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The Effect of High Intensity Interval Training on Inflammatory Status and Cardiovascular Risk Factors in Females with Rheumatic Diseases

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Abbreviations

1-minHRR	One minute Heart Rate Recovery
ACPA	Anti Cyclic Citrullinated Peptide Antibodies
ADL	Activities of Daily Living
AIT	Aerobic Interval Training
ANA	Anti-Nuclear Antibodies
Anti-CCP	Anti Cyclic Citrullinated Peptide Antibodies
BMI	Body Mass Index
CAD	Coronary Artery Disease
COPM	Human Cartilage Oligometric Matrix Protein
CPK	Creatine Phosphokinase
CRP	C-reactive Protein
CVD	Cardiovascular Diseases
DAS28	Disease Activity Score
DMARD	Disease Modifying Anti-rheumatic Drugs
ELISA	Enzyme-Linked Immunosorbent Assay
ESR	Erythrocyte Sedimentation Rate
HIT	High Intensity Training
HIIT	High Intensity Interval Training
HLA	Human Lymphocyte Antigens
HR_{max}	Maximal Heart Rate
HRR	Heart Rate Recovery
hsCRP	High sensitive CRP
IgG	Immunoglobulin G
IL-1	Interleukin-1

IL-1ra	Interleukin-1 receptor antagonist
IL-6	Interleukin 6
IL-10	Interleukin 10
IMT	Intima-media Thickness
JIA	Juvenile Idiopathic Arthritis
MHAQ	Modified Stanford Health Assessment Questionnaire
MI	Myocardial Infarction
mRNA	Messenger RNA
MTX	Methotrexate
NTNU	Norwegian University of Science and Technology
PTX3	Human Pentraxin 3
qPCR	Quantitative Polymerase Chain Reaction
RA	Rheumatoid Arthritis
RNA	Ribonucleic acid
RF	Rheumatoid Factor
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAP	Serum Amyloid P Components
SD	Standard Deviation
SF-36	36-item Short Form survey
TLR	Toll-like Receptor
TNF-α	Tumor Necrosis Factor-alpha
TSG-14	Tumor Necrosis Factor-stimulated Gene 14
VAS	Visual analogue scales
VO_{2max}	Maximal Oxygen Uptake
VO_{2peak}	Peak Oxygen Uptake

Abstract

BACKGROUND: Arthritis and other rheumatic conditions are a growing health problem, in terms of prevalence, disability and cost. Rheumatism is classified as chronic, systemic, and autoimmune diseases. Major symptoms are synovial inflammation and swollen joints, autoantibody production, deformation of cartilage and bone structures, and systematic features such as cardiovascular, pulmonary, psychological and skeletal disorders. The mortality rate in patients with rheumatoid arthritis (RA) is 1.5 – 1.6 compared to that of the general public, and cardiovascular diseases (CVD) account for 40-50% of the deaths. In addition, a five-fold increased CVD-risk, was observed in female patients with RA who were diagnosed at a young age. Scientific studies have shown that aerobic interval training of high intensity is effective in improving general physical status and cardiovascular health, but is not yet used as a treatment option for people with rheumatism. The aim of the present study was to find out if ten weeks of high intensity interval training was well tolerated by people with rheumatic diseases, and if it would improve activity of the disease, quality of life, and traditionally risk factors for cardiovascular diseases.

METHODS: Eighteen women with RA and juvenile idiopathic arthritis (JIA), aged 20-50 years, were recruited to this cross-over study. Participants performed supervised interval training 2 times a week for 10 weeks on a spinning bike. The exercises consisted of four 4-minute intervals at ~90% of maximal heart rate (HR_{max}), interspersed with 3 minute recovery periods at ~70% of HR_{max}. Maximal oxygen uptake (VO_{2max}), HR_{max}, one minute heart rate recovery (1-minHRR), blood pressure, body composition, blood analysis, molecular status (TNF-α, Interleukin-6 (IL-6), Human Cartilage Oligometric Matrix Protein (COMP) and Pentraxin-3), disease

activity status, and questionnaires (Visual analogue scales (VAS), Modified Stanford Health Assessment Questionnaire (MHAQ), and SF-35) were measured before and after both exercise and control periods. Statistical analyses were performed using the software program SPSS.

RESULTS: As a consequence of high intensity interval training (HIIT), the results showed that; VO_{2max} increased by 12.2% ($p < 0.001$), 1-minHRR decreased by 3.7% ($p = 0.02$), BMI, body fat, visceral fat and waist circumference decreased by 1.2% ($p= 0.04$), 1.0% ($p= 0.05$), 0.2% ($p= 0.08$) and 1.6% ($p=0.004$), respectively, whereas muscle percentage increased by 0.6% ($p=0.03$). Significant differences were found for serum glucose (increased 6.3%, $p=0.05$), haemoglobin (decreased 2.2%, $p= 0.03$) and ferritin (decreased 24.0%, $p= 0.006$). High sensitive CRP (hsCRP) level decreased by 41.9% ($p= 0.08$). IL-6 mRNA increased by 32.6% ($p=0.02$), COMP levels increased by 12.6% ($p=0.06$), and Pentraxin-3 levels decreased by 27.3% ($p=0.14$). MHAQ score decreased by 24.6% ($p=0.13$), self reported global health VAS-score decreased by 33.8% ($p=0.13$), and pain VAS-score decreased by 34.8% ($p=0.12$). The participants scored themselves better for bodily pain and emotional role in SF-36.

