariances was violated. There was homogeneity of variances at all treatment time points ($p > 0.05$). The assumption of sphericity was violated ($X^2(5) = 17.67, p = 0.003$).

There was no statistically significant interaction between the group and time on IL-13 concentration ($F(2.07, 57.90) = 1.61, p = 0.21, \text{partial } \eta^2 = 0.05, \epsilon = 0.69$ [G-G]). There was no significant main effect of time, demonstrating no difference in mean IL-13 concentration at the different time points ($F(2.07, 57.90) = 0.45, p = 0.65, \text{partial } \eta^2 = 0.02, \epsilon = 0.69$). Nor was there a main effect of group, showing that there was no difference in mean IL-13 concentration between the RF/PF groups ($F(1, 28) = 0.09, p = 0.76, \text{partial } \eta^2 = 0.003$).

Interferon-gamma (IFN- γ)

One patient from each group was outside of the cut-off and thus excluded as outliers. With these outliers excluded, 17 RF and 9 PF patients were included in the analysis. In the Resolved Fatigue group, distribution of IFN- γ was non-normal at all time points. In the Persistent Fatigue group, IFN- γ levels at TW0 were non-normal, but were normally distributed at TW4, 24 and at follow-up. The assumption of homogeneity of covariances was violated. The assumption of homogeneity of variance was met at treatment time points TW0, 24 and six-month follow-up, but violated at TW4. The assumption of sphericity was met ($X^2(5) = 5.30, p = 0.38$).

There was no statistically significant interaction between group and time on IFN- γ concentration ($F(3,72) = 1.67, p = 0.18, \text{partial } \eta^2 = 0.07$). There was no significant main effect of time, demonstrating no difference in mean IFN- γ concentration at the different treatment time points ($F(3,72) = 2.09, p = 0.11, \text{partial } \eta^2 = 0.08$). Nor was there a main effect of group, showing that there was no difference in mean IFN- γ concentration between the RF/PF groups ($F(1,24) = 0.31, p = 0.58, \text{partial } \eta^2 = 0.01$).

Interleukin (IL)-2

Two participants in the Resolved Fatigue group, and three in the Persistent Fatigue groups had at least one IL-2 result exceeding 3 times the interquartile range for each time point. With these results excluded, 19 RF and 11 PF patients were included in the analysis. Data in the RF group was not normally distributed at any time point. In the Persistent Fatigue group, data was non-normal at TW0, but normal at TW4, TW24 and at follow-up. The assumption of homogeneity of covariance was met, as was the assumption of homogeneity of variance at all time points. The assumption of sphericity was violated ($\chi^2(5) = 23.86, p < 0.001$).

There was no statistically significant interaction between the group and time on IL-2 concentration, $F(1.94, 54.29) = 1.26, p = 0.29$, partial $\eta^2 = 0.04$, $\epsilon = 0.65$ [G-G]. There was a significant main effect of time, demonstrating a difference in mean IL-2 concentration at the different time points, $F(1.94, 54.29) = 14.05, p < 0.001$, partial $\eta^2 = 0.33$, $\epsilon = 0.65$). There were significant increases from TW0 to TW4 (Mean \pm SEM pg/ml; 0.20 ± 0.04 vs. $0.27 \pm 0.04, p < 0.001$), and TW0 to TW24 (0.20 ± 0.04 vs. $0.35 \pm 0.06, p < 0.001$). There were also significant decreases in levels of IL-2 when measured during treatment, to the follow-up visit: from TW4 (0.27 ± 0.04 vs. $0.21 \pm 0.03, p = 0.023$) and TW24 (0.35 ± 0.06 vs. $0.21 \pm 0.03, p = 0.001$). There was no main effect of group, showing that there was no difference in mean IL-2 concentration between the RF/PF groups ($F(1, 28) = 0.02, p = 0.88$, partial $\eta^2 = 0.001$). See Figure 3.38 (page 226).

Interleukin (IL)-6

Four participants, two from each group, had at least one result that was considered an outlier. There were 19 RF and 12 PF patients included in the analysis. Data in the RF group was only normal distributed at TW0. Data in the PF group was normal at all time points. The assumption of homogeneity of covariance was violated. The assumption of homogeneity of variances was violated at all time points except for follow-up. The assumption of sphericity was violated ($\chi^2 (5) = 22.13, p < 0.001$).

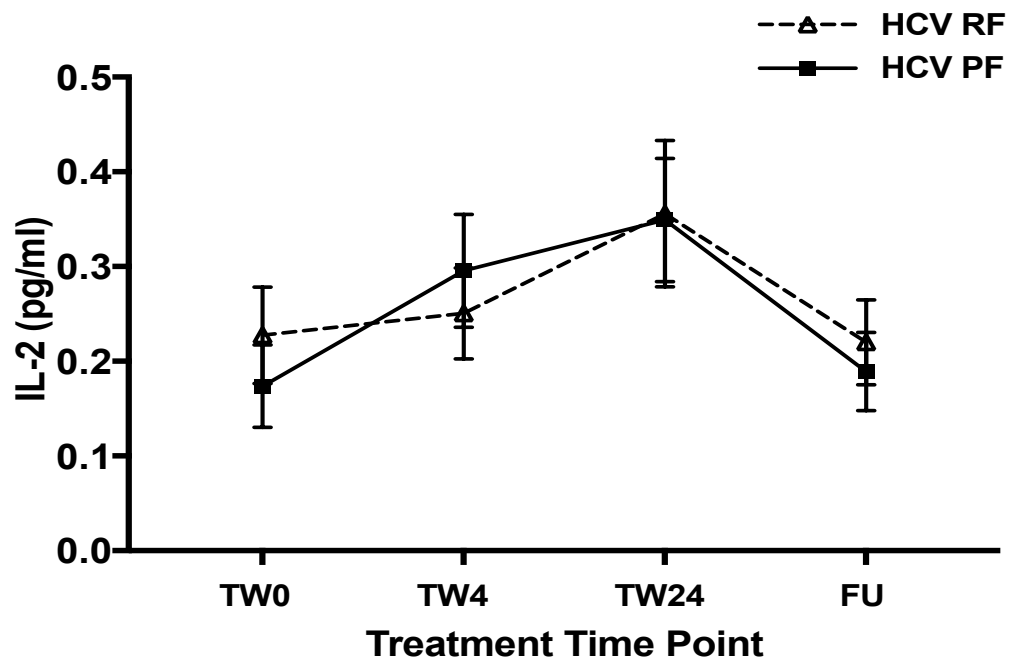
There was a trend towards a significant interaction between the group and time on IL-6 concentration ($F (2.24, 64.61) = 2.62, p = 0.075$, partial $\eta^2 = 0.08$, $\epsilon = 0.74$ [G-G]). There was a significant main effect of time, demonstrating a difference in mean IL-6 concentration at the different time points ($F (2.23, 64.61) = 10.14, p < 0.001$, partial $\eta^2 = 0.26$, $\epsilon = 0.74$). There were significant increases from TW0 to TW4 (Mean \pm SEM pg/ml; 0.89 ± 0.10 vs. $1.90 \pm 0.24, p = 0.003$), and from TW0 to TW24 (0.89 ± 0.10 vs. $1.84 \pm 0.22, p = 0.001$). In addition, there was a significant decrease from TW4 to the follow-up visit (1.90 ± 0.24 vs. $1.13 \pm 0.14, p = 0.042$) and TW24 to follow-up (1.84 ± 0.22 vs. $1.13 \pm 0.14, p = 0.040$). There was also a significant main effect of group, showing that there was a difference in mean IL-6 concentrations between RF and PF groups ($F (1,29) = 8.78, p = 0.006$, partial $\eta^2 = 0.23$). The estimated marginal means showed increased levels in PF patients (Mean \pm SEM pg/ml; 1.80 ± 0.20 vs. 1.03 ± 0.16). See Figure 3.38 (page 226).

Independent t-tests indicated that there were significant differences in IL-6 levels between the two groups at TW0 and TW4, with a trend towards higher levels at TW24. However, it should be noted that the equality of variance was

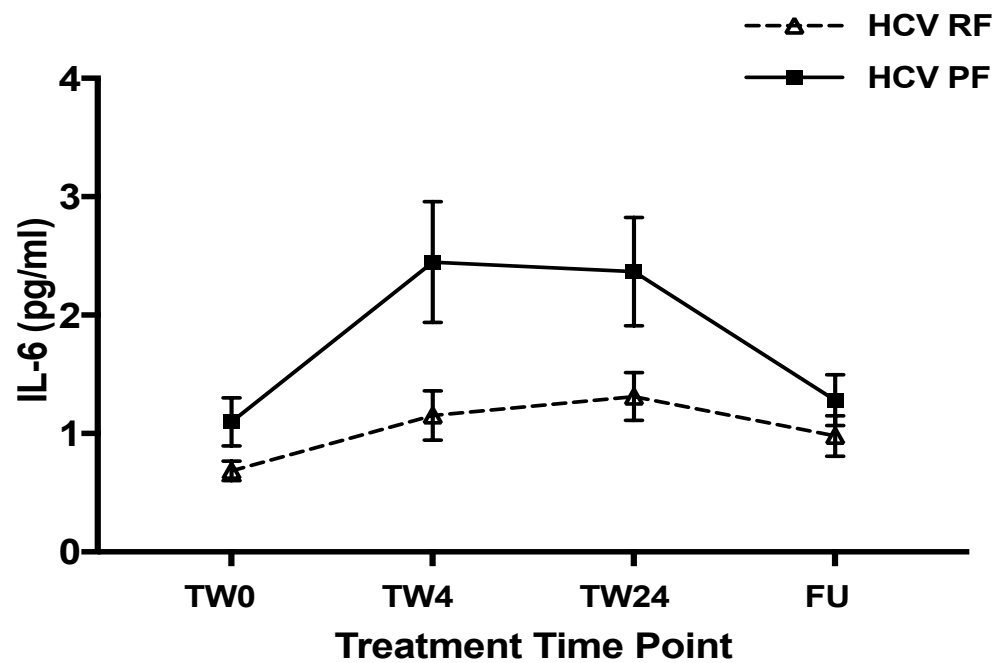
violated ($p < 0.05$) for TW4 and TW24 (data shown in Table 3.44, including adjusted results). Non-parametric Mann Whitney U tests showed a trend towards a group difference in IL-6 levels at TW0 and TW24, and a statistically significant difference at TW4. Thus it appears that the difference at TW4, and to a lesser extent TW24, is driving the difference between groups. Interestingly, the data also suggest that there may already be a difference in IL-6 pre-treatment between the two groups.

Table 3.44 IL-6: post-hoc parametric and non-parametric comparisons of HCV PF vs. RF groups

Time point	HCV RF	HCV PF	Test and statistic
	Mean±SEM		
TW0	0.69±0.08	1.10±0.20	<i>t</i> (29) = -2.17, <i>p</i> = 0.039
TW4	1.15±0.21	2.45±0.51	<i>t</i> (14.73) = -2.35, <i>p</i> = 0.033
TW24	1.31±0.20	2.37±0.46	<u><i>t</i> (15.34) = -2.11, <i>p</i> = 0.052</u>
FU	0.98±0.17	1.28±0.21	<i>t</i> (29) = -1.10, <i>p</i> = 0.28
	Median		
TW0	0.64	1.01	<u><i>U</i> = 71, <i>z</i> = -1.74, <i>p</i> = 0.081</u>
TW4	0.80	2.25	<i>U</i> = 54, <i>z</i> = -2.43, <i>p</i> = 0.015
TW24	1.07	1.96	<u><i>U</i> = 68, <i>z</i> = -1.87, <i>p</i> = 0.062</u>
FU	0.72	1.17	<i>U</i> = 82, <i>z</i> = -1.30, <i>p</i> = 0.19



IL-2 ANOVA: Group*Time: $p = 0.29$; Time: $p < 0.001$; Group: $p = 0.88$



IL-6 ANOVA: Group*Time: $p = 0.08$; Time: $p < 0.001$; Group: $p = 0.006$

Figure 3.38 Baseline levels, and changes in IL-2 and IL-6 during treatment and at FU in HCV PF vs. RF groups

Interleukin (IL)-7

Examination of the boxplots showed two participants in the RF group, and one in the PF group to have at least one result that could be considered an outlier ($3 \times \text{IQR}$). With these participants excluded, 19 RF and 13 PF patients were included in the analysis. Data in the RF group was non-normal at all time points. In the PF group, data was more normally distributed at TW0, TW4 and TW24, but not at follow-up. The assumption of homogeneity of covariance was not met. There was homogeneity of variances at all treatment time points. The assumption of sphericity was met ($\chi^2 (5) = 2.38, p = 0.80$).

There was a statistically significant interaction between the group and time on IL-7 concentration ($F (3, 90) = 3.27, p = 0.025$, partial $\eta^2 = 0.10$). Group differences at each time point were further explored (data shown in Table 3.45). Though levels changed differently in response to IFN- α in each group, levels were not significantly different at any time point measured.

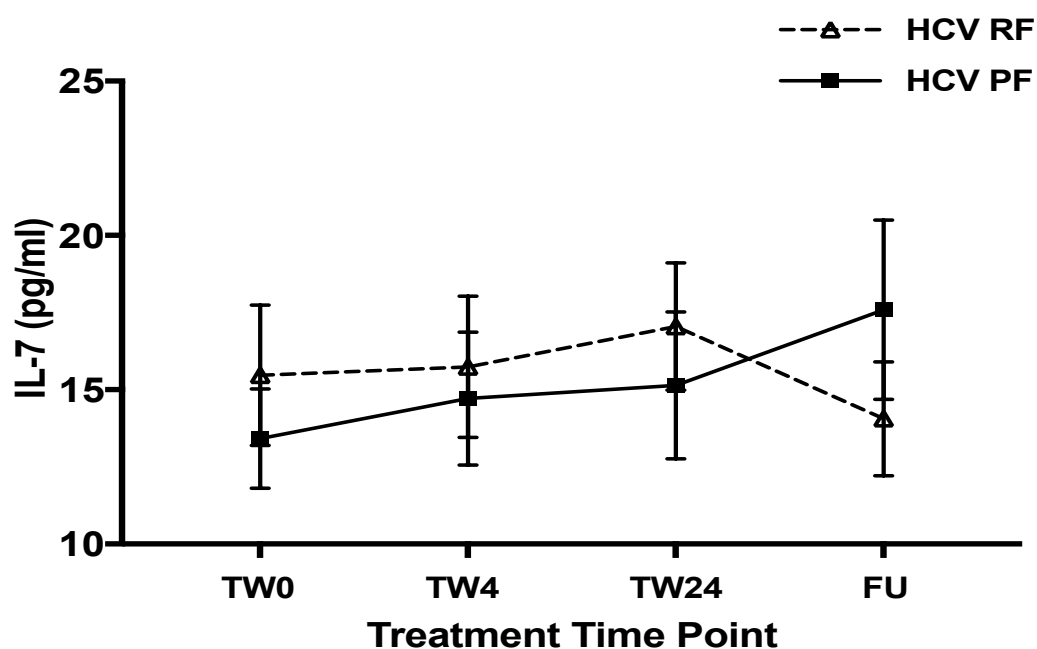
Table 3.45 IL-7: post-hoc parametric and non-parametric comparisons of HCV PF vs. RF groups

	HCV RF	HCV PF	
Time point	Mean±SEM		Test and statistic
TW0	15.47±2.28	13.41±1.61	$t(30) = 0.67, p = 0.51$
TW4	15.74±2.29	14.71±2.16	$t(30) = 0.31, p = 0.76$
TW24	17.05±2.06	15.14±2.38	$t(30) = 0.60, p = 0.55$
FU	14.06±1.85	17.59±2.90	$t(30) = -1.08, p = 0.29$
	Median		
TW0	14.09	13.55	$U = 121, z = -0.10, p = 0.92$
TW4	12.38	11.57	$U = 115, z = -0.33, p = 0.74$
TW24	13.98	12.44	$U = 105, z = -0.71, p = 0.48$
FU	12.41	13.59	$U = 99, z = -0.94, p = 0.35$

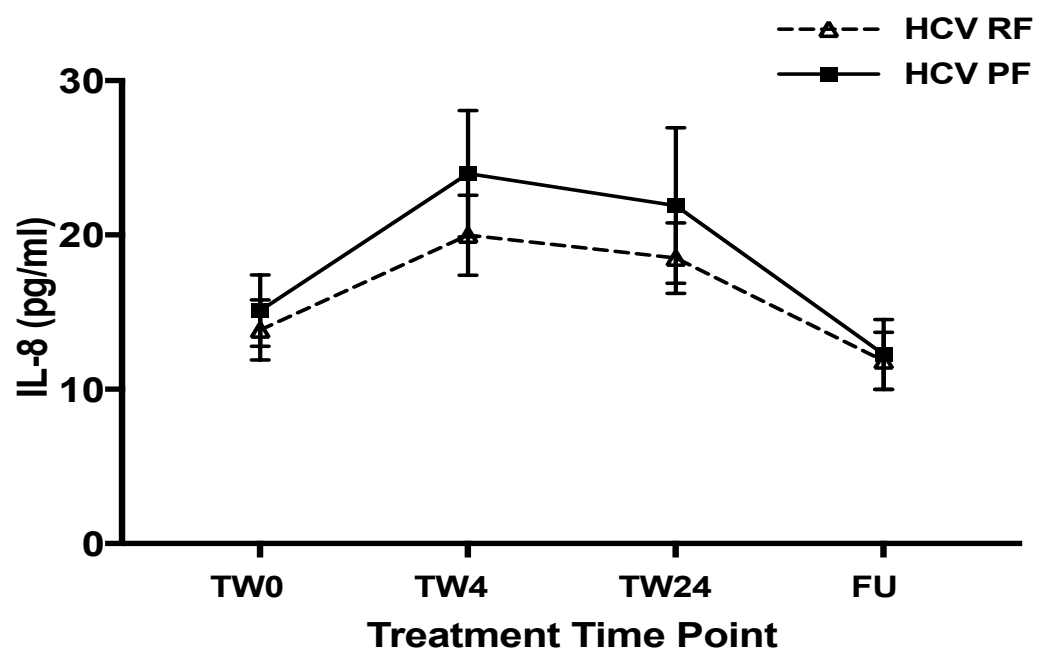
Interleukin (IL)-8

One patient in each group had at least one result in four that could be considered as an outlier, and were therefore excluded from the analysis of IL-8 levels. There were 20 RF and 13 PF patients included in the analysis. Data was not normally distributed in any of the groups, at any time points. The assumption of homogeneity of covariance was violated. The assumption of homogeneity of variance was met at all treatment time points. The assumption of sphericity was violated ($\chi^2(5) = 20.96, p < 0.001$).

There was no statistically significant interaction between the group and time on IL-8 concentration, ($F(2.00, 61.91) = 0.39, p = 0.68$, partial $\eta^2 = 0.013$, $\epsilon = 0.67$ [G-G]). There was a significant main effect of time, demonstrating a difference in mean IL-8 concentration at the different time points ($F(2.00, 61.91) = 11.90, p < 0.001$, partial $\eta^2 = 0.28$, $\epsilon = 0.67$). There was a significant increase from TW0 to TW4 (Mean \pm SEM pg/ml; 14.47 ± 1.53 vs. $21.98 \pm 2.30, p < 0.001$), and significant decreases from TW4 to the follow-up visit (21.98 ± 2.30 vs. 12.05 ± 1.46), and TW24 to follow-up (20.21 ± 2.46 vs. $12.05 \pm 1.46, p = 0.007$). There was no main effect of group, showing that there was no difference in mean IL-8 concentration between the RF/PF groups ($F(1, 31) = 0.50, p = 0.49$, partial $\eta^2 = 0.02$).



IL-7 ANOVA: Group*Time: $p = 0.03$



IL-8 ANOVA: Group*Time: $p = 0.68$; Time: $p < 0.001$; Group: $p = 0.49$

Figure 3.39 Baseline levels, and changes in IL-7 and IL-8 during treatment and at FU in HCV PF vs. RF groups

Interleukin (IL)-12p70

Two individuals had at least one score identified as an outlier, both in the Resolved Fatigue group. With these outliers removed, 10 RF and 9 PF patients were included in the analysis. In the RF group, data was non-normal at TW0 and TW24, but was more normally distributed at TW4 and at follow-up. In the Persistent Fatigue group, IL-12p70 levels were more normally distributed at TW0 but non-normal at TW4, 24 and at follow-up. The assumption of homogeneity of covariance was violated. The assumption of homogeneity of variance was met only at TW24, and otherwise violated at TW0, TW4 and at follow-up. The assumption of sphericity was violated ($X^2(5) = 17.03, p = 0.005$).

There was no statistically significant interaction between the group and time on IL-12p70 concentration ($F(2.19, 37.21) = 1.62, p = 0.21, \text{partial } \eta^2 = 0.09, \epsilon = 0.73$ [G-G]). There was no significant main effect of time, demonstrating no difference in mean IL-12p70 concentration at the different time points ($F(2.19, 37.21) = 0.98, p = 0.39, \text{partial } \eta^2 = 0.05, \epsilon = 0.73$). Nor was there a main effect of group, showing that there was no difference in mean IL-12p70 concentration between the RF/PF groups ($F(1, 17) = 2.32, p = 0.15, \text{partial } \eta^2 = 0.12$).

Interleukin (IL)-17A

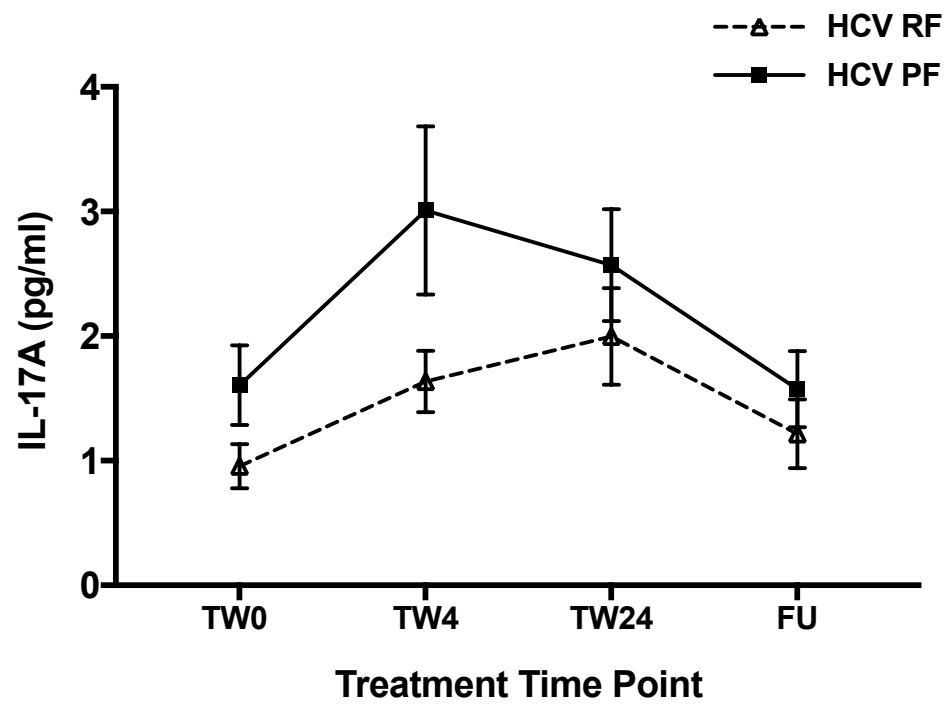
Examination of the boxplots revealed four participants in the Resolved Fatigue group, and two in the Persistent Fatigue group who had at least one value which exceeded the 3 times the interquartile range cut-off. With these participants excluded, 17 RF and 13 PF patients were included in the analysis. In the RF group IL-17A levels were non-normal at all time points. Levels in the PF group were also not normally distributed at all points except TW24, when they were more normally distributed. The assumption of homogeneity of covariance was violated. The assumption of homogeneity of variance was met at treatment time points TW0, TW24 and follow-up, but violated at TW4. The assumption of sphericity was violated ($\chi^2(5) = 24.13, p < 0.001$).

There was no statistically significant interaction between the group and time on IL-17A concentration ($F(1.93, 52.21) = 1.16, p = 0.32$, partial $\eta^2 = 0.04$, $\epsilon = 0.65$ [G-G]). There was a significant main effect of time, demonstrating a difference in mean IL-17A concentration at the different time points ($F(1.93, 52.21) = 7.48, p = 0.002$, partial $\eta^2 = 0.22$, $\epsilon = 0.65$). There were significant increases from TW0 to TW4 (Mean \pm SEM pg/ml; 1.28 ± 0.17 vs. $2.32 \pm 0.32, p = 0.009$), and from TW0 to TW24 (1.28 ± 0.17 vs. $2.28 \pm 0.30, p = 0.006$). In addition, there was a significant decrease from TW24 to the follow-up visit (2.28 ± 0.30 vs. $1.40 \pm 0.21, p = 0.001$). Finally, there was a trend towards a decrease from TW4 to follow-up (2.32 ± 0.32 vs. $1.40 \pm 0.21, p = 0.056$). There was a trend towards a significant main effect of group ($F(1, 27) = 4.00, p = 0.056$, partial $\eta^2 = 0.13$). Though this was not statistically significant, there was a trend towards increased levels of IL-17A in the PF group versus the RF group (Mean \pm SEM pg/ml; 2.19 ± 0.28 vs. 1.45 ± 0.24). See Figure 3.40.

As a result of the non-normal distribution at several time points, and the trend towards a group difference observed, post-hoc analyses were still conducted to further explore group differences in IL-17A levels. Independent t-tests revealed a trend towards higher mean IL-17A levels at TW0 and TW4, with the latter p-value adjusted to reflect the violation of the assumption of equal variance. The Mann Whitney U Test showed higher levels of IL-17A in the Persistent Fatigue group at TW0. The test results also showed a trend towards higher levels at TW4, and at six-months follow-up as well (data shown in Table 3.46). In comparing the two tests, although the results are not consistent, increased levels are again suggested early on in treatment, at TW0 and TW4, in those who go on to experience persistent fatigue post-treatment.

Table 3.46 IL-17A: post-hoc parametric and non-parametric comparisons of HCV PF vs. RF groups

Time point	HCV RF	HCV PF	Test and statistic
	Mean±SEM		
TW0	0.95±0.18	1.61±0.32	<u>t (27) = -1.91, p = 0.066</u>
TW4	1.64±0.25	3.01±0.67	<u>t (13.95) = -1.91, p = 0.077</u>
TW24	2.00±0.39	2.57±0.45	t (27) = -0.96, p = 0.35
FU	1.22±0.28	1.57±0.30	t (27) = -0.86, p = 0.40
<hr/>			
	Median		
TW0	0.74	1.29	U = 51, z = -2.26, p = 0.024
TW4	1.42	2.07	<u>U = 64, z = -1.68, p = 0.092</u>
TW24	1.30	2.39	U = 78, z = -1.06, p = 0.29
FU	0.94	1.03	<u>U = 63, z = -1.72, p = 0.084</u>



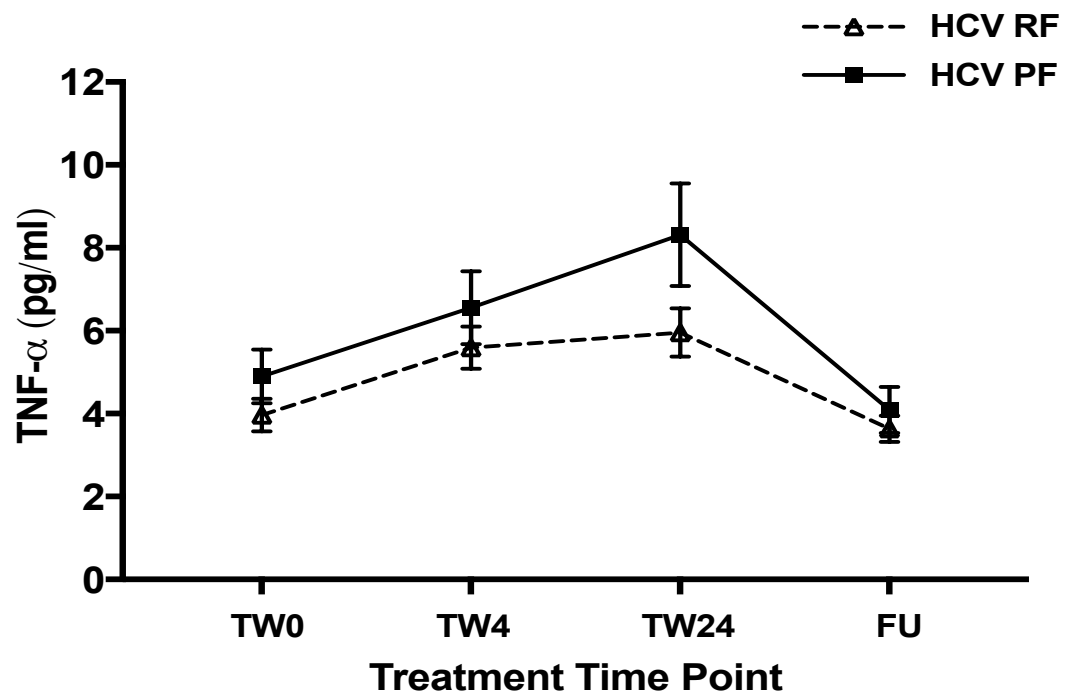
IL-17A ANOVA: Group*Time: $p = 0.32$; Time: $p = 0.026$; Group: $p = 0.06$

Figure 3.40 Baseline levels, and changes in IL-17A during treatment and at FU in HCV PF vs. RF groups

Tumour Necrosis Factor-alpha (TNF- α)

Two patients in the RF group, and one patient in the PF group had at least one result that exceeded the criteria for outliers, and were subsequently removed from analysis of TNF- α levels. There were 19 RF and 13 PF patients included in the analysis. Data was not normally distributed at any treatment time point, in any group. There was homogeneity of covariances. The assumption of homogeneity of variance was met at treatment time points TW0, 4 and six-month follow-up, but violated at TW24. The assumption of sphericity was violated ($\chi^2 (5) = 32.24, p = 0.001$).

There was no statistically significant interaction between the group and time on TNF- α concentration ($F (1.79, 53.68) = 2.25, p = 0.12$, partial $\eta^2 = 0.07$, $\epsilon = 0.60$ [G-G]). There was a significant main effect of time, demonstrating a difference in mean TNF- α concentration at the different time points, ($F (1.79, 53.68) = 30.09, p < 0.001$, partial $\eta^2 = 0.50$, $\epsilon = 0.60$ [G-G]). There was a significant increase from TW0 to TW4 (Mean \pm SEM pg/ml; 4.43 ± 0.36 vs. 6.08 ± 0.48 , $p < 0.001$), and TW0 to TW24 (4.43 ± 0.36 vs. 7.14 ± 0.62 , $p < 0.001$). There were significant decreases from TW0 to six-month follow-up, or 'FU' (4.43 ± 0.36 vs. 3.86 ± 0.30 , $p = 0.048$), TW4 to FU (6.08 ± 0.48 vs. 3.86 ± 0.30 , $p < 0.001$) and TW24 to FU (7.14 ± 0.62 vs. 3.86 ± 0.30 , $p < 0.001$). There was no main effect of group, showing that there was no difference in mean TNF- α concentration between the RF/PF groups ($F (1, 30) = 2.30, p = 0.14$, partial $\eta^2 = 0.07$). See Figure 3.41.



TNF α ANOVA: Group*Time: $p = 0.12$; Time: $p < 0.001$; Group: $p = 0.14$

Figure 3.41 Baseline levels, and changes in TNF- α during treatment and at FU in HCV PF vs. RF groups

Vascular Endothelial Growth Factor (VEGF)

There was one outlier, in the RF group. This left 17 RF and 10 PF patients who were included in the analysis. Data was not normally distributed at any time point in the RF group, but was more normally distributed in the PF group. The assumption of homogeneity of covariance was violated. The assumption of homogeneity of variance was met at all treatment time points. The assumption of sphericity was violated ($\chi^2(5) = 12.11, p = 0.033$). There was no statistically significant interaction between the group and time on VEGF concentration ($F(2.31, 57.70) = 0.24, p = 0.82, \text{partial } \eta^2 = 0.01, \epsilon = 0.77$ [G-G]). There was no significant main effect of time, demonstrating no difference in mean VEGF concentration at the different time points, ($F(2.31, 57.70) = 1.51, p = 0.23, \text{partial } \eta^2 = 0.06, \epsilon = 0.77$). Nor was there a main effect of group ($F(1, 25) = 0.55, p = 0.46, \text{partial } \eta^2 = 0.02$).

Conclusion

Results are summarised in Table 3.47 overleaf. There was no effect of IFN- α treatment on levels of IFN- γ , IL-12p70, IL-13 or VEGF. There was an effect of treatment on levels of IL-2, IL-8 and TNF- α , though no difference in the RF/PF groups, For levels of IL-10 and IL-6, there was an effect of IFN- α in both groups, though levels were higher in the Persistent Fatigue group. For IL-17A, the same pattern of results was found, though there was only a trend towards higher levels in the PF group. Different patterns of change in IL-7 were found in the RF and PF groups, though at no point were levels significantly different. Comparisons of the groups at different time points yielded mixed results, but for those markers where group differences were found, the early response to treatment appeared key.

Table 3.47 Summary: cytokine changes in HCV PF vs. RF groups

Cytokine	Group*Time	Time	Group
IL-10	$(p = 0.05)$	$p = 0.001$ TW0>FU; TW4>FU; TW24>FU	$p = 0.008$ (↑ PF)
IL-2	-	$p < 0.001$ TW0<4; TW0<24; TW4>FU; TW24>FU	-
IL-6	$(p = 0.08)$	$p < 0.001$ TW0<4; TW0<24; TW4>FU; TW24>FU	$p = 0.006$ (↑ PF)
IL-7	$p = 0.03$		
IL-8	-	$p < 0.001$ TW0<4; TW4>FU; TW24>FU	-
IL-17A	-	$p = 0.026$ TW0<4; TW0<24; TW24>FU	$(p = 0.06)$ (↑ PF)
TNFα	-	$p < 0.001$ TW0<4; TW0<24; TW0>FU; TW4>FU; TW24>FU	-
IL-13	-	-	-
IFN-γ	-	-	-
IL-12p70	-	-	-
VEGF	-	-	-

Kynurenine pathway: longitudinal changes in persistent versus resolved fatigue

To explore differences in levels of the metabolites, and patterns of change in response to IFN- α treatment between the Resolved Fatigue (RF) and Persistent Fatigue (PF) groups, repeated measures ANOVAs were performed. Where data from any time point was non-normal, analyses were repeated with transformed data, using the logarithmic method (Log_{10}). For this data set, follow-up samples had not been analysed in all patients, and so this time point was not included so as not to reduce numbers further still. In addition, for the kynurenine pathway analysis, samples had been analysed at a separate time point to baseline and treatment measurements in some individuals. Therefore, the time points included were baseline (TW0), and treatment weeks (TW)-8, and 24. There were 36 participants who had data available for all three treatment time points: 25 in the RF group, and 11 in the PF. See Table 3.48 and Table 3.49 for sub-sample characteristics.

Table 3.48 Socio-demographic characteristics of the kynurenine pathway analysis subsample (repeated measures analysis)

	RF (n = 25)	PF (n = 11)	Test and statistic
Age (years)			
Mean±SEM	45.9±2.6	46.3±4.0	t (34) = -0.08, p = 0.94
Gender			
Male	18 (72%)	9 (82%)	X ² (1) = 0.39, p = 0.53
Ethnicity			X ² (3) = 2.53, p = 0.47
White (British/European)	12 (48%)	7 (64%)	
Asian	4 (16%)	0	
Black	1 (4%)	1 (9%)	
Other (incl. mixed)	8 (32%)	3 (27%)	
Education Level			
Degree	7 (28%)	2 (18%)	X ² (1) = 0.39, p = 0.53
Employment status			
Unemployed	16 (64%)	9 (82%)	X ² (1) = 1.14, p = 0.29
Relationship status			
Married/ Living with someone	10 (40%)	5 (46%)	X ² (1) = 0.09, p = 0.76
Fatigue 'case' (TW0 CFQ score >18)	2 (8%)	1 (9%)	X ² (1) = 0.01, p = 0.91
History of Depression*	7 (28%)	7 (64%)	X² (1) = 4.08, p = 0.043
Family history	7 (28%)	5 (46%)	X ² (1) = 0.59, p = 0.44
IFN-α induced Depression	8 (32%)	7 (64%)	X ² (2) = 3.33, p = 0.19
Drug use			
<i>Opioid</i>			
Current (medical)**	6 (24%)	1 (10%)	X ² (2) = 1.35, p = 0.51
History of abuse***	12 (48%)	5 (50%)	X ² (1) = 0.01, p = 0.92
Current smoking**	8 (32%)	7 (64%)	X ² (3) = 4.27, p = 0.23
Current alcohol**	8 (32%)	8 (73%)	X² (2) = 6.04, p = 0.049

* vs. no/baseline depression; ** vs. past/never; some missing; *** (incl current) vs. never

Table 3.49 Treatment and virus characteristics in the kynurenine pathway analysis subsample (longitudinal analysis)

	RF (<i>n</i> = 25)	PF (<i>n</i> = 11)	Test and statistic
HCV genotype			$\chi^2 (3) = 1.75, p = 0.63$
1	4 (16%)	2 (18%)	
2	3 (12%)	3 (27%)	
3	17 (68%)	6 (55%)	
4	1 (4%)	0	
HCV viral load, millions			
Mean±SEM	2.54±0.67	2.97±1.21	<i>t</i> (34) = -0.33, <i>p</i> = 0.74
Liver stiffness, kPa*			
Mean±SEM	8.1±0.7	6.9±0.8	<i>t</i> (28) = 1.05, <i>p</i> = 0.30
Treatment duration, wks			$\chi^2 (1) = 1.05, p = 0.31$
≤24	18 (72%)	6 (55%)	
>24	7 (28%)	5 (46%)	
Treatment type			$\chi^2 (1) = 0.31, p = 0.58$
IFN-α + ribavirin	21 (84%)	10 (91%)	
Triple therapy	4 (16%)	1 (9%)	
RVR			
Yes	15 (60%)	5 (46%)	$\chi^2 (1) = 0.66, p = 0.42$
SVR			
Yes	21 (84%)	10 (91%)	$\chi^2 (1) = 0.31, p = 0.58$
Blood time taken			
AM	11 (44%)	6 (55%)	$\chi^2 (1) = 0.34, p = 0.56$

Tryptophan

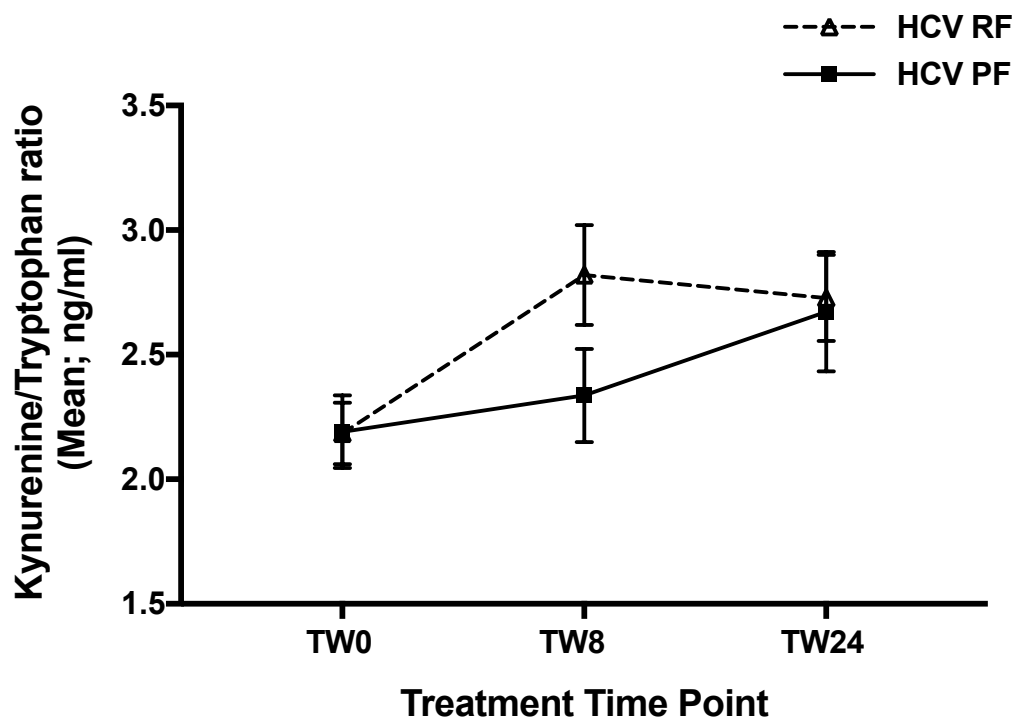
Data was normally distributed at all three time points. Visual examination of the boxplots showed that there were no outliers. The assumption of the equality of covariances was met. So too was the assumption of the homogeneity of variances at all time points. The assumption of sphericity was violated ($X^2(2) = 6.26, p = 0.044$).

There was no interaction between time and group on Tryptophan levels ($F(1.71, 57.98) = 0.72, p = 0.47, \text{partial } \eta^2 = 0.02, \epsilon = 0.85$ [Greenhouse-Geisser correction applied]). There was a trend towards a main effect of time ($F(1.71, 57.98) = 2.59, p = 0.092, \text{partial } \eta^2 = 0.07, \epsilon = 0.85$). There was no significant main effect of group ($F(1,34) = 0.56, p = 0.46, \text{partial } \eta^2 = 0.02$). As tryptophan levels were normally distributed at all three treatment time points, this data was not transformed.

Kynurenine/Tryptophan ratio

The ratio was calculated by dividing the level of kynurenine by the level of tryptophan, and multiplying the number by 100. Data was normally distributed at baseline, but not at TW8 or 24. Visual examination of the boxplots showed that there were no outliers. The assumption of homogeneity of covariances was met. The assumption of homogeneity of variances was also met at all time points. The assumption of sphericity was met ($X^2(2) = 1.16, p = 0.56$).

