

Sabine J.M. de Brouwer

Psychophysiological stress reactivity in chronic inflammatory diseases

Stress exposure and stress management
in rheumatoid arthritis and psoriasis



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rheumatoid arthritis and psoriasis

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‘The real voyage of discovery
consists not in seeking new landscapes
but in having new eyes’

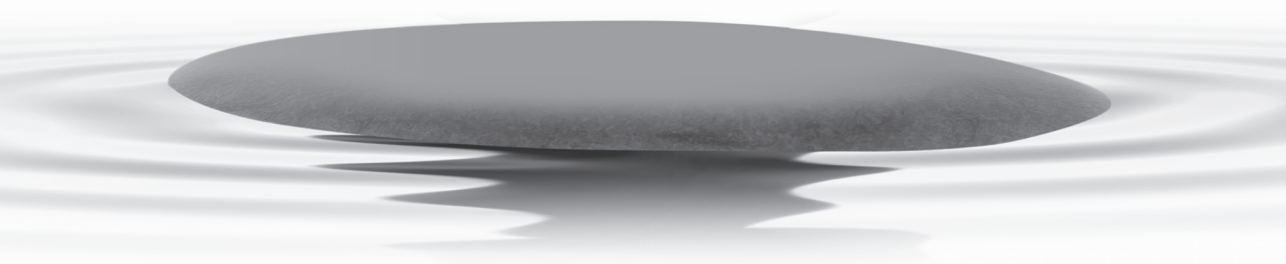
Marcel Proust

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Chapter 1

General introduction



General Introduction

The chronic inflammatory diseases rheumatoid arthritis (RA) and psoriasis affect 1 to 3 percent of the worldwide population and continue to be subject of investigation, because the disabling and destructive processes that are triggered by ongoing inflammation are significant and costly. Patients often suffer from physical disability, physical symptoms, such as pain, itch, or fatigue, and psychological difficulties that are associated with a chronic invalidating or stigmatizing disease. Many factors contribute to the etiology or maintenance of chronic inflammatory diseases, one of them being stress. "Stress" is a broad concept for a complex process including the exposure to psychological or physical triggers (i.e., stress exposure), the evaluation or perception of these stimuli as being stressful (i.e., stress evaluation), and the subsequent activation of a psychophysiological cascade (i.e., stress response) in the organism in order to re-establish homeostasis. This cascade has two main pathways: the autonomic nervous system (ANS), which includes the sympathetic-adrenal-medullary (SAM) axis, and the neuroendocrine system, which includes the hypothalamus-pituitary-adrenal (HPA) axis. Both pathways are connected with the immune system and therefore may influence disease processes (Figure 1).

As the relationship between the stress response system and the immune system is known to be bidirectional, the ANS and HPA axis may become compromised or altered in patients with a chronic inflammatory disease, due to system 'exhaustion' of ongoing inflammatory input. These alterations may add to the harmful effects of stress on disease severity. Furthermore, there is a growing body of evidence that suggests that psychological interventions are able to change various psychophysiological parameters of stress and/or disease, which indicates that it may be possible to counteract the harmful effects of stress on health with an intervention aimed at reducing the psychophysiological response to stress.

This thesis aims to provide greater insight into the nature and reactivity of the psychophysiological stress response system of patients with different chronic inflammatory diseases. The first aim of this thesis is to investigate the psychophysiological stress response of patients with the chronic inflammatory diseases RA and psoriasis, with a focus on parameters of the main stress response system; the ANS and the HPA axis, and on indicators of the immune system. The second aim of this thesis is to investigate whether a short-term stress management intervention for patients with RA is able to alter the psychophysiological response to an acute stressor.

Chronic inflammatory diseases

Chronic inflammatory diseases are defined by long-term inflammatory processes directed at a particular pathogen [1]. They include not only (systemic or organ-specific) autoimmune diseases, such as systemic lupus erythematosus or type 1 diabetes, but also auto-inflammatory conditions, such as ulcerative colitis, or mixed pattern diseases, such as RA, psoriasis or ankylosing spondylitis [2-4].

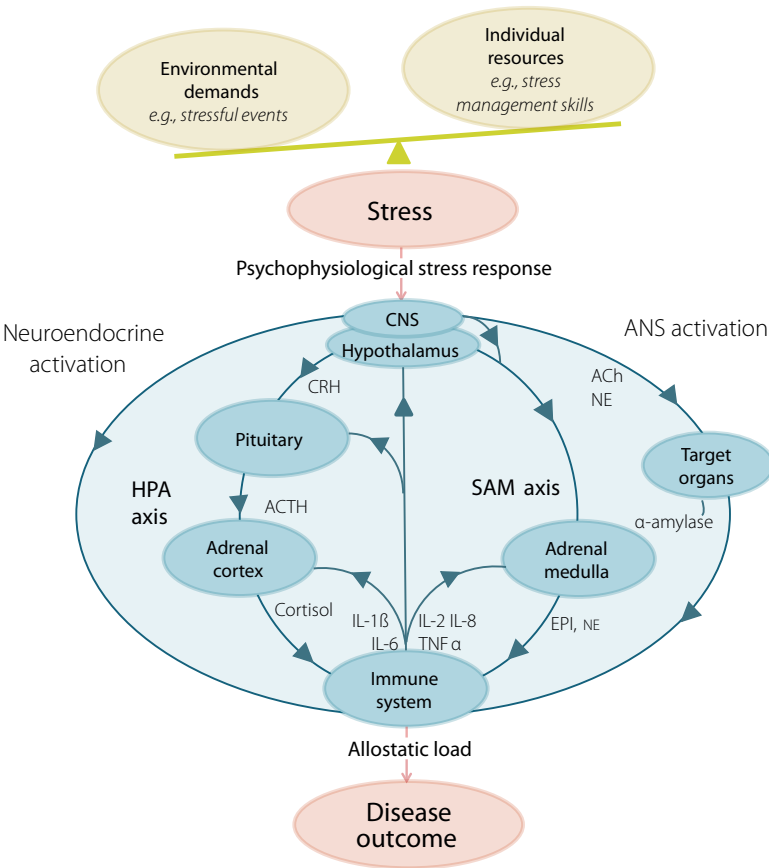


Figure 1 The psychophysiological construct of stress with possible links to disease outcome. ACh, acetylcholine; ACTH, adrenocorticotropin hormone; ANS, autonomic nervous system; CNS, central nervous system; CRH, corticotropin-releasing hormone; EPI, epinephrine; HPA axis, hypothalamus-pituitary-adrenal axis; IL, interleukin; NE, norepinephrine; SAM axis, sympathetic-adrenal-medullary axis.

Chronic inflammation

Inflammation, deduced from the Latin *inflammare* or ‘to ignite, set on fire’, is a complex cascade of biochemical events of the immune system, which are ignited in response to an endogenous or exogenous harmful stimulus, such as a pathogen, tissue damage, or infection [5]. A wide range of immune cells, such as neutrophils, basophils, mast cells, T-cells, and B-cells are sent to the site of injury by a host of extracellular mediators and

regulators, including cytokines, growth factors, and peptides, to start a tissue healing process and restore homeostasis. Although inflammation is initially a beneficial process, the inflammatory response can cause unnecessary tissue damage when it is not terminated in time [6]. When a chronic state of inflammation develops, parallel processes of tissue healing and cell death occur, underlying a major part of human diseases, including RA, psoriasis, asthma, celiac disease, and inflammatory bowel syndrome [7]. In these chronic inflammatory states, there is a shift in the type of cells that are present at the site of inflammation and inflammation may spread to become systemic. In this thesis, two prototypical diseases of chronic inflammation, RA and psoriasis, were subject of research.

Rheumatoid arthritis

Rheumatoid arthritis is a chronic systemic musculoskeletal joint disorder that is characterized by persistently inflamed synovial tissues. This persistent synovial inflammation is associated with damage to articular cartilage and underlying bone, which can lead to bone destruction and loss of function [8]. Synovial inflammation is characterized by the presence of many interacting immune cells and various pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF α), interleukin (IL)-1, IL-6, and IL-17 [9]. In some patients, other organs apart from the joints, such as the lungs and pericardium, can be affected by the systemic inflammatory process [8].

Approximately 1% of the world-wide population is affected by RA, with the age of disease onset peaking at 58 years and women being affected twice as much as men [10-12]. Frequently, patients suffer from severe pain, fatigue, and disability and decreased psychological functioning, especially when they are not treated adequately. The etiology of RA contains a complex and multi-factorial framework including genetic, stochastic, and environmental factors, which is not yet fully understood. RA has a strong genetic component, with a relative contribution of about 50%, and is thought to be associated with specific major histocompatibility complex (MHC) HLA antigens; therefore, family history is an important risk factor. Furthermore, smoking and being rheumatoid factor (RF) positive are amongst some of the risk factors for developing RA [10].

Living with a chronic invalidating disease such as RA is often accompanied by adjustment problems with substantially increased levels of distress, such as anxiety and depressed mood [13, 14]. Because stress is one of the factors believed to play a role in the progression of RA [15-18], the distressing consequences of the disease as well as other stress factors can negatively influence disease severity.

Psoriasis

Psoriasis, a papulosquamous skin disease, is one of the most common chronic immune-mediated disorders [19]. The disease is associated with well-delineated red or pink papulosquamous plaques with a white scale, most commonly distributed symmetrically on the extensor aspects of elbows and knee, scalp, lumbosacral region, and umbilicus

[20]. There is strong evidence that cell-mediated adaptive immunity is crucial in psoriasis. Pro-inflammatory cytokines of the Th1 pathway, such as interferon gamma (IFN γ), IL-2, IL-12 and IL-17 dominate plaques [19]. Therefore, psoriasis is typically classified as a Th1-disease, just like RA. Debate continues as to whether psoriasis is an autoimmune disorder, with an epidermal factor, for example keratin, being the most likely antigen candidate. Psoriasis is most common in Caucasian people, with a prevalence of 1.5-3% of the general population of Northern Europe, with men and women equally affected. Although psoriasis can appear at any time of life, the mean age at which it occurs is estimated at 33 years. Patients often suffer from physical symptoms of itch and fatigue and limitations in daily life, including stigmatization, which can increase psychological distress and impair quality of life [19].

Despite advances in understanding the pathogenesis of psoriasis, little is known about the natural history of the disease. Psoriasis clearly has a genetic component, with one pathway that follows the Mendelian pattern, although in general, psoriasis arises through multiple genetic risk factors interacting with each other and with environmental triggers (e.g., streptococcal infection). In parallel with observations in RA, stress is one of the factors involved in aggravation of symptom severity in psoriasis [21, 22].

In sum, patients with RA or psoriasis frequently have to cope with disabling physical and psychological limitations. RA and psoriasis represent two prototypic chronic inflammatory diseases with, in general, auto-immune processes and systemic inflammation occurring in RA, whereas psoriasis mainly has an auto-inflammatory profile with local inflammation of the skin. Although the multifactorial etiology of these diseases is a reason that they continue to be a subject of investigation, psychological factors, such as stress, have been documented to aggravate disease severity in both conditions [16-18].

Stress

In contemporary language, the term 'stress' is used abundantly to refer to a wide variety of situations and responses. In order to fully include all aspects of this broad construct, the term 'stress' has gone through some major revisions in scientific literature over the years.

The psychophysiological construct of stress

Since Selye first identified the concept of stress (the 'general adaptation syndrome') in the late thirties of the previous century many definitions have been proposed to describe and fully comprise this broad construct. In one of his first writings in 1936 Selye spoke about stress being 'the non-specific (neuroendocrine) response of the body' to an external agent, i.e., the 'stressor', that could either be physical, chemical, or psychological in nature, and the internal bodily changes these stressors produced were called the 'stress response' [23]. In the mid 1960s, when the focus in stress research shifted to cognitive and behavioral factors influencing the stress response, it became widely accepted that psychological

processes, such as the interpretation and appraisal of a situation, and our judgments as to whether we would be able to cope with a situation successfully, were decisive in making a situation a 'stressor'. Nowadays, these views are integrated in a psychophysiological construct of stress in which it is acknowledged that stress arises from the interaction between environmental demands on the one hand and individual resources on the other hand, resulting in a physiological response to deal with the stressor. Consequently, depending on the focus one chooses, there are different approaches to study the concept of stress; a focus on the environmental demands, such as the prevalence of major life events, daily hassles, and interpersonal stressors; a focus on individual resources, i.e., how does a person appraise a situation, and what coping mechanisms are available; and a focus on physiological responses to a stressor, i.e., what happens with the autonomic and neuroendocrine systems that are activated when stress is perceived [23-25]. Although these three domains are closely linked to each other and cannot be separated when trying to fully understanding the concept of stress, this thesis will mainly focus on the psychophysiological processes that are activated in response to a stressful stimulus, in our attempt to delineate the correlates linking stress and disease in greater depth.

The physiological stress response and the immune system

When an organism perceives an external stimulus as stressful, the physiological stress response system of the body is activated in order to re-establish homeostasis; a state of constant and appropriate internal conditions and functioning in the face of ever changing environmental demands [26]. The sensory input in the face of stress is processed by higher and limbic brain regions. The hypothalamus responds to stress exposure by activating at least two parallel pathways; the first is the fast responding ANS, which consists of the sympathetic nervous system (SNS) and includes the SAM axis, and of the parasympathetic nervous system (PSNS). Both branches of the ANS connect the organs and tissues with the central nervous system (CNS). In response to stimulation of preganglionic (cholinergic) sympathetic nerves originating in the spinal cord and receiving their input from the hypothalamus, the neurosecretory chromaffin cells of the adrenal medulla synthesize and secrete the catecholamines epinephrine and (to a smaller amount) norepinephrine into the circulation. The physiological effects of catecholamines are manifold, all more or less aimed at preparing for 'fight or flight', with actions including increased heart rate, stroke volume, blood pressure, dilated bronchi, the mobilization of glucose and stimulated lipolysis. All these processes are mediated by β - and α -adrenergic receptors (ARs) found on a wide variety of tissues [25, 27]. The second pathway that is activated in response to stress is the hypothalamus-pituitary-adrenal (HPA) axis. The neurosecretory cells in the paraventricular nucleus of the hypothalamus release corticotropin-releasing hormone (CRH) in the portal capillaries that run to the anterior lobe of the pituitary gland, where it stimulates the synthesis and secretion of adrenocorticotrophic hormone (ACTH), which -in turn- stimulates the release of the glucocorticoid cortisol by the adrenal cortex. Cortisol

activates intracellular glucocorticoid receptors (GRs), which are present in many tissues of the body. Therefore, cortisol has a very wide action range, including on the cardiovascular system and the central nervous system. Glucocorticoids are essential steroids to life, not only initiating many processes within the body, but also creating a healthy environment for these processes to occur by increasing enzyme activity, or augmenting or inhibiting the action of other hormones. Homeostasis of the HPA axis occurs by negative feedback actions from cortisol on various levels of the HPA axis [25, 27, 28].

Recent advances in psychoneuroimmunology have provided a more detailed insight into how the major stress hormones of the body, catecholamines and cortisol, influence inflammatory and immune responses [29-32]. The immune system is highly integrated with other physiological systems, including the nervous and endocrine systems. Autonomic (sympathetic, parasympathetic, and sensory) nerves innervate organs of the immune system, and cells and tissues of the immune system, including spleen, thymus, and other lymphoid organs, possess glucocorticoid as well as α - and β -adrenergic receptors, which are sensitive to glucocorticoids and catecholamines and other stress response mediators [28]. Overall, these mediators affect lymphocyte traffic, circulation, and proliferation, and modulate cytokine production and functional activity of various lymphoid cells in order to resist infection or challenge and enhance the effectiveness of the immune response [33]. In general, glucocorticoids and catecholamines inhibit the production of pro-inflammatory prostaglandins and cytokines, such as TNF α , IFN γ , and IL-1 [34], whereas they stimulate the production of anti-inflammatory cytokines, such as IL-10 and IL-4 [33]. Through inhibition and stimulation of type 1 and type 2 cytokine secretion, respectively, they cause selective suppression of Th1-mediated cellular immunity and a shift toward Th2-mediated humoral immunity [34]. Stress response mediators can either exert immunosuppressive or immunostimulatory effects in a dose-dependent manner, with generally, high doses being related to immunoinhibitory effects and low doses to immunostimulatory actions [16, 35]. Conversely, the immune system can also communicate with the brain and influence the ANS and HPA axis, emphasizing the bidirectional relationship between autonomic, neuroendocrine, and immune interactions. During an excessive immune response, cytokines - particularly those that are pro-inflammatory, such as IL-1 and IL-6,- stimulate the HPA axis, thereby down-regulating immune and inflammatory processes to prevent an "overshoot" of pro-inflammatory cytokines [36].

All in, because stress modulates the body's physiology, with multifaceted interactions between the autonomic, endocrine, and immune system, it seems clear that stress may affect the course of immune-mediated diseases.

Stress and chronic inflammatory diseases

Stress has been linked to affect a multitude of somatic diseases, ranging from infectious diseases like HIV and Epstein-Barr virus (EBV), to chronic inflammatory diseases, such as RA and psoriasis [16, 18, 37-42]. In patients with RA and patients with psoriasis, stressful events

have been associated with increases in disease activity, tender joints, pain, physical disability, and markers of inflammation [43-56]. This is in line with patients' reports; with 45%, stress is the most attributed cause for disease flare-ups by patients with RA [57], while 37 to 88 percent of patients with psoriasis believe psychological stress to be a factor in the manifestations of their condition [21]. Research supports the view that stress can be a disease permissive or aggravating factor in these patient groups, but mechanisms underlying the influence of stress on inflammatory processes are largely unknown.

Stress and chronic inflammation

Allostatic responses to stress, which are mainly mediated by glucocorticoids and catecholamines, are protective as they try to re-establish homeostasis. However, negative consequences can occur when adaptive systems are out of balance, or when responses persist (e.g., in case of chronic stress), are terminated inefficiently, or occur inappropriately or inadequately (e.g., when the HPA axis is hypoactive) [58]. A general hypocortisolism has been documented in various psychiatric disorders, such as clinical depression or social anxiety, in stress-related bodily disorders, and incidentally also in chronic inflammatory conditions [59, 60]. Low basal cortisol levels and low cortisol responses to stress in patients with chronic inflammatory conditions may facilitate or sustain the pro-inflammatory Th1 shift that is observed in these patients. Moreover, under specific conditions and in certain local responses, stress hormones can actually boost immune activity, thereby sustaining or stimulating a pro-inflammatory state and influencing the onset and/or course of disease [61]. For example, in RA and psoriasis, stress activates the CRH-mast-cell-histamine axis, with the peripherally produced corticotropin-releasing hormone (CRH) acting as a local pro-inflammatory agent, and stimulating cutaneous and synovial mast cells to release histamine and the pro-inflammatory cytokines TNF α and IL-6, which may boost manifestations of inflammation. In addition, the pro-inflammatory load of circulating cytokines that is associated with chronic inflammation may lead to sustained feedback stimulation of the hypothalamus and this may alter ANS or HPA axis function [16].

Therefore, specifically in chronic inflammatory states, stress may increase allostatic load and aggravate disease activity through modulation of the systemic or local pro/anti-inflammatory cytokine balance. Moreover, the physiological pathways of the stress response system may be altered as a consequence of over-activity due to chronic pro-inflammatory load [62]. Indeed, there is evidence that both the ANS and HPA axis may be dysfunctional in patients with RA and psoriasis.

The HPA axis

A few decades ago, most knowledge about mechanisms linking stress and RA came from experimental work with animals. The HPA axis was one of the main physiological pathways identified to be involved. For example, Lewis rats failed to realise an appropriate HPA axis response resulting in increased vulnerability to auto-immune and inflammatory

disturbances such as adjuvant-induced arthritis [62]. Chronic inflammation appeared to be associated with a dysfunction of the HPA axis, with altered secretion patterns of CRH, ACTH and glucocorticoids that modulated immune functions. In humans, there are indications that the diurnal rhythm of plasma cortisol could be absent or at the lower limit of normal in patient with long-standing RA, while the diurnal rhythms may still be intact in recently diagnosed patients [63-66]. Overall, basal cortisol levels appear to be increased in patients with recently diagnosed RA [67, 68] and may decrease during the progression of RA, especially during flare-ups [69, 70]. Similarly, a lower daily cortisol output and lower basal cortisol levels have been observed in patients with psoriasis [71].

In order to detect possible alterations on specific levels of the HPA axis in RA and incidentally also in psoriasis, pharmacological function tests (e.g., dexamethasone suppression test (DST), insulin tolerance tests, or infusions with acetylcholine (ACh), CRH, or epinephrine) were set up, with contrasting results. Some studies suggest that patients have a relatively hypofunctional axis with defective central (hypothalamic or pituitary) and/or peripheral (adrenal) components [37], while other studies hardly found any deviations from healthy subjects [72-74]. However, pharmacological function tests do not provide information about HPA axis function in response to real-life stressors; this has hardly been investigated in patients with chronic inflammatory diseases such as RA and psoriasis. Some studies on experimental acute stress reported patients with RA to show an impaired stress-induced cortisol increase [49, 75], but others did not find changes in cortisol reactivity [76-78] nor in ACTH responses to acute stress [75, 76, 78]. In psoriasis, only two studies reported lower cortisol levels after acute stress [79] or diminished cortisol increase in a subgroup of patients with psoriasis [80].

All in, chronic inflammation may be associated with a dysfunction of the HPA axis, which could reduce glucocorticoid output and pave the way for other systems, such as the immune system, to show elevated activity and increase allostatic load, because of to the inability of the HPA axis to contain its activity [62]. Consequently, HPA axis function may be too 'normal' considering the degree of inflammation present in patients with a chronic inflammatory disease, with cortisol levels not being sufficiently high to dampen inflammation [81]. Whether this supposed HPA axis hypofunction is primary or secondary has not been established yet.

The ANS

Involvement of branches of the ANS, including the sympathetic nervous system (SNS) and adrenoceptor-mediated mechanisms, in the chronic inflammatory diseases RA and psoriasis have also been first documented in animal research [62]. For example, early studies reported that eliminating small afferents through capsaicin administration and depleting catecholamines by peripheral sympathectomy attenuates joint injury [82]. In contrast to this disease-exacerbating effect of the SNS, later studies also reported protecting effects of the SNS, for example, when treatment with β -adrenergic agonists in

experimentally induced arthritis in mice significantly reduced disease symptoms [83]. These differential effects of the SNS mainly seem to depend on the type of receptor stimulation, either α -adrenergic (pro-inflammatory) or β -adrenergic (anti-inflammatory) [16]. In humans, histological studies revealed that patients with RA show a functional loss of sympathetic nerve fibers in inflamed synovial tissue, while sensory innervation, i.e., substance P-positive pro-inflammatory nerve fibers, into the joints is increased, creating an imbalance between sensory and sympathetic nerve fibers [84]. This may induce a local β - to α -adrenergic shift that could enhance a pro-inflammatory state [16, 85]. Parallel to the loss of sympathetic nerve fibers observed in inflamed synovial tissue of patients with RA, patients with psoriasis show fewer β -adrenoceptors on keratinocytes in skin lesions compared to normal skin, risking insufficient down-regulation of inflammatory and immune processes [86]. Because receptor densities often decrease in response to elevated ligand levels, this finding may indicate patterns of systemic sympathetic hyperactivity (secondary to stressors) [28]. Increased sympathetic tone at rest as well as higher basal plasma catecholamines levels have been documented regularly in patients with RA [87]. Autonomic function tests have provided some insight into possible ANS alterations in RA and -to a lesser extent- in psoriasis [88-92]. They indicate that at least one fifth of patients with RA show general hypo- or hyperfunction of the ANS in standardized function tests that include the Valsalva maneuver, deep breathing, orthostatic tests, and sustained handgrip [91]. The few studies that evaluated the effects of experimental acute physical or psychological stress exposure on autonomic responses reveal either hypo- or hyper-activation of (branches of) the ANS in patients or subgroups of patients with RA [78, 93, 94] or psoriasis [52, 95-97].

Therefore, although there are indications of systemic or local SNS dysfunction, it has not been fully elucidated yet to what extent the ANS or branches of the nervous system are dysfunctional in patients with RA or psoriasis when encountering a stressful situation, and what the specific consequences are for chronic inflammation.

The immune system

The immune system of patients with the Th1-mediated chronic inflammatory diseases RA and psoriasis is obviously affected, evidenced by the high amounts of various inflammatory markers, such as IL-1 β , TNF α and IL-6, observed both locally, in the joints or skin, as well as systemically in some patients [98-100]. In addition, immune cells of patients with RA show changes in the communication pathways with the ANS and HPA axis; alterations in corticosteroid receptor densities were observed in circulating lymphocytes (PBMCs), as well as changes in β -adrenergic receptors on lymphocytes in the synovial fluid [101-104]. Together with indications that receptor sensitivity for G-protein coupled receptors in lymphocytes is altered, leading to a disturbed intracellular signaling cascade in lymphocytes, these findings suggest that the influence of cortisol and catecholamines on lymphocyte function may be compromised [105, 106].

Therefore, the systemic pro-inflammatory state observed in chronic inflammation and possible disturbances in the signaling cascade between stress response mediators and immune cells may generate the basis for a disease-specific immune response to stress that contributes to the perpetuation of the disease process [37, 62]. The few prospective studies that have attempted to shed light on the effects of stress on immune function in RA or psoriasis indicate that minor stressful events have been positively associated with an overall enhancement of immune function, such as higher B cell numbers [44, 54, 107], while major life events have been related to immune suppression, such as lower T-helper/T-suppressor cell ratios [43, 54]. Experimental studies of acute stress showed that short-term stressors can lead to acute alterations in leukocyte, lymphocyte, helper T cell, cytotoxic T cell, and NK cell numbers and pro-inflammatory cytokine activity and release (e.g., TNF α , IL-6) in patients with RA [49-51, 77, 78] or psoriasis [52, 53, 97] compared to healthy controls. However, those studies did not investigate the psychophysiological response to experimental real-life psychological stress in both patient groups.

Because there are only few experimental stress studies investigating short-term immune responses, little is known about how psychophysiological stress response mediators acutely influence immune function in patients with the chronic inflammatory diseases RA and psoriasis.

Stress exposure

Building on knowledge of how stress affects the ANS, HPA axis and immune response in healthy populations, the understanding of how stress influences psychophysiological systems in various patient populations, including those suffering from chronic inflammation, is still increasing [29, 30, 108, 109]. Possible dysfunctions in the two main branches of the stress response system, the ANS and HPA axis, could increase stress-induced aggravation of chronic inflammatory conditions, such as RA and psoriasis [36, 87, 91, 92, 110]. Laboratory studies investigating the psychophysiological effects of short-term real-life stressors may be the best way to investigate these mechanisms. Although they do not create a *natural* context, they allow control of key factors in the delivery of stress and the observation of its immediate effects.

Stress research has a wide variety of experimental real-life stress tests at its disposal, ranging from physical exercise to psychological stress tasks, and all applied to evoke a general stress response involving central hypothalamic activation and stimulation of both the SAM and HPA axis [87]; these types of experimental stressors may give a more comprehensive insight into stress physiology than physiological infusion studies that inject, for example, epinephrine or autonomic function tests, which are both particularly aimed at stimulating and/or measuring specific parts of the stress system. Physical stress tests not only include physical exercise, such as treadmill and bicycle training, but also sensory stressors, such as exposure to thermal stimuli -either cold or heat- and acoustic stimuli [29, 87]. Psychological stressors include tasks with a cognitive component, such as

the Stroop task or mental arithmetic, as well as psychosocial tasks with a social-evaluative component, such as the Trier Social Stress Test (TSST) [29, 87]. In fact, the TSST is a combination of both a psychosocial evaluation and a cognitive challenge, because it combines a mock job interview with mental arithmetic in front of a critical audience. The TSST is one of the most widely-used psychological stress tasks, which has been applied for over two decades, and consistently activates both autonomic and neuroendocrine parameters of stress [111, 112], and influences immune system function in healthy populations as well as in patients with various psychological or somatic disorders [50, 78, 113-118]. Therefore, this task may be particularly efficient to elucidate and compare psychophysiological stress responses in patients with RA, patients with psoriasis and healthy controls, and to investigate how other factors, such as sex, age, medication, menopausal status and psychological functioning may influence this response. Until now, the TSST has only been used incidentally in these patient groups [50, 52, 53, 78].

Experimental studies of stress may provide valuable information about possible alterations in the stress response system, either on the level of the ANS, the HPA axis or the immune system, in patients with inflammatory diseases; alterations that may add to aggravation of disease severity. Moreover, studies that compare psychophysiological stress response patterns of different patient groups with chronic inflammation in order to reveal possible disease-specificity in ANS, HPA axis and immune system reactivity are still rare.

Psychological stress-reducing intervention

In an attempt to alleviate both the physical and psychological burden associated with a chronic inflammatory disease such as RA, psychological interventions are frequently used adjuncts to pharmacotherapy [119, 120]. Nevertheless, psychological interventions that are specifically aimed to alter psychophysiological parameters of stress and disease are still scarce.

Psychophysiological effects of psychological intervention

Numerous studies have been investigating the effects of multimodal therapies that are usually based on cognitive behavioral principles, such as patient education, cognitive training, relaxation therapy, physical exercise, biofeedback, emotional disclosure, and stress management training, on psychological and physical functioning in patients with RA. Interventions vary in duration, format, and primary outcome, but overall, different types of interventions have small but significant effects on physical outcomes, such as pain, joint swelling, and functional disability, and on psychological outcomes, such as anxiety and depression [119-124]. In line with this, psychological interventions for patients with psoriasis have sometimes generated effects on disease severity [125]. In general, there has been a growing interest in the ability of intervention studies to specifically alter physiological mediators of the stress response system. Stress-reducing interventions could eventually lead to decreased anxiety and dampening of the stress response as

evidenced by diminished autonomic arousal and neuroendocrine activity. For example, basal norepinephrine levels, urinary free cortisol output, serum cortisol levels, and dehydroepiandrosterone sulphate (DHEA-S) decrease in patients with HIV and cancer after stress management interventions [126-129]. In line with altered ANS and HPA axis function, intervention-related changes in immune parameters have also been observed in healthy controls and a number of patient groups with immune-related disorders, such as patients with HIV, cancer, delayed type hypersensitivity, or EBV or Herpes Simplex virus (HSV). For example, psychological interventions, such as stress management or relaxation training, can increase total lymphocyte numbers, neutrophil adherence, natural killer cell cytotoxicity (NKCC), and total sIgA concentration, and decrease EBV or HSV-2 antibody titres and T helper lymphocyte numbers [126, 130-133]. To what extent these types of interventions can also alter immune function in patients with RA and psoriasis has not been fully investigated. Although some studies with these patients have reported improvements in disease activity and/or joint swelling after intervention [134-136], these were hardly mirrored by changes in biological parameters of disease status. Studies in patients with RA patients that investigate changes in physiological parameters mostly focused on circulating levels of the pro-inflammatory C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [134, 137-147], with only incidental and temporary reports of alterations in those parameters [145]. Only very few studies with RA patients reported changes in circulating cytokine levels, such as IL-6 or IFN γ [148, 149]. Therefore, until now little research has been conducted to see whether psychological treatments can alter specific parameters of disease and immune function in patients with the chronic inflammatory conditions RA or psoriasis.

Psychological intervention and psychophysiological effects after stress exposure

Despite the interest in the effects of psychological interventions on psychophysiological parameters and the clear evidence that stress can aggravate symptoms of disease through activation of the stress axes, there are hardly any studies designed to specifically investigate the effects of psychological interventions on the acute psychophysiological response to stress. Whereas basal post-intervention measures of autonomic, neuroendocrine, or immune function can be informative, the investigation of the psychophysiological response to a natural stressful situation will specifically challenge participants to cope with stress; it is only then that the benefits of psychological interventions may in particular become evident. To our knowledge only one study previously assessed the effects of a psychological intervention for healthy participants on the psychophysiological stress response to a laboratory stress task [150, 151]. Subjects attended group-based cognitive-behavioral stress management training following the principles of stress inoculation training. After the training, treatment and control participants were subjected to the previously described TSST [111, 112]. In contrast to the control group, the treatment group showed reduced endocrine salivary cortisol responses to the TSST and a lower stress

appraisal. Because basal cortisol levels after the training did not differ between groups, it seems evident that the effects of the intervention become specifically visible when investigating the acute psychophysiological response to stress. This specific approach, using a widely-used stress induction paradigm to test the effects of stress management training, would be very well-suited to investigate whether similar psychophysiological effects as found in healthy participants may be obtained in patients with RA, who may be particularly prone to the detrimental effects of stress on health. Psychological treatment aimed at decreasing stress levels in patients and reducing the psychophysiological stress response may limit the possible detrimental effects of stress on immune and inflammatory processes.

Stress vulnerability

Although psychological interventions for patients with rheumatic and other chronic inflammatory diseases have generated moderate improvements in indicators of psychological and physical functioning, there is a large individual variability in treatment outcome, which is usually attributed to the heterogeneity of the patient population.

Specifically for RA research has shown the importance of evaluating psychological risk factors for disease progression and treatment outcomes, such as the personality trait neuroticism, psychological distress, or other cognitive-behavioral factors [45, 47, 107, 152-164]. Although a substantial part of patients is relatively well-adjusted, up to 42 percent of patients with RA show heightened levels of distress, such as (sub)clinical levels of depression or anxiety [165]. In particular these vulnerable patients will probably benefit most from psychological interventions, emphasizing the need to customize care to patients with psychological risk profiles. In fact, there is increasing evidence that psychological treatment tailored to patients with specific risk profiles of heightened levels of distress particularly increases treatment effectiveness [15, 166, 167]. The rationale of tailored psychological interventions for patients psychologically at risk is also evidenced by research in other chronic inflammatory diseases, such as psoriasis, indicating that certain patient characteristics, such as patients' reports that stress influences their disease severity, are associated with altered psychophysiological stress responses [80].

Therefore, with the focus shifting to individual differences and tailored or personalized care, the largest treatment effects can be expected in patients with a psychological profile of heightened levels of distress, which eventually may reduce possible harmful effects of stress on disease severity.

Focus and outline of the thesis

The aim of this thesis is to further elucidate the nature and reactivity of the psychophysiological stress response system of patients with two classical forms of chronic inflammatory

diseases, RA and psoriasis. The main objectives of the studies presented in this thesis were 1) to gain a greater insight into the psychophysiological stress response of patients with these different chronic inflammatory diseases, and to compare the psychophysiological stress response pattern of these patients with that of a healthy population, and 2) to investigate whether a stress management intervention can alter psychophysiological responses to stress in patients with RA.

Part I concerns the empirical background on and an experimental study of psychophysiological stress reactivity in patients with various chronic inflammatory diseases. In order to obtain a comprehensive understanding of psychophysiological responses to stress in rheumatic patients, **Chapter 2** includes a review offering a summary of the existing literature on self-reported, autonomic, neuroendocrine, and immune responses to various experimental psychological and physical stressors in patients with inflammatory rheumatic diseases, such as RA and SLE. **Chapter 3** covers an experimental study in which we investigate the psychophysiological responses to a psychosocial stress task, the TSST, of patients with the chronic inflammatory diseases RA and psoriasis. We compare patients' subjective, autonomic, and neuroendocrine response patterns with the stress response of healthy participants. The aim is to investigate whether autonomic and/or neuroendocrine stress reactivity is specifically altered in different patient groups with chronic inflammation. In **Chapter 4** we explore the effects of the TSST on parameters of the immune system, that is to say, serum cytokine levels such as IL-6 and TNF- α , in the two patient groups and healthy controls, and we examine whether the stress test leads to a specific cytokine response in the three groups.