CONCLUSION: Ten weeks of HIIT was well tolerated by females with RA and JIA, and improved the disease activity, quality of life, and traditionally risk factors for cardiovascular diseases. HIIT might be recommended as a safe treatment for persons with RA and JIA.

Contents

1. Introduction

1.1 Inflammatory rheumatic diseases

Inflammatory rheumatic diseases are often just called rheumatism. Rheumatism includes more than 100 rheumatic diseases and conditions that affect joints, and is typically characterized by pain and stiffness in or around one or more joints in addition to systemic symptoms such as fever, fatigue and loss of energy (Hunder, 2001, Brady et al., 2003). The pattern, severity and location of symptoms can vary depending on the specific form of the disease. The symptoms can develop gradually or suddenly, and the rheumatic conditions most often decrease the quality of life and function. The disease has a significant impact on a nation's economy in terms of great amounts and/or expensive medication, as well as hospitalization (Hunder, 2001). Rheumatism and its related musculoskeletal disorders constitute the most common chronic illness in the western world (Hunder, 2001). Arthritis and other rheumatic conditions are an important and growing health problem, whether measured in terms of prevalence, disability or costs (Brady et al., 2003). This study focuses on subjects with rheumatoid arthritis (RA), the most common inflammatory rheumatic disease, and juvenile idiopathic arthritis (JIA) among adults.

1.1.1 Rheumatoid arthritis (RA)

RA is the most common inflammatory arthritis, affecting approximately 0.5-1% of the world's population (Alamanos et al., 2006). The incidence varies by age, race and geographic location, and females are affected 3-times more often than males (Hunder, 2001). The cause of RA is unknown, but there is strong evidence that the disease occurs in genetically predisposed individuals, probably after exposure to one

or several yet unknown antigens (Hunder, 2001). There are probably multiple genetic factors involved, and the disease involves a complex interplay of genotype, environmental triggers and chance (McInnes and Schett, 2011). RA is classified as a chronic, systemic, autoimmune disease. The major symptoms are synovial inflammation and swollen joints, autoantibody production, deformation of cartilage and bone structures, and systematic features such as cardiovascular, pulmonary, psychological and skeletal disorders (McInnes and Schett, 2011). The 1987 American College of Rheumatology (ACR) criteria has until very recently been used in order to classify patients. For the diagnosis of RA a patient must fulfill 4 out of 7 criteria: Morning stiffness ≥1 hour, arthritis of ≥3 joints areas, arthritis of hand/wrist joints, symmetrical arthritis, rheumatic nodules, serum rheumatoid factor (RF) and radiographic changes (Arnett et al., 1988). The new classification system published in 2010 redefines the current paradigm of RA by focusing on features at earlier stages of disease that are associated with persistent and/or erosive disease, rather than defining the disease by its late-stage features (Table 1). To be classified as having RA, patients must have synovitis > 1 joint, absence of alternative diagnosis that better explain the synovitis, total score ≥ 6 from the individual scores in 4 domains (Aletaha et al., 2010).

Table 1.1: The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for RA

	Score
Target population (Who should be tested?): Patients who 1) have at least 1 joint with definite clinical synovitis (swelling) 2) with the synovitis not better explained by another disease	
Classification criteria for RA (score-based algorithm: add score of categories A-D; a score of ≥6/10 is needed for classification of a patient as having definite RA)	
A. Joint involvement	
1 large joint	0
2−10 large joints	1
1−3 small joints (with or without involvement of large joints)	2
4−10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)	5
B. Serology (at least 1 test result is needed for classification)	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)	
Normal CRP *and* normal ESR 0	0
Abnormal CRP *or* normal ESR 1	1
D. Duration of symptoms	
<6 weeks	0
≥6 weeks	1

RF= Rheumatoid Factor, ACPA= Anti Cyclic Citrullinated Peptide Antibodies, CRP= C - reactive protein, ESR= Erythrocyte Sedimentation Rate. (Aletaha et al., 2010)

1.1.2 Juvenile idiopathic arthritis (JIA)

Juvenile idiopathic arthritis (JIA) is not a single disease, but a term that encompasses all forms of arthritis that begin before a person is 16 years of age, that persist for more than 6 weeks, and are of unknown origin. It is the most common chronic rheumatic disease in children and causes much disability. In high-income countries it has a yearly incidence of 2-20 cases per 100.000 and a prevalence of 16-150 cases per 100.000 (Prakken et al., 2011). JIA is not only a disease of childhood, as more than a third will continue to have active disease as adults (Foster et al., 2003). As children who have JIA reach adulthood, they face possible continuing disease activity, medication-associated morbidity, and life-long disability and risk for

emotional and social dysfunction (Moorthy et al., 2010). The disease is poorly described in adult patients, and there is not much literature dealing with adult JIA. The JIA-patients do not receive the same focus and attention as RA patients, due to absence of research and understanding of the disease. There are few adolescent JIA clinics, and there is a need to develop care services with the aim of improving the long-term global outcome of JIA (Foster et al., 2003).