There was a trend towards a significant interaction between time and group on the kynurenine/tryptophan ratio level ($F(2,68) = 2.71, p = 0.074, \text{partial } \eta^2 = 0.07$). There was a significant effect of time on the ratio measured ($F(2,68) = 11.22, p < 0.001, \text{partial } \eta^2 = 0.25$), demonstrating a change in the ratio over the treatment course. Examination of the estimated marginal means showed levels to increase during treatment, with lower levels at baseline (Mean \pm SEM ng/ml; 2.18 ± 0.10), increasing by TW8 (2.58 ± 0.17) and then being highest at TW24 (2.69 ± 0.15). There was a significant increase from baseline to TW8 ($p = 0.002$) and TW24 ($p < 0.001$). There was no main effect of group on kynurenine/tryptophan ratio levels calculated ($F(1,34) = 0.48, p = 0.49, \text{partial } \eta^2 = 0.01$). See Figure 3.42. Log-transformed data was all normally distributed. The assumption of the homogeneity of covariances was met. The assumption of the homogeneity of variances was met at all time points. The assumption of sphericity was met ($X^2(2) = 1.05, p = 0.59$). As with the raw data, analysis of the transformed data showed that there was a trend towards an interaction between time and group on levels of the Kynurenine/Tryptophan ratio ($F(2, 68) = 2.82, p = 0.067, \text{partial } \eta^2 = 0.08$). Likewise, there was a significant effect of time ($F(2, 68) = 12.51, p < 0.001, \text{partial } \eta^2 = 0.27$). Analysis of the transformed data confirmed that there was no main effect of group ($F(1,34) = 0.30, p = 0.59, \text{partial } \eta^2 = 0.009$).



Kyn/Trp ANOVA: Group*Time: $p = 0.074$; **Time:** $p < 0.001$; Group: $p = 0.49$

Figure 3.42 Baseline levels, and changes in the Kyn/Trp ratio during treatment in HCV PF vs. RF groups

Kynurenic acid

Data was not normally distributed at TW0 or TW8, but was normally distributed at TW24. There was one outlier, in the RF group. With this individual excluded, there was homogeneity of covariances. The assumption of homogeneity of variances was also met at all time points. The assumption of sphericity was met ($X^2(2) = 3.10, p = 0.21$).

There was no significant interaction between time and group on kynurenic acid levels ($F(2,66) = 0.74, p = 0.48$). There was a main effect of time ($F(2,66) = 4.57, p = 0.01$), demonstrating a change in kynurenic acid levels across the treatment duration. Examination of the estimated marginal means showed a decrease throughout treatment, with levels highest at baseline (7.90 ± 0.59), and lower at TW8 (7.56 ± 0.54) and TW24 (6.72 ± 0.53). Pairwise comparisons showed the decrease from TW0 to TW24 to be significant ($p = 0.043$). There was no main effect of group ($F(1,33) = 0.80, p = 0.38$). Log-transformed data was all normally distributed. There were no outliers. The assumption of the homogeneity of covariances was met, as was the assumption of the homogeneity of variances. On the transformed data, there was no significant interaction between time and group on levels of kynurenic acid ($F(2, 68) = 0.75, p = 0.48, \text{partial } \eta^2 = 0.02$). There was a main effect of time ($F(2, 68) = 4.27, p = 0.02, \text{partial } \eta^2 = 0.11$). Analysis of the transformed data also confirmed that there was no main effect of group ($F(1, 34) = 0.72, p = 0.40, \text{partial } \eta^2 = 0.02$).

Quinaldic Acid

Data was not normally distributed at TW0 and TW24, but was normally distributed at TW8. Visual examination of the boxplots showed no outliers outside of +/- three times the interquartile range. The assumption of the homogeneity of covariance was met. The assumption of homogeneity of variance was met at all time points. The assumption of sphericity was also met ($X^2(2) = 3.11, p = 0.21$).

There was no significant interaction between the group and time on levels of quinaldic acid ($F(2,68) = 0.37, p = 0.69, \text{partial } \eta^2 = 0.01$). There was a main effect of time on quinaldic acid levels ($F(2,68) = 6.62, p = 0.002, \text{partial } \eta^2 = 0.16$), indicating that levels of quinaldic acid changed over the course of treatment. Examination of the estimated marginal means showed levels to be highest at baseline (Mean \pm SEM ng/ml; 2.16 ± 0.23), decreasing by TW8 (1.81 ± 0.45) and again by TW24 (1.48 ± 0.15). Pairwise comparisons showed the decrease from baseline to TW24 to be statistically significant ($p = 0.005$). There was no main effect of group ($F(1,34) = 0.05, p = 0.82, \text{partial } \eta^2 = 0.002$). Log-transformed data was normally distributed at TW0 and 8, but not at TW24. Other attempts to transform the data (square root; inverse) had not resulted in a normal distribution, and so the analysis was still repeated with the log-transformed data. The assumption of the homogeneity of covariances was met. The assumption of the homogeneity of variances was met at all time points. The assumption of sphericity was met ($X^2(2) = 2.333, p = 0.31$). Analysis of the transformed data confirmed that there was no significant interaction between time and group on levels of ($F(2, 68) = 0.58, p = 0.56, \text{partial } \eta^2 = 0.02$). There was a main effect of time ($F(2, 68) = 9.46, p < 0.001, \text{partial } \eta^2 = 0.22$).

Post-hoc pairwise comparisons of the transformed data also showed significant a decrease from TW0 to TW24 ($p = 0.001$). In addition, unlike the raw data, the decrease from TW8 to 24 was also significant ($p = 0.029$). There was no main effect of group ($F(1, 34) = 0.000$, $p = 0.999$, partial $\eta^2 = 0.000$).

3-hydroxykynurenine (3-HK)/Kynurenine ratio

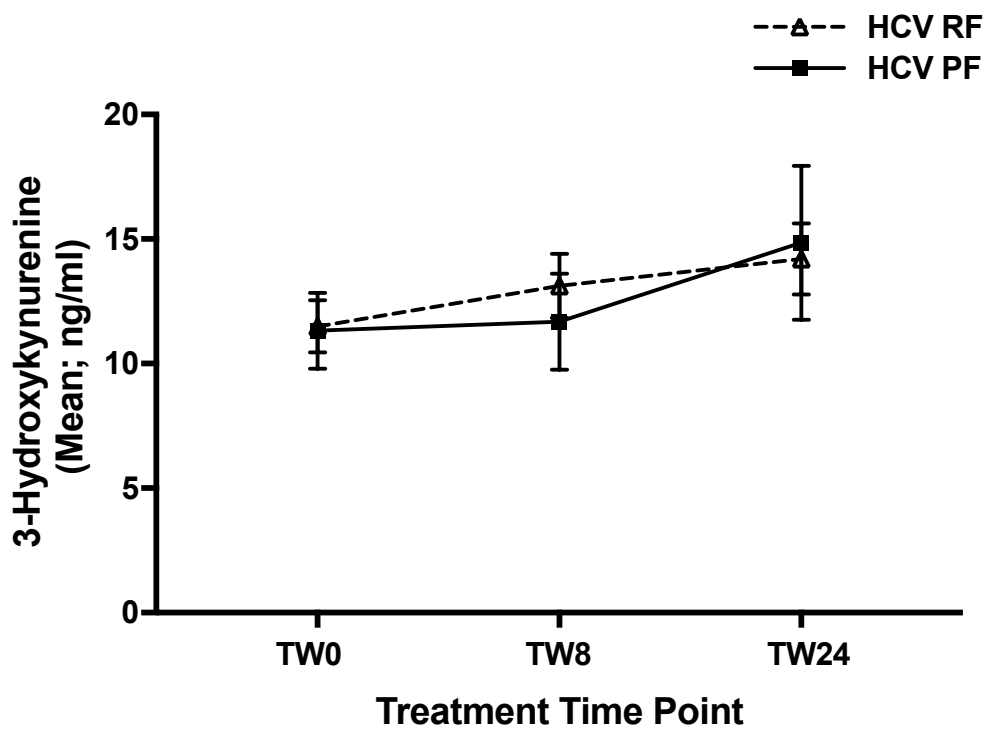
The ratio was calculated by dividing the level of 3-hydroxykynurenine (3-HK) by the level of kynurenine, and multiplying the number by 100. Data was normally distributed at baseline, but not at TW8 or 24. Visual examination of the boxplots showed that there were no outliers. The assumption of homogeneity of covariances was met. The assumption of homogeneity of variances met at all time points. The assumption of sphericity was met ($X^2(2) = 2.45$, $p = 0.29$).

There was no significant interaction between time and group on levels of the 3-HK/Kyn ratio ($F(2,68) = 0.34$, $p = 0.71$, partial $\eta^2 = 0.01$). Nor was there a significant effect of time ($F(2,68) = 1.50$, $p = 0.23$, partial $\eta^2 = 0.04$). Lastly, there was no main effect of group on the ratio calculated for 3-HK/Kynurenine ($F(1,34) = 0.23$, $p = 0.64$, partial $\eta^2 = 0.007$). Log-transformed data was normally distributed at all treatment time points. The assumption of the homogeneity of covariances was met. The assumption of the homogeneity of variances was met at all time points. The assumption of sphericity was met ($X^2(2) = 3.30$, $p = 0.19$). Analysis of the transformed data confirmed that there was no significant interaction between time and group on levels of ($F(2, 68) = 0.26$, $p = 0.77$, partial $\eta^2 = 0.008$). Nor was there a main effect of time ($F(2, 68) = 1.79$, $p = 0.17$, partial $\eta^2 = 0.05$). Lastly, there was no main effect of group ($F(1, 34) = 0.10$, $p = 0.75$, partial $\eta^2 = 0.003$).

3-hydroxykynurenine (3-HK)

Data was normally distributed at baseline ($p > 0.05$), but not at treatment weeks 8 or 24. Visual examination of the boxplots showed that there were no outliers. The assumption of the homogeneity of covariance was met. The assumption of homogeneity of variance was met at all time points. The assumption of sphericity was met ($X^2 (2) = 3.10, p = 0.21$).

There was no significant interaction between the group and time on levels of 3-HK ($F (2,68) = 0.41, p = 0.66, \text{partial } \eta^2 = 0.01$). There was a main effect of time ($F (2,68) = 3.86, p = 0.03, \text{partial } \eta^2 = 0.10$), indicating that levels of 3-HK changed over the course of treatment. Examination of the estimated marginal means showed levels to be lowest at baseline (Mean \pm SEM ng/ml; 11.41 \pm 0.93), with increases at TW8 (12.41 \pm 1.16) and TW24 (14.52 \pm 1.48). Pairwise comparisons showed the increase from baseline to TW24 to be significant ($p = 0.04$). There was no main effect of group ($F (1,34) = 0.03, p = 0.88, \text{partial } \eta^2 = 0.001$). See Figure 3.43. Log-transformed data was normally distributed at all three time points. There were no outliers \pm three times the interquartile range. Homogeneity of covariances was met. Analysis of the transformed data confirmed that there was no significant interaction between time and group on levels of 3-HK ($F (2, 68) = 0.26, p = 0.77, \text{partial } \eta^2 = 0.008$). In contrast to the analysis of the raw data, there was no effect of time ($F (2, 68) = 1.79, p = 0.17, \text{partial } \eta^2 = 0.05$). Like the raw data, there was no effect of group ($F (1, 34) = 0.10, p = 0.75, \text{partial } \eta^2 = 0.003$).



3-HK ANOVA: Group*Time: $p = 0.66$; Time: $p = 0.026$; Group: $p = 0.88$

Figure 3.43 Baseline levels, and changes in levels of 3-HK during treatment in HCV PF vs. RF groups

Xanthurenic acid

Data was not normally distributed at any of the three time points. Visual examination of the boxplots showed that there was one outlier at TW24 \pm three times the interquartile range, in the HCV RF group. With this individual excluded, a repeated measures ANOVA was performed. The assumption of homogeneity of covariances was violated. The assumption of homogeneity of variance was met at all time points. The assumption of sphericity was also met ($X^2 (2) = 4.30, p = 0.12$).

There was no significant interaction between time and group on xanthurenic acid levels ($F (2, 66) = 1.29, p = 0.28, \text{partial } \eta^2 = 0.04$). Nor was there a main effect of time ($F (2, 66) = 1.10, p = 0.34, \text{partial } \eta^2 = 0.03$). Lastly, there was no main effect of group ($F (1, 33) = 1.32, p = 0.26, \text{partial } \eta^2 = 0.04$). Due to the distribution of data for levels of xanthurenic acid, data was transformed using the logarithmic method. Transformed data was normally distributed at TW0 and TW8, but not at TW24. Other attempts to transform the data had not resulted in a normal distribution, and so the analysis was still repeated with the log-transformed data. There were no outliers. The assumption of the homogeneity of covariances was still violated. The assumption of the homogeneity of variances was met at all time points. So too was the assumption of sphericity ($X^2 (2) = 0.98, p = 0.71$). Analysis of the transformed data confirmed that there was there was no significant interaction between time and group on levels of xanthurenic acid ($F (2, 68) = 1.74, p = 0.18, \text{partial } \eta^2 = 0.05$). Nor was there a main effect of time ($F (2, 68) = 2.29, p = 0.11, \text{partial } \eta^2 = 0.06$). Finally, there was no main effect of group ($F (1, 34) = 2.46, p = 0.13, \text{partial } \eta^2 = 0.07$).

Picolinic Acid

Data was normally distributed at TW8 and TW24, but not at baseline. Visual examination of the boxplots showed that there were no outliers. The homogeneity of covariances was met. There was homogeneity of variances at all time points. The assumption of sphericity was met ($X^2(2) = 2.61, p = 0.27$).

There was no significant interaction between time and group on picolinic acid levels ($F(2, 68) = 0.09, p = 0.91, \text{partial } \eta^2 = 0.003$). There was a trend towards a main effect of time ($F(2, 68) = 2.49, p = 0.091, \text{partial } \eta^2 = 0.068$). There was no main effect of group ($F(1, 34) = 0.77, p = 0.39, \text{partial } \eta^2 = 0.02$). Log-transformed data was normally distributed at all three treatment time points. The assumption of the homogeneity of covariances was met. The assumption of the homogeneity of variances was met at all time points. Analyses of the transformed data confirmed that there was no significant interaction between time and group on levels of picolinic acid ($F(2, 68) = 0.526, p = 0.593, \text{partial } \eta^2 = 0.015$). The trend towards a main effect of time was also replicated ($F(2, 68) = 2.398, p = 0.099, \text{partial } \eta^2 = 0.066$). Finally, as before, there was no main effect of group ($F(1, 34) = 1.265, p = 0.269, \text{partial } \eta^2 = 0.036$).

Quinolinic Acid

Data was not normally distributed at any time point. Visual examination of the boxplots showed that there were no outliers. The assumption of homogeneity of covariances was met. So too was the assumption of the homogeneity of variances at all time points. The assumption of sphericity was met ($X^2(2) = 5.06, p = 0.08$).

There was no significant interaction between time and group on quinolinic acid levels ($F(2, 68) = 2.31, p = 0.11, \text{partial } \eta^2 = 0.06$). Nor was there a main effect of time ($F(2, 68) = 1.37, p = 0.26, \text{partial } \eta^2 = 0.07$). Lastly, there was no main effect of group ($F(1, 34) = 0.28, p = 0.60, \text{partial } \eta^2 = 0.008$). Log-transformed data was normally distributed at all three treatment time points. The assumption of the homogeneity of covariances was met. The assumption of the homogeneity of variances was met at all time points. The assumption of sphericity was met ($X^2(2) = 5.19, p = 0.08$). There was no significant interaction between time and group on levels of quinolinic acid, though there was a trend ($F(2, 68) = 3.04, p = 0.054, \text{partial } \eta^2 = 0.08$). Nor was there a significant effect of time ($F(2, 68) = 1.55, p = 0.22, \text{partial } \eta^2 = 0.08$). Finally there was no main effect of group ($F(1, 34) = 0.12, p = 0.73, \text{partial } \eta^2 = 0.004$).

Conclusion

See Table 3.50 overleaf for a summary of these results. To summarise the results of the longitudinal analyses, some kynurenine pathway metabolites did change in response to IFN- α treatment, though there was no difference in how they did so between those in whom fatigue persisted, versus those in whom fatigue resolved. Specifically, the markers that did change were quinaldic acid; kynurenic acid; 3-HK, and the ratio of kynurenine to tryptophan. Others did not alter in response to treatment. Results were the same when performed on raw or transformed data, with one exception; for 3-HK, analysis of the raw data found an effect of IFN- α treatment, with levels changing over the treatment course, but this was not replicated on the transformed data.

Table 3.50 Summary table: kynurenine pathway repeated measures ANOVA

Metabolite	Group*Time	Time		Group
<i>Tryptophan</i>	-	-	-	-
<i>KYN/TRP</i>	-	$p < 0.001$	TW0<4; TW0<24	-
<i>Kynurenic acid</i>	-	$p = 0.014$	TW0>24	-
<i>Quinaldic acid</i>	-	$p = 0.002$	TW0>24	-
<i>3-HK/KYN</i>	-	-	-	-
<i>3-HK</i>	-	$p = 0.026^*$	TW0<24	-
<i>Xanthurenic acid</i>	-	-	-	-
<i>Picolinic acid</i>	-	-	-	-
<i>Quinolinic acid</i>	-	-	-	-

* Result not replicated in log-transformed data

Kynurenine pathway: cross-sectional and delta analyses in persistent versus resolved fatigue

To further explore any difference in the metabolites in Persistent versus Resolved Fatigue patients in a larger sample, independent samples t-tests were conducted on all available data. In addition, independent samples t-tests were conducted on delta scores to explore differences in how levels changed, relative to baseline, in HCV RF and PF patients. Because of how this data was analysed in the laboratory (see Methods), at Time 1 and Time 2, for the delta score analyses only time points that had been measured at the same time within individuals were compared. Data from the follow-up visit six-months post-treatment is presented as part of the cross-sectional analyses including CFS patients and healthy controls. Summaries of results (direction of relationship only) are presented in Table 3.51 and Table 3.52.

At TW8, data was available in 46 patients: 32 RF and 14 PF. There were no significant differences between the two groups in metabolites measured at TW8. However, interestingly, there was a trend towards lower levels of quinolinic acid in Persistent Fatigue patients (44.52 ± 4.81 vs. 57.76 ± 4.41 , $t(44) = 1.79$, $p = 0.081$), and a lower KYN/TRP ratio in the same patients (2.26 ± 0.15 vs. 2.76 ± 0.16 , $t(44) = 1.91$, $p = 0.063$). At TW24, data was available in 43 patients: 28 RF and 15 PF. There were no significant differences in any of the metabolites measured at this treatment time point. For 'end of treatment' (TW24, 36 or 48), data was available in 40 patients: 25 RF and 15 PF. Levels of quinolinic acid were now significantly higher in PF patients (75.84 ± 8.00 vs. 56.78 ± 4.98 , $t(38) = -2.14$, $p = 0.039$). However, there were no differences in any other metabolites.

Looking at delta values, somewhat surprisingly there was a significantly higher increase in the KYN/TRP ratio in the Resolved Fatigue patients than the Persistent Fatigue patients, from baseline to TW8 (0.58 ± 0.10 vs. 0.17 ± 0.14 , $t(42) = 2.21$, $p = 0.033$). Otherwise there were no other group differences in changes in the first eight weeks of treatment. Nor were there any group differences in changes from baseline to TW24. Lastly, patterns of change from the end of treatment to six-months post treatment were examined. There was a trend towards a difference in the change in picolinic acid between the two groups; patients in the RF group experienced a decrease, while patients in the PF group had an increase (-10.93 ± 7.44 vs. 16.83 ± 13.51 , $t(31) = -1.96$, $p = 0.059$).

For a summary of results, see Table 3.51 and Table 3.52 overleaf. In conclusion, in keeping with the results from the longitudinal analyses, levels of kynurenine pathway metabolites were largely the same across HCV patients. However, there were trends towards lower levels of quinolinic acid, and the KYN/TRP ratio after eight weeks of treatment. The only significant difference occurred at the end of treatment, when levels of quinolinic acid were now significantly higher in those in whom fatigue subsequently persisted. For changes in the metabolites, there was a greater increase in the KYN/TRP ratio from TW0 to 8 in those in the Resolved Fatigue group, in keeping with the trend towards higher levels at TW8 described above. Finally, there was a trend towards a difference during the six-months post-treatment, with an increase in the PF patients, and a decrease in the RF group.

Table 3.51 Summary table: RF/PF group differences, analysis per time point

	TW0	TW8	TW24	END
Tryptophan	=	=	=	=
Kyn/Trp	=	↘	=	=
Kynurenic acid	=	=	=	=
Quinaldic acid	=	=	=	=
3-HK/Kyn	=	=	=	=
3-HK	=	=	=	=
Xanthurenic acid	=	=	=	=
Picolinic acid	=	=	=	=
Quinolinic acid	=	↘	=	↑

Table 3.52 Summary table: RF/PF group differences, delta analysis

	Δ 0.8	Δ 0.24	Δ END.FU
Tryptophan	=	=	=
Kyn/Trp	↓	=	=
Kynurenic acid	=	=	=
Quinaldic acid	=	=	=
3-HK/Kyn	=	=	=
3-HK	=	=	=
Xanthurenic acid	=	=	=
Picolinic acid	=	=	↖
Quinolinic acid	=	=	=

Key: ↘trend towards PF<RF; ↓PF<RF; ↖trend towards PF>RF; ↑PF>RF

3.1.3.5 IFN- α induced persistent fatigue: a summary

For the first part of aim three of this thesis, I explored possible risk factors of IFN- α induced persistent fatigue. A summary of these results can be found overleaf, in Figure 3.44. As reported, there were few baseline characteristics that were associated with the subsequent experience of persistent fatigue post-IFN- α treatment. For clarity, apart from noting the trend towards an effect of the duration of treatment, only the significant findings are shown.

For the second part of the third aim, baseline levels, as well as changes in clinical and biological measures, as well as health status, were examined to investigate whether patients in each group had a different response to IFN- α treatment, which contributed to the persistence of fatigue. A summary of these results can be found on page 260, in Figure 3.45. Notably, again there were no differences between groups in baseline measures. However, during treatment patients in the Persistent Fatigue group experienced higher fatigue, depressive symptoms and perceived stress, and a greater decline in health status. In addition to an increased response early on, these patients tended to be on an upward trajectory of worsening symptoms and disability. Unexpectedly, the change in these symptoms and health status scores over the six-month follow-up period was similar in both groups; however, the PF group were at a significant disadvantage from this higher symptomatology during treatment. Not reported in the figure, there were also some interesting differences in patterns of change in cytokine levels. There were differences in how IL-7 changed in response to IFN- α , though no differences between the two groups in levels at any time point. Levels of IL-10 and IL-6 were significantly higher in the PF group, while there was a trend towards higher levels of IL-17A.

Prospective HCV (IFN-α) Cohort Study	
Aim 3	Persistent Fatigue (PF) vs. Resolved Fatigue (RF) (CFQ FU>TW0) (CFQ FU ≤ TW0)
	Risk factors for Persistent Fatigue:
	There were few baseline characteristics associated with PF: Significantly lower current use of opioid drug replacement therapy Trends: longer exposure to IFN-α (more 'RF' treated for ≤ 24 weeks)
	X Past experience of psychosocial stress or trauma
	X Baseline illness perceptions
	XXX Baseline Inflammation; kynurenine pathway; cortisol

Figure 3.44 Summary of results for Aim 3 (i): IFN-α induced persistent fatigue

Prospective HCV (IFN-α) Cohort Study				
Aim 3	Persistent Fatigue (PF) vs. Resolved Fatigue (RF) (CFQ FU>TW0) (CFQ FU ≤ TW0)			
	HCV PF vs. RF: Baseline levels & Changes:			
		Clinical	Health Status	Biological
	TW0	=	=	(see predictors)
	4	↑Δ Fatigue	↓Δ Phys Function ↓Δ Vitality ↓Δ Social Function	↑ IL-10; IL-6 (Kyn not measured)
	8	↑Depression/Δ ↑Stress/Δ	↓ΔVitality ↓Social Function/Δ ↓Δ Mental Health	↓Δ Kyn/Trp (CytK not measured)
	12	↑ Δ Depression	↓Δ Phys Function ↓ΔVitality	(no measurement)
	24	↑Fatigue/Δ ↑Depression /Δ ↑Stress/Δ	↓Δ Phys Function ↓Vitality/Δ ↓Social Function/Δ ↓ Limitations to role	=
	6mFU	↑Fatigue/Δ ↑Stress/Δ ↑Δ Depression ↓ Sleep	↓Δ Phys Function ↓ Vitality/Δ ↓Δ Soc. Function ↑ Pain	=

Key: = no difference; ↑increase; ↓decrease; Δ change relative to baseline (TW0);
/Δ significant difference in level at time point and significant difference in change

Figure 3.45 Summary of results for Aim 3 (ii): IFN-α induced persistent fatigue

3.2 Cross-sectional study

To address the final aim of my thesis, I compared each of the clinical and biological measures in turn, in healthy controls, HCV Resolved Fatigue (RF) and Persistent Fatigue (PF) groups, and CFS patients. For HCV, data analysed was that obtained at the follow-up visit six-months post-treatment. Chi-squared tests were used for categorical variables. For continuous variables, a series of one-way ANOVAs were performed, with additional post-hoc analyses where indicated.

3.2.1 Socio-demographic characteristics

The four groups studied were different across most of the socio-demographic characteristics measured. Age was normally distributed in all four groups, with no outliers. All assumptions required were met. There was a significant difference between the four groups (see Table 3.53, page 263); post-hoc Tukey's HSD tests showed that CFS patients were younger than patients in the HCV RF and PF groups. Healthy controls fell in between the HCV and CFS groups. There were also significant differences in gender between groups. Additional post-hoc chi-squared tests showed that there were a greater proportion of males in the HCV groups than the CFS and Control groups. There was a trend towards a higher proportion of males in the Control group versus the CFS group, which was predominantly female. There were also differences in ethnicity between groups. Because of the complexities of these differences across categories (White; Asian; Black; Other), it was not possible to perform post-hoc tests, but the descriptives presented in the summary table provide some information on the breakdown of ethnicities within each group. With regards to education, CFS patients and healthy controls were more highly educated than the HCV groups. There were lower rates of unemployment in the

healthy control group, though rates were similar across the CFS and the two HCV groups. Lastly, there was no difference in the proportion of participants in each group who were either married or living with their partners.

For characteristics relating to the clinical presentation, as expected, there were a greater number of fatigue cases in the CFS group compared with the other three groups, with 89% of patients having fulfilled the Chalder Fatigue Scale criteria for fatigue 'caseness' (a score of >18) based on problems with fatigue in the last month. There were also higher rates in the HCV PF group (33%) than the Control or HCV RF groups. There were significant differences in the personal history of depression among groups, with the highest rates in the CFS sample. They also had the highest rates of family history of mental illness (71%), closely followed by the healthy controls (55%), with lower rates in the two HCV groups. The CFS patient group also had a greater proportion of individuals who were currently depressed. The HCV patient groups were much more likely to have past opioid use, and be current smokers, particularly the HCV PF group. Current alcohol use was highest in the CFS and healthy control groups, then lower in the HCV groups, with slightly higher rates in the PF versus RF group. Regarding the AUDIT scores, tests showed that they were not normally distributed in any of the four groups. Visual examination of the histograms showed a positive skew towards lower scores in all of the groups. There were no outliers \pm three times the interquartile range in any of the groups, and the assumption of homogeneity of variances was met. The ANOVA showed that there were no differences between groups (see Table 3.53, second page), with low scores across the four groups, indicating no problem with alcohol use.

Table 3.53 Socio-demographic characteristics of healthy control (CTRL), HCV Resolved (RF) and Persistent Fatigue (PF), and CFS groups

	CTRL (n = 57)	HCV RF (n = 37)	HCV PF (n = 18)	CFS (n = 54)	Test and statistic
Age (years)					
Range	18-67	23-68	18-63	18-60	
Mean±SEM	40.8±1.6	43.7±2.0	46.4±2.8	37.2±1.5	F(3,162)=3.88, p=0.01 CFS < PF, p = 0.022 CFS < RF, p = 0.047 CFS = CTRL, p = 0.37 PF = CTRL, p = 0.29 RF = CTRL, p = 0.63
Gender					
Male	28 (49.1)	28 (75.7)	16 (88.9)	17 (31.5)	X² (3)= 27.35, p<0.001 CFS vs PF, p<0.001 CFS vs RF, p<0.001 CFS = CTRL, p=0.06 PF vs CTRL, p=0.003 RF vs CTRL, p=0.010
Ethnicity					X² (9)= 25.20, p=0.003
White	45 (78.9)	26 (70.3)	16 (88.9)	43 (79.6)	
Asian	3 (5.3)	7 (18.9)	0	0	
Black	7 (12.3)	1 (2.7)	1 (5.6)	2 (3.7)	
Other	2 (3.5)	3 (8.1)	1 (5.6)	9 (16.7)	
Education					
Degree	41 (71.9)	14 (37.8.)	4 (22.2)	35 (66.0)	X² (3)= 21.37, p<0.001
Employment					
Unemployed	9 (15.8)	12 (32.4)	7 (38.9)	20 (37.7)	X² (3)= 7.85, p=0.049
Relationship					
Married/living	22 (38.6)	15 (40.5)	7 (38.9)	18 (34.0)	χ² (3)= 0.47, p = 0.93

Cont. overleaf.

	CTRL (n = 57)	HCV RF (n = 37)	HCV PF (n = 18)	CFS (n = 54)	Test and statistic
	Count (%)				
Fatigue Case	0	1 (2.7)	6 (33.3)	48 (88.9)	X² (3)=117.76, p<0.001
History of Depression	24 (42.1)	12 (32.4)	8 (44.4)	33 (62.3)	X² (3)=8.71, p=0.033
Family History	31 (54.4)	11 (33.3)	6 (35.3)	36 (70.6)	X² (3)=12.97, p=0.005
Depression Current	0	2 (5.7)	2 (12.5)	17 (32.1)	X² (3)= 27.14, p<0.001
Drug use					
Past opioid	1 (1.8)	15 (41.7)	9 (56.3)	1 (1.9)	X² (3)= 53.26, p<0.001
Smoking	10 (17.5)	13 (36.1)	9 (52.9)	10 (18.9)	<u>X² (6)= 12.26, p=0.056</u>
Alcohol					
Current use, Yes	47 (82.5)	15 (41.7)	11 (64.7)	44 (83.0)	X² (6)= 29.67, p<0.001
	Mean±SEM				
AUDIT score	3.1±0.4	3.0±0.7	4.1±0.9	3.6±0.4	F(3,161)=1.31, p =0.27

3.2.2 Experience of psychosocial stress

As before, measures of psychosocial stress examined were: experience of one or more stressful life events in the last six-months; experience of one or more intrusive life event in their lifetime; and experience of any childhood trauma, as measured by the CECA questionnaire. There was a significant difference between groups in the proportion of individuals who had experienced a stressful life event in the six months before the assessment. Surprisingly, CFS patients were least likely to have experienced such an event recently, followed by identical proportions of each of the HCV groups. Unexpectedly, levels were highest in the healthy controls. There were no differences between groups in the lifetime experience of intrusive life events. Nor was there a difference in reports of childhood trauma between groups. Data is presented in Table 3.54.

Table 3.54 Experience of psychosocial stress in the healthy control (CTRL), HCV Resolved (RF) and Persistent Fatigue (PF), and CFS groups

	CTRL (<i>n</i> = 57)	HCV RF (<i>n</i> = 37)	HCV PF (<i>n</i> = 18)	CFS (<i>n</i> = 54)	Test and statistic
	count (%)				
Recent Stressful life events					
Any, six-months	45 (78.9)	21 (60.0)	9 (60.0)	21 (39.6)	X²(3)=17.7, <i>p</i><.001
Intrusive life events					
Any, lifetime	41 (74.5)	23 (63.9)	14 (87.5)	41 (77.4)	X ² (3)=3.8, <i>p</i> =0.29
<i>Injury/assault</i>	23 (41.8)	16 (44.4)	10 (62.5)	22 (41.5)	
<i>Bullying</i>	21 (38.2)	9 (25.0)	7 (43.8)	32 (60.4)	
<i>Homeless</i>	8 (14.5)	8 (22.2)	6 (37.5)	3 (5.7)	
<i>Violence (home)</i>	9 (16.4)	8 (22.2)	5 (31.3)	14 (26.4)	
<i>Sexual abuse</i>	7 (12.7)	4 (11.1)	3 (18.8)	5 (9.4)	
<i>Running away</i>	7 (12.7)	7 (19.4)	4 (25.0)	8 (15.1)	
<i>Expelled</i>	6 (10.9)	4 (11.1)	4 (25.0)	2 (3.8)	
<i>Children's Institution</i>	2 (3.6)	4 (11.1)	2 (12.5)	1 (1.9)	
<i>Violence (work)</i>	6 (10.9)	3 (8.3)	2 (12.5)	8 (15.1)	
<i>Taken into care</i>	3 (5.5)	4 (11.1)	1 (6.3)	3 (5.7)	
Childhood trauma					
Any, yes	25 (43.9)	18 (48.6)	9 (50.0)	22 (41.5)	X ² (3)=0.7, <i>p</i> =0.89
Forms of trauma					
<i>Separation</i>	15 (26.3)	10 (27.0)	6 (33.3)	15 (28.3)	
<i>Loss of parent</i>	1 (1.8)	7 (18.9)	2 (11.1)	2 (3.8)	
<i>Physical abuse</i>	9 (15.8)	7 (18.9)	2 (11.1)	7 (13.2)	
<i>Sexual abuse</i>	7 (12.3)	2 (5.4)	4 (22.2)	3 (5.7)	

3.2.3 Clinical symptoms

Next, current clinical symptoms were examined for any differences between groups. For each measure, a one-way ANOVA was performed. Where the Levene's test result indicated that the assumption of the homogeneity of variances could be assumed ($p>0.05$), an ANOVA and Tukey's HSD post-hoc tests were performed. Where it was violated ($p<0.05$), results for the Welch's ANOVA are reported, and Games-Howell post-hoc comparisons. Results are presented in Table 3.55 (see page 270).

Fatigue data is presented in Figure 3.46 (see page 271). Scores were normally distributed in HCV PF patients, but not in the HCV RF or healthy control groups, where most individuals reported that they had experienced fatigue 'no more than usual' in the last month, resulting in a score of '11', or in the CFS group, where data was negatively skewed, with more patients reporting higher scores. There were no outliers \pm times the interquartile range in the HCV PF, CFS or healthy control groups. However, in the HCV RF group, such was the tendency for patients to report scores of '11', visual examination of the boxplot revealed seven individuals to be outliers: three with higher scores, and four with lower scores. The assumption of the homogeneity of variances was violated and so the Welch's ANOVA was used, which showed a significant group difference. Games-Howell post-hoc tests showed that the CFS patients reported higher fatigue than all other groups, and that HCV PF patients had higher fatigue than the HCV RF and healthy control groups. The HCV RF and healthy controls reported similar levels of fatigue.

Depression data is presented in Figure 3.47 (see page 271) Scores were normally distributed in the CFS patients, but not in the HCV or healthy control groups. Histograms of scores showed scores in the healthy control and HCV RF groups to be positively skewed. So too were scores in the HCV PF group, though with a higher majority of patients reporting scores around '10', indicating some degree of low mood or associated problems. There were no outliers +/- three times the interquartile range. The assumption of homogeneity of variances was violated and so the Welch's ANOVA was used, which showed a significant difference between groups in depressive symptoms. Games-Howell post-hoc tests showed a trend towards a difference between CFS and HCV PF patients. CFS patients reported higher depressive symptoms than HCV RF and healthy controls, as did the HCV PF patients. There was no difference between HCV RF and healthy controls.

Anxiety data is presented in Figure 3.48 (see page 272). Scores were normally distributed in HCV PF patients, but not in the other three groups. Histograms per group showed that scores were negatively skewed. There was one individual, in the HCV RF group, whose higher score was more than three times more than the interquartile range. The assumption of the homogeneity of variances was not met. Welch's ANOVA showed that there was a significant difference between groups, with Games-Howell post-hoc tests showing that this was due to higher levels of anxiety in the CFS patients versus all three other groups. There was also a trend towards higher levels in HCV PF patients versus healthy controls.

Perceived Stress data is presented in Figure 3.49 (see page 272). Scores were normally distributed in the CFS and healthy control groups, but not in the HCV groups. Patients in the RF group more often reported lower levels of perceived stress, resulting in positively skewed data, while the PF patients reported higher levels, which were therefore negatively skewed. There were no outliers. The assumption of homogeneity of variances was violated and so the Welch's ANOVA was used, which showed a significant difference in levels of perceived stress between the four groups.

Sleep quality was measured in a smaller group of HCV patients. Group sizes are noted in Table 3.55 overleaf. Data is presented in Figure 3.50 (see page 273). Scores were normally distributed in CFS patients, but only just ($p = 0.051$). They were not normally distributed in any of the other three groups. In all samples, there was some degree of positive skew, with the majority of participants reporting lower scores indicating fewer problems with sleep quality. There were only two outliers, in the healthy control group, who reported higher scores than others in their group. The assumption of homogeneity of variance was met. The ANOVA showed that there was a significant difference between the four groups. Tukey's HSD post-hoc tests were performed, which showed that patients with CFS reported more problems with sleep quality than any of the other three groups. HCV PF patients reported higher scores, and more problems, than HCV RF and healthy control participants. There was no difference between HCV RF patients and healthy controls.