Part II of this thesis describes the effect of a brief stress management training for patients with RA on the psychophysiological response to the TSST. In this randomized controlled trial, patients are assigned to the intervention or the control group and subjected to the TSST twice, immediately after the intervention and at a follow-up assessment 9 weeks later. In **Chapter 5**, the overall effects of the stress management training on psychological and physical functioning are compared between patients in the intervention and control groups. We also compare the subjective, autonomic, and neuroendocrine response to the TSST post-treatment and at the follow-up between intervention and control groups. In addition, we explored whether the effects of the training on psychophysiological stress reactivity were particularly observed in patients psychologically at risk, i.e, showing heightened levels of distress. In **Chapter 6**, we examine the effects of the stress management training on immune responses to the TSST. We explored the effects of the training on circulating cytokine levels, such as IL-6, TNF α , and IL-8. In the concluding chapter of this thesis, **Chapter 7**, the main results of the studies are summarized and the theoretical, empirical, and clinical implications of our findings are discussed. We give directions for future research based on the existing literature and recent developments in stress research.

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Part I

The Psychophysiological Stress Response in Chronic Inflammatory Diseases



Chapter 2

Experimental stress in inflammatory rheumatic diseases: a review of psychophysiological stress responses



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Abstract

Introduction Stressful events are thought to contribute to the etiology, maintenance and exacerbation of rheumatic diseases. Given the growing interest in acute stress responses and disease, this review investigates the impact of real-life experimental psychosocial, cognitive, exercise and sensory stressors on autonomic, neuroendocrine and immune function in patients with inflammatory rheumatic diseases.

Methods Databases Medline, PsychINFO, Embase, Cinahl and Pubmed were screened for studies (1985 to 2009) investigating physiological stress responses in inflammatory rheumatic diseases. Eighteen articles met the inclusion criteria.

Results Results suggest that immune function may be altered in response to a stressor; such alterations could contribute to the maintenance or exacerbation of inflammatory rheumatic diseases during stressful events in daily life.

Conclusions This review emphasizes the need for more experimental research in rheumatic populations with controlled stress paradigms that include a follow-up with multiple evaluation points, simultaneous assessment of different physiological stress systems, and studying factors contributing to specific physiological responses, such as stress appraisal.

Introduction

Stress is widely recognized as an important risk factor in the etiology of inflammatory rheumatic diseases [1-5]. An adaptational stress response involves the activation of both the hypothalamus-pituitary-adrenal axis (HPA axis) [6] and the autonomic nervous system (ANS) [7], and both stress axes are thought to communicate bidirectionally with the immune system [7-10]. Because many rheumatic diseases are characterized by immune-mediated joint inflammation, stressful events might contribute to the etiology, maintenance and exacerbation of rheumatic diseases [11,12]. Recent advances in psychoneuroimmunology have provided insight into the complex mechanisms by which stressors might acutely affect the body's immune system [13-16]. However, little attention has been paid to whether and how different short-term experimental stressors influence the separate pathways of the physiological stress response system (ANS, HPA axis, immune system) in patients with inflammatory rheumatic diseases.

Perception of an external stressful stimulus prompts the activation of various physiological systems that together define the body's stress response, which is aimed at re-establishing homeostasis. The physiological stress response is mainly coordinated by the hypothalamus, with activation of the ANS and the pituitary and adrenal glands (HPA axis) resulting in the release of catecholamines and cortisol, respectively [1,9,17]. These stress hormones, supposedly acting via β - and α -adrenergic as well as glucocorticoid receptors, down-regulate immune and inflammatory processes; however, these processes also influence the central nervous system (CNS) [7,18-20]. Circulating cytokines (for example, tumor necrosis factor α (TNF α), interleukin (IL)-6 and IL-1) and activated immune cells, markers of inflammation, activate both (intermediates of) the HPA axis and the ANS. Chronically elevated levels of cytokines, as occur during long-term inflammation, might lead to changes in HPA axis and ANS activity [21]. Moreover, the bidirectional relationship between the CNS and immune system implies that the physiological response to real-life stressors could contribute to the pathophysiology of inflammatory diseases [1-5]. How these three systems, the ANS, the HPA axis and the immune system, act in response to a stressful event in rheumatic disorders is not well understood.

Although the laboratory setting is not a natural environment, it allows control of key factors in the delivery of stress and observation of its effects and reduces many sources of bias and individual differences [16,22]. The literature on acute psychoneuroimmunological and psychoendocrinological responses to experimental stress in healthy individuals is still increasing. Studies of healthy populations suggest that experimental psychological and physical stressors not only activate the ANS [23] and the HPA axis [24], but also influence the immune system by activating innate immunity, as reflected by increased numbers of natural killer (NK) cells and the production of pro-inflammatory IL-6 [15,16]. Moreover, these different physiological systems (ANS, HPA axis and immune system) seem to work in an interdependent fashion [25].

Despite the possible detrimental physiological effects of stress in patients with inflammatory rheumatic diseases, such as an altered disease course, little is known about acute-phase reactants of experimentally induced stress (both autonomic, neuroendocrine and immune). Reviews of acute physiological stress responses have either focused on one [16,24] or two [2] stress response systems only (for example, ANS and/or neuroendocrine system), and included either only patients with rheumatoid arthritis [2] or a heterogeneous group of both healthy participants and various patient populations [16]. In addition, studies of the relationship between stress and inflammatory rheumatic diseases have often used experimental stressors that do not necessarily mimic real-life stressors. Different types of time-limited experimental stressors have been identified, namely, physical stressors (autonomic function tests, exercise), physiological stressors (corticotropin-releasing hormone (CRH) and (nor)epinephrine infusions, insulin tolerance test and dexamethasone suppression test) and psychological stressors (cognitive tests, public speaking) [2]. Many studies have investigated the effects of these types of stress on components of the stress response system, such as the ANS or the HPA axis, but external validity of these studies of stress is questionable. The prevalence of cardiovascular dysfunction is high after standard tests of autonomic function [26], such as the Valsalva maneuver, deep breathing, orthostatic tests, and sustained handgrip. While these tests may trigger autonomic responses, it is not known whether they activate the stress response system and alter neuroendocrine or immune function. HPA axis function has been investigated extensively by challenging specific parts of the HPA axis by means of infusion of CRH, synthetic glucocorticoids, or cytokines [27,28]. Although alterations in HPA axis responsiveness at a hypothalamic, pituitary or adrenal level have been reported, more subtle changes in HPA functioning have also been suggested to occur [27,28]. While injection studies might shed some light on possible altered neuroendocrine responses, the anti-inflammatory effects of exogenously administered glucocorticoids are not necessarily mirrored by increased secretion of endogenous glucocorticoids in response to a real-life stressor. Thus the question remains to what extent different types of experimental stressors that mimic real-life stressful events (for example, psychological stressors and physical exercise) are able to induce an autonomic, neuroendocrine and immune response in patients with inflammatory rheumatic diseases.

To the best of our knowledge this is the first review to investigate whether and how different experimental stressors mimicking real-life stressful events (psychosocial, cognitive, exercise and sensory stressors) influence physiological responses at the three levels (ANS, HPA-axis, immune system) in patients with prototypic inflammatory rheumatic diseases (for example, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

Materials and methods

This review is limited to studies involving patients with inflammatory rheumatic diseases who were exposed to a time-limited experimental stressor to assess the autonomic, neuroendocrine, and/or immune responses to stress.

Search strategy

To identify studies, the electronic bibliographic databases MEDLINE, PsychINFO, Embase, Cinahl and Pubmed databases were searched, using the key words rheum*, (idiopathic or psoriatic) arthr*, spondylitis, sclerosis and lupus in combination with the terms stress and either cortisol or immun* or epinephrine or endocrin* or autonom* or hypothalam* or HPA. In addition, reference sections of the articles and review papers were hand-searched for relevant articles on psychological and physical stressors and inflammatory rheumatic diseases.

Inclusion criteria were studies published after 1985 in English peer-reviewed journals; evaluation of an experimental laboratory stress task that induces psychological and/or physical (exercise) stress and/or sensory stress (for example, thermoceptive (cold/heat), visual (light), auditive (noise)) by means of a time-limited experimental stressor; patients diagnosed with systemic inflammatory rheumatic diseases, such as RA, juvenile idiopathic arthritis (JIA), ankylosing spondylitis, systemic sclerosis, or SLE; control group consisting of either healthy participants or participants without a systemic inflammatory rheumatic disease, such as osteoarthritis; use of (neuro)endocrine variables (for example, cortisol levels), ANS variables (for example, heart rate, skin conductance, (nor)epinephrine levels), or immune variables (for example, number of leucocytes or lymphocytes, subsets of lymphocytes, interleukin levels) as outcomes. Exclusion criteria were pharmacological studies involving CRH, glucocorticoids, insulin or (nor)epinephrine infusions; studies evaluating a battery of standard autonomic function tests, which include deep breathing, the Valsalva maneuver, posture changes, and sustained handgrip, unless they were part of a paradigm with a psychological and/or exercise and/or sensory stressor [29-33]; assessment of anaerobic threshold, peak oxygen consumption, lactate threshold [34,35], fibrinogen and prothrombin time [36]. If a research group published more than one article on the same experimental study but evaluated different outcome measures, both articles were included in the review [29,30,36,37].

Conclusions were based on (uni- or multivariate) statistics for within-group and/or between-groups differences. If studies reported significant between-group differences in repeated measures ANOVAs, baseline values between groups were assumed to be different, unless stated otherwise. If studies did not report significant (within- or between-) group differences, it was assumed that none were found. If studies did not provide statistical analyses, findings were based on mean (\pm standard error of the mean (SEM) or standard deviation (SD)) or median values. If those values were ambiguous, no conclusions were drawn.

Results

Participants

Patient groups

Sixteen studies (18 articles) met the inclusion criteria. The study sample characteristics are summarized in Table 1. Nine studies included patients with RA [29,30,34-42] and seven patients with SLE [31,33,35,40,43-45]. Two of these studies involved both types of patients [35,40]. In addition, one study included patients with JIA [46] and one study included a heterogeneous group of patients with inflammatory arthritis (RA, psoriatic arthritis, ankylosing spondylitis and fibrositis) [32]. Fifteen studies included healthy participants without systemic inflammatory rheumatic disease as control and one study, patients with osteoarthritis [36,37]. In three studies a second control group was added, consisting of either patients with myofascial pain [32], patients with sarcoidosis [43], or participants taking corticosteroids [44]. Most studies have relatively small sample sizes ($N = 10$ to 20 per group), with even smaller sample sizes ($N < 10$) in two studies [35,40]. In addition, patient samples are probably heterogeneous because, for example, strict exclusion criteria regarding comorbidities are lacking in half of all studies [32,33,35,38,42-44,46]. With regard to diagnostic criteria, one study even included also patients with non-inflammatory arthritis [32]. There is also a high variability in pharmacotherapy profiles, but overall these profiles were well-defined for the various classes of drugs (see Table 1), except in two studies [32,33]. To control for the effects of pharmacotherapy some studies excluded patients using specific medication (for example, corticosteroids [35,38-40,46], opioids [39,41], antidepressants [39,40,45], or adrenoceptor antagonists [29,40,45]), or the intake of medication was regulated prior to and on the day of testing [39,41]. In addition, two studies performed subgroup analyses with patients on different medical regimes [41,45].

Stress paradigms

The stressors included in this review were experimental manipulations of stressful experiences, either of a psychological, exercise and/or sensory nature, and lasted between one minute and two hours.

Psychological stressors

Ten of 16 studies used a psychological stressor, applied for one minute to two hours [29-32,36-38,40,41,43-45].

Cognitive stressors Seven studies used cognitive stressors [29-32,36-38,42,44], namely, the Stroop Color-Word Interference test [29,30,38]; a two-minute cognitive discrimination task [29,30]; the Attention and Concentration Test, Syndrome Short Test, computerized-controlled Reaction Time Task and three parts of the Hamburg Wechsler Adult Intelligence Test (comprehension, digit span, block design) [42,44]; Multiple Choice Word Fluency; and the Benton Visual Memory Scale [44]. Mental arithmetic was assessed in

two studies, either with a one- or two-minute serial subtraction task [31,32], or with a two-minute paced auditory serial addition test performed while patients were tilted to a head-up-tilt of 64 degrees [36,37].

Psychosocial stressors Three studies used a psychosocial stressor, namely, a ten-minute public speaking task [40]; the Trier Social Stress Test consisting of a five-minute public speech and a five-minute public mental subtraction task [41]; or a ten-minute public speech (including a five-minute preparation period) on the topic 'Children in today's society' [45].

Exercise stressors

Three studies used exercise stressors, namely, a bicycle ergometer task, which involved cycling at a rate of 50 revolutions per minute against a resistance of 12.5 watt for three minutes [38]; or an ergospirometric exercise test on a treadmill to the limit of subject's tolerance for several minutes [34,35].

Sensory stressors

Eight studies assessed sensory stressors, most of which used thermal stimuli, usually experienced as painful. Five studies assessed responses to a cold stressor [31,33,38,39,46], in which participants either had to put their hand in a bowl filled with (ice) cold water for several minutes [38,39,46], or endure an ice-pack on their hand [31]. One study did not describe the cold pressor task [33]. Heat pulses [39], an acoustic stress test (several minutes) [42,43] and a pupillary light flash test [32] were also used.

Outcome measures

Outcome measures were self-reported distress, autonomous nervous system responses, neuroendocrine responses, or immune responses (see Tables 2, 3 and 4).

Self-reported distress

Stress-induced self-reported distress was assessed in only two of 16 studies [41,45]. Subjective distress levels were either measured on a visual analogue scale (VAS) ranging from 0 to 100 [41] or on a VAS tension and excitement together with the State Trait Anxiety Inventory (STAI) [45].

Measures of the autonomous nervous system

Eleven studies assessed ANS responses (see Table 2) [29,31-33,37,40-43,45,46], namely, heart rate [29,31,32,37,41,45], diastolic and/or systolic blood pressure [29,31,33,41,45], mean arterial pressure, systemic vascular resistance, plasma volume and cardiac output [36,37], and pre-ejection period (PEP) [41]. Furthermore, plasma (nor)epinephrine levels [31,40,42,44-46] and skin conductance [29,32] were assessed.

Table 1 Study sample characteristics

Study	Patient sample	Control sample
Dekkers <i>et al.</i> , 2001 [38]	N = 29 RA patients; No exclusion criteria given	N = 30 HC; Age and sex matched; Exclusion: chronic disease, chronic pain, heart problems, hypertension
Edwards <i>et al.</i> , 2009 [39]	N = 19 RA patients; Exclusion: history of myocardial infarction or cardiovascular disease, peripheral neuropathy, Raynaud syndrome, vasculitis, peripheral vascular disease, other autoimmune or rheu- matic disorders, current infection, recent history of substance abuse or dependence, pregnancy, mood or anxiety disorder	N = 21 HC; No age and sex difference with patients; Same exclusion criteria as patients;
Geenen <i>et al.</i> , 1996/1998 [29,30]	N = 21 RA patients; Exclusion: other serious diseases	N = 20 HC; Age and sex matched; Exclusion: chronic pain, cardiovascular complaints, chronic diseases
Hinrichsen <i>et al.</i> , 1989 [43]	N = 14 SLE patients; No exclusion criteria given	N = 14 HC, N = 12 sarcoidosis patients; Age and sex matched;
Hinrichsen <i>et al.</i> , 1992 [44]	N = 14 SLE patients; No exclusion criteria given	N = 14 HC, N = 10 HC taking corticosteroids; Age and sex matched
Hogarth <i>et al.</i> , 2002 [31]	N = 23 SLE patients; Exclusion: diabetes mellitus, hypertension Remark: some patients have comorbid Sjogren syndrome, Raynaud's phenomenon, history of cerebral lupus	N = 23 HC; Age and sex matched; Exclusion: diabetes mellitus, hypertension
Jacobs <i>et al.</i> , 2001 [40]	N = 9 RA patients, N = 7 SLE patients; Exclusion: significant cardiovascular diseases, concurrent infections, history of other auto- immune disorders, drug or alcohol abuse; no exacerbation of disease < 4 weeks	N = 15 HC; Age and sex matched; Same exclusion criteria as patients
Kurtais <i>et al.</i> , 2006 [34]	N = 19 RA patients; Exclusion: severe illness, other general con- traindications for graded exercise test	N = 14 HC; Age and sex matched; Inclusion: sedentary Exclusion: chronic systemic disease, chronic pain, contraindication for exercise test

Pharmacotherapy of patient sample					
NSAIDs	DMARDs	Corticosteroids	Biologicals	Other	Pharmacotherapy comments:
+	+	- ¹	o	o	¹ patients taking oral prednisone (5-15 mg) or corticosteroid injections < 3 months prior to study onset excluded
+ ²	+	-	+	- ³	² 24h prior to study onset no NSAIDs intake; ³ Opioids, antidepressants excluded; Stable medication regime of >1 months
+	+	o	o	- ⁴	⁴ α and β adrenoceptor antagonists excluded (only for autonomic response evaluation)
o	o	+ ⁵	o	o	⁵ 24h prior to study onset no corticosteroid therapy;
o	o	+ ⁶	o	o	⁶ 4-10 mg; 24h prior to study onset no corticosteroid therapy
+	+	+ ⁷	o	- ⁸	⁷ 1-30 mg; ⁸ Antihypertensive medication <1 week prior to study excluded
o	+	-	-	- ⁹	⁹ adrenoceptor antagonists, antidepressants and benzodiazepines taken < 4 weeks prior to study onset excluded
+	+	+ ¹⁰	o	o	¹⁰ 7.5-15 mg;

Table 1 Continued

Study	Patient sample	Control sample
Motivala <i>et al.</i> , 2008 [41]	N = 21 RA patients; Exclusion: cardiovascular disease, endocrine-related other immune disorders, acute or chronic infections, pregnancy, current psychiatric mood or anxiety disorder	N = 20 HC; Age and sex matched; Same exclusion criteria as patients
Palm <i>et al.</i> , 1992 [42]	N = 18 RA patients; No exclusion criteria given	N = 14 HC; Age and sex matched; Half of all controls had been hospitalized because of coronary heart disease or hypertension
Pawlak <i>et al.</i> , 1999 [45]	N = 15 SLE patients (N < 10 for subanalyses: NK cytotoxicity and # β -adrenoceptors); Exclusion: significant cardiovascular diseases, concurrent infections, history of other autoimmune disorders, drug or alcohol abuse	N = 15 HC (N < 10 for subanalyses: NK cytotoxicity and # β -adrenoceptors); Age and sex matched; Same exclusion criteria as patients
Perry <i>et al.</i> , 1989 [32]	N = 19 heterogeneous group of arthritis patients (RA, psoriatic arthritis, ankylosing spondylitis, fibrositis); No exclusion criteria given	N = 38 HC, 17 patients with myofascial pain; No exclusion criteria given
Pool <i>et al.</i> , 2004 [35]	N = 7 RA patients, N = 6 SLE patients; No exclusion criteria given	N = 10 HC; HC recruited from hospital staff; No exclusion criteria given
Roupe <i>et al.</i> , 2000 [46]	N = 20 JIA patients (N = 15 for <i>in vivo</i> analyses); No exclusion criteria given	N = 20 HC (N = 14 for <i>in vivo</i> analyses); Age and sex matched;
Shalimar <i>et al.</i> , 2006 [33]	N = 51 SLE patients; No exclusion criteria given	N = 30 HC; Age and sex matched
Veldhuijzen <i>et al.</i> , 2005/2008 [36,37]	N = 21 RA patients; Exclusion: previously confirmed acute coronary syndrome, DM, serious psychiatric diseases	N = 10 OA patients; Same exclusion criteria as patients

RA= rheumatoid arthritis, SLE= systemic lupus erythematosus, HC= healthy controls, JIA= juvenile idiopathic arthritis, OA= osteoarthritis, DM = diabetes mellitus. + = Included; - = Excluded; o = Not mentioned in article. ANS = autonomic nervous system, BP = blood pressure, DMARDs=disease-modifying antirheumatic drugs, HR = heart rate, NSAIDs = nonsteroidal anti-inflammatory drugs, PRL = prolactin.

Pharmacotherapy of patient sample					
NSAIDs	DMARDs	Corticosteroids	Biologicals	Other	Pharmacotherapy comments:
+ ¹¹	+	+ ¹²	+	- ¹³	¹¹ 24h prior to study onset no NSAIDs taken; ¹² < 10 mg (> 10 mg excluded); ¹³ Opioids, oral contraception excluded; Stable medication regime >2 months
+	+	+ ¹⁴	o	o	¹⁴ 2,5-10 mg
o	+	+ ¹⁵	o	- ¹⁶	¹⁵ 5-10 mg; ¹⁶ Adrenoceptor antagonists, antidepressants, benzodiazepines taken < 4 week prior to study onset excluded
+	o	o	o	+ ¹⁷	¹⁷ Benzodiazepines, psychotropics, and other medication affecting ANS, etc; 12h prior to onset study no medication taken known to affect ANS
+	+	-	o	- ¹⁸	¹⁸ Phenothiazines (e.o. drugs influencing PRL levels) excluded
+	+	-	o	o	
o	o	o	o	- ¹⁹	¹⁹ Medication known to affect HR, BP excluded
+	+	+	+	+ ²⁰	²⁰ Analgesics included; no use of oral contraception

Endocrine measures

Eight studies assessed neuroendocrine responses (see Table 3) [30,34,35,38,39,41,42,44], namely plasma [30,34,38,41,42,44] or serum [35,39] cortisol levels and plasma ACTH levels [34,38,41]. Two studies measured hormones other than those involved in the HPA axis, namely, plasma growth hormone (GH) and insulin-like growth factor I (IGF-I) levels [34] and serum prolactin levels [35].

Immunological measures

Twelve studies assessed immune responses (see Table 4) [30,35,36,38-46], namely, the total number of leucocytes [36,40,42-45], and the number of lymphocytes and/or subsets of lymphocytes, including B cells, T cells and NK cells [30,35,40,42-45]. Two studies assessed NK cell cytotoxicity [40,45]. Six studies reported on cytokine levels [38-42,46], namely, plasma levels of cytokine IL-1 β [42], IL-6 [39,41,42] and TNF α [39], *ex vivo* stimulated mononuclear cell production of IL-2 [40], IL-4 [38,40], IL-6 [40,41,46], IL-8 [46], IL-10 [40], interferon (IFN) γ [38,40] and TNF α [41], or intracellular concentrations of IL-4, IL-6, IL-10 and IFN γ [40]. Other inflammation markers were also assessed, such as C-reactive protein (CRP) [36], the number of β -adrenoceptors on monocytes [45], β -adrenoceptor sensitivity [45], and plasma soluble IL-2 receptor levels [42].

Time points of outcome measures

All studies reported baseline values for the outcome measures, and all, except two [32,34], reported a resting period of 3 to 45 minutes. During administration of the experimental stressor, physiological reactivity was either assessed at specific time points (in three studies) [35,41,42], or continuously throughout the stress procedure (only for autonomic measures such as heart rate, blood pressure and/or skin conductance; in four studies) [29,32,37,45]. All studies reported immediate post stress measurements, except two studies that only assessed stress reactivity at 30 minutes [34] or 60 minutes [35] after cessation of the stressor. Eight of 16 studies have one [29,30,36-38,40,45] or more [39,41,46] additional follow-up measurement points, ranging from 5 to 60 minutes after cessation of the stressor.

Baseline characteristics

Autonomic, neuroendocrine, and immune functions were assessed at baseline in patients with rheumatic disorders and controls. Results for the separate outcome measures are summarized in Tables 2, 3 and 4.

ANS variables

ANS function at baseline was not different between patients with rheumatic disorders and controls in most studies. Cardiovascular variables, skin conductance, and catecholamine levels did not differ in nine studies [29,31,36,37,40-42,44-46], whereas three studies reported

significantly higher levels of autonomic activity at baseline (heart rate and pupil area [32]; epinephrine levels [46]) or lower levels ((nor)epinephrine [31]) in patients with rheumatic disorders compared with controls (see Table 2).

Neuroendocrine variables

Neuroendocrine function at baseline was not significantly different between patients with rheumatic disorders and controls. Five of eight studies reported similar baseline levels of cortisol, ACTH, GH, IGF-I, and prolactin [34,35,39,41,42]. Three studies reported higher levels of cortisol at baseline (in patients with RA) [30,38] or lower levels (in patients with SLE) [44] than in controls (see Table 3). Lower cortisol levels at baseline were also observed in one study in which control subjects were taking corticosteroids [44].

Immune variables

Baseline leucocyte counts [36,40,42] were not different between patients with RA and controls. However, two of three studies found patients with RA to have lymphopenia at baseline [30,42]. Levels of cytokines and other inflammatory factors (IL-2, IL-4, IL-6, IFN γ , TNF α , and CRP) were similar in patients with RA and controls in five studies [36,38-41]. Three studies reported higher baseline levels of IL-6 [39] and soluble IL-2 receptor [42] and lower basal levels of IL-10 and IFN γ [40] in patients with RA compared with controls.

Baseline leucocyte counts were not significantly different between patients with SLE and controls in most (three of four) studies [43-45]. One study reported baseline leucopenia in patients with SLE [40]. All studies reported lower lymphocyte counts (including lower subsets of lymphocytes) in patients with SLE than in controls [40,43,45]. Despite this baseline lymphopenia, a higher percentage of B cells (from the total lymphocyte count) was found once [43]. Only one study reported similar numbers of helper T and cytotoxic T cells in patients with SLE and controls [35]. Cytokine (IL-2, IL-4 and IL-6) levels at baseline were not significantly different between patients with SLE and controls [40]. However, cytokine levels of IL-10 and IFN γ at baseline and the sensitivity of β -adrenoceptors, which are involved in the transduction of autonomic signals to immune cells, were lower in patients with SLE than in controls [45]. The one study with immunological data on patients with JIA reported higher cytokine levels (IL-6 and IL-8) than in controls at baseline [46] (see Table 4).

In summary, at baseline autonomic function is similar in patients with RA and SLE and controls, with only limited evidence for heightened autonomic function in (a subgroup of) patients with rheumatic diseases. Neuroendocrine function at baseline is also comparable in patients with RA and SLE and controls, with only three studies reporting altered cortisol levels in patient groups. Specific immune variables, mainly (subsets of) lymphocyte counts, appear to be lower in patients with SLE than in controls.

Psychophysiological responses to stress

An overview of findings is given in Tables 2, 3 and 4.

Table 2 Autonomic function in patients with systemic inflammatory rheumatic diseases

Parameter	Studies & patients (N)	Baseline patients vs. controls	
Heart rate	[29] 21 RA vs. 20 HC [41] 21 RA vs. 20 HC [36,37] 21 RA vs. 10 OA [31] 23 SLE vs. 23 HC [45] 15 SLE vs. 15 HC [32] 19 Arthr vs. 38 HC, 17 MFP	RA:	No difference [29,36,37,41]
		SLE:	No difference [31,45]
		Arthr:	Altered (↑) [32]
Blood pressure (diastolic/systolic)	[29] 21 RA vs. 20 HC [41] 21 RA vs. 20 HC [36] 21 RA vs. 10 OA [31] 23 SLE vs. 23 HC [45] 15 SLE vs. 15 HC [33] 51 SLE vs. 30 HC	RA:	No difference [29,36,41]
		SLE:	No difference [31,45] Not reported [33]
Mean arterial pressure (MAP)	[37] 21 RA vs. 10 OA	RA:	No difference
Systemic vascular resistance (SVR)	[37] 21 RA vs. 10 OA	RA:	No difference
Plasma volume (PV)	[36] 21 RA vs. 10 OA	RA:	No difference
Cardiac output (CO)	[37] 21 RA vs. 10 OA	RA:	No difference
Pre-ejection period (PEP)	[41] 21 RA vs. 20 HC	RA:	No difference
Plasma catecholamines (nor)epinephrine	[42] 18 RA vs. 14 HC [40] 9 RA, 7 SLE vs. 15 HC [44] 14 SLE vs. 14 HC, 10 HC [31] 23 SLE vs. 23 HC [45] 15 SLE vs. 15 HC [46] 15 JIA vs. 14 HC	RA:	No difference [40] [42] *
		SLE:	No difference [40,45] Altered (↓) [31] Not reported [44]
		JIA:	No difference (NE) [46] Altered (↑) (EPI) [46]
Skin conductance (SC)	[29] 21 RA vs. 20 HC [32] 19 Arthr vs. 38 HC, 17 MFP	RA:	No difference [29]
		Arthr:	No difference [32]
Pupillary constriction	[32] 19 Arthr vs. 38 HC, 17 MFP	Arthr:	Altered (↓)

* Findings assumed after inspection of descriptive data.

↑ = altered response pattern is more pronounced compared to a control group; ↓ = altered response pattern is diminished compared to a control group; RA= rheumatoid arthritis, SLE= systemic lupus erythematosus, JIA = juvenile idiopathic arthritis, Arthr= heterogeneous group of arthritis patients, HC= healthy controls, OA= osteoarthritis, MFP= patients with myofascial pain, NE= norepinephrine, EPI= epinephrine.

Stress reactivity within patients		Stress reactivity patients vs. controls	
RA:	Increase [29,36,37,41]	RA:	No difference [36,37,41] Altered (↓) [29]
SLE:	Increase [45] Not reported [31]	SLE:	No difference [31] (cogn.) [45] Altered (↓) (cold) [31]
Arthr:	Increase [32]	Arthr:	No difference [32]
RA:	Increase [29,36,41]	RA:	No difference [36,41] Altered (↓) [29] / (↑) [41]
SLE:	Increase [45] Not reported [31,33]	SLE:	No difference [31,33,45]
RA:	Increase	RA:	No difference
RA:	Increase severe subgroup	RA:	Altered (↑) severe subgroup
RA:	Decrease	RA:	No difference
RA:	No response	RA:	No difference
RA:	Decrease	RA:	No difference
RA:	Increase [40] No response [42]	RA:	No difference [40,42]
SLE:	Increase [40,44,45]	SLE:	No difference [40,45] [44] *
JIA:	Increase (NE)[46] No response (EPI)[46]	JIA:	No difference [46]
RA:	Increase [29]	RA:	Altered (↓) [29]
Arthr:	Increase [32]	Arthr:	Altered (↑) [32]
Arthr:	Not reported	Arthr:	Altered (↓)

Table 3 Autonomic function in patients with systemic inflammatory rheumatic diseases

Parameter	Studies & patients (N)	Baseline patient vs. control	
ACTH	[38] 29 RA vs. 30 HC [34] 19 RA vs. 14 HC [41] 21 RA vs. 20 HC	RA:	No difference [34,38,41]
Cortisol	[30] 21 RA vs. 20 HC [38] 29 RA vs. 30 HC [39] 19 RA vs. 21 HC [34] 19 RA vs. 14 HC [41] 21 RA vs. 20 HC [42] 18 RA vs. 14 HC [35] 7 RA, 6 SLE vs. 10 HC [44] 14 SLE vs. 14 HC, 10 HC	RA: SLE:	No difference [34,35,39,41,42] Altered (↑) [30,38] No difference [35] Altered (↓) [44]
Growth hormone (GH)	[34] 19 RA vs. 14 HC	RA:	No difference
Insulin-like growth factor (IGF-I)	[34] 19 RA vs. 14 HC	RA:	No difference
Prolactin	[35] 7 RA, 6 SLE vs. 10 HC	RA: SLE:	No difference No difference

↑ = altered response pattern is more pronounced compared to a control group; ↓ = altered response pattern is diminished compared to a control group; RA= rheumatoid arthritis, SLE= systemic lupus erythematosus, HC= healthy controls, ACTH= adrenocorticotropin hormone.

Table 4 Immune function in patients with systemic inflammatory rheumatic diseases

Parameter	Studies & patients (N)	Baseline patient vs. control	
Leucocytes	[42] 18 RA vs. 14 HC [36] 21 RA vs. 10 OA [40] 9 RA, 7 SLE vs. 15 HC [43] 14 SLE vs. 14 HC, 12 SD [44] 14 SLE vs. 14 HC, 10 HC [45] 15 SLE vs. 15 HC	RA: SLE:	No difference [36,40,42] No difference [43,45] [44] * Altered (↓) [40]
Total lymphocytes	[30] 21 RA vs. 20 HC [42] 18 RA vs. 14 HC [40] 9 RA, 7 SLE vs. 15 HC [43] 14 SLE vs. 14 HC, 12 SD [44] 14 SLE vs. 14 HC, 10 HC [45] 15 SLE vs. 15 HC	RA: SLE:	Altered (↓) [30,42] No difference [40] Altered (↓) [40,43,45] Not reported [44]

Stress reactivity within patients		Stress reactivity patients vs. controls	
RA:	Increase [34,41] Not reported [38]	RA:	No difference [34,38,41]
RA:	Decrease [34,35,42] Change [30] Increase [39,41]	RA:	No difference [30,34,39,41,42] Altered (↓) [35,38] No difference [44]
SLE:	Decrease [35] No response [44]	SLE:	Altered (↓) [35]
RA:	Increase	RA:	No difference
RA:	No response	RA:	No difference
RA:	No response	RA:	Altered (↓)
SLE:	No response	SLE:	Altered (↓)

Stress reactivity within patients		Stress reactivity patients vs. controls	
RA:	Increase [36,40,42]	RA:	No difference [36,40] [42] *
SLE:	Increase [40,43-45]	SLE:	Altered (↓) [40,43,45] No difference [44] *
RA:	Increase [30,40] No response [42]	RA:	No difference [30,40] Altered (↓) [42]
SLE:	Increase [40,45] No response[43,44]	SLE:	Altered (↓) [43-45] No difference [40]

Table 4 Continued

Parameter	Studies & patients (N)	Baseline patient vs. control	
Total T cells (CD3+)	[30] 21 RA vs. 20 HC [40] 9 RA, 7 SLE vs. 15 HC [43] 14 SLE vs. 14 HC, 12 SD [44] 14 SLE vs. 14 HC, 10 HC [45] 15 SLE vs. 15 HC	RA:	No difference [40] Altered (↓) [30]
		SLE:	Altered (↓) [40,45] No difference (%) [43] [44] *
Helper T cells (CD4+)	[40] 9 RA, 7 SLE vs. 15 HC [35] 7 RA, 6 SLE vs. 10 HC [43] 14 SLE vs. 14 HC, 12 SD [44] 14 SLE vs. 14 HC, 10 HC [45] 15 SLE vs. 15 HC	RA:	No difference [35,40]
		SLE:	Altered (↓) [40,45] No difference [35] / (%) [43] Not reported [44]
Cytotoxic T cells (CD8+)	[40] 9 RA, 7 SLE vs. 15 HC [35] 7 RA, 6 SLE vs. 10 HC [43] 14 SLE vs. 14 HC, 12 SD [44] 14 SLE vs. 14 HC, 10 HC [45] 15 SLE vs. 15 HC	RA:	No difference [35,40]
		SLE:	Altered (↓) [40,45] No difference [35] / (%) [43] Not reported [44]
B cells (CD19+)	[30] 21 RA vs. 20 HC [43] 14 SLE vs. 14 HC, 12 SD [44] 14 SLE vs. 14 HC, 10 HC	RA:	Altered (↓) [30]
		SLE:	Altered (↓) (%) [43] No difference (%) [44] *
NK cells (CD56+)	[30] 21 RA vs. 20 HC [40] 9 RA, 7 SLE vs. 15 HC [44] 14 SLE vs. 14 HC, 10 HC [45] 15 SLE vs. 15 HC	RA:	No difference [40] Altered (↓) [30]
		SLE:	Altered (↓) [40,45] Not reported [44]
NK cell cytotoxicity	[40] 9 RA, 7 SLE vs. 15 HC [45] 4 SLE vs. 8 HC	RA:	No difference [40]
		SLE:	No difference [40,45]
Cytokines			
IL-6	[39] 19 RA vs. 21 HC [41] 21 RA vs. 20 HC [42] 18 RA vs. 14 HC [40] 9 RA, 7 SLE vs. 15 HC [46] 15 JIA vs. 14 HC	RA:	No difference [40,41] Altered (↑) [39]
		SLE:	No difference [40]
		JIA:	Altered (↑) [46]
IL-2	[40] 9 RA, 7 SLE vs. 15 HC	RA:	No difference
		SLE:	No difference
IL-4	[38] 29 RA vs. 30 HC [40] 9 RA, 7 SLE vs. 15 HC	RA:	No difference [38,40]
		SLE:	No difference [40]
IL-8	[46] 15 JIA vs. 14 HC	JIA:	Altered (↑)
IL-10	[40] 9 RA, 7 SLE vs. 15 HC	RA:	Altered (↓) (not intracell.)
		SLE:	Altered (↓) (not intracell.)