1.2 Molecular mechanisms associated with RA/JIA

The pathogenesis of RA and JIA implicates involvement of various cellular populations strictly connected by an intricate web of cytokines, chemokines, and growth factors leading into cartilage destruction and bone damage (Perricone et al., 2011). JIA is poorly described in the literature, RA, however, is given more attention. About 50% of the risk of developing RA is attributable to genetic factors, and more than 30 genetic regions are associated with the disease (Scott et al., 2010). Genome-wide analyses make it clear that immune regulatory factors underlie the disease (Scott et al., 2010). Human lymphocyte antigens (HLA) are strongly associated with RA-patients, especially HLA-DRB1, which has been established as a marker gene (Adebajo, 2010). Alleles that contain a common amino acid motif in the HLA-DRB1 region, termed the shared epitope, confer particular susceptibility (McInnes and Schett, 2011). HLA are only associated with patients who have positive Rheumatoid Factor (RF-positive) or have antibodies against citrullinated antigens (anti-CCP-/ACPA-positive). Genetic risk factors for ACPA-negative disease appear to be no less important than for ACPA-positive disease. However, they are less well established and involve different HLA alleles, regulatory factors and lecithin-binding proteins. Patients with ACPA-positive disease have a less favorable prognosis than those with

ACPA-negative disease (McInnes and Schett, 2011). Many cellular and chemical markers have been studied aiming to find a solution for autoimmunity. The activated T-cell is believed to be the key effector cell, which orchestrates the immune response and mediate a release of cytokines. The key cytokines involved in the pathogenesis of RA are Tumor Necrosis Factor-alpha (TNF-α), interleukin-1 (IL-1) and interleukin 6 (IL-6) (Adebajo, 2010). The mechanisms of JIA are not well described. JIA comprises a heterogeneous group of diseases with distinct subtypes, and all subtypes have different phenotypes, course and prognoses. However, JIA-patients respond to the same treatment as RA-patients, such as TNF-inhibitors and IL-6-inhibitors, thus the underlying mechanisms of adult JIA therefore have to be quite similar to those in RA.

1.2.1 Biomarkers in RA/JIA and cardiovascular diseases

Biomarkers are quantifiable biological parameters which serve as indices of healthy or pathological processes, and also reflect response to pharmacological intervention. Biomarkers have the potential to help predict which individual is at risk of developing RA, and when the arthritis is present, they can indicate bad prognosis such as persistent erosive disease, loss of function or early death (Provan, 2011).

ANA screening (Anti-nuclear antibodies)

ANA is a group of autoantibodies against constituents in the nucleus. A positive test for ANA indicates presence of antibodies against the patient's own gene products. ANA screening is an Enzyme-Linked Immunosorbent Assay (ELISA)-test of seven important autoantibodies (IgG), subgroups, which are connected to chronic connective tissue diseases and other autoimmune diseases. A low-titer ANA without subgroups occur in 1-5% of healthy individuals as well, and increase with age. Of

people with RA, 25 % are ANA positive, without presenting any specific subgroups (Gran, 2009).

Serum rheumatoid factor (RF)

Rheumatoid factor (RF) is antibodies against a patient's own immunoglobulin G (IgG). The structure of the antibody class may also be IgM or IgA. About 80% of the patients with RA have such autoantibodies (Gran, 2009). The RF was until recently considered the most important serological biomarker of RA, but since several other inflammatory diseases also give a positive test, RF is now explained as a by-product of inflammatory activity (Gran, 2009, Provan, 2011).

Anti Cyclic Citrullinated Peptide Antibodies (Anti-CCP/ACPA)

Proteins with changed arginine to citrulline are called citrullinated. Antibodies against citrullinated antigens (Anti-CCP) are found in 50-70% of patients with RA (Gran, 2009). Anti-CCP, also called ACPA can be detected many years preceding the onset of the disease, and assign nearly 70% risk of developing the disease in patients who have a strong family history of RA (Provan, 2011). ACPA seems to be more specific and sensitive for diagnosis and seems to be a better predictor of prognostic features such as progressive joint destruction than RF. Most, but not all, ACPA-positive patients are also RF-positive, and 50-80% of individuals with RA have RF, ACPA, or both (Scott et al., 2010).