Table 3.55 Clinical symptoms in in the healthy control (CTRL), HCV Resolved (RF) and Persistent Fatigue (PF), and CFS groups

	CTRL (n = 57)	HCV RF (n = 37)	HCV PF (n = 18)	CFS (n = 54)	Welch's ANOVA / Games-Howell post-hoc statistic
Fatigue	11.6±0.3	10.6±0.5	17.0±0.8	26.0±0.6	<i>F(3,58.4)=154.4, p<.001</i> <i>CFS > PF, p<0.001</i> <i>CFS > RF, p<0.001</i> <i>CFS > CTRL, p<0.001</i> <i>PF > CTRL, p<0.001</i> <i>PF > RF, p<0.001</i> <i>RF = CTRL, p = 0.30</i>
Depression	7.5±0.7	8.9±1.4	19.9±2.9	28.3±1.7	<i>F(3,55.9)=42.7, p<.001</i> <i>CFS > PF, p=0.064</i> <i>CFS > RF, p<0.001</i> <i>CFS > CTRL, p<0.001</i> <i>PF > CTRL, p=0.004</i> <i>PF > RF, p=0.017</i> <i>RF = CTRL, p = 0.83</i>
Anxiety	2.1±0.3	3.7±0.6	4.1±0.7	8.2±0.8	<i>F(3,60.0)=18.6, p<.001</i> <i>CFS > PF, p= 0.002</i> <i>CFS > RF, p<0.001</i> <i>CFS > CTRL, p<0.001</i> <i>PF = CTRL, p = 0.085</i> <i>PF = RF, p = 0.98</i> <i>RF = CTRL, p = 0.13</i>
Stress	10.2±0.7	8.8±1.2	13.7±1.8	20.9±1.1	<i>F(3,58.9)=26.9, p<.001</i> <i>CFS > PF, p = 0.009</i> <i>CFS > RF, p<0.001</i> <i>CFS > CTRL, p<0.001</i> <i>PF = CTRL, p = 0.29</i> <i>PF = RF, p = 0.12</i> <i>RF = CTRL, p = 0.72</i>
	(n = 57)	(n = 27)	(n = 13)	(n = 52)	ANOVA / Tukeys HSD post-hoc statistic
Sleep	4.14±0.5	3.3±0.8	8.0±1.5	13.3±0.7	<i>F(3,145)=51.1, p<.001</i> <i>CFS > PF, p = 0.001</i> <i>CFS > RF, p<0.001</i> <i>CFS > CTRL, p<0.001</i> <i>PF > CTRL, p = 0.022</i> <i>PF > RF, p = 0.008</i> <i>RF = CTRL, p = 0.82</i>

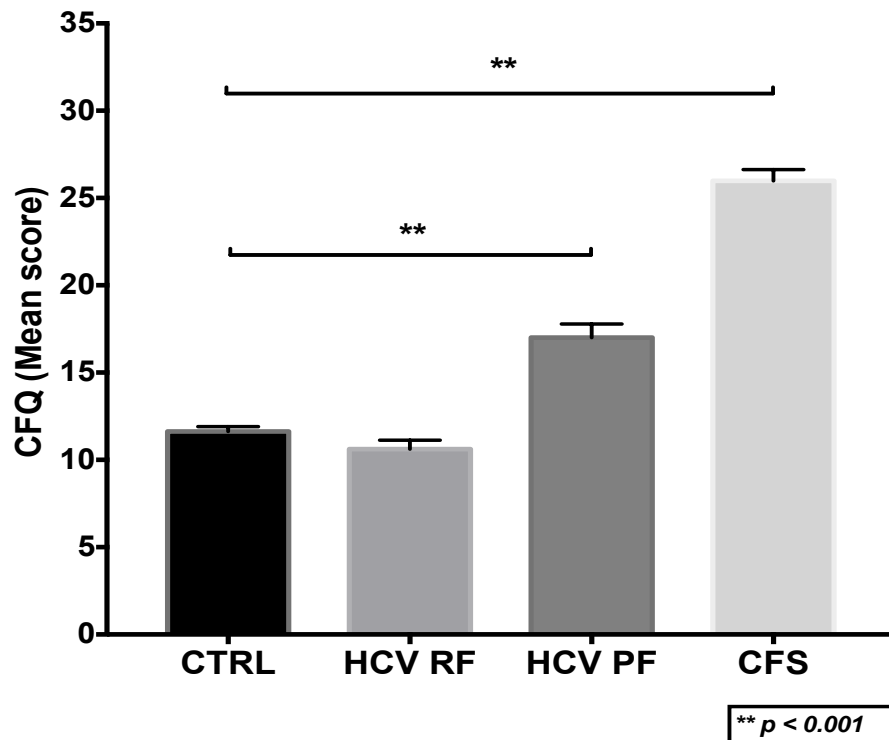


Figure 3.46 Fatigue scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

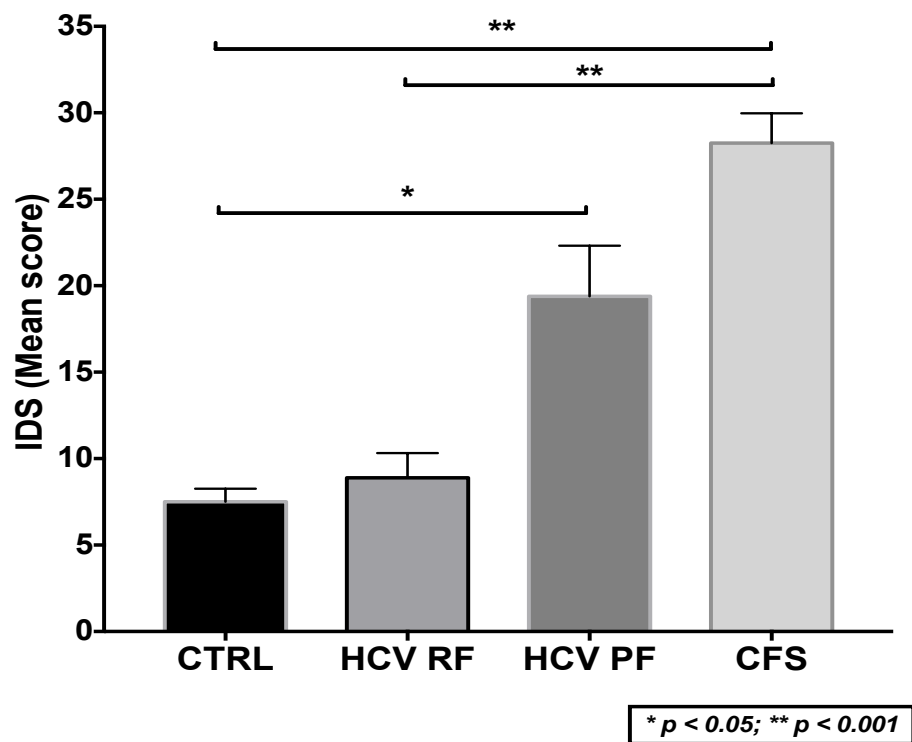


Figure 3.47 Depression scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

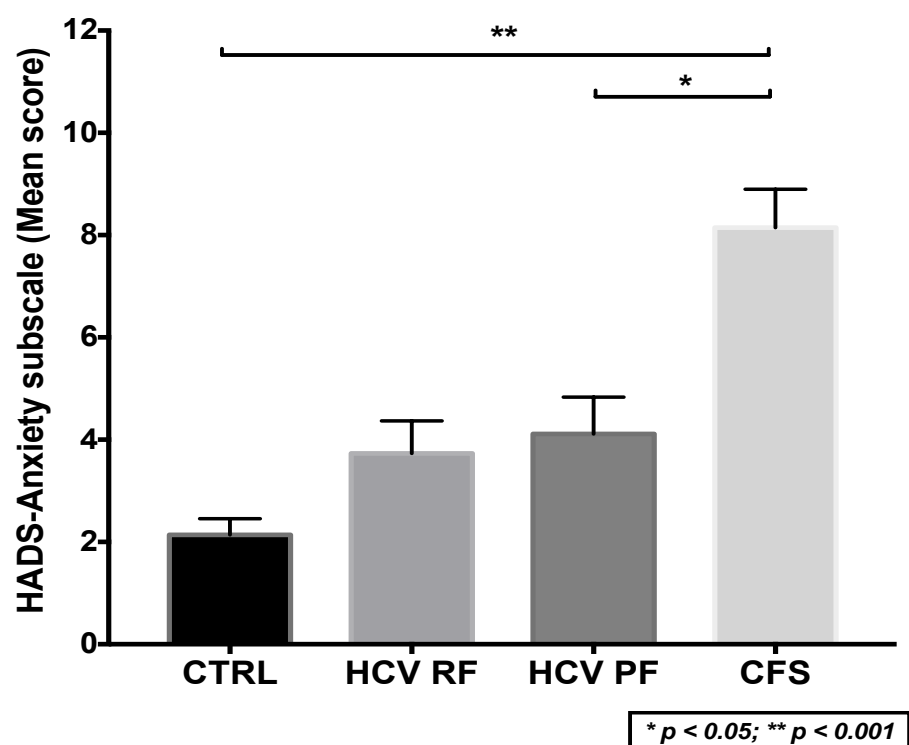


Figure 3.48 Anxiety scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

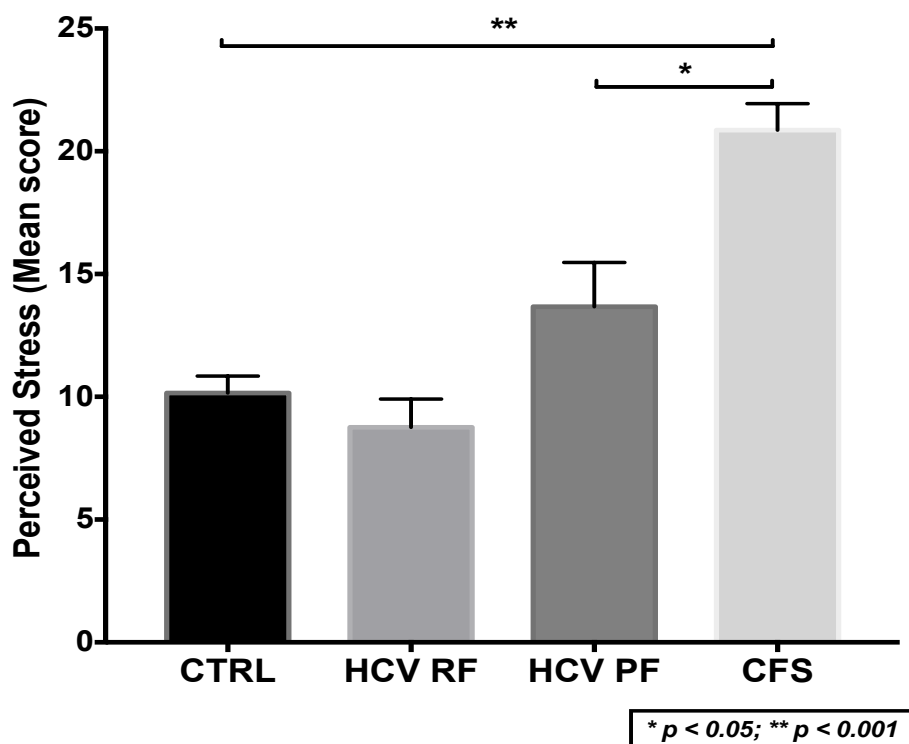


Figure 3.49 Perceived Stress scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

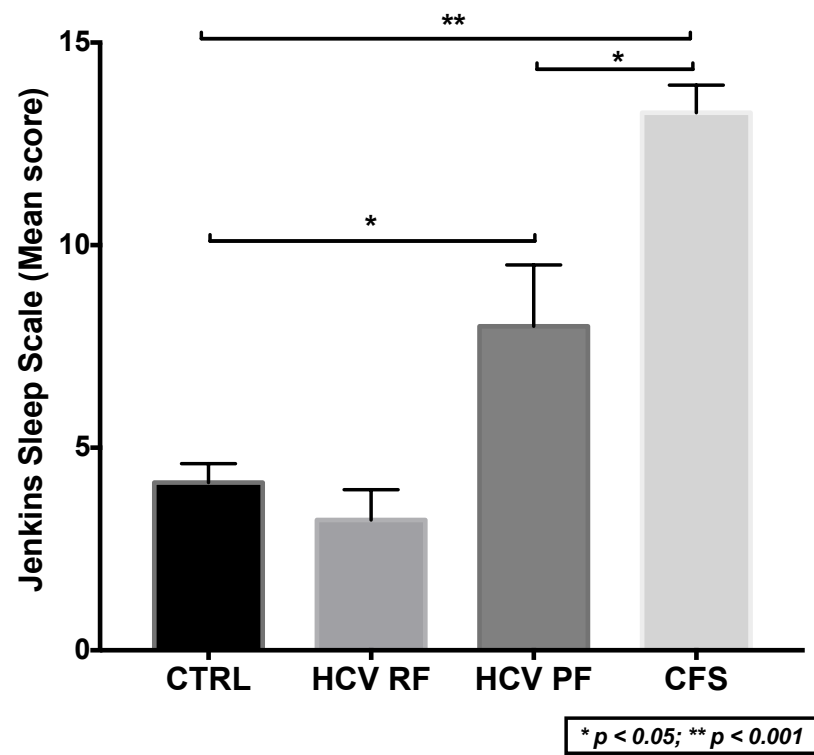


Figure 3.50 Sleep scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

3.2.4 Health status

Health status, as measured using the Medical Outcomes Survey SF-36, was then explored, again using a series of one-way ANOVAs. As before, where the assumption of homogeneity of variances was met, as assessed by Levene's test ($p>0.05$), ANOVA and, where indicated, Tukey's HSD post-hoc tests were used. Otherwise, if violated, Welch's ANOVA and Games-Howell post-hoc tests were performed. For this measure, higher scores reflect better functioning in each area. Scores (Mean \pm SEM) and test results are shown in Table 3.56 (see page 279). Due to the difference in the distribution of these scores versus the other data, all figures for SF-36 subscales instead show the distribution of scores (with Mean \pm SEM).

Emotional Role Limitation subscale scores represent the degree to which the individual feels limited in every day life or in their roles by any 'emotional problems'. Data is presented in Figure 3.51 (see page 281) Scores were not normally distributed in any of the four groups. Visual examination of the histograms per group revealed that scores were negatively skewed in all groups, though there was a slightly greater spread of scores in the HCV PF and CFS groups. This was reflected in the HCV RF and healthy control groups, where any individual who did not score '100' was an outlier. The assumption of homogeneity of variances was not met, and Welch's ANOVA showed that there was a significant difference between groups. Post-hoc tests showed that CFS patients reported being significantly more limited than HCV RF and healthy control participants. There were no significant differences between any other groups.

Physical Functioning data is presented in Figure 3.52 (see page 284). Subscale scores for were normally distributed in CFS patients, but not in the other three groups. Scores were negatively skewed, reflecting fewer problems with physical functioning, though to a lesser degree in the HCV PF patients, where a greater spread of scores was seen. There were four outliers in the HCV RF group, each reporting lower scores, and greater problems, than their peers. In the healthy control group, there was one individual who fulfilled the same criteria. The assumption of the homogeneity of variances was violated. Welch's ANOVA showed a significant difference between groups in levels of physical functioning. Games-Howell post-hoc tests showed that CFS patients reported lower levels of physical functioning than any other group. The two HCV groups reported similar levels of functioning. HCV PF patients had significantly worse functioning than healthy controls, and there was a trend towards the same pattern in HCV RF patients.

Pain data is presented in Figure 3.53 (see page 282). Subscale scores were normally distributed in the HCV PF patients, but not in the other three groups. Scores in the HCV RF and healthy control groups were negatively skewed. In the CFS group, the distribution was less well defined on account of the spread of scores, but slightly more positively skewed in favour of scores indicating more problems with pain. There were no outliers. The assumption of the homogeneity of variances was violated. Welch's ANOVA revealed a significant difference between groups. Post-hoc Games-Howell tests showed that CFS patients reported more pain than the HCV RF and healthy control participants, but similar levels to the HCV PF group. HCV PF patients on the other hand were not significantly different to HCV RF and healthy control participants.

Mental Health data is presented in Figure 3.54 (see page 283). Subscale scores were normally distributed in the HCV PF and CFS groups, but not in the HCV RF or healthy control groups, where more frequent higher scores resulted in data being negatively skewed. There was one outlier, in the HCV RF group, who reported a lower score more than three times outside of the interquartile range. The assumption of homogeneity of variances was violated. Welch's ANOVA revealed a significant difference in ratings of Mental Health between groups. Games-Howell post-hoc tests showed that CFS patients' self-report ratings of their mental health were significantly higher than HCV RF and healthy control participants, indicating greater problems. There was a trend towards higher scores than HCV PF patients. There were no other significant differences between groups.

The **Vitality** subscale reflects the balance between energy and fatigue. Data is shown in Figure 3.55 (see page 283). Subscale scores were normally distributed in the HCV groups. They were not in the CFS group, where there was a slight positive skew, reflecting a propensity towards lower (worse) scores, and a maximum score of 55/100. In the healthy controls data was negatively skewed. There were, however, no outliers. The assumption of the homogeneity of variances was violated. Welch's ANOVA revealed a significant difference in Vitality scores between groups. Post-hoc Games-Howell tests showed CFS patients to have significantly higher problems with energy/fatigue than any other group. The HCV PF group scores were significantly lower, indicating poorer functioning, than the HCV RF and healthy control groups. Scores not significantly different in the HCV RF patients versus healthy controls.

Social Functioning data is presented in Figure 3.56 (see page 283). Scores were not normally distributed in any group. Scores in the HCV groups and healthy controls were negatively skewed, albeit to a lesser degree in HCV PF. In the CFS group there were fewer patients with scores from 70-90, indicating that most patients experienced some degree of disability in this area, and resulting in a slightly negatively skewed distribution. In the HCV RF group, there was one outlier. There were no outliers in the HCV PF or CFS groups. In the healthy controls, anyone reporting a score under 100 was considered an outlier, totalling 8 individuals. The assumption of the homogeneity of variances was violated. The Welch's ANOVA showed a significant difference in levels of social functioning between groups. Games-Howell tests showed CFS patients to experience significantly poorer social functioning than any other group. Again, HCV PF patients fell somewhere in between, with similar levels to their HCV RF peers, but worse than healthy controls. HCV RF and healthy controls were no different.

General Health data is presented in Figure 3.57 (see page 284). Data was normally distributed in the HCV PF group, but not in the other three groups. In the CFS group, scores were positively skewed, with patients more often reporting higher scores, indicative of poorer general health. In the HCV RF and healthy control groups, scores were negatively skewed. There were no outliers. The assumption of homogeneity of variance was violated. Welch's ANOVA showed that there was a difference in scores between groups. Games-Howell post-hoc tests showed significant differences in ratings of general health between CFS patients and all three other groups. The two HCV groups had similar ratings of their general health, with both groups having higher (poorer) scores than the healthy controls.

Physical Role Limitation subscale scores represent the degree to which the respondent feels limited in their every day activities by their physical health. Data is presented in Figure 3.58 (see page 284). Scores were not normally distributed in any group. Scores were negatively skewed in HCV PF, RF and healthy control groups, and positively skewed in CFS patients. As with the Emotional Role Limitation measure, in the HCV RF and healthy control groups, there were 7 and 6 individuals respectively who did not score '100' and were therefore outliers. The assumption of the homogeneity of variances was violated. Welch's ANOVA showed that scores were significantly different in the four groups. Games-Howell post-hoc tests confirmed that CFS patients reported being much more limited in their ability to perform their regular roles and daily activities than any other group. The HCV RF patients fell between the ratings of HCV PF and healthy control participants, and so were not significantly different to either group. HCV PF patients reported being more limited than healthy controls.

Table 3.56 Health status in the healthy control (CTRL), HCV Resolved (RF) and Persistent Fatigue (PF), and CFS groups

SF-36	CTRL (n = 56)	HCV RF (n = 37)	HCV PF (n = 18)	CFS (n = 50)	Welch's ANOVA / Games-Howell post-hoc statistics
Emotional Role Limitation	91.1±3.0	88.3±5.0	68.5±10.3	60.0±5.8	$F(3,56.73)=8.4, p<0.001$ <i>CFS = PF, $p=0.89$</i> $CFS < RF, p=0.002$ $CFS < CTRL, p<0.001$ <i>PF = CTRL, $p=0.18$</i> <i>PF = RF, $p=0.33$</i> <i>RF = CTRL, $p=0.96$</i>
Physical Functioning	97.5±0.7	88.2±3.6	75.3±5.9	53.6±3.5	$F(3,49.3)=53.1, p<0.001$ <i>CFS < PF, $p = 0.018$</i> $CFS < RF, p<0.001$ $CFS < CTRL, p<0.001$ $PF < CTRL, p=0.008$ $PF < RF, p=0.26$ <u>$RF = CTRL, p=0.071$</u>
Pain	88.2±1.7	82.7±3.7	69.0±5.4	53.9±4.1	$F(3,56.1)=21.7, p<0.001$ <i>CFS = PF, $p=0.13$</i> $CFS < RF, p<0.001$ $CFS < CTRL, p<0.001$ $PF < CTRL, p=0.013$ <i>PF = RF, $p=0.18$</i> <i>RF = CTRL, $p=0.54$</i>
Mental Health	81.7±1.6	81.7±2.5	71.3±4.9	56.2±3.0	$F(3,55.4)=20.6, p<0.001$ <i>CFS = PF, $p=0.064$</i> $CFS < RF, p<0.001$ $CFS < CTRL, p<0.001$ <i>PF = CTRL, $p=0.21$</i> <i>PF = RF, $p=0.26$</i> <i>RF = CTRL, $p=1.0$</i>

SF-36	CTRL (n = 56)	HCV RF (n = 37)	HCV PF (n = 18)	CFS (n = 50)	Welch's ANOVA / Games-Howell post-hoc statistics
Vitality	72.8±1.7	69.7±3.5	53.9±4.3	21.6±2.2	$F(3,58.4)=113.0, p<0.001$ $CFS < PF, p<0.001$ $CFS < RF, p<0.001$ $CFS < CTRL, p<0.001$ $PF < CTRL, p=0.002$ $PF < RF, p=0.032$ $RF = CTRL, p=0.86$
Social Functioning	95.3±1.6	91.2±2.9	79.9±5.3	43.0±3.4	$F(3,56.8)=64.3, p<0.001$ $CFS < PF, p<0.001$ $CFS < RF, p<0.001$ $CFS < CTRL, p<0.001$ $PF < CTRL, p=0.049$ $PF = RF, p=0.26$ $RF = CTRL, p=0.62$
General Health	81.2±1.7	67.2±4.2	55.3±5.2	35.4±2.9	$F(3,56.2)=64.0, p<0.001$ $CFS < PF, p = 0.012$ $CFS < RF, p<0.001$ $CFS < CTRL, p<0.001$ $PF < CTRL, p = 0.001$ $PF = RF, p=0.29$ $RF < CTRL, p=0.018$
Physical Role Limitation	94.2±2.6	83.1±5.7	66.7±8.8	14.0±3.6	$F(3,56.2)=108.7, p<0.001$ $CFS < PF, p<0.001$ $CFS < RF, p<0.001$ $CFS < CTRL, p<0.001$ $PF < CTRL, p=0.033$ $PF = RF, p=0.41$ $RF = CTRL, p=0.30$

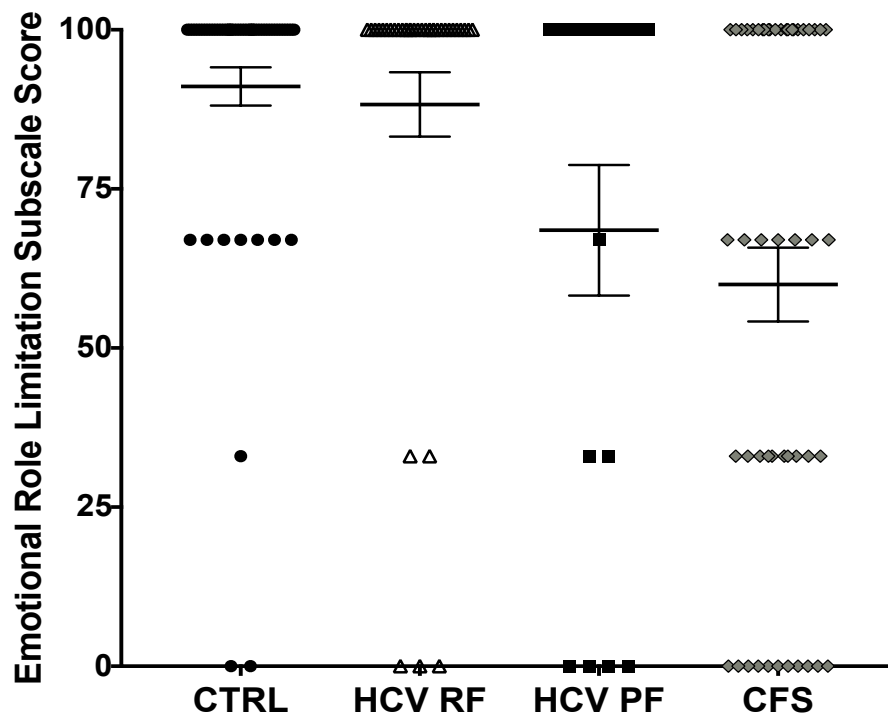


Figure 3.51 SF-36 Emotional Role Limitation scores in healthy controls (CTRL), HCV Resolved Fatigue (RF) and Persistent Fatigue (PF) groups, and CFS patients

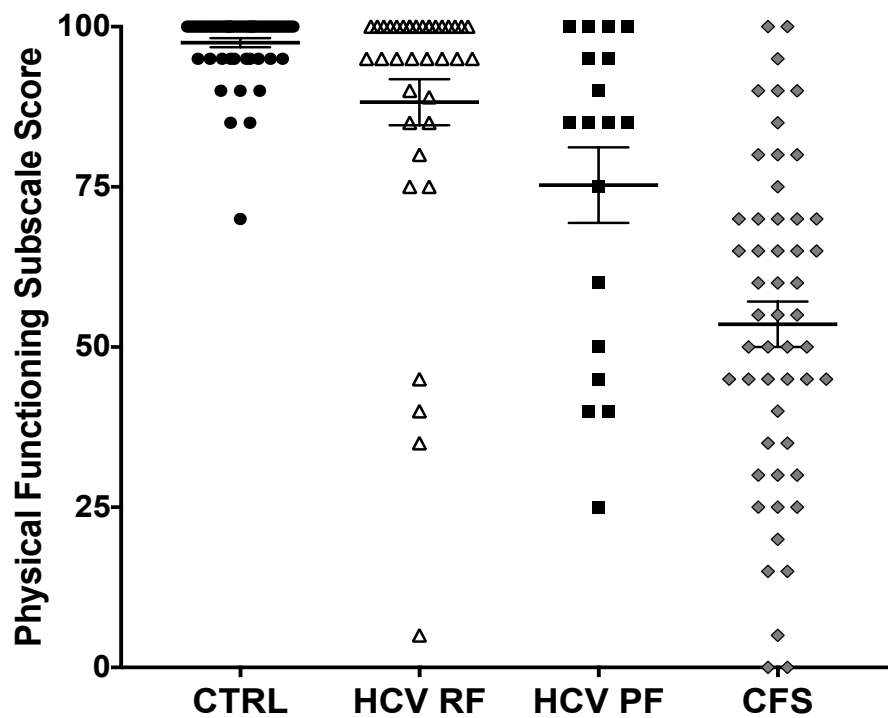


Figure 3.52 SF-36 Physical Functioning scores in healthy controls (CTRL), HCV Resolved Fatigue (RF) and Persistent Fatigue (PF) groups, and CFS patients

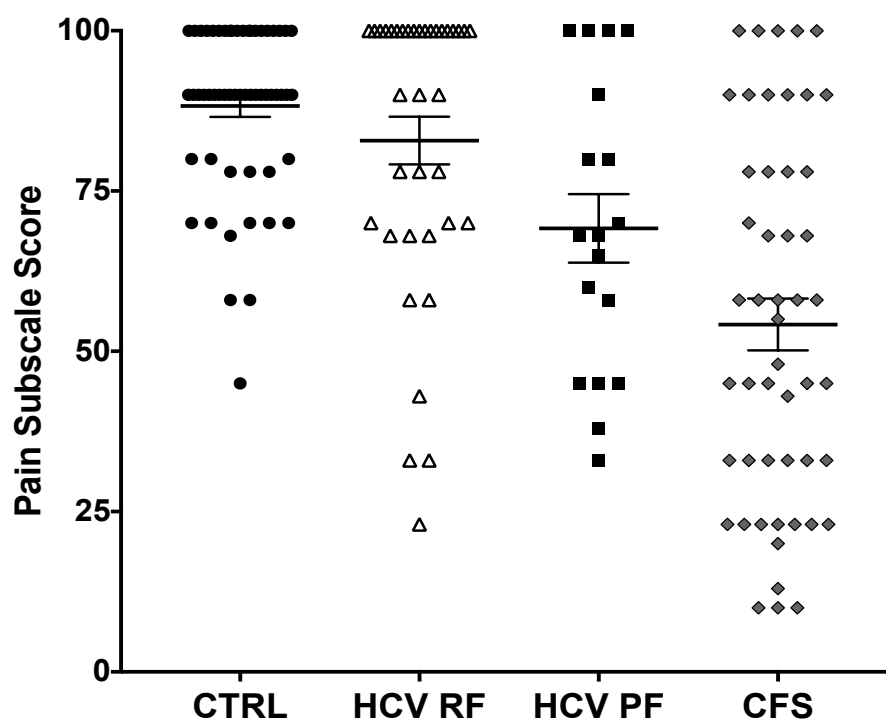


Figure 3.53 SF-36 Pain scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

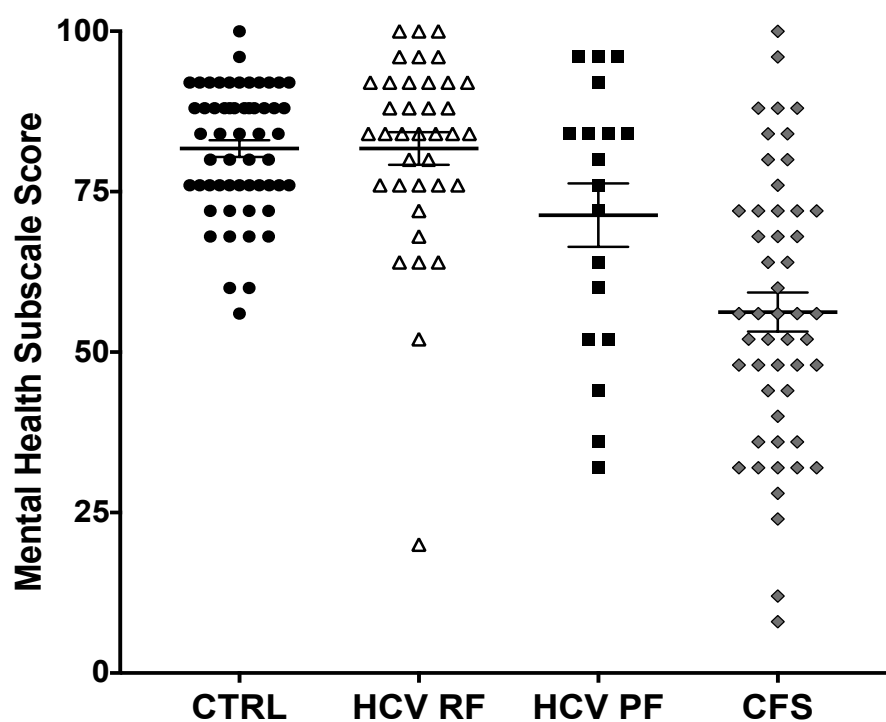


Figure 3.54 SF-36 Mental Health scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

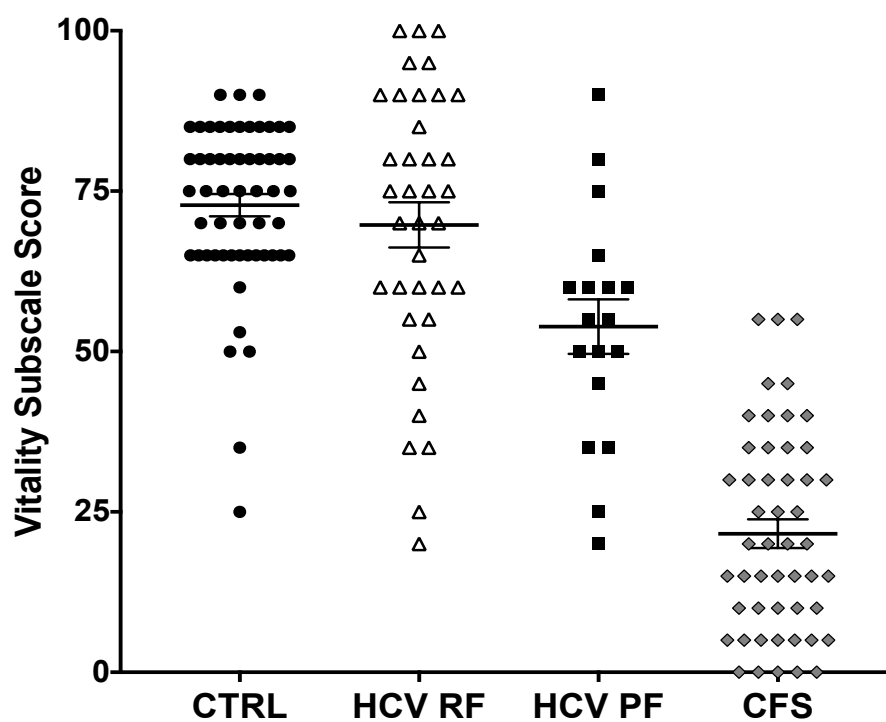


Figure 3.55 SF-36 Vitality scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

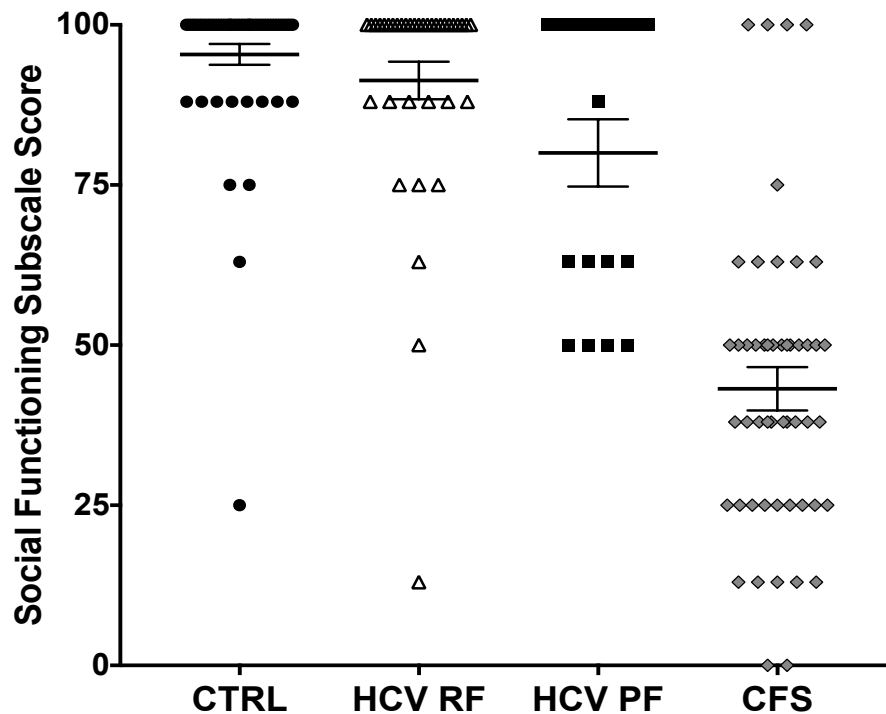


Figure 3.56 SF-36 Social Functioning scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

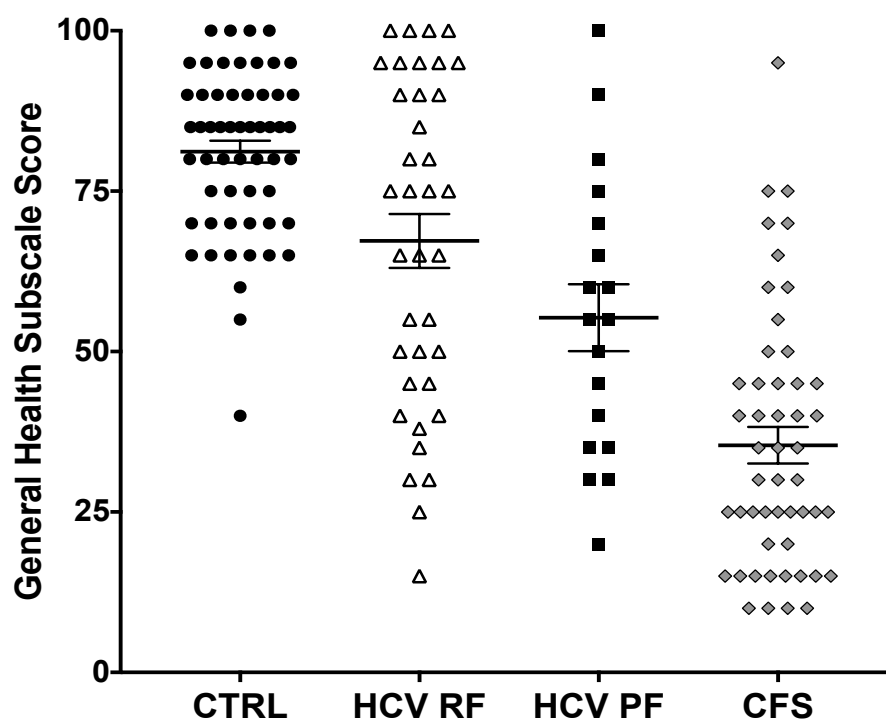


Figure 3.57 SF-36 General Health scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

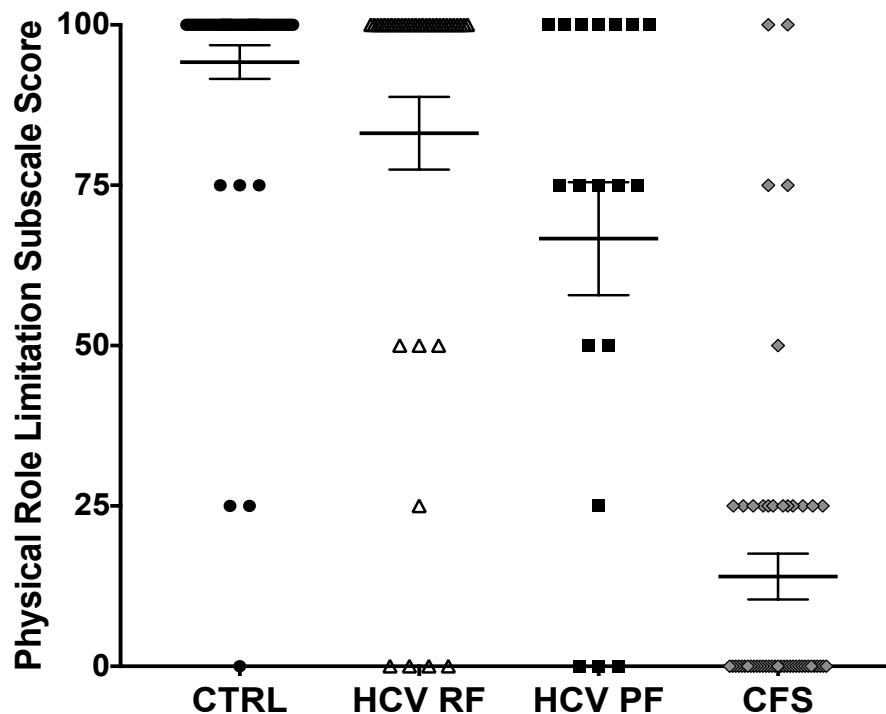


Figure 3.58 SF-36 Physical Role Limitation scores in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

3.2.5 Biological measures

Next, I explored similarities and differences between the four groups in the biological measures: cytokines, the metabolites in the kynurenine pathway and cortisol (Cortisol Awakening Response; diurnal output). As before, a series of one-way ANOVAs were performed for each marker or measure. Where the Levene's test result indicated that the assumption of the homogeneity of variances could be assumed ($p>0.05$), an ANOVA and Tukeys HSD post-hoc tests were performed. Where it was violated ($p<0.05$), results for the Welch's ANOVA are reported, and Games-Howell post-hoc comparisons.

3.2.5.1 Cytokines: levels

Not all cytokines measured in the prospective cohort study has been measured in CFS and healthy control participants. The analytes measured were IL-10, IL-13, IL-2, IL-6, IL-7, IL-8, IL-17A and TNF- α . For all cytokines, boxplots were examined for HCV patients, CFS patients and healthy controls, and any result that was identified as being outside of the range of \pm three times the interquartile range for each marker was excluded from the analyses. Since a series of one-way ANOVAs were performed, participants were excluded on a test-by-test basis only; further details are provided below. Since earlier attempts at transforming the cytokine data were not successful in addressing the positive skew of the data, the same strategy of additional post-hoc testing using parametric and non-parametric methods was used, and the results compared. Where a significant main effect of group was found, graphs displaying Mean \pm SEM are shown.

Interleukin (IL)-10

One patient in each of the HCV groups (RF/PF) was identified as outliers using the method described above. Four patients in the CFS group and three in the healthy control (CTRL) group were also shown to be outside of the cut-off. With these outliers removed, data was not normally distributed in any of the four groups. The assumption of homogeneity of variances was violated. As a result of this violation, the Welch ANOVA was used. This showed that there was a significant difference in levels of IL-10 between groups (Welch's $F(3,44.74) = 4.29$, $p = 0.010$). Examination of the mean scores showed that levels were lowest in CFS patients (Mean \pm SEM pg/ml; 0.27 ± 0.02), similarly low in healthy controls (0.29 ± 0.01), and then higher in the HCV RF (0.52 ± 0.09) and HCV PF groups (0.57 ± 0.12). Games-Howell post-hoc tests showed a trend towards lower IL-10 levels in CFS patients (0.27 ± 0.02) versus HCV PF (0.57 ± 0.12 ; $p = 0.089$) and HCV RF patients (0.52 ± 0.09 ; $p = 0.056$). There was also a trend towards lower levels in healthy controls compared with HCV RF patients (0.29 ± 0.01 vs. 0.52 ± 0.09 , $p = 0.080$). See Figure 3.59.

Additional post-hoc analysis was conducted. Results are shown in Table 3.57 overleaf. There were significant differences between both HCV groups and CFS patients. Only the difference between HCV PF patients and controls was significant across both tests. The HCV groups, and Control and CFS groups, were no different. See also Figure 3.60 for the distribution of IL-10 levels (with the median and interquartile range, or IQR).

Table 3.57 IL-10: Post-hoc parametric and non-parametric comparisons

Comparison	Mean±SEM		Test and statistic
HCV RF vs PF	0.52±0.09	0.57±0.12	$t(46) = -0.33, p = 0.74$
HCV RF vs CFS	0.52±0.09	0.27±0.02	$t(34.37) = 2.65, p = 0.012$
HCV RF vs CTRL	0.52±0.09	0.29±0.01	$t(33.45) = 2.49, p = 0.018$
HCV PF vs CFS	0.57±0.12	0.27±0.02	$t(14.67) = 2.58, p = 0.021$
HCV PF vs CTRL	0.57±0.12	0.29±0.01	$t(14.41) = 2.45, p = 0.028$
CFS vs CTRL	0.27±0.02	0.29±0.01	$t(101) = -0.73, p = 0.47$
	Median		Test and statistic
HCV RF vs PF	0.29	0.39	$U = 179, z = -1.52, p = 0.13$
HCV RF vs CFS	0.29	0.25	$U = 581, z = -2.15, p = 0.031$
HCV RF vs CTRL	0.29	0.27	$U = 767, z = -1.09, p = 0.28$
HCV PF vs CFS	0.39	0.25	$U = 153, z = -3.40, p = 0.001$
HCV PF vs CTRL	0.39	0.27	$U = 180, z = -3.27, p = 0.001$
CFS vs CTRL	0.25	0.27	$U = 1100, z = -1.47, p = 0.14$

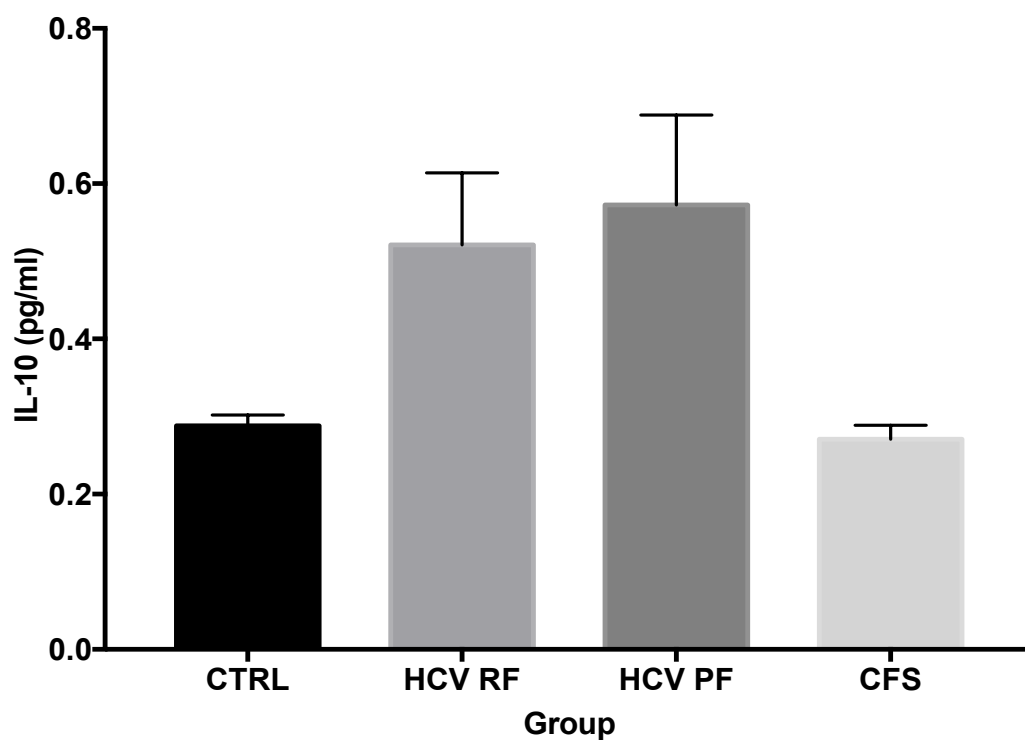


Figure 3.59 Mean Interleukin-10 levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

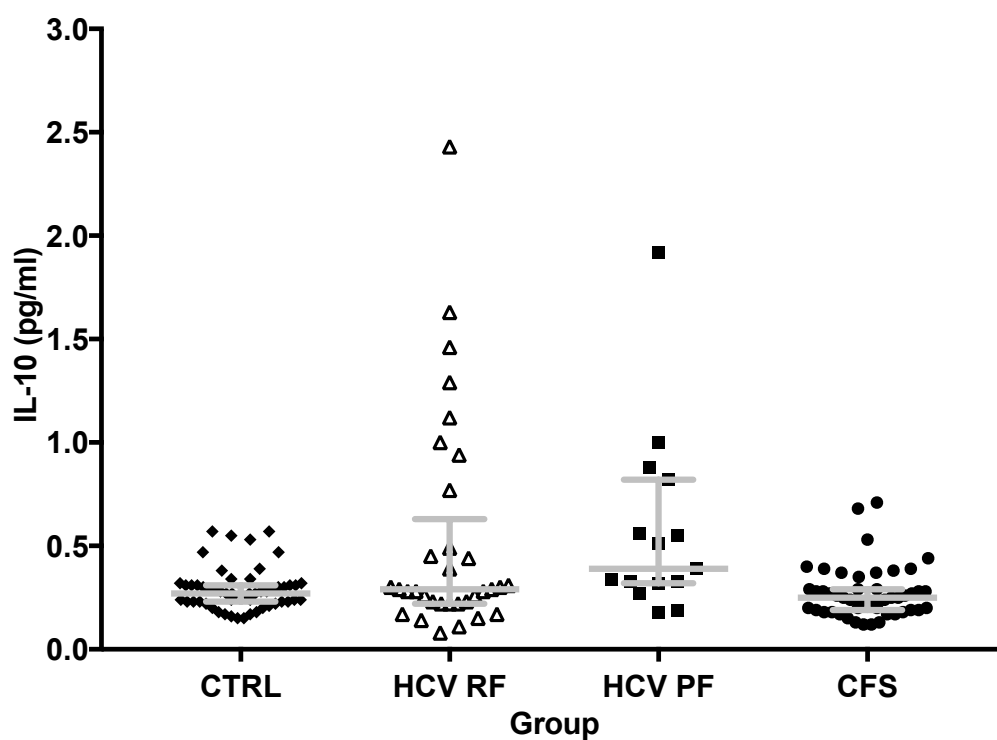


Figure 3.60 Interleukin-10 levels (with Median and IQR) in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

Interleukin (IL)-13

A total of three individuals who had at least once score outside of the cut-off of three times the interquartile range, one in the Resolved Fatigue group and two in the Persistent Fatigue group, were removed from the analysis of IL-13 levels. No CFS patients exceeded the cut-off, though one healthy control participant did. Excluding these values, data was non-normal in all groups. The assumption of homogeneity of variances was met. There was no statistically significant difference between the groups in IL-13 concentration ($F(3, 152) = 1.79, p = 0.15, \text{partial } \eta^2 = 0.03$).