Stress reactivity within patients		Stress reactivity patients vs. controls	
RA:	Increase [40] No response [30]	RA:	No difference [30,40]
SLE:	Increase [40,45] No response (%) [43,44]	SLE:	No difference [40,43] Altered (↓) [45] / (%) [44]
RA:	Increase [40] Decrease [35]	RA:	Altered (↑) [35,40]
SLE:	No response [40,45] / (%) [44] Decrease [35] / (%) [43]	SLE:	Altered (↓) [45] (%) [43,44] / () [35] No difference [40]
RA:	Increase [40] No response [35]	RA:	No difference [40] Altered (↓) [35]
SLE:	Increase [40,45] / (%) [43,44] Decrease [35]	SLE:	No difference [40,45] [44] * Altered (↓) [35] / (%) [43]
RA:	Increase [30]	RA:	No difference [30]
SLE:	Increase (%) [43] No response (%) [44]	SLE:	Altered (↓) (%) [43,44]
RA:	Increase [30,40]	RA:	No difference [30,40]
SLE:	Increase [40,45] No response [44]	SLE:	Altered (↓) [40,45] No difference [44]
RA:	No response [40]	RA:	Altered (↓) [40]
SLE:	No response [40,45]	SLE:	Altered (↓) [40,45]
RA:	No response [40] Increase [39] Decrease (not plasma) [41]	RA:	No difference [39-41]
SLE:	No response [40]	SLE:	No difference [40]
JIA:	Increase [46]	JIA:	Altered (↑) [46]
RA:	No response	RA:	No difference
SLE:	No response	SLE:	No difference
RA:	No response [40]	RA:	No difference [40]
SLE:	Increase [40]	SLE:	Altered (↑) [40]
JIA:	No response	JIA:	No difference
RA:	No response	RA:	No difference
SLE:	No response	SLE:	No difference

Table 4 Continued

Parameter	Studies & patients (N)	Baseline patient vs. control	
IFN γ	[38] 29 RA vs. 30 HC	RA:	No difference [38]
	[40] 9 RA, 7 SLE vs. 15 HC	SLE:	Altered (\downarrow) (not intracell.) [40] Altered (\downarrow) (not intracell.) [40]
TNF α	[39] 19 RA vs. 21 HC	RA:	No difference [39,41]
	[41] 21 RA vs. 20 HC		
β -adrenoceptors	[45] 7 SLE vs. 8 HC	SLE:	No difference
β -adrenoceptor sensitivity	[45] 7 SLE vs. 8 HC	SLE:	Altered (\downarrow)
sIL-2 receptor	[42] 18 RA vs. 14 HC	RA:	Altered (\uparrow)
C-reactive protein (CRP)	[36] 21 RA vs. 10 OA	RA:	No difference

* Findings assumed after inspection of descriptive data.

\uparrow = altered response pattern is more pronounced compared to a control group; \downarrow = altered response pattern is diminished compared to a control group; RA= rheumatoid arthritis, SLE= systemic lupus erythematosus, JIA = juvenile idiopathic arthritis, HC= healthy controls, OA= osteoarthritis, SD=sarcoidosis patients. IL= interleukin, IFN γ = interferon γ , TNF α = tumor necrosis factor α , sIL-2 receptor=soluble interleukin-2 receptor, intracell. = intracellular interleukin concentration on the single-cell level.

Self-reported distress

Self-reported distress was increased significantly from baseline by psychosocial stress in patients with RA [41] and SLE [45] and in controls. This increase was greater in patients with RA [41] than in controls. This was not the case for patients with SLE [45].

ANS variables

The autonomic response to stress was assessed in patients with RA, SLE, JIA and in a heterogeneous group of patients with inflammatory arthritis. Results are summarized in Table 2. In patients with RA, experimental stress increased autonomic activity (heart rate [29,36,37,41], blood pressure [29,37,41], systemic vascular resistance [37], pre-ejection period (PEP) [41], plasma volume [36], skin conductance [29], and plasma (nor)epinephrine levels [40]), with the increase being similar to that seen in controls in most studies. However, three studies reported either diminished [29] or more pronounced [37,41] autonomic responses in (a subgroup of) patients. In patients with SLE, experimental stress increased autonomic activity (heart rate [45], blood pressure [45], and (nor)epinephrine

Stress reactivity within patients		Stress reactivity patients vs. controls	
RA:	No response [40]	RA:	Altered (↓) [40]
SLE:	No response [40]	SLE:	Altered (↓) [40]
RA:	Increase [39,41]	RA:	Altered (↑) [39,41]
SLE:	No response	SLE:	Altered (↓)
Not assessed		Not assessed	
RA:	No response	RA:	No difference
RA:	Increase	RA:	Altered (↑)

levels [40,44,45]), the increase often being similar to that seen in controls [31,33,40,45]. Only one study observed a diminished autonomic response (heart rate), but only during one specific type of stressor (cold) [31]. The one study involving patients with JIA showed experimental stress to increase norepinephrine levels to a similar extent in patients and healthy controls [46]. In one study with a heterogeneous group of patients with inflammatory arthritis, experimental stress increased autonomic activity (heart rate, skin conductance, and pupillary constriction) [32], but this increase was smaller (pupillary constriction) or greater (galvanic response) than that of controls.

In summary, patients with rheumatic disorders respond to experimental stress with increased cardiovascular, galvanic and catecholamine responses. Whereas most autonomic responses to stress are similar to those of a control group, there is partial evidence (five studies) for altered stress-induced autonomic responses.

Neuroendocrine variables

Neuroendocrine reactivity, mainly measured as changes in cortisol levels, was assessed in patients with RA and SLE and in controls (see Table 3). Experimental stress elicited both an increase [39,41] and (more often) a decrease [34,35,38,42] in cortisol levels in patients with RA. ACTH, which is activated upstream of cortisol, increased in response to stress [34,41]. In addition to the assessment of HPA axis hormones, one study also reported an increase in GH levels in response to a stressor [34]. The neuroendocrine response to stress of patients with RA was not significantly different from that of controls in most studies, but two studies reported that cortisol responses were diminished in patients with RA compared with controls [35,38]. In one study, experimental stress increased prolactin levels in controls but not in RA patients [35]. The effect of stress on neuroendocrine function in patients with SLE was inconsistent, with stress eliciting a decrease [35] or no change [44] in cortisol levels. A lack of responsiveness was also reported in control subjects [35,44]. Experimental stress did not increase prolactin levels in patients with SLE [35].

In summary, although cortisol and ACTH levels change in response to experimental stress, differences between patients with rheumatic diseases (RA and SLE) and control groups have been reported in only two studies. There is preliminary evidence (one study) that the prolactin response to stress is different in patients than in controls.

Immune variables

Because several immune variables were used in the various studies to assess the effect of experimental stress on immune function, we have classified results on the basis of changes in leucocyte and lymphocyte counts and inflammatory markers (see Table 4).

Leucocytes and (subgroups of) lymphocytes In patients with RA, experimental stress consistently increased the number of leucocytes [36,40,42], and increased the number of lymphocytes [30,40], with increases in the number of B cells [30], total T cells [40] and cytotoxic T cells [40] and either an increase [40] or a decrease [35] in helper T cells. The stress-induced increase in NK cell numbers [30,40] did not result in an increase in NK cell activity [40]. Most studies did not detect a difference in the immune response (leucocyte and lymphocyte counts) to stress of patients with RA and controls, but one study reported lower lymphocyte counts after stress in patients with RA [42]. Changes compared with controls were noted for helper T cells [35,40] and cytotoxic T cells [35]. In patients with SLE, stress induced a consistent increase in the number of leucocytes [40,43-45], and a less consistent increase in the number of lymphocytes [40,45]. Stress increased the number of total T cells [40,45], number [40,45] and percentage [43,44] of cytotoxic T cells, and the percentage of B cells [43], and decreased the number of helper T cells [35,43]. Again, although NK cell numbers increased after stress [40,45], NK cell activity did not increase [40,45]. Comparison showed that the immune response to stress was often smaller in patients with SLE than in controls: the increase in total leucocyte counts in patients with SLE was smaller than that of controls [40,43,45], as was the increase in total lymphocyte

count [43-45]. Moreover, the stress-induced change in subsets of lymphocytes was often smaller for B cells [43,44], total T cell numbers [44,45], helper T cells [43-45], cytotoxic T cells [35,43], and NK cells [40,45].

Thus, total leucocyte counts and lymphocyte subsets change in response to stress in patients with rheumatic diseases, with patients with SLE having a smaller response (in addition to their baseline lymphopenia) than controls; no consistent alterations were found for patients with RA. NK cell cytotoxicity is diminished in patients with RA and SLE compared with control.

Inflammatory markers In patients with RA, experimental stress did not change levels of various inflammatory markers (IL-2, soluble IL-2 receptor, IL-4, IL-10 and IFN γ) [40,42], although levels of CRP [36] and TNF α increased after stress [39,41]. Results for IL-6 were inconsistent, with stress inducing a decrease [41], an increase [39] or no change [40] in levels. Patients differed from controls, having smaller IL-10 and IFN γ responses [40], and larger TNF α [39,41] and CRP responses [36]. In patients with SLE, experimental stress did not change levels of cytokines and other inflammatory markers (IL-2, IL-6, IL-10, IFN γ , β -adrenoceptors) [40,45], although levels of IL-4 increased after stress [40]. Patients with SLE differed from controls in their response to stress, having a larger increase in IL-4, smaller IL-10 and IFN γ responses [40], and fewer β -adrenoceptors on monocytes [40,45]. In patients with JIA, stress did not affect IL-8 production, but increased IL-6 production [46]. This increase was not observed in controls. Thus, although in general experimental stress does not seem to influence levels of certain cytokines and inflammatory markers in patients with rheumatic diseases (for example, sIL-2, IL-8, IL-10, IFN γ , β -adrenoceptors), it does cause specific changes in CRP and TNF α in patients with RA, changes in IL-4 in patients with SLE, and changes in IL-6 in patients with JIA. These changes are not observed in controls.

In summary, the leucocyte and lymphocyte responses to stress are smaller in patients with SLE than in controls but consistent differences with controls are not seen in patients with RA. Experimental stress does not seem to affect NK cell cytotoxicity in either patient group. However, specific stress-induced changes in inflammatory markers are reported in patients with RA (CRP, TNF α), SLE (IL-4) and JIA (IL-6) that are not observed in controls.

Physiological stress reactivity for specific types of stressors

As seen above, experimental stress has a distinct effect on the autonomic, neuroendocrine, and immune systems. Below, we summarize the effect of the different types of experimental stress (cognitive, psychosocial, physical, and sensory), to determine whether different types of stress elicit different responses in different patients groups (see Table 5). Studies in which more than one type of stressor was used, were not included [38,42] unless outcome measures were reported separately for the different types of stressors [31,32].

Table 5 Autonomic (ANS), neuroendocrine (NE), and immune responses to different stressors in patients with systemic inflammatory rheumatic diseases

Stress paradigm	Studies	ANS		NE system		Immune system	
		Measure	Response	Measure	Response	Measure	Response
Psychological stress		HR	↑ RA[29,37] ↑ Arthr[32]	Cortisol	↓/↑ RA[30] 0 SLE[44]	Leucocytes	↑ RA[36] ↑ SLE[44]
Cognitive tasks	[38]* [29,30,44] [31]** [42]* [32,36,37]	SC	↑ RA[29] ↑ Arthr[32]			Lymphocytes	↑ RA[30] 0 SLE[44]
		CO	↑ RA[37]			T	0 RA[30] 0 SLE[44]
		PV	↓ RA[36]			B	↑ RA[30] 0 SLE[44]
		SVR	↑ RA[37]			NK	↑ RA[30] 0 SLE[44]
		BP	↑ RA[29,36,37]			Th	0 SLE[44]
		CA	↑ SLE[44]			Tcyt	↑ RA[30] 0 SLE[44]
						CRP	↑ SLE[44] ↑ RA[36]
Psychosocial tasks	[40,41,45]	HR	↑ RA[41] ↑ SLE[45]	Cortisol	↑ RA[41] ↑ RA[41]	Leucocytes	↑ RA[40] ↑ SLE[40,45]
		BP	↑ RA[41]	ACTH		Lymphocytes	↑ RA[40] ↑ SLE [40,45]
		PEP	↓ RA[41]			T	↑ RA[40] ↑ SLE[40,45]
		CA	↑ RA[40] ↑ SLE[40,45]			Tcyt	↑ RA[40] ↑ SLE[40,45]
						NK	↑ RA[40] ↑ SLE[40,45]
						Th	↑ RA[40] 0 SLE[40,45]
						NK toxicity	0 RA[40] 0 SLE[40,45]
						IL-6	↓ [41] / 0 [40] RA 0 SLE[40]

Cognitive stressors

Five studies investigated a cognitive stressor in patients with RA, SLE, or inflammatory arthritis [29-32,36,37,44]. In all patient groups, cognitive stress consistently increased autonomic function: heart rate [29,32,37], blood pressure [29,36,37], skin conductance [29,32], cardiac output and systemic vascular resistance [37], plasma volume [36], and catecholamines [44]. Patients with RA and SLE had different cortisol responses to cognitive stress [30,44] (see Table 5). Cognitive stress elicited changes in leucocyte counts, lymphocyte counts, subsets of lymphocytes, and CRP levels in patients with RA [30,36], but only changes in leucocyte counts and cytotoxic T cell numbers in patients with SLE [44].

Psychosocial stressors

A psychosocial stressor was used in three studies of patients with RA and SLE [40,41,45]. Psychosocial stress consistently increased autonomic activity (heart rate [41,45], blood pressure [41], pre-ejection period [41] and catecholamine levels [40,45]) in patients with RA and SLE and increased neuroendocrine variables (cortisol and ACTH [41]) in patients with RA. Some immune responses were similar in the two patient groups (leucocyte counts, lymphocyte counts, (cytotoxic) T cells, NK cells and NK cell cytotoxicity [40,45]), whereas others were different (helper T cells [40], IL-4 [40]) (see Table 5). Cytokine levels were not influenced by psychosocial stress, with the exception of an increase in IL-4 in patients with SLE, an increase in TNF α in patients with RA, and inconsistent findings for IL-6.

Exercise stressors

The stress of exercise was investigated in two studies with patients with RA and SLE [34,35]. These studies did not assess autonomic function; however, cortisol levels decreased in both groups of patients [34,35] whereas ACTH levels and GH levels increased [34]. Exercise stress elicited a different cytotoxic T cell response in patients with RA and SLE [35] (see Table 5).

Sensory stressors

Sensory stressors were used in six studies involving patients with SLE and JIA [31-33,39,43,46]. Sensory stress increased catecholamine levels [46] and cortisol levels [39]. It also increased leucocyte counts, changed percentages of certain subsets of lymphocytes [43], and increased levels of IL-6 [39,46] and TNF α [39] (see Table 5).

In summary, although few data are available about the effects of specific types of stress, results suggest that psychosocial, cognitive and sensory stress consistently increase autonomic activity. Changes in neuroendocrine variables in response to psychosocial, cognitive and sensory stressors are observed in patients with RA only. Psychosocial stress seems to elicit the strongest immune response in all patient groups studied.

Discussion

A better understanding of the acute physiological response to stress in patients with inflammatory rheumatic diseases might further our knowledge of how stress affects the health of these patients. We reviewed the effects of time-limited stressors on autonomic, endocrine, and immune variables in patients with chronic inflammatory rheumatic diseases. Results suggest that autonomic and neuroendocrine responses to stress are not consistently altered in patients with rheumatic disorders compared with controls, although patients do appear to show a distinct immune response to stress. Psychosocial stress might prove to be the best tool to evaluate these specific immune responses to stress.

Physiological stress response systems

ANS

Although previous studies that used regular autonomic function tests showed autonomic reactivity to be altered in different populations of patients with rheumatic diseases compared with controls [2,47-50], we failed to find consistent evidence for these alterations in studies investigating psychological, exercise, or sensory stress. The autonomic function of patients with rheumatic disorders was not only similar to that of controls at baseline but also after experimental stress, with cardiovascular, galvanic and catecholaminergic responses largely comparable to those of controls. Only a minority of studies reported autonomic function to be altered in these patients (as compared with controls) [29,31,32,37]. Studies have shown that alterations in autonomic function in response to stress are correlated with disease severity [36,37,48,51], which suggests that only subgroups of patients with severe disease show distinct alterations in autonomic function. Changes in autonomic function might also be linked to physical deconditioning, vascular inflammation and accelerated atherosclerosis [52], which could explain the inconsistencies in the literature on this subject. It should also be noted that alterations in autonomic function might reflect a change in sympathetic or parasympathetic activity or in the balance of the two systems [53,54]. Autonomic measures used in experimental stress paradigms (for example, the heart rate response) do not always differentiate between these systems, in contrast to regular autonomic function tests [55], which specifically aim to distinguish between sympathetic dysfunction and parasympathetic neuropathy [49,51,56].

HPA axis

The expected increase in neuroendocrine variables in response to a stressor was only observed in two studies with patients with RA [39,41]. This increase is consistent with previous studies reporting significant and consistent endocrine responses to psychosocial stress both in healthy subjects and in various patient populations without rheumatic disorders [24,25,41,57-60,60-62]. The change in cortisol levels is consistent with the

endocrine response to real-life anticipation stress before hip or knee surgery in patients with RA [63,64]. However, most studies evaluated in this review reported a decrease in cortisol, levels after stress, both in patients and controls, which might reflect the normal diurnal rhythmic decline in cortisol levels instead of a stress response [34,35,38,42]. All studies, except the two reporting an increase in cortisol levels, assessed patients in the early morning hours, when cortisol levels are known to decrease sharply.

Fuelling the ongoing debate on whether or not HPA axis responses are diminished in patients with rheumatic disease compared with healthy subjects, we did not find consistent changes in HPA axis function in response to psychological and exercise stress in patients with rheumatic diseases. Indeed, only two studies showed a different neuroendocrine response to stress in patients and controls [35,38]. Previous studies involving pharmacological stimulation of the HPA axis reported either similar or slightly lower responses in patients with rheumatic diseases compared with controls [27,28]. However, results should be interpreted with caution because stress manipulation may have been ineffective. Variations across studies in disease duration and activity, age, sex and sampling time might contribute to the variability in HPA axis responses in patients with rheumatic diseases. Moreover, attention should be paid to the role of pharmacotherapy (for example, corticosteroids), which could influence the endocrine response to stress [65].

Immune system

Most studies reported that total leucocyte counts and specific subsets of lymphocytes (not total number per se) increased after stress in patients with rheumatic diseases. The most consistent finding was an increase in the number of NK cells, as reported in earlier studies showing that the most robust effect of acute time-limited stressors in healthy subjects is a marked increase in the number of NK cells, which suggest an activation of innate immunity [15]. However, NK cell cytotoxicity after stress was lower in patients than in controls, possibly due to permanent activation as a consequence of the inflammatory disease [40,45]. The change in cytokine levels elicited by stress in patients with rheumatic disorders was small or inconsistent, for example, for the most frequently measured cytokine IL-6 [40,41,46]. Previous semi-experimental studies with patients with RA reported ambiguous results, reporting either significantly increased [63] or decreased levels of IL-6 [64] during anticipation of planned knee or hip arthroplasty. In addition, in a study evaluating responses to cryotherapy, patients with RA not treated with glucocorticoids responded with an increase in IL-6 levels [65]. A recent meta-analysis showed IL-6 levels to be consistently increased after psychosocial stress in healthy individuals [16]. The contradictory findings regarding cytokine responses in patients with rheumatic diseases might be due to methodological caveats, such as failed stress manipulation or small sample sizes (for example, in Jacobs *et al.* (2001)). However, it is also possible that cytokines do not effectively regulate immune function systemically, but instead near the effector site [40]. Future studies involving patients with rheumatic diseases should measure local

immune variables in samples obtained from joint tissue or synovial fluid [66,67]. Another explanation is that patients or subgroups of patients might have different cytokine responses (for example, TNF α) because of differences in disease severity [68] or pharmacotherapy [41,65]. In general, as could be expected for immune-mediated diseases, the immune response to stress was different in patients and controls, and also differed between the various types of inflammatory rheumatic diseases.

Differences in type of stressor, rheumatic disease and (time points of) outcome measures

Findings of this review should be interpreted with caution due to differences in the types of stress induced, the various rheumatic diseases evaluated, and the variability in outcome measures and assessment times. In addition, the highly variable methodological quality of the studies, the small number of studies, and the relatively small patient samples hinder an unequivocal interpretation of findings. Future research urgently requires well-designed studies with larger numbers of patients. These studies should, for example, systematically take into account physiological baseline levels.

Stress paradigms

Only the studies of psychosocial stress evaluated all three physiological stress response systems (autonomic, neuroendocrine and immune) and, moreover, consistently reported stress-induced increases at all three levels. This is in line with previous studies showing significant and consistent autonomic, neuroendocrine, and immune responses to psychosocial stress in both healthy subjects and patient groups [25,41,57-60,60-62,69]. In contrast, cognitive stressors did not consistently alter neuroendocrine variables, and immune responses were small in patients with SLE. The stress of exercise was found not to activate the HPA axis in patients with rheumatic diseases, in contrast to what has previously been observed in healthy subjects [70], although studies suggest that neuroendocrine changes are only observed after exercise of longer duration [71]. Data on immune responses were very limited. The studies that assessed sensory stress reported stress-induced increases in autonomic, neuroendocrine, and immune function, but data were limited. Because most sensory stressors are also frequently applied to induce pain [72,73], future studies of stress should take into account whether a stressor is pain-inducing in order to unravel the relationship between stressors, pain and physiological responses [74]. Overall, more research into the effects of psychosocial stress on physiological function is needed, especially because these stress paradigms seem to generate the strongest response at all three physiological levels and probably have the greatest ecological validity. Importantly, insight in physiological responses to acute stressors cannot automatically be translated to the field of daily stressors, major stress events or even chronic stressors [5,75]. For example, studies in healthy and other disease populations

suggest that chronic stress might induce hypocortisolism [6,76] and global immunosuppression of both innate and specific immunity [15]. Overall, there is a lack of studies examining the association between physiological parameters and chronic stressors in rheumatic populations [2]. Studies investigating stressors in daily life reported an association between stressors, mainly of an interpersonal nature, and concentrations of cytokines and lymphocytes (for example, IL-6, soluble IL-2 receptors and T cell numbers) [77-79]. However, results are inconclusive [80] and future studies are needed to elucidate how acute and chronic stressors differentially affect inflammatory rheumatic diseases.

Disease and treatment characteristics

In contrast to autonomic and neuroendocrine responses, the immune response elicited seemed to be specific to the type of inflammatory rheumatic disorder involved, and this was especially evident for psychosocial stress. Patients with SLE often have baseline lymphopenia and this review showed that stress led to diminished cell mobilization and consequently less pronounced stress-induced changes in lymphocyte (sub)populations in these patients compared with controls and patients with RA. In contrast, patients with RA did not consistently show these alterations, but only alterations in certain subpopulations of lymphocytes. Furthermore, the cytokine response to stress might be distinct for different rheumatic populations. The effect of pharmacotherapy (for example, corticosteroids and biologics) on neuroendocrine and immune responses to stress in these populations is unclear. Only two of 16 studies conducted subgroup analyses to reveal possible effects of pharmacotherapy on response patterns. Motivala *et al.* (2008) found significant stress-induced increases in TNF α only in patients with RA not treated with TNF α antagonists (and no effect of corticosteroid treatment), and Pawlak *et al.* (1999) reported no significant difference in physiological variables between patients with SLE treated with corticosteroids and/or disease-modifying anti-rheumatic drugs (DMARDs) and non-treated patients [45]. Four other studies also suggest no effect of pharmacotherapy (more specifically, DMARDs [38], nonsteroidal anti-inflammatory drugs (NSAIDs) [34,38], and corticosteroids [34,43,44]) on stress reactivity patterns based on descriptive data. The other studies do not mention possible effects of pharmacotherapy [29-33,35-37,39,40,42,46].

Clearly, patient samples are often too small to draw conclusions. More studies comparing different inflammatory diseases and healthy participant are needed to address the question whether the physiological response to stress is disease-specific, knowledge which might facilitate our understanding of factors contributing to the maintenance and exacerbation of rheumatic disorders.

Outcome measures

The heterogeneity of outcome measures, especially with regard to immune variables, makes it difficult to compare studies of the stress response. Moreover, stressors that activate the physiological stress system via the hypothalamus and subsequent down-stream cascades

might induce alterations in hormones, peptides and cytokines other than discussed and assessed in this review (for examples, dehydroepiandrosterone (sulfate) (DHEA-S), neuro-peptide Y (NPY), substance P). Previous studies suggest that alterations in the stress response in patients with rheumatic disorders might be located in the interaction between the two stress axes (HPA and ANS) and the immune system, more specifically in the number or signaling capacity of β -adrenoceptors [45,48,66,81] or glucocorticoid receptors [82-84] on lymphocytes.

Although the HPA axis, ANS, and immune system are thought to function in an interdependent fashion, only four of 16 studies in this review investigated outcome variables that evaluate all three stress response systems. Cardiovascular reactivity during a mental stress task has been shown to be associated with the subsequent cortisol response [25], and cardiovascular responses have been associated with post stress levels of cytokines such as IL-6 and TNF α [85]. In addition, HPA axis variables are correlated with immune responses after stress [53]. Future studies should try to integrate the responses of different systems by simultaneously assessing (para)sympathetic responses, neuroendocrine variables, and immune factors, to increase our knowledge of the coordination and possible dysregulation of these systems [59].

Psychological variables

In addition to assessing the physiological response to experimental real-life stressors, it is important to consider other key elements of the stress response, such as the individual's appraisal of the threat of the event, perceived distress, coping behavior and personality characteristics [4]. As the appraisal of threat might partly determine the biological stress response of an individual, self-reported measures of distress appear to be a simple and adequate manipulation check in studies of psychosocial stress. Furthermore, acute stress responses are known to be correlated with individual differences in psychological factors, such as coping styles [86], trait anxiety [87,88], depressed mood [59,89,90], perfectionism [91], social inhibition [92] and anticipatory cognitive appraisal [93-96]. However, none of the studies, except one [74], assessed individual psychological characteristics. In addition, only two studies in this review assessed stress-induced self-reported distress [41,42] and only three studies [36,37,39,41] explicitly excluded affective disorders. Although half of all studies excluded patients with (severe or chronic) comorbidity, these were mainly physical in nature. We cannot rule out the possibility that patients with psychiatric disorders are included in these studies, obscuring the major research question whether inflammatory rheumatic diseases are related to a specific physiological stress responses profile. Future studies of stress responses in patients with rheumatic diseases should include individual psychological characteristics (for example, personality, coping styles) and affective responses to help identify whether patients differ with regard to psychophysiological responses to stress.

Time points

Some consideration should be given to when outcomes are measured. Almost all the studies included at least one immediate post stress measurement, but only half of the studies included an additional measurement (mostly 30 or 60 minutes after cessation of the stressor), with only two studies including more than two follow-up measurements. Cytokine responses may be delayed for minutes or hours relative to ANS and HPA responses, because cytokines are produced by activated lymphocytes and macrophages [97-99]. This makes it difficult to detect the peak immune response when collecting only one or two samples, for example for the increase in IL-6 and IL-1 receptor agonists following psychosocial stress [85].

Additionally, in accordance with the allostatic load concept [100] the ability and time taken by the activated stress system to return to baseline (recovery period) might be an important factor in the link between stress and disease [99,101-103]. Moreover, repeated mental stress has been linked to a lack of habituation of the cortisol response [89,104] and plasma interleukin-6 [99] in subgroups of individuals. Thus future studies should have more frequent evaluation time points and investigate individual differences in the return-to-baseline values and in habituation patterns after repeated stress.

Conclusions

In summary, this review shows that there is limited evidence that autonomic and neuroendocrine function is altered after physical or psychological stress in patients with inflammatory rheumatic diseases compared with healthy subjects. In contrast, there is evidence that immune function is altered by stress in a manner specific to different rheumatic diseases, and thus real-life stressors could contribute to the maintenance or exacerbation of rheumatic diseases.

Future studies of stress, and particularly psychosocial stress, should have a follow-up with multiple measurement points, assess different physiological stress systems, and take into account stress appraisal. As individual differences in stress appraisal and stress response patterns might be important prognostic factors for disease progression, therapies that focus on stress management may be important adjuncts to traditional pharmacotherapy in the treatment of inflammatory rheumatic diseases. Stress induction studies could prove to be helpful for evaluating the effectiveness of these interventions [105,106].

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Chapter 3

The psychophysiological stress response in psoriasis and rheumatoid arthritis



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Abstract

Background Psychosocial stress can be a risk factor for the maintenance and exacerbation of chronic inflammatory diseases, such as psoriasis and rheumatoid arthritis (RA).

Objectives To gain insight into the specificity of the psychophysiological stress response during chronic inflammation, we assessed autonomic and neuroendocrine responses to stress in different chronic inflammatory diseases.

Methods Thirty patients with psoriasis (nine women, mean age 58.5 years \pm 12.4), 34 patients with RA (16 women, mean age 60.8 years \pm 9.2) and 25 healthy controls (16 women, mean age 55.6 years \pm 8.7) underwent a standardized psychosocial stress task (Trier Social Stress Test). Salivary levels of α -amylase and cortisol and self-reported tension levels were measured before and after the stress test.

Results The cortisol response to stress was heightened in patients with psoriasis compared with patients with RA and healthy controls, whereas there were no differences in the autonomic and self-reported measures.

Conclusions The altered neuroendocrine stress response in patients with psoriasis suggests that stressful events might have different physiological consequences for specific patient groups with chronic inflammatory conditions, possibly adversely affecting disease status.

Introduction

Psychosocial stress can be a risk factor for the maintenance and exacerbation of chronic inflammation in, for example, patients with psoriasis and rheumatoid arthritis (RA) [1–8]. The stress response system, mainly comprising the autonomic nervous system (ANS) and the hypothalamus–pituitary–adrenal axis (HPA axis), is activated by stress and induces the secretion of (nor)adrenaline and cortisol. Both stress axes interact with the immune system [9,10] and are able to influence skin and joint inflammation in patients with psoriasis and RA, respectively [11–17]. For example, cortisol activates cutaneous and synovial mast cells, which are rich in the proinflammatory cytokines tumor necrosis factor- α and interleukin-6, and which might exacerbate disease severity [18,19].

Moreover, chronic inflammation may compromise the flexibility of the branches of the stress response system [20–23], possibly with clinical consequences. With regard to autonomic function, patients with psoriasis have fewer β -adrenoceptors on keratinocytes in skin lesions than in normal skin, risking insufficient down regulation of immune and inflammatory processes [23]. In addition, the decreased sympathetic (sudomotor) activity in patients with psoriasis reduces perspiration and increases skin dryness, which may evoke itch and aggravate disease activity [22]. In parallel, patients with RA show a functional loss of sympathetic nerve fibres in inflamed synovial tissue and a decrease in β -adrenergic receptors on synovial fluid lymphocytes [24,25]. With regard to neuroendocrine function, blunted cortisol suppression after dexamethasone administration in patients with psoriasis suggests that the negative feedback action of corticosteroids could be attenuated in psoriasis [26]. In RA, subtle alterations in cortisol secretion may occur during the diurnal rhythm or in certain disease stages [27].

Studies with real-life experimental stressors might shed light on the extent to which stress triggers the ANS and HPA axis in patients with different chronic inflammatory diseases, such as psoriasis and RA, and whether autonomic and neuroendocrine function is systemically altered in these patients. So far, results have been inconclusive. Although some experimental studies have reported changes in autonomic [28–31] and cortisol [30] responses to psychological stress in patients with psoriasis, these changes were not observed in all autonomic parameters [28,31–34] nor was cortisol reactivity always altered [28,29,32,34,35]. Likewise, some studies have shown patients with RA to have altered basal or stress-induced autonomic or neuroendocrine function [20,27,36–41], but not all [41].

In order to gain insight into possible disease-specific changes in HPA axis and ANS function during chronic inflammation, we investigated whether the response to experimental real-life stress was associated with alterations in autonomic and neuroendocrine reactivity in patients with the chronic inflammatory diseases psoriasis and RA. To this end, we assessed tension levels and measured α -amylase and cortisol levels in response to an acute psychosocial stress task in patients with psoriasis and RA and in healthy subjects.

Materials and methods

Participants

Patients with psoriasis and RA were recruited from the Departments of Dermatology and Rheumatology at the Radboud University Medical Center and the Sint Maartenskliniek in Nijmegen, The Netherlands. Healthy participants were recruited through a local newspaper announcement and on the website of the Radboud University Medical Center. Patients with RA were receiving regular care and were in the control group of a trial, the data of which have been described elsewhere [42]. Patient inclusion criteria were a diagnosis of psoriasis [43] or RA [44], respectively, and a minimum age of 18 years. Exclusion criteria were: severe physical comorbidity (e.g. major cardiac problems, psoriatic arthritis, malignancies, severe respiratory or renal insufficiency, hepatitis B, HIV and insulin-dependent diabetes mellitus); severe psychiatric disturbances that might interfere with the study protocol; pregnancy; illiteracy; use of antidepressants, anxiolytics or antipsychotics. The study protocol was approved by the regional medical ethics committee and conducted according to the Helsinki principles. Written informed consent was obtained from all participants.

Procedure

Stress test sessions were run between 13.00 and 15.30 h at the Radboud University Medical Center. All participants were asked to refrain from using caffeine, alcohol, nicotine or physical exercise on the test day, and from eating 2 h before the first saliva sample was taken. Psychophysiological parameters (tension and saliva) were measured at baseline (i.e. after 20 min of rest; $t = 0$ min), immediately after the stress test ($t = 20$ min) and 10, 20, 40 and 60 min after the test ($t = 30, 40, 60$ and 80 min). During periods of rest participants looked at a natural history documentary.

Stress test

The Trier Social Stress Test (TSST) is a 15-min standardized laboratory stress task that consists of a public-speaking task and mental arithmetic in front of a critical audience [45]. The TSST consistently induces self-reported, neuroendocrine and autonomic nervous system responses [46].

Measures

Demographic, psychological and clinical measures

Demographic variables were assessed with a general checklist for age, sex, marital status, education and medical history. Psychological functioning was measured with the state anxiety and negative and positive mood scales of the Impact of Rheumatic Diseases on General Health and Lifestyle (IRGL) questionnaire [47]. Disease severity in patients with psoriasis was assessed with the Psoriasis Area and Severity Index (PASI) [48], and disease activity in RA patients was measured with the Disease Activity Score 28 (DAS28) [49].

Self-reported tension

Participants rated how tense they were on a visual analogue scale (VAS; 0–10).

α -Amylase

Saliva samples were collected with salivettes (Sarstedt, Rommelsdorf, Germany) and stored at -35°C until analysed. Salivary α -amylase was measured with the Aeroset (Abbott Laboratories, Libertyville, IL, U.S.A.). The rate of CPNP (2-chloro-4-nitrophenol) formation was detected spectrophotometrically at 404 nm to give a direct measurement of amylase in saliva.

Cortisol

Salivary cortisol was measured with a commercial Luminescence Enzyme Immunoassay (IBL, Hamburg, Germany). At levels of 3.3 and 27.3 nmol L⁻¹, within-assay coefficients of variation were 8.7% and 3.6% respectively, and between-assay coefficients of variation were 12.3% and 7.7%. To reduce error variance caused by between-run variation, all samples from one participant were analysed in the same run.