Tumor necrosis factor alpha (TNF-α)

TNF-α is the prototype ligand of the TNF superfamily. This molecule plays a central role in inflammation and immune system development. TNF-α is also involved in a number of pathological conditions, including RA and autoimmunity. It is produced by

a wide variety of immune, epithelial, endothelial and tumor cells (Idriss and Naismith, 2000). TNF-α is considered to be a proinflammatory cytokine. In inflammatory diseases TNF-α expression is increased in the affected tissue as a result of innate and adaptive immune responses. TNF-α then mediates a variety of direct pathogenic effects and induces production of other mediators of inflammation and tissue destruction, placing TNF-α at the top of a proinflammatory cytokine cascade. Since TNF-α is at the top of the cascade, many of the hallmarks of chronic inflammation are reduced by TNF-α antagonist therapy (Tracey et al., 2008). Blockade of actions from TNF-α by the human TNF-α receptor 2-immunoglobulin constant region fusion protein, etanercept, resulted in the first success of biological-response-modifying therapy in RA. Since then, monoclonal antibodies to human TNF-α have come into use. These agents have been demonstrated to be effective in the treatment of RA in clinical, radiological and laboratory settings, particularly when used in combination with Methotrexate (Adebajo, 2010).

Human pentraxin 3 (PTX3)

Pentraxin 3 (PTX3), also known as Tumor necrosis factor-stimulated gene 14 (TSG-14), is a long pentraxin belonging to the pentraxin superfamily. Unlike the classic, short pentraxins, C-reactive protein (CRP) and serum amyloid P components (SAP), PTX3 is highly conserved in evolution, and it differs from the short pentraxins by presence of an unrelated long N-terminal domain. Short pentraxins are mainly produced in the liver in response to inflammatory cytokines, in particular as a direct response to interleukin-6 (IL-6) stimulation. PTX3 is produced by a variety of cells in response to primary inflammatory signals and Toll-like Receptor (TLR) engagement. It increases rapidly in plasma during inflammatory and infectious conditions (Bottazzi et al., 2006). PTX3 also has a role in the regulation of the innate immune response,

and interacts with selected viral, fungal and bacterial components, providing protection from infection and may act as an opsonin (Bottazzi et al., 2006).

PTX3 is a novel candidate immunoinflammatory marker that has been reported to be associated with cardiovascular risk factors and to predict adverse outcomes in individuals with CVD (Jylhava et al., 2011). High-intensity training (HIT) shares similarities with acute phase responses of inflammatory disease (Nakajima et al., 2010). Nakajimi and colleges found that plasma levels of PTX3 and high sensitive CRP (hsCRP) increased during acute HIT in untrained healthy subjects. Daily regular exercise is known to induce anti-inflammatory effects and protect against the risk of CVD by decreasing plasma levels of PTX3 and hsCRP. Plasma PTX3, as an inflammatory marker, increases acutely during HIT, but decreases below pre-training levels during cardiac rehabilitation, following the improvement of exercise capacity in patients with CVD (Fukuda et al., 2011).

C-reactive protein (CRP)

Among various markers of inflammation, CRP is a powerful predictor of cardiovascular diseases (CVD) (Pahor et al., 2006). CRP belongs to the pentraxin family of proteins and its production is mainly mediated by IL-6. In pathological situations such as inflammation or infections, CRP levels can increase substantially. Increased levels of CRP in the high sensitive range (hsCRP) are associated with higher risk of cardiovascular morbidity and mortality in the general population (Jeppesen et al., 2008). RA patients have often higher CRP/hsCRP levels than healthy persons (Dundar et al., 2008).

Interleukin 6 (IL-6)

IL-6 is a multifunctional cytokine involved in the inflammatory response and modulation of immune responses, including B-cell and T-cell differentiation. IL-6 is a B-cell differentiation cytokine that induces the final maturation of activated B cells into immunoglobulin-secreting plasma cells, and is over-expressed in the affected tissues of RA patients (Lee et al., 2012). IL-6 appears to be involved in a long series of deleterious actions, and it was previous classified as a “bad” interleukin (Fisman et al., 2003). New research has found that IL-6, besides its pro-inflammatory properties, also has anti-inflammatory activity (Petersen and Pedersen, 2005). Regular physical exercise has been shown to promote anti-inflammatory effects by decreasing some inflammatory cytokines (Bruunsgaard, 2005). The mechanism by which regular exercise reduces inflammation is not completely understood, but IL-6 has been shown to be a part of it (Mitchell et al., 2011). IL-6 mRNA is upregulated in contracting skeletal muscle, and the transcriptional rate of the IL-6 gene is markedly enhanced by acute exercise (Jonsdottir et al., 2000, Keller et al., 2001). The increase in circulating levels of IL-6 after exercise is consistent and proportional to exercise duration, intensity, muscle mass involved, and endurance capacity (Fischer, 2006, Leggate et al., 2010). Physiological concentrations of IL-6 stimulate the appearance in the circulation of the anti-inflammatory cytokines interleukin-1 receptor antagonist (IL-1ra) and interleukin-10 (IL-10), and inhibit the pro-inflammatory cytokine TNF-α (Steensberg et al., 2003, Keller et al., 2004).