Interleukin (IL)-2

Two participants in the Resolved Fatigue group, and one in the Persistent Fatigue groups had IL-2 levels outside of the applied cut-off of 3 times the interquartile range for each time point. One CFS patient also did, as well as three healthy control participants. With these results excluded, data in all groups was non-normal. The assumption of homogeneity of variance was violated. As a result of this violation, the Welch ANOVA was used. This showed that there was no significant difference in levels of IL-2 between groups (Welch's $F(3, 45.99) = 1.90, p = 0.14$).

Interleukin (IL)-6

No patients in either HCV group (RF/PF) had IL-6 levels outside of the applied cut-off of 3 times the interquartile range for each time point. Three CFS patients did, as well as one healthy control participant. With these results excluded, data in the HCV PF group was normally distributed, but non-normal in all other groups. The assumption of homogeneity of variance was violated.

As a result, the Welch's ANOVA was used. This showed that there was a significant difference in levels of IL-6 between groups (Welch's $F(3,47.48) = 10.94$, $p < 0.001$). Examination of the mean levels showed that levels were lowest in CFS patients (0.44 ± 0.03), and healthy control participants (0.53 ± 0.03), then higher in HCV RF (1.21 ± 0.22) and HCV PF patients (1.40 ± 0.21). Games-Howell post-hoc tests revealed that levels were significantly higher in HCV RF patients versus CFS ($p = 0.007$) and healthy controls ($p = 0.018$). Levels were likewise significantly higher in HCV PF patients ($p = 0.002$ and 0.004 respectively). There were no differences between CFS/CTRL groups, or the two HCV groups. See Figure 3.61.

Post-hoc parametric and non-parametric comparisons also yielded similar results, though also found lower levels in CFS patients versus healthy controls, attributable to the less conservative tests (see Table 3.58). See also Figure 3.62 for the distribution of IL-6 levels, and the median and IQR.

Table 3.58 IL-6: Post-hoc parametric and non-parametric comparisons

Comparison	Mean±SEM		Test and statistic
HCV RF vs PF	1.21±0.22	1.40±0.21	$t(48) = -0.56, p = 0.58$
HCV RF vs CFS	1.21±0.22	0.44±0.03	$t(34.55) = 3.51, p = 0.001$
HCV RF vs CTRL	1.21±0.22	0.53±0.03	$t(34.18) = 3.12, p = 0.004$
HCV PF vs CFS	1.40±0.21	0.44±0.03	$t(15.77) = 4.58, p < 0.001$
HCV PF vs CTRL	1.40±0.21	0.53±0.03	$t(15.58) = 4.17, p = 0.001$
CFS vs CTRL	0.44±0.03	0.53±0.03	$t(104) = -2.01, p = 0.047$
	Median		Test and statistic
HCV RF vs PF	0.69	1.17	$U = 191, z = -1.69, p = 0.092$
HCV RF vs CFS	0.69	0.40	$U = 483, z = -3.34, p = 0.001$
HCV RF vs CTRL	0.69	0.51	$U = 684, z = -2.23, p = 0.026$
HCV PF vs CFS	1.17	0.40	$U = 79, z = -4.80, p < 0.001$
HCV PF vs CTRL	1.17	0.51	$U = 110, z = -4.58, p < 0.001$
CFS vs CTRL	0.40	0.51	$U = 1032, z = -2.33, p = 0.02$

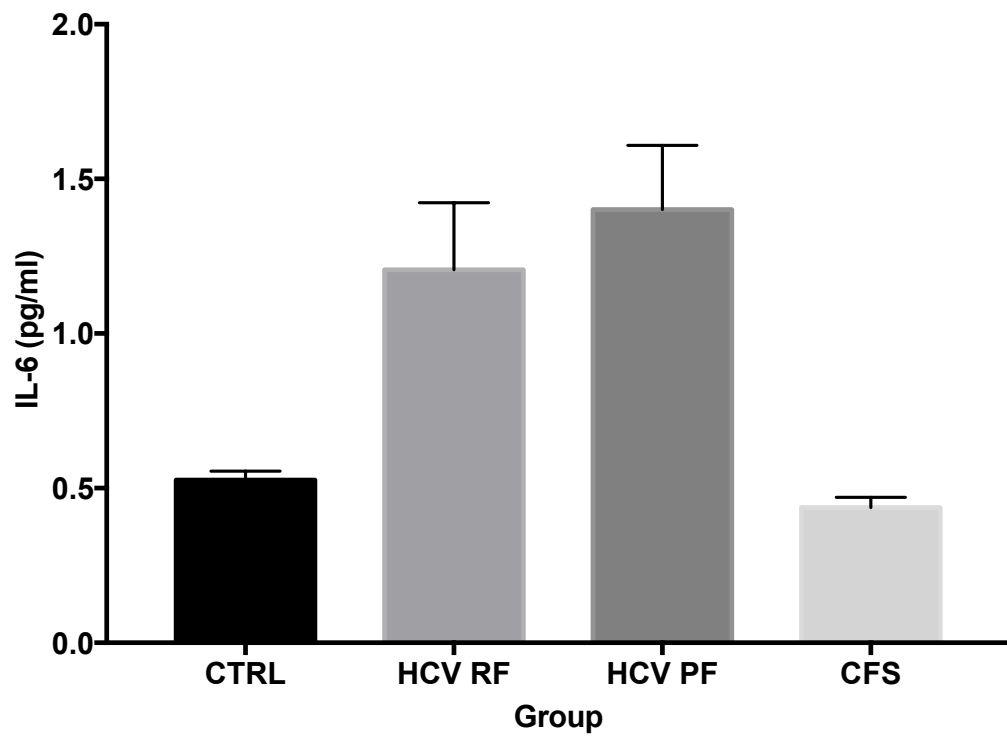


Figure 3.61 Mean Interleukin-6 levels healthy controls (CTRL), HCV RF and PF groups, and CFS patients

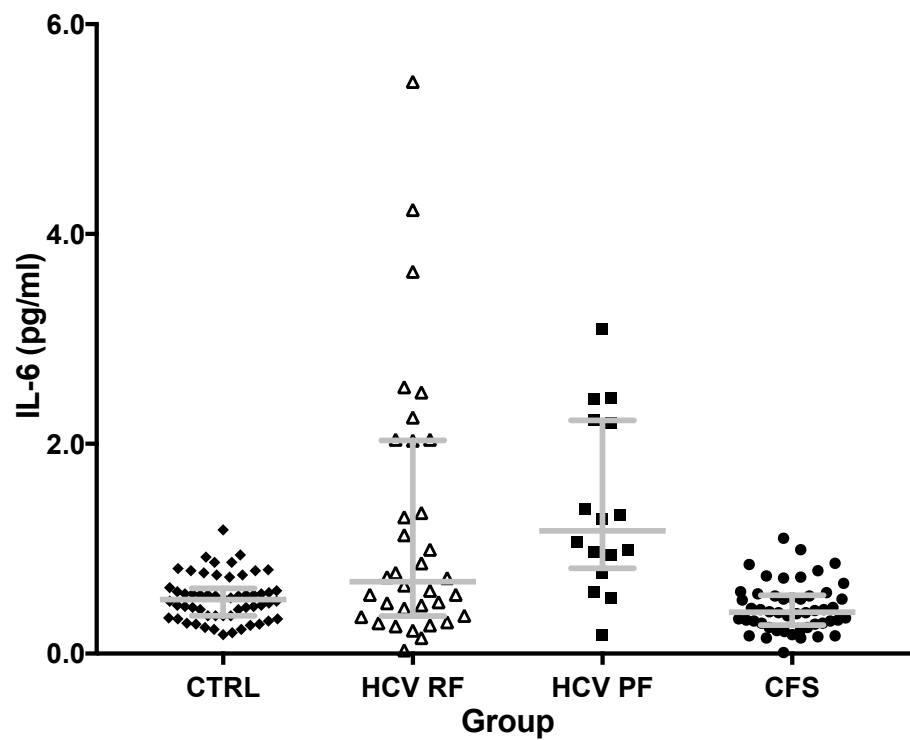


Figure 3.62 Interleukin-6 levels (with Median and IQR) in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

Interleukin (IL)-7

There were five patients in total across the HCV patients, three in the RF group, and two in the PF group that met the criteria set for outliers. No patients in either the CFS or healthy control groups were identified. After exclusion of these individuals from the subsequent analysis, Data in the HCV RF group was not normally distributed. The HCV PF group was only slightly so ($p = 0.054$), and data in the CFS and Control groups were normal. The assumption of homogeneity of variances was met. An ANOVA showed a significant difference between groups ($F(3,151) = 4.61$, $p = 0.004$, partial $\eta^2 = 0.08$). Levels were highest in controls (20.98 ± 0.94) and then HCV PF patients (16.59 ± 2.87). Next were levels in HCV RF patients (15.77 ± 2.00), and CFS patients (15.85 ± 0.87). Post-hoc Tukeys HSD tests showed significantly higher levels in healthy control participants versus the HCV RF ($p = 0.025$) and CFS patients ($p = 0.007$). See Figure 3.63.

Post-hoc parametric and non-parametric tests also showed the healthy controls to have higher levels of IL-7 than HCV RF and CFS patients (see Table 3.59). See also Figure 3.64 for the distribution of IL-7 levels, and the median and IQR.

Table 3.59 IL-7: Post-hoc parametric and non-parametric comparisons

Comparison	Mean±SEM		Test and statistic
HCV RF vs PF	15.77±2.00	16.59±2.87	$t(43) = -0.23, p = 0.82$
HCV RF vs CFS	15.77±2.00	15.85±0.87	$t(82) = -0.04, p = 0.97$
HCV RF vs CTRL	15.77±2.00	20.98±0.94	$t(86) = -2.67, p = 0.009$
HCV PF vs CFS	16.59±2.87	15.85±0.87	$t(15.45) = 0.25, p = 0.81$
HCV PF vs CTRL	16.59±2.87	20.98±0.94	$t(69) = -1.86, p = 0.067$
CFS vs CTRL	15.85±0.87	20.98±0.94	$t(108) = -4.00, p < 0.001$
	Median		Test and statistic
HCV RF vs PF	13.13	13.56	$U = 208, z = -0.22, p = 0.83$
HCV RF vs CFS	13.13	15.34	$U = 674, z = -1.37, p = 0.17$
HCV RF vs CTRL	13.13	20.63	$U = 444, z = -3.84, p < 0.001$
HCV PF vs CFS	13.56	15.34	$U = 356, z = -0.23, p = 0.82$
HCV PF vs CTRL	13.56	20.63	$U = 253, z = -2.11, p = 0.035$
CFS vs CTRL	15.34	20.63	$U = 918, z = -3.54, p < 0.001$

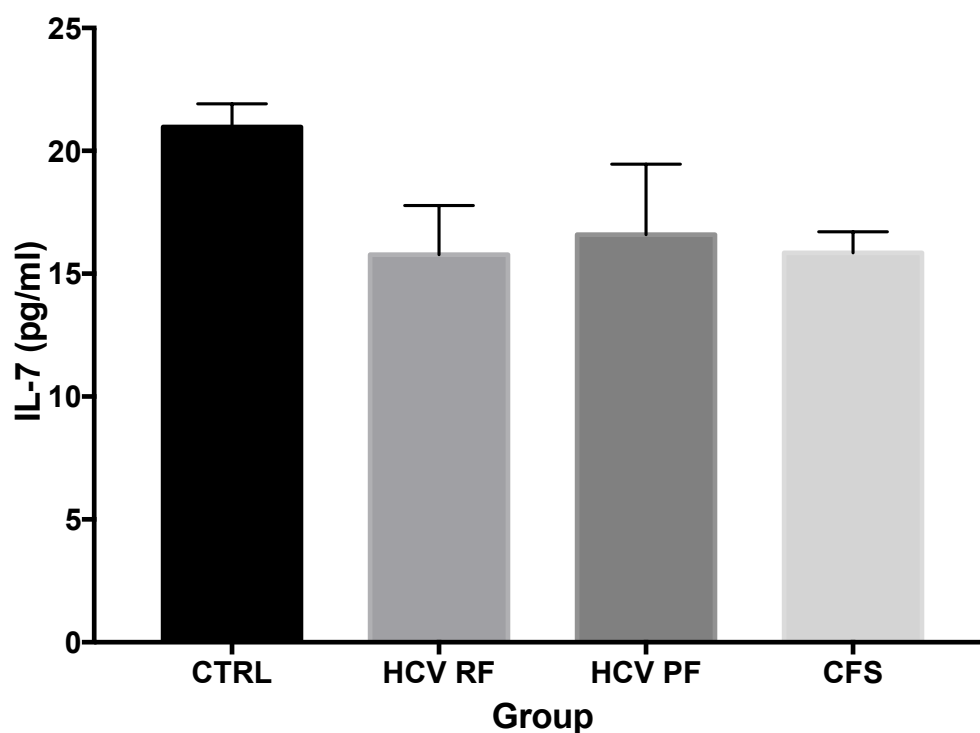


Figure 3.63 Mean Interleukin-7 levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

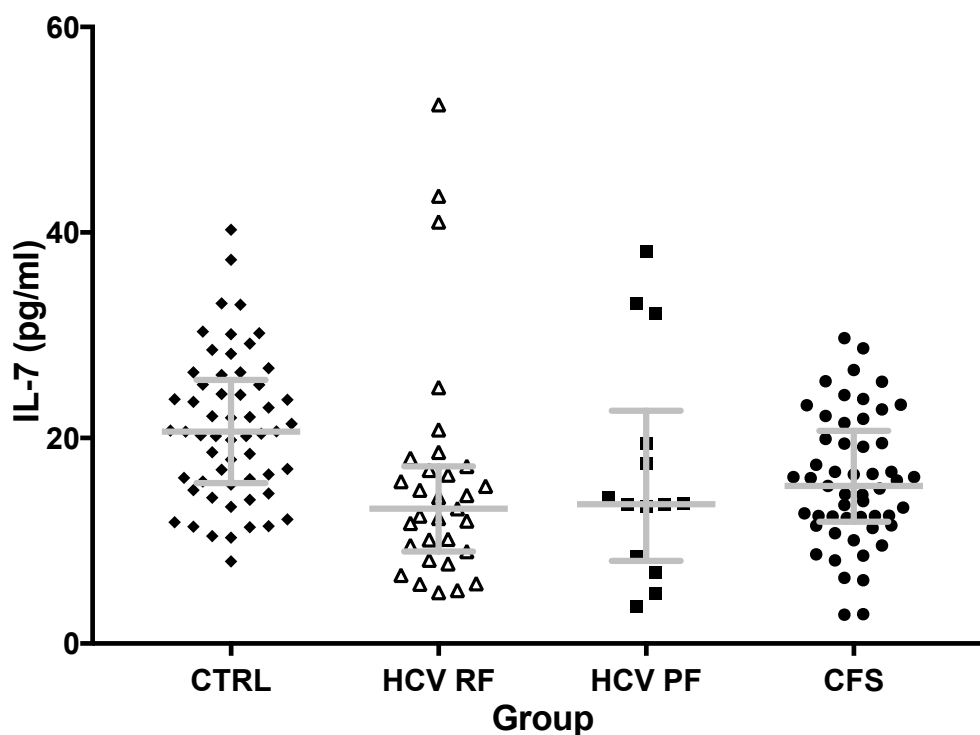


Figure 3.64 Interleukin-7 levels (with Median and IQR) in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

Interleukin (IL)-8

One patient in the HCV RF group, and two in the CFS group had IL-8 levels that could be considered as an outlier ($\pm 3 \times \text{IQR}$), and were therefore removed from this analysis. Levels were non-normal in each of the two HCV groups, as well as the CFS patient group. Levels in the healthy control group were normally distributed ($p = 0.11$). The assumption of homogeneity of variance was violated. As a result, Welch's ANOVA was again used. This showed that there was a significant difference in mean IL-8 levels between groups (Welch's $F(3,47.55) = 5.50$, $p = 0.003$). Consistent with the other cytokines, levels were lowest in the CFS patients (8.47 ± 0.39), and healthy control participants (9.74 ± 0.38), and then higher in the HCV RF (13.64 ± 1.73) and PF patients (14.94 ± 2.55). Post-Hoc Games-Howell tests showed that levels were significantly different between the HCV RF and CFS patients ($p = 0.030$), and there was a trend towards higher levels in the PF versus CFS groups ($p = 0.098$). There was also a trend towards increased levels in the healthy controls versus CFS patients ($p = 0.098$). See Figure 3.65.

Post-hoc comparisons using parametric and non-parametric methods also confirmed the higher levels in the HCV RF group versus CFS patients (see Table 3.60). The higher levels in the PF group versus CFS patients was also significant, perhaps since results were not now adjusted for multiple comparisons. Unlike the more conservative Games-Howell results above, CFS patients had significantly lower values than healthy controls across both methods. See also Figure 3.66 for the distribution of IL-8 levels, and the median and IQR.

Table 3.60 IL-8: Post-hoc parametric and non-parametric comparisons

Comparison	Mean±SEM		Test and statistic
HCV RF vs PF	13.64±1.73	14.94±2.55	$t(47) = -0.43, p = 0.67$
HCV RF vs CFS	13.64±1.73	8.47±0.39	$t(35.34) = 2.91, p = 0.006$
HCV RF vs CTRL	13.64±1.73	9.74±0.38	$t(35.09) = 2.20, p = 0.034$
HCV PF vs CFS	14.94±2.55	8.47±0.39	$t(15.72) = 2.51, p = 0.024$
HCV PF vs CTRL	14.94±2.55	9.74±0.38	$t(15.67) = 2.015, p = 0.061$
CFS vs CTRL	8.47±0.39	9.74±0.38	$t(106) = -2.33, p = 0.022$
	Median		Test and statistic
HCV RF vs PF	10.25	10.25	$U = 248, z = -0.34, p = 0.73$
HCV RF vs CFS	10.25	8.18	$U = 593, z = -2.28, p = 0.023$
HCV RF vs CTRL	10.25	9.26	$U = 840, z = -0.84, p = 0.40$
HCV PF vs CFS	10.25	8.18	$U = 250, z = -2.32, p = 0.02$
HCV PF vs CTRL	10.25	9.26	$U = 352, z = -1.39, p = 0.17$
CFS vs CTRL	8.18	9.26	$U = 1066, z = -2.39, p = 0.017$

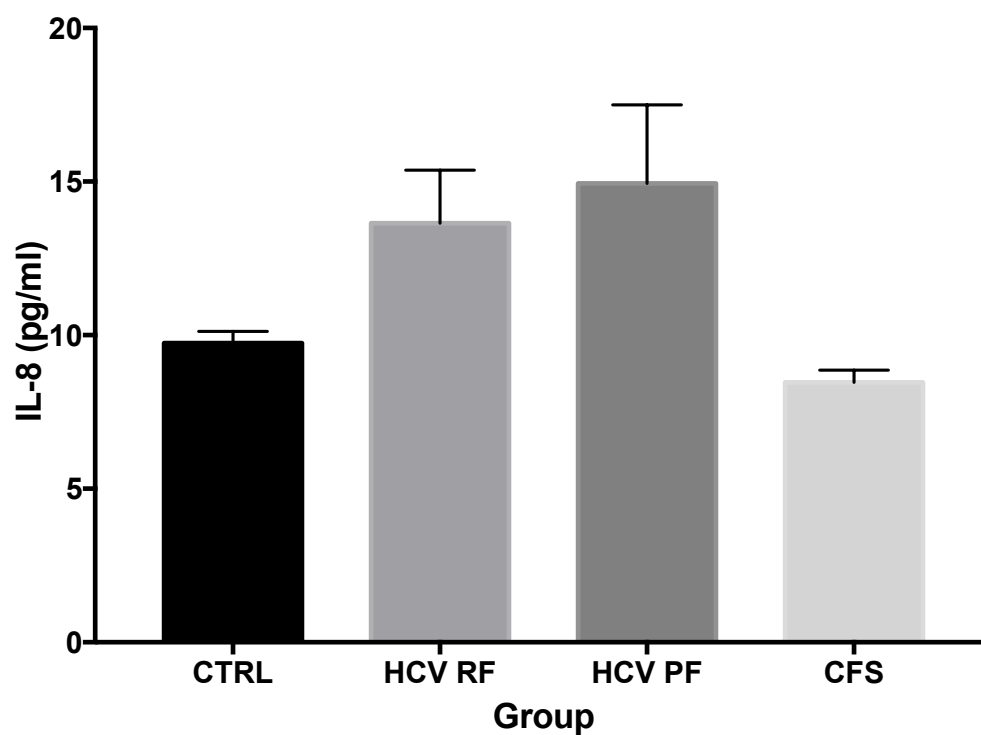


Figure 3.65 Mean Interleukin-8 levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

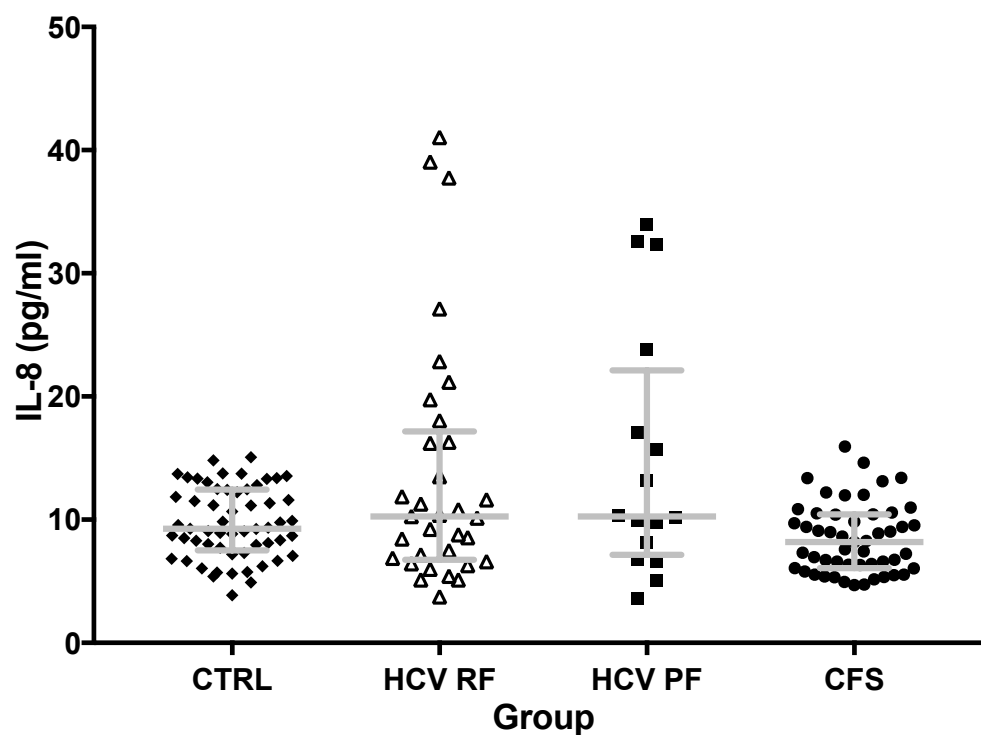


Figure 3.66 Interleukin-8 levels (with Median and IQR) in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

Interleukin (IL)-17A

There were three patients in the HCV RF group, one in the PF group, four in the CFS group and four in the Control group who had results outside of the cut-off of three times the interquartile range. Data was not normally distributed in any of the four groups. The assumption of homogeneity of variances was violated. Welch's ANOVA showed that there was a difference between the four groups in mean levels of IL-17A (Welch's $F(3,43.37) = 5.27$, $p = 0.003$). Levels were lowest in the healthy control participants (0.81 ± 0.04) and CFS patients (0.88 ± 0.06), and higher in the HCV RF (1.89 ± 0.37) and PF patient groups (2.04 ± 0.45). Post-hoc comparisons, conducted with the Games-Howell test, indicated that there were significantly higher IL-17A levels in the HCV RF group versus healthy controls ($p = 0.032$), and a trend towards higher levels versus CFS patients ($p = 0.052$). In the HCV PF group, there was a trend towards higher levels than healthy controls ($p = 0.066$) and CFS patients ($p = 0.090$), but no significant differences from any group. See Figure 3.67.

Post-hoc parametric and non-parametric tests confirmed that the two HCV groups each had significantly higher levels of IL-17A than both CFS patients and healthy controls. The Two HCV groups had similar levels, as did the CFS patients and healthy controls (see Table 3.61). See also Figure 3.68 for the distribution of IL-17A levels, and the median and IQR.

Table 3.61 IL-17A: Post-hoc parametric and non-parametric comparisons

Comparison	Mean±SEM		Test and statistic
HCV RF vs PF	1.89±0.37	2.04±0.45	$t(44) = -0.24, p = 0.81$
HCV RF vs CFS	1.89±0.37	0.88±0.06	$t(31.71) = 2.70, p = 0.011$
HCV RF vs CTRL	1.89±0.37	0.81±0.04	$t(30.78) = 2.91, p = 0.007$
HCV PF vs CFS	2.04±0.45	0.88±0.06	$t(14.55) = 2.57, p = 0.022$
HCV PF vs CTRL	2.04±0.45	0.81±0.04	$t(14.25) = 2.75, p = 0.015$
CFS vs CTRL	0.88±0.06	0.81±0.04	$t(100) = 0.988, p = 0.33$
	Median		Test and statistic
HCV RF vs PF	1.04	1.04	$U = 195, z = -0.88, p = 0.38$
HCV RF vs CFS	1.04	0.85	$U = 504, z = -2.53, p = 0.012$
HCV RF vs CTRL	1.04	0.76	$U = 494, z = -3.04, p = 0.002$
HCV PF vs CFS	1.04	0.85	$U = 172, z = -3.10, p = 0.002$
HCV PF vs CTRL	1.04	0.76	$U = 152, z = -3.63, p < 0.001$
CFS vs CTRL	0.85	0.76	$U = 1254.5, z = -0.30, p = 0.77$

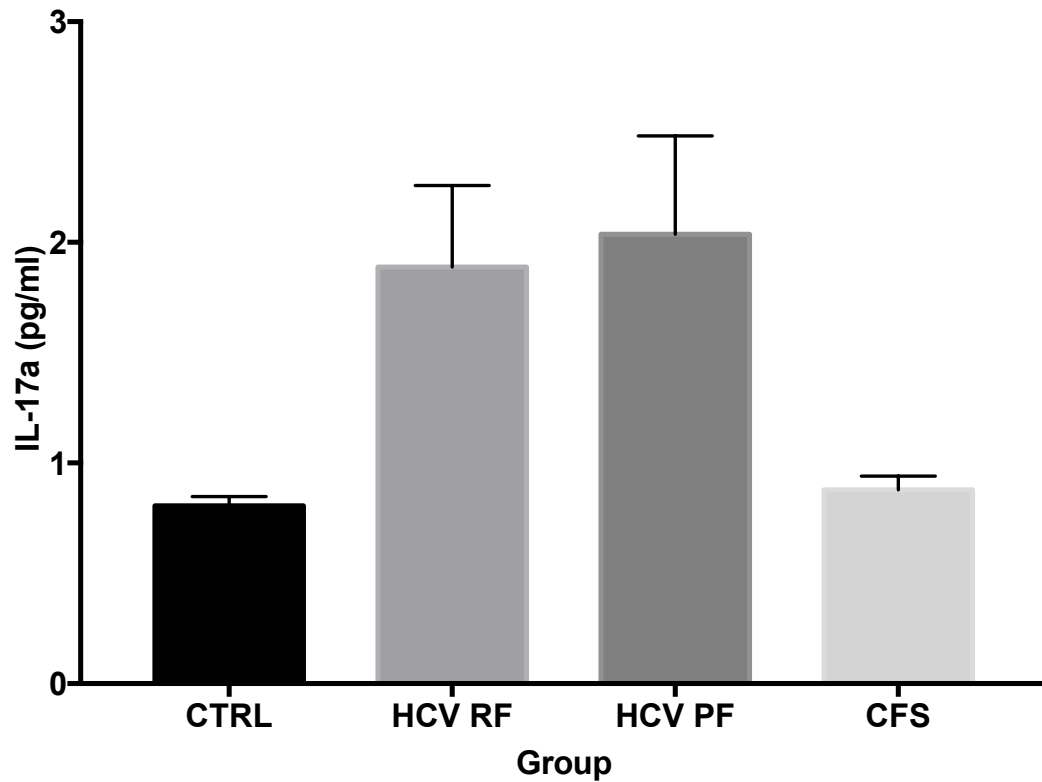


Figure 3.67 Mean Interleukin-17A levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

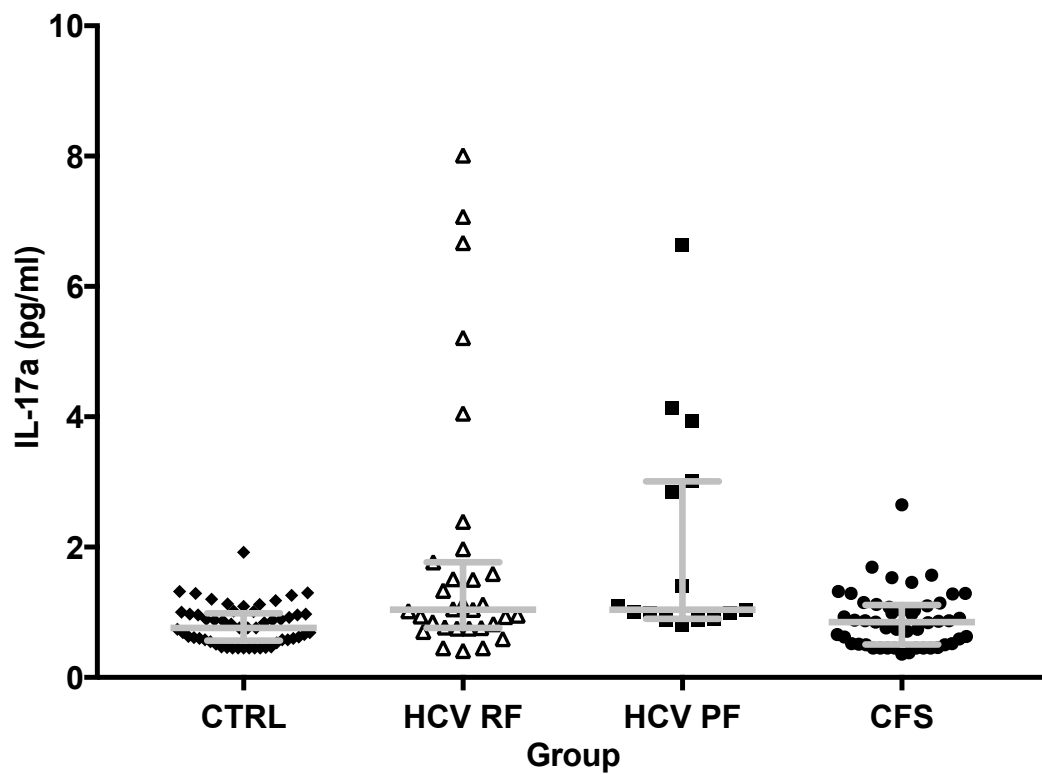


Figure 3.68 Interleukin-17A levels (with Median and IQR) in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

Tumour Necrosis Factor-alpha (TNF- α)

There were no results in any of the four groups that fell outside of the cut-off of three times the interquartile range. Data was not normally distributed in any of the four groups. The assumption of the homogeneity of variance was violated. Welch's ANOVA showed that there was a significant difference in mean TNF- α levels between groups (Welch's $F(3,47.26) = 9.41, p < 0.001$). Levels were lowest in the healthy control group (2.54 ± 0.07) and CFS patient group (2.61 ± 0.09), and then higher in the HCV RF (4.57 ± 0.49) and PF groups (5.09 ± 0.71). Post-hoc Games-Howell tests showed that levels were significantly higher in HCV RF patients versus CFS ($p = 0.002$) and healthy control participants ($p = 0.001$). Likewise, levels were higher in PF patients when compared with the same groups ($p = 0.015$ and 0.013 respectively). There were no differences between CFS and Control groups, or between the two HCV groups (see Figure 3.69). Further post-hoc comparisons yielded the same results (see Table 3.62). See also Figure 3.70 for the distribution of TNF- α levels, and median and IQR.

Table 3.62 TNF- α : Post-hoc parametric and non-parametric comparisons

Comparison	Mean \pm SEM		Test and statistic
HCV RF vs PF	4.57 \pm 0.49	5.09 \pm 0.71	$t(48) = -0.60, p = 0.55$
HCV RF vs CFS	4.57 \pm 0.49	2.61 \pm 0.89	$t(35.05) = 3.91, p < 0.001$
HCV RF vs CTRL	4.57 \pm 0.49	2.54 \pm 0.07	$t(34.25) = 4.07, p < 0.001$
HCV PF vs CFS	5.09 \pm 0.71	2.61 \pm 0.89	$t(15.46) = 3.48, p = 0.003$
HCV PF vs CTRL	5.09 \pm 0.71	2.54 \pm 0.07	$t(15.28) = 3.59, p = 0.003$
CFS vs CTRL	2.61 \pm 0.89	2.54 \pm 0.07	$t(108) = 0.63, p = 0.53$
Median			Test and statistic
HCV RF vs PF	3.39	3.73	$U = 241, z = -0.64, p = 0.52$
HCV RF vs CFS	3.39	2.48	$U = 372, z = -4.60, p < 0.001$
HCV RF vs CTRL	3.39	2.38	$U = 356, z = -5.03, p < 0.001$
HCV PF vs CFS	3.73	2.48	$U = 152, z = -3.87, p < 0.001$
HCV PF vs CTRL	3.73	2.38	$U = 142, z = -4.19, p < 0.001$
CFS vs CTRL	2.48	2.38	$U = 1416, z = -0.57, p = 0.57$

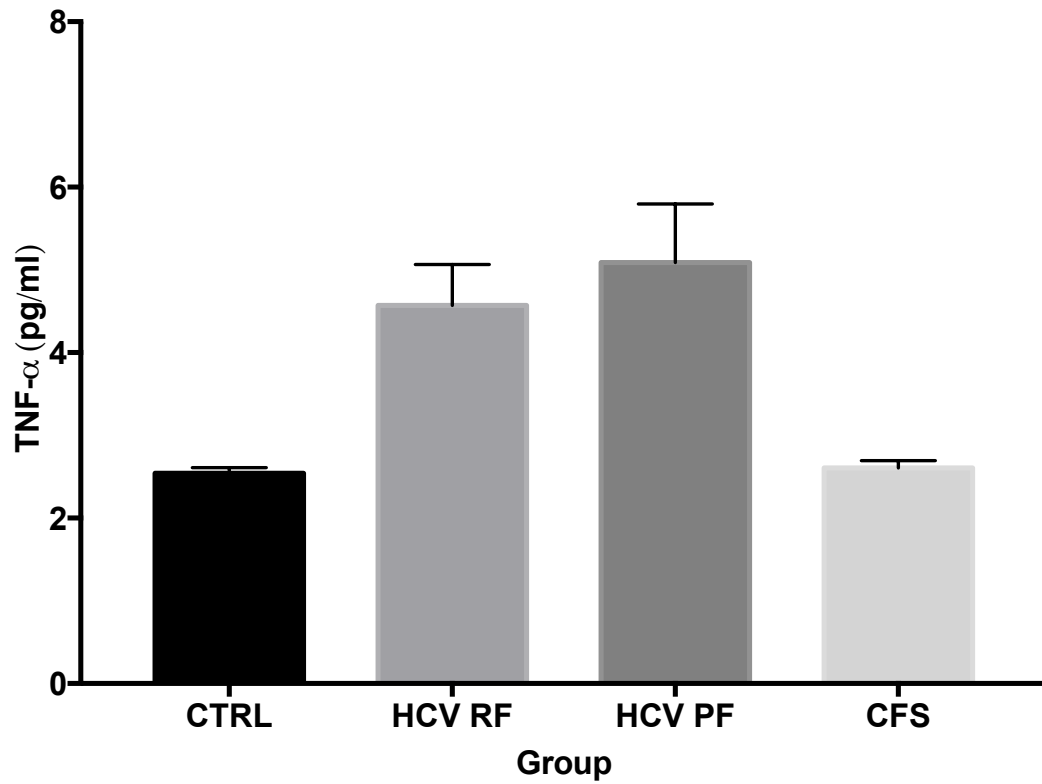


Figure 3.69 Mean TNF-α levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

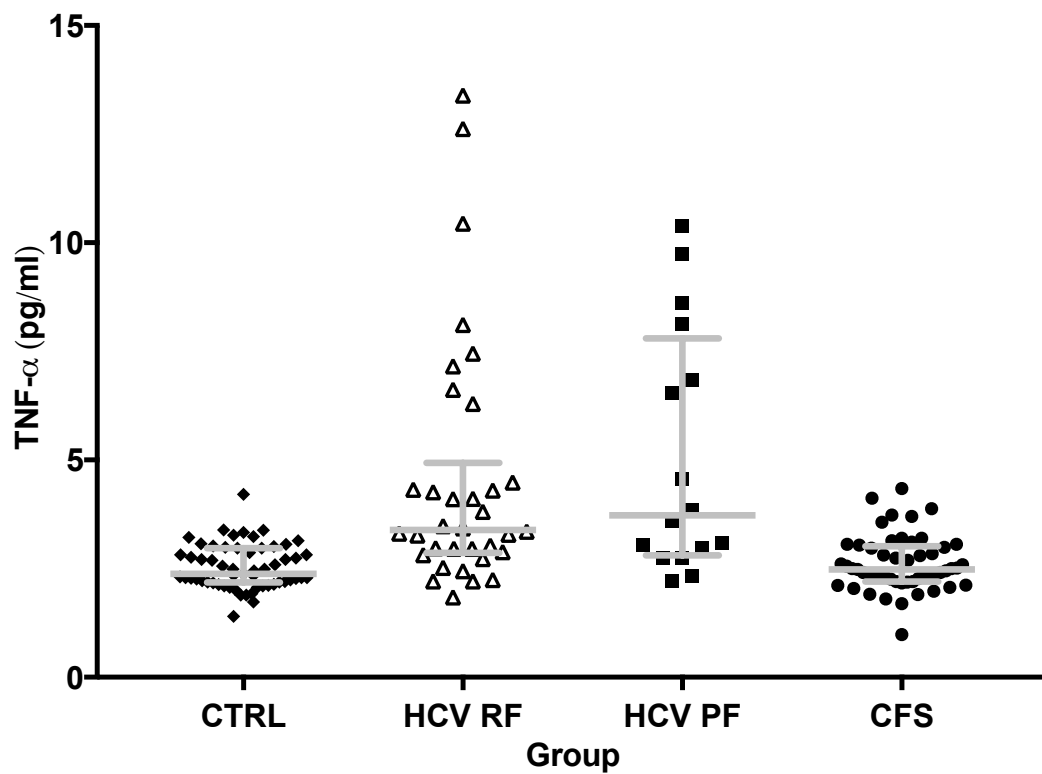


Figure 3.70 TNF-α levels (with Median and IQR) in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

3.2.5.2 Cytokines: effect of CFS specific illness characteristics

Previous studies had found an effect of the duration of CFS symptoms on cytokine levels. Because of the non-normal distribution of the cytokine levels in this group, Spearman's Rho correlations were performed. With outliers excluded, as described above, there was no significant association between the duration of symptoms (months) and any of the markers examined. There were, however, a trend towards lower levels of IL-17A ($r_s = -0.24$, $p = 0.099$) and higher levels of IL-7 ($r_s = 0.24$, $p = 0.085$) in patients who had been ill for longer. A second analysis using independent samples t-tests, which dichotomised CFS patients into those who reported having experienced symptoms for three years or less ('recent onset') versus those who reported having had symptoms for longer than three years did not find any differences in any of the cytokines measured (data not shown).

3.2.5.3 Cytokines: conclusion

With regards to the Cross-Sectional analysis of cytokines, data was available for eight markers. Group differences were found in six cytokines: IL-10, IL-6, IL-8, TNF- α , IL-17A and IL-7. There was no difference in IL-2 or IL-13. Where group differences were observed, this was largely attributable to two clusters: HCV PF and RF patients, where levels of inflammatory markers (both those typically considered 'pro-' and 'anti-') were higher, though not dissimilar from each other, and then healthy control and CFS patients, where levels were lower, and again were similar between these two groups. Finally, there was no link between cytokine levels and the duration of symptoms reported by CFS patients.

3.2.5.4 Kynurenine pathway: levels

All of the kynurenine pathway metabolites were compared in the four groups, each in a one-way ANOVA. First, boxplots were examined for HCV patients, CFS patients and healthy controls, and any result that was identified as being outside of the range of \pm three times the interquartile range for each marker was excluded from the analyses. Since a series of one-way ANOVAs were performed, participants were excluded on a test-by-test basis only; further details are provided below. Where non-normal distribution in one or more groups was identified, attempts were made to transform the data. Logarithmic transformation (Log_{10}) was again found to be the most appropriate method for this data set, as assessed by the Shapiro-Wilk normality test. Some exceptions are noted below.

Tryptophan

There were no outliers for levels of tryptophan in any of the groups. Data was normally distributed in all groups except for the healthy control group. The assumption of homogeneity of variance was met. There was a significant difference between the groups in levels of tryptophan ($F(3,148) = 11.73$, $p < 0.001$, partial $\eta^2 = 0.19$). Examination of the mean levels showed levels to be highest in the HCV Resolved Fatigue (18605.52 ± 556.06), and HCV Persistent Fatigue groups (18285.62 ± 941.48), then CFS (15966.20 ± 396.82) and healthy control groups (15140.19 ± 347.49). Post-hoc Tukeys HSD tests showed the two HCV groups to have similar levels, as were CFS and healthy control participants. Levels were higher in HCV RF versus CFS ($p = 0.001$) and healthy controls ($p < 0.001$), and in HCV PF versus CFS ($p = 0.047$) and healthy controls ($p = 0.003$). See Figure 3.71.

As described above, data was not normally distributed in the healthy control group. After logarithmic transformation, levels of tryptophan were normally distributed in all four groups. There were no outliers \pm three times the interquartile range. The assumption of the homogeneity of variances was met. Repeating the ANOVA on the transformed data confirmed the difference between groups ($F(3,148) = 11.75, p < 0.001$).