Statistical analysis

All outcome parameters were logarithmically transformed to render unskewed data distributions. Between-group differences in age, sex and education were tested with independent Student's *t*-tests and χ^2 analyses. Baseline differences in psychophysiological parameters (VAS tension, α -amylase and cortisol at $t = 0$ min) and total cortisol output (area under the curve with respect to ground; AUC_G) [50] in the three groups were evaluated with analyses of (co)variance [AN(CO)VA]. Psychophysiological responses to the TSST were evaluated using a linear mixed model (LMM); the three psychophysiological parameters were used as dependent variables, and group (psoriasis, RA, healthy controls), baseline measurements of the dependent variable ($t = 0$ min) and time levels ($t = 20, 30, 40, 60$ and 80 min) were used as independent variables. The following factors that are known to impact psychophysiological responses [46,51,52] were separately introduced as covariates in the ANCOVA and LMM models: medical treatment [i.e. biologics, topical or systemic corticosteroids, disease-modifying antirheumatic drugs (DMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs)], medication known to affect the ANS [β -blockers, angiotensin-converting enzyme inhibitors, Ca²⁺-blockers, α_1 -blockers, thiazides (or related), acetylcholine-receptor antagonists or β_2 -adrenergics], sex, age, body mass index (BMI), smoking status, menopausal status and psychological well-being (state anxiety, positive and negative mood). When a significant correlation with the outcome variable was found, covariate analyses were reported. Furthermore, analyses were performed with and without the participants using contraceptives. Psychophysiological data of the 89 participants were 93% complete. Analyses were performed using SPSS 16.0 for Windows (IBM Corp., Armonk, NY, U.S.A.). The significance level was $\alpha = 0.05$ (two-tailed).

Results

Patient characteristics

The baseline demographic characteristics and disease status of 30 patients with psoriasis, 34 patients with RA and 25 healthy controls are presented in Table 1. Groups did not significantly differ in age ($F = 1.823$, $p = 0.168$), educational level ($\chi^2 = 1.859$, $p = 0.162$), smoking status ($\chi^2 = 4.700$, $p = 0.319$) or psychological functioning (anxiety $F = 0.854$, $p = 0.429$; positive mood $F = 0.732$, $p = 0.484$; trend for negative mood $F = 3.109$, $p = 0.050$, patients with psoriasis less than patients with RA and healthy controls), but among patients with psoriasis, there were more men ($\chi^2 = 6.366$, $p = 0.041$) and the BMI was significantly higher than in patients with RA and controls ($F = 5.522$, $p = 0.006$) (see Table 1). Three patients with psoriasis took biologics (etanercept, adalimumab and ustekinumab) vs. 16 patients with RA (etanercept, adalimumab, abatacept and infliximab), and six patients with psoriasis received DMARDs [methotrexate (MTX) or sulfasalazine] vs. 23 patients with RA (MTX, sulfasalazine, hydroxychloroquine, leflunomide and/or azathioprine). In addition, four patients with psoriasis and 16 patients with RA received NSAIDs, and five patients with RA took glucocorticoids [(methyl-)prednisolone]. Of the 15 patients with psoriasis using topical medication, 14 used corticosteroid creams or ointments. Seven patients with psoriasis, nine patients with RA and three healthy controls received medication known to affect the ANS. One patient with RA and one healthy woman used oral contraceptives, and one healthy woman had a hormonal intrauterine device. Three women with psoriasis, one woman with RA and four female healthy controls were premenopausal.

Psychophysiological response to stress

Baseline differences in psychophysiological parameters

There were no significant baseline differences between the three groups with regard to subjective tension ($F = 1.188$, $P = 0.310$) or α -amylase ($F = 1.336$, $p = 0.268$) and cortisol levels ($F = 0.007$, $p = 0.993$) (Table 2).

None of the covariates investigated were significantly related to the (baseline) outcome variables, except for sex, as men showed higher basal cortisol levels than women ($p = 0.001$), and age, which was negatively associated with basal subjective tension levels ($p = 0.011$) and positively associated with α -amylase levels ($p = 0.007$); incorporation of these confounders as a covariate into the models did not change the results (cortisol $F = 0.477$, $p = 0.622$; tension $F = 1.338$, $p = 0.268$; α -amylase $F = 2.148$, $p = 0.123$).

Psychophysiological stress reactivity

The stress test induced significant changes in subjective tension (time effect $F_{1,88} = 105.969$, $p < 0.001$), α -amylase (time effect $F_{1,146.981} = 24.068$, $p < 0.001$) and cortisol levels (time effect $F_{1,86.159} = 43.279$, $p < 0.001$; Fig. 1) across groups, with significant increases in the three parameters as measured from baseline ($P < 0.001$ for all outcomes; Table 2).

Table 1 Demographic characteristics, disease severity, and medical regimen for patients with psoriasis (PS), patients with rheumatoid arthritis (RA) and healthy controls

	Patients with PS (n = 30)	Patients with RA (n = 34)	Healthy controls (n = 25)
No. females/males	9/21	16/18	16/9
Age (years), mean \pm SD	58.5 \pm 12.4 (range 26-76)	60.8 \pm 9.2 (range 26-80)	55.6 \pm 8.7 (range 39-71)
Education level (%)			
Primary	0%	3%	0%
Secondary	70%	71%	52%
Tertiary	30%	27%	48%
BMI, mean \pm SD	28.0 \pm 5.5	25.7 \pm 3.6	23.9 \pm 4.6
Smoking status (%)			
5-20 per day	17%	24%	4%
1/week to 5/day	7%	3%	4%
No or quit	77%	74%	92%
PASI, mean \pm SD	7.8 \pm 5.3 (range 0.6-19)	-	-
DAS28, mean \pm SD	-	2.6 \pm 1.0 (range 0.8-4.5)	-
Disease duration (years), mean \pm SD	21.2 \pm 14.5 (range 4-53)	12.6 \pm 7.6 (range 3-37)	-
Systemic medication, n	12/30	32/34	-
Biologics	3	16	
DMARDs	6	23	
NSAIDs	4	16	
Corticosteroids	0	5	
Topical medication, n	15/30	-	-
Corticosteroids	14		
RA/VitD3 analogs	3		
Coal tar	1		
Medication affecting autonomic nervous system ^a , n	7/30	9/34	3/25

BMI, body mass index; DAS28, Disease Activity Score 28; DMARDs, disease-modifying antirheumatic drugs; NSAIDs, nonsteroidal anti-inflammatory drugs; PASI, Psoriasis Area and Severity Index; SD, standard deviation; VitD3, vitamin D3. ^ae.g. β -blockers, thiazides.

Table 2 Mean (\pm SEM) untransformed baseline and stress-induced levels of VAS tension, salivary α -amylase and cortisol in patients with psoriasis (PS; $n = 30$), patients with rheumatoid arthritis (RA; $n = 34$), and healthy controls (HC; $n = 25$).

	$t = 0$ min	$t = \text{TSST} / 20$ min	$t = 30$ min	$t = 40$ min	$t = 60$ min	$t = 80$ min
VAS tension (0-10)						
PS	1.0 (0.3)	5.8 (0.5)	1.4 (0.3)	0.6 (0.1)	0.6 (0.2)	0.6 (0.2)
RA	1.2 (0.2)	5.7 (0.4)	1.7 (0.3)	1.2 (0.3)	0.9 (0.3)	0.8 (0.2)
HC	1.3 (0.2)	5.9 (0.5)	1.6 (0.3)	1.3 (0.2)	1.0 (0.2)	1.1 (0.2)
α -Amylase (U mL ⁻¹)						
PS	241 (37)	373 (45)	259 (29)	241 (29)	237 (28)	258 (30)
RA	221 (37)	323 (46)	244 (43)	240 (39)	264 (43)	291 (48)
HC	271 (47)	375 (56)	249 (36)	305 (52)	294 (47)	295 (39)
Cortisol (nmol L ⁻¹) ^a						
PS	5.5 (0.5)	8.7 (0.9)	12.2 (1.7)	10.8 (1.4)	6.8 (0.7)	4.9 (0.5)
RA	6.7 (1.4)	8.5 (1.7)	8.2 (1.6)	7.3 (1.2)	5.6 (0.9)	4.9 (0.8)
HC	6.1 (1.0)	7.8 (1.3)	8.4 (1.6)	7.7 (1.6)	5.7 (1.0)	4.6 (0.6)

TSST, Trier Social Stress Test; VAS, visual analogue scale. ^aSignificant group \times time and group effects; analyses performed on ln-transformed data.

Stress-induced tension responses (group \times time $F_{8,86.000} = 1.854$, $p = 0.078$; group $F_{2,84.193} = 1.205$, $p = 0.305$) and α -amylase responses (group \times time $F_{8,83.550} = 1.328$, $p = 0.241$; group $F_{2,84.429} = 0.022$, $p = 0.978$) did not significantly differ between the three groups (Table 2). In contrast, groups significantly differed in their cortisol response over time (group \times time $F_{8,83.903} = 2.802$, $p = 0.008$; group $F_{2,83.276} = 3.241$, $p = 0.044$), with patients with psoriasis showing a larger increase in cortisol levels than healthy controls and patients with RA (Table 2 and Fig. 1). The total cortisol output with respect to ground (AUC_G) did not significantly differ between the groups ($F = 1.818$, $p = 0.169$), with patients with psoriasis, patients with RA and healthy controls having a mean AUC_G (\pm SD) of 72 (\pm 7), 62 (\pm 11) and 60 (\pm 9), respectively.

None of the covariates investigated were significantly related to the (stress-induced) outcome variables, except for the use of systemic corticosteroids, which was negatively associated with the cortisol response and the AUC_G ($P = 0.017$ and $p = 0.019$, respectively), and sex, for which the AUC_G was larger in men ($p < 0.001$); in addition, the use of biologics ($p = 0.023$) and psychological functioning (positive mood $p = 0.055$; negative mood

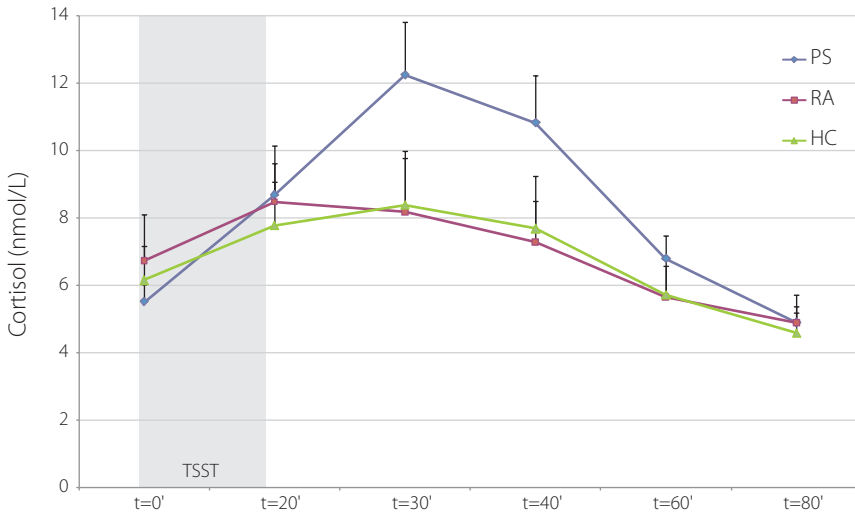


Figure 1 Endocrine response to stress. Mean stress-induced salivary cortisol levels (\pm SEM) in patients with psoriasis (PS), patients with rheumatoid arthritis (RA) and healthy controls (HC).

$p = 0.052$; anxiety $p = 0.035$) were or tended to be positively associated with amylase reactivity. Incorporation of these confounders into the models as covariates did not change the results (cortisol response: group \times time $F_{8,83.872} = 2.806$, $p = 0.008$; group $F_{2,84.186} = 2.372$, $p = 0.100$ and $AUC_G F = 0.439$, $p = 0.646$; amylase response: group \times time $F_{8,83.585} = 1.326$, $p = 0.242$; group $F_{2,83.029} = 0.565$, $p = 0.570$).

Discussion

Although it is known that stress can maintain or exacerbate chronic inflammatory conditions, for example psoriasis and RA, it is still debated whether the response of the ANS and HPA axis to stress is altered in these patients [53–55]. Different psychopathological (e.g. clinical depression and social anxiety) and somatic diseases (e.g. atopic dermatitis and asthma) have been associated with characteristic stress response profiles, which are often suggestive of blunted cortisol reactivity, although enhanced cortisol responses have also been observed in certain psychopathologies [46,56–58]. We provide preliminary evidence that the cortisol response to acute psychosocial stress in patients with psoriasis is increased compared with patients with RA and healthy controls, indicating that the neuroendocrine stress response system is altered in this population with chronic

inflammation. Salivary α -amylase responses, as an indicator of sympathetic reactivity, were not different in the three groups. Because general psychological functioning and stress-induced tension levels did not significantly differ between groups, the heightened cortisol response in patients with psoriasis might not have been caused by underlying psychopathology or perceived stressfulness, but could reflect a disease-specific difference in neuroendocrine stress reactivity.

The general hypothesis for chronic inflammatory conditions is that of hypofunctional HPA axis activity and reactivity that fuels inflammation through inadequate suppression of immune function [6,59]. Our finding of heightened cortisol reactivity in psoriasis does not support this idea. Of the limited number of studies available on endocrine responses to experimental stressors in patients with psoriasis [28–30,32,34,35], most reported no significant difference in cortisol reactivity between patients with psoriasis and controls [28,29,32,34,35]. Only one study reported a trend for a larger cortisol response [32]. In line with this, dexamethasone-induced cortisol suppression, although still within the normal range, was blunted in patients with psoriasis compared with healthy controls [26]. Possibly, the negative feedback action of corticosteroids is attenuated in psoriasis, although alterations in adrenocorticotrophic hormone and cortisol output were not observed in corticotropin-releasing hormone tests [26]. Nevertheless, pharmacological tests trigger specific parts of the HPA axis and are not necessarily comparable with the effects of real-life stressors, which activate the stress response system at various psychophysiological levels. For example, it is suggested that endocrine activity occurs not only inside the classical HPA axis, but also at the peripheral level in the skin; this ‘brain–skin HPA axis’ probably coordinates peripheral responses to stress and maintains cutaneous homeostasis [60], and may underlie inflammatory skin diseases, such as psoriasis, that are triggered or aggravated by stress [18]. However, it is as yet unclear whether this local HPA axis is activated after systemic stress induction, and whether and to what extent skin–HPA axis mediators are released into the circulation. Furthermore, a distinction should be made between the effects of chronic and acute stressors on neuroendocrine function [60]. In chronic stress and pain conditions, the HPA axis is thought to be continuously activated, which can increase feedback sensitivity of corticosteroids [59,61]. This might lead to hypofunction and hyporeactivity of the HPA axis and desensitization of the immune system to glucocorticoids, increasing inflammation [59,61]. For example, patients with psoriasis persistently experiencing high levels of daily stressors had significantly lower basal cortisol levels than patients continuously experiencing low levels of daily stressors [11]. During peak levels of stress, daily stressors were significantly associated with an increase in disease severity 1 month later, suggesting that stress might exert its negative effects on psoriasis severity specifically when stress levels are high [62]. Another study reported basal and stress-induced cortisol levels to be lower in patients with psoriasis who considered themselves sensitive to stress [34]. In all, stress-related and disease-specific mechanisms might be at work at the same time. On the one hand, chronic stress might

lead to reduced HPA axis reactivity, while on the other hand, possible disease-specific mechanisms that increase cortisol responses might be present. The result of these processes – and what this means for stress response patterns in case of acute stress – needs further investigation. We did not find patients with RA to have a specific neuroendocrine profile, which is in line with literature suggesting that patients with RA show no or only subtle alterations in HPA axis function after stress induction [41,54].

Our findings do not support there being an altered ANS response to stress in patients with psoriasis and RA compared with healthy controls. The few studies investigating autonomic function in patients with psoriasis do not equivocally demonstrate sympathetic dysfunction [22,63]. Most studies of experimentally induced psychological stress did not detect differences in autonomic reactivity between patients with psoriasis and controls [28,30–34], although increased [28,29,31] or decreased [30,31] responses have also been reported. Our results on autonomic stress reactivity in patients with RA are consistent with those of most controlled studies investigating short-term experimental stressors in these patients [37,38,54,64–66], although some studies reported diminished [36] or more pronounced [37,38] autonomic stress reactivity. However, those alterations were either observed in subgroups of patients with severe disease activity [38], in patients using biologics [37] or in response to only one type of stress [36]. Our finding supports the idea that the autonomic response to acute stress is not altered in patients with psoriasis and RA.

This study has some limitations. Firstly, because patients with relatively mild disease activity participated in the study, the study sample might not be representative of patient populations and the results cannot be generalized to patients with psoriasis or RA with more severe disease activity. Moreover, as we included two different patient groups on disease-specific medication regimens, we could not investigate the role of type of pharmacotherapy on the physiological response to the stress task. However, most pharmacotherapy regimens were not related to the psychophysiological outcome measures studied, and statistically adjusting for use of systemic corticosteroids and biologics did not change the results. Nevertheless, the results of this study should be interpreted with caution and replicated in larger groups of patients. Secondly, there was a significant difference in sex distribution across the three groups. It is well known that sex [51,67], menstrual cycle, menopause and oral contraceptives [52] influence the cortisol response to laboratory stress paradigms. Therefore, we explored the possible influence of several sex (hormone)-related confounders, but this did not alter our findings. Thirdly, the measurement of α -amylase and cortisol may be accompanied by methodological issues [68], because, for example, low blood glucose levels [69,70] and smoking [68,71–73] are associated with blunted cortisol responses and lower α -amylase activity. We limited the effects of these factors by asking patients not to drink alcohol, use caffeine, eat, smoke or exercise 2 h before the start of our experiment. Fourthly, although salivary α -amylase is an easy-to-use indirect measure of sympathetic stress reactivity and levels correspond with

those of cardiovascular measures, its secretion is clearly both sympathetic and parasympathetic in nature and does not give insight into possible peripheral ANS mechanisms [74,75]. Future studies should try to unravel the effects of stress on different branches of the ANS, including both central and peripheral processes.

In conclusion, patients with psoriasis showed an increased cortisol response to experimental psychosocial stress compared with patients with RA and healthy controls. Possibly they have a more pronounced neuroendocrine response to stressful events, which could increase their vulnerability to the adverse effects of stress on psoriasis activity. Results should be replicated in a larger, more homogeneous cohort of patients with chronic inflammatory conditions. This would allow subgroup analyses on medically treated and untreated patients, and investigate stress response patterns of stress-sensitive patients. It could eventually lead to the development of guidelines for clinical practice to make clinicians more alert to the effects of stress in patients with psoriasis and pay special attention to those patients who report being sensitive to stress in their daily lives.

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Chapter 4

Immune responses to stress in rheumatoid arthritis and psoriasis



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Abstract

Objective Stress is one of the factors that may exacerbate the progression of chronic inflammatory diseases such as RA and psoriasis. We exploratively compared the effects of acute stress on levels of circulating cytokines involved in disease progression and/or the stress response in patients with RA, patients with psoriasis and healthy subjects.

Methods Patients with RA, patients with psoriasis and healthy controls underwent a standardized psychosocial stress test (Trier Social Stress Test). Levels of circulating cytokines (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFN γ and TNF α) were measured before and after the stress test.

Results The baseline levels of all cytokines, except IL-8, were significantly higher in patients with RA. After correction for baseline levels, patients with RA showed higher stress-induced levels of IL-1 β and IL-2 than patients with psoriasis and healthy controls.

Conclusion The results suggest that patients with RA have a different immune response to stress than patients with psoriasis or healthy controls. More needs to be learned about the complex interaction between stress, immune parameters and chronic inflammation.

Introduction

The etiology of chronic inflammatory diseases such as RA and psoriasis is complex and disease progression may be adversely affected by several factors, including stress [1, 2]. The main physiological pathways by which stress exerts its effects on the immune system, namely, the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis, are also critically involved in disease progression [3]. In the case of chronic inflammation, these axes might be altered [4]. Accompanying local and systemic changes in immune function can lead to disease-specific immune responses to stress that differ among inflammatory conditions and might increase negative health effects [5]. Various prospective studies of stress [6-8] and research with experimental stressors [9-11] or psychological interventions [12] have shown a link between stress and measures of immune function or disease activity in RA and psoriasis. For example, we previously showed that stress management training not only diminished levels of subjective tension and cortisol reactivity [13], but also influenced the IL-8 response to acute stress in patients with RA [14], indicating that psychological interventions can alter immune function.

To identify patients with a chronic inflammatory disease who might be especially vulnerable to the effects of stress on inflammatory activity and disease progression, we explored the effects of an acute real-life laboratory stressor on levels of circulating cytokines involved in disease progression and/or the stress response (e.g., IL-6, IL-8 and TNF α) in patients with RA, patients with psoriasis and healthy subjects.

Patients and methods

Patient cohort

Patients with RA or psoriasis were recruited from the Departments of Rheumatology and Dermatology at the Radboud University Medical Center and Sint Maartenskliniek in Nijmegen, the Netherlands. Healthy participants were recruited by means of an announcement in a local newspaper and/or on a website.

The study was approved by the regional medical ethics committee (CMO Regio Arnhem-Nijmegen) and informed consent was obtained from all participants according to the Declaration of Helsinki. As reported previously in the study on autonomic and endocrine responses, the study involved 34 RA patients [16 women, mean age 60.8 years (S.D. 9.2)], 30 psoriasis patients [9 women, mean age 58.5 years (S.D. 12.4)] and 25 healthy controls [HCs, 16 women, mean age 55.6 years (S.D. 8.7)] [11]. The groups differed significantly in sex distribution ($p = 0.004$), with relatively fewer women than men with psoriasis. The mean 28-joint DAS (DAS28) and Psoriasis Area and Severity Index scores were 2.6 (S.D. 1.0) and 7.8 (S.D. 5.3), respectively. Patients were treated with biologics ($n = 16$ RA, $n = 3$ psoriasis), DMARDs ($n = 23$ RA, $n = 6$ psoriasis), NSAIDs ($n = 16$ RA, $n = 4$

psoriasis), glucocorticoids (systemic: $n = 5$ RA; topical: $n = 14$ psoriasis) and medication known to affect the autonomic nervous system (ANS), including β -blockers, angiotensin-converting enzyme (ACE) inhibitors, Ca^{2+} -blockers, α_1 -blockers, thiazides or related drugs), acetylcholine receptor antagonists or β_2 -adrenergics ($n = 9$ RA, $n = 7$ psoriasis, $n = 3$ HCs). Three women used hormonal (oral or intrauterine) contraception ($n = 1$ RA, $n = 2$ HC) and eight women were premenopausal ($n = 1$ RA, $n = 3$ psoriasis, $n = 4$ HCs).

Stress test

Participants underwent the Trier Social Stress Test (TSST), a standardized laboratory stress task that consists of a mock job interview and mental arithmetic in front of a critical audience [11, 13, 15]. Sessions were run between 1:00 and 3:30 P.M. Blood (for cytokines) was collected at baseline (i.e. after 20 min of rest; $t = 0$ min), immediately after the stress test ($t = 20$ min) and at 20 and 60 min thereafter ($t = 40$ and $t = 80$ min). Saliva (for cortisol and α -amylase) and self-reported visual analogue scale (VAS) tension scores (0-10) were collected at $t = 0$, $t = 20$ [tension levels during the TSST ($t = \text{TSST}$) were reported here], $t = 30$, $t = 40$, $t = 60$ and $t = 80$ min.

Cytokine assay

Human IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFN γ and TNF α were measured in serum using human cytokine multiple kits (Invitrogen, Carlsbad, CA, USA). Samples were analysed with a Luminex 100 system (Luminex, Austin, TX, USA).

Statistics

Baseline cytokine levels were evaluated with analyses of variance or covariance (ANOVA or ANCOVA). Cytokine responses to the TSST were explored using a linear mixed model, taking into account the specific design of the study. Lognormal-transformed cytokine levels (except skewed IL-5 and IFN γ data, which were excluded from further analyses) were used as dependent variables; group (RA, psoriasis, HC), time ($t = 0$, $t = 20$, $t = 40$, $t = 80$ min) and group \times time were used as independent variables. Treatment (i.e. biologics, topical or systemic corticosteroids, DMARDs and NSAIDs), use of medication known to affect the ANS, sex, age, hormonal contraceptives and menopausal status were tested one by one as potential covariates using dummy variables. When a significant correlation with the outcome variable was found, covariate analyses were also reported, which happened to include at most two covariates per model. In additional analyses, baseline levels of the dependent variable ($t = 0$ min) were used as the covariate. Based on significant between-group differences in cytokine responses, Pearson correlation coefficients of cytokine parameters [area under the curve with respect to ground (AUC_G)] with physiological (AUC_G of α -amylase and cortisol) and subjective [peak stress-induced tension levels ($\text{tension}_{t = \text{TSST}}$)] parameters were explored. Because physiological indices showed interindividual variability in the response to the stressor, the AUC_G was used to

obtain uniform stress-induced output parameters [16]. Blood samples from six psoriasis patients and two RA patients were missing. Data from one psoriasis patient and IL-8 levels from one control participant were excluded because cytokine levels were more than 4 S.D. higher than the mean. Consequently, data from 32 RA patients, 23 psoriasis patients and 25 healthy controls were analysed. Undetectable cytokine levels were set to zero and included in all analyses. The Bonferroni correction for multiple testing was not applied due to the high intercorrelation of most cytokines and the explorative nature of this study of small sample size [17]. Analyses were performed using SPSS 16.0 for Windows (IBM, Armonk, NY, USA). The significance level was $\alpha = 0.05$ (two-tailed).

Results

Baseline cytokine levels

Baseline levels of all cytokines except IL-8 were significantly different among the three groups ($p < 0.005$ for IL-1 β , IL-2, IL-4, IL-6, IL-7, IL-10 and TNF α), with levels being significantly higher in RA patients than in psoriasis patients and HCs (except for IL-10; RA did not significantly differ from HCs; $p = 0.14$) (Table 1). Results remained (non-)significant after correction for covariates that were significantly associated with the baseline level of specific cytokines (i.e. NSAIDs, biologics, corticosteroids and medication known to affect the ANS).

Stress manipulation check

As reported in a previous paper, the stress test induced significant increases in tension, α -amylase and cortisol levels ($p < 0.001$ for all). Patients with RA, patients with psoriasis and HCs showed mean peak tension levels during stress of 5.7 (S.E.M. 0.4), 5.8 (S.E.M. 0.5) and 5.9 (S.E.M. 0.5), respectively. Stress-induced tension and α -amylase responses did not differ significantly between the three groups, but patients with psoriasis showed a larger increase in cortisol levels than HCs and patients with RA [11].

Cytokine responses to stress

The cytokine response to stress did not differ among the three groups (group \times time interaction; $p > 0.14$ for all cytokines) (Table 1), also after correction for covariates significantly associated with specific cytokine responses (i.e. age, biologics, DMARDs, NSAIDs, corticosteroids and medication affecting the ANS). However, after correction for baseline differences in cytokine level, stress-induced IL-1 β and IL-2 levels were significantly different in the three groups [group effect for IL-1 β , $F(2, 76) = 3.323$, $p = 0.04$; group effect for IL-2, $F(2, 76) = 3.553$, $p = 0.03$], with IL-1 β (Fig. 1A) and IL-2 (Fig. 1B) levels being significantly higher in RA patients than in psoriasis patients 5 ($p = 0.03$ and $p = 0.01$, respectively) and HCs [$p = 0.03$ and $p = 0.10$ (trend), respectively]. In addition, the group difference in the IL-7

Table 1 Mean untransformed baseline and stress-induced cytokine levels in patients with RA, patients with psoriasis and healthy controls

	<i>t</i> = 0 min	<i>t</i> = 20 min	<i>t</i> = 40 min	<i>t</i> = 80 min
IL-1 β				
RA	114.3 (38.6)	130.9 (43.5)	116.6 (37.0)	136.7 (59.0)
PS	8.688 (5.06)	13.66 (8.30)	10.48 (7.52)	11.60 (8.50)
HC	10.20 (6.18)	10.58 (6.38)	12.10 (7.58)	10.60 (6.92)
IL-2				
RA	32.60 (14.0)	42.86 (19.2)	38.77 (17.3)	47.51 (27.0)
PS	2.726 (2.20)	4.944 (4.33)	4.243 (3.61)	4.291 (3.76)
HC	4.439 (2.47)	4.910 (2.89)	5.139 (3.01)	4.854 (2.76)
IL-4				
RA	60.18 (29.3)	72.79 (30.1)	65.76 (28.4)	65.39 (29.9)
PS	8.354 (2.91)	10.72 (4.14)	9.602 (3.60)	10.13 (3.87)
HC	4.195 (1.97)	4.575 (1.99)	4.339 (1.87)	4.035 (1.76)
IL-5				
RA	5.129 (2.17)	7.887 (3.72)	7.006 (2.94)	8.948 (4.51)
PS	0.112 (0.07)	0.018 (0.01)	0.074 (0.04)	0.142 (0.08)
HC	0.188 (0.07)	0.249 (0.08)	0.253 (0.08)	0.103 (0.04)
IL-6				
RA	30.18 (9.36)	41.18 (15.2)	33.54 (10.7)	39.57 (16.2)
PS	4.652 (1.20)	4.344 (1.14)	5.043 (1.21)	5.301 (1.30)
HC	6.340 (2.29)	7.221 (2.78)	6.768 (3.18)	7.076 (2.61)
IL-7				
RA	52.69 (16.5)	63.65 (20.5)	55.94 (18.5)	60.73 (21.8)
PS	4.061 (2.55)	3.107 (2.08)	3.440 (2.35)	2.605 (2.24)
HC	2.641 (1.61)	3.561 (1.93)	1.900 (1.21)	1.783 (1.17)
IL-8				
RA	29.55 (6.50)	34.04 (7.81)	29.45 (6.75)	28.18 (7.14)
PS	18.95 (4.92)	11.95 (3.20)	11.55 (3.25)	9.236 (2.75)
HC	22.04 (5.51)	20.03 (5.03)	13.74 (4.53)	10.17 (3.87)
IL-10				
RA	49.33 (25.9)	57.07 (31.8)	73.55 (48.1)	71.02 (45.9)
PS	0.855 (0.22)	0.898 (0.23)	0.788 (0.20)	0.852 (0.22)
HC	8.407 (4.37)	8.834 (4.63)	8.114 (4.12)	8.500 (4.45)

Table 1 Continued

	$t = 0$ min	$t = 20$ min	$t = 40$ min	$t = 80$ min
IFN γ				
RA	1.545 (0.45)	8.328 (4.98)	6.623 (4.91)	7.591 (5.70)
PS	0.177 (0.15)	0.000 (0.00)	0.000 (0.00)	0.000 (0.00)
HC	0.082 (0.05)	0.146 (0.10)	0.255 (0.22)	0.184 (0.16)
TNF α				
RA	34.56 (12.4)	39.35 (12.7)	36.00 (11.1)	35.45 (11.2)
PS	5.642 (2.94)	6.742 (3.50)	6.688 (3.38)	6.599 (3.56)
HC	0.435 (0.23)	0.680 (0.25)	0.368 (0.18)	0.329 (0.17)

Cytokine levels expressed in pg/ml (S.E.M.). RA, $n = 32$; PS, $n = 23$; HCs, $n = 25$. PS, patients with psoriasis; HC, healthy controls.

response approached statistical significance [group effect, $F(2, 76) = 2.883$, $p = 0.06$], with levels being higher in RA patients than in psoriasis patients ($p = 0.02$) and HCs (trend; $p = 0.08$). The other cytokine responses to stress did not differ among the groups ($p > 0.23$ for IL-4, IL-6, IL-8, IL-10 and TNF α). Based on significant group differences in the IL-1 β and IL-2 response to stress, correlations with other physiological and subjective parameters were explored. IL-1 β and IL-2 output (AUC_G) were not significantly related to peak stress-induced tension levels, nor with α -amylase or cortisol output, in the total group and in the RA group specifically ($p > 0.24$ for all tests).

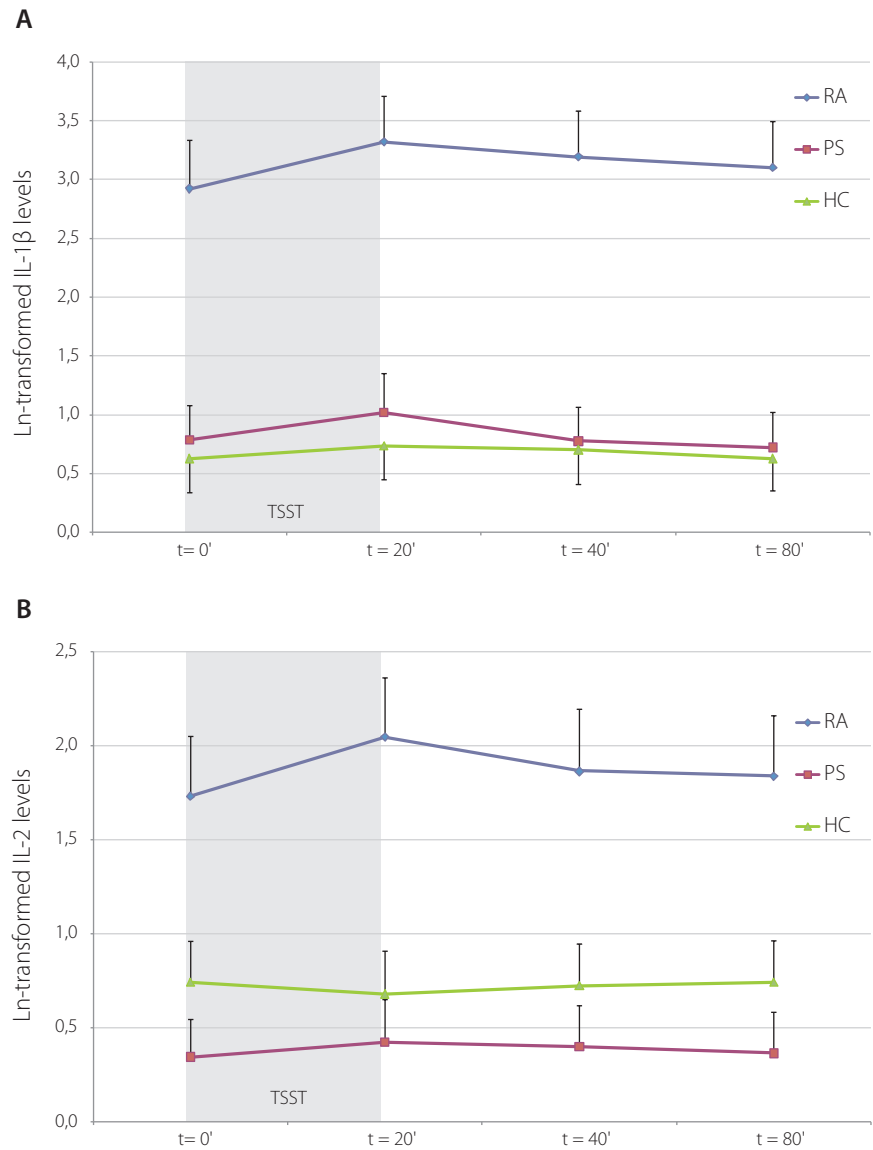


Figure 1 Mean lognormal-transformed (A) IL-1 β and (B) IL-2 levels [in pg/ml (\pm S.E.M.)] at $t = 0$ (baseline/pre-TSST), $t = 20$, $t = 40$ and $t = 80$ min (post-TSST) of patients with RA ($n = 32$), patients with psoriasis (PS; $n = 23$) and healthy controls (HC; $n = 25$).

Discussion

Basal levels of cytokines were significantly higher in RA patients than in patients with psoriasis or HCs, and after correction for baseline levels, patients with RA showed higher stress-induced levels of IL-1 β and IL-2 than in patients with psoriasis or HCs. Stress has previously been found to have a robust effect on circulating levels of IL-1 β and IL-6 in healthy and clinical populations, whereas less consistent results have been found when measuring cytokine production after cell stimulation *in vitro* [18, 19]. While acute experimental stress has been found to have an inconsistent (for IL-6) or no (for IL-2, IL-4, IL-8 and IFN γ) effect on cytokine levels in RA patients, it consistently increased TNF α to a greater extent in RA patients than in controls [4]. The only study to investigate the effects of stress on cytokine levels in patients with psoriasis found that psychosocial stress increased IFN γ and decreased IL-10 and IL-4 levels more in patients than in controls [10]. Thus a possible disease-specific immune response to stress is indicated.