Human Cartilage Oligometric Matrix Protein (COMP)

Human Cartilage Oligometric Matrix Protein (COPM) is a member of the Thrombospondin protein family, calcium-binding extracellular glycoproteins. COMP has been demonstrated to be a biomarker for cartilage breakdown. COMP is

subjected to proteolytic cleavage in the cartilage of patients suffering from arthritis (Neidhart et al., 1997, Morozzi et al., 2007). These fragments are detectable in the circulation and may serve as diagnostic and prognostic indicators and as biomarkers for disease severity and response to treatment (Posey and Hecht, 2008). In rat studies serum COMP levels correlate with the degree of cartilage destruction in arthritis rats (Vingsbo-Lundberg et al., 1998). In RA, serum COMP levels decreases in patients who respond to treatment (Crnkic et al., 2003).

1.3 Cardiovascular risk in patients with RA/JIA

Cardiovascular diseases (CVD) is the global number one cause of mortality, and 30% of global deaths are attributed to one or other form of CVD (WHO, 2011). The general mortality rate in patients with RA is 1.5 – 1.6 compared to that of the general public, and CVD accounts for 40-50% of the deaths (Avina-Zubieta et al., 2008). An especially high risk was observed in female patients with RA who were diagnosed at a young age. Those being 20-39 years of age at first discharge had a more than 5-fold increased risk of death from coronary artery diseases (CAD) (Bjornadal et al., 2002). Patients with RA therefore have a shorter life expectancy compared to the general population. Many people die every year from CVD, but in people with RA, CVD related death often occurs at an earlier age (Pahor et al., 2006).

Mechanisms underlying increased CVD risk in RA patients are not yet elucidated. RA patients may have an unfavourable genetic profile, and there is evidence that they are more likely to have a close relative who has suffered from a myocardial infarction (MI) (Solomon et al., 2003). Common risk factors for CVD and RA such as smoking, lack of physical activity or high body mass index (BMI), which are consequences of RA and risk factors for CVD, may also be of importance (Solomon et al., 2003).

Patients with RA have been shown to be at a considerably increased risk of developing MI already in the two years prior to fulfilling the 1987 American College of Rheumatology (ACR) diagnostic criteria for RA (Maradit-Kremers et al., 2005). RA itself is an important risk factor for early atherosclerosis, demonstrated by intima-media thickness (IMT) and plaque formation (Pahor et al., 2006). Carotid artery IMT is associated with cardiovascular risk factors and atherosclerosis and is a strong predictor of MI and stoke (Pahor et al., 2006).

1.4 Treatment of RA/JIA

1.4.1 Medication

Until some decades ago gold-components, sulphasalazine and hydroxychloroquine were the preferred treatment drugs for RA (Provan, 2011). However, the introduction of Methotroxate (MTX) in the 1980's did indeed bring great changes to the treatment. More than a decade later, in the late 1990 and start of year 2000, the introduction of TNF-α inhibitors further enhanced the treatment of RA. TNF-α inhibitors, termed biologic treatment, is given to the patients that do not have sufficient effect of MTX. The last years other biologic treatments targeting cytokines or molecules involved in the RA disease have become available, such as antibodies against IL-1 and IL-6, antibodies against B-cells and T-cells modulators (Hoff, 2009). TNF-α inhibitors are now, in combination with MTX, the most effective drug treatment for patients with RA (Seymour et al., 2001, Barrera et al., 2002).

1.4.2 Exercise

Not more than 10-20 years ago, patients with arthritis were told to rest and to avoid exercise involving the affected joints (Brady et al., 2003, Baillet et al., 2010). Now the recommendations are different. RA-patients are told to exercise 4-7 days a week with moderate intensity aerobic activity such as cycling, cross-country skiing, walking, pool training, dance or strength training with low weights (Helsedirektoratet, 2009). Scientific studies have shown that regular physical activity is just as important for people with arthritis or other rheumatic conditions as it is for the general population (Brady et al., 2003, CDC, 2011). Participation in physical moderate-intensity activities improves pain, function, mood, and quality of life without worsening symptoms or disease severity. Being physically active can also delay the onset of disability in patients with arthritis (CDC, 2011, Baillet et al., 2010). However, literature on the effect of exercise at high intensity in this patient group is sparse. Several studies have indicated that aerobic interval training (AIT) with high intensity is effective in improving general physical status and cardiovascular health in people with and without CVD (Tjonna et al., 2009, Wisloff et al., 2007).

1.5 The aim of the study

The primary aim of this study was to investigate if 10 weeks of high intensity interval training influenced the disease activity in patients with RA and JIA, and to find out if high-intensity aerobic exercise can be considered safe and used as therapy in stabile RA/JIA.

Since inflammatory rheumatic diseases increase the risk for CVD, and the mortality rate in patients with RA and JIA is higher compared to that of the general public, the secondary aim of this study was to investigate how HIIT is affecting cardiovascular

risk factors in RA/JIA patients. This study also aimed to elucidate if HIIT improved outcomes in function, quality of life and pain in patients with RA and JIA.