Kynurenine/Tryptophan ratio

No results were identified as outliers. Data was normally distributed in the HCV PF and healthy control groups ($p > 0.05$), but not in the HCV RF or CFS groups ($p < 0.05$). The assumption of homogeneity of variance was met ($p > 0.05$). There was a significant difference between the four groups in the ratio of Kynurenine to Tryptophan ($F(3,148) = 4.68, p = 0.004, \text{partial } \eta^2 = 0.09$). Levels were highest in the HCV RF (2.26 ± 0.10), and healthy control groups (2.16 ± 0.06), then the HCV PF groups (2.14 ± 0.10), and CFS patients (1.90 ± 0.07). Tukeys HSD showed levels to be significantly lower in CFS patients versus healthy controls ($p = 0.020$) and HCV RF patients ($p = 0.006$), but not compared with HCV PF patients. See Figure 3.72. Log-transformed data was normally distributed in all four groups. There were no outliers \pm three times the interquartile range. The assumption of the homogeneity of variances was met. The analysis of the transformed data confirmed the difference between groups in levels of the KYN/TRP ratio ($F(3,148) = 5.60, p = 0.001$).

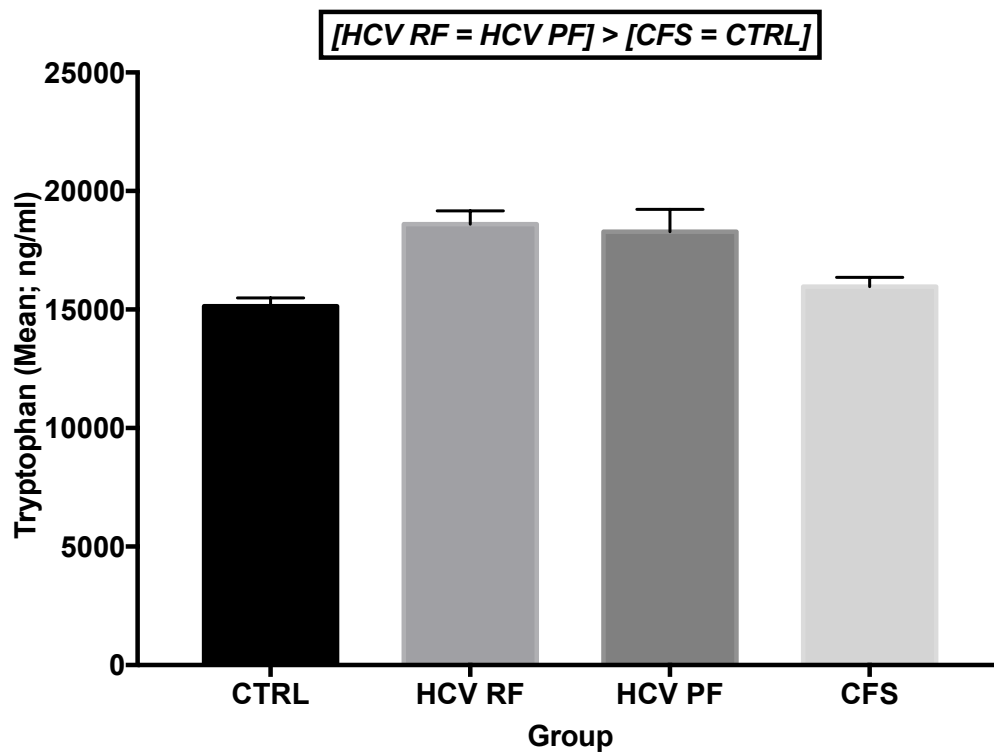


Figure 3.71 Mean tryptophan levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

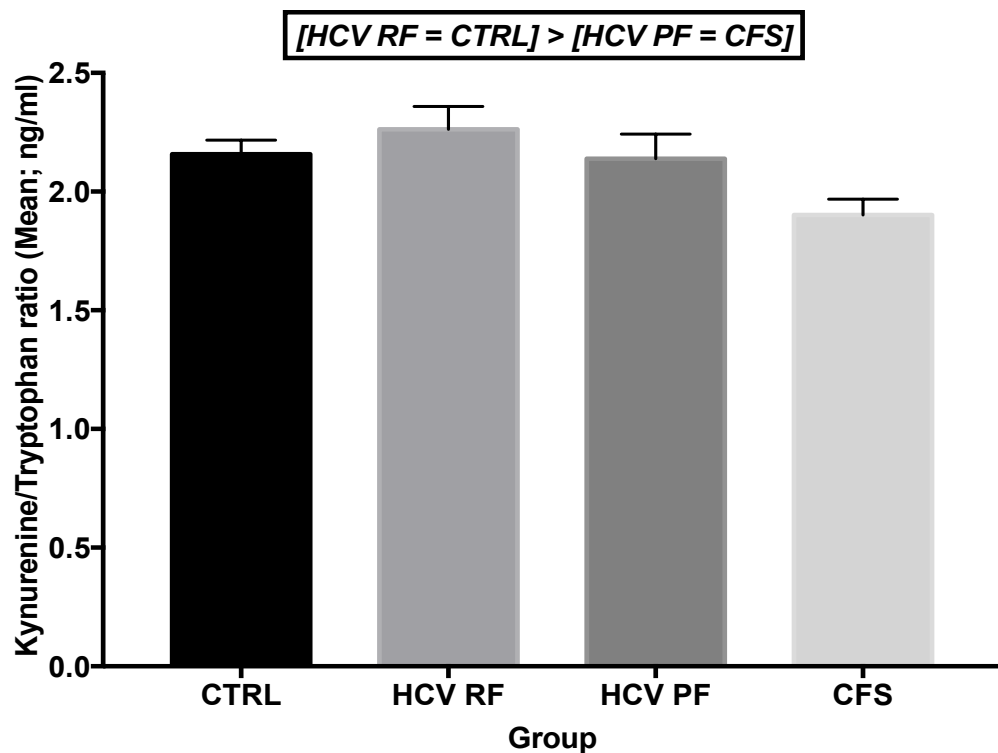


Figure 3.72 Mean values for the KYN/TRP ratio in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

Kynurenic acid

There were no patients in any of the groups that were identified as outliers, according to the criteria of \pm three times the interquartile range. Data was normally distributed in all groups, except for the healthy control group. The assumption of homogeneity of variance was met. Results from the ANOVA showed that there was a significant difference in kynurenic acid between the four groups ($F(3,148) = 6.04$, $p = 0.001$, partial $\eta^2 = 0.11$). Examination of the means showed that levels were highest in the HCV Resolved Fatigue group (Mean \pm SEM ng/ml; 9.60 ± 0.57), followed by the HCV Persistent Fatigue (8.63 ± 0.85) and the healthy control participants (8.08 ± 0.38). The lowest levels were found in the CFS patients (6.93 ± 0.34). Post-hoc Tukeys HSD tests showed the difference between the HCV RF group and the CFS patients to be significant ($p<0.001$). There was a trend towards higher levels in the HCV RF versus healthy control participants ($p = 0.082$). See Figure 3.73. Log-transformed data was normally distributed. There were no outliers. The assumption of the homogeneity of variances was met. The ANOVA on the transformed data confirmed the difference between groups ($F(3,148) = 6.15$, $p = 0.001$, partial $\eta^2 = 0.11$).

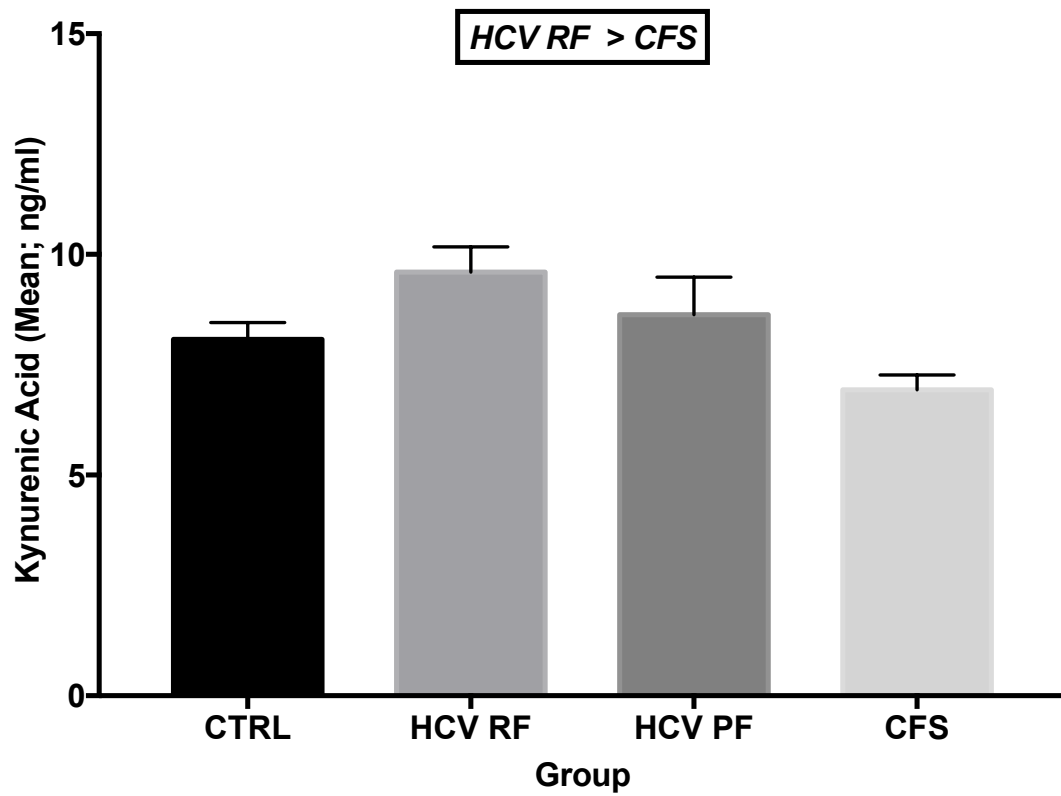


Figure 3.73 Mean kynurenic acid levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

Quinaldic Acid

No patients in the HCV groups were identified as outliers. Nor were there any outliers in the CFS data, though one individual was identified as an outlier in the healthy control group. Data was normally distributed in the HCV PF group, but not normally distributed in the HCV RF, CFS or healthy control groups. An ANOVA excluding the outlier was run. The assumption of homogeneity of variance was met. There was no significant difference between groups in levels of quinaldic acid ($F(3,147) = 0.05$, $p = 0.99$, partial $\eta^2 = 0.001$). Due to concerns about distribution, data was transformed. Though different methods were applied, levels of quinaldic acid in the healthy controls remained positively skewed, and so logarithmic transformation was performed as the method chosen for its success with other variables. After transformation, data was normally distributed in the other three groups (HCV RF; PF; CFS patients). There were no outliers \pm three times the interquartile range in any group. The assumption of the homogeneity of variances was met. The ANOVA on the transformed data again showed levels of quinaldic acid to be similar across the four groups ($F(3,148) = 0.04$, $p = 0.99$, partial $\eta^2 = 0.001$).

3-Hydroxykynurenine (3-HK)/Kynurenine ratio

There were no outliers in any of the groups. Data was normally distributed in the HCV PF group, but not in the HCV RF, CFS or healthy control groups. The assumption of homogeneity of variance was violated. There was no significant difference in levels of the 3-HK//Kyn ratio (Welch's $F(3,151) = 2.91$, $p = 0.083$). Due to the positive skew of the data, the logarithmic transformation was applied. Transformed data was normally distributed in all four groups. There were no outliers \pm three times the interquartile range. The assumption of the

homogeneity of variances was still violated. In this case, the Welch's ANOVA of the transformed data showed that there was a significant difference between groups in levels of the ratio of 3-HK to Kynurenine (Welch's $F(3, 41.87) = 3.01$, $p = 0.041$). Games-Howell post-hoc tests on the transformed data showed the only significant difference to be between the CFS and healthy control participants, with higher levels of the ratio in healthy controls (raw data: Mean \pm SEM ng/ml; 2.00 ± 0.09 vs. 1.71 ± 0.11).

3-Hydroxykynurenine (3-HK)

One HCV patient was identified as an outlier, in the Resolved Fatigue group. So too was one CFS patient and one healthy control participant. With these outliers excluded, data was normally distributed in the CFS patients, but not normally distributed in the HCV groups or the healthy control participants. The assumption of homogeneity of variance was violated. Welch's ANOVA showed that there was a significant difference between groups (Welch's $F(3, 39.92) = 9.31$, $p < 0.001$). Examination of the means showed levels to be highest in HCV RF (8.95 ± 0.96) and PF patients (8.88 ± 1.57), then healthy controls (6.30 ± 0.28) and CFS patients (4.82 ± 0.29). Games-Howell post-hoc tests revealed levels in HCV RF patients to be significantly higher than CFS patients ($p = 0.001$), and a trend towards higher levels than healthy control participants ($p = 0.058$). There was a trend towards higher levels in the HCV PF group than CFS patients ($p = 0.099$). Levels of 3-HK were also significantly higher in healthy controls versus CFS patients ($p = 0.002$). See Figure 3.74. Log-transformed data was normally distributed. There were no outliers. The assumption of the homogeneity of variances was still violated. A Welch's ANOVA on the transformed data confirmed the difference in 3-HK levels between the four groups (Welch's $F(3, 42.496) = 8.603$, $p < 0.001$).

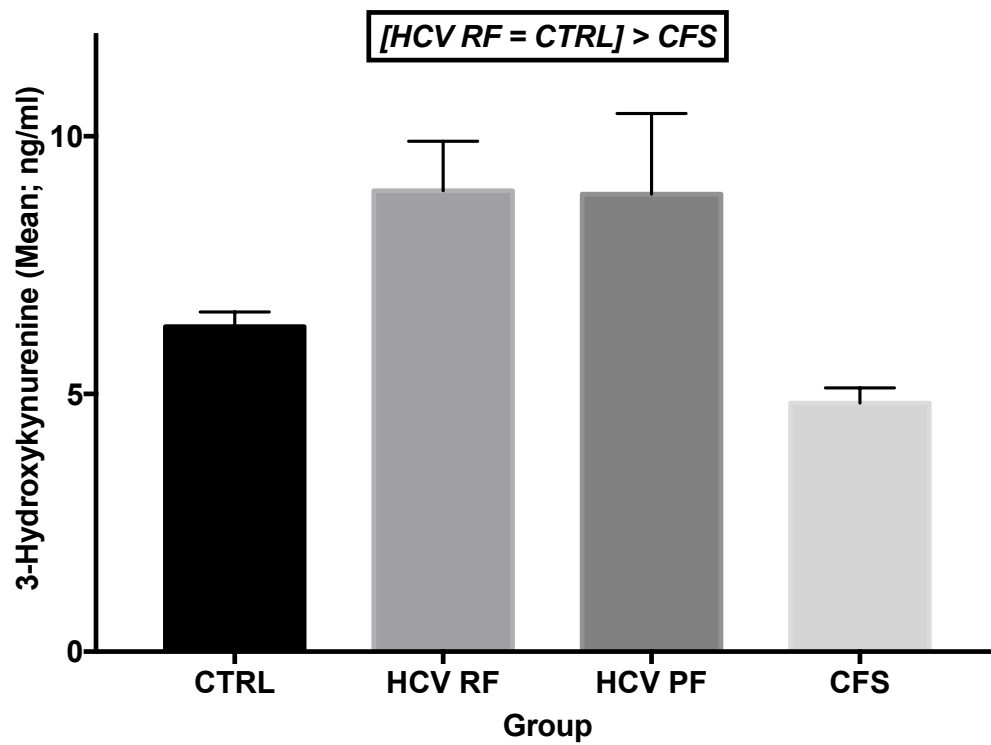


Figure 3.74 Mean 3-HK levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

Xanthurenic acid

There were no participants who were identified as outliers. Data was not normally distributed in any of the four groups. The assumption of homogeneity of variance was met. The ANOVA showed that there were no significant differences in xanthurenic acid levels between the four groups ($F(3,148) = 4.45, p = 0.41, \text{partial } \eta^2 = 0.02$). Log-transformed data was normally distributed in all four groups. There were no outliers. The assumption of the homogeneity of variances was met. The ANOVA on the transformed data confirmed that there was no group difference ($F(3,148) = 0.82, p = 0.49, \text{partial } \eta^2 = 0.02$).

Picolinic acid

No outliers were identified. Data was normally distributed in the HCV PF group but not in the HCV RF, CFS or healthy control groups. The assumption of homogeneity of variance was violated. Welch's ANOVA was used, and showed that picolinic acid levels were different between the four groups (Welch's $F(3,39.84) = 14.80, p > 0.001$). Examination of the means showed levels to be highest in HCV PF patients (68.77 ± 9.31), then HCV RF patients (54.97 ± 5.94), and healthy control participants (26.52 ± 1.20) and CFS patients (24.99 ± 0.99). Post-hoc Games-Howell tests indicated that levels were significantly higher in HCV PF patients than healthy controls ($p = 0.003$) and CFS patients ($p = 0.002$). The same was true of HCV RF (both results $p < 0.001$). See Figure 3.75. Log-transformed data was normally distributed in the HCV groups and CFS patients, though remained positively skewed in healthy controls. Other methods tried were not successful. There were no outliers. The assumption of the homogeneity of variances was violated. The Welch's ANOVA of transformed data confirmed a group difference (Welch's $F(3, 41.86) = 23.58, p < 0.001$).

Quinolinic acid

There were no outliers identified in the HCV or CFS patient groups. There was one individual in the healthy control group who had a result outside of the criteria for outliers. With the outlier excluded from the analysis, data was normally distributed in the HCV RF group and the healthy controls, but not in the HCV PF or CFS groups. The assumption of homogeneity of variance was violated. The Welch's ANOVA showed that levels of quinolinic acid were significantly different between the four groups (Welch's $F(3,42.91) = 19.93$, $p < 0.001$). Examination of the means showed levels to be highest in the HCV PF (68.85 ± 5.33) and RF patients (64.70 ± 2.83), then CFS patients (46.30 ± 1.83) and healthy controls (43.32 ± 1.43). Post-hoc Games-Howell tests showed levels to be higher in HCV PF patients versus CFS ($p = 0.006$) and healthy control participants ($p = 0.002$). The same was true of RF patients (both $p < 0.001$ respectively). Levels were similar in the two HCV groups, and also in the CFS and healthy control groups. See Figure 3.76. Log-transformed data was normally distributed, and there were no outliers. The assumption of the homogeneity of variances was met. Analysis of the transformed data confirmed the group difference in levels quinolinic acid ($F(3,149) = 21.53$, $p < 0.001$).

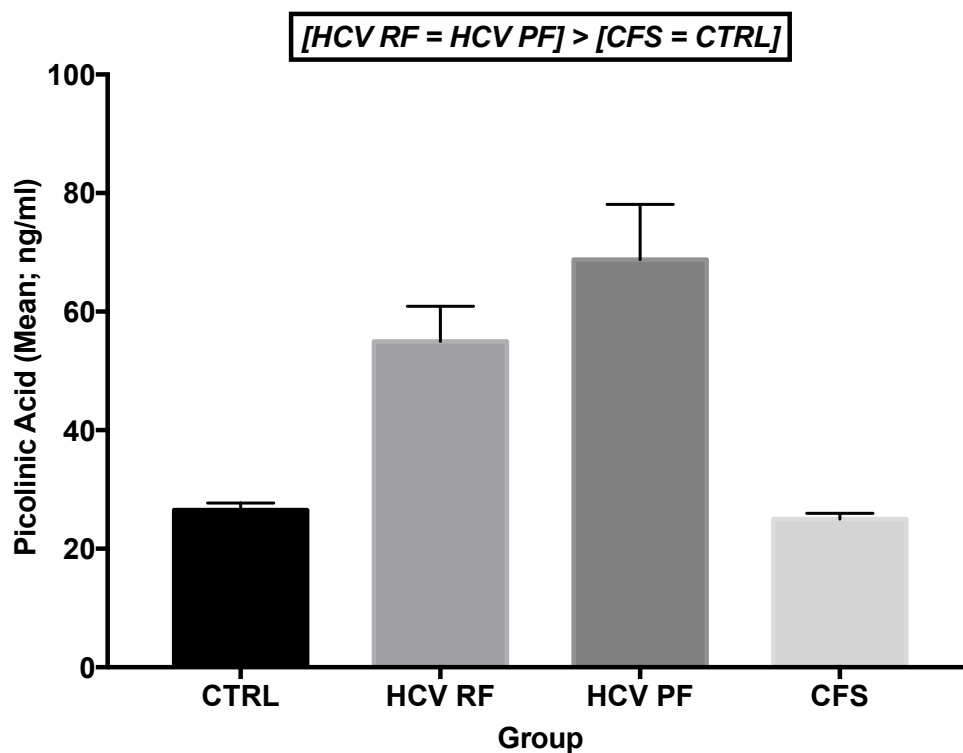


Figure 3.75 Mean picolinic acid levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

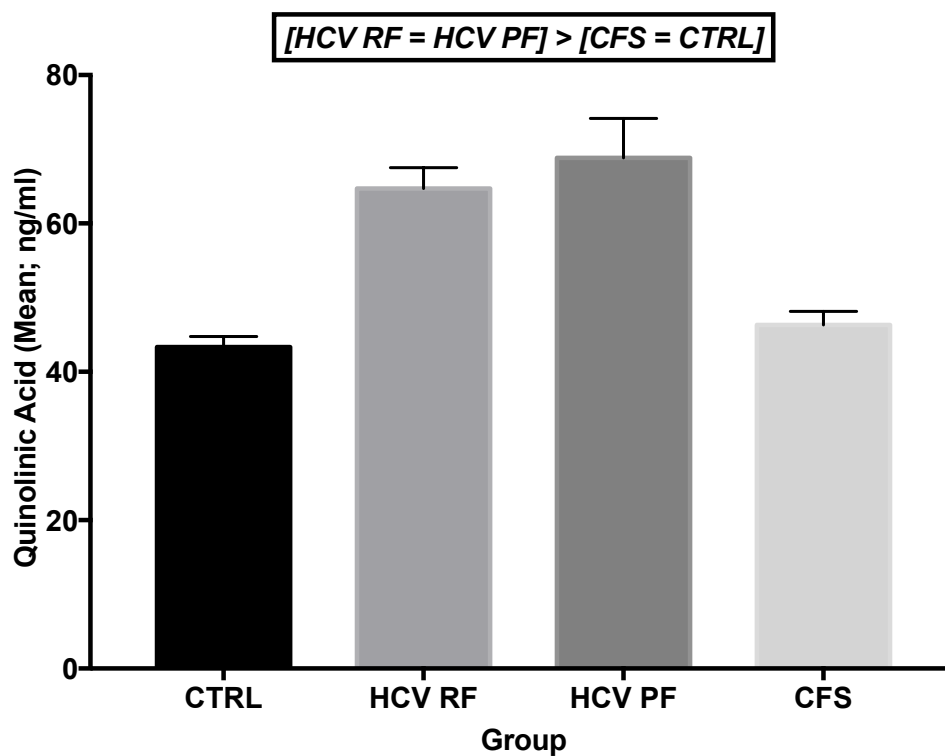


Figure 3.76 Mean quinolinic acid levels in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

3.2.5.5 Kynurenine pathway: effect of CFS specific illness characteristics

Due to the significance of illness duration on cytokine patterns found by other authors, I also explored an effect of CFS illness duration on kynurenine pathway metabolites. First I conducted correlational analyses with each marker and duration of symptoms in months. There were no significant associations. There were trends towards higher levels of the ratio of kynurenine to tryptophan ($r_s = 0.26$, $p = 0.07$) and quinaldic acid ($r_s = 0.27$, $p = 0.05$) in those with a longer duration of illness. Data was available in 20 patients with an illness lasting three years or less, and 31 patients with an illness lasting longer than three years. Again, there were no significant differences between these two groups. There was a trend towards higher levels of picolinic acid in those with a more recent onset (27.5 ± 2.1 vs. 23.3 ± 1.0 , $t(49) = 1.84$, $p = 0.08$). The result was adjusted since equal variance could not be assumed; data was normally distributed in those with a shorter illness duration, though slightly positive skewed in those ill for longer than three years.

3.2.5.6 Cortisol: levels

Finally, I examined group differences in the two cortisol measurements: awakening response and diurnal output. As per the methods reported earlier, where samples were missed or empty, or there was poor compliance with the protocol either in general, or at the relevant time points for each calculation, these patients were excluded from the analysis. This resulted in some patients being included for only one analysis of either the Awakening Response (AUCi) or diurnal output (AUCg) calculations. Therefore, relevant mean group cortisol levels have been reported separately, as they relate to each measure. To depict the response to awakening, delta values were calculated per time point (+15; +30; +60) relative to the level of the sample collected on awakening for each individual (see Figure 3.77). There were no differences between groups in the Cortisol Awakening Response ('CAR'; see Table 3.63), as calculated using the AUCi method. However, there was a trend towards an effect of group on diurnal cortisol output ($p = 0.065$) (see also Table 3.63; Figure 3.78). Post-hoc tests revealed a significant difference between the CFS and Control groups, with a lower diurnal cortisol output in CFS patients (Mean \pm SEM; 2993.4 \pm 220.7 vs. 4438.4 \pm 473.4).

Table 3.63 Cortisol measurements in healthy control (CTRL), HCV Resolved Fatigue (RF) and Persistent Fatigue (PF), and CFS groups

Cort measure	CTRL (n = 48)	HCV RF (n = 13)	HCV PF (n = 10)	CFS (n = 37)	Test and statistic
Awakening Response (AUC _i nmol min/l)	80.6 ± 35.8	185.3 ± 105.2	65.8 ± 75.1	129.6 ± 35.8	$F(3,100)=0.74, p=0.53$
<i>Levels (nmol/L)*</i>					
Awakening	11.8±0.8	10.2±1.6	9.3±2.3	8.5±0.8	
+15 min	14.5±0.8	12.7±1.2	10.5±2.1	11.6±0.7	
+30 min	14.5±0.9	15.3±1.8	11.2±1.8	12.2±0.6	
+60 min	10.3±0.6	12.5±3.0	9.6±1.5	8.4±0.5	
Diurnal Output (AUC _g nmol h/l)	4438.4 ± 473.4	3743.8 ± 427.8	4089.1 ± 439.3	2993.4 ± 220.7	$F(3,100)=2.48, p=0.065$
					(CTRL > CFS, p=0.008)
<i>Levels (nmol/L)*</i>					
Awakening	11.5±0.8	9.2±1.4	10.1±2.5	8.3±0.8	
12pm	6.3±1.1	4.6±0.6	5.3±1.0	4.7±0.5	
8pm	2.4±0.3	2.9±1.0	2.8±0.5	1.7±0.2	

* **Note** – levels reported reflect group in which (i) awakening response and (ii) diurnal output were measured, which differ slightly (n = 46; 13; 8; 37 and 46; 13; 10; 35 respectively)

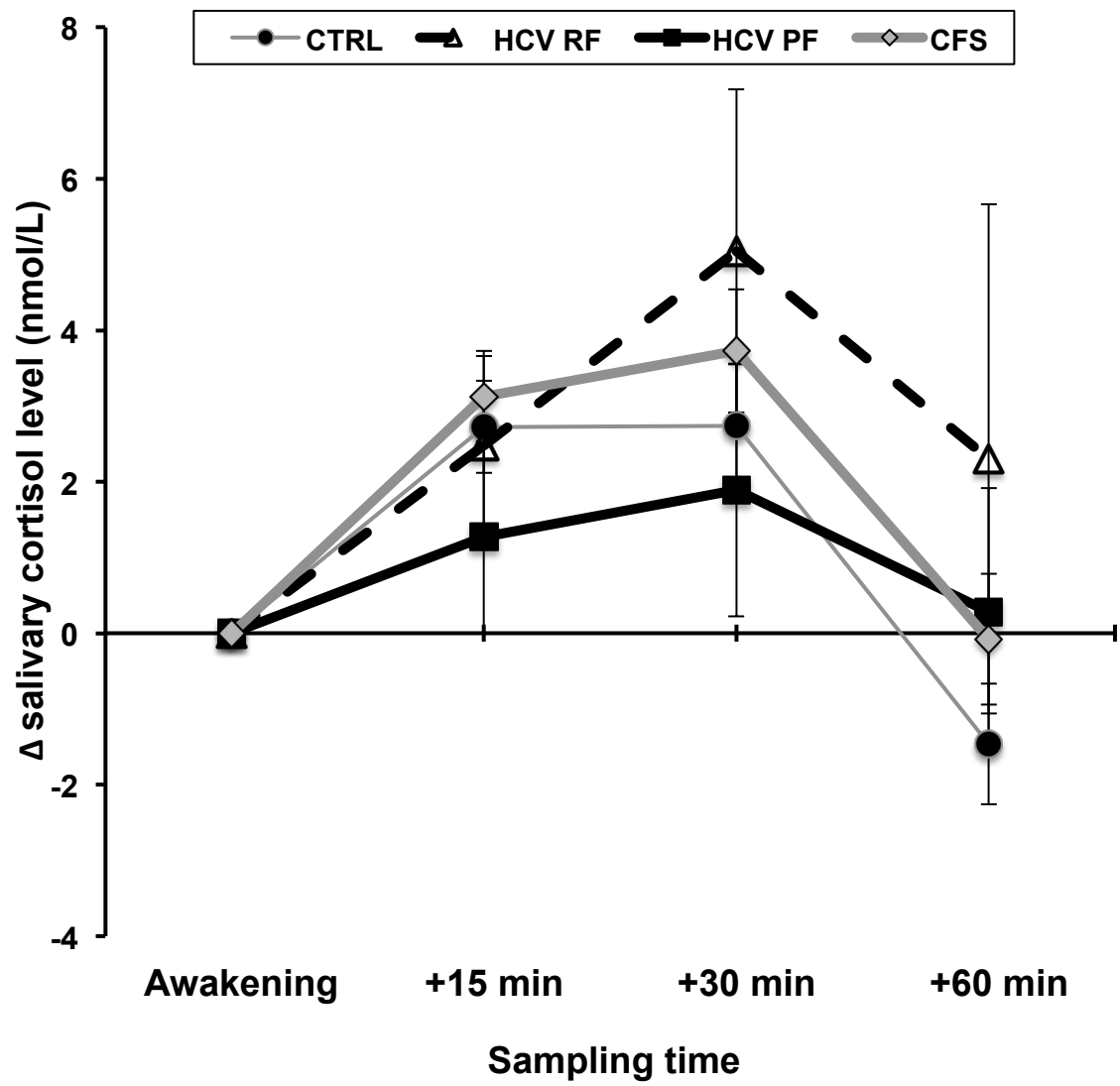


Figure 3.77 The change in cortisol levels from awakening ('CAR'), in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

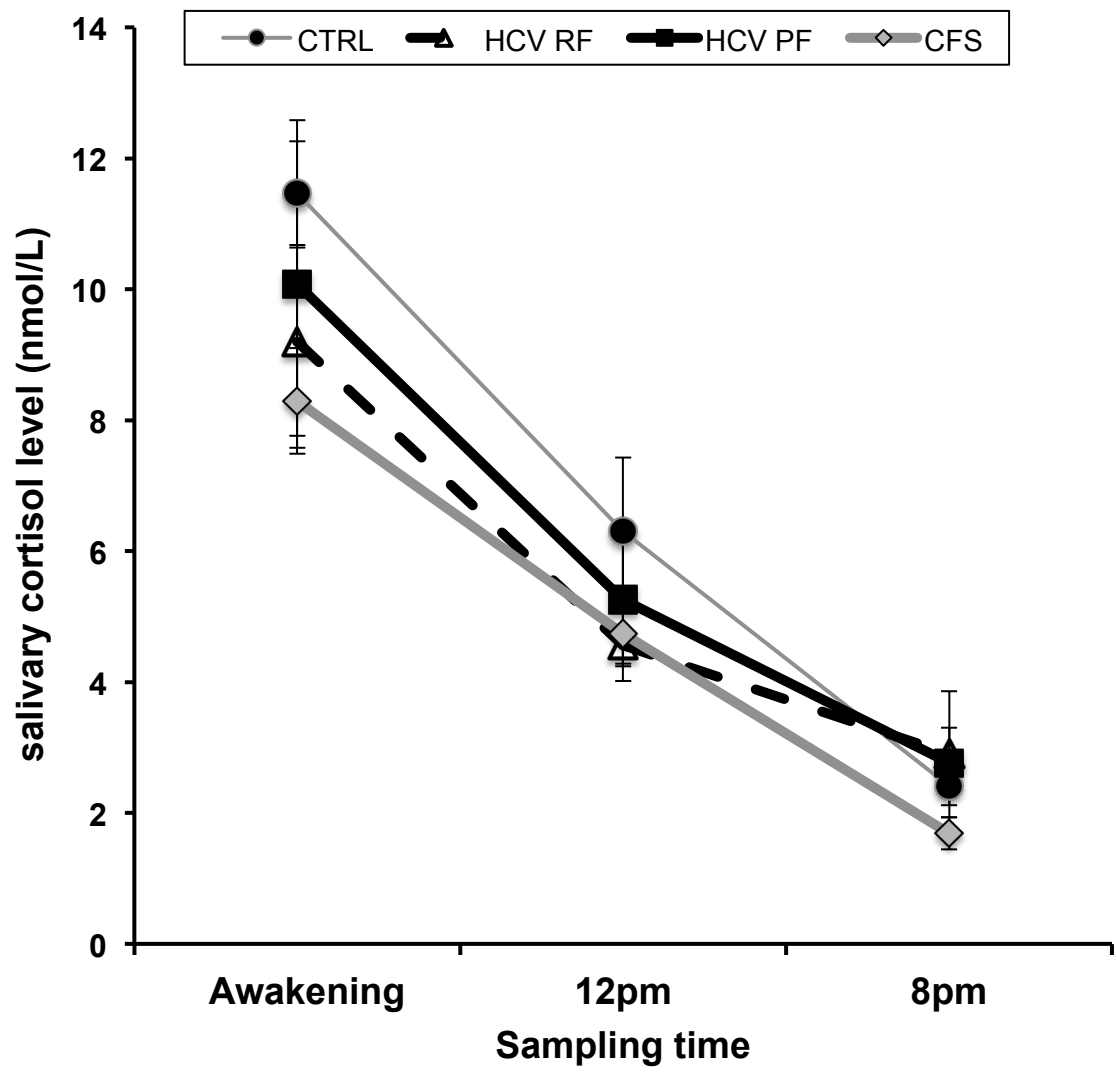


Figure 3.78 Diurnal cortisol in healthy controls (CTRL), HCV RF and PF groups, and CFS patients

3.2.5.7 Cortisol: effect of CFS specific illness characteristics

Again, in light of the recent findings of differential patterns of biological markers according to illness duration, I also explored an association between the two measures of cortisol and (i) CFS illness duration in months (ii) illness duration dichotomised ≤ 3 years $>$. Data was available for CAR in 15 patients who had had CFS for three years or less, and 21 patients who had been ill for longer. Data on the diurnal output was available for 14 and 20 patients respectively. Interestingly, there was no association between illness duration in months and either the cortisol awakening response ($r_s = 0.10$, $p = 0.58$) or the diurnal output ($r_s = -0.17$, $p = 0.35$). Nor was there a difference in either measure between those with three years of less illness, or more than three years (CAR: ≤ 3 ys vs. > 3 ys, Mean \pm SEM; 121.6 ± 54.5 , $t(34) = -0.21$, $p = 0.84$; DAY: 3369.9 ± 450.0 vs. 2793.9 ± 209.7 , $t(32) = 1.28$, $p = 0.21$).

3.2.6 Psychological responses to fatigue

Two measures were used to explore cognitions and behaviours surrounding fatigue: acceptance of fatigue, measured in all four groups, and the Cognitive and Behavioural Responses to Symptoms Questionnaire (CBSQ), which being symptom focussed was only completed by the patient groups. Both measures were introduced later on, and so the small sample size should be noted, particularly with the CBSQ. All data is shown in Table 3.64. As before, a series of one-way ANOVAs were performed for each marker or measure. Where the Levene's test result indicated that the assumption of the homogeneity of variances could be assumed ($p>0.05$), an ANOVA and Tukeys HSD post-hoc tests were performed. Where it was violated ($p<0.05$), results for the Welch's ANOVA are reported, and Games-Howell post-hoc comparisons.

'Acceptance of fatigue' scores were not normally distributed in the HCV groups or healthy controls, where data was positively skewed. In the CFS group, data was more normally distributed, though there was a slight negative skew towards higher scores in these patients. There were no outliers in the HCV or CFS groups. In the healthy controls, four participants reported higher scores that could be considered as outliers, since they were higher than three times the interquartile range. The assumption of the homogeneity of variances was violated. Welch's ANOVA showed that scores were significantly different between groups. Games-Howell tests showed that CFS patients had significantly higher scores on the measure than any other group, indicating a lack of acceptance of fatigue. There were no other significant differences. However, it should be noted that the mean difference between HCV PF and healthy controls was 10.1 points on the scale, which may have been significant in a larger sample.

For the first of the cognitive domains of the **CBSQ**, '**Fear avoidance**' beliefs, scores were normally distributed in all three groups. There were no outliers. The assumption of homogeneity of variances was met. There was a difference between groups, with higher fear avoidance beliefs in CFS versus both HCV patient groups. '**Catastrophising**' beliefs scores were also normally distributed in all groups. There was one outlier, in the HCV RF group, with a higher score than their peers. The assumption of the homogeneity of variances, however, was violated ($p = 0.036$). Welch's ANOVA revealed a difference between groups, with higher catastrophising beliefs in CFS patients compared to the other two groups. '**Damage beliefs**' were normally distributed in HCV RF patients, but not in the PF or CFS groups. In the PF patients, data was positively skewed, and in the CFS patients, slightly negatively skewed. There were no outliers. The assumption of the homogeneity of variances was, however, met. In this case, there was a group difference, though only between CFS patients and the HCV RF group, with HCV PF patients in between.

For scores relating to '**embarrassment avoidance**', data was normally distributed in the HCV PF and CFS groups, but not in the HCV RF groups, where data was slightly positively skewed. There were no outliers. The assumption of homogeneity of variances was met, and a group difference was found, with higher embarrassment avoidance beliefs in the CFS group versus HCV RF patients. Scores for '**symptom focussing**' were normally distributed in all three groups. There were no outliers. The assumption of homogeneity of variances was met, and a group difference found. CFS patients had significantly higher levels of symptom focussing than HCV RF patients, and there was a trend towards higher levels than HCV PF patients.

The first of the two behavioural subscales was **‘All or nothing behaviour’**. Scores were normally distributed in all three patient groups. There were no outliers. The assumption of homogeneity of variance was met. There was a significant difference between groups, with significantly higher scores in the CFS versus HCV RF group, and HCV PF patients in the middle. Scores relating to the second behavioural domain, **‘Avoidance/Resting behaviours’** were also normally distributed across the three groups. There were no outliers. The assumption of homogeneity of variances was met. There was a significant difference between groups, and post-hoc tests showed levels to be significantly higher in CFS versus HCV PF and RF groups. Levels were similar across the two HCV groups.

In summary, CFS patients had higher scores across the measures of cognitive and behavioural responses to fatigue, as measured by the CBSQ and the acceptance of fatigue questionnaire. In this smaller sample, HCV PF patients often reported scores in the middle, revealing stronger beliefs and some degree of behaviour adaptation to their symptoms and/or side effects, but not enough to distinguish them from either their HCV peers or the CFS patient group. Examples were ‘damage’ and ‘embarrassment avoidance’ beliefs, as well as ‘all or nothing’ behaviour. Other measures, ‘catastrophising’ and ‘fear avoidance’ beliefs, to some extent ‘symptom focussing’ and ‘avoidance/resting behaviours’ appeared to be specific to the experience of CFS in particular, where CFS patients reported significantly higher scores than both HCV groups.

Table 3.64 Acceptance of and responses to fatigue symptoms in the healthy control (CTRL), HCV Resolved (RF) and Persistent Fatigue (PF), and CFS groups

Measure	CTRL (<i>n</i> = 57)	HCV RF (<i>n</i> = 25)	HCV PF (<i>n</i> = 13)	CFS (<i>n</i> = 51)	Test and statistic
Acceptance of Fatigue*	4.3±1.3	9.4±2.3	14.4±4.2	31.8±1.2	<i>F</i>(3,40.9)=83.6, <i>p</i><.001 <i>CFS</i> > <i>PF</i>, <i>p</i>=0.007 <i>CFS</i> > <i>RF</i>, <i>p</i><0.001 <i>CFS</i> > <i>CTRL</i>, <i>p</i><0.001 <i>PF</i> = <i>CTRL</i> , <i>p</i> =0.15 <i>PF</i> = <i>RF</i> , <i>p</i> =0.73 <i>RF</i> = <i>CTRL</i> , <i>p</i> =0.22
Measure	CTRL n/a	HCV RF (<i>n</i> = 13)	HCV PF (<i>n</i> = 5)	CFS (<i>n</i> = 53)	Test and statistic
CBSQ					
Cognitive					
Fear avoidance	-	5.5±0.9	8.2±1.8	15.6±0.6	<i>F</i>(2,68)=35.1, <i>p</i><0.001 <i>CFS</i> > <i>PF</i>, <i>p</i>=0.001
Catastrophising*	-	3.5±0.6	3.8±1.2	8.6±0.5	<i>F</i>(2,10.6)=23.8, <i>p</i><.001 <i>CFS</i> > <i>PF</i>, <i>p</i>=0.033
Damage beliefs	-	8.5±0.6	8.4±1.6	11.5±0.5	<i>F</i>(2,68)=5.22, <i>p</i>=0.008 <i>CFS</i> = <i>PF</i> , <i>p</i> =0.14
Embarrassment Avoidance	-	5.9±1.6	9.2±3.0	12.8±0.7	<i>F</i>(2,68)=8.2, <i>p</i>=0.001 <i>CFS</i> = <i>PF</i> , <i>p</i> =0.37
Symptom Focussing	-	7.5±1.4	9.0±2.5	14.7±0.7	<i>F</i>(2,68)=11.3, <i>p</i><0.001 <u><i>CFS</i> = <i>PF</i>, <i>p</i>=0.059</u>
Behavioural					
All or nothing	-	4.7±1.1	6.8±1.4	9.9±0.7	<i>F</i>(2,68)=7.3, <i>p</i>= 0.001 <i>CFS</i> = <i>PF</i> , <i>p</i> =0.32
Avoidance/ resting	-	4.1±1.1	6.4±1.4	13.1±0.7	<i>F</i>(2,68)=18.9, <i>p</i><0.001 <i>CFS</i> > <i>PF</i>, <i>p</i>=0.016

* Welch's ANOVA and Games-Howell test results reported

3.2.7 Attribution of symptoms

The first question asked was 'which of the following best describes the nature of your symptoms' and this question had not been asked of healthy volunteers. Results given below are descriptive only (see Table 3.65). The second, set of questions was 'which of the factors do you think was important in having caused your fatigue or made it worse' (see appendix S; Table 3.66). Where at least one of the areas of fatigue detailed in the Chalder Fatigue Questionnaire has been experienced 'more' or 'much more than usual' in the last month, patients were asked to complete these questions. Where data is recorded as 'missed', these individuals did experience at least one problem with fatigue 'more than usual', but the attribution questionnaire was not completed, either because it was introduced later or because the measure was not completed. If no problem was identified, responses were recorded as 'not applicable'. Participants were asked to tick at least one response per item (not; maybe; definitely), but some data was missing (see below). The categories 'definitely' and 'might be a factor' were collapsed.