Our results suggest that the immune response to acute stress, and specifically circulating IL-1 β and IL-2 levels, might be altered in certain chronic inflammatory conditions. It has been postulated that acute stress predominantly up-regulates innate immunity, increasing the number of natural killer cells and neutrophils and the production of cytokines associated with acute inflammation [18], such as IL-1 β . IL-2, a component of the adaptive immune system that mainly activates cellular responses (e.g. T cell activation), was specifically elevated in response to stress in RA patients. Since stress-induced acute changes in circulating cytokines probably reflect redistribution processes, the response may be greater when baseline cytokine levels are already elevated. The effect of stress on immune parameters was relatively small, although the study participants reported finding the stress test stressful, as was also evidenced by a significant increase in salivary α -amylase and cortisol levels [11]. Probably, cytokines do not regulate immune function systemically, but instead at the effector site. Functional studies that measure cytokine production by local immune cells can test this. Alternatively, patients with different disease activity or on different medications, or participants who are specifically sensitive to stress [20], might respond differently. This study was limited by the relatively small number of patients with RA or psoriasis, the rather heterogeneous groups and patients with mild disease activity, which prevented analysis of subgroups of patients on different medications or with different disease activity, or a more detailed analysis of possible confounders. Our finding that the cytokines IL-1 β and IL-2 seem to increase more in patients with RA than in patients with psoriasis and HCs could indicate that stress might specifically have altered effects on immune function in those patients and possibly increases negative effects on disease severity. Nevertheless, the clinical significance of stress-induced changes in circulating cytokine levels and their possible association with disease exacerbation remain to be investigated.

In conclusion, basal levels of cytokines were significantly higher in patients with RA than in patients with psoriasis or controls, and stress-induced levels of IL-1 β and IL-2 were higher in patients with RA than in patients with psoriasis or controls after correction for baseline differences in cytokine levels. The results suggest that there is a complex interaction between stress, immune parameters and chronic inflammation that should be investigated further.

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Part II

Stress Management Training in Rheumatoid Arthritis



Chapter 5

Psychophysiological responses to stress after stress management training in patients with rheumatoid arthritis



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Abstract

Background Stress management interventions may prove useful in preventing the detrimental effects of stress on health. This study assessed the effects of a stress management intervention on the psychophysiological response to stress in patients with rheumatoid arthritis (RA).

Methods Seventy-four patients with RA, who were randomly assigned to either a control group or a group that received short-term stress management training, performed a standardized psychosocial stress task (Trier Social Stress Test; TSST) 1 week after the stress management training and at a 9-week follow-up. Psychological and physical functioning and the acute psychophysiological response to the stress test were assessed.

Results Patients in the intervention group showed significantly lower psychological distress levels of anxiety after the training than did the controls. While there were no between-group differences in stress-induced tension levels, and autonomic (α -amylase) or endocrine (cortisol) responses to the stress test 1 week after the intervention, levels of stress induced tension and cortisol were significantly lower in the intervention group at the 9-week follow-up. Overall, the response to the intervention was particularly evident in a subgroup of patients with a psychological risk profile.

Conclusion A relatively short stress management intervention can improve psychological functioning and influences the psychophysiological response to stress in patients with RA, particularly those psychologically at risk. These findings might help understand how stress can affect health and the role of individual differences in stress responsiveness.

Trail registration The Netherlands National Trial Register (NTR1193)

Introduction

The etiology of rheumatoid arthritis (RA), a chronic inflammatory systemic disease that affects 1% of the general population [1,2], remains poorly understood. Despite the growing spectrum of pharmacological therapies aimed at reducing disease activity [3], many patients continue to suffer from pain, fatigue, functional disability, and an overall poor quality of life [4]. One of the factors believed to play a role in the initiation, maintenance, and exacerbation of RA is psychological stress [5,6]. Evidence is accumulating that stress-evoked physiological changes, brought about by activation of the two main branches of the stress response system, the autonomic nervous system (ANS) and the hypothalamus-pituitary-adrenal (HPA) axis, might have detrimental effects on disease activity and health [7–10]. This has led to growing interest into the effects of stress management interventions on physiological outcomes. Stress-reducing psychological interventions aimed at modifying stress appraisal and decreasing subjective anxiety might alter autonomic arousal (e.g., decrease heart rate and galvanic responses, and increase tonic vasodilation) and influence neuroendocrine activity (e.g., lower cortisol levels) [11–14]. Alleviating the physiological response to a stressor could be particularly relevant in clinical populations, specifically in patients with immune-mediated diseases, such as RA. Although evidence is limited, there are indications that stress management interventions might affect basal autonomic or endocrine parameters, such as norepinephrine levels, urinary free cortisol output, serum dehydroepiandrosterone sulphate, or testosterone levels in patients with HIV and cancer [15–21].

Psychological interventions, such as multimodal cognitive-behavioral therapy (CBT), biofeedback, stress management training, or emotional disclosure, have generally led to modest improvements in psychological and physical functioning in patients with RA, with similar effects for the different types of interventions [4,22–25]. Only incidental effects have been found on biological measures of disease, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [26–28]. Medical and methodological explanations have been searched for this lack of uniform effects of psychological interventions on biological measures, such as disease status, medication regimen, and used time frame to assess physiological stress measures. However, there is also relatively consistent support that inter-individual variation in psychological risk factors also play a role [29,30]. Specifically, previous research increasingly indicates the importance of evaluating psychological risk factors when investigating treatment outcome, such as the experience of interpersonal stress and levels of depression [29,31]. For instance, there is increasing evidence that patients at risk, for example those who report being sensitive to stress or who have heightened levels of distress (e.g., heightened anxiety and depression), are especially prone to the detrimental effects of stress on disease activity and accompanying physical symptoms [32,33]. Moreover, stress-induced changes in physiological function are particularly observed in these groups of patients psychologically at risk [29,31,34].

Although there is preliminary evidence that stress management interventions can influence the acute psychophysiological response to stress in healthy individuals [35,36], it is not known whether such interventions alter the acute-phase psychophysiological response to a stressful event in immune-compromised patients with chronic inflammatory diseases, such as RA.

In this study, we examined the effects of a short-term individual stress management intervention on the self-reported, sympathetic, and neuroendocrine response to a validated psychosocial stress test (Trier Social Stress Test, TSST) in patients with RA and in a subsample of patients at risk of heightened anxiety and depression. We hypothesized that patients in the intervention group, particularly those at risk, would show reduced levels of distress and a diminished psychophysiological response to acute psychosocial stress compared with controls both after the intervention and at the 9-week follow-up after prolonged use of the stress management techniques.

Materials and Methods

Ethics statement

The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1 and Protocol S1. The study protocol was approved by the regional medical ethics committee (CMO Regio Arnhem-Nijmegen) and registered in The Netherlands National Trial Register (NTR 1193). Written informed consent was obtained from all participants.

Participants

Patients with RA were recruited from the Department of Rheumatology at the Radboud University Nijmegen Medical Centre and the St Maartenskliniek in Nijmegen, the Netherlands. Inclusion criteria were a diagnosis of RA according to the American Rheumatism Association 1987 classification criteria [37] and a minimum age of 18. Exclusion criteria were severe physical comorbidity (e.g., major cardiac problems, psoriasis, malignancies, severe respiratory or renal insufficiency, hepatitis B, HIV, and insulin-dependent diabetes mellitus); severe psychiatric disturbances that might interfere with the study protocol; pregnancy; illiteracy; use of antidepressants, anxiolytics, or antipsychotics; and psychological treatment.

Procedure

Ninety-six eligible patients were enrolled (see Figure 1) and randomized through simple randomization with an equal allocation ratio to one of two parallel groups, the control or the treatment condition, in accordance with the fixed therapist's time schedule and using a computerized random generator scheme made by an independent researcher.

Allocation was concealed for the participant enroller until the moment that participants were scheduled into the treatment program. After randomization, 19 participants ($n = 8$ intervention, $n = 11$ control) withdrew from the study prematurely (prior to the first stress test), because of physical comorbidity ($n = 3$ intervention, $n = 6$ control), severe illness or death of a significant other ($n = 3$ intervention, $n = 1$ control), a change in pharmacotherapy ($n = 1$ control), or lack of motivation ($n = 2$ intervention, $n = 3$ control). In addition, 3 participants ($n = 1$ intervention, $n = 2$ control) reported taking antidepressants or anxiolytics after randomization and were excluded based on our predefined exclusion criteria. Seven of 74 participants withdrew from the second stress test ($n = 4$ intervention, $n = 3$ control) because of physical comorbidity ($n = 2$ intervention), death of a significant other ($n = 1$ intervention), and lack of motivation ($n = 1$ intervention, $n = 3$ control). There were no differences in sociodemographic variables (sex, age, education level) and

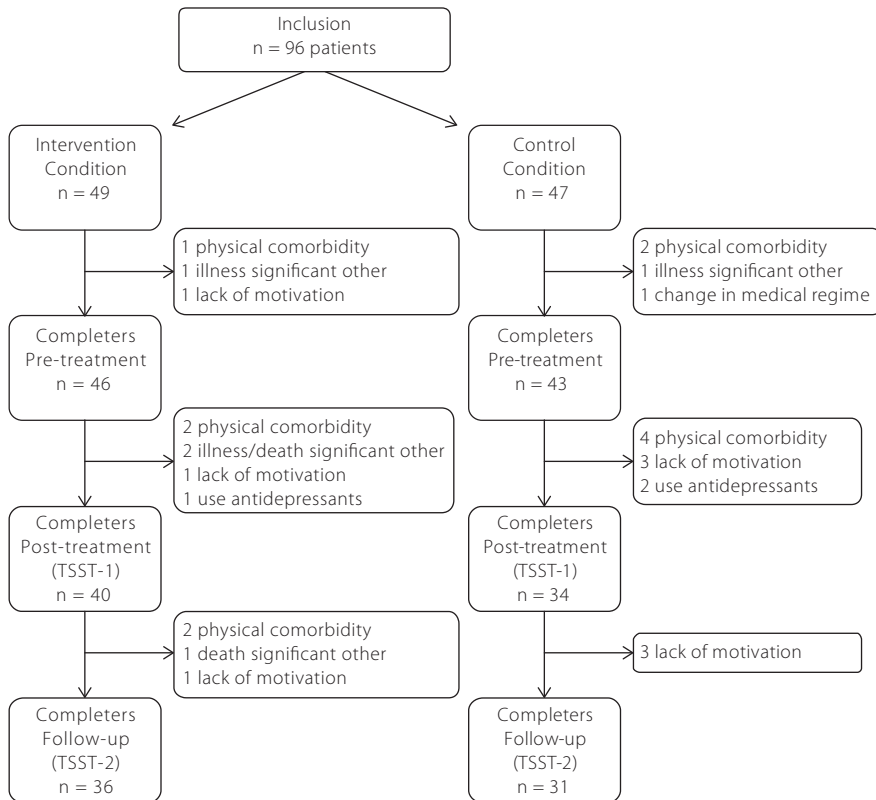


Figure 1 Flow chart showing participant selection and drop-out.

psychological and physical functioning at baseline (anxiety, negative mood, positive mood, Disease Activity Score 28 (DAS28)) between the drop-outs and the completers.

Participants were *post hoc* divided into 2 subgroups based on the participant's risk status by means of a median split on a composite score of baseline anxiety and negative mood assessed with the IRGL (see Measures) [30,32].

Study design

At the first assessment, the medical history and current disease activity of all participants were evaluated at the University Medical Centre, and in the subsequent two weeks half of the participants started the individual stress management training program. All participants performed a stress test three weeks after the first assessment (i.e., second assessment) and 9 weeks thereafter (i.e., third assessment). Stress test sessions were run between 13.00 and 15.30 hours. Participants were asked to refrain from using caffeine, alcohol, nicotine, or physical exercise on the test day, and from eating 2 hours before the first blood sample was drawn. Forty minutes before the stress test, a venous catheter was inserted into the non-dominant arm (immunological data presented elsewhere) and participants were asked to rest for 20 minutes. They then performed the stress test. During periods of rest, participants looked at a natural history documentary. Psychophysiological parameters (tension, saliva, and blood) were measured at baseline (i.e., after 20 minutes of rest), immediately after the stress test, and 10, 20, 40, and 60 minutes after cessation of the test.

Stress task

The Trier Social Stress Test (TSST) is a standardized laboratory stress task that consists of a mock job interview and mental arithmetic in front of an audience. The persons conducting the TSST were unaware of group allocation of the participants. The TSST lasts 15 minutes, including introduction to the job interview and a 5-minute preparation phase, and has repeatedly been found to induce self-reported, neuroendocrine, and autonomic nervous system responses [38].

Stress management training

Participants in the intervention group received individual stress management training with a focus on psycho-education and the principles of applied relaxation, including progressive, cue controlled, and differential relaxation [30,39–42]. In addition, patients were taught breathing and visualization exercises. Participants attended 4 individual 1-hour sessions with a trained therapist over 2 consecutive weeks. Patients received an MP3-player with relaxation exercises and, at the end of each session, a training manual containing a summary of the information and stress-reducing techniques introduced in that session. As consolidating homework, participants assessed stress-relevant situations and behaviors in their daily life and used relaxation exercises for 1 hour at least twice a day during the 2 weeks of the stress management intervention. Subsequently, patients were

encouraged to continue the homework assignments, to use the relaxation exercises, to focus on long-term goals, and to stick to a relapse-prevention checklist during the 2-month follow-up period.

Measures

Demographic, clinical, and self-report measures at baseline, post-treatment, and follow-up

Demographic variables were assessed with a general checklist for age, sex, marital status, education, and medical history. Educational level was measured using seven categories that can be classified as primary, secondary, and tertiary education, representing on average 7, 12, and 17 years of education, respectively.

Physical functioning was assessed in terms of disease activity. Disease activity of patients was measured with the DAS28, which is a validated composite score for swelling and tenderness of 28 joints, a Visual Analogue Scale (VAS) of the patients' general health, and the ESR (mm/h) [43].

Psychological functioning was measured with the state anxiety and negative and positive mood scales of the IRGL [44,45]. The IRGL is derived from the Arthritis Impact Measurement Scales (AIMS) [46]. The 10-item anxiety scale is a shortened version of the Dutch State Anxiety Scale [47,48] and assesses anxiety over the last 2 weeks (sample item: "I worry too much about unimportant matters."); the 6-item negative mood scale assesses various negative mood states over the previous 2 weeks (sample item: "How depressed were you during the past 2 weeks?"); and the 6-item positive mood scale assesses various positive mood states over the previous 2 weeks (sample item: "How cheerful were you during the past 2 weeks?").

Patients' evaluation of stress management training

After training ended, patients were asked to indicate their satisfaction with the training and its usefulness (score range 0–10, ranging from "not at all" to "very"), and to what extent their distress and tension had improved (score range 1–4, ranging from "not" to "very").

Psychophysiological measures during the stress test at post-treatment and follow-up

VAS tension Participants rated how tense they were on a VAS at baseline (after 20 minutes of rest), during the stress test (retrospectively), and 10, 20, 40, and 60 minutes after cessation of the stress test.

α -Amylase as a measure of autonomic reactivity. Saliva samples were collected with salivettes (Sarstedt, Rommelsdorf, Germany) and stored at -35°C until further biochemical analyses. After saliva samples were thawed, centrifuged, and diluted, α -amylase (AA) was measured with the Aeroset (Abbott). According to the procedure, α -amylase hydrolyses

the reagent CNPG3 (2-chloro-4-nitrophenyl- α -D-maltotrioside) to CPNP (2-chloro-4-nitrophenol), CNPG2 (2-chloro-4-nitrophenyl- α -D-maltoside), maltotriose, and glucose. The rate of CPNP formation was detected spectrophotometrically at 404 nm to give a direct measurement of amylase in saliva.

Cortisol as a measure of endocrine reactivity. Salivary cortisol was measured with a commercial Luminescence Enzyme Immunoassay (IBL, Hamburg, Germany). After samples were thawed and centrifuged, 20 μ l aliquots of the supernatant were pipette into anti-cortisol (rabbit-) antibody-coated microtitre plate wells, followed by 100 μ l of enzyme conjugate (horseradish peroxidase). After 3-hour incubation at room temperature, the plate was washed and luminescence reagent (luminol/peroxide) was added to each well, with subsequent reading of the signal in a luminometer. At levels of 3.3 and 27.3 nmol/l, within-assay coefficients of variation (CV) were 8.7 and 3.6% respectively, and between-assay CVs were 12.3 and 7.7%. To reduce error variance caused by between-run variation, all samples from one participant were analyzed in the same run.

Statistics

Analyses were performed on the 74 participants completing the study protocol. Skewed data (i.e., negative mood and all physiological parameters) were logarithmically transformed to render unskewed data distributions before statistical analysis. Between-group differences in age, sex, education, and psychological measures at baseline were tested with independent Student's *t*-tests and Chi-square analyses. For cortisol, the area under the curve (AUC_G) was calculated using the trapezoid formula [49]. Baseline differences in psychophysiological outcome parameters (VAS tension, cortisol, and α -amylase) ($t = 0$ minutes) and AUC_G in the intervention and control groups were evaluated with analyses of covariance (ANCOVA). Effects of the stress management training (i.e., psychological/physical functioning and psychophysiological responses to the stress test) were evaluated using a linear mixed model taking into account the specific design features of the study. The primary outcome measure was state anxiety as a measure of psychological distress. The effects on secondary outcomes of psychological and physical functioning (positive and negative mood, and DAS28) and psychophysiological stress parameters (tension, cortisol, and α -amylase measured during the stress test) at the post-treatment and follow-up assessments were also assessed. In analyses of the effects of the stress management training on psychological and physical functioning, measures of psychological and physical functioning were used as dependent variables, and group, baseline measurement of the dependent variable (pretreatment), and time levels (post-treatment and follow-up) were used as independent variables. With regard to the psychophysiological response to the stress test, the three psychophysiological outcome measures (tension, cortisol, and α -amylase) were used as dependent variables, and group, baseline measurement of the dependent variable ($t = 0$ minutes), and time levels ($t = 20$; $t = 30$; $t = 40$; $t = 60$; and $t = 80$ minutes) were used as independent variables. Explorative subgroup analyses were performed to test whether

effects were stronger or only held in patients at risk as compared to patients not at risk (also see Procedure) by incorporating risk group and risk group by treatment interactions into the analysis models. A significant interaction was interpreted as an indication of subgroup differences with respect to the effect of the treatment. Stratified analyses were performed to gain a better understanding of the nature of the responses in the subgroups of patients.

For every outcome measure, an unstructured covariance matrix was used to model the dependence between repeated measurements of the dependent variable. Owing to a slightly unequal distribution of sex across the two groups ($p = 0.08$) and a trend towards higher anxiety scores at baseline in the intervention group ($p = 0.09$) (see Results section, Patient characteristics), all analyses were performed with the covariates sex and baseline (pretreatment) anxiety. In addition, cortisol analyses were also performed with the additional covariate hormonal contraceptives [36] (see Results section, Patient characteristics).

A priori power calculation resulted in an optimal sample size of $N = 64$ (expected adjusted effect size of $f = 0.45$ of the primary outcome measure psychological distress (state anxiety), a power of 0.90, and $\alpha = 0.05$). However, because there were missing blood samples (a venous catheter could not be inserted in $n = 15$ patients during one or two stress tests) and the high drop-out rate before the start of the first stress test was high ($n = 22$; see procedure), we increased the earlier estimate of 64 patients to 96. In total, data of the 74 patients included in the analyses were 95% complete regarding psychological and physical outcomes at baseline, post-treatment, and follow-up, and 97% complete regarding psychophysiological parameters at post-treatment and follow-up. Physiological data for three participants at one of the assessment moments (cortisol levels in two participants and amylase levels in one participant) were excluded from analyses because levels were four standard deviations higher than the mean for at least one of the six time points during the stress test. All analyses were performed using SPSS 16.0 for Windows. For all analyses, the significance level was $\alpha = 0.05$ (two-sided). Unless indicated, all results are means \pm standard deviation (SD).

Results

Patient characteristics

Baseline demographic and disease-related characteristics of the 74 participants are presented in Table 1. The two groups did not differ significantly regarding age, education level, mean disease activity, and mean disease duration. However, there tended to be more women in the intervention group ($\chi^2 = 3.155$, $p = 0.08$). Thirty-three of 74 patients were taking biologics (including etanercept, adalimumab, abatacept, and infliximab), 54 patients were taking DMARDs (including methotrexate (MTX), sulfasalazine, hydroxychloroquine, leflunomide, and/or azathioprine), 47 patients were taking NSAIDs, and 14

patients were taking prednisone (<10 mg/day). Twenty-four patients received medication known to affect the ANS (including β -blockers, ACE-inhibitors, Ca^{2+} -blockers, α_1 -blockers, thiazides (or -related), ACh-receptor antagonists, β_2 -adrenergics, and anti-histamines), and 7 patients used hormonal contraceptives (6 intervention, 1 control; $\chi^2 = 3.120$, $p = 0.08$). There were no significant group differences in the use of biologics, DMARDs, steroids, and medication known to influence the ANS, except for the use of NSAIDs, which was significantly higher in the intervention group ($\chi^2 = 7.349$, $p = 0.01$). There were no significant group differences in pretreatment measures of negative and positive mood, and disease activity, but anxiety scores tended to be higher in the intervention group than in the control group ($t(67.835) = 21.715$, $p = 0.09$) (Table 2). Consequently, all further analyses were performed with covariates sex and baseline anxiety, with the additional covariate hormonal contraceptives for endocrine analyses.

Table 1 Demographic characteristics, disease severity, and medical regime of patients with rheumatoid arthritis in the intervention and control groups^a

	Intervention (<i>n</i> = 40)	Control (<i>n</i> = 34)	<i>p</i> -value
No. females/males	27/13	16/18	.08
Age (years \pm SD)	57.2 \pm 11.8 (range 24-75)	60.7 \pm 9.2 (range 26-80)	.17
Education level (%)			.56
Primary	7.5%	2.9%	
Secondary	60.0%	70.6%	
Tertiary	32.5%	26.5%	
Disease Activity (DAS28)	2.6 \pm 1.0 (range 0.8-4.5)	2.6 \pm 1.1 (range 0.5-5.1)	.81
Disease duration (years \pm SD)	15.7 \pm 10.9 (range 5-51)	12.4 \pm 7.6 (range 3-37)	.15
No. of patients currently under treatment for RA	38	32	
Biologics	17	16	.69
DMARDs	31	23	.43
NSAIDs	31	16	.007
Steroids (< 10mg/day)	9	5	.39

^a Values are means \pm SD. RA, rheumatoid arthritis; DMARDs, disease-modifying anti-rheumatic drugs; NSAIDs = nonsteroidal anti-inflammatory drugs.

Psychological and physical functioning

Satisfaction and usefulness of the training

Patients rated their satisfaction with the intervention with a score of $8.1 \pm \text{SD } 1.2$ and its usefulness with a score of $7.6 \pm \text{SD } 2.0$. Approximately 87% of patients in the intervention group reported an improvement in stress and tension after the training (little improvement by 42%, moderate improvement by 32%, and strong improvement by 13%).

Psychological functioning in intervention and control condition

Means and estimated marginal means (EMM; i.e., means corrected for the covariates) ($\pm \text{SEM}$) of the psychological and physical outcomes are presented in Table 2. A significant group effect was found for anxiety ($F(1,69.887) = 5.579, p = 0.02$); the intervention group had a significantly lower anxiety score than the control group after the intervention. Furthermore, patients in the intervention group had significantly higher levels of positive mood after the intervention than did patients in the control group (group effect, $F(1,67.436) = 4.851, p = 0.03$). No overall group effect was observed for negative mood ($F(1,68.389) = 0.028, p = 0.87$). Subgroup analyses showed a significant interaction effect between condition (intervention/control) and risk group (high/low) for anxiety ($F(1,68.002) = 7.820, p < 0.01$) and negative mood ($F(1,66.893) = 11.509, p < 0.01$), but not for positive mood ($F(1,65.985) = 0.205, p = 0.65$), indicating that high-risk patients responded differently to the stress management training with regard to anxiety and negative mood than did low-risk patients. Inspection of the data by *post hoc* tests revealed that lower anxiety scores (group effect, $F(1,32.725) = 8.128, p < 0.01$) and lower negative mood scores ($F(1,31.473) = 4.021, p = 0.05$) were present in the subgroup of high-risk patients in the intervention group compared to high-risk controls, but not in low-risk patients (group effect anxiety, $F(1,33.898) = 0.019, p = 0.89$; reverse group effect negative mood, $F(1,31.677) = 8.644, p < 0.01$). In addition, a trend towards higher positive mood scores was observed in high-risk patients in the intervention group compared to controls ($F(1,31.578) = 3.548, p = 0.07$), but not in low-risk patients ($F(1,31.256) = 0.691, p = 0.41$).

Physical functioning in intervention and control condition

There were no differences in disease activity (DAS28) between control and intervention groups after the stress management intervention ($F(1,61.610) = 0.004, p = 0.95$). Subgroup analyses showed no interaction effect between condition (intervention/control) and risk group (high/low) ($F(1,59.864) = 0.051, p = 0.82$).

Psychophysiological stress reactivity

Stress manipulation check

Both after treatment and at follow-up, the stress test induced a significant increase in tension (time effect, $F(1,73) = 304.899, p < 0.001$, and $F(1,66) = 182.031, p < 0.001$, respectively; Figure 2), α -amylase (time effect, $F(1,69.211) = 46.003, p < 0.001$, and $F(1,65) = 21.404$,

Table 2 Means (\pm SEM) and estimated marginal means (\pm SEM) of psychological and physical outcomes of patients in the intervention condition (IC: $n = 40$) and the control condition (CC: $n = 34$) pre= and post-treatment, and at follow-up

		Means (\pm SEM)		Estimated marginal means (\pm SEM)	
		Pre-treatment	Post-treatment	Follow-up	Follow-up
<i>Psychological functioning</i>					
Anxiety	IC	17.69 (0.94)	17.15 (0.76)	16.78 (0.74)	15.95 (0.39)*
	CC	15.68 (0.70)	16.64 (0.79)	16.06 (0.72)	17.14 (0.42)
Negative Mood	IC	3.23 (0.66)	2.97 (0.53)	2.17 (0.46)	0.79 (0.08)
	CC	1.94 (0.40)	2.00 (0.51)	1.77 (0.43)	0.78 (0.08)
Positive Mood	IC	12.00 (0.68)	12.10 (0.64)	13.00 (0.53)	13.35 (0.40)*
	CC	12.97 (0.61)	12.18 (0.75)	12.48 (0.64)	12.14 (0.43)
<i>Physical functioning</i>					
DAS28	IC	2.62 (0.16)	2.81 (0.16)	2.51 (0.20)	2.43 (0.10)
	CC	2.56 (0.19)	2.56 (0.19)	2.48 (0.19)	2.43 (0.11)

*Significant between-group effect ($p \leq 0.05$). Means of outcomes pre- and post-treatment, and at follow-up; and estimated marginal means of post-treatment and follow-up, corrected for pretreatment measures (and other covariates).

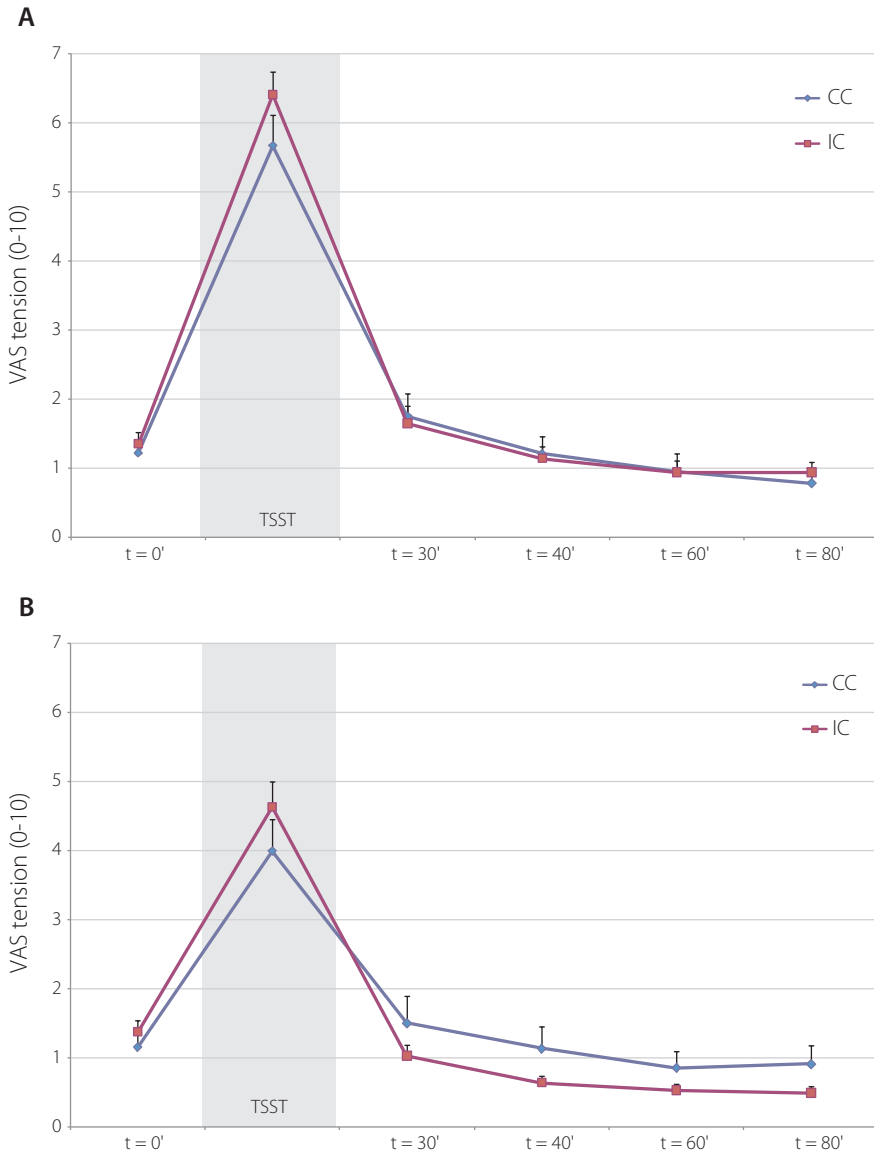


Figure 2 Self-reported response to stress. Mean stress-induced VAS tension levels (\pm SEM) in the intervention (IC) and control (CC) conditions post-treatment (**A**; IC, $n = 40$; CC, $n = 34$) and at follow-up (**B**; IC, $n = 36$; CC, $n = 31$).

$p < 0.001$, respectively; Figure 3), and cortisol levels (time effect, $F(1,69.041) = 29.566$; $p < 0.001$, and $F(1,63.003) = 9.688$, $p < 0.01$, respectively; Figure 4) in all patients.

Baseline differences between intervention and control condition

Both after treatment and at follow-up, there were no significant differences between the intervention and control groups in baseline levels ($t = 0$ minutes) of tension ($F = 0.230$, $p = 0.63$ and $F = 0.444$, $p = 0.51$, respectively), α -amylase ($F = 0.007$, $p = 0.93$ and $F = 0.326$, $p = 0.57$, respectively) and cortisol ($F = 1.530$, $p = 0.22$ and $F = 1.729$, $p = 0.19$, respectively).

Post-treatment psychophysiological stress reactivity

After treatment, levels of self-reported tension in response to the stress task were similar in the intervention and control groups (group effect, $F(1,69.000) = 0.340$, $p = 0.56$, Figure 2), as was autonomic reactivity (group effect α -amylase, $F(1,66.359) = 0.068$, $p = 0.80$, Figure 3), and endocrine reactivity (group effect cortisol, $F(1,64.287) = 0.315$, $p = 0.58$, Figure 4; and AUC_G : $F(1,66) = 0.734$, $p = 0.40$, Table 3), indicating that patients in the intervention group did not have an altered psychophysiological response to stress compared to patients in the control group after the intervention. Subgroup analyses also showed no interaction effect between condition (intervention/control) and risk group (high/low) for psychophysiological measures of stress, indicating that high-risk and low-risk patients did not respond differently to the stress management training with regard to stress-induced levels of tension, α -amylase, and cortisol.

Follow-up psychophysiological stress reactivity

At the follow-up assessment, self-reported tension elicited by the stress test was significantly lower in patients in the intervention group than in patients in the control group (group effect, $F(1,62.000) = 6.092$, $p = 0.02$, Figure 2). In addition, there was a significantly diminished cortisol response (group effect, $F(1,59.010) = 4.877$, $p = 0.03$, Figure 4) and a trend towards a lower total cortisol output (AUC_G) in the intervention group compared with the control group (AUC_G , $F(1,60) = 3.689$, $p = 0.06$, Table 3). The autonomic response was similar in the two groups (group effect α -amylase, $F(1,61.085) = 0.301$, $p = 0.59$, Figure 3). Subgroup analyses showed no interaction effect between condition (intervention/control) and risk group (high/low) for tension ($F(1,60.000) = 1.919$, $p = 0.17$), but a trend towards an interaction effect for α -amylase ($F(1,58.996) = 2.752$, $p = 0.10$) and cortisol ($F(1,57.100) = 3.682$, $p = 0.06$), indicating that high-risk patients tended to respond differently to the stress management training with regard to physiological measures of stress than did low-risk patients. Inspection of the data by *post hoc* tests revealed that high-risk patients in the intervention group had or tended to have lower overall levels of tension, α -amylase, and cortisol than did high-risk patients in the control group (group effect tension, $F(1,28.000) = 6.768$, $p = 0.02$; group effect α -amylase, $F(1,28.052) = 3.495$, $p = 0.07$; group effect cortisol, $F(1,25.384) = 7.450$, $p = 0.01$; and AUC_G $F(1,27) = 5.264$,

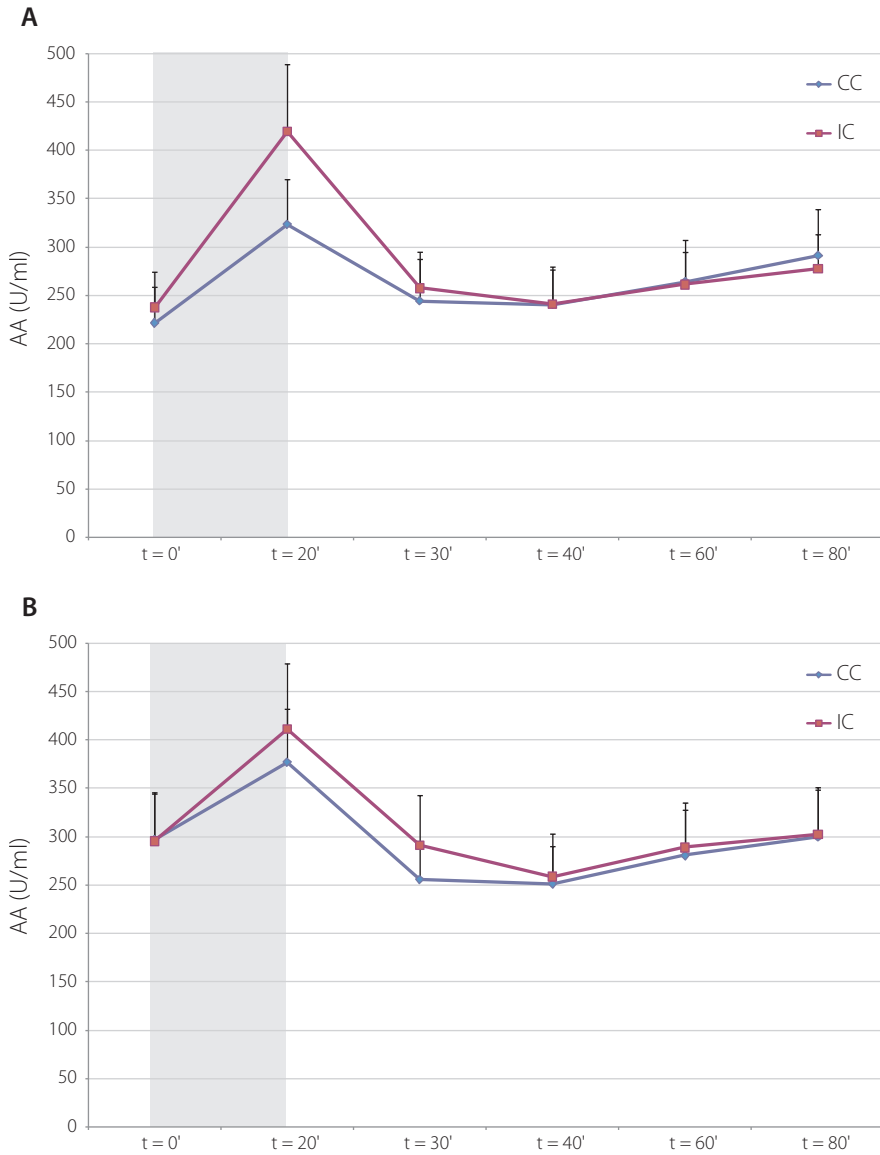


Figure 3 Autonomic response to stress. Mean stress-induced α -amylase levels (\pm SEM) in the intervention (IC) and control (CC) conditions post-treatment (**A**; IC, $n = 39$; CC, $n = 33$) and at follow-up (**B**; IC, $n = 35$; CC, $n = 31$).