Hypothesis

Ten weeks of high intensity interval training will be well tolerated by RA and JIA patients, and will improve the disease activity, quality of life, and traditionally risk factors for CVD.

2. Methods and experimental procedures

2.1 The study

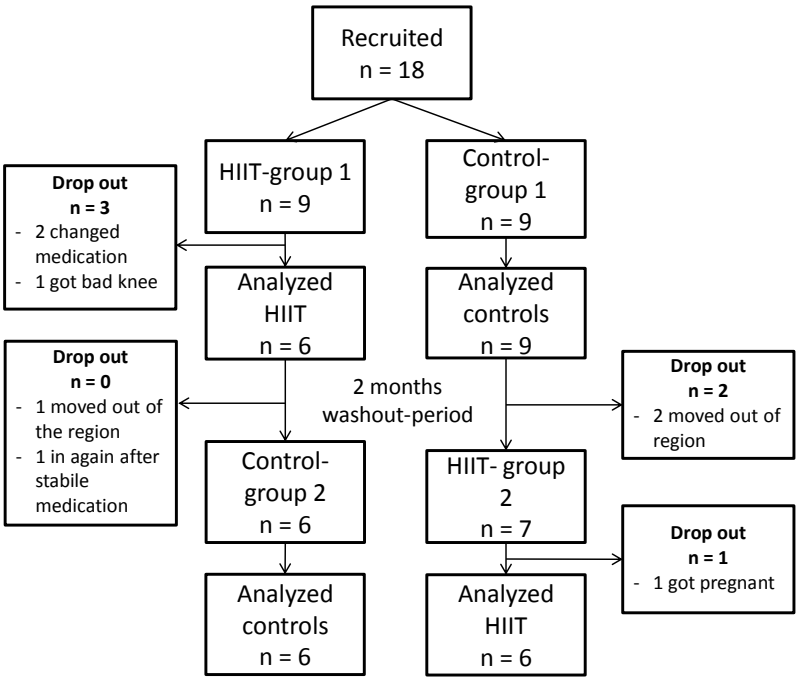
2.1.1 Subjects

Eighteen women with RA and JIA, aged 20-50 years, were recruited through advertising in a local newspaper (Adresseavisen), via the webpage at NTNU, and via notes in Department of Rheumatology at St. Olavs Hospital, Trondheim. Before inclusion a rheumatologist ensured that the subjects were qualified, with correct diagnosis, stable health and were free of other complications. The exclusion criteria were heart disease, lung disease, or unstable disease. All subjects provided written informed consents. The regional committee for medical research ethics approved the protocol (REK 2010/3347).

2.1.2 Study design

This was a cross-over study, meaning that the participants were their own controls for the effect of the intervention. The participants were randomized into two different groups; interval training or control. The randomisation was carried out by The Clinical Research unit at NTNU. It was stratified for RA and JIA, to secure equal numbers of both diseases in both groups. After the first period of either training or control, the participants had a two months “washout-period”. Then the groups changed place, crossed over, so that the first training group became the new control group, and the first control group became the new training group (Figure 2.1). In the control period the subjects were instructed to live their lives as normal. All measurements and tests were

conducted pre and post the 10 weeks of either interval training or control-period, a total of 4 tests for each person.



Total: 12 participants finished the HIIT-period. 15 partisipants finished the control-period. 11 participants did both HIIT and control period.

Figure 2.1: Flow chart of the study design.
n = numbers of subjects, HIIT = high intensity interval training.

2.2 Characteristics and fitness of the participants

2.2.1 Maximal oxygen uptake and heart rate

The subjects' maximal oxygen uptake (VO_{2max}) was tested by using the ergospirometry system Oxycon Pro (Jaeger, Oxycon Pro, Hoechberg, Germany). An individually adjusted test bike (Monark 839 E, Monark Exercise AB, Sweden) was used. During warm-up the resistance of the bike was individually adjusted, based on the subject's fitness level. After the 10 min warm-up the test started by increasing the resistance of

the bike. The resistance was increased by 20 watts every time the level of the oxygen uptake stabilised, meaning approximately once every minute. This was done until exhaustion. The best way to ensure that the true VO_{2max} was reached, was to see that the oxygen (O_2) curve had a straightening out or drop, in spite of increased resistance of the bike. This, in combination with a respiratory exchange ratio (volume of exhaled carbondioxide (CO_2) divided by volume of inspired O_2 per minute) higher than 1.05, was used as criteria for true VO_{2max}.

Knowing the individual's maximal heart rate (HR_{max}) was necessary since the intervals during 10 weeks of exercise training were to be performed at an intensity of ~90% of HR_{max}. The heart frequency was registered continuously during the VO_{2max}-test using a heart rate monitor (Polar Electro, Kempele, Finland). The highest heart rate during the test was ascribed 5 additional beats, to find the HR_{max}. When the HR_{max} was obtained it was used to calculate individual heart rate at which exercise was to be performed. After stopping at exhaustion the subjects were told to sit still on the bike for one minute, and the one-minute heart rate recovery (1-minHRR) was found by measuring the heart rate one minute after the end of the test.