For the first measure regarding the nature of symptoms numbers were very low in the HCV groups as the questionnaire had again been introduced later in the study. However, somewhat unexpectedly, the largest proportion of CFS patients (54%) felt that their symptoms were evenly weighted between psychological and physical. **For the second measure**, 22/57 healthy controls (just under 40%) reported experiencing one or more problems with fatigue in the last month. Of the HCV RF group, 43% of patients had experienced at least one problem with fatigue, and 100% of the HCV PF had, though data was only available for 27% and 67% respectively. All CFS patients completed the questionnaire.

For attributions to infections, 78% of CFS patients felt that their problems with fatigue could be related to a viral infection, 20% to a current infection, and 35% to another (non-viral) infection. In the HCV PF group, the proportions were 33, 25 and 7 respectively. In the HCV RF group, the proportion of patients attributing fatigue to an infectious factor was higher, at 50, 40 and 20% respectively. **Treatment attributions** were highest in the HCV groups, with 40% of RF patients, and 33% of PF patients attributing fatigue to treatment. In the CFS group, 9% felt that treatment could have contributed to their fatigue. However, for the HCV group, the wording of the item as 'current treatment' may have led to an underestimation of the number of individuals attributing their fatigue to IFN- α , since they were no longer under treatment.

With regards to **emotional stress**, the proportion of patients in both HCV groups was around the same, at 40-42%. In the control group, levels were also similar at 32%. In the CFS group, the only group in which a diagnosis of depression had not been an exclusion criterion, rates were higher at 70%. Interestingly, this is similar to the proportion attributing fatigue to a previous viral infection. Measures of **social or environmental stress** included work and relationship stress. High proportions of the control, HCV Resolved Fatigue and CFS groups (64-72%) believed that their fatigue could be attributed to work stress. A much lower proportion of patients did in the HCV Persistent Fatigue group (33%). Rates for attribution of fatigue to relationship stress varied widely between groups, though CFS patients were most likely to believe this was a factor (52%). Based on the socio-demographic characteristics of the groups, there were no differences in unemployment or relationship status. Apart from one or two HCV patients, only CFS patients attributed their fatigue to **allergic causes** (food 33%; other 19%).

Table 3.65 'Nature of symptoms' responses in HCV Resolved Fatigue (RF) and Persistent Fatigue (PF), and CFS groups

<i>'Nature of symptoms'</i>	CTRL (<i>n</i> = 57)	HCV RF (<i>n</i> = 12)	HCV PF (<i>n</i> = 5)	CFS (<i>n</i> = 54)
<i>Physical</i>	-	3 (25%)	2 (40%)	9 (17%)
<i>Mainly physical</i>	-	2 (17%)	0	13 (25%)
<i>Both physical and Psychological</i>	-	5 (42%)	2 (40%)	28 (54%)
<i>Mainly psychological</i>	-	2 (17%)	1 (20%)	2 (4%)
<i>Psychological</i>	-	0	0	0

Table 3.66 Attribution scale responses in healthy controls (CTRL), HCV Resolved Fatigue (RF) and Persistent Fatigue (PF), and CFS groups

<i>Response</i>		CTRL (<i>n</i> = 57)		HCV RF (<i>n</i> = 37)		HCV PF (<i>n</i> = 18)		CFS (<i>n</i> = 54)	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
<i>Missed</i>	-	0	-	6	16	6	33	0	-
<i>N/A</i>	-	35	61	21	57	0	-	0	-
Respondents	-	22	39	10	27	12	67	54	100
<i>Previous Viral Infection</i>		5	23	5	50	4	33	42	78
<i>Current Infection</i>		0	-	4	40	3	25	11	20
<i>Other Infection</i>		2	9	2	20	1	7	19	35
<i>Current Treatment</i>		0	-	4	40	4	33	5	9
<i>Work Stress</i>		14	64	7	70	4	33	39	72
<i>Relationship Stress</i>		7	32	1	10	3	25	28	52
<i>Emotional Stress</i>		7	32	4	40	5	42	38	70
<i>Food Allergy</i>		0	-	1	10	1	16	18	33
<i>Other Allergy</i>		0	-	0	-	2	16	10	19
<i>Hormonal Disorder</i>		4	18	2	20	4	33	18	33

Note – responses were made to each item and so totals exceed 100%

3.2.8 Cross-Sectional comparisons: a summary

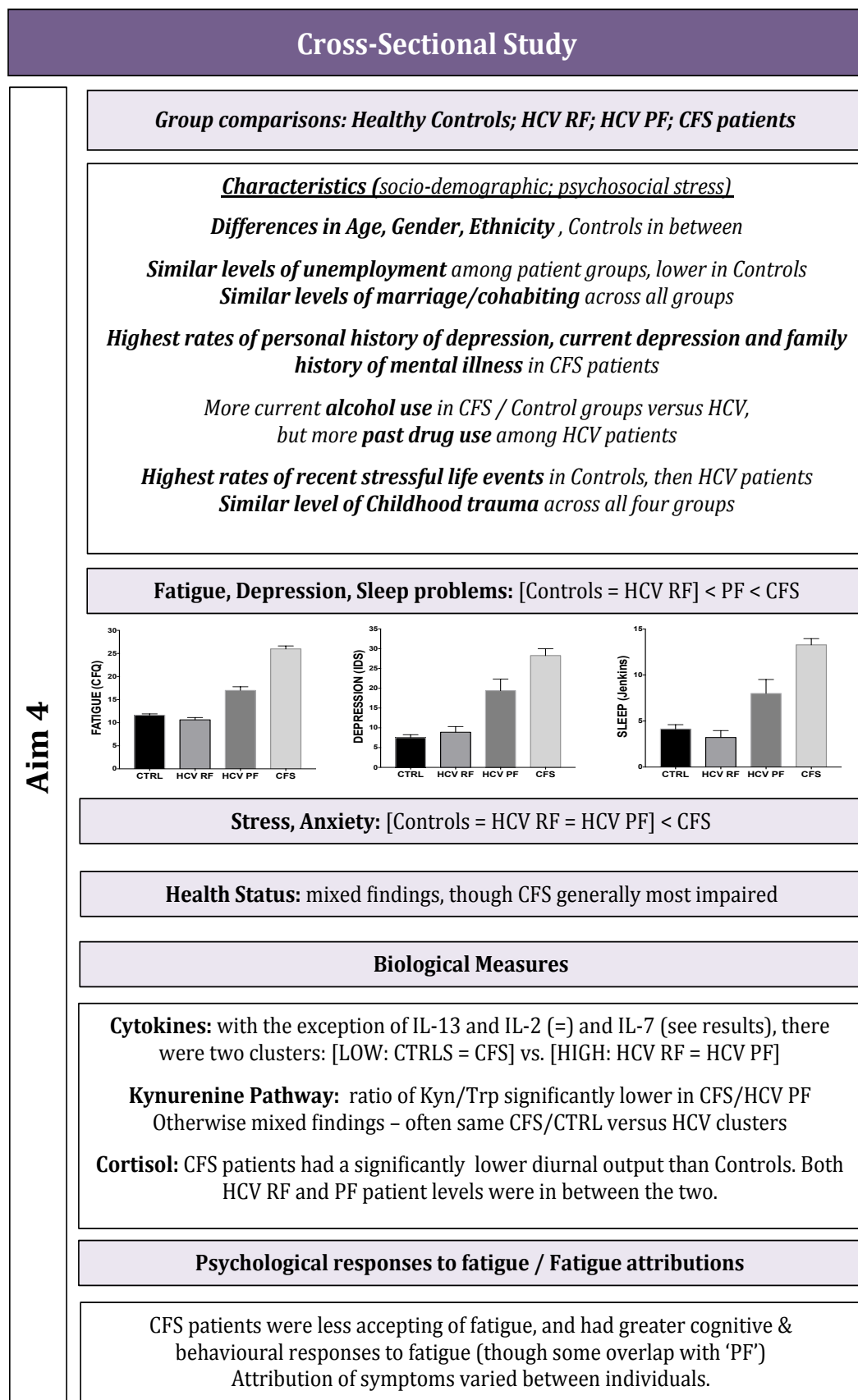


Figure 3.79 Summary of findings for Aim 4: Cross-sectional comparison

4 Discussion

4.1 Summary of findings

This is the first study to investigate IFN- α induced persistent fatigue as a proxy model of Chronic Fatigue Syndrome. This thesis has explored the relevance of a wide range of measures, covering psychosocial and disease specific characteristics, clinical symptoms, health status, biological markers related to inflammatory processes and HPA axis function, and psychological factors associated with the experience of fatigue. As well as obtaining measures of baseline function, changes have been prospectively monitored throughout IFN- α treatment. I have explored the effect of IFN- α administration on the whole HCV cohort, as well as differences in patients in whom fatigue persists post-treatment, versus those in whom it resolves. I have also identified differences and similarities between HCV patients with persistent fatigue induced by IFN- α and Chronic Fatigue Syndrome patients, and made comparisons with HCV patients who recovered post-trigger, and healthy volunteers.

4.1.1 Aim 1. To examine the effect of IFN- α on the whole HCV cohort.

To achieve this, I measured baseline levels, and changes in the following:

- ***Clinical symptoms:*** fatigue, depression, anxiety, stress, sleep;
- ***Health status,*** including measures of general and mental health, as well as functioning;
- ***Biological measures:*** cytokines and the kynurenine pathway.

As has been found in previous studies, IFN- α treatment for chronic Hepatitis C induced increases in fatigue, symptoms of depression and anxiety, and levels of perceived stress. There was no consistent pattern relating to IFN- α induced sleep disturbances in this sample, though the measure was introduced later in the study and therefore smaller numbers may limit interpretation. IFN- α also induced a steady decline in their health status, incorporating measures of overall health and a decline in functioning associated with poorer health.

First, for the biological measures examined, there were IFN- α induced changes in some of the peripheral cytokines measured: IL-2; -6; -7; -12p70; -17A and TNF- α . There were no significant changes detected in IFN- γ , IL-8 and IL-10. Next, indicative of increased Indoleamine 2,3 dioxygenase (IDO) activity, there were decreases in levels of tryptophan (TRP), while levels of the ratio of kynurenine (KYN) to TRP increased in response to IFN- α treatment. There were also decreases in kynurenic acid (KYN-A), and its metabolite quinaldic acid. Levels of 3-Hydroxykynurenine (3-HK) were increased, though no difference in the ratio of 3-HK to KYN was detected. There was no significant effect of IFN- α on the metabolites of 3-HK, xanthurenic acid (XAN) or quinolinic acid (QUIN) or picolinic acid (PIC),.

4.1.2 Aim 2. To identify factors associated with the severity of fatigue

The second aim was to explore associations between the severity of fatigue and the other variables examined, in an attempt to better characterise HCV patients who experience greater problems with fatigue, at three key time points. Overall, the relevant factors differed according to the type of fatigue examined: baseline, acute or persistent. **Baseline fatigue** has been shown to be predictive

of subsequent severity of IFN- α induced fatigue in other samples. Patients more likely to experience problems with baseline fatigue included those with the more common HCV genotypes 2 and 3. I found some evidence of an effect of life stressors: while there was no association with childhood trauma or experience of more traumatic 'intrusive' life events, patients who had experienced at least one stressful life event in the six-months before treatment had greater problems with fatigue. I also found some evidence for an association with illness perceptions, in that individuals who expressed a stronger illness identity, and those who described having a greater emotional response to their HCV reported more severe baseline fatigue. More severe fatigue at baseline was also associated with higher symptoms of depression and anxiety, and more perceived stress, as well as lower ratings of health and functioning. Finally, more severe baseline fatigue was associated with higher levels of pro-inflammatory cytokines.

For **IFN- α induced acute fatigue**, as measured at TW4, there was no association with any of the characteristics examined. Interestingly, baseline fatigue did not predict acute fatigue in this sample, though levels of depression, anxiety and stress did, as did poorer baseline health status. Negative illness perceptions at baseline were also predictive of worse problems with acute fatigue, across almost all dimensions. The picture for biological predictors was less clear; fatigue was higher in those with higher baseline IL-10 and TNF- α , with a trend towards a link with higher baseline IL-6. There was also an association with lower baseline KYN and XAN. Interestingly, I found no link with concurrent inflammation, as measured by cytokine levels at the same visit, TW4, indicating that 'pre-IFN- α ' immune status is more relevant to acute fatigue than immune status during IFN- α .

For **post-treatment fatigue**, measured six-months post-treatment, there were no 'pre-IFN- α ' socio-demographic or HCV specific characteristics associated with an increased risk of more severe fatigue later on. However, fatigue was higher in those who had developed IFN- α induced depression during treatment. Patients who had an earlier response to treatment, clearing the virus by week four ('RVR'), were less likely to have high fatigue levels post-treatment. However, there was no association with virus clearance at the same visit, the definition of successful treatment ('SVR'). Stress experienced during treatment was also associated with more severe fatigue, though again there was no association with childhood trauma. There was a trend towards an association with baseline fatigue, though not with other baseline clinical symptoms such as depression or anxiety. Interestingly, there was a link to current clinical symptoms, suggesting that, though likely overlapping, there may be two slightly distinct groups of vulnerable patients at baseline versus post-treatment; that is to say, some patients who are psychologically vulnerable at baseline have an effective recovery from IFN- α -induced fatigue, while some who are seemingly psychologically healthy at baseline develop a vulnerability during IFN- α which persists post-treatment. Only the measures of baseline health status connected to mood were predictive of post-treatment fatigue, though by six-months post-treatment all measures of health status were connected. Of the baseline biological markers examined, lower IL-13 and higher IL-17A were predictive of more severe fatigue. In regard to biological measures post-treatment, there were few associations with inflammation overall, though patients with higher IL-6 had slightly higher fatigue. The cortisol awakening response was blunted in those with a higher post-treatment fatigue, the reverse pattern to earlier measures of fatigue.

4.1.3 Aim 3. To identify risk factors for IFN- α induced persistent fatigue, and examine the effects of IFN- α in persistent versus resolved fatigue

To achieve this aim, I explored possible risk factors for persistent fatigue, as well as investigating whether patients who would go on to experience persistent fatigue post-treatment had a different response to IFN- α compared with those whose fatigue resolved. I found that around 30% of patients reported worse fatigue at the follow-up visit, six-months post-treatment than they had at baseline. These individuals were therefore defined in this thesis as the 'persistent fatigue' patient group. The stratification of patients was based on relative changes in fatigue from baseline to follow-up rather than absolute levels of fatigue (which are commented upon in the paragraphs above). I have chosen this theoretical approach in order to identify mechanisms specifically underlying sensitivity to the immune trigger, to mimic the experience described by many patients with CFS.

The only socio-demographic characteristic that might have predicted this outcome was related to opioid use, with current users as part of rehabilitation programs less likely to experience persistent fatigue. With regard to the effect of IFN- α , patients who would later experience persistent fatigue had a more exaggerated response to IFN- α , with increased fatigue, depressive symptoms and perceived stress, a greater decline in health status, and some evidence of increased inflammation in levels of IL-10 and IL-6. There was also some evidence for a role for dysfunction of the kynurenine pathway, with a greater decrease in the KYN/TRP ratio at TW8 relative to baseline, versus RF patients.

Of note, I found that PF was not due to non-recovery from IFN- α treatment per se, since the change in scores over the six-months post-treatment showed a similar rate of recovery in both groups; however, greater disability by the end of treatment in the PF group put them at a significant disadvantage.

4.1.4 Aim 4. A cross-sectional comparison with CFS patients and controls

Finally, to achieve this aim I compared data obtained from patients in both HCV groups six-months post-treatment, with the data obtained at a one-off assessment of healthy controls and CFS patients. **With regard to socio-demographic characteristics**, I found that the HCV and CFS patients groups differed quite widely in relation to age (older vs. younger) and gender (male vs. female), with controls that had been matched for age and gender somewhere in between. There were similar levels of unemployment across groups, and similar rates were married or cohabiting. CFS patients had more of a background of mental illness, in that they were more likely to report a family history, and had higher rates of both past and current depression, though again this should be considered in the context of the exclusion of depressed individuals in the other groups. There was more current alcohol use in the CFS and control groups, though more past drug use in HCV patients.

For **experience of psychosocial stress**, controls were, surprisingly, more likely to have experienced a recent stressful event (within the past six-months), with CFS patients least likely. There was no expected increased incidence of childhood trauma in the fatigued patient groups, with similar levels reported in all four groups.

The comparison of **clinical symptoms** showed that CFS patients reported higher fatigue, depression scores and greater sleep disturbances than all other groups, though HCV PF patients were worse than their HCV RF peers, who reported similar levels to the controls. In relation to perceived stress and anxiety, however, there was no difference between the HCV patient groups or controls, while CFS patients had significantly higher levels.

For the measures of **health status**, results were mixed, though CFS patients were generally the most impaired. Levels of problems with mental health, limitations to everyday activities due to emotional problems and pain were similar in CFS and HCV PF patients. However, on measures related to physical functioning and limitations due to physical health, as well as vitality (energy/fatigue), social functioning and ratings of general health, CFS patients were significantly more impaired than any other group.

For the **biological measures**, I did not find evidence of increased peripheral inflammation, either in relation to cytokines or the kynurenine pathway, in CFS patients. In fact, two clusters were usually apparent, linking the two HCV groups ('high inflammation'), and the CFS patients with controls ('low inflammation'). Exceptions included the ratio of KYN/TRP, which was lower in CFS patient and HCV PF patients (truly, the only biological marker that is similar in the two fatigue groups), and diurnal cortisol output, which was lower in CFS patients, and significantly lower than levels obtained from controls (a finding described before and that I was therefore able to replicate).

Finally, with regard to the **psychological responses to fatigue**, CFS patients were less accepting of fatigue than the other groups, with some indication of higher scores in PF versus RF patients too. There was also some overlap between the cognitive and behavioural responses to fatigue from the CFS and HCV PF patients, though on some measures CFS patients did have stronger cognitive and behavioural responses to their condition.

4.2 Effect of IFN- α in the whole HCV cohort

4.2.1 Changes in clinical symptoms in response to IFN- α treatment

IFN- α led to increases in fatigue, as well as symptoms of depression and anxiety, and levels of perceived stress. The greatest changes in fatigue occurred earlier, in the first four weeks of treatment, while depressive symptoms peaked after twelve weeks. This is in line with previous findings that neurovegetative side effects appear earlier in treatment and persist, while depressive symptoms and clinically relevant depression develop later on (Capuron & Miller, 2004). I did not find an effect of IFN- α on self-report sleep disturbances. The smaller sample sizes, caused by the late introduction of the measure, should be noted. However, though previous studies have suggested that IFN- α does typically induce sleep disturbances, they also suggest that the effect may be minimised by the lower doses received in HCV, and that any effect on sleep may be more short-lived than is observed for other clinical symptoms (Dowell et al., 2016; Schaefer et al., 2002). Furthermore, a more detailed study incorporating polysomnography found that while IFN- α induced changes in the objective measures of sleep, it did not increase subjective, self-report measures of sleepiness (Raison, Rye, et al., 2010).

4.2.2 Changes in health status in response to IFN- α treatment

In this study I also observed a decline in health status throughout treatment. As a result of treatment, patients experienced a particularly significant increase in the degree to which they felt limited in performing their everyday activities as a result of their physical health. The most notable change occurred during the first four weeks, in line with decreases in energy ('vitality') over the same period. This reflects the results from existing studies which have also observed a decline in health status and function across all dimensions, using the same measure (SF-36); similar to the findings in my study, the authors described the greatest changes as being in energy, social functioning, and the degree to which patients were limited in performing their everyday roles due to physical health or emotional problems (McHutchison et al., 2001; Ware et al., 1999).

4.2.3 Biological changes in response to IFN- α treatment

I investigated changes in cytokine levels, as well as TRP and kynurenine pathway metabolites. I found an effect of IFN- α treatment on levels of most of the cytokines measured. Levels of IL-2 and -12p70 decreased over the first four weeks of IFN- α administration, with no significant difference in levels from TW4 to TW24. Levels of IL-6, -7 and -17A increased initially, then there were no differences in levels from TW4 to TW24. Levels of TNF- α increased throughout treatment. This is in keeping with previous research relating to increases in cytokines, and differences in levels in response to acute versus chronic administration of IFN- α (Raison, Borisov, et al., 2010). However, I did not detect an effect of IFN- α treatment on levels of IFN- γ , IL-8 or IL-10 in the whole sample. The latter negative finding could be related to the time points at which the cytokines were measured. For example, one study also measuring

peripheral cytokines in serum did observe IFN- α induced increases in IL-8 and IL-10 after one week of treatment, with subsequent decreases thereafter (Wichers et al., 2007). Another study, also in serum, found no effect of IFN- α on levels of IFN- γ , IL-8 or IL-10 when examined using longitudinal analyses including levels at each treatment time point, as I had conducted here. However, an alternative strategy taking the peak value obtained after 2-4 weeks of treatment, and 4-6 months (weeks 20-24) did reveal IFN- α induced increases in IL-8 and IL-10 relative to baseline levels (Bonaccorso et al., 2001). Of note, both studies examined fasting bloods, and bloods were collected at the same time for each individual, which was beyond the scope of this study.

I observed an overall decrease in TRP. In line with increased IDO activity, I found that IFN- α also induced an increase in the KYN/TRP ratio. I also found decreases in both KYN-A, and its metabolite quinaldic acid. There were increases in 3-HK, though I did not detect any changes in its metabolites, XAN, QUIN or PIC. The findings regarding TRP and the KYN/TRP ratio have been reported elsewhere, while the reduction in KYN-A has previously been reported indirectly, as an increase in the ratio of KYN to KYN-A (Bonaccorso et al., 2002; Wichers et al., 2005). However, few studies, if any, have measured such an extensive range of metabolites in the context of IFN- α treatment. One such study did a case-control comparison of IFN- α treated patients after twelve weeks of treatment, and they too found no significant effect of IFN- α on QUIN levels (Raison, Dantzer, et al., 2010).

4.3 Factors associated with the severity of fatigue

4.3.1 Severity of baseline fatigue

Baseline fatigue has been found to predict the severity of fatigue during IFN- α treatment (Dowell et al., 2016). A review on HCV and fatigue suggested that due to the complex nature of the symptom, a direct link between HCV infection per se and increased fatigue was questionable; indeed, in the earlier stages of illness markers of disease severity are rarely associated with fatigue (Wessely & Pariente, 2002). It is therefore likely that the baseline fatigue in this sample is also a representation of general mental health, which may be related to factors associated with increased risk of HCV transmission (e.g. drug use), or distress deriving from the chronic, life-threatening diagnosis.

In my study, I aimed to clarify which variables were associated with more severe baseline fatigue, which in turn may put patients at greater risk of IFN- α induced fatigue. Despite investigating a wide range of socio-demographic and virus/treatment characteristics, the only significant association found was with genotypes 2 and 3. This is in contrast to a large-scale study of HCV patients in France, which found no link between fatigue and genotype (Poynard et al., 2002). However, in this case the authors examined incidence of fatigue, that is to say the proportion of patients experiencing fatigue, yes or no, as opposed to fatigue severity. I did not find any association with female gender, nor with age, as had been observed in the same study and others (Poynard et al., 2002; Sarkar et al., 2012). It should be noted, however, that there were few women in my sample. I did not find the expected association between a history of childhood trauma and more severe baseline fatigue, though there was an association with recent stressful events. Under-studied in the context of HCV fatigue, a cross-sectional study of HIV fatigue did find more severe fatigue in

those with a history of childhood trauma, as well as those who had experienced recent stressful life events (Leserman et al., 2008). The same group later showed that the same variables could predict severity of fatigue after a one-year follow-up; though there was also a direct association between stressful life events and fatigue severity, the effect was largely mediated by current symptoms of depression and anxiety (Barroso et al., 2010).

I observed a link between the severity of baseline fatigue and illness perceptions. Those patients with a stronger illness identity and more emotional representations of their illness, indicating a greater emotional response to their HCV, had higher baseline fatigue. Illness identity is calculated by totalling the number of symptoms attributed by the individual to HCV; the fewer symptoms patients experience generally, the less there are to be attributed to the condition. Therefore it is possible that these are indeed patients who are more symptomatic. However, since fatigue itself is subjective, both attributions and ratings of severity are based largely on patients representations of their illness (Dantzer et al., 2014). Moreover, patients with HCV may attribute fatigue to their condition as the most plausible explanation, without considering other possible causes (Zalai et al., 2016).

I found an association between symptoms of depression and anxiety, as well as levels of perceived stress, and the severity of baseline fatigue. The link between such symptoms is a common one, both in general, as well as in the context of HCV (Wessely & Pariente, 2002). Another study of the severity of HIV fatigue also found an association with higher levels of perceived stress (Phillips et al., 2004).

I did not find any association with pre-treatment measures of sleep, which has been linked with increased risk for IFN- α induced depression (Prather et al., 2009). In my study, poorer health status was also associated with greater baseline fatigue.

Finally, there was an association with elevated levels of peripheral pro-inflammatory cytokines, though not with anti-inflammatory cytokines. Indeed, it is pro-inflammatory cytokines, such as IL-6 and IL-1 β , which are responsible for the induction of sickness behaviours, including fatigue (Dantzer et al., 2014). I did not observe an association with the kynurenine pathway, nor with salivary cortisol. This is in contrast to previous studies that have found an association between increased IDO activity, as indicated by increases in the KYN/TRP ratio, and higher fatigue in untreated HCV patients (Gess et al., 2009).

4.3.2 Severity of fatigue at TW4: acute fatigue

There were few characteristics that might have identified those at greater risk of IFN- α induced acute fatigue. Baseline fatigue 'caseness' was only moderately associated with subsequent fatigue severity, and, discussed later, there was no association with baseline fatigue, which suggests that IFN- α induced fatigue was also affecting the 'asymptomatic' HCV patients. As in the case of baseline fatigue, I did not find an association between age and the acute response. This is in contrast to a study of post-infective fatigue syndrome (PIFS), that had observed greater fatigue in older participants in the acute sickness phase (Hickie et al., 2006). Nor did I find an association with a past history of depression and the acute response, though I did find an association with higher depressive symptoms measured at baseline. This is consistent with a study of

IFN- α treated patients, which found no link between past depression and IFN- α induced acute fatigue (Majer et al., 2008). The aforementioned PIFS study also found no association with pre-morbid psychiatric disorders, though did find an association between current psychiatric disorders and the severity of the acute sickness (e.g. headaches, fever) (Hickie et al., 2006). I found no association with gender. A study of predictors of symptom severity in the DIOS PIFS cohort found that female gender was associated with more severe fatigue, as well as more severe neurocognitive symptoms and mood disturbance during the acute sickness phase (Piraino et al., 2012).

In support of my hypothesis that negative baseline illness perceptions would predict worse acute fatigue, I found an association with all but one of the measures of pre-treatment illness perceptions. Beliefs not associated with the severity of acute fatigue related to the potential success of treatment in controlling/managing symptoms. Under-studied in the context of IFN- α induced fatigue, this finding adds to previous work in the same cohort of HCV patients which found a predictive effect of illness perceptions on subsequent IFN- α induced depression and anxiety (Hepgul et al., 2016). Though I have examined illness perceptions in relation to HCV, similar findings have been observed for beliefs about the trigger itself. In one such study of infectious mononucleosis (IM), patients who believed early on in the illness that IM would have greater consequences were more likely to meet criteria for fatigue 'caseness' while unwell (Candy et al., 2003).

There was no relationship between baseline fatigue and the severity of fatigue after four weeks. This is somewhat in contrast to previous studies in HCV IFN- α treated patients, though in this case the authors measured the change in fatigue over the same period, while I measured absolute levels of fatigue after four

weeks (Dowell et al., 2016). Nor did I find an association with baseline reports of sleep disturbances. Though unfortunately they did not measure fatigue, a previous study found that pre-treatment sleep problems predicted the subsequent development of IFN- α induced depression (Prather et al., 2009). I did, however, find an association with baseline measures of symptoms of depression, anxiety and perceived stress. Baseline measures of health status also predicted the severity of IFN- α induced fatigue after four weeks.

With respect to biological markers, I found an association between more severe acute fatigue and higher levels of baseline IL-10 and TNF- α , as well as higher levels of IL-6. These markers have been implicated in IFN- α induced neuropsychiatric effects before; baseline levels of IL-6 and IL-10 have both been found to predict IFN- α induced depression (Prather et al., 2009; Wichers et al., 2006). I also found that lower baseline KYN-A and XAN were associated with subsequent higher levels of acute fatigue. KYN-A may reduce neurotoxicity associated with this pathway, through its action as an NMDA receptor antagonist (Müller & Schwarz, 2007). Therefore, lower levels may indicate less protection against neurotoxic processes. While my finding related to the predictive effect of baseline levels, higher levels of the ratio of KYN to KYN-A during treatment have been associated with IFN- α induced depressive symptoms, and lower energy specifically, measured in the context of depression (Wichers et al., 2005). For XAN, however, less is known about its relevance in the case of IFN- α induced fatigue. Finding an association with lower levels somewhat contradicts findings from a recent study, which suggested that increases in XAN may inhibit the activity and bioavailability of tetrahydrobiopterin (BH₄) (Haruki et al., 2016). As discussed earlier in the introduction, BH₄ is essential for the conversion of tyrosine to dopamine, and

acute IFN- α induced fatigue has been linked to a lower tyrosine, and lower dopamine in cerebrospinal fluid (Felger et al., 2013).

I also found an association between higher levels of acute fatigue at TW4, and lower baseline diurnal cortisol output, as measured in saliva. Studies of IFN- α and HPA axis function have typically focussed on the change in levels of the hormones and their association with symptoms, as opposed to baseline levels as a predictor to identify those at greater risk. One such study in plasma found an association between the change in HPA-axis measures in the first three hours after the first injection, and the later development of cognitive, depressive and anxiety symptoms, but not somatic and neurovegetative symptoms, incorporating fatigue. Another study in plasma found that the change in fatigue in the first twelve weeks of treatment was associated with the change in cortisol over the same period, and the flattening of the cortisol slope (Raison, Borisov, et al., 2010). However, a previous study of PIFS following IM did not find any link between measures of salivary cortisol and acute fatigue severity (Candy et al., 2003).

Contrary to expectations, I did not find an association between current inflammation and the severity of acute fatigue. One study in HCV IFN- α treated patients did find an association between changes in TNF- α and its receptor soluble TNF-Receptor-2 (sTNF-R2) and increases in fatigue, though did not find an association with IL-6 or the soluble IL-6 receptor (sIL-6R) (Raison, Borisov, et al., 2010). Another study of PIFS, of the DIOS cohort, also failed to find an association with the peripheral cytokines IL-6 and IL-1 β , measured in serum. However, they did observe associations with 'spontaneous production' of the cytokines by unstimulated peripheral blood mononuclear cells (PBMCs) (Vollmer-Conna et al., 2004). While both markers were associated with fatigue,

IL-1 β was also associated with physical symptoms, and IL-6 with cognitive and neuropsychiatric symptoms; however, the authors note that while this might be so, since they are so highly related it is difficult to tease apart the specific contributions of each. Nonetheless, results did suggest that the severity of the symptoms experienced was related to the strength of the immune signalling, emphasising the significance of the host response to the infection (Vollmer-Conna et al., 2004).

4.3.3 Severity of fatigue post-treatment

As is the theme, there were few baseline characteristics that might have identified patients at greater risk of more severe fatigue post-IFN- α -treatment. Patients who were diagnosed with IFN- α induced depression during treatment experienced more severe fatigue post-treatment, despite no longer meeting criteria for a major depressive episode. This is in line with the association with mood symptoms, discussed further below in the context of persistent fatigue. Patients that had not achieved a 'rapid virological response' after four weeks were also more likely to experience more problems with fatigue later on. This association may be due to a range of factors, including the biological changes associated with the persistent infection, and the increased stress placed on the patient in not having achieved this milestone, which is a good indicator of overall treatment success. Indeed, in the absence of an RVR, the nature of the viral response means that it is often difficult to predict precisely what the outcome may be, which is particularly difficult for patients undergoing treatment. Though not directly comparable due to the continued effect of the trigger 'IFN- α ', longer illness duration does also predict a later diagnosis of PIFS, as is also

discussed below (Vollmer-Conna et al., 2007). Interestingly, I did not observe the expected association with the failure of treatment, defined as a failure to achieve a 'sustained virological response', whereby the virus is detectable in the blood six-months post-treatment. The few studies that have been conducted on post-IFN- α -fatigue have consistently reported higher levels of fatigue, or less improvement relative to baseline function, in those who had relapsed, or not responded to treatment in the first instance (Huckans et al., 2015; Sarkar et al., 2012; Ware et al., 1999). In my sample, 88% of patients achieved an SVR, leaving only seven individuals who had not. I do not have information on the patients lost to follow-up, who I speculate may have been slightly more likely to have failed treatment, and therefore less willing to engage with the final visit. As discussed in the introduction, success rates of combination therapy, as well as Simeprevir are estimated at around 80%, with Telaprevir and Boceprevir ($n = 6$; 11%) lower, at 65%. Therefore, in this study, I perhaps had a higher proportion of responders. Nonetheless, I have demonstrated clearly that in some individuals, fatigue persists despite clearance of the virus, and in the absence of the initial immune trigger, as is seen in CFS.

Patients who had experienced a stressful event during treatment had higher fatigue levels post-treatment than those who had not, though interestingly there was no difference for events experienced more recently, in the follow-up period. This suggests that the event(s) may have had an added effect on top of the acute response to treatment, which contributed to the persistence of symptoms post-treatment.

In support of the hypothesis made that they would not be associated, I did not find an effect of baseline illness perceptions on the severity of fatigue post-treatment. However, there were weak associations between the severity of

fatigue and more negative beliefs about the consequences of HCV, as well as stronger emotional representations, the latter of which was consistently associated with fatigue severity throughout. This has not been studied in the context of IFN- α treatment before, though in PIFS, as discussed below in the context of persistent fatigue, illness perceptions were only found to be important for fatigue 'caseness' during the acute sickness, not for fatigue reported later (Candy et al., 2003).

Interestingly, and as highlighted before, there was no effect of baseline clinical symptoms, with only a trend towards an association with baseline fatigue levels. This again suggests that not all patients experiencing problems with fatigue post-treatment had been symptomatic before IFN- α . Nor was there an association with baseline health status, apart from measures of limitations to everyday activities due to emotional problems specifically, and to some extent, mental health. Since there was no association with baseline depressive or anxiety symptoms, these more functional measures of mental health point towards the need for a broader assessment of pre-morbid mood/psychiatric disorders; this may be more beneficial in identifying those at greater risk of more severe fatigue following an immune trigger.

4.4 IFN- α induced persistent fatigue

In this study, just over 30% of patients reported experiencing worse fatigue post-IFN- α treatment than they had at baseline, despite the fact that the original immune trigger (IFN- α) was no longer present, nor in most cases was the HCV (discussed further below). A large cohort study of post-viral fatigue observed that fatigue and neurocognitive symptoms, including poor memory and concentration, continued long after the initial acute sickness symptoms, suggesting that a different mechanism underlies these persistent symptoms after an immune challenge (Hickie et al., 2006). Therefore it was important to consider the risk factors and longitudinal responses to the immune trigger (IFN- α) in those in whom fatigue persisted, in addition to the earlier measures of severity in response to the acute challenge.

4.4.1 Risk factors for IFN- α induced persistent fatigue

I examined a wide range of possible risk factors for IFN- α induced persistent fatigue, again considering: socio-demographic characteristics; virus/treatment characteristics; experience of psychosocial stress; illness perceptions; and baseline biological markers including cytokines, TRP and kynurenine pathway metabolites, and salivary cortisol. Because of the wide range of risk factors examined, I will limit the discussion to those that were found to be associated with increased risk of IFN- α induced persistent fatigue, or those that were not, contrary to expectations based on previous research.

I was not able to identify any pre-IFN- α characteristics as risk factors for the persistence of IFN- α induced fatigue post-treatment, apart from the decreased use of opioids as part of drug rehabilitation programs. One reason for this unexpected finding may be that patients accepted for IFN- α treatment who were on drug rehabilitation programs would have had access to a greater support network. Though no patients in this study were recruited from outreach services, most of such patients regularly visited key workers - who were often involved in the multidisciplinary package of care - to obtain drugs. Moreover, additional support was provided where necessary from the HCV clinical nurse specialists to ensure adherence, in line with practices employed to treat PWID (Arain & Robaeys, 2014).

The lack of clear baseline predictors is in line with the aforementioned PIFS study, which found no association with any particular socio-demographic characteristics, or with pre-morbid psychiatric disorders. (Hickie et al., 2006). Nor was a past psychiatric history predictive of post-infectious mononucleosis (IM) fatigue in another cohort study, though patients who met the criteria for CFS following infection with IM were more likely to have a history of a mood disorder (White et al., 2001). Moreover, a further study of IM, which examined fatigue 'caseness' (based on the Chalder Fatigue Questionnaire cut-off), did find that patients who met this criterion 3-4 months after the onset of the condition were more likely to have had a history of 'emotional problems'. Caseness 6-7 months post-onset was associated with female gender, and caseness 12-13 months post-onset with female gender and older age (Candy et al., 2003). That said, in this particular study, only the 12-13 month follow-up represented 'post-infection' in all participants; for measures obtained at the earlier follow-ups, 55% and 62% of patients had already recovered respectively, a key methodological

difference between our studies which may have influenced results. The association with gender was also found in IM patients who had not recovered six-months post-onset (Buchwald et al., 2000).

Though not a significant relationship, patients exposed to the trigger for longer, with a longer duration of treatment, were more likely to experience persistent fatigue post-treatment. A similar finding was observed in the DIOS cohort, in that individuals who developed PIFS (CFS) had a significantly longer duration of illness than those who recovered (Vollmer-Conna et al., 2007). Furthermore, the aforementioned study of IM also found an association between 'caseness' 3-4 months post onset, and a longer convalescence (Candy et al., 2003). Also connected may be the pattern observed that patients who cleared the HCV more quickly were less likely to experience persistent fatigue; that is to say, patients who had cleared the virus within four weeks of treatment initiation, known clinically as the 'rapid virological response', were less likely to experience persistent fatigue post-treatment. Intriguingly, though similar levels were found across the two groups, patients who experienced persistent fatigue were slightly more likely to have achieved a 'sustained virological response', meaning that the virus was still not detected when assessed post-treatment. As already highlighted in the context of the severity of fatigue, this goes against previous findings in IFN- α treated patients (Huckans et al., 2015; Sarkar et al., 2012). However, the methodological difference should be acknowledged, that this result relates to the persistence of fatigue within an individual, regardless of severity.

There was no effect of a history of childhood trauma on the persistence of fatigue post-IFN- α treatment. This is in contrast to what was hypothesised, based on studies of CFS (see Borsini, Hepgul, Mondelli, Chalder, & Pariante,

2013). However, in the present study, there was no association with CFS either (see section 4.5.1). Instead, though not a significant relationship, I did find that patients who experienced persistent fatigue were slightly more likely to have reported at least one intrusive life event. This measure of stressful events covered a wider range of 'intrusive', and more traumatic life events, experienced at any point during the participant's lifetime. A similar finding was observed following infectious mononucleosis, with patients who had experienced a greater number of significant life events historically, more than six-months before illness onset, more likely to remain ill six-months post-onset (Buchwald et al., 2000). Of note, this study was based on 'non-recovery' versus chronic fatigue, though fatigue was one of the main complaints by such individuals.

I did not find any effect of baseline illness perceptions on later development of persistent fatigue. Mentioned previously in the context of the severity of acute fatigue, colleagues conducting a study of fatigue 'caseness' following IM reported a similar finding. They observed that 'caseness' at both 3-4 and 6-7 months post-onset was associated with stronger negative beliefs about the consequences of IM, but this effect was no longer present for 'caseness' determined 12-13 months post-onset (Candy et al., 2003). It should be noted however that, as described above, some patients were no longer infected at the 3 and 6-month follow-ups. As such, for some individuals, illness perceptions may have been associated with persistent fatigue after the original stimulus was no longer present. Illness perceptions may also be connected with personality traits. The study of PIFS previously mentioned found no association between personality traits and a subsequent diagnosis (Hickie et al., 2006). It should again be noted that, unlike in these disease models of CFS, illness perceptions in this sample relate to treatment and HCV, as opposed to the trigger itself.

Finally, in addition to the longitudinal analyses of the response to IFN- α reported below, I also examined baseline biological markers in both groups as possible biomarkers of persistent fatigue post-treatment. In this case, I did not find any significant differences in cytokines, kynurenine pathway metabolites or in cortisol. Baseline levels of IL-6 were slightly higher in PF patients, as were levels of IFN- γ . For levels of cortisol, as mentioned previously, overall levels in the PF group dipped 15 minutes after awakening, as opposed to increased as one would expect. Since peak production would usually occur 30 minutes after awakening, one explanation for such a pattern would be that the individual has in fact not adhered to the timing advised. However, the expected pattern is known to be influenced by various factors and pathologies so this is not necessarily the case. Indeed, a recent review of the topic highlights that in patients with a clinical picture in keeping with a reduced CAR, an absent or negative CAR may be expected on occasion (Stalder et al., 2016). Furthermore, in the present study, samples were only included where sampling diaries indicated that there had been compliance with the protocol.

4.4.2 Baseline clinical symptoms, and changes in persistent versus resolved Fatigue

Next, I examined the effect of IFN- α in patients in whom fatigue persisted (Persistent Fatigue; PF), versus patients in whom fatigue resolved (Resolved Fatigue; RF), in order to mimic the onset of CFS. There were no differences in baseline clinical symptomatology. Clinical symptoms were increased in PF patients, which supports the hypothesis made that they would have an exaggerated response to IFN- α . However, in contrast to what was expected post-treatment, PF and RF patients typically recovered at similar rates, as measured in the change in scores from end of treatment to six-months post-treatment. Instead, I found that the PF group were at a disadvantage due to higher symptomatology at the end of treatment.