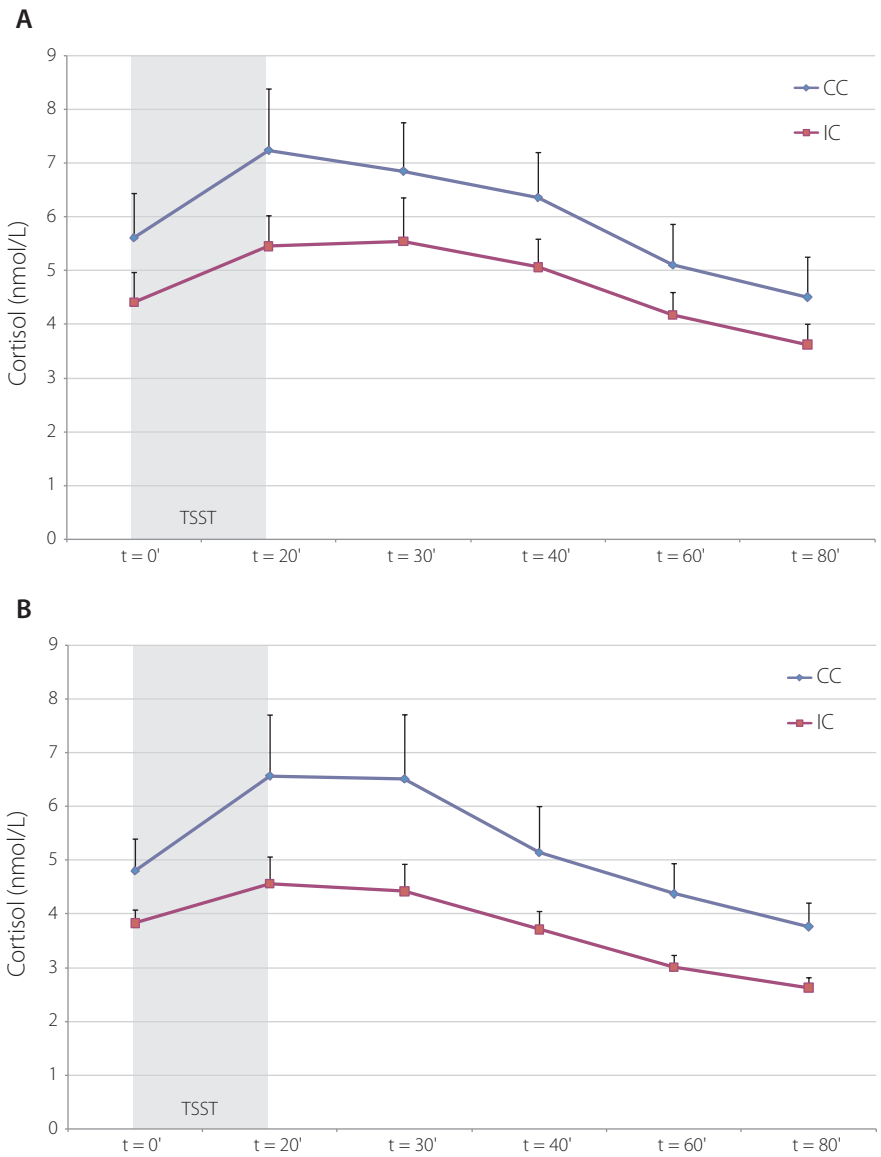


Figure 4 Endocrine response to stress. Mean stress-induced cortisol levels (\pm SEM) in the intervention (IC) and control (CC) conditions post-treatment (**A**; IC, $n = 39$; CC, $n = 32$) and at follow-up (**B**; IC, $n = 34$; CC, $n = 31$).

$p = 0.03$); this was not the case for the low-risk patients (group effect tension, $F(1,29.000) = 1.965$, $p = 0.17$; group effect α -amylase, $F(1,28.000) = 1.277$, $p = 0.27$; group effect cortisol, $F(1,27.000) = 0.818$, $p = 0.37$; and AUC_G ($F(1,28) = 0.548$, $p = 0.47$).

Table 3 Area under the curve (AUC_G) for cortisol (means \pm SEM) in the intervention (IC) and control (CC) conditions post-treatment and at follow-up

	Post-treatment	Follow-up
Intervention condition	42.59 (4.50)	33.46 (2.73)
Control condition	54.04 (7.30)	47.05 (6.96)

Discussion

This is the first study to assess psychological functioning and psychophysiological responsiveness (subjective, autonomic, and neuroendocrine) to a psychosocial stress task in patients with RA who had received training in stress management. Results indicated high satisfaction and perceived usefulness of the training, and a lower anxiety and higher positive mood after the training in the stress management than in the control group. No effect on disease activity or post-treatment psychophysiological stress responsiveness was found, but at follow-up (9 weeks after the training) the stress management group showed a lower tension and cortisol response to stress than the control group. These results were particularly evident in a subgroup of patients psychologically at risk, supporting previous findings of increased treatment effects in at-risk patients [32,50]. Results of this study suggest that short-term individual stress management training is not only able to improve psychological functioning by the level of tension, but may also alter psychophysiological responses to stress by reducing levels of cortisol.

Stress might have detrimental effects on health, particularly in clinical populations. Over the last decade, there has been an increasing interest in the physiological effects of stress management interventions for patient groups [15–21]. Studies of various forms of stress management or cognitive-behavioral therapy in patients with RA have only incidentally reported changes in overall disease activity or biological indicators of disease after the intervention, such as a decrease in overall disease activity [51,52], self-reported disease flare-ups [24], and joint tenderness [53] in the intervention group compared with the control group. Changes in cortisol values [54], cytokine INF-c [54], C-reactive protein [28], and ESR [51] have also been reported. In a response to the aforementioned studies, the current study uniquely investigated the effects of a stress management intervention on the acute-phase physiological response to stress. It seems apparent that alterations on

the physiological level might particularly occur when interventions are successful in changing the appraisal or perception of stressors [55]. We found that anxiety was significantly, but modestly, reduced after 2 weeks of individual stress management training. After an interval of 9 weeks, during which participants practiced the stress management exercises at home, focusing on long-term stress management and relapse prevention, stress-induced tension was slightly lower and there was a lower stress-evoked cortisol response in the intervention group compared with patients in the control group. The effect of stress management training on psychophysiological stress responsiveness appears to be delayed, possibly because repeated exercise during two months might have stronger effects than exercise of two weeks; it takes time to integrate the learned exercises into the daily lives of participants and to help them cope with stress-provoking situations. Results are in line with preliminary evidence suggesting that intervention-related physiological changes, particularly those related to the immune system, might become more pronounced with time [52,56].

To our knowledge, only two other studies assessed the acute-phase physiological response to a laboratory stressor after stress management [35,36]. Healthy males participating in a group-based cognitive-behavioral stress management training showed a significantly diminished cortisol response to the TSST 2 weeks after the intervention [35], and this pattern, although less pronounced, was also observed 4 months after a similar training in male and female subjects [36]. Our results provide preliminary evidence that, in line with recent findings in healthy populations, stress management might also alter endocrine responsiveness to a stress task in a clinically comprised population of patients with RA. Our findings on endocrine responsiveness extend recent results suggesting that basal cortisol levels and stress-induced cortisol reactivity in patients with RA might not be significantly different from those of healthy participants [8,57]. This implies that the endocrine stress response system could be a target for stress management interventions not only in healthy subjects, but also in patients with immune-mediated diseases such as RA. These interventions might prevent the possible negative physiological consequences of stress on health. Although a reduced psychophysiological stress reaction was found at the follow-up in the stress management group as compared to the control group, this was not accompanied by a simultaneous decrease in disease activity. Because the psychophysiological results were only found at the longer term, this could imply that the effects on disease activity may have occurred even later. Theoretically, a lowered cortisol response might reflect a decreased psychological stress level and/or an improvement in the functioning of all physiological regulatory systems [e.g., 54]. However, no studies have yet reliably shown the consequences of non-pharmacological cortisol changes in rheumatoid arthritis and future studies with a longer-term follow-up are needed to provide insight into this question.

In contrast to altered responses on self-reported tension and cortisol, autonomic reactivity to stress was similar in the two patient groups, as evidenced by the similar levels

of α -amylase levels in saliva, an indicator of sympathetic activity [58,59]. The stress management intervention included principles and techniques that are mainly aimed at reducing tension and negative emotion by inducing a generalized relaxation response [60], which is hypothesized to dampen sympathetic activity [61]. Several studies investigating the effects of relaxation on autonomic changes at baseline or in stress-provoking situations have reported reduced galvanic and cardiovascular reactivity, but evidence of altered autonomic responsiveness is not unequivocal [11–13,62–64]. Our results suggest that the responses of the ANS and HPA axis to (repeated) stress are not necessarily synchronous; a phenomenon that has also been documented after recurrent exposure to the same stressful stimulus, both in animal and human research [65]. Whereas (social-evaluative) threat and uncontrollability might be the most important components contributing to an endocrine response to a laboratory stressor [66], autonomic reactivity could be an α -specific response to more generalized arousal, such as the effort to do well [67,68]. As the cortisol response to a stressor is sensitive to emotions and appraisals that are associated with threats of the social self, such as rumination and submissiveness [69], we hypothesize that the training specifically influenced the endocrine response to stress due to changes in specific emotions.

Overall, subgroup analyses showed that the effects of the stress management training on specific psychological outcomes and physiological stress responses (anxiety and cortisol levels) were particularly evident in a subgroup of patients at risk. Previous studies have shown that particularly patients with RA with heightened levels of anxiety and depression benefit from cognitive-behavioral therapy, not only after treatment but also at follow-up assessments [32]. The importance of subgroup analyses has also been acknowledged in other patient populations [70–72]. The lower anxiety and cortisol levels that were observed in the intervention group at follow-up might be attributed to the subgroup of high-risk patients. Additional subgroup effects were found for negative mood and α -amylase levels at follow-up in the subgroup of high-risk patients only. The latter findings support the idea that beneficial effects of treatment might be particularly observed in dysfunctional groups of patients and highlights the importance of identifying subgroups of patients most likely to benefit from a specific intervention in future studies of stress.

This study has several limitations. First, exclusion criteria with regard to physical and psychological comorbidity may have resulted in a homogenous sample of patients showing relatively mild disease activity at baseline. In addition, the sample size was relatively small for the subgroup analyses, particularly when considering multiple testing. Therefore, the results of this study, particularly those regarding subgroups of patients, should be interpreted with caution and should be replicated in larger groups of patients. Secondly, there were marginal baseline differences between the intervention and control groups, with a trend towards a higher female-to-male ratio in the intervention group and higher anxiety scores. We statistically controlled for differences by adding these

confounders as a covariate in all analyses, in addition to the use of oral contraceptives for endocrine analyses. It is well-documented that not only has a person's sex differential effects on physiological stress response patterns [73,74], but also the menstrual cycle, menopause, and the use of oral contraceptives of females influence the cortisol response to laboratory stress paradigms [75], which makes it difficult to control for these effects in a heterogeneous group of patients with arthritis. Thirdly, due to the character of the study, which included a no-treatment control condition, it was impossible to blind patients and researchers for the treatment status of the participants. However, by blinding the persons conducting the Trier Social Stress Test for the treatment status of participants, we tried to limit possible bias on the psychophysiological stress response as much as possible. Lastly, we decided against pre- and post-treatment assessment of psychophysiological stress reactivity, because repeated exposure to the stress test has been found to elicit small habituation effects [76,77]. In addition, the small effects found on psychophysiological measures at the follow-up assessment might have been larger if the stress test would have been performed only once, at the follow-up assessment.

This is the first study to provide preliminary evidence that a relatively short stress management intervention not only improves psychological functioning, but may also influence the psychophysiological response to stress (self-reported tension and cortisol reactivity) in patients with RA, particularly those psychologically at risk. Our study highlights the need to look at individual differences in stress responsiveness and psychological factors that are able to influence stress response patterns. Interventions such as the current stress management training, alone or as a part of a more comprehensive treatment programme, may prove useful in preventing the detrimental effects of stress on patients with systemic inflammatory diseases, such as RA.

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Chapter 6

Immune responses to stress after stress management training in patients with rheumatoid arthritis



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Abstract

Introduction Psychological stress may alter immune function by activating physiological stress pathways. Building on our previous study, in which we report that stress management training led to an altered self-reported and cortisol response to psychological stress in patients with rheumatoid arthritis (RA), we explored the effects of this stress management intervention on the immune response to a psychological stress task in patients with RA.

Methods In this study, 74 patients with RA, who were randomly assigned to either a control group or a group that received short stress management training, performed the Trier Social Stress Test (TSST) 1 week after the intervention and at a 9-week follow-up. Stress-induced changes in levels of key cytokines involved in stress and inflammatory processes (for example, interleukin (IL)-6 and IL-8) were assessed.

Results Basal and stress-induced cytokine levels were not significantly different in patients in the intervention and control groups one week after treatment, but stress-induced IL-8 levels were lower in patients in the intervention group than in the control group at the follow-up assessment.

Conclusion In line with our previous findings of lower stress-induced cortisol levels at the follow-up of stress management intervention, this is the first study to show that relatively short stress management training might also alter stress-induced IL-8 levels in patients with RA. These results might help to determine the role of immunological mediators in stress and disease.

Introduction

Psychological stress may alter immune function by activating physiological pathways of stress, such as the autonomic nervous system and the hypothalamus–pituitary–adrenal axis, which in turn interact with the immune system [1-4]. Consequently, stress could have negative effects on health, particularly in populations with immune dysfunction, such as patients with rheumatoid arthritis (RA). The pathophysiological mechanisms involved in stress and disease exacerbation have not yet been elucidated.

Psychological responses to stress that might lead to immune dysregulation can be altered by interventions aimed at reducing psychological stress [1,5]. As yet there is no consensus about whether and to what extent stress management interventions are able to alter immune function. In an extensive meta-analysis by Miller and Cohen there was only modest evidence that different types of stress management interventions change basal immune function in healthy and clinical populations, with most consistent changes being found in basal total leukocyte counts and secretory immunoglobulin A levels [6]. More recent studies reported that psychological interventions for patients with HIV or cancer changed basal lymphocyte proliferation and basal levels of proinflammatory cytokines [7-10]. Even though the effects of psychological interventions in patients with RA have been extensively studied and reviewed [11-16], there are only incidental reports of immune changes after psychological interventions in patients with RA, such as changes in interleukin (IL)-6 or interferon-gamma (IFN γ) [17,18], or in immune measures indicative of disease status, such as C-reactive protein and erythrocyte sedimentation rate [19-24]. Potentially, previous effects in RA might be limited because changes in immune function in response to a real-life stressor have not yet been investigated combining both a stress management intervention and a stress induction paradigm. Particularly then, the benefits of stress management training can become evident because patients are challenged to cope with a stressful situation.

We previously showed that a short course of stress management training decreased the subjective distress response and stress-induced cortisol levels in patients with RA at a follow-up assessment, and especially in those patients psychologically at risk [5]. In the present study, we explored the effects of the intervention on stress-induced levels of key cytokines involved in disease progression (for example, IL-6 and IL-8) in patients with RA, with stress being elicited by the Trier Social Stress Test. Building on our previous findings [5], we expected that patients in the intervention group would show an altered cytokine response to acute psychosocial stress compared with controls at the 9-week follow-up assessment. We also explored immune effects specifically in patients psychologically at risk.

Materials and methods

This study was part of a larger trial for which the methods and CONSORT statement have been described extensively elsewhere [5]. The study protocol was approved by the regional medical ethics committee (CMO Region Arnhem-Nijmegen) and was registered in The Netherlands National Trial Register (NTR 1193). Written informed consent was obtained from all participants.

Participants and procedure

Participants

Ninety-six eligible patients with RA [25] were randomized to one of two parallel groups: the control or the intervention condition. After randomization, 19 participants withdrew before the first stress test and three participants were excluded based on our predefined exclusion criteria (that is, use of psychiatric medication). In addition, seven out of 74 participants withdrew before the second stress test. Reasons for withdrawal were physical comorbidity, severe illness or death of a significant other, a change in pharmacotherapy, or lack of motivation (for more information on completers and dropouts, see the flowchart in [5]). There were no differences in sociodemographic variables and psychological and physical functioning at baseline between the dropouts and the completers. For explorative subgroup analyses of patients psychologically at risk, participants were *post hoc* divided into two subgroups using a median split of a composite score for baseline anxiety and negative mood [5].

Study design

Participants performed a stress test 3 weeks after the first assessment (post treatment) and 9 weeks thereafter (follow-up). One-half of the participants had participated in an individual stress management training program between the first and second assessments. The control group received care as usual. Stress test sessions were run between 13:00 and 15:30 hours. Participants refrained from using caffeine, alcohol, nicotine, or physical exercise on the test day, and from eating 2 hours before the first blood sample was drawn. Forty minutes before the stress test, a venous catheter was inserted into the nondominant arm and participants rested for 20 minutes. Blood samples were taken at baseline (that is, after 20 minutes of rest), immediately after the stress test, and 20 and 60 minutes later ($t = 0$, $t = 20$, $t = 40$, and $t = 80$ minutes, respectively).

Stress task

The Trier Social Stress Test is a standardized laboratory stress task consisting of a mock job interview and mental arithmetic, and induces self-reported, neuroendocrine, and autonomic nervous system responses [26,27].

Stress management training

Participants in the intervention group received individual stress management training as described previously [5]. The program consisted of four individual 1-hour sessions of stress management with a trained therapist over 2 consecutive weeks and included applied, progressive, cue-controlled, and differential relaxation techniques, as well as psycho-education, breathing and visualization exercises. After the training, patients were encouraged to stick to a relapse-prevention checklist during the 9-week follow-up period.

Measures

This study builds on a previous study [5], in which general psychological (for example, anxiety), physical (28-joint Disease Activity Score), autonomic (α -amylase) and neuroendocrine (cortisol) outcomes are reported, by further exploring immune responses to stress through measurement of various circulating cytokines.

Cytokine assay

The blood samples that were collected during the two stress tests (post treatment and follow-up) were stored at -35°C until analysis. Based on the literature of psychophysiological stress reactivity in healthy populations and chronic inflammatory diseases, such as RA [1,28–30], human IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFN γ and tumor necrosis factor alpha (TNF α) were measured in serum using human cytokine multiple kits (Invitrogen Corporation, Camarillo, CA, USA) according to the manufacturer's instructions. Samples were analyzed with a Luminex[®] 100 TM instrument (Luminex Corporation, Austin, TX, USA). The sensitivity of the cytokine assay was <5 pg/ml for all cytokines measured. To reduce error variance caused by between-run variation, all samples from one participant were analyzed in the same run.

Statistical analysis

Data for the 74 participants who completed the study protocol were analyzed. Skewed data were logarithmically transformed to generate unskewed data distributions before statistical analysis. Normal distributions and residuals were not obtained after logarithmic transformation of data for IL-5 and IFN γ levels. Between-group differences in age, sex, education, and psychological measures at baseline were tested with independent Student's t tests and chi-square analyses. Baseline cytokine levels ($t = 0$ minutes) were compared between intervention and control groups with analyses of covariance. Cytokine responses to the Trier Social Stress Test (post treatment and follow-up) were evaluated using a linear mixed model taking into account the specific design features of the study. Cytokine levels (IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFN γ , and TNF α) were used as dependent variables; and group, baseline measurement of the dependent variable ($t = 0$ minutes), and time ($t = 20$ minutes, $t = 40$ minutes, and $t = 80$ minutes) were used as independent variables. As group by time interactions were not observed, the final model

contained only main effects. Explorative subgroup analyses were performed to test whether effects were particularly detected in patients psychologically at risk as compared with patients not at risk [5] by incorporating risk group and risk group by treatment interactions into the models. A significant interaction was interpreted as indicating that there were subgroup differences in the effect of the treatment. Stratified analyses were performed to gain a better understanding of the nature of the responses in the patient subgroups. For each outcome measure, an unstructured covariance matrix was used to model the dependence between repeated measurements of the dependent variable. Owing to (a tendency towards) an unequal sex distribution, use of hormonal contraceptives, baseline anxiety scores, and the use of nonsteroidal anti-inflammatory drugs across the two groups [5], all analyses were performed with these four covariates.

Because participants dropped out mostly prior to the first stress test (see previous section and [5]), and consequently no stress test data were available for these participants 1 week after treatment and at follow-up, intention-to-treat analyses were not performed [31]. In total, the data for the 74 patients (post treatment) and 67 patients (follow-up) included in the analyses were 85% complete, mainly because a venous catheter could not be inserted in a number of patients during one or both stress tests. Cytokines were significantly intercorrelated with at least five to nine of the other cytokines, and significant correlations ranged from 0.20 to 0.80. Undetectable levels (in percentage of available samples) of IL-1 β (33%), IL-2 (41%), IL-4 (37%), IL-5 (43%), IL-6 (27%), IL-7 (41%), IL-8 (13%), IL-10 (25%), IFN γ (70%) and TNF α (16%) were set to zero and included in all analyses. The Bonferroni correction for multiple testing was not applied due to the explorative nature of this study, the small sample size, and the high intercorrelation of most cytokines, which makes the method even more conservative than in other applications [32]. Analyses were performed using SPSS 16.0 for Windows (SPSS Inc, Chicago, IL, USA). For all analyses, the significance level was $\alpha = 0.05$ (two-sided).

Results

Psychophysiological stress reactivity

Cytokine levels at baseline

Both after the intervention and at follow-up there were no significant differences between the intervention and control groups in baseline levels ($t = 0$ minutes) of all cytokines ($p > 0.05$) (Table 1 and Additional file 1).

Post-treatment stress-induced cytokine levels

Immediately after the intervention, stress-induced cytokine levels were similar in the intervention and control groups (group effect, $p > 0.10$ for all cytokines), indicating that patients in the intervention group did not have an altered immune response to

Table 1 Baseline and stress-induced cytokine levels (pg/ml) in the intervention and control conditions post-treatment and at follow-up

		<i>t</i> = 0 min	<i>t</i> = 20 min	<i>t</i> = 40 min	<i>t</i> = 80 min
IL-1 β					
Post-treatment	IC	92.39 (33.6)	106.7 (41.4)	111.3 (46.4)	111.6 (45.4)
	CC	114.3 (38.6)	130.9 (43.5)	116.6 (37.0)	136.7 (59.0)
Follow-up	IC	66.48 (18.8)	77.43 (23.7)	69.49 (20.1)	70.89 (19.9)
	CC	119.0 (43.0)	100.7 (32.5)	98.13 (34.1)	117.6 (41.2)
IL-2					
Post-treatment	IC	28.11 (11.1)	30.85 (11.9)	34.83 (15.6)	31.58 (13.4)
	CC	32.60 (14.0)	42.86 (19.2)	38.77 (17.3)	47.51 (27.0)
Follow-up	IC	20.28 (7.20)	25.27 (10.3)	17.37 (7.23)	19.32 (7.15)
	CC	35.89 (18.4)	27.82 (14.1)	28.12 (14.0)	32.24 (15.4)
IL-4					
Post-treatment	IC	48.22 (24.9)	48.30 (21.0)	48.98 (20.3)	52.90 (25.7)
	CC	60.18 (29.3)	72.79 (30.1)	65.76 (28.4)	65.39 (29.9)
Follow-up	IC	33.22 (13.9)	35.16 (14.9)	33.62 (12.6)	33.08 (13.6)
	CC	56.48 (23.2)	51.79 (20.0)	50.91 (21.5)	56.99 (24.8)
IL-5					
Post-treatment	IC	20.94 (15.2)	21.89 (15.3)	23.24 (16.5)	21.49 (15.6)
	CC	5.129 (2.17)	7.887 (3.72)	7.006 (2.94)	8.948 (4.51)
Follow-up	IC	16.23 (12.8)	15.89 (12.1)	15.14 (12.1)	17.60 (14.5)
	CC	7.368 (3.48)	6.048 (2.95)	5.929 (2.60)	6.969 (3.11)
IL-6					
Post-treatment	IC	36.46 (11.2)	34.94 (9.69)	38.80 (11.1)	40.36 (11.2)
	CC	30.18 (9.36)	41.18 (15.2)	33.54 (10.7)	39.57 (16.2)
Follow-up	IC	25.79 (7.51)	23.39 (7.90)	23.63 (6.41)	23.18 (6.68)
	CC	31.20 (14.1)	30.18 (12.0)	29.22 (11.3)	30.43 (11.6)
IL-7					
Post-treatment	IC	70.11 (26.0)	70.27 (24.8)	73.62 (24.7)	69.51 (27.2)
	CC	52.69 (16.5)	63.65 (20.5)	55.94 (18.5)	60.73 (21.8)
Follow-up	IC	52.54 (23.4)	54.54 (23.5)	49.13 (19.6)	53.32 (22.2)
	CC	52.80 (18.4)	53.69 (18.0)	48.64 (18.2)	52.89 (19.1)
IL-8					
Post-treatment	IC	22.14 (3.67)	16.19 (3.70)	17.02 (3.53)	14.47 (3.41)
	CC	29.55 (6.50)	34.04 (7.81)	29.45 (6.75)	28.18 (7.14)
Follow-up	IC	19.51 (4.28)	13.97 (4.67)	12.53 (4.00)	10.37 (3.38) ^a
	CC	33.63 (6.68)	33.46 (8.33)	29.31 (7.83)	23.64 (5.72)

Table 1 Continued

			<i>t</i> = 0 min	<i>t</i> = 20 min	<i>t</i> = 40 min	<i>t</i> = 80 min
IL-10						
Post-treatment	IC		172.0 (75.2)	183.7 (81.0)	175.5 (80.0)	164.7 (75.8)
	CC		49.33 (25.9)	57.07 (31.8)	73.55 (48.1)	71.02 (45.9)
Follow-up	IC		72.67 (35.6)	115.6 (54.6)	85.90 (41.9)	78.49 (37.4)
	CC		46.83 (26.8)	43.63 (24.2)	39.90 (20.9)	50.27 (28.0)
IFN γ						
Post-treatment	IC		1.063 (0.39)	1.070 (0.43)	0.821 (0.33)	0.706 (0.31)
	CC		1.545 (0.45)	8.328 (4.98)	6.623 (4.91)	7.591 (5.70)
Follow-up	IC		0.857 (0.39)	0.772 (0.32)	0.925 (0.36)	1.062 (0.38)
	CC		4.389 (2.87)	8.535 (6.37)	6.597 (4.98)	7.377 (5.94)
TNF α						
Post-treatment	IC		31.40 (8.54)	34.98 (8.95)	34.82 (8.35)	35.60 (10.7)
	CC		34.56 (12.4)	39.35 (12.7)	36.00 (11.1)	35.45 (11.2)
Follow-up	IC		23.90 (5.75)	26.44 (6.94)	24.74 (5.89)	25.19 (6.44)
	CC		26.26 (8.74)	23.84 (7.17)	25.04 (9.44)	25.93 (8.92)

Data presented as mean (\pm standard error of the mean). CC, control condition; IC, intervention condition; IFN γ , interferon gamma; IL, interleukin; TNF α , tumor necrosis factor alpha. ^aSignificant between-group effect ($p \leq 0.05$). Means at the 4 time points. Statistical analyses performed on ln-transformed data.

stress compared with patients in the control group. Subgroup analyses also showed no interaction between condition (intervention/control) and psychological risk group (high/low) ($p > 0.20$ for all cytokines) (Table 1 and Additional file 1).

Follow-up stress-induced cytokine levels

At the follow-up assessment, stress-induced IL-8 levels were significantly lower in patients in the intervention group than in patients in the control group (group effect, $F(1, 54.273) = 5.421$, $p = 0.02$) (Figure 1). Exploration of IL-8 responses in subgroups of patients psychologically at risk and not at risk showed a tendency towards an interaction effect between condition (intervention/control) and risk group (high/low) (interaction effect, $F(1, 51.990) = 3.244$, $p = 0.08$), indicating that high-risk patients tended to respond differently to stress management training than low-risk patients. *Post hoc* tests revealed that IL-8 levels were more decreased in high-risk patients in the intervention group than in the low-risk intervention group. Omission of the data for patients with undetectable IL-8 levels from analyses did not change the main result (group effect, $F(1, 48.178) = 8.226$, $p = 0.01$), and

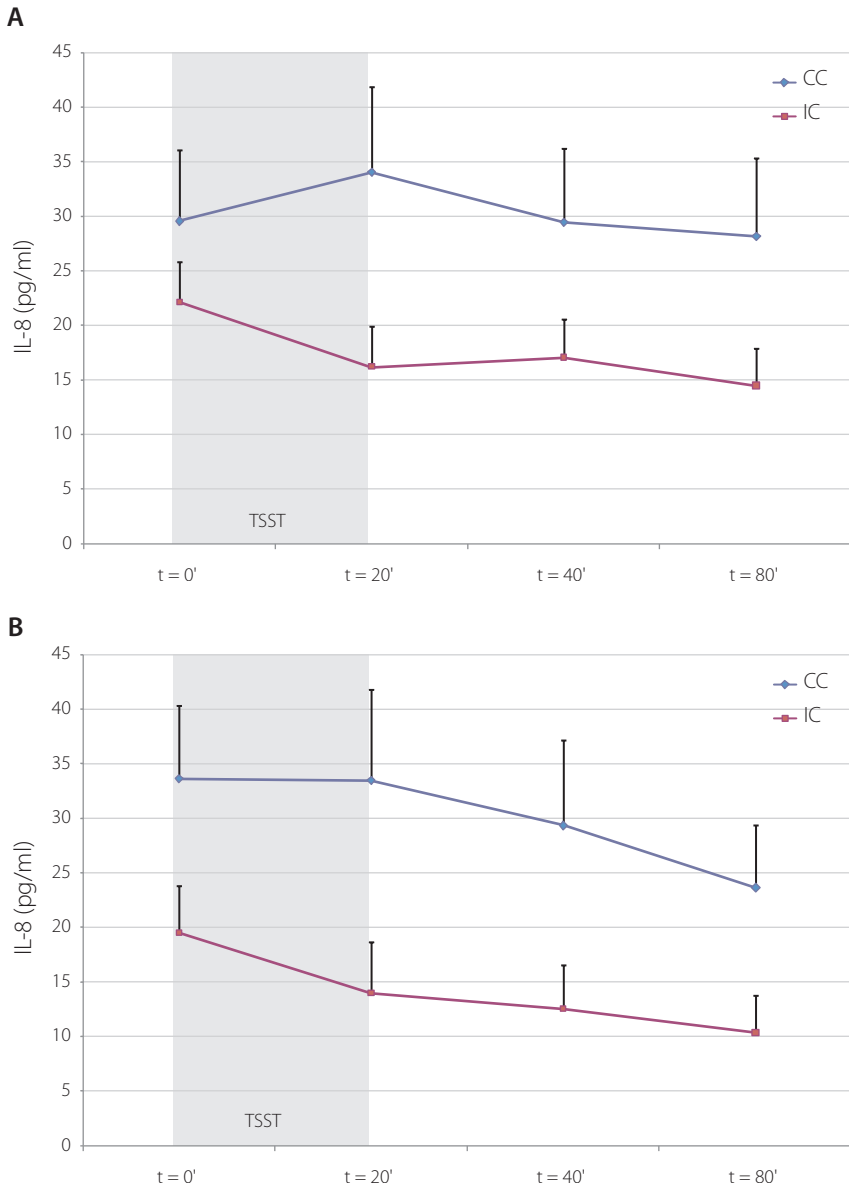


Figure 1 Interleukin-8 response to stress. Mean \pm standard error of the mean interleukin (IL)-8 levels (pg/ml) at $t = 0$ minutes (baseline/pre-Trier Social Stress Test (TSST)), $t = 20$ minutes, $t = 40$ minutes, and $t = 80$ minutes (post TSST) of patients in the intervention conditions (IC) and control conditions (CC) immediately after the intervention (**A**: IC, $n = 35$; CC, $n = 32$) and at follow-up (**B**: IC, $n = 33$; CC, $n = 28$).

high-risk patients still showed a (significantly) different response to the training than low-risk patients (interaction effect condition and risk status, $F(1, 46.212) = 4.472, p = 0.04$). For all other cytokines, there were no significant differences in levels after stress induction between the intervention and control groups at follow-up ($p > 0.10$) (Table 1 and Additional file 1).

Discussion

This is the first study to explore the response of circulating cytokines to a psychosocial stress test after stress management training in patients with RA. Although no differences in basal and stress-induced levels of key cytokines were observed immediately after the intervention, patients in the intervention group had lower stress-induced IL-8 levels than patients in the control group at the follow-up assessment. Results suggest that a short individual training in stress management might alter immune parameters after a psychosocial stress task in a population with immune dysfunction; namely, patients with RA. This finding is in line with our previous report indicating that the stress management training improves psychological functioning and influences subjective and endocrine parameters of stress (that is, distress and cortisol levels) at the follow-up assessment [5].

Stress-induced immune effects after a stress management intervention have not so far been investigated in rheumatic patients, including patients with RA. Stress induction paradigms using only a single stress exposure have yielded relatively robust effects on IL-6, IL-1 β , and IFN γ levels in various healthy and patient populations [28,29]. Stress exposure also changes levels of these and other cytokines in rheumatic patients, but results are much less consistent [2]. For example, IL-6 levels increased in response to a cold pressor task in patients with RA and juvenile idiopathic arthritis [33,34], but IL-6 and IFN γ levels remained unchanged after psychological stress was induced in patients with RA and systemic lupus erythematosus [35-37]. Differences in stress induction paradigms and detection methods used and differences in the heterogeneity of patient samples might explain the inconsistent findings. Immune function after stress management training has only been measured incidentally in patients with RA and, moreover, has not been investigated in combination with stress exposure. One study reported altered basal IFN γ levels after emotional disclosure therapy for patients with RA [17], while lower basal IL-6 levels were observed after cognitive behavioral therapy compared with meditation and education groups [18]. Several other studies also reported other types of biological markers, mostly erythrocyte sedimentation rate and/or C-reactive protein, often as part of assessing overall disease activity, but did not find intervention-related changes [21,22,24,38-46]. In our study, the stress management intervention did not change basal or stress-induced cytokine levels, except for a decrease in stress-induced IL-8 levels at follow-up.

Chemotactic IL-8 is a key player in the acute exacerbation of inflammatory conditions, directing neutrophils and other cell types (for example, monocytes and lymphocytes) to sites of inflammation when homeostasis is disrupted [47]. Blocking the actions of IL-8 has been shown to prevent acute inflammation in animal models [48]. The lipopolysaccharide-stimulated production of IL-8 has been found to be positively correlated with perceived stress in healthy adults, and this could be primarily attributed to negative affect [49,50]. However, IL-8 levels did not change after the induction of stress with the cold pressor task in patients with juvenile idiopathic arthritis and healthy controls [34]. Whether IL-8 acts as a more general marker of stress or whether it is specifically involved in the physiological stress response of patients with RA is not yet clear. Consequently, future studies should compare IL-8 responses to stress and stress management training in both healthy and clinical populations. Interestingly, the effect of the stress management training on stress-induced IL-8 levels tended to be particularly evident in patients with heightened levels of anxiety and negative mood. We found comparable effects for self-reported levels of tension and cortisol levels in our previous report [5], but these measures were not related to IL-8 levels in this study. In addition, the effectiveness of psychological treatment for RA patients at risk was reported previously [51], which warrants further research into the benefits of stress management on different types of psychophysiological parameters in high-risk patients.