2.2.2 Blood pressure

Blood pressure was measured at pre- and post-testing using sphygmomanometer Microlife BPA 100 Plus (Microlife AG, 9435 Heerbrugg, Switzerland). The sphygmomanometer used had an integrated stethoscope, and all measurements were performed automatically. The subjects rested in a quiet room for about 10 min prior to the measurements. The cuff was placed at the upper arm, and it was quickly inflated to ~200 mmHg, and then the pressure was gradually released. Systolic and diastolic

pressures were measured. This was done two to three times, two times if the measurements were similar, three times if the variation coefficient was more than 15%. The first measurement was rejected. The second, or the average of the second and third measurements was used.

2.2.3 Body composition

To assess body fat percentage, muscle mass percentage, weight and BMI, the bioelectrical impendence analyzer HBF-352-W (Omron Healtcare Co, Kyoto, Japan) was used. Patients were weighed after an overnight fasting, in light clothing and without shoes. Body weight was recorded to the nearest 0.1 kg, and body fat percentage and muscle mass percentage were measured to the nearest 0.1%. It was ensured that the measurements during post-testing were taken at the same time of the day as the pre-testing.

2.3 Blood analysis

Overnight fasting blood was taken from the antecubal vein, both at pre and post-test. Blood samples taken for the post-test were done 48-96 hours after the last exercise session. Blood was collected into both PAXgene (Qiagene, Germantown, MD), EDTA and serum tubes (Greiner-Bio One GmbH, Frickenhausen, Germany). EDTA plasma and serum were centrifuged at 3000 rcf for 10 minutes at 20°C. For later analysis the serum and plasma were stored at -80°C. The PAXgene tubes were handled according to the manufacturer's instructions. In addition: S-ferritin, triglyceride, total cholesterol, HDL-cholesterol, glucose, leukocytes, neutrophil granulocytes, haemoglobin,

thrombocytes, high sensitive CRP, eosinophils, basophiles, white blood cell count, insulin C-peptide and IGF-1 were analysed at the Department of Medical Biochemistry at St. Olavs University Hospital, Trondheim, Norway.

2.4 Disease activity

All patients were examined by a rheumatologist before and after the training and the control period. Clinical and demographic data were collected by the treating rheumatologists and by patient self-report. Counting of swollen and tender joints was performed by the rheumatologist.

2.4.1 Disease activity score (DAS28)

DAS28 is a measurement of disease activity in RA calculated for a total of 28 joints. The score is calculated by a complex mathematical formula, which includes the number of tender and swollen joints (out of a total of 28), the patient's VAS-score for global health (indicated by marking a 10 cm line between very good and very bad), and the erythrocyte sedimentation rate (ESR, a blood marker of inflammation) or the CRP rate. In this study CRP levels were used, since the CRP is a more sensitive and direct measurement of inflammation than ESR (Crowson et al., 2009). High disease activity is defined as DAS28 >5.1, moderate disease activity between 3.2-5.1, and low disease activity is below 3.2 (Prevoo et al., 1995).

DAS28 was calculated from the following formula:

DAS28-CRP = 0.56*√(TJC28) + 0.28 *√(SJC28) + 0.36*ln(CRP+1) + 0.014*GH + 0.96

TJC28: 28 Tender joint count; SJC28: 28 Swollen joint count; CRP: C-reactive protein (mg/l); GH: General Health on a 100mm Visual Analogue Scale (VAS).

2.4.2 Questionnaires

Visual analogue scales (VAS)

VAS has been used in the social and behavioral sciences to measure a variety of subjective phenomena. A VAS is a straight line, whose end anchors indicate the extreme boundaries of the sensation, feeling, or responses to be measured. For example a VAS to measure pain could be labeled “no pain” on one end and “pain as bad as it could possibly be” on the other end. Subjects respond to the VAS by placing a mark through the line at a position which best represents their current perception of a given phenomenon between the labeled extremes. Although a VAS may be horizontal or vertical and of any length deemed appropriate, its most common form is a 100 mm horizontal line. The VAS is scored by measuring the distance, usually in millimeters, from one end of the scale to the subject’s mark on the line (Wewers and Lowe, 1990). In the present study, a score from 1-100 was used for measuring self reported global health, total pain and fatigue. While a score from 1-18 was used for self reported joint pain.

Modified Health Assessment Questionnaire (MHAQ)

Patient satisfaction in performing activities of daily living (ADL) was assessed by using a self-administered questionnaire modified from the Stanford Health Assessment Questionnaire (MHAQ). The MHAQ includes questions to determine a patient's degree of difficulty and need for help and assistive devices in ADL. The patients answer if they

have difficulty performing ADL from 0= no difficulty; 1= some difficulty; 2= much difficulty, and 4 is unable to do (Pincus et al., 1983).

SF-36

The patients also answered the medical outcome study 36-item short form survey (SF-36) The SF-36 consists of eight scaled scores, which are the weighted sums of the questions in their section. Each scale is directly transformed into a 0-100 scale on the assumption that each question carries equal weight. The eight sections are; vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health.