Specifically, I found increased fatigue, and a greater increase in fatigue scores at various points during treatment, most notably after four weeks of treatment, and by the end of treatment, and post-treatment. Longitudinal studies in the DIOS cohort also found that fatigue was higher across the illness phase as well as follow-up, in those diagnosed with PIFS versus those who recovered (Hickie et al., 2006; Vollmer-Conna et al., 2007). Another study of PIFS following IM also observed that the severity of baseline fatigue (measured after IM onset, following presentation to primary care) predicted fatigue 'caseness' at one-year follow-up, after patients had otherwise recovered (Candy et al., 2003). In this study, both patient groups experienced an increase in depressive symptoms in the first four weeks, such that there was no difference between groups. However, in line with previous findings for the effect of IFN- α treatment generally, greater changes were apparent later, after twelve weeks (Capuron & Miller, 2004). PF patients experienced a steady increase in symptoms

throughout the first six-months of treatment, with higher levels, as well as a greater increase in symptoms relative to baseline function throughout. Furthermore, while RF patients' mood had improved post-treatment compared to baseline symptoms, PF patients also experienced persistent mood disturbances. The association between current depression and fatigue following an infective trigger has also been seen in an IM cohort (White et al., 2001).

4.4.3 Baseline health status, and changes in persistent versus resolved fatigue

As in clinical symptoms, there were no differences in baseline measures of health status between the two groups which may act as predictors of who would be more vulnerable to persistent fatigue post-IFN- α -treatment. Again there was evidence of an exaggerated response to IFN- α in the PF group, with more prominent differences by the end of treatment. Specifically, there were no differences in the level of problems reported by patients at each treatment time point until later on in treatment. However, examination of the rate of decline in function relative to baseline revealed early differences between the groups that continued throughout, specifically in physical functioning, energy/fatigue ('vitality') and social functioning. Reflecting changes in depressive symptoms, PF patients also experienced a greater decline in mental health after twelve weeks of treatment. After 24 weeks of treatment, as well as experiencing greater decline relative to baseline, PF patients also had significantly worse problems in those areas, in addition to limitations to their roles/everyday activities due to physical health and emotional problems, and feeling worse off in terms of their overall, general health. Post-treatment, although ratings of general health had improved, they still reported significantly worse problems in

vitality and pain. There were also trends towards worse functioning physically and socially, limitations to their activities due to emotional problems, and overall problems with mental health versus RF patients. Though not measured as such in this study, pain is among the symptoms that form the sickness behaviours, which typically appear earlier on in treatment. Therefore it was surprising that PF patients, who in many ways had an exaggerated response to IFN- α , did not experience more problems with pain during treatment, and that the difference should be evident so far into the treatment course. Indeed, a study of the DIOS cohort found that patients who were subsequently diagnosed with PIFS had experienced greater pain associated with the infective illness versus those who recovered promptly. In addition, those patients also experienced a significantly longer time “out of role”. Though this is a different measure to that administered in this study, it also highlights the importance of the decline in functioning during the acute sickness phase for predicting who may continue to suffer later on (Vollmer-Conna et al., 2007). In a study of ‘non-recovery’ post-IM, those who reported that they were not fully recovered six-months after illness onset had a pattern of significantly worse functioning than those who had recovered on all measures of health status, bar limitations to their activities as a result of emotional problems (Buchwald et al., 2000). Indeed, a systematic review of risk factors for PIFS following IM found that the most consistent factor contributing to ‘non-recovery’, usually relating to fatigue, was earlier, poorer physical functioning (Candy et al., 2002). To the best of my knowledge, no study has previously examined differences in longitudinal changes in health status in response to IFN- α , related to the persistence of fatigue post-IFN- α treatment.

4.4.4 Biological changes in persistent versus resolved fatigue

There were mixed findings with regard to the biological markers examined, with very few differences in the kynurenine pathway. However, in support of the hypothesis made regarding inflammatory markers, there was some evidence for an exaggerated inflammatory response. Specifically, longitudinal analysis of levels of peripheral cytokines in serum revealed higher overall levels of IL-6 and IL-10 in patients who experienced persistent fatigue, versus those whose fatigue resolved. For levels of IL-10, an anti-inflammatory cytokine, the difference was driven largely by the initial response to IFN- α , during the first four weeks. For levels of IL-6, a pro-inflammatory cytokine, a similar pattern in those first four weeks was observed, though there was also some evidence for higher baseline levels, as well as higher levels after six-months of treatment. That these two cytokines – anti- and pro- - should be those related to the persistence of fatigue likely reflects the initial increase in inflammatory processes in general, in response to IFN- α . Indeed, IL-10 would typically be thought of as inhibiting the effects of pro-inflammatory cytokines on behaviour, in its role as a key negative immunoregulatory cytokine. As referenced in the introduction, the genetic polymorphism associated with increased levels of IL-10 (-592, C allele) has been shown to be protective against neurocognitive and mood symptoms associated with the acute response to infection in a PIFS cohort (Piraino et al., 2012). Interestingly, there was no significant change in levels of IL-10 in the whole sample, nor was there a change in those patients in whom fatigue resolved. This suggests that such changes in IL-10 only occur in those who experience an overall, exaggerated inflammatory response to IFN- α , and associated increases in IFN- α induced side effects. The relevance of IL-6 for fatigue and IFN- α induced neuropsychiatric effects in general has been

discussed extensively throughout (Dantzer et al., 2014). It is also of interest that increases in levels of IL-6 in the PF patients should persist for longer than the inhibitory IL-10. With regard to the overall findings concerning changes in cytokine levels, that I found a difference is somewhat at odds with previous work conducted in the DIOS cohort. In a study of acute fatigue already mentioned, there was no association between acute fatigue and peripheral cytokines in serum, but there was an association with levels produced spontaneously by PBMCs in culture (Vollmer-Conna et al., 2004). The authors then used the same methods to examine changes in peripheral cytokines in PIFS patients versus those patients whose illness had resolved, but found no difference between the two groups in either measure over time (Vollmer-Conna et al., 2007). However, this may be attributable to the greater increases in cytokines in response to therapeutic doses of IFN- α , versus what would be expected as part of the natural immune response (Pollmacher et al., 2002). As had been found in the same PIFS study, and as reported earlier in the context of the severity of fatigue, any inflammation resolved after treatment. Therefore, as also hypothesised, there was no evidence of a persistence of peripheral inflammation in the PF cohort.

Finally, with respect to the kynurenine pathway, there were limited differences between groups on any of the measures. Of note, however, there was a trend towards a lower KYN/TRP ratio after eight weeks of treatment, and a lower change in the KYN/TRP ratio relative to baseline in those patients who would later experience persistent fatigue versus those in whom fatigue would resolve. Interestingly, lower levels of the ratio were also seen in CFS patients, as discussed in section 4.5.3. This would suggest that persistent fatigue specifically may not be associated with IFN- α induced increases in IDO

activation, as has been seen in IFN- α induced depression and somatic symptoms (Anderson et al., 2014; Leonard & Maes, 2012). However, the strong association with changes in mood in this group means that this is not completely clear. Nor was there any indication of a difference between groups in levels of TRP. Finally, it is important to note that in this case, a possible connection has been made between earlier changes in response to the acute immune trigger, and the later persistence of symptoms. This is unlike earlier studies, such as one that found an opposite trend towards an association between a higher KYN/TRP ratio and more severe symptoms in chronic activated EBV. In this case the authors reported an association with levels measured at the same time as the symptoms. They did follow patients up 4-8 months later, but they did not report any findings regarding the predictive value of the measures obtained on the persistence of symptoms at follow-up. Of note, 25% of patients were still reporting persistent problems with severe tiredness, however this represented only five patients (Bellmann-Weiler et al., 2008).

4.5 Cross-sectional study

4.5.1 Characteristics

Characteristic of HCV and CFS patients, there were differences between groups in age and gender, which are likely to have influenced the results to some degree. Specifically, HCV patients were typically older males, and CFS patients were middle-aged women. The recruitment strategy for healthy volunteers had been to match them by age and gender to each group, resulting in a sample which fell somewhere in between the HCV and CFS patient groups. Incidentally, there were no differences in rates of unemployment, or in the rates of those who were either married or living with their partner.

I did not find the expected higher rates of childhood trauma in the CFS group, as has been found previously, suggested to be related to inflammation and the neuroendocrine dysfunction observed in CFS patients (Borsini et al., 2013; Heim et al., 2009). A study of clinically confirmed cases recruited from the community found a 3-8 fold increased risk for CFS according to the different type of trauma, versus non-fatigued controls (Heim et al., 2006). Interestingly, the authors also found that the risk was increased if patients reported current psychopathology, as was often the case in my sample. The authors also used moderate-severe cut-offs, similarly to the present study; however, the greatest difference was in emotional neglect, which I did not measure.

A further study of American patients found similar results; again using the Childhood Trauma Questionnaire, incorporating measures of neglect, they found that just under 50% of patients met the criteria for at least one category of childhood trauma, not dissimilar from the rates observed in this study (42%) (Johnson et al., 2010). In this case, patients were also recruited from tertiary care, as in the present study. The similarity in the rates reported highlight that

the lack of a difference may be more attributable to high rates in our healthy controls (44%). Indeed, a large UK survey study found that rates of reported childhood maltreatment were 16% overall, with 7% reporting having experienced physical abuse, and 11% sexual abuse including contact (May-Chahal & Cawson, 2005). However, important differences include the age at which participants were interviewed (18-24), versus an average age of 40 years old in my sample, as well as the environment in which they were interviewed, at home, where they may have been less forthcoming about experiences.

I did find a connection with a personal history of depression, current depression and a family history of mental illness. However, with regard to the latter items these findings are biased by the recruitment strategy, which excluded HCV patients and controls with current depression, or in the case of controls, recurrent episodes.

4.5.2 Clinical symptoms and health status

I found that patients with CFS typically reported higher levels of clinical symptoms including fatigue and sleep disturbances than any of the other groups. I also found higher depression scores in this group, though as mentioned earlier, this should be also be interpreted with the exclusion of other depressed individuals in mind. The HCV Persistent Fatigue patients, while not as impaired as the CFS patients, had higher fatigue, depression, and sleep problems than the HCV Resolved Fatigue patients and healthy volunteers. Levels in the latter two groups were similar. Levels of stress and anxiety on the other hand were similar across the healthy controls and HCV groups, with only CFS patients reporting higher symptoms and perceived stress. For measures of

health status, CFS patients were impaired across almost all categories. Despite higher levels of diagnosed depression in the CFS group, HCV PF and CFS patients reported similar levels of limitations due to emotional problems. Though not a significant difference, there was a trend towards greater problems with mental health in the CFS patients. Levels of pain while slightly higher in the CFS patients were not significantly different from the HCV PF patients. That the CFS patients were overall more impaired in relation to clinical symptoms and health status supports the hypothesis made for this study. It also corresponds to findings concerning the increased level of disability reported by patients in comparison with other chronic health conditions (Moss-Morris & Chalder, 2003; Nacul et al., 2011).

4.5.3 Biological measures

I typically found two 'clusters' for cytokine levels: the two groups of HCV patients (RF and PF; 'high') and CFS patients and healthy volunteers ('low'). Though measured six-months post-treatment, when IFN- α would no longer be having a direct effect, they had only recently undergone an intensive period of immunotherapy for a difficult to treat chronic viral infection. Though in this case the majority of patients had cleared the HCV virus, both the treatment and the virus have been shown to impact on levels of circulating cytokines (Pollmacher et al., 2002). Levels among HCV patients may therefore have been too recently influenced by other factors for a difference to be evident. However, few studies if any have compared a persistently fatigued cohort against both resolved patients exposed to the same trigger, and healthy volunteers, for comparison with the present results. Nonetheless, the findings disprove my hypothesis that HCV PF patients would have somewhat higher levels of inflammation than their

HCV RF peers, and comparable levels to the CFS group. That peripheral cytokines measured in serum were not associated with the persistence of symptoms does fit with the PIFS literature, and to some extent results from CFS studies also (Blundell et al., 2015; Vollmer-Conna et al., 2007). Interestingly I did not find an association with the duration of symptoms in CFS patients, as had been reported previously (Hornig et al., 2015; Russell et al., 2016). The earlier study, in plasma, was of 52 early onset patients, 246 with a longer duration of illness, and 348 healthy volunteers. Hence the study may have been more adequately powered to detect slight differences in what are relatively low levels of cytokines versus those seen in response to an acute challenge. The later study was also relatively small, and studied cytokine levels in three groups: 18 years and over, 2 years or less of illness; 18-50, 7 years of illness and 50 years and over, 11 years average illness, drawn from different samples. I did not control for age, which may have influenced my results. All participants in this study were female, unlike the present study (70%). Other considerations include the measurement of cytokines in plasma, using a different assay. Finally, in this analysis, since I had already conducted case-control analyses, I conducted a CFS patient only comparison of the two groups. The absence of significant findings did not warrant further case-control analyses.

Findings concerning the kynurenine pathway were mixed, with some evidence of similarities between PF and CFS at least for the ratio of KYN/TRP, indicative of decreased IDO activation in comparison to the two other groups. To the best of my knowledge, kynurenine pathway measurements have not previously been reported post-IFN- α treatment, and certainly not from the perspective of IFN- α induced persistent fatigue. However, I can assume that any residual increases in the kynurenine pathway metabolites may also be an after effect of chronic

immune activation during IFN- α treatment. The finding of interest relates to the finding mentioned above of the lower KYN/TRP ratio in both the HCV PF and CFS groups. This is again opposite to the direction of the association observed between higher levels of the ratio and the persistence of symptoms in CAEBV (Bellmann-Weiler et al., 2008). In this study, that levels of the KYN/TRP ratio are low in both patient groups suggests that low levels of the ratio may relate to the persistence of fatigue and experience of chronic fatigue in CFS. The same issues as discussed before, in the context of lower levels of the ratio in HCV PF patients at treatment week 8, are relevant here. In the absence of higher levels of tryptophan, or measures of tryptophan access to the brain, it is difficult to ascertain what this finding means. Future studies of levels of TRP, together with further examination of the kynurenine pathway measures presented here are warranted to further understand the relationship with CFS specifically.

I found that CFS patients had significantly lower cortisol diurnal output versus controls, with both HCV patient groups falling somewhere in between. I did not find a difference between the groups in relation to the cortisol awakening response; mean levels did appear to be lower in HCV PF patients, though there was variability between patients. The under activity of the HPA axis in CFS has been well documented, and this replication reassures me that this CFS sample is similar to other samples previously studied. Of note, it remains unclear to what extent the low cortisol output is a causative factor, or a result of symptoms such as deconditioning and sleep disturbances (Papadopoulos & Cleare, 2011). Though I did not measure deconditioning specifically, I did measure physical functioning and limitations to everyday activities as a result of physical health. I found that patients in the HCV PF group were still experiencing a decline in these areas of health relative to baseline, at the follow-up visit six-months post-

treatment. However, while by the follow-up HCV PF patients were experiencing greater problems with sleep than their HCV RF peers, this was not consistent throughout the study. It should be noted, however, that the measure was introduced later in the study and as such numbers were much lower for in-treatment measurements of sleep.

4.5.4 Psychological responses to fatigue, and fatigue attributions

These measures were introduced later in the study, and as such it was not possible to examine the effect of early cognitions and behaviours in the prospective cohort study on later fatigue. However, I conducted a cross-sectional comparison to explore differences and similarities between the groups. I had a particular interest in whether the beliefs held and behaviours adopted by the HCV PF group at this stage might already be similar to individuals with CFS. Unfortunately the numbers for these measures were lower, due to the later introduction of the relevant questionnaires. For acceptance of fatigue, given the nature of their illness, CFS patients unsurprisingly showed the greatest lack of acceptance. HCV PF patients too showed some indication of higher scores than healthy volunteers. For the measure of cognitive and behavioural responses to fatigue, numbers were smaller still. However, beliefs regarding symptoms reflecting physical damage, symptom focussing and embarrassment about symptoms were not significantly different between the two groups. All or nothing behaviour was similarly a feature of both the CFS and HCV PF groups. What distinguished CFS patients from the HCV PF (and RF) patients, were fear avoidance beliefs, concerning avoidance for fear of increasing symptoms, catastrophising about symptoms, and avoidance/resting behaviours. Such cognitions and behaviours are likely

more typical of chronic illness, as part of learned response to more severe symptoms, adopted over time. Finally, I also measured fatigue attributions. For the purpose of this study, analysis was descriptive only, and I did not examine differences between groups statistically. In my study, CFS patients were more likely to describe their symptoms as equally physical and psychological in nature (54%). Few patients (17%) described their symptoms as purely physical. This is in contrast to previous findings, which have suggested that CFS patients have a tendency to attribute their symptoms to more somatic causes (Butler et al., 2001). For the second measure of attribution used, participants could select more than one answer relating to factors which might have caused their fatigue or made it worse. HCV patient groups were understandably more likely to attribute their symptoms to treatment, in their case IFN- α . However, HCV PF patients were much less likely to attribute their fatigue to work or relationships stress than other groups, a difference that could not be explained by their work status. Studies have found that attribution of symptoms to changeable, transient causes such as social factors are protective against the subsequent persistence of fatigue later (Chalder et al., 1996). Interestingly, CFS patients in my sample were almost equally as likely to attribute their symptoms to a previous viral infection (78%) as they were to emotional and work stress (70 and 72% respectively).

4.6 Methodological considerations

There are some limitations to the study, and methodological considerations to be discussed. Firstly, the sample size for the IFN- α treated HCV group is smaller than originally planned. Despite my best efforts in adding additional sites during the study, and a no-cost extension to the wider study grant, the rapid, unprecedented changes made by NHS England gave me a much smaller pool of patients receiving IFN- α from which to recruit. In addition to patients not meeting the eligibility criteria, particularly on the level of English language, an increasing number of patients at the study sites, key London university hospitals, were treated through clinical trials. In addition, my primary outcome measure for the study was assessed six-months post-treatment, at what was usually the eighth visit for participants enrolled, and six to twelve months after the baseline visit. Although I did have a reasonable retention rate, (70% of patients who completed treatment), there was a loss to follow-up which may have introduced some bias into my study, in that only patients who remained engaged for the full study were included. However, my results show that the severity of the acute response alone is not sufficient to identify those who may subsequently experience persistent fatigue, emphasising the importance of this measurement post-treatment. In addition, I was unable to collect information concerning the characteristics of patients who declined to take part in the study, and had incomplete information on the number of patients that declined or were not suitable for the study.

The prospective cohort study of IFN- α treated HCV patients was observational, resulting in some limitations typical of studies conducted in naturalistic settings. For example, the characteristics of the target patient sample meant that some individuals had co-morbid health conditions, which may have impacted on

results, independent of the effect of IFN- α . Due to a smaller sample size, and the scope of the project, it was not possible to further examine the relevance of any other underlying conditions. However, efforts were made to exclude patients with conditions known to significantly impact fatigue and inflammation, both at study initiation, and if diagnoses were made during the study period. Furthermore, by measuring the change in individuals in addition to fatigue severity, this gave me some indication of the effect of IFN- α , independent of baseline (pre-morbid) symptoms. In addition, some participants were taking adjunctive medications, such as opioids as part of drug rehabilitation programs, and statins, that may independently affect levels of inflammation and possibly fatigue. However, I found in this study that more patients on opioids recovered post-treatment. In addition, though originally I had excluded patients who were on antidepressants at study initiation, and/or met criteria for a depressive episode, because of the aforementioned changes in the prescription of IFN- α , it became necessary to widen the criteria. However, only four such patients were included, representing a small minority of patients.

Blood samples were non-fasting, and the time of blood draw was not standardised. However, patients attended the same half-day clinic throughout, and where possible were allocated similar appointment times. Therefore approximate times were at least standard within patients, across longitudinal measures. In addition, as presented in the results section, distribution of patients in the RF/PF groups was approximately equal for AM/PM blood draws.

I did not collect complete information on disease severity, specifically the onset of the illness, or duration of untreated HCV. Previous studies have found differences in patients' responses to their illness, relevant to illness perceptions, as well as the severity of fatigue. However, fatigue in HCV is typically only

related to disease severity after patients become cirrhotic, and severely ill patients were excluded from this study. Moreover, duration of untreated HCV and illness onset may be approximate, since many patients do not know they have contracted HCV until it is identified either through routine blood testing, or they become symptomatic later on in the disease.

Since the longitudinal data was collected over many time points, there was some missing data for some participants. In the case of the reports of clinical symptoms and health status, this meant that at each time point, the sample may have differed slightly and this should be considered in the interpretation of the results. However, in the context of persistent fatigue, as well as examining the difference in severity I also explored differences in the change relative to baseline at each point, which provides useful information on the decline and recovery of function at discrete time points. For measures of biological markers, the analyses conducted included only those individuals who had all time points of interest, and so missing data was less important, though of course it did reduce the sample size further.

I measured levels of cytokines and tryptophan and kynurenine pathway metabolites in the periphery, representing a snapshot of levels at the given time. This may have led to an over- or underestimation of their relationship with the different factors explored, due to the various methodological considerations that may affect their levels, for example diurnal and temporal variability. However, the repeated measures analyses examined longitudinal changes within individuals. The measure of salivary cortisol would be similarly affected, though the protocol used is well validated, and the strict protocols for data collection and data cleaning should restrict some confounding factors. Furthermore, attempts to measure IL-1 β and IL-4 were not successful, with levels

undetectable, or recorded but below the lower limit of detection, in too great a proportion of patients. For levels of IL-4, this is in keeping with the manufacturers protocols. For IL-1 β , though it is significant for the effects of cytokines on behaviour, it is often difficult to detect in human blood even in cases of infection, and so this finding too is not unexpected (Pollmacher et al., 2002). Finally, for both the prospective cohort and cross-sectional studies, due to cost constraints it was not possible to measure all analytes in all patients. Therefore, I selected analytes according to those that had been of interest in the prospective cohort results, as well as the best fit according to those markers available to be measured together in standard kits. In doing so, I may have missed some markers of interest.

There were also some issues to consider with the questionnaires selected for my study. Some data was obtained from an earlier study, which I was also a part of, with a focus on IFN- α induced depression. As such, some measures more specific to chronic fatigue and relevant risk factors were introduced later. Participant numbers for the reviewed package of measures were also limited by the decline in participant numbers later in the study, and the range of measures that could be added was limited. For example, it would have been advantageous to obtain additional measures of CFS-like symptoms apart from fatigue, such as flu-like symptoms, as well as a more detailed assessment of neurocognitive symptoms and aches and pains. Another example is the Cognitive and Behavioural responses to Symptoms Questionnaire (CBSQ). In addition to the reduced sample size, it had also originally been included in the baseline pack, but was subsequently withdrawn. This was because its design assumes that patients are symptomatic, with no options available for patients who are asymptomatic, which was true of many HCV patients. Therefore, to

avoid disengagement and reduce the time of an already lengthy interview, following patient feedback it was removed. However, in doing so I may have missed the opportunity to obtain data on the responses of symptomatic patients to pre-treatment fatigue. With regard to the measure of fatigue, the Chalder Fatigue Questionnaire, the measure has been used successfully in many studies of Chronic Fatigue Syndrome and other associated conditions where fatigue is one of the main complaints. However, the wording of the questionnaire (e.g. 'less than usual'/'more than usual') did not always feel well suited to this population, resulting in patients typically responding with all 'no more than usual' answers, and score of '11', which reduced the ability of the scale to detect subtle differences longitudinally. Perhaps a scale that includes a response of 'no fatigue' or 'no problems' would have been better suited to this particular study. However, it should be noted that it was possible to detect changes in acute fatigue scores using the measure and differences between groups.

With regard to the characterisation of patients as 'persistently fatigued', this was based on a somewhat crude measure based on patients having a fatigue score that was one point or higher 'worse' than they had reported at baseline. There is no comparable model to understand what a meaningful change in score might look like, and it is true that for this scale one point indicates at least one problem experienced 'more' or 'much more' than usual (a score of 1 vs. 2; 2 vs. 3), or alternatively, not less than usual, (1 vs. 0), which may still be relevant for the patient experience. However, given the smaller sample size, more advanced characterisation of patients was not viable. With a larger sample size, plotting of individual fatigue scores longitudinally could help identify clusters of patients who responded similarly, to further examine 'phenotypes' in relation to their

response to the immune trigger, IFN- α , and baseline fatigue. In addition, six of the PF individuals, or one third, reported debilitating fatigue, which would classify them as 'cases' (Cella & Chalder, 2010). A similar proportion of patients could be considered 'cases' six to twelve months after the onset of infectious mononucleosis (38-40%) (Candy et al., 2003). A larger sample would have also allowed for a sub-analysis of these individuals. Nonetheless, the characterisation strategy used did identify a subset of patients in whom fatigue was worse than reported at baseline, who exhibited an exaggerated clinical response, greater functional decline, and some evidence of an increased inflammatory response to the trigger, IFN- α .

With regard to the cross-sectional study, perhaps the greatest limitation was in the differences in the study populations. Indeed, one of the key findings was the incidence of depression in the CFS sample. However, this finding was biased by the recruitment strategy, with depressed HCV patients only included much later in the study, and healthy controls screened for psychiatric conditions. It may also have been further biased, since individuals generally attended if they did not have work commitments. I had intended to attempt to recruit a non-depressed CFS sample, however the high rates of depression in patients referred for specialist care, and difficulties in identifying patients who were able to commit to a study visit meant that I had to widen my inclusion criteria. Furthermore, volunteers were offered evening appointments, though uptake was understandably limited. Therefore, it was not within the scope of this project to disentangle the effects of CFS versus depression, which in any case are highly interrelated. The same considerations for widening the criteria were true regarding the onset of CFS; PF patients had experienced persistent fatigue for the minimum timeframe required by the CDC diagnosis (Fukuda et al., 1994).

This is also a consideration for the risk factors identified, since previous studies of PIFS have suggested that psychosocial factors may be of greater relevance to more chronic presentations (White et al., 2001). In order to achieve a more similar group, ideally I wanted to recruit recent onset CFS patients only. However, patients with CFS often had a longer duration of illness by the time they had been granted funding for an assessment at a specialist service, and so this was not a viable strategy in the time available. Of note, however, I did not observe an effect of illness duration on the biological markers examined.

As highlighted previously, I focussed on fatigue as a symptom, and did not capture additional information on flu-like symptoms, and more detailed information on neurocognitive symptoms, which might have also yielded some interesting results. I did not incorporate home visits, to include severely affected patients. However, for the primary aim of the cross-sectional study, to compare HCV PF versus CFS patients, the degree of disability for those patients able to attend the unit for an assessment was more appropriate. Finally, as well as the aforementioned concerns about fatigue onset, recruiting solely from specialist CFS services may well have resulted in a CFS sample which was not representative of CFS patients overall, due to the underrepresentation of ethnic minorities in tertiary care (Euba et al., 1996; Jason et al., 2003). However, in doing so we ensured that all patients had been diagnosed in a standardised manner, by trained doctors who were experienced in conducting such assessments.

Another difference between the HCV PF patients and CFS patients that is of interest was the relevance of the perceived cause of their symptoms, and prognosis. Not captured completely by the attribution measures reported, patients with CFS had endured months or even years of diagnostic tests to

investigate a possible explanation for their condition, with no clear 'end' in sight. Anecdotally, patients with HCV tended to attribute their symptoms to either their (treatable) condition, or a transient effect of IFN- α treatment. Anecdotally, no patient expressed concern about on-going symptoms or that they were concerned symptoms would not resolve. This is in stark contrast to CFS and such beliefs may have influenced results.

Finally, there are critics of the use of such proxy models of chronic fatigue for the application of knowledge to Chronic Fatigue Syndrome. Indeed, one such comment highlights the high concentrations of the protein (IFN- α) administered, higher than would occur in the natural sickness response, as well as the potential for the observed effects to be confounded by the underlying medical illness itself (Vollmer-Conna, 2001). However, as highlighted earlier, though this may be true, I did investigate changes relative to baseline function. In addition, I focussed on fatigue as a symptom, which was not associated with the available measures of the severity of HCV.

In summary, while there are some limitations to the proxy model, and the way in which I conducted this study specifically, I have also highlighted some of the strengths of this study throughout. In recent years, despite some advances, the field of CFS has made slow progress in understanding the pathogenesis of this complex illness. Studies such as this, which examine the development of chronic fatigue following an immune trigger from a different angle, are not designed to replace large cohort studies, but to supplement them. My study utilised the IFN- α model to examine baseline risk factors, and prospectively monitor longitudinal changes in response to the trigger within a well-defined timeframe with much fewer resources than a larger cohort study would require. Furthermore, I examined a wide range of variables within one study.

4.7 Clinical implications

There are some findings from this study that may have relevance for clinical practice. A key finding in this regard was that it was difficult to identify patients at the greatest risk of persistent fatigue following an immune trigger, or indeed more severe fatigue, based on socio-demographic characteristics alone. Indeed, there was a group of individuals who were seemingly psychologically healthy who, for some reason not fully elucidated here, had an increased vulnerability to the effects of the trigger, which resulted in the persistence of symptoms later on. Part of the story involved inflammation-induced mood disturbances, and increases in perceived stress. Recent experience of a stressful event, specifically during exposure to the trigger was also associated with the persistence of symptoms post-treatment. These findings emphasise the need to monitor changes in mood, fatigue, and external stressors during exposure to the trigger to identify those in need of further intervention.

In addition, as suggested earlier, more commonly used measures of baseline mental health, for example of depressive symptoms, were not associated with the later severity of fatigue post-IFN- α . However, measures of functioning associated with poorer mental health were, specifically the degree to which the individual felt limited in their usual role or everyday activities by emotional problems. Therefore, more functional assessments of the impact of low mood could also be integrated into any assessment of pre-morbid psychopathology.

In this study, levels of IL-10 only increased in those individuals who would later experience persistent fatigue, not in those in whom fatigue resolved. While IL-6 was also significantly higher in PF patients, it was also elevated in the RF group to some degree. Therefore the measurement of IL-10 in the acute sickness phase may prove more useful as a biomarker for later persistent fatigue.

Though they did not predict persistent fatigue, or more severe fatigue post-treatment, illness perceptions were strongly associated with the experience of acute fatigue during treatment. As opposed to non-recovery, it was the increased fatigue and other symptoms in response to the trigger (IFN- α) that put PF patients at a disadvantage for their subsequent recovery. Therefore, the measurement of illness perceptions prior to IFN- α treatment, or early on in the illness course of an infectious disease may identify those who would benefit from psychoeducation to challenge any negative beliefs. As an additional point, the only illness perception dimension found not to be associated with subsequent acute fatigue was beliefs relating to the potential success of treatment in controlling their symptoms. Anecdotally, the services provide a great deal of information about treatment, such as what to expect and the likely chance of success based on the individual's clinical presentation. This helps ensure patients are ready for the challenge that IFN- α will present, and adherence to treatment. For IFN- α specifically, or indeed other treatments for HCV where side effects include fatigue, interventions to address other negative perceptions could be integrated as part of the existing package of care.

Importantly, there was some indication that HCV PF patients had started to adopt some behaviours in response to the persistent symptoms, and were showing some evidence of a lack of acceptance of fatigue. However, there were some characteristics, for example catastrophising and fear avoidance beliefs, as well as avoidance/resting behaviours, which were specific to CFS. These findings highlight the need to identify individuals who report persistent fatigue soon after exposure to the immune trigger, as early intervention and advice may prevent these individuals from engaging in behaviours, or developing cognitions that might contribute towards deconditioning and further decline.

Though not the main aim of this study, in light of the continued use of IFN- α regimens in some populations, the findings should also be borne in mind for the general clinical management of IFN- α treated patients. For example, as previously discussed in the introduction, there is such a focus on treatment response and managing the patient through the acute symptoms that there may be little advice provided on the potential longer term outcomes and persistence of side effects post-treatment. The monitoring of patient reported symptoms of low mood and fatigue would give clinical nurse specialists and consultants some indication of which patients may require additional follow-up. Clinicians should also note that some patients continued to experience worse fatigue than baseline six-months post-treatment, despite having cleared the virus.

4.8 Conclusions and future research

In conclusion, the findings from this study highlight differential, often exaggerated responses to an immune trigger, IFN- α immunotherapy, in patients who continue to experience persistent fatigue long after the stimulus is no longer present, versus those in whom fatigue resolves. Furthermore, persistent fatigue was not attributable to the failure of treatment. As opposed to non-recovery, both groups experienced a similar rate of improvement post-treatment, but the persistently fatigued patients were at a significant disadvantage, having experienced higher levels of fatigue, depressive symptoms, and perceived stress, and a greater decline in health status.

Understudied in the context of IFN- α induced side effects, I found that baseline illness perceptions were predictive of subsequent acute fatigue. However, unlike has been seen in post-viral fatigue, they were not associated with the persistence of fatigue post-treatment. Indeed, few characteristics or baseline risk factors were evident, which may have otherwise identified patients at greater risk of persistent fatigue, or indeed more severe fatigue post-treatment. For example, I did not find the expected association with past experience of childhood trauma, though traumatic events across the lifespan were to some extent associated with the persistence of fatigue. Although baseline inflammatory markers were in general not associated with persistent fatigue, there was a trend towards higher levels of IL-6 and IFN- γ , which warrant further investigation as predictors in other disease models.

With regard to longitudinal changes, mood and fatigue were highly related, and persistence of fatigue was associated with higher clinical symptoms, and a greater decline in health status throughout, with particular points of interest after

four weeks, as well as after six-months of treatment. Levels of the cytokines IL-10 and IL-6 were also elevated in response to IFN- α treatment in these patients.

Future research would need to further examine possible mechanisms underlying an exaggerated immune response, for example genetic polymorphisms among patients who continue to experience persistent fatigue. In addition, longitudinal analysis of changes in gene expression would be beneficial, as has been conducted in relation to acute fatigue and IFN- α induced depression. Specifically, analysis of genes related to immune system functioning, as well as those related to the neuroendocrine system would aid understanding of the specific pathways underlying the persistence of such a side effect in the absence of the original stimulus.

With regard to cross-sectional comparisons of HCV RF and PF patients, healthy volunteers and CFS patients, differences in age and gender should be noted between HCV and CFS samples. There were higher rates of depression among CFS patients, though this is a descriptive point only, since any comparison was biased by the exclusion of such individuals in other samples. CFS patients were more impaired across measures of fatigue and perceived stress, and various measures of health status and functioning. For some clinical measures, HCV PF too experienced greater problems than their HCV RF peers, as well as healthy volunteers. However, across the range of peripheral markers examined, there was very little evidence of increased inflammation in CFS patients, comparable to controls, while HCV PF and RF patients too had similarly higher levels. Notably, lower levels of the ratio of kynurenine to tryptophan were found in HCV PF and CFS patients, suggestive of an association with the experience of chronic fatigue, and worthy of further exploration.

The findings from this study support previous evidence from disease models of post-viral or post-infective fatigue syndrome, that the most notable differences which distinguish patients in whom fatigue persists, versus those in whom fatigue resolves, occur during the 'acute sickness' phase, in the host response to the trigger. Few clear socio-demographic or psychosocial risk factors were apparent, suggesting that biological differences not elucidated here may be more heavily weighted in determining outcomes. In addition, they replicate previous findings concerning the disparity between the levels of disability experienced by CFS patients, and the measurement of peripheral cytokines. This study incorporated a wide range of biological and clinical measures to examine in detail the response to an immune trigger which in some leads to persistent fatigue and other associated symptoms. Though not so closely related to CFS as the PIFS disease models, this study also provides valuable insights to enhance our understanding of the relevant factors to pursue to better understand this terrible, disabling condition.

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Appendix A: Schedule of measures

Measure	Baseline (TW0)	TW4	TW8	TW12	TW24	[TW36]	[TW48]	Six-months post-treatment
Questionnaires								
Social Data Schedule (SDS)	X							
MINI International Neuropsychiatric Interview (MINI)	X	X*	X*	X*	X*	X*	X*	X*
Family Interview for Genetic Studies (FIGS)	X							
Cannabis Experience Questionnaire, Section II (CEQ)	X							
Alcohol Use Disorders Identification Test (AUDIT)	X				X	X	X	X
Brief Life Events Questionnaire (BLE)	X				X	X	X	X
Intrusive Life Events Schedule (ILES)	X							
Childhood Experience of Care and Abuse Questionnaire (CECA-Q)	X							
Illness Perception Questionnaire (IPQ)	X							
Chalder Fatigue Questionnaire (CFQ)	X	X	X	X	X	X	X	X
Inventory of Depressive Symptomatology (IDS)	X	X	X	X	X	X	X	X
Hospital Anxiety & Depression Scale (HADS)	X	X	X	X	X	X	X	X
Perceived Stress Scale (PSS)	X	X	X	X	X	X	X	X
Jenkins Sleep Scale [^]	X	X	X	X	X	X	X	X
Short-Form 36 Medical Outcomes Survey (SF-36)	X	X	X	X	X	X	X	X
Acceptance of Fatigue Questionnaire [^]	X			X	X	X	X	X
Cognitive and Behavioural responses to Symptoms (CBSQ) [^]								
Attribution scale (1) [^]								X
Attribution scale (2) [^]								X
Biological measures								
Salivary cortisol	X							X
Cytokines	X	X			X			X
Kynurenine pathway	X		X		X	X	X	X

* Only completed if either HADS subscale ≥ 7 ; [^] subsample only (see main text)

Appendix B: Social Data Schedule

Social Data Schedule

1) Sex

- 0 Male
- 1 Female

--

2) Date of birth

--	--	--	--	--	--

3) Age

--	--

4) From the list below, how would you describe your ethnicity?

- 0 White British
- 1 Mixed
- 2 Indian
- 3 Pakistani
- 4 Bangladeshi
- 5 Other Asian
- 6 Black Caribbean
- 7 Black African
- 8 Black Other
- 9 Chinese
- 10 Other

--	--

5) Where did you live for the first 16 years of your life, starting with the place you were born?

Country	City/Town	Street	No. of years
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....

6) With whom do you live now?

- 0 Alone
- 1 Alone, with children
- 2 Partner/Spouse
- 3 Partner/Spouse and children
- 4 Parents
- 5 Other family
- 6 Friends
- 7 Other (specify)

☐

.....

7) With whom did you live one year ago?

- 0 Alone
- 1 Alone, with children
- 2 Partner
- 3 Partner and children
- 4 Parents
- 5 Other family
- 6 Friends
- 7 Other (specify)

☐

.....

8) With whom did you live five years ago?

- 0 Alone
- 1 Alone, with children
- 2 Partner
- 3 Partner and children
- 4 Parents
- 5 Other family
- 6 Friends
- 7 Other (specify)

☐

.....

9) Since leaving your parents' home, have you ever lived with others?

- 0 No
- 1 Yes

☐

10) What is your relationship status now?

- 0 Single
- 1 Married/Living with someone
- 2 In steady relationship
- 3 Divorced, Separated
- 4 Widowed

☐

11) What was your relationship status one year ago?

- 0 Single
- 1 Married/Living with someone
- 2 In steady relationship
- 3 Divorced, Separated
- 4 Widowed

☐

12) What was your relationship status five years ago?

- 0 Single
- 1 Married/Living with someone
- 2 In steady relationship
- 3 Divorced, Separated
- 4 Widowed

☐

13) Have you ever been in a long-term relationship (1 or more years)?

- 0 No
- 1 Yes

☐

14) What was the highest level of education you reached?

- 0 No qualifications
- 1 GCSE/O' levels
- 2 A' levels
- 3 Vocational/college (B. Tecs/NVQs etc.)
- 4 University/Professional Qualifications

☐

15) Are you employed now?

- 0 No, unemployed
- 1 No, student
- 2 Yes, full-time
- 3 Yes, part-time

☐

16) Were you employed one year ago?

- 0 No, unemployed
- 1 No, student
- 2 Yes, full-time
- 3 Yes, part-time

☐

17) Were you employed five years ago?

- 0 No, unemployed
- 1 No, student
- 2 Yes, full-time
- 3 Yes, part-time

☐

18) Have you ever been employed?

- 0 No
- 1 Yes

☐

19) What is your first language?

- 0 English
- 1 Other (please state) _ _ _ _ _

☐

Appendix C: MINI International Neuropsychiatric Interview

[selected questions]

MAJOR DEPRESSIVE EPISODE

➞ MEANS: GO TO THE DIAGNOSTIC BOXES, CIRCLE **NO** IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

A1	Have you ever been consistently depressed or down, most of the day, nearly every day, for the past two weeks?	NO	YES
A2	In the past two weeks, have you been less interested in most things or less able to enjoy things that you used to enjoy most of the time?	NO	YES
	IS A1 or A2 CODED YES?	➞	NO
	YES		

A3 Over the past two weeks, when you have depressed or uninterested:

a.	Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e., by $\pm 5\%$ of body weight or ± 8 lbs. or ± 3.5 kgs., for a 160 lb./70 kg. person in a month)?	NO	YES
b.	Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?	NO	YES
c.	Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still almost every day?	NO	YES
d.	Did you feel tired or without energy almost every day?	NO	YES
e.	Did you feel worthless or guilty almost every day?	NO	YES
f.	Did you have difficulty concentrating or making decisions almost every day?	NO	YES
g.	Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead?	NO	YES

A4 ARE 3 OR MORE A3 ANSWERS CODED YES?
(OR 4 A3 ANSWERS IF A1 OR A2 ARE CODED NO)?

NO YES

**MAJOR DEPRESSIVE
EPISODE CURRENT**

IF PATIENT MEETS CRITERIA FOR MAJOR DEPRESSIVE EPISODE CURRENT:

A5	a. During your lifetime, did you have other periods of two weeks or more when you felt depressed or uninterested in most things, and had most of the problems we just talked about?	➞	NO	YES
	b. Was there an interval of at least 2 months without depression/loss of interest between your current episode and your last episode of depression?		NO	YES

IS A5b CODED YES?

NO YES

**MAJOR DEPRESSIVE
EPISODE PAST**

Appendix D: Family Interview for Genetic Studies (FIGS)

[selected questions]

I am asking you to keep in mind all members of your family including Grandparents, parents, siblings and offspring aged 18 or above.

Did anyone:

- a) Feel very low for a couple of weeks or more, or have a diagnosis of depression?**

YES / NO If YES, who? _____

- b) Attempt or complete suicide?**

YES / NO If YES, who? _____

- c) Seem overexcited (or manic) day and night, or have a diagnosis of mania?**

YES / NO If YES, who? _____

- d) Have visions, hear voices, or have beliefs that seem strange or unreal?**

YES / NO If YES, who? _____

- e) Have unusual or bizarre behavior, or have a diagnosis of schizophrenia?**

YES / NO If YES, who? _____

- f) Was anyone hospitalized for psychiatric problems?**

YES / NO If YES, who? _____

Appendix E: Cannabis Experience Questionnaire, Section II

Please indicate in the table below any drug(s) (cannabis, amphetamines, cocaine, ecstasy, acid, LSD, tranquilisers, crack, heroin) **including alcohol and tobacco** which you use/have used recreationally, the frequency with which you use/have used this drug, your age when you first tried the drug(s) and whether you are a past or current user. Use a new box for each additional drug: Circle your response(s) as appropriate.