This study had several limitations. The relatively homogeneous and small sample of patients with mild RA prevents generalization of our findings. The normal range for many immune parameters is very broad and psychological interventions, especially of short duration, might not induce physiological changes of sufficient magnitude or duration to move cytokine levels beyond this range [6]. Nevertheless, intervention studies have demonstrated that immune alterations occur when people display a change in cognition [52] and emotion [7]. Moreover, intervention-related immune changes could have been masked by biological forces, such as disease flare-ups and biological treatments that affect the patients' immune system [6]. Although we tried to limit effects of disease flare-ups by monitoring the patients' disease status and ruled out that treatment effects were caused by differences in biological treatment protocols through covariate analyses, we cannot preclude that this problem might have influenced our results. Prompted by earlier unequivocal findings of stress-induced changes to immune function in rheumatic patients [2], the high intercorrelation of most cytokines, and the small sample size, Bonferroni correction for multiple testing was not applied in this explorative study. Future research should try to replicate our findings and, if possible, apply the Bonferroni correction to data with large sample sizes. Moreover, the direction of other cytokine responses observed in this study (for example, IFN γ) seems consistent with the stress literature and tentatively suggests a broader effect of stress management training on immune function, but larger studies are needed to validate this effect. Furthermore, no statements can be made about the clinical relevance of our results, especially since the intervention was of

short duration (four 1-hour sessions over 2 weeks) and disease activity did not improve over the course of our study [5]. A longer intervention that may produce more pronounced effects might overcome these problems. Another general problem concerning immune markers in stress research, particularly circulating cytokines, is the ambiguity regarding the interpretation of findings. Circulating levels of cytokines are thought to reflect levels of systemic inflammation and are correlated with disease activity and radiographic progression [53]; however, changes in cytokine concentrations from baseline might not indicate *de novo* cytokine production or clearance, but a redistribution of existing cytokines from or into the periphery [54]. To what extent these alterations represent adaptive or maladaptive immune processes is not well understood and needs further investigation.

Conclusions

Patients with RA who received training in stress management not only show changes in the subjective and cortisol response to stress [5], but might also be characterized by an altered immune response to stress; that is, lower IL-8 levels. Although results of this and our previous study need validation in larger studies, they provide preliminary evidence that a short psychological intervention is not only able to improve psychological functioning, but also acts on the neuroendocrine and immune systems and therefore might have the potential to ameliorate the possible harmful effects of stress on health in patients with RA. Stress management training might prove to be beneficial as an adjunct to standard therapy to control arthritis symptoms.

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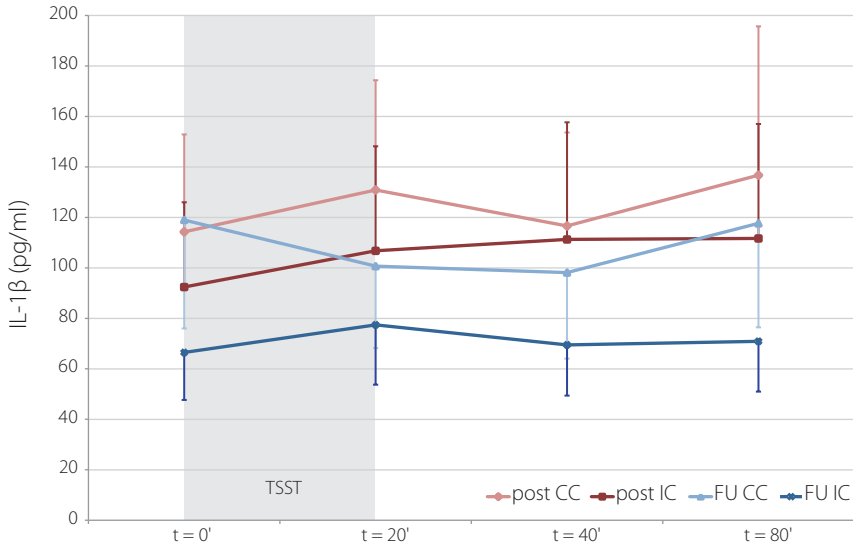
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Appendix

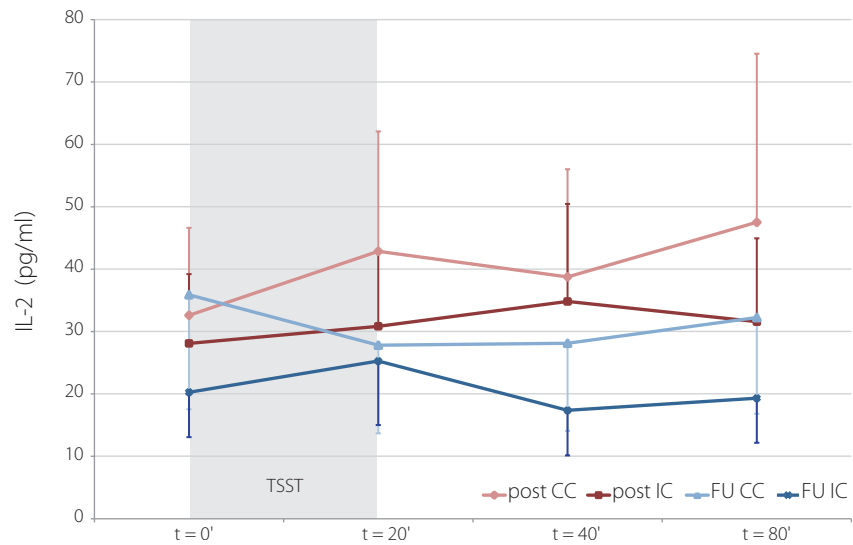
Additional file 1

Additional file 1 the mean response to stress of **(A)** IL-1 β , **(B)** IL-2, **(C)** IL-4, **(D)** IL-5, **(E)** IL-6, **(F)** IL-7, **(G)** IL-8, **(H)** IL-10, **(I)** IFN γ , and **(J)** TNF α (in pg/ml \pm standard error of the mean) at $t = 0$ minutes (baseline/pre TSST), $t = 20$ minutes, $t = 40$ minutes, and $t = 80$ minutes (post TSST) for patients in the intervention condition (IC) and control condition (CC) immediately after the intervention (post; red) and at follow-up (FU; blue).

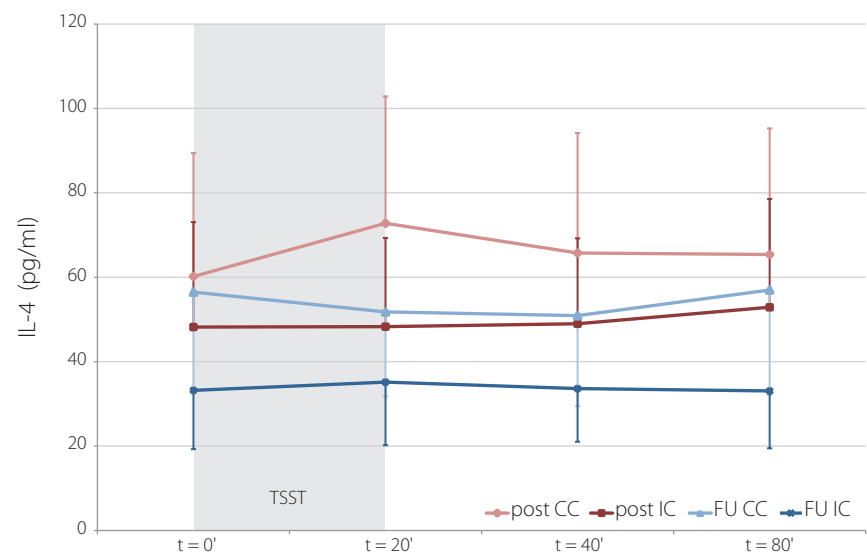
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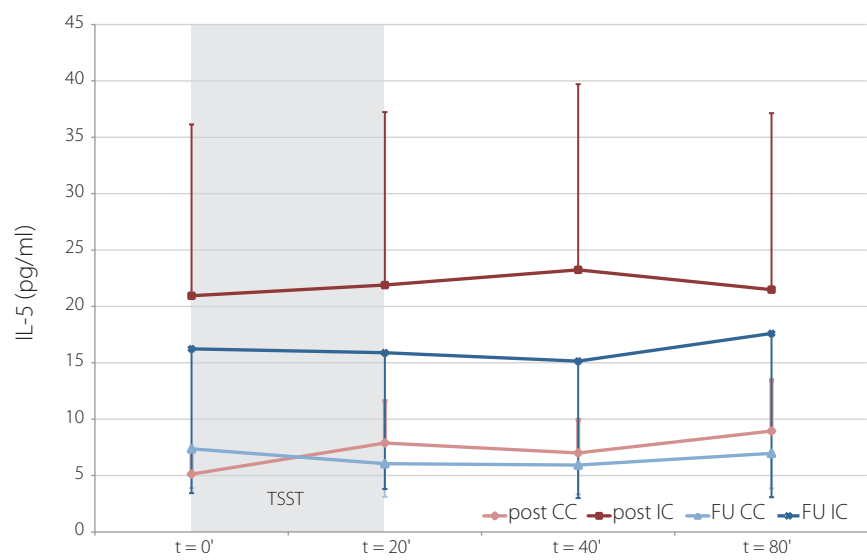
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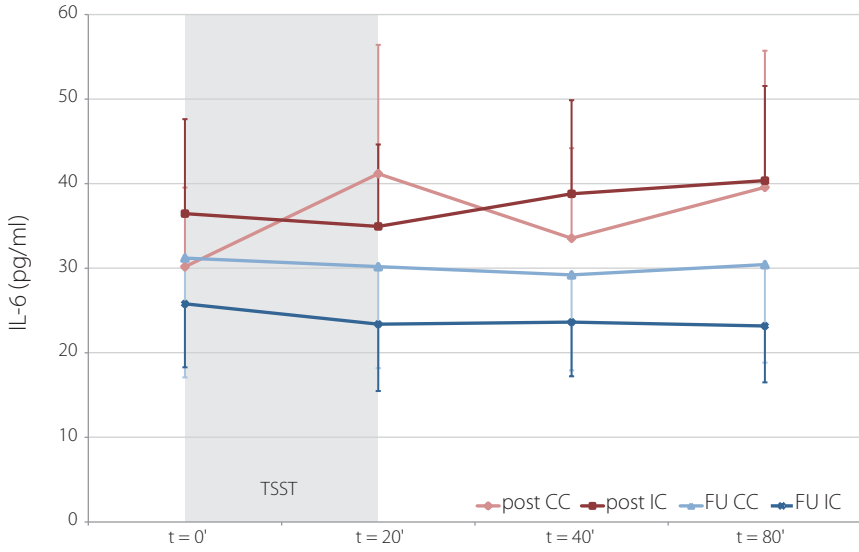
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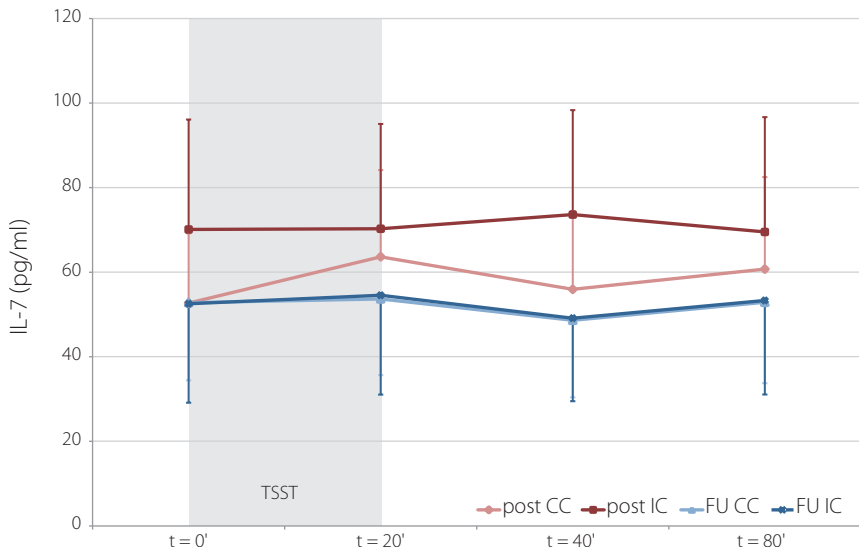
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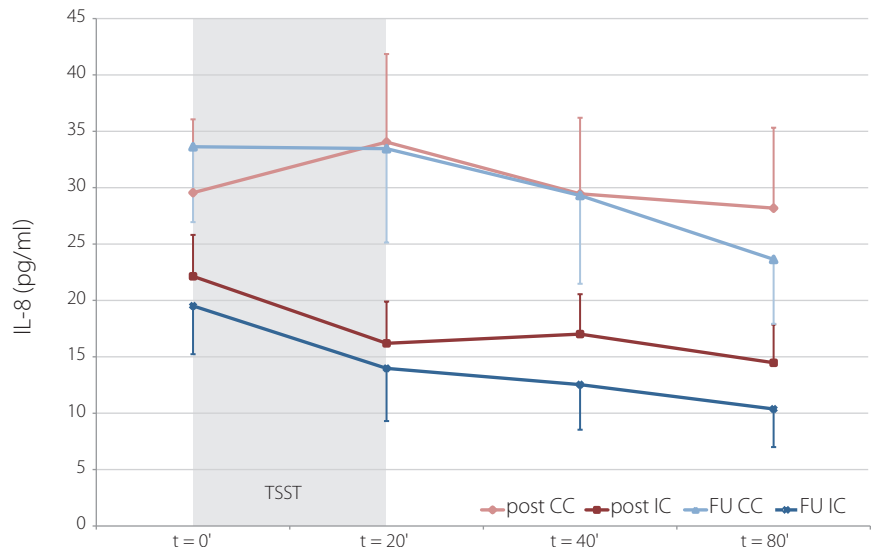
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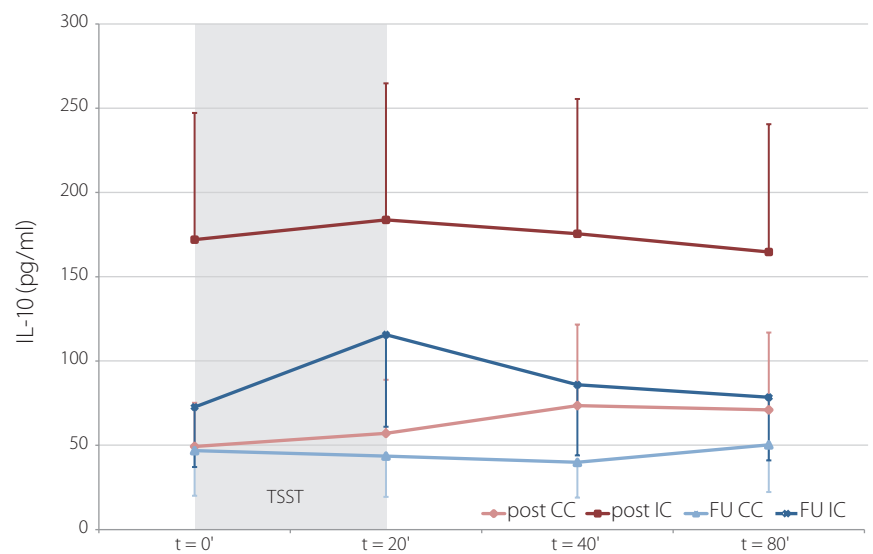
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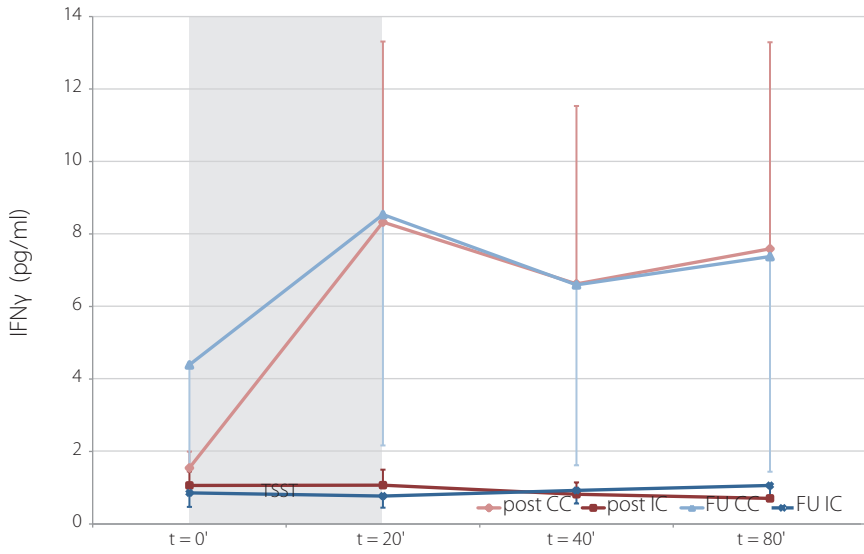
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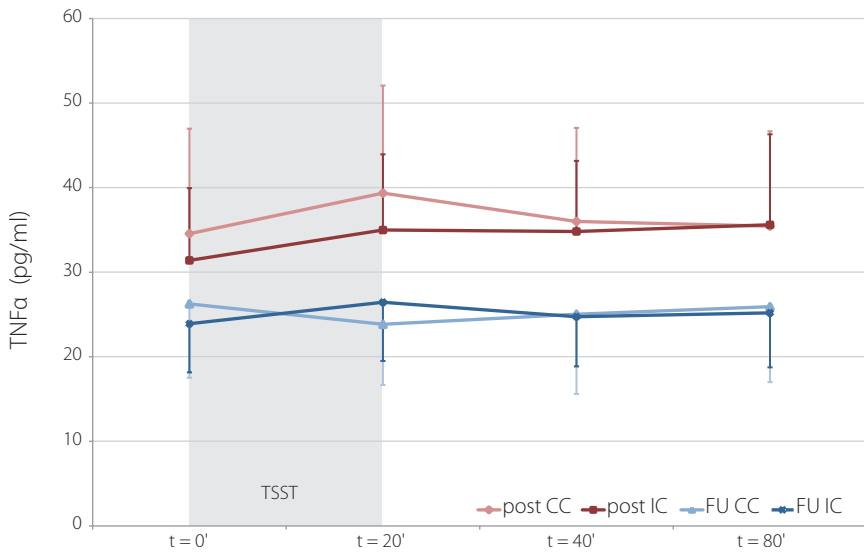
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Chapter 7

Summary



Summary

Stress is one of the factors believed to play a role in the maintenance or exacerbation of chronic inflammatory diseases, such as rheumatoid arthritis (RA) and psoriasis. The physiological stress response system, that is, the ANS and HPA axis, is activated when a stressful situation is encountered. Its bidirectional relationship with the immune system mediates an important link between stress and chronic inflammatory diseases. How stress activates these physiological pathways in patients with inflammatory conditions and to what extent they are altered in the light of chronic inflammation is not yet fully understood. The overall goal of this thesis was to further elucidate the nature of the psychophysiological stress response system of patients with chronic inflammatory diseases. Furthermore, this thesis adds insight into the psychophysiological effects of stress-reducing interventions that are aimed at counteracting the possible detrimental effects of stress on health. This chapter will summarize the main findings of the experimental studies that were conducted.

The first part of this thesis aimed to give a greater insight into the psychophysiological stress response of patients with chronic inflammatory conditions, such as RA and psoriasis, and to investigate whether and how the stress response system might be altered in these patients. We investigated whether patients with different chronic inflammatory conditions differ in their physiological response to stress, and whether patient groups differed in their response to stress from healthy individuals without chronic inflammatory condition.

In Chapter 2, we reviewed the literature on psychophysiological stress reactivity in patients with various inflammatory rheumatic diseases. We extensively summarized and discussed autonomic, neuroendocrine, and immune responses to different types of short-term experimental physical and psychological stress tasks in patients with RA, systemic lupus erythematosus (SLE) and juvenile idiopathic arthritis (JIA). Sixteen studies published between 1985 and 2009 complied with the inclusion criteria and were evaluated. These studies assessed a wide variety of stress induction paradigms, including cognitive stress tasks (e.g., Stroop, arithmetic, memory test), psychosocial stressors (e.g., Trier Social Stress Test), physical exercise (ergometer or treadmill test), or sensory stressors (e.g., cold pressor, heat pulses, acoustic test, or pupillary light flash) in patients and control subjects without inflammatory condition. Overall, there was hardly any evidence that autonomic and neuroendocrine basal function and stress-induced responses were altered in patients compared to control subjects. However, patients showed disease-specific alterations in the immune response to stress compared to control subjects. Clearly, the large heterogeneity of studies with regard to outcome parameters, stress tests, measurement points and patient characteristics thwarted the interpretation of findings and an adequate comparison of studies. In particular, the role of pharmacotherapy, age, sex, and disease severity should be taken into account when evaluating psychophysiological stress responses in future studies.

In Chapter 3 and 4, psychophysiological responses to stress were investigated in patients with two prototypic chronic inflammatory diseases, RA and psoriasis, and in healthy controls. Participants were subjected to a psychosocial stress task (Trier Social Stress Test) and self-reported tension levels, indices of SAM and HPA axis function (salivary α -amylase and cortisol levels, respectively), and immune system parameters (circulating cytokine levels) were measured at baseline and up to 1 hour after cessation of the stress test. In Chapter 3, we found that the salivary cortisol response to stress was significantly increased in patients with psoriasis compared to patients with RA and healthy controls. There were no differences in subjective tension and α -amylase responses to stress among the three groups. Covariate analyses with possible confounders, such as various pharmacotherapies (e.g., biologics, topical or systemic corticosteroids, DMARDs, and NSAIDs), age, sex, BMI, smoking status, menopausal status, and psychological well-being, did not yield different results. In Chapter 4, we reported that all basal cytokine levels, except IL-8, were significantly elevated in patients with RA compared to patients with psoriasis and controls. When controlling for baseline differences in cytokine levels, the IL-1 β and IL-2 response to stress was significantly heightened in patients with RA compared to the other groups. Again, statistically controlling for pharmacotherapies, age, sex, hormonal contraceptives or menopausal status did not change the results. The results of Chapters 3 and 4 suggest a distinct cortisol response to stress in psoriasis and a distinct cytokine response to stress in RA compared to the other groups, but results should be interpreted with caution due to the explorative nature of this study.

The second aim of this thesis was to investigate whether a stress management intervention was able to alter the psychophysiological response to stress in patients with RA. In Chapter 5 and 6, the effects of individual short-term stress management training for patients with RA on overall psychological and physical functioning and on the psychophysiological response to a psychosocial stress task (Trier Social Stress Test) were evaluated. Patients with RA were randomized to either the intervention or the control group, and subjected to the TSST immediately after the intervention and at a 9-weeks follow-up. Measures of psychological and physical functioning were assessed before and after the stress management training and indices of sympathetic, neuroendocrine and immune function (salivary α -amylase, salivary cortisol, and circulating cytokine levels) were measured during and after the two stress tests. After the baseline assessment half of the participants enrolled in the training program. Patients in the intervention group received four individual training sessions in stress management over two consecutive weeks. The sessions were based on psycho-education and the principles of applied relaxation, including progressive, cue controlled, and differential relaxation. In addition, patients were taught breathing and visualization exercises. During the training, participants were encouraged to make homework assignments on a daily basis. After the training, participants were encouraged to continue this practice, as well as to stick to a relapse-prevention program during the time of the study.

In Chapter 5, we showed that the short-term stress management training overall improved psychological functioning (anxiety and positive mood) in patients in the intervention group compared to the control group (post-treatment and follow-up combined). In addition, the training decreased subjective tension and cortisol levels in response to the stress test at the follow-up assessment, but not immediately after the intervention. The effect of the intervention on psychophysiological stress reactivity (e.g., cortisol levels) was particularly evident in patients psychologically at risk (i.e., with heightened levels of psychological distress). In Chapter 6, lower stress-induced IL-8 levels at the follow-up assessment (not immediately after treatment) were reported in patients in the intervention group compared to patient controls, in line with our results on tension and cortisol in Chapter 5. Again, the effect of the training tended to be more pronounced in patients psychologically at risk compared to low-risk patients. When combined, the results of this study indicate that a brief training in stress management is able to improve psychological functioning and alter the neuroendocrine and immune response to acute stress in patients with RA.

To conclude, the results of the studies in this thesis suggest that patients with different chronic inflammatory diseases show a disease-specific psychophysiological response to stress and that brief training in stress management can alter this psychophysiological stress response in patients with RA.

Chapter 8

General Discussion



General discussion

A growing body of evidence shows a link between stress factors and exacerbation of disease severity in a multitude of conditions, including infectious diseases and chronic inflammatory diseases [1-8]. Patients with chronic inflammatory conditions, such as rheumatoid arthritis (RA) or psoriasis, are in danger of slipping into a vicious disease-distress cycle; the pain or itch and limitations of living with a chronic inflammatory disease can result in substantial psychological distress, which may in turn enhance inflammatory activity through activation of the stress response system. This may result in even greater levels of psychological distress with the above-mentioned consequences. An understanding of the mechanisms linking stress and disease and knowledge on how to intervene in this process may eventually help limit the psychophysiological consequences of stressful experiences on health. In the following paragraphs, we will elaborate on the findings presented in this thesis. Methodological considerations will be discussed and directions for future research will be given.

Stress exposure and chronic inflammatory diseases

Chronic inflammation has been suggested to compromise the flexibility of the branches of the stress response system, that is, the autonomic nervous system (ANS) with its sympathetic-adrenal-medullary (SAM) axis and the neuroendocrine system with its hypothalamic-pituitary-adrenal (HPA) axis [2, 9]. As yet, there is no consensus about whether the physiological response to stress is altered in patients with chronic inflammatory diseases compared to healthy controls, and to what extent these potential alterations may be specific for a certain chronic inflammatory condition.

Psychophysiological effects of stress exposure

In this thesis, we report that a brief psychosocial stress task alters the physiological stress response in patients with RA, patients with psoriasis, and a control group of participants without chronic inflammatory condition. Not only did we find an increase in salivary α -amylase levels (an indicator of ANS activation) in the three groups, we also reported a significant rise in salivary cortisol levels (an indicator of HPA axis activation) with a higher increase in patients with psoriasis than in patients with RA and controls (Chapter 3). The stress task was perceived as being stressful, evidenced by significantly increased tension levels, and it activated both the ANS and the HPA axis. Although the stress response system is known to interact with the immune system, circulating cytokine levels were not significantly affected by the stress task. Only when controlling for baseline differences in cytokine levels, we found that patients with RA had heightened stress-induced levels of interleukin (IL)-1 β and IL-2 compared to patients with psoriasis and controls (Chapter 4). Our results suggest that specific markers of the physiological response to stress are altered in chronic inflammatory diseases, which may adversely affect disease status. In our review

in Chapter 2, a consistent increase in cardiovascular and galvanic activity and a rise in catecholamines after stress induction in patients with inflammatory rheumatic diseases was not necessarily followed by changes in immune parameters. Nevertheless, changes in leukocyte and (subsets of) lymphocyte counts, and inflammatory markers C-reactive protein (CRP), tumor necrosis factor (TNF) α , IL-4 and IL-6 were sometimes reported after stress induction (Chapter 2). Apart from these unequivocal effects of stress on immune function in rheumatic populations, little is known about immune effects of acute stress in other chronic inflammatory conditions, such as psoriasis or atopic dermatitis (AD); studies have reported changes in subsets of lymphocytes, immunoglobulin, Th1 cytokines (interferon gamma [IFN γ], IL-2) and Th2 cytokines (IL-4, IL-10) [10-13]. In a meta-analysis combining data of both patient and healthy populations, general effects of acute stress suggest some activation of natural immunity and suppression of specific immunity. In general, the most consistent changes across groups were an increase in natural killer cells and large granular lymphocytes in the circulation, decreased proliferative responses, and increases in pro-inflammatory cytokines IL-6 and IFN γ [14]. How these stress-induced changes in immune function eventually affect disease severity is a question that is complex to answer in studies investigating acute experimental stress, such as ours. Due to ethical reasons, stressors are generally mild in nature and of short duration, and the time that it takes to see alterations in disease severity is usually longer than the time span of the study protocol. Although prospective studies of stress are very well-suited to show a possible association between stress and exacerbation of disease severity [15], controlled experimental studies can infer causal relations between stress exposure and markers of disease progression, but not disease progression itself.

Stress paradigms

When investigating the physiological effects of acute stress in controlled experimental studies, an important decision is which stress paradigm should be applied. The degree of external validity of the stress test, as well its ability to consistently activate the physiological stress response system, should be an important criterion in this decision. For example, in our review covering all controlled experimental studies of acute stress in inflammatory rheumatic diseases, we included 16 studies that applied different types of stressors, including psychological tests (e.g., cognitive tasks), exercise stress (e.g., treadmill), and sensory stressors (e.g., thermal, acoustic or visual stimuli). Psychosocial stress was mostly induced by the short-term Trier Social Stress Test (TSST) or a derivative of this test. We demonstrated that not all stress paradigms necessarily activate the HPA axis in the same way and increase cortisol levels (Chapter 2). Instead, especially stress paradigms containing both uncontrollable and social-evaluative elements have previously been associated with the largest cortisol changes [16]. In our review, psychosocial stress paradigms such as the TSST most consistently activated different levels of the stress response system in rheumatic patients; in our experimental studies, the TSST was also able to effectively activate both

the ANS and HPA axis (Chapter 3), in line with previous reports [17]. With regard to immunological reactivity to acute stress, the effects of different types of stress paradigms on immune function in rheumatic patients were more inconsistent, possibly due to the high heterogeneity in stress induction paradigms, outcome measures, and time points of measurements. However, the paradigms were usually able to generate at least some changes in immune function in rheumatic patients, either in leukocyte or lymphocyte (subset) counts, or specific cytokine levels. These findings are in line with previous reviews suggesting that acute stressors can elicit similar patterns of immune change across a wide spectrum of durations ranging from 5 through 100 minutes, and irrespective of whether they involved social (e.g., public speaking), cognitive (e.g., mental arithmetic), or experiential (e.g., sky diving) forms of stressful experiences across populations [14]. Future studies may further explore possible specificity in the psychophysiological stress response of various stress designs.

Markers of stress and temporal kinetics

When investigating physiological intermediates of stress and disease, there is a wide array of stress markers to choose from. In this thesis, we selected widely-used markers of activation of the ANS and HPA axis, namely, salivary α -amylase and salivary cortisol, respectively. These markers are non-invasive and easily assessed, and possible confounders could be controlled for by asking participants to refrain from using alcohol or caffeine, and from eating, smoking and exercising prior to the experimental session. Nevertheless, these two markers have their weaknesses; even though α -amylase is mainly sympathetic in nature and correlates with cardiovascular measures, it also receives parasympathetic input [18]. In order to differentiate between the parasympathetic and sympathetic branches of the ANS different and separate measures will be required, such as the sympathetically mediated pre-ejection period (PEP) and the parasympathetically mediated high-frequency heart rate variability (HF-HRV) [19, 20]. Moreover, salivary cortisol does not necessarily correlate with plasma cortisol levels [21]. With regard to immunological measures, we measured circulating cytokine levels that are known to be associated with stress and/or disease severity in chronic inflammatory diseases, namely IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFN γ and TNF α . Other cytokine studies of acute stress have measured either similar well-known cytokines in the circulation, or focused on *in vitro* stimulation of immune cells and their cytokine production, or measured activity or numbers of local (e.g., synovial or skin) immune cells [14, 22]. Our choice to explore circulating levels was in part based on the idea that systemic changes may be a general measure of the overall response of the immune system.

Associated with the choice of a specific marker of stress are the temporal kinetics of the chosen parameter; these will determine the time frame of the response and return-to-baseline and are a guideline for the choice of measurement points after stress exposure. While ANS responses, especially cardiovascular or galvanic in nature, will happen

within seconds after perception of the stressor, neuroendocrine changes in HPA axis activity usually take about 20 minutes. In our study, both α -amylase and cortisol showed peak levels 20 minutes (first measurement point after the TSST) to 30 minutes after initiating the stress task. Previous studies are less conclusive about the time frame for immunological parameters, such as circulating cytokines, to respond to stress; a rise in circulating levels of interleukins has been reported to occur within minutes to hours after cessation of a stressor [22]. Overall, cytokine levels in our study usually did not show a clearly defined peak increase or decrease in response to the stress task during our measurement period of up to 1 hour after stress induction. Future studies may examine the response profile of sensitive markers of acute stress in more detail.

Disease-specific effects of stress

Both disease-specific and generic effects of stress on health may aggravate disease severity in patients with a chronic inflammatory disease. In this thesis, we have reported some disease-specific differences in physiological stress reactivity across chronic inflammatory diseases. These could either represent differences in the stress response between patients and controls, or differences between patient groups. In our review, we were not able to delineate consistent disease-specific differences in autonomic or neuroendocrine markers of stress in patients with various rheumatic disorders, but immune reactivity to stress seemed to be specific in these populations; patients with systemic lupus erythematosus (SLE) showed less pronounced changes in lymphocyte (sub)populations compared to controls, and patients with RA, SLE and juvenile idiopathic arthritis (JIA) had increases in CRP and TNF α (RA), IL-4 (SLE) and IL-6 (JIA), that were not observed in control subjects. In contrast, patients with RA or SLE lacked the stress-induced increase in IFN γ and IL-10 observed in controls. Nevertheless, most studies did not compare patient groups, but only included a control group. Only once it was suggested that patients with RA and SLE may differ in their IL-4 response to stress. In our stress exposure study, the cortisol response to stress was heightened in patients with psoriasis compared to RA and controls, while patients with RA showed heightened immune reactivity (IL-1 β and IL-2) compared to the other two groups, but only when controlling for baseline differences in cytokine levels. These and other studies with patients with chronic inflammatory diseases, such as psoriasis and atopic dermatitis (AD) [10-13] support the hypothesis of possible disease-specific physiological stress reactivity patterns, which may in turn affect the course of chronic inflammation. However, a lack of consistency in study designs, patient samples, outcome measures, and measurement points thwarts generalization of the findings.

To our knowledge, our study was the first to report a heightened cortisol response to a stress task in psoriasis. Usually, chronic inflammatory or psychopathological conditions have been associated with blunted neuroendocrine stress response profiles. In chronic stress and pain conditions, the HPA axis is thought to be activated continuously, which

may increase feedback sensitivity of corticosteroids. This could lead to hypo-function and -reactivity of the HPA axis and desensitization of the immune system to glucocorticoids, increasing inflammation. It is complex to determine the exact immune effects of hypo- or hyperactivity of the stress response system, because they may differ as a function of location (local versus systemic) and type of immune measure. For example, although cortisol is known to be immunosuppressive, a rise in cortisol in response to stress may lead to local activation of the immune system; activation of the corticotropin-releasing-hormone (CRH)-mast-cell-histamine axis leads to heightened levels of peripherally produced CRH. CRH can act as a local pro-inflammatory agent and stimulate mast cells to release histamine and the pro-inflammatory cytokines TNF α and IL-6, which can boost inflammation [23]. When cortisol reactivity to stress is heightened compared to the response of healthy controls, as we observed in our psoriasis sample, this may result in heightened local activation of the CRH-mast-cell-histamine axis, a more pronounced local pro-inflammatory state, and a larger release of pro-inflammatory cytokines TNF α and IL-6. However, differences in stress-induced TNF α and IL-6 levels were not found in our study, which suggest that other mediators and/or pathways may be involved in the link between stress and disease.