Drug	Frequency	Age	Use	When
	<input type="checkbox"/> Everyday <input type="checkbox"/> More than once a week <input type="checkbox"/> A few times each month <input type="checkbox"/> A few times each year <input type="checkbox"/> Only once or twice	<input type="checkbox"/> Started <input type="checkbox"/> Stopped	<input type="checkbox"/> Current <input type="checkbox"/> Past	<input type="checkbox"/> Day <input type="checkbox"/> Night <input type="checkbox"/> Both day/night
	<input type="checkbox"/> Everyday <input type="checkbox"/> More than once a week <input type="checkbox"/> A few times each month <input type="checkbox"/> A few times each year <input type="checkbox"/> Only once or twice	<input type="checkbox"/> Started <input type="checkbox"/> Stopped	<input type="checkbox"/> Current <input type="checkbox"/> Past	<input type="checkbox"/> Day <input type="checkbox"/> Night <input type="checkbox"/> Both day/night
	<input type="checkbox"/> Everyday <input type="checkbox"/> More than once a week <input type="checkbox"/> A few times each month <input type="checkbox"/> A few times each year <input type="checkbox"/> Only once or twice	<input type="checkbox"/> Started <input type="checkbox"/> Stopped	<input type="checkbox"/> Current <input type="checkbox"/> Past	<input type="checkbox"/> Day <input type="checkbox"/> Night <input type="checkbox"/> Both day/night

Drug	Frequency	Age	Use	When
	Everyday More than once a week A few times each month A few times each year Only once or twice	Started Stopped	Current Past	Day Night Both day/night
	Everyday More than once a week A few times each month A few times each year Only once or twice	Started Stopped	Current Past	Day Night Both day/night
	Everyday More than once a week A few times each month A few times each year Only once or twice	Started Stopped	Current Past	Day Night Both day/night
	Everyday More than once a week A few times each month A few times each year Only once or twice	Started Stopped	Current Past	Day Night Both day/night

Appendix F: Alcohol Use Disorders Identification Test (AUDIT)

This questionnaire asks about your alcohol use **in the last year**. Please read each item and circle the number next to the response that best describes your use of alcohol.

1. How often do you have a drink containing alcohol?
 - 0 Never
 - 1 Monthly or less
 - 2 Two or four times a month
 - 3 Two to three times a week
 - 4 Four or more times a week

2. How many drinks containing alcohol do you have in a typical day when you are drinking?
 - 0 1 or 2
 - 1 3 or 4
 - 2 5 or 6
 - 3 7 to 9
 - 4 10 or more

3. How often do you have six or more drinks on any one occasion?
 - 0 Never
 - 1 Less than monthly
 - 2 Monthly
 - 3 Weekly
 - 4 Daily or almost daily

4. How often during the last year have you found that you were not able to stop drinking once you had started?
 - 0 Never
 - 1 Less than monthly
 - 2 Monthly
 - 3 Weekly
 - 4 Daily or almost daily

5. How often during the last year have you failed to do what was normally expected of you because of drinking?
 - 0 Never
 - 1 Less than monthly
 - 2 Monthly
 - 3 Weekly
 - 4 Daily or almost daily

[continued overleaf]

6. How often during the last year have you needed a drink in the morning to get yourself going after a heavy drinking session?

- 0 Never
- 1 Less than monthly
- 2 Monthly
- 3 Weekly
- 4 Daily or almost daily

7. How often during the last year have you had a feeling of guilt or remorse after drinking?

- 0 Never
- 1 Less than monthly
- 2 Monthly
- 3 Weekly
- 4 Daily or almost daily

8. How often during the last year have you been unable to remember what happened the night before because you had been drinking?

- 0 Never
- 1 Less than monthly
- 2 Monthly
- 3 Weekly
- 4 Daily or almost daily

9. Have you or someone else been injured because of your drinking?

- 0 No
- 2 Yes, but not in the last year
- 4 Yes, during the last year

10. Has a relative, friend, doctor or other health worker been concerned about your drinking or suggested that you should cut down?

- 0 No
- 2 Yes, but not in the last year
- 4 Yes, during the last year

Appendix G: Brief Life Events Questionnaire (BLE)

The following questions are about events or problems which may have happened to you **in the last 6 months** which might have caused you distress and to seek help.

1. Did you suffer from a serious illness injury or an assault?	Yes/No	Date: _____
2. Did a serious illness, injury or assault happen to a close relative?	Yes/No	Date: _____
3. Did a parent, spouse, partner, child, brother or sister of yours die?	Yes/No	Date: _____
4. Did a close family friend or other relative die?	Yes/No	Date: _____
5. Did you have a separation due to marital difficulties or break off a steady relationship?	Yes/No	Date: _____
5b. Did you end a long lasting friendship with a close friend or relative?	Yes/No	Date: _____
6. Did you have serious problem with a close friend, neighbour or relative?	Yes/No	Date: _____
7. Were you made redundant or sacked from your job?	Yes/No	Date: _____
8. Were you seeking work without success for more than 1 month?	Yes/No	Date: _____
9. Did you have a major financial crisis such as losing the equivalent of three months income?	Yes/No	Date: _____
10. Did you have problems with the police involving a court appearance?	Yes/No	Date: _____
11. Was something you valued lost or stolen?	Yes/No	Date: _____
12. Did you/your wife or partner give birth to a child?	Yes/No	Date: _____

Appendix H: Intrusive Life Events Schedule (ILES)

I would now like to ask you about things that may have happened to you or problems you may have faced throughout your life. [SHOW CARD] Looking at the card, can you tell me if you have ever suffered from any of the problems or events shown on the card, at any time in your life.

0	No
1	Yes

- 1) Serious injury or assault to yourself _____
- 2) Bullying _____
- 3) Violence at work _____
- 4) Violence in the home _____
- 5) Sexual abuse _____
- 6) Being expelled from school _____
- 7) Running away from home _____
- 8) Being homeless _____
- 9) Taken into local authority care _____
- 10) Time in children's institution _____

Appendix I: Childhood Experiences of Care & Abuse

Questionnaire (CECA-Q)

[Selected items]

1) WHO BROUGHT YOU UP BEFORE AGE 17?

Write below the PARENT FIGURES who brought you up in childhood. List each family arrangement with different types of parent figures which lasted a year or longer. Consider natural parents, step-parents (including parents' live in partners), aunt, friends of the family, adoptive parents, foster parents, etc.

If you have only lived in one arrangement, then fill in the first family arrangement and leave the other boxes blank. For example, if this was with your natural parents, write in 'Mother' and 'Father' and age '0'.

Family arrangement	Mother figure	Father figure	Your age at start
First (ALL)			

If you have lived in other arrangements such as with mother alone or mother and step-father, then list them below together with age you were when the arrangement began.

Family arrangement	Mother figure	Father figure	Your age at start
Second (If applicable)			
Third (If applicable)			

Were you ever in a children's home or institution prior to age 17?

0 No
1 Yes

☐

If NO, go to question x.

If yes: Type of institution e.g. local authority care; hospital, etc.	Age entered	Age left

[continued overleaf]

2) PARENTAL LOSS AND SEPARATION [Please circle or write in answer]

Did either parent die before you were aged 17?

If YES, what age were you?

Have you ever been separated from either parent for 6 months or more before age 17?

Mother		Father	
No	Yes	No	Yes
Age		Age	
No	Yes	No	Yes

If NO separation, then go to question x.

If YES separated:

At what age were you first separated?	Age	Age
How long was this separation for? Years Years
What was the reason for separation?		
Parental illness	No Yes	No Yes
Parental divorce, separation	No Yes	No Yes
Abandoned by parent or never knew parent	No Yes	No Yes
Other reason (please specify below)	No Yes	No Yes

Please describe your experience

.....

.....

.....

[continued overleaf]

3) PHYSICAL PUNISHMENT BEFORE THE AGE OF 17 BY A PARENT FIGURE OR OTHER HOUSEHOLD MEMBER

When you were a child or a teenager were you ever hit repeatedly with an implement such as a belt or stick) or punched, kicked or burnt by someone in the household?

0 No
1 Yes

☐

If NO, go to question x.

If YES:

	Mother Figure	Father Figure
How old were you when it began?	Age	Age
Did the hitting happen on more than one occasion?	No Yes	No Yes
How were you hit?	0 Belt or stick 1 Punched/kicked 2 Hit with hand 3 Other	0 Belt or stick 1 Punched/kicked 2 Hit with hand 3 Other
Were you ever injured, e.g. bruises, black eyes, broken limbs?	No Yes	No Yes
Was this person ever so angry they seemed out of control?	No Yes	No Yes

Please describe your experience

.....

.....

.....

.....

Did you experience this from anyone else in the household?

0 No
1 Yes

☐

Please describe your experience

.....

.....

.....

.....

[continued overleaf]

4) UNWANTED SEXUAL EXPERIENCES BEFORE AGE 17 [Please circle as appropriate]

When you were a child or teenager did you ever have any unwanted sexual experiences?

- 0 No
- 1 Yes
- 2 Unsure

☐

Did anyone force you or persuade you to have sexual intercourse against your wishes before age 17?

- 0 No
- 1 Yes
- 2 Unsure

☐

Can you think of any upsetting sexual experiences before age 17 with a related adult or someone in authority, e.g. teacher?

- 0 No
- 1 Yes
- 2 Unsure

☐

If NONE, end interview.

If YES or UNSURE to any of the above, then complete the following:

	First Experience		Second Experience	
How old were you when it began?	Age		Age	
Was the other person someone you knew?	No	Yes	No	Yes
Was the other person a relative?	No	Yes	No	Yes
Did this person do it on more than one occasion?	No	Yes	No	Yes
Did it involve touching private parts of your body?	No	Yes	No	Yes
Did it involve sexual intercourse?	No	Yes	No	Yes

Please describe your experience

.....

.....

.....

.....

Appendix J: Illness Perception Questionnaire (IPQ)

Your views about your illness

Listed below are a number of symptoms that you may or may not have experienced since your illness. Please indicate by circling yes or no, whether you have experienced any of these symptoms since your illness, and whether you believe that these symptoms are related to your illness.

	<i>I have experienced this symptom since my diagnosis</i>	<i>This symptom is related to my illness</i>
Pain	Yes / No	Yes / No
Sore Throat	Yes / No	Yes / No
Nausea	Yes / No	Yes / No
Breathlessness	Yes / No	Yes / No
Weight Loss	Yes / No	Yes / No
Fatigue	Yes / No	Yes / No
Stiff Joints	Yes / No	Yes / No
Sore Eyes	Yes / No	Yes / No
Wheeziness	Yes / No	Yes / No
Headaches	Yes / No	Yes / No
Upset Stomach	Yes / No	Yes / No
Sleep Difficulties	Yes / No	Yes / No
Dizziness	Yes / No	Yes / No
Loss of Strength	Yes / No	Yes / No

[continued overleaf]

We are interested in your own personal views of how you see your current illness.

Please indicate how much you agree or disagree with the following statements about your illness by ticking the appropriate box.

	Views about your Illness	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
IP1 *	My illness will last a short time					
IP2	My illness is likely to be permanent rather than temporary					
IP3	My illness will last for a long time					
IP4 *	This illness will pass quickly					
IP5	I expect to have this illness for the rest of my life					
IP6	My illness is a serious condition					
IP7	My illness has major consequences on my life					
IP8 *	My illness does not have much effect on my life					
IP9	My illness strongly affects the way others see me					
IP10	My illness has serious financial consequences					
IP11	My illness causes difficulties for those who are close to me					
IP12	There is a lot which I can do to control my symptoms					
IP13	What I do can determine whether my illness gets better or worse					
IP14	The course of my illness depends on me					
IP15 *	Nothing I do will affect my illness					
IP16	I have the power to influence my illness					
IP17 *	My actions will have no affect on the outcome of my illness					
IP18 *	My illness will improve in time					
IP19 *	There is very little that can be done to improve my illness					

	Views about your Illness	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
IP20	The treatment will be effective in curing my illness					
IP21	The negative effects of my illness can be prevented by my treatment					
IP22	My treatment can control my illness					
IP23 *	There is nothing which can help my condition					
IP24 *	The symptoms of my condition are puzzling to me					
IP25 *	My illness is a mystery to me					
IP26 *	I don't understand my illness					
IP27 *	My illness doesn't make any sense to me					
IP28	I have clear picture or understanding of my condition					
IP29	The symptoms of my illness change a great deal from day to day					
IP30	My symptoms come and go in cycles					
IP31	My illness is very unpredictable					
IP32	I go through cycles in which my illness gets better and worse					
IP33	I get depressed when I think about my illness					
IP34	When I think about my illness I get upset					
IP35	My illness makes me feel angry					
IP36 *	My illness does not worry me					
IP37	Having this illness makes me feel anxious					
IP38	My illness makes me feel afraid					

[continued overleaf]

Causes of my illness

We are interested in what you consider may have been the cause of your illness. As people are very different, there is no correct answer for this question. We are most interested in your own views about the factors that caused your illness rather than what others, including doctors of family, may have suggested to you. Below is a list of possible causes for your illness. Please indicate how much you agree or disagree that they were causes for you by ticking the appropriate box.

	Possible Causes	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
C1	Stress or worry					
C2	Hereditary – runs in my family					
C3	A germ or virus					
C4	Diet or eating habits					
C5	Chance or bad luck					
C6	Poor medical care in my past					
C7	Pollution in the environment					
C8	My own behaviour					
C9	My mental attitude e.g. thinking about life negatively					
C10	Family problems or worries caused my illness					
C11	Overwork					
C12	My emotional state e.g. feeling down, lonely, empty, anxious					
C13	Ageing					
C14	Alcohol					
C15	Smoking					
C16	Accident or injury					
C17	My personality					
C18	Altered immunity					

In the table below, please list in rank order the three most important factors that you now believe caused your illness. You may use any of the items from the box above, or you may add additional ideas of your own.

The most important causes for me: -

1. _____
2. _____
3. _____

Appendix K: Chalder Fatigue Questionnaire (CFQ)

We would like to know more about any problems you have had with feeling tired, weak or lacking in energy in the **LAST MONTH**. Please answer all the questions by ticking the answer which applies to you most closely. Please choose only one option per question.

	Less than usual (0)	No more than usual (1)	More than usual (2)	Much more than usual (3)
Do you have problems with tiredness?				
Do you need to rest more?				
Do you feel sleepy or drowsy?				
Do you have problems starting things?				
Do you lack energy?				
Do you have less strength in your muscles?				
Do you feel weak?				
Do you have difficulty concentrating?				
Do you make slips of the tongue when speaking?				
Do you find it more difficult to find the correct word?				
Do you have problems with your memory?				

Appendix L: Inventory of Depressive Symptomatology

Please read each group of statements carefully and then pick out the **one statement** in each group that best describes the way that you have been feeling during the **past seven days, including today**. Circle the number beside each statement that you have picked.

1. Falling Asleep

- 0 I never take longer than 30 minutes to fall asleep.
- 1 I take at least 30 minutes to fall asleep, less than half the time.
- 2 I take at least 30 minutes to fall asleep, more than half the time.
- 3 I take more than 60 minutes to fall asleep, more than half of the time.

2. Sleep During the Night

- 0 I do not wake up at night.
- 1 I have restless, light sleep with few brief awakenings each night.
- 2 I wake up at least once a night, but I go back to sleep easily.
- 3 I awaken more than once a night and stay awake for 20 minutes or more, more than half the time.

3. Waking up too Early

- 0 Most of the time, I awaken no more than 30 minutes before I need to get up.
- 1 More than half the time, I awaken more than 30 minutes before I have to get up.
- 2 I almost always awaken at least one hour or so before I need to, but I go back to sleep eventually.
- 3 I awaken at least one hour before I need to, and can't go back to sleep.

4. Sleeping Too Much

- 0 I sleep no longer than 7-8 hours/night, without napping during the day.
- 1 I sleep no longer than 10 hours in a 24 hour period including naps.
- 2 I sleep no longer than 12 hours in a 24 hour period including naps.
- 3 I sleep longer than 12 hours in a 24 hour period including naps.

5. Feeling Sad

- 0 I do not feel sad.
- 1 I feel sad less than half the time.
- 2 I feel sad more than half the time.
- 3 I feel sad nearly all of the time.

6. Feeling Irritable

- 0 I do not feel irritable.
- 1 I feel irritable less than half the time.
- 2 I feel irritable more than half the time.
- 3 I feel extremely irritable nearly all of the time.

7. Feeling Anxious or Tense

- 0 I do not feel anxious or tense.
- 1 I feel anxious/tense less than half the time.
- 2 I feel anxious/tense more than half the time.
- 3 I feel extremely anxious/tense nearly all of the time.

8. Response of Your Mood to Good Events

- 0 My mood brightens to a normal level which lasts for several hours.
- 1 My mood brightens but I do not feel like my normal self when good events occur.
- 2 My mood brightens only somewhat to a rather limited range of desired events.
- 3 My mood does not brighten at all, even when very good or desired events occur in my life.

9. Mood in Relation to Time of Day

- 0 There is no regular relationship between my mood and the time of day.
- 1 My mood often relates to the time of day because of environmental events (e.g. being alone, working).
- 2 In general, my mood is more related to the time of day than to environmental events.
- 3 My mood is clearly and predictably better or worse at a particular time each day.

9A. Is your mood typically worse in the morning, afternoon or night (circle one).

9B. Is your mood variation attributed to the environment? Yes / No (circle one).

10. The Quality of your Mood

- 0 The mood (internal feelings) that I experience is very much a normal good mood.
- 1 My mood is sad, but this sadness is pretty much like sad mood I would feel if someone close to died or left.
- 2 My mood is sad, but this sadness has a rather different quality to it than the sadness I'd feel if someone close to me died or left.
- 3 My mood is sad, but this sadness is different from they type of sadness associated with grief or loss.

Please complete either 11 or 12 (not both)

11. Decreased Appetite

- 0 There is no change in my usual appetite.
- 1 I eat somewhat less often or lesser amounts
- 2 I eat much less than usual and only with personal effort.
- 3 I rarely eat within a 24hour period, and only with extreme personal effort or when other persuade me to eat.

12. Increased Appetite

- 0 There is no change in my usual appetite.
- 1 I feel a need to eat more frequently than usual,.
- 2 I regularly eat more often and/or greater amounts of food than usual.
- 3 I feel driven to overeat both at mealtimes and in between meals.

Please complete either 13 or 14 (not both)

13. Decreased Weight (within the last 2 weeks)

- 0 I have not had a change in my weight.
- 1 I feel as if I've had a slight weight loss.
- 2 I have lost 2 pounds or more.
- 3 I have lost 5 pounds or more.

14. Increased Weight (within the last 2 weeks)

- 0 I have not had a change in my weight.
- 1 I feel as if I've had a slight weight gain.
- 2 I have gained 2 pounds or more
- 3 I have gained 5 pounds or more

15. Concentration/Decision Making

- 0 There is no change in my usual capacity to concentrate or make decisions.
- 1 I occasionally feel indecisive or find that my attention wanders.
- 2 Most of the time, I struggle to focus my attention or to make decisions.
- 3 I cannot concentrate well enough to read or cannot make even minor decisions,

16. View of Myself

- 0 I see myself as equally worthwhile and deserving as other people.
- 1 I am more self-blaming than usual.
- 2 I largely believe that I cause problems for others.
- 3 I think almost constantly about major and minor defects in myself.

17. View of my future

- 0 I have an optimistic view of my future.
- 1 I am occasionally pessimistic about my future, but mostly I believe things will get better.
- 2 I'm pretty certain that my immediate future does not hold much promise of good things for me.
- 3 I see no hope of anything good happening to me anytime in the future.

18. Thought of Death or Suicide

- 0 I do not think of suicide or death.
- 1 I feel that life is empty or wonder if it's worth living.
- 2 I think of suicide or death several times a week for several minutes.
- 3 I think of suicide or death several times a day in some detail, or I have made specific plans for suicide or have actually tried to take my life.

19. General Interest

- 0 There is no change from usual in how interested I am in other people or activities.
- 1 I notice that I am less interested in people or activities.
- 2 I find I have interest in only one or more of my formerly pursued activities.
- 3 I have virtually no interest in formerly pursued activities.

20. Energy Level

- 0 There is no change in my usual level of energy.
- 1 I get tired more easily than usual.
- 2 I have to make a big effort to start or finish my usual daily activities
- 3 I really cannot carry out most of my usual daily activities because I don't have the energy.

21. Capacity for Pleasure of enjoyment (excluding sex)

- 0 I enjoy pleasurable activities just as much as usual.
- 1 I do not feel my usual sense of enjoyment from pleasurable activities.
- 2 I rarely get a feeling of pleasure from any activity.
- 3 I am unable to get any pleasure or enjoyment from anything.

22. Interest in Sex (please rate interest, not activity)

- 0 I'm just as interested in sex as usual.
- 1 My interest in sex is somewhat less than usual or I do not get the same pleasure from sex as I used to.
- 2 I have little desire for or rarely derive pleasure from sex.
- 3 I have absolutely no interest in or derive no pleasure from sex.

23. Feeling Slowed Down

- 0 I think, speak, and move at my usual rate of speed.
- 1 I find that my thinking is slowed down or my voice sounds dull or flat.
- 2 It takes me several seconds to respond to most questions and I'm sure my thinking is slowed.
- 3 I am often unable to respond to questions without extreme effort.

24. Feeling restless

- 0 I do not feel restless
- 1 I'm often fidgety, wringing my hands or need to shift how I am sitting.
- 2 I have impulses to move about and am quite restless.
- 3 At times, I am unable to stay seated and need to pace around.

25. Aches and Pains

- 0 I don't have any feeling of heaviness in my arms or legs and don't have any aches or pains.
- 1 Sometimes I get headaches or pains in my stomach, back or joints but these pains are only sometimes present and they don't stop me from doing what I need to.
- 2 I have these sorts of pains most of the time.
- 3 These pains are so bad that they stop what I am doing.

26. Other bodily symptoms

- 0 I don't have any of these symptoms: heart pounding fast, blurred vision, sweating, hot and cold flashes, chest pain, heart turning over in my chest, ringing in my ears, or shaking
- 1 I have some of these symptoms but they are mild and are present only sometimes
- 2 I have several of these symptoms and they bother me quite a bit.
- 3 I have several of these symptoms and when they occur I have to stop doing whatever I am doing

27. Panic/Phobic Symptoms

- 0 I have no spells of panic or specific fears/phobia
- 1 I have mild panic episodes or fears that do not usually change my behaviour or stop me from functioning.
- 2 I have significant panic episodes or fears that force me to change my behaviour but do not stop me from functioning.
- 3 I have panic episodes at least once a week or severe fears that stop me from carrying on my daily activities.

28. Constipation/Diarrhoea

- 0 There is no change in my usual bowel habits.
- 1 I have intermittent constipation or diarrhoea which is mild.
- 2 I have diarrhoea or constipation most of the time but it does not interfere with my day to day functioning.
- 3 I have constipation or diarrhoea for which I take medicine or which interferes with my day to day functioning.

29. Interpersonal Sensitivity

- 0 I have not felt easily rejected, slighted, criticised or hurt by others at all.
- 1 I have occasionally felt rejected, slighted, criticised or hurt by others.
- 2 I have often felt rejected, slighted, criticised or hurt by others, but these feelings have had only slight effects on my relationships or work.
- 3 I have often felt rejected, slighted, criticised or hurt by others and these feelings have impaired my relationships or work.

30. Leadon Paralysis/Physical Energy

- 0 I have not experienced the physical sensation of feeling weighted down and without physical energy.
- 1 I have occasionally experienced periods of feeling physically weighted down and without physical energy, but without a negative effect on work, school, activity level.
- 2 I feel physically weighted down and without physical energy, more than half the time.
- 3 I feel physically weighted down and without physical energy, most of the time, several hours per day, several days per week.

Appendix M: Hospital Anxiety & Depression Scale (HADS)

[Anxiety subscale used only – relevant questions considered highlighted in grey]

Instructions: Read each item and place a firm tick in the box opposite the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies: your immediate reaction to each item will probably be more accurate.

I feel tense or 'wound up':		A	I feel as if I am slowed down:	D	
Most of the time		3	Nearly all of the time	3	
A lot of the time		2	Very often	2	
Time to time, occasionally		1	Sometimes	1	
Not at all		0	Not at all	0	
I still enjoy the things I used to enjoy:	D		I get a sort of frightened feeling like 'butterflies in the stomach':		A
Definitely as much	0		Not at all		0
Not quite so much	1		Occasionally		1
Only a little	2		Quite often		2
Not at all	3		Very often		3
I get a sort of frightened feeling like something awful is about to happen:		A	I have lost interest in my appearance:	D	
Very definitely and quite badly		3	Definitely	3	
Yes, but not too badly		2	I don't take as much care as I should	2	
A little, but it doesn't worry me		1	I may not take quite as much care	1	
Not at all		0	I take just as much care as ever	0	
I can laugh and see the funny side of things:	D		I feel restless as if I have to be on the move:		A
As much as I always could	0		Very much indeed		3
Not quite so much now	1		Quite a lot		2
Definitely not so much now	2		Not very much		1
Not at all	3		Not at all		0
Worrying thoughts go through my mind:		A	I look forward with enjoyment to things:	D	
A great deal of the time		3	As much as I ever did	0	
A lot of the time		2	Rather less than I used to	1	
From time to time but not too often		1	Definitely less than I used to	2	
Only occasionally		0	Hardly at all	3	
I feel cheerful:	D		I get sudden feelings of panic:		A
Not at all	3		Very often indeed		3
Not often	2		Quite often		2
Sometimes	1		Not very often		1
Most of the time	0		Not at all		0
I can sit at ease and feel relaxed:		A	I can enjoy a good book or radio or TV programme:	D	
Definitely		0	Often	0	
Usually		1	Sometimes	1	
Not often		2	Not often	2	
Not at all		3	Very seldom	3	

Total A: ____ Total D: ____

Appendix N: Perceived Stress Scale (PSS)

The questions in this scale ask you about your feelings and thoughts during the **last month**. In each case, please indicate with a check how often you felt or thought a certain way.

Please write here the time of the day at which this questionnaire was completed: _____

1. In the last month, how often have you been upset because of something that happened unexpectedly?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

2. In the last month, how often have you felt that you were unable to control the important things in your life?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

3. In the last month, how often have you felt nervous and "stressed"?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

4. In the last month, how often have you felt confident about your ability to handle your personal problems?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

5. In the last month, how often have you felt that things were going your way?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

6. In the last month, how often have you found that you could not cope with all the things that you had to do?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

7. In the last month, how often have you been able to control irritations in your life?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

8. In the last month, how often have you felt that you were on top of things?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

9. In the last month, how often have you been angered because of things that were outside of your control?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?

___0=never ___1=almost never ___2=sometimes ___3=fairly often ___4=very often

Appendix O: Jenkins Sleep Scale

Instructions: These questions are about sleep problems in the **LAST MONTH**. For each question, please tick the box that applies to you. Please tick ONE box only for each question.

How many times in the past month did you	Not at all	1-3 days	4-7 days	8-14 days	15-21 days	22-31 days
1. Have trouble falling asleep?						
2. Wake up several times each night?						
3. Have trouble staying asleep (including waking too early)?						
4. Wake up after your usual amount of sleep feeling tired and worn out?						

Appendix P: Short-Form 36 (SF-36) Medical Outcomes Survey

1 In general how would you say your health is?

Excellent	<input type="checkbox"/>
Very Good	<input type="checkbox"/>
Good	<input type="checkbox"/>
Fair	<input type="checkbox"/>
Poor	<input type="checkbox"/>

2 Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago?	<input type="checkbox"/>
Somewhat better now than one year ago?	<input type="checkbox"/>
About the same	<input type="checkbox"/>
Somewhat worse now than one year ago?	<input type="checkbox"/>
Much worse now than one year ago?	<input type="checkbox"/>

3 The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so how much?

	<i>Yes limited a lot</i>	<i>Yes limited a little</i>	<i>No not limited at all</i>
Vigorous activities such as running, lifting heavy objects, participating in strenuous sports	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Moderate activities such as moving a table, pushing a vacuum cleaner, bowling or playing golf	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Lifting or carrying groceries	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Climbing several flights of stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

[continued overleaf]

	<i>Yes limited a lot</i>	<i>Yes limited a little</i>	<i>No not limited at all</i>
Climbing one flight of stairs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bending, kneeling or stooping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking more than a mile	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking half a mile	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Walking 100 yards	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bathing or dressing yourself	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

4 During the past 4 weeks have you had any of the following problems with your work or other regular daily activities as result of you physical health?

	<i>Yes</i>	<i>No</i>
Cut down the amount of time you spent on work or other activities.	<input type="checkbox"/>	<input type="checkbox"/>
Accomplished less than you would like	<input type="checkbox"/>	<input type="checkbox"/>
Were limited in the kind of work or other activities	<input type="checkbox"/>	<input type="checkbox"/>
Had difficulty performing the work or other activities (for example, it took extra effort)	<input type="checkbox"/>	<input type="checkbox"/>

5 During the past 4 weeks have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	<i>Yes</i>	<i>No</i>
Cut down the amount of time you spent on work or other activities.	<input type="checkbox"/>	<input type="checkbox"/>
Accomplished less than you would like	<input type="checkbox"/>	<input type="checkbox"/>
Didn't do work or other activities as carefully as usual	<input type="checkbox"/>	<input type="checkbox"/>

[continued overleaf]

6 During the past 4 weeks to what extent have your physical health or emotional problems interfered with your social activities with family, friends, neighbours or groups?

- Not at all ☐
- Slightly ☐
- Moderately ☐
- Quite a bit ☐
- Extremely ☐

7 How much bodily pain have you had during the past 4 weeks?

- None ☐
- Very mild ☐
- Mild ☐
- Moderate ☐
- Severe ☐
- Very severe ☐

8 During the past 4 weeks how much did pain interfere with your normal work (including both work outside the home and housework)?

- Not at all ☐
- A little bit ☐
- Moderately ☐
- Quite a bit ☐
- Extremely ☐

[continued overleaf]

The following questions are about how you feel and how things have been with you during the past 4 weeks. For each question please give the one answer that comes closest to the way you have been feeling.

9 How much of the time during the past 4 weeks...

	<i>All of the time</i>	<i>Most of the time</i>	<i>A Good bit of the time</i>	<i>Some of the time</i>	<i>A little of the time</i>	<i>None of the time</i>
Did you feel full of life?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Have you been a very nervous person?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Have you felt so down in the dumps that nothing could cheer you up?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Have you felt calm and peaceful?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Did you feel worn out?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Have you felt downhearted and low?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Did you have a lot of energy?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Have you been a happy person?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Did you feel tired?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Has your health limited your social activities (like visiting your friends or relatives)?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

[continued overleaf]

10 Please choose the answer that best describes how true or false each of the following statements is for you.

	<i>Definitely true</i>	<i>Mostly true</i>	<i>Not sure</i>	<i>Mostly false</i>	<i>Definitely false</i>
I seem to get ill more easily than other people	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I am as healthy as anybody I know	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
I expect my health to get worse	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
My health is excellent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Appendix Q: Acceptance of Fatigue Questionnaire

	Never True	Very Rarely True	Seldom True	Sometimes True	Often True	Almost Always True	Always True
I would gladly sacrifice important things in my life to control my fatigue better							
I need to concentrate on getting rid of my fatigue							
My thoughts and feelings about fatigue must change before I can take important steps in my life							
Keeping my fatigue level under control takes first priority whenever I'm doing something							
Before I can make any serious plans, I have to get some control over my fatigue							
I have better control over my life if I can control my negative thoughts about fatigue							
I avoid putting myself in situations where my fatigue might increase							
My worries and fears about what fatigue will do to me are true							
I have to struggle to do things when I have fatigue							

Appendix R: Cognitive and Behavioural responses to Symptoms Questionnaire (CBSQ)

Please indicate how much you agree or disagree with the following statements about your current symptoms by ticking the appropriate box.

	IEWS ABOUT YOUR SYMPTOMS	STRONGLY DISAGREE	DISAGREE	NEITHER AGREE NOR DISAGREE	AGREE	STRONGLY AGREE
FA1	I am afraid that I will make my symptoms worse if I exercise					
FA2	My symptoms would be relieved if I were to exercise					
FA3	Avoiding unnecessary activities is the safest thing I can do to prevent my symptoms from worsening					
FA4	The severity of my symptoms must mean there is something serious going on in my body					
FA9	Even though I experience symptoms, I don't think they are actually harming me					
FA10	When I experience symptoms, my body is telling me that there is something seriously wrong.					
FA12	Physical activity makes my symptoms worse					
FA14	Doing less helps symptoms					
FA15	Symptoms are a signal that I am damaging myself					
FA16	I am afraid I will have more symptoms if I am not careful					
FA17	I should avoid exercise when I have symptoms					
C1	I worry that I may become permanently bedridden because of my symptoms					
C2	I think that if my symptoms get too severe they may never decrease					

	VIEWS ABOUT YOUR SYMPTOMS	STRONGLY DISAGREE	DISAGREE	NEITHER AGREE NOR DISAGREE	AGREE	STRONGLY AGREE
C4	My illness is awful and I feel that it overwhelms me					
C6	I will never feel right again					
SF1	When I experience symptoms, I think about them constantly					
SF2	I worry when I am experiencing symptoms					
SF3	When I am experiencing symptoms it is difficult for me to think of anything else					
SF5	I think a great deal about my symptoms					
SF9	My symptoms are always at the back of my mind					
SF12	I spend a lot of time thinking about my illness					
EA1	I am embarrassed about my symptoms					
EA2	I worry that people will think badly of me because of my symptoms					
EA3	The embarrassing nature of my symptoms prevents me from doing things					
EA4	I avoid social situations because I am scared my symptoms will get out of control					
EA5	I am ashamed of my symptoms					
EA6	My symptoms have the potential to make me look foolish in front of other people					

We are interested in how you respond to or manage your symptoms at the moment. Listed below are a number of different responses that people may have to their symptoms. Please indicate how often you respond in the following ways by ticking the appropriate box. Choose the most accurate answer for YOU

	MANAGING SYMPTOMS	Never	Sometimes	Quite often	Very Often	All the time
L2	I stay in bed to control my symptoms					
L3	When I experience symptoms, I rest.					

	MANAGING SYMPTOMS	Never	Sometimes	Quite often	Very Often	All the time
L4	I tend to avoid activities that make my symptoms worse					
L7	I tend to nap during the day to control my symptoms					
AL1	I tend to overdo things when I feel energetic					
AL2	I find myself rushing to get things done before I crash					
AL3	I tend to overdo things and then rest up for a while					
AL4	I tend to do a lot on a good day and rest on a bad day					
L9	I sleep when I'm tired in order to control my symptoms					
L10	I avoid making social arrangements in case I'm not up to it.					
L11	I avoid exerting myself in order to control my symptoms					
AL5	I'm a bit all or nothing when it comes to doing things					
L13	I avoid stressful situations					

Appendix S: Attribution scale (1)

Which of the following do you consider important in having caused your fatigue or made it worse (tick as many as you wish)?

	Not a factor	Might be a factor	Definitely a factor
Current treatment (give details)			
Previous viral infections			
Current/continuing infections			
Other infections			
Work stress			
Stress from relationship difficulties			
Emotional upset or distress			
Food allergy			
Other allergy			
Hormonal disorder			

Appendix T: Attribution scale (2)

Which of the following best describes the nature of your symptoms (please tick one):

My symptoms are physical	My symptoms are mainly physical	Both physical and psychological factors are involved in my symptoms	My symptoms are mainly psychological	My symptoms are psychological in nature

Appendix U: Cortisol information sheet (total 4 pages)

Measuring your biological levels of Stress:

A step by step guide for the saliva collection

DHEP ID: _____

TIMEPOINT: _____

****Please record date of saliva collection**:** _____

****IMPORTANT – READ INSTRUCTIONS ON BACK PAGE FIRST****

Wake up (before 10 a.m.).

Immediately after waking up collect your saliva by chewing on the cotton wool pad **for 2 minutes**, then put it back in the tube **marked 0**.

Write here the **EXACT TIME OF AWAKENING**: _____

Try to sit down and relax in the next hour. **YOU CANNOT BRUSH YOUR TEETH AND CANNOT HAVE ANYTHING TO EAT OR DRINK FOR THE NEXT HOUR.** If you need, you can drink water, but only immediately AFTER you have taken the sample.

15 minutes after waking up, collect your saliva using the **tube marked 15**.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here: _____

30 minutes after waking up, collect your saliva using the **tube marked 30**.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thought or any kind of pain before taking this sample? If yes, please describe it here: _____

60 minutes (1 hour) after waking up collect your saliva using the **tube marked 60**.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thought or any kind of pain before taking this sample? If yes, please describe it here: _____

******* You can now have breakfast and brush your teeth! *******

At 12, noon - before lunch collect your saliva using the **tube marked 12**.

You should not eat or drink anything, or do not brush your teeth in the 30 minutes before noon.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thought or any kind of pain before taking this sample? If yes, please describe it here: _____

At 8 pm - before dinner collect your saliva using the **tube marked 8**.

You should not eat or drink anything, or do not brush your teeth in the 30 minutes before 8pm.

- What time is it now? _____
- What were you doing before giving the sample? _____

- Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here: _____
- Did you have any difficult or tense situation, unpleasant thought or any kind of pain before taking this sample? If yes, please describe it here: _____

Store the tubes away from the heat and direct sunlight and put them into the fridge as soon as possible.

Please note name and time of any **medication taken today** (including the contraceptive pill):

Do you have any **medical problem**? If so, please list them here _____

If you are female: Please indicate the age of your first menstrual cycle: _____

And please indicate the date of the first day of your last menstrual cycle: _____

If you have any questions about any of these instructions please call **Alice Russell**

0207 8480726/07580915755

Collecting the saliva samples

1. Take the salivette marked with the appropriate number and carefully remove the lid (the part on the end with ridges on it).
2. Tip the cotton wool swab into the lid of the tube, then use this to place the swab in your mouth. **Do not touch the swab with your fingers.**
3. Gently chew the swab, repeatedly turning and moving it around in your mouth, for two minutes, so that it is saturated with saliva. This may seem longer than you expect, but the people in the laboratory need a lot of saliva for their analyses!
4. Take the swab out of your mouth with the help of the lid (so you are not touching it with your fingers) – it may be easier if you are looking in a mirror to do this. Tip the cotton bud back into the inner tube, again without touching the swab with your fingers.
5. Put the lid back on firmly.
6. Store the finished samples in the fridge.



Appendix V: levels of biological markers in whole HCV cohort

<i>Biological Marker</i>	<i>TW0 Mean±SEM</i>
<u>Cytokines</u> (<i>n</i> = 49 ^{*#})	
IL-10	1.21±0.26
IL-13	0.48±0.1
IFN-γ	9.06±1.26
IL-2	0.63±0.26
IL-6	0.95±0.13
IL-7	15.56±1.36
IL-8	24.31±5.95
IL-12p70	0.26±0.07
IL-17A	2.13±0.33
TNF-α	4.64±0.36
VEGF	201.81±23.28
<u>Kynurenine pathway</u> (<i>n</i> = 49)	
Tryptophan	17836.4±424.71
Kyn/Trp ratio	2.19±0.08
Kynurenic acid	7.78±0.41
Quinaldic acid	2.18±0.18
3Hk/Kyn ratio	2.75±0.16
3-HK	10.44±0.68
Xanthurenic acid	3.28±0.3
Picolinic acid	71.88±4.81
Quinolinic acid	52.01±3.63
<u>Cortisol</u> (<i>n</i> = 25; 27)	
<i>Awakening Response (CAR AUC_i)</i>	81.69±48
<i>Diurnal (DAY AUC_g)</i>	2987.91±249.81

**n* = 39; # *n* = 29

Continued overleaf

Biological Marker	TW4 Mean±SEM
<u>Cytokines</u> (<i>n</i> = 46 ^{*#})	
IL-10	1.01±0.12
IL-13	0.48±0.1
IFN-γ*	8.05±1.09
IL-2	0.44±0.1
IL-6	1.63±0.22
IL-7	17.21±1.86
IL-8	26.87±5.3
IL-12p70 [#]	0.19±0.06
IL-17A	3.78±0.8
TNF-α	5.95±0.4
VEGF*	179.08±22.52

^{*}*n* = 36; [#] *n* = 28

Biological Marker	TW8 Mean±SEM
<u>Kynurenine pathway</u> (<i>n</i> = 46)	
Tryptophan	17189.31±590.83
Kyn/Trp ratio	2.61±0.13
Kynurenic acid	8.14±0.66
Quinaldic acid	1.71±0.12
3Hk/Kyn ratio	11.77±0.88
3-HK	2.78±0.2
Xanthurenic acid	3.24±0.34
Picolinic acid	67.84±3.65
Quinolinic acid	53.73±3.49

Continued overleaf

Biological Marker	TW24 Mean±SEM
<u>Cytokines</u> (<i>n</i> = 39 ^{*#})	
IL-10	1.09±0.18
IL-13	0.53±0.11
IFN-γ*	10.96±2.23
IL-2	1.14±0.57
IL-6	2.04±0.28
IL-7	17.78±1.77
IL-8	22.75±3.55
IL-12p70 [#]	0.21±0.07
IL-17A	3.82±0.7
TNF-α	7.26±0.61
VEGF*	162.43±21.26
<u>Kynurenine pathway</u> (<i>n</i> = 43)	
Tryptophan	17043.53±668.05
Kyn/Trp ratio	2.7±0.13
Kynurenic acid	6.95±0.41
Quinaldic acid	1.5±0.13
3Hk/Kyn ratio	13.6±1.17
3-HK	3.03±0.22
Xanthurenic acid	2.84±0.31
Picolinic acid	68.24±4.31
Quinolinic acid	58.12±3.99
* <i>n</i> = 32; [#] <i>n</i> = 22	

Continued overleaf

Biological Marker	FU Mean±SEM
<u>Cytokines</u> (<i>n</i> = 47 ^{*#})	
IL-10	0.52±0.07
IL-13	0.48±0.1
IFN-γ*	10.7±1.47
IL-2	0.8±0.42
IL-6	1.12±0.14
IL-7	18.3±2.17
IL-8	15.44±2.75
IL-12p70 [#]	0.31±0.09
IL-17A	2.49±0.46
TNF-α	4.4±0.36
VEGF*	175.62±22.91
<u>Kynurenine pathway</u> (<i>n</i> = 43)	
Tryptophan	18506.5±476.07
Kyn/Trp ratio	2.22±0.07
Kynurenic acid	9.3±0.48
Quinaldic acid	2.26±0.16
3Hk/Kyn ratio	9.39±0.92
3-HK	2.29±0.21
Xanthurenic acid	4.26±0.39
Picolinic acid	59.14±5.05
Quinolinic acid	65.95±2.53
<u>Cortisol</u> (<i>n</i> = 22; 21)	
Awakening Response (CAR AUC _i)	139.76±70.92
Diurnal (DAY AUC _g)	3693.79±238.72

**n* = 37; [#] *n* = 27