In contrast, patients with RA did not show altered cortisol reactivity, but disease-specific alterations in certain inflammatory markers after stress when controlling for basal cytokine levels; i.e., heightened levels of IL-1 β , involved in innate immune responses, and IL-2, a cytokine of the adaptive immune system. Results suggest that a stress-induced rise in cortisol may be accompanied by a relatively bigger increase in specific immune markers in RA than in controls and patients with psoriasis; an increase that may further fuel the chronic inflammatory state observed in RA. A possible explanation could be that part of the signaling cascade from cortisol to the immune system, for example corticosteroid receptor sensitivity or density, may be altered in patients with RA [24-26]. The significance of alterations in these cytokine responses to stress and their possible clinical value still remains to be elucidated. Acute stress-induced changes in cytokine levels probably do not only reflect *de novo* protein synthesis or clearance, especially when responses occur within minutes after stress exposure, but may also indicate a redistribution process of immune cells releasing these cytokines from or into the periphery, with fluctuations being either adaptive or maladaptive [22].

In sum, preliminary data show disease-specific changes in the acute psychophysiological response to stress in patients with RA and patients with psoriasis; changes that may augment the possible damaging effects of stress on health. The significance of (disease-specific) alterations in stress physiology and their link to aggravating disease severity clearly requires further elucidation, but interventions that decrease psychological distress in patients may be fruitful to moderate these effects.

Psychological stress-reducing intervention and stress exposure

Although a substantial part of patients with a chronic inflammatory condition face psychological and physical problems, such as physical disability, pain and fatigue, intervention studies have proven to be modestly successful in improving psychological and physical functioning in these patients as adjunct to pharmacotherapy [27-31]. As stress factors have been related to disease flare-ups, specific interventions aimed at reducing stress reactions may prove an adjunct therapy in relieving symptoms of disease. Several studies in healthy participants showed that interventions aimed at decreasing levels of distress, such as muscle relaxation and guided imagery, can also alter autonomic and neuroendocrine activity [32, 33]. The effect of psychological interventions on autonomic, neuroendocrine, and immune function was also evidenced in several clinical populations, such as in patients with HIV and cancer [34-40]. In addition, there have been reports of changes in basal physiological parameters after psychological interventions in patients with RA, such as in cortisol, levels of interleukin (IL)-6 or IFN γ , or immune measures indicative of disease status, such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) [41-44]. To our knowledge, the study in this thesis is the first to investigate stress-induced autonomic, neuroendocrine, and immune responses after stress management training in patients with RA.

Psychophysiological effects of a stress management intervention

After a brief stress management training, patients with RA showed a significant overall reduction in levels of distress (specifically anxiety) and higher levels of positive mood compared to the control group (Chapter 5). Moreover, after stress exposure, stress-induced subjective tension, and cortisol and IL-8 levels were significantly lower in the intervention than in the control group, but only at the follow-up assessment 9 weeks after the intervention (Chapter 5 and 6). These results suggest that a brief psychological intervention is able to influence the psychophysiological response to stress and may help prevent the possible detrimental effects of stress on health.

Stress arises when the environmental demands outsource individual resources, resulting in the activation of the stress response system in order to deal with the stressor and restore homeostasis. In order to change the physiological response to a stressor or situation, intervening on the level of individual resources may prove rewarding to shift the balance between demands and resources. We specifically designed a short-term stress management training of four individual sessions during two consecutive weeks with a trained therapist, in which different protocols to deal with stress were put forward. The first focus of the brief intervention was on psycho-education; patients were informed on how stress and tension may exacerbate physical symptoms and disease severity. Secondly, the principles of applied relaxation were taught, including progressive, cue-controlled, and differential relaxation. Breathing exercises and visualization exercises were also added to the repertoire of relaxation exercises. Homework assignments and relaxation exercises

were regularly practiced during the two weeks of the intervention. Home work assignments included the assessment of stress-relevant situations and behaviors in daily life. During the two-month follow-up period, participants were encouraged to keep practicing their exercises and assignments, as well as to stick to a relapse-prevention program and focus on long-term goals in order to consolidate what they had learnt. Most patients (87 percent) in the intervention group reported improvement in their stress and tension levels after the training; 45 percent of patients even reported moderate to strong improvements. In addition, patients in the intervention group had significantly lower anxiety scores and scored higher on positive mood after the training than patients in the control group, demonstrating that the intervention positively affected distress levels.

With a relatively small number of intervention studies investigating the physiological effects of psychological treatment in patients with RA, the study in this thesis aimed to add knowledge on this topic. Importantly, and as we hypothesized, the stress management training did not alter parameters of basal physiological activity, but only diminished stress-induced physiological reactivity. Although we did not see alterations in the psychophysiological stress response immediately after treatment, patients in the intervention group were significantly less tense and had a lower cortisol response to stress at follow-up. In line with this, stress-induced IL-8 levels were lower in these patients at the follow-up. The delayed effect at the follow-up on stress physiology observed in our study may have occurred because patients were encouraged to keep practicing their exercises and stick to a relapse-prevention program during the two months prior to the last assessment; this time could have been necessary to integrate all that was learnt in daily life and to consistently and substantially change the way of coping with stressful situations.

With the design presented in this thesis, we were able to demonstrate that a brief stress management intervention was able to alter both subjective and neuroendocrine parameters of acute stress, namely tension and cortisol levels, as well as change stress-induced immune parameters, namely circulating IL-8 levels. To our knowledge, the application of a Trier Social Stress Test after a psychological intervention has rarely been investigated [45, 46]. The combination of stress management training followed by stress exposure enables investigating the effects of a psychological intervention on the stress response when patients are challenged to cope with a real-life stressful situation; this study design offers the opportunity for new insights into the link between stress and disease. In the few studies that used this design, healthy students were randomized to receive a two-day group-based stress inoculation training before or after a TSST. Participants of the training reported having a lower stress appraisal and lower cortisol levels in response to the stress task; an effect that was also observed when the stress test was performed four months after the training. Results of these and our studies suggest that cortisol responses to stress can be altered through training in stress management, both in healthy subjects and in patients. Moreover, to our knowledge our study was the first to investigate and show potential intervention-related changes in immune function.

Stress-induced tension and cortisol levels were reduced by the training, but not the α -amylase response to stress. This is unexpected, because the stress management training was aimed at reducing overall arousal and sympathetic activity. Previously, no significant association between cortisol and α -amylase reactivity to stress was reported [47, 48], suggesting that (different types of) stress not necessarily activate(s) parameters of stress in a similar way. The training may have specifically lowered the cortisol response to stress by changing negative emotions and/or cognitions that are associated with social threats, such as fear of losing social approval [16]. Future studies of stress should experiment with modulating negative as well as positive emotions and cognitions to establish more pronounced autonomic and neuroendocrine changes in the psychophysiological response to stress.

The implication of the decrease in IL-8 levels is as yet unknown. IL-8 acts as an acute player in exacerbation of inflammation and is related to perceived levels of stress attributed by negative affect [49, 50]. Although cortisol and IL-8 levels were not significantly related, the decrease in levels of IL-8 could indicate that it is a general marker of stress reactivity, and not necessarily a disease-specific marker of RA. This is supported by results of our first study that showed that patients with RA, patients with psoriasis, and healthy controls did not differ in basal IL-8 levels and in the IL-8 response to stress (Chapter 4), which suggests that IL-8 may respond in a similar fashion across different (healthy and medical) populations.

Proof of principle vs. clinical relevance

Our findings evoke the question of the clinical relevance of the observed alterations in stress physiology, especially since disease activity did not significantly change during the course of the study in both groups. In fact, so far, there has been ample evidence of psychological interventions that change clinical measures, such as overall disease activity [41, 51], disease flare-ups [30] and joint tenderness [52]. With four 1-hour sessions during two consecutive weeks, our intervention was very limited in time and content, but even with this brief training we were able to induce psychological and physiological changes in patients with RA. Since there are many reports of a temporal relationship between elevated levels of pro-inflammatory cytokines and symptoms of RA, such as morning stiffness and radiographic progression [53, 54], it seems likely that changes in psychophysiological stress reactivity (e.g., cortisol and cytokines) can eventually affect the course of disease activity in these patient groups. However, this study was meant as a proof of principle to see whether it would be possible to influence various physiological parameters of stress and disease by means of psychological intervention. Treatment effects could have easily been masked by biological forces, such as disease flare-ups and pharmacological treatments that affect the neuroendocrine and immune system, although we tried to limit these effects through randomization and by taking into account several possible confounders, including the patient's disease status. Most likely, longer and more intense interventions will produce more pronounced effects on stress physiology and possibly also on disease progression.

Risk groups

The stress management training had more pronounced effects in patients with a psychological risk profile showing with heightened levels of distress, than in patients without this profile. These effects were found on general psychological functioning (anxiety and negative mood) and physiological stress reactivity at a follow-up assessment; levels of stress-induced subjective tension, α -amylase and cortisol decreased more in high-risk patients than in low-risk patients in the intervention group. There also tended to be a risk group effect for the response of IL-8 at follow-up; stress-induced IL-8 levels had decreased more in high-risk patients than in low-risk patients in the intervention group. Thus, stress management training may be particularly beneficial for patients psychologically at risk. Previous research already indicated the importance of evaluating psychological risk factors when evaluating treatment outcome [55-58]. Prospective studies with patients with RA or with another chronic inflammatory condition, such as psoriasis, revealed that patients with high levels of worrying were most vulnerable to the impact of stressors on their disease severity, especially in highly stressful circumstances [15, 59, 60]. If patients with psoriasis reported their disease to be stress-reactive, they scored higher on disease activity than patients who did not consider themselves reactive to stress [7, 61]. Moreover, psychological risk factors, such as depression, influenced immune function in patients with RA when they felt stressed [56]. Overall, these studies, including our own, suggest that especially patients vulnerable to psychological stress factors may particularly benefit from psychological treatment and its effects on psychophysiological stress reactivity.

Methodological considerations

The studies presented in this thesis were subject to a number of limitations and methodological considerations.

Threats to external validity: generalization across types of stressors

In this thesis we used the TSST, an acute time-limited psychosocial experimental stressor that activates the ANS, HPA axis and immune system [14], to investigate acute physiological responses to stress. The most obvious critical note that can be put forward is to what extent the effects of acute stress are comparable to the wide variety of stressors encountered in daily life, including major life events (for example, death or divorce), chronic stressors (for example, living with a disabling disease) or minor or more acute daily stressors, such as daily hassles (for example, losing your keys). Stress research can take different conceptual and measurement approaches, which include a wide range of psychosocial stressors varying on important dimensions such as major or minor events, acute or chronic stressors, or a sudden or gradual onset [14, 62]. In the case of major events, intense release of stress mediators over a large time interval will occur, while in the case of minor events, only short-lived surges of neurotransmitters and hormones are expected [2]. Consequently, different types of stressors are associated with specific stress physiology

profiles, which may have different or even opposite effects on health status in patients with chronic inflammatory conditions. Although laboratory stressors allow control of key factors in the delivery of stress and observation of its effects, they have their limitations. Those limitations can be reduced by choosing a stress paradigm that simulates a real-life stressful situation, such as the psychosocial evaluation that is part of the Trier Social Stress Test.

Threats to external validity: generalization across patient samples

Patients in our studies were recruited from the participating departments of two regional hospitals. Participating patients had longstanding and relatively mild disease activity compared to the general population of outpatients with RA or psoriasis. Patients in this study were monitored by specialists of hospitals who aim to stabilize the disease or bring it into remission. In addition, all participants were self-selected volunteers of a study that was quite demanding in terms of repeated visits (especially for RA patients in the stress management condition), time spent at the hospital, medical assessment, blood drawings, saliva collection, and stress tasks. Moreover, we applied rather strict exclusion criteria with regard to physical and psychological co-morbidity. All in, this may have resulted in a bias of relatively mobile patients with well-managed disease activity or severity. Consequently, the patient samples of our study are not representative of regular outpatients with RA or psoriasis and therefore results may not be generalizable to patients with more severe disease activity. Patients with severe disease activity will have more inflammatory activity, local and/or systemic, which may be associated with altered ANS and HPA axis function [63, 64] and altered psychophysiological responses to stress [65-67]. In addition, because patients with recently diagnosed RA may show basal HPA axis function different from patients with longer-standing RA [64, 68], differences in the physiological response to stress can be expected. In order to fully comprehend psychophysiological stress responses in patients with chronic inflammatory diseases, including RA and psoriasis, patients with a wider range of disease activity and other characteristics of representative patient groups should be investigated.

Threats to internal validity: confounding factors and pre-test differences

When studying a population or comparing groups of patients, there is the following dilemma: if homogenous and comparable groups are created in order to rule out possible confounders, for example by using strict exclusion criteria, this will result in a study population that is not representative. Alternatively, when aiming for a representative and ecologically valid study sample, groups will be highly heterogeneous and differ on a wide range of possible confounders. In our studies, there were some significant pre-test differences between groups. In our study investigating the stress response of patients with RA, patients with psoriasis and healthy controls, there was a significant difference in the female-to-male ratio and the BMI across the three groups. Moreover, patients with RA

or psoriasis used different types of treatment medication that are designed to influence the immune system. For example, many more patients with RA than patients with psoriasis used biologics, DMARDs, and NSAIDs. Half of the patients with psoriasis used topical corticosteroids, which probably influence systemic physiological processes to a lesser extent than systemically administered corticosteroids, as used in RA. In addition, there were some small differences between groups in our stress management study; the female-to-male ratio, basal levels of anxiety, and the use of hormonal contraceptives tended to be different between the two groups, and there was a significant difference in the use of NSAIDs. It is well-known that all these factors can influence neuroendocrine and/or immune function [17]. In order to control for the possible influence of pre-existing differences between groups on outcome measures we used the following strategies. In our study investigating disease-specific differences in physiological stress reactivity, we tested all possible confounders that could affect an outcome measure by introducing them one-by-one as a covariate into the statistical models. Those confounders included all types of medications, sex, age, BMI, smoking status, menopausal status, and different indicators of psychological well-being. Results of the covariate analyses were reported when a confounder was significantly related to the outcome variable. In addition, the randomized nature of our stress management study made us decide to include all confounders that tended to differ or differed significantly between the two groups as covariates in the statistical model. The results of both studies indicate that, despite some pre-existing differences between groups and some significant correlations between these particular confounders and outcome measures, controlling for the confounders did not change our main findings. Although results of our studies suggest no or limited effects of confounders, it should be considered to use a matching strategy or a stratified randomization procedure in future studies of stress in order to yield more homogeneous groups with regard to known confounding factors and eliminate as much bias as possible.

Statistical analyses

In our studies, we separately investigated group effects on basal physiological activity and stress-induced physiological reactivity. First, we analyzed whether there were possible differences in basal levels of physiological outcome parameters (i.e., did patients and controls differ in basal physiological activity (Chapter 3 and 4) or did the stress management training result in altered basal physiological activity (Chapter 5 and 6)). Second, we specifically compared groups with regard to their physiological reactivity to a stress task. For example, in our stress management study we hypothesized that the benefits of the training would become particularly evident when participants were challenged to cope with a stressful situation, with subsequent changes in physiological reactivity. Depending on the specific characteristics and assessment methods of the outcome measures, we tested whether groups differed on the combined post-stress measurement points (immune measures) or with regard to the response over time (other psychophysiological

measures), with basal levels as a covariate in the statistical model. With this model we compared the level of the response after stress, while controlling for possible differences in baseline values.

In addition, the sample size of our studies was relatively small to perform statistical subgroup analyses, particularly considering multiple testing. Prompted by the small sample size and the high intercorrelation of most cytokines, corrections for multiple testing, such as Bonferroni, were not applied in our explorative cytokine analyses. Overall, the studies in this thesis, especially those investigating cytokine responses, were explorative in nature and -clearly - results should be interpreted with caution and need replication in larger-sized samples.

Study design

In our stress management study, we applied the TSST two times in the same patient population. Because repeated exposure to the TSST leads to habituation of the neuroendocrine response to stress in the majority of people [69, 70], we decided against assessment of stress reactivity prior to the stress management training. Nevertheless, the effect of the stress management on cortisol and IL-8 levels were relatively small. Considering that the benefits of the training seem to become visible over time, future studies may need to apply stress induction paradigms such as the TSST only once, at a follow-up assessment, in order to capture the effects of the training most effectively [46]. In addition, we were unable to blind patients and researchers for the treatment status (control or intervention group) of participants. We could only blind the research members that conducted the stress test for the treatment status of the participant. Furthermore, our stress management training was relatively short in duration and limited in content. Although even this brief intervention was able to alter physiological parameters of stress, physiological changes of a sufficient magnitude may be established by interventions with a larger content and number of sessions.

Future directions

Our studies aimed to further elucidate how stress may be linked to chronic inflammatory diseases. They suggest that patients with a chronic inflammatory condition, such as RA and psoriasis, may show specific neuroendocrine and immune responses to stress. In addition, we showed that short-term training in stress management not only improves psychological functioning in patients with RA, but is also able to alter the acute psychophysiological response to a stressor. Based on the results of our studies, future studies may clarify this relationship in more detail.

Stress test measures and design

First of all, future studies of experimental stress should replicate findings in larger cohorts of patients in order to disentangle the role of specific pharmacotherapy regimens, sex

(hormone)-related factors, and psychological risk profiles on physiological stress reactivity. Those larger studies would also allow correction for multiple testing. In addition, a wider variety of outcome measures should be assessed within a larger time window to measure stress responses that include a return-to-baseline.

With regard to physiological parameters, studies should include not only measures of both the sympathetic and parasympathetic branches of the nervous system, such as pre-ejection period (PEP), total peripheral resistance (TPR), and HF-HRV, but also neuroendocrine measures other than those of the HPA axis, such as prolactin, growth hormone (GH), or parameters representing communication between the neuroendocrine-immune system or the ANS-immune system, such as the number or sensitivity of corticosteroid or β -adrenergic receptors. The selection of immune measures should largely depend on the subject population being studied, with measures being relevant for disease progression [71]. It may prove fruitful to broaden the scope beyond measuring the major and well-known cytokines involved in many immune processes, such as IL-1 and TNF α , and integrate recent immunological insights. For example, IL-17A and IL-23 are two important cytokines involved in the disease process of psoriasis and RA, but these more recently discovered cytokines have remained largely unexplored in psychological stress research so far [72, 73]. Furthermore, immune system function can be measured by means of *in vitro* techniques and/or local measurements, in addition to more systemic approaches. For example, synovial cytokine levels or production by *in vitro* stimulated immune cells collected from the synovium and synovial fluid after stress induction may give insight into the effects of stress on local physiology. *In vitro* cell stimulation may especially be appropriate for parameters that are difficult to measure in the circulation, such as levels of IL-1 in healthy participants.

With the growing interest in the physiological effects of psychological interventions for all kinds of patients, the large heterogeneity of studies -mainly in terms of composition of the study groups, outcome measures and assay methods, timing of samples, type of stress paradigm, and type of intervention used - require reviews and meta-analyses that summarize and string together data to help us decide which steps to take next.

Psychophysiological effects of stress management and its clinical relevance

The effects of our brief intervention on psychophysiological stress reactivity may be amplified in full-blown interventions of longer duration and larger content. These more extensive interventions may not only have increased effects on ANS and HPA axis measures, but may also affect the immune system more effectively, for example, by producing changes in cytokine levels or other immunological parameters beyond their broad normal range. Moreover, selecting parameters of physiological processes that unfold over a longer period of time and are not susceptible to short-term perturbations may prove fruitful specifically for training in stress management [71]. Longer follow-ups may be needed to discern possible changes in disease activity in the long run.

Various cortical and limbic regions are involved in the processing of potentially threatening stimuli, such as psychosocial stressors. Modifying these processes may therefore affect the psychophysiological response to a potentially stressful situation [45]. For example, meta-analyses have demonstrated that cortisol and immune alterations particularly emerge when people show relevant changes in specific cognitions and emotions that are associated with imminent stressors [16]. Future research should use this information to build specific intervention modules that will produce clinically relevant and significant changes in psychophysiological stress reactivity. In line with this, acute physiological stress responses have also been related to individual differences in psychological distress, coping styles, and cognitive appraisal [70, 74-77]. Future research needs to disentangle which factors are most relevant to identify subgroups of patients at risk for heightened stress reactivity, who are most likely to benefit most from stress management interventions. An important new step in stress research will be to determine the clinical relevance of alterations in physiological stress reactivity among patients with chronic inflammatory diseases and after psychological intervention. This is best determined in longitudinal prospective studies that can test whether patients with specific psychophysiological responses are at a higher risk for future disease exacerbations and whether interventions are able to limit these effects on the disease status. Moreover, a greater insight into which specific subgroups of patients are most at risk and may benefit most from psychological interventions could eventually lead to the development of screening tools for these patients and the implementation of this approach in clinical guidelines. All in, new and challenging steps have to be taken to increase our knowledge on how stress affects chronic inflammation and whether and how this process can be counteracted.

Pathways to influence psychophysiological stress reactivity

In this thesis, we aimed to influence the psychophysiological stress response in patients with a chronic inflammatory condition through training in stress management. This training helped to reduce the perception of stress and diminish the activation of markers of the physiological stress response system. Another entrance to influence the psychophysiological stress response could be to tackle the possible inadequate release of hormones, for example stress-induced cortisol output. The aim subsequently shifts from reducing the perception of a stimulus as being stressful -and thereby indirectly reducing a physiological response to stress - to directly influencing the physiological stress response. For example, single high cortisol administration in humans is suggested to facilitate coping with stress through adaptive regulation of automatic cognitive processing [78]. Future studies of stress may focus on directly altering the physiological stress response in patients with chronic inflammatory conditions. This could, for example, be tested in an experimental setting by administering cortisol prior to a challenging situation, such as the TSST. In clinical studies, however, it has to be taken into account that patients with chronic inflammatory conditions may show disturbances in HPA axis function or neuroendo-

crine-immune interactions, and that exogenous cortisol administration prior to stress may not necessarily have beneficial effects on the disease process, especially since both too much and too little HPA axis (re)activity is linked to disease and health complaints [79].

Since preliminary evidence also suggests that endocrine and immune function are subject to the effects of conditioning [80], future studies of stress may eventually investigate whether conditioning paradigms can help in modulating psychophysiological stress reactivity in patients. Altogether, combining pharmacological treatment with psychological intervention seems a promising line of future research that may help to elucidate the complex puzzle of psychophysiological stress reactivity and disease outcome.

Concluding remarks

The goal of this thesis was to further elucidate the nature of the psychophysiological stress response system of patients with chronic inflammatory diseases. First, we aimed to increase knowledge on possible disease-specific patterns in the psychophysiological response to stress of patients with the prototypic chronic inflammatory diseases RA and psoriasis; patterns that may add to an aggravation of disease symptoms in times of stress. We reported that patients with psoriasis showed an increased cortisol response to a psychosocial stress task compared to patients with RA and controls, whereas patients with RA showed higher stress-induced IL-1 β and IL-2 levels compared to the other groups when controlling for basal cytokine levels. Disease-specific immune reactivity to stress was also reported in our review on patients with inflammatory arthritis. Second, we aimed to gain a greater insight into the psychophysiological effects of stress management interventions. A stress management intervention was overall able to decrease psychological distress in patients with RA, and decrease the psychophysiological response to a psychosocial stress task at follow-up; stress-induced tension, cortisol, and IL-8 levels were lower in the intervention than in the control group, with effects being particularly evident in patients with RA who are psychologically at risk. Results suggest that patients with a chronic inflammatory condition have a disease-specific stress response profile and that a psychological intervention is capable of altering the psychophysiological response to stress in patients. The findings reported in this thesis may be substantiated by further research into the psychophysiological effects of stress on chronic inflammatory conditions and how to counteract these effects with psychological stress-reducing treatments. In the future, this may lead to the development of psychological interventions that are a valuable adjunct to regular medical treatment to control the effects of stress on chronic inflammation for patients psychologically at risk.

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List of Abbreviations

(s-)AA	(Salivary) alpha amylase
ACh	Acetylcholine
ACTH	Adrenocorticotropin hormone
AIMS	Arthritis impact measurement scales
AN(C)OVA	Analyses of (co-)variance
ANS	Autonomic nervous system
AR	Adrenergic receptor
AUCG	Area under the curve with respect to ground
BP	Blood pressure
CA	Catecholamines
CBT	Cognitive-behavioral therapy
CC	Control condition
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
CRP	C-reactive protein
CV	Coefficients of variation
DAS28	Disease activity score 28
DHEA(-S)	Dehydroepiandrosterone (sulfate)
DM	Diabetes mellitus
DMARDs	Disease-modifying antirheumatic drugs
EMM	Estimated marginal means
EPI	Epinephrine
ESR	Erythrocyte sedimentation rate
GH	Growth hormone
HC	Healthy controls
HF-HRV	High-frequency heart rate variability
HPA axis	Hypothalamus-pituitary-adrenal axis
HR	Heart rate
IC	Intervention condition
IFNγ	Interferon gamma
IGF-I	Insulin-like growth factor 1
(s-)IL	(Soluble) interleukin
IRGL	Impact of rheumatic diseases on general health and lifestyle
JIA	Juvenile idiopathic arthritis
LMM	Linear mixed model
MTX	Methotrexate
NE	Norepinephrine
NK cell	Natural killer cell
NPY	Neuropeptide Y
NSAIDs	Nonsteroidal anti-inflammatory drugs
NTR	Netherlands trial register
OA	Osteoarthritis
PASI	Psoriasis area and severity index
PEP	Pre-ejection period
PRL	Prolactin
PS	Psoriasis
PSNS	Parasympathetic nervous system
RA	Rheumatoid arthritis
SAM axis	Sympathetic-adrenal-medullary axis
SC	Skin conductance
SD	Standard deviation
SEM	Standard error of the mean
SLE	Systemic lupus erythematosus
SNS	Sympathetic nervous system
SUD	Subjective unit of distress
SVR	Systemic vascular resistance
TNFα	Tumor necrosis factor alpha
TPR	Total peripheral resistance
TSST	Trier Social Stress Test
VAS	Visual analogue scale

Samenvatting

Chronische ontstekingsziekten, zoals reumatoïde artritis (RA) en psoriasis, gaan gepaard met met langdurige ontstekingsprocessen in het lichaam. RA is een chronische ontstekingsziekte van de gewrichten. Deze gewrichtsontstekingen leiden vaak tot schade aan kraakbeen en botweefsel en de ziekte gaat dan ook vaak gepaard met lichamelijke, psychische en sociale klachten, zoals pijn, moeheid, functionele beperkingen, angst en depressieve klachten. Psoriasis is een chronische ontstekingsziekte van de huid. De ziekte kenmerkt zich door afgebakende rode plaques met witte huidschilders. Patiënten met psoriasis hebben vaak lichamelijke symptomen, zoals jeuk en moeheid, maar ook andere beperkingen in het dagelijks functioneren, waaronder psychische klachten, zoals angst en depressieve stemming. Een van de factoren die een rol speelt bij het instandhouden en verergeren van chronische ontstekingsziekten zoals RA en psoriasis is stress. Wanneer iemand stress ervaart, wordt het psychofysiologische stresssysteem geactiveerd. Dit systeem bestaat uit twee takken: het autonome zenuwstelsel wordt geactiveerd (de hartslag versnelt, je gaat transpireren, etc.) en de afgifte van stresshormonen wordt gestimuleerd. De beide takken van het psychofysiologische stresssysteem communiceren met het immuunstelsel en kunnen op deze manier de relatie tussen stress en chronische ontstekingsziekten verklaren.

Het is nog niet precies duidelijk hoe stress het psychofysiologische stresssysteem activeert in patiënten met chronische ontstekingsziekten en in hoeverre dit stresssysteem -wellicht ziektespecifiek- is veranderd in patiënten met een chronische ontstekingsziekte en ten opzichte van mensen zonder deze aandoening. In deze thesis werd een bijdrage geleverd aan de beantwoording van deze vragen. Bovendien werd er onderzocht in hoeverre het mogelijk is om de psychofysiologische effecten van stress te verminderen door patiënten met een chronische ontstekingsziekte een interventie te geven die gericht is op het verminderen van stress. De belangrijkste bevindingen uit deze thesis worden hieronder beschreven en bediscussieerd.

Na een inleidend hoofdstuk (Hoofdstuk 1) werd in deel 1 van deze thesis (Hoofdstuk 2, 3 en 4) ingegaan op de vraag hoe de psychofysiologische stressreactie eruit ziet van patiënten met verschillende chronische ontstekingsziekten. In Hoofdstuk 2 vatten we de literatuur over stressreacties van patiënten met reumatische ontstekingsziekten samen. We keken naar de autonome, neuroendocriene en immuunreacties op verschillende typen kortdurende fysieke en psychologische stresstaken patiënten met RA, systemische lupus erythematoses (SLE) en patiënten met juveniele ideopathische artritis (JIA). De 16 studie die voldeden aan onze inclusiecriteria lieten zien dat er weinig bewijs was voor veranderde basale of stress-gerelateerde autonome en neuroendocriene waarden. Echter, patiënten lieten wel ziekte-specifieke veranderingen zien in de immuunreactie op stress vergeleken met controles. De vergelijking tussen de verschillende studies werd echter

bemoeilijkt door de grote heterogeniteit van de studies, onder meer qua uitkomstmaten, stresstesten, meetpunten en patiëntengroepen.

In Hoofdstuk 3 en 4 werd de psychofysiologische stressreactie onderzocht van patiënten met de prototypische chronische ontstekingsziekten RA en psoriasis en van gezonde controles. Vóór, gedurende en na een psychosociale stresstaak (de Trier Social Stress Test; TSST) werden zelf-gerapporteerde spanning en maten voor autonome (speeksel α -amylase), neuroendocriene (speekselcortisol) en immuunactiviteit (cytokines in het bloed) afgenomen. In Hoofdstuk 3 rapporteerden we de resultaten van de autonome en endocriene reacties en vonden we een significant verhoogde cortisolreactie op de stresstaak in patiënten met psoriasis, terwijl de andere parameters niet verschilden tussen de drie groepen. De resultaten veranderden niet als er gecontroleerd werd voor verschillende farmacotherapieën, leeftijd, sexe, BMI, roken en menopauze. In Hoofdstuk 4 rapporteerden we de immuunreacties en vonden we als belangrijkste resultaat dat de IL-1 β en IL-2 waarden na stress verhoogd waren in patiënten met RA vergeleken met de andere twee groepen. Ook hier veranderden de resultaten niet nadat we hadden gecontroleerd voor farmacotherapieën, leeftijd, sexe of menopauze. De resultaten van hoofdstuk 3 en 4 suggereren dat patiënten met psoriasis een specifieke cortisolreactie op stress laten zien, terwijl patiënten met RA een specifieke cytokinereactie na stress laten zien vergeleken met de andere groepen. Terughoudendheid in de interpretatie van deze resultaten is echter gewenst, gezien het exploratieve karakter van deze studie.

Het tweede doel van deze thesis (Hoofdstuk 5 en 6) was te onderzoeken in hoeverre een stressmanagement training in staat is om de psychofysiologische stressreactie te beïnvloeden in patiënten met RA. Patiënten met RA werden gerandomiseerd naar de interventiegroep of de controlegroep. Er werd gekeken naar de algemene effecten van een individuele korte stressmanagement training voor RA patiënten door het algemeen psychologisch en lichamelijk functioneren te meten vóór de stressmanagement training, direct na de training en negen weken later. Ook werd de psychofysiologische reactie op een stresstaak (TSST) bepaald direct na de training en negen weken later. Maten voor autonome, neuroendocriene en immuunactiviteit (speeksel α -amylase, speekselcortisol en cytokines in het bloed) werden afgenomen vóór, tijdens en na de stresstaak. Na de eerste algemene meting nam de helft van de participanten deel aan de training. Patiënten in de interventiegroep kregen vier individuele trainingen in stressmanagement gedurende twee weken. De sessies bevatten modules psycho-educatie en ademhalings-, ontspannings- en visualisatieoefeningen. Tijdens de training werden participanten aangemoedigd dagelijks huiswerkoefeningen te maken en dit voort te zetten na de training.

De effecten van de stressmanagement training worden beschreven in Hoofdstuk 5 en 6. De resultaten laten zien dat een korte individuele stressmanagement training het psychologisch functioneren van patiënten met RA verbeterde. Angstklachten namen af

en de positieve stemming verbeterde. Ook rapporteerden patiënten die de training hadden gevolgd minder spanning en hadden ze lagere cortisolspiegels en lagere IL-8 waarden bij het uitvoeren van de stresstaak negen weken na de training, maar niet bij de stresstaak direct na de training. De effecten op de stressreactie waren het sterkst in de subgroep patiënten met verhoogde basale zelfgerapporteerde stresswaarden. De resultaten van deze studie laten zien dat een korte stressmanagement interventie invloed kan hebben op psychofysiologische maten van het endocriene en immuunsysteem. Vooral risico-patiënten die meer stress rapporteren lijken baat te hebben bij een dergelijke interventie. Uitgebreidere interventies zullen naar verwachting nodig zijn om de hiergenoemde effecten te versterken, uitspraken te doen over mogelijke klinische relevantie en wellicht op termijn ook veranderingen in ziekteactiviteit zichtbaar te kunnen maken.

Uit de studies van dit proefschrift kan geconcludeerd worden dat patiënten met verschillende chronische ontstekingsziekten zich kenmerken door een specifieke psychofysiologische stressreactie en dat een korte stressmanagement training deze psychofysiologische stressreactie kan beïnvloeden. Meer onderzoek op dit terrein kan op de lange termijn wellicht leiden tot de ontwikkeling van psychologische interventies als additionele therapie op de reguliere medische zorg voor patiënten met een chronische ontstekingsziekte, om zodoende de effecten van stress op chronische ontsteking te verminderen, vooral in patiënten met een psychologisch risico-profiel.

List of Publications

De Brouwer SJM, van Middendorp H, Stormink C, Kraaimaat FW, Joosten I, Radstake TRDJ, de Jong EMGJ, Schalkwijk J, Donders ART, Eijsbouts A, van de Kerkhof PCM, van Riel PLCM, Evers AWM. Immune responses to stress in rheumatoid arthritis and psoriasis. *Rheumatology* 2014;pii: keu221.

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Curriculum Vitae

Sabine de Brouwer werd geboren op 30 november 1979 te Tilburg. In 1998 behaalde zij haar gymnasiumdiploma aan het Pauluslyceum te Tilburg en startte zij haar vervolgopleiding aan de Radboud Universiteit Nijmegen. Na het behalen van de propedeuse Psychologie begon ze in 1999 met de opleiding Medische Biologie, die ze begin 2006 *cum laude* afrondde. Tijdens haar studietijd liep zij een jaar stage op de afdeling Gynaecologie en Obstetrie van het Radboudumc, waar zij celbiologisch onderzoek verrichtte naar DNA-fouten in de muiszygote. Ook liep zij een wetenschapsjournalistieke stage op de redactie van Medisch Contact te Utrecht en deed ze toegepast onderzoek op de Sint Maartenskliniek te Nijmegen. Eind 2006 startte zij als junior onderzoeker op de afdeling Medische Psychologie van het Radboudumc. Daar deed zij onderzoek naar de effecten van een experimentele sociale stresstaak en stressmanagement training op de psychofysiologie van patiënten met de chronische ontstekingsziekten reumatoïde artritis en psoriasis. Tijdens haar onderzoek verrichtte zij ook onderwijstaken, waaronder het begeleiden van Masterstudenten Psychologie in het kader van hun onderzoeksstage. Haar promotieonderzoek maakte deel uit van de onderzoeksschool Experimentele Psychopathologie (EPP) en het onderzoeksinstituut Radboud Institute for Health Science (voorheen Nijmegen Centre for Evidence Based Practice). Eind 2011 ontving zij de prijs voor beste posterpresentatie op de Najaarsdagen van de Nederlandse Vereniging voor de Reumatologie. Naast het onderzoek was zij tevens enige tijd lid van de onderdeelcommissie van de ondernemingsraad van het Radboudumc. Momenteel is zij werkzaam als pharmacovigilance manager bij Synthon te Nijmegen en als onderzoeker aan de Universiteit Leiden.