2.5 Molecular analysis

2.5.1 Enzyme-linked immunosorbent assay (ELISA)

The ELISA technique can detect an amount of a given protein, antibody, or antigen in a fluid sample. Plasma levels of TNF-α were measured before and after both the exercise and control periods by Quantikine Human TNF-α Immunoassay kit (R&D Systems, Minneapolis, USA). Plasma levels of Pentraxin 3 were measured before and after both the exercise and control periods by Quantikine Human Pentraxin 3/TGS-14 Immunoassay kit (R&D Systems, Minneapolis, USA). Serum levels of COMP were measured before and after both the exercise and control periods by Quantikine Human COMP Immunoassay kit (R&D Systems, Minneapolis, USA). All samples were treated according to the manufacturer's instructions, and the analyses were automated and performed by use of a DS2 Two-plate Automated ELISA Processing System (Dynex Technologies, Chantilly, USA).

2.5.2 Isolation of RNA from full blood

DNA is stored in a cells nucleus, and since only the white blood cells have nucleus, they contain DNA. DNA is transcribed to messenger RNA (mRNA), which again are translated to specific proteins. At a given time, the mRNA level encoding different proteins reflects the cellular response to different stimuli. In this study, RNA was isolated from whole blood, collected in PAXgene tubes (Qiagene, Germantown, MD). Total RNA was isolated using the PAXgene Blood RNA Kit (Qiagene, Germantown, MD) according to the manufacturer's protocol (Blood RNA Kit Handbook, version 2), and the analyses were done automatically in QIAcube (Qiagene, Germantown, MD). To ensure high mRNA quality and purity all samples was measured on a Nanodrop 2000, NanoDrop Technologies, Baltimore, MD). The Nanodrop instrument applies ultraviolet spectrophotometry at 260/280 nm to assess the concentration and quality of total RNA in the sample. Only samples with a 260/280 ratio > 1.8 were used for further analysis.

2.5.3 Real-time quantitative polymerase chain reaction (Real-time qPCR)

Reverse transcriptase PCR is also called quantitative PCR (qPCR). Traditional PCR is used for end-point analysis to distinguish gene products. In real-time qPCR, the amount of PCR product is measured at each cycle. The amount of RNA is measured by the use of fluorescent markers that are incorporated into the PCR product. This ability to monitor the reaction during its exponential phase enables users to determine the initial amount of target with great precision. One step qPCR combines the first-strand cDNA synthesis reaction and qPCR in the same tube. In multiplex real-time PCR more than one set of gene-specific primers are used to amplify separate genes in a single tube. Typically, multiplex reactions are used to amplify the gene of interest

and a housekeeping gene. Different fluorescent reporter dyes are used to label and distinguish the separate primers or probes for each gene.

Isolated RNA from before and after the exercise period was used to determine the levels of IL-6 by using one-step multiplex real-time qPCR. The analysis was done by using the Hs-IL6-1-FAM QuantiFast Probe Assay, One-step RT-PCR, Duplex kit (Qiagen, Germantown, MD) according to the manufacturer's instructions (QuantiFast Probe Assay Handbook 05/2011). The reference gene was Hs-B2M-2-Max. The analysis was performed by the use of the Bio-Rad, CFX 96 Real-Time system (Bio-Rad laboratories Ldt., UK).

2.6 Exercise training protocol

Participants performed supervised exercise training 2 times a week for 10 weeks on a spinning bike. Individual speed and resistance of the bikes were adjusted to fit the participant's fitness level. The exercises started with a warm-up for 10 minutes at ~70% of HR_{max} followed by four 4-minute intervals at ~90% of HR_{max}, interspersed with 3 minute recovery periods at ~70% of HR_{max}. Total exercise duration was 40 minutes. All subjects used a heart rate monitor (Polar Electro, Kempele, Finland) during all exercise sessions to ensure that the assigned exercise training intensities were obtained. The speed and resistance of the bike was adjusted continuously to ensure that each exercise session was carried out at the assigned intensity throughout the exercise training period. The HIIT program "4x4", is found to be an interval program that most people are able to manage, despite their fitness level (Helgerud et al., 2007).

2.7 Statistical analyses

Statistical analyses were performed using the software program SPSS, version 19.0 (SPSS Inc.). Means and standard deviations (SD) for all variables were computed using descriptive statistics. All values are expressed as mean ± SD. All tests had a significant level set to $p \leq 0.05$. Trend levels were set to $p \leq 0.15$. Figures were made using GraphPad (GraphPad Prisma 5, LaJolla, CA, USA). To test the normality of the data, the Smirnov Kolmogorov test was used. All the data were found to have normal distribution. The values at the start of a period (pre values) and the values at the end of the period (post values) for the different variables were used for calculating the significant change in variables during the ten week period. Paired Student's T-test was used for pre/post comparisons. Linear mixed model was used for calculating the group comparisons of the study. This type of analysis was used since the same subjects were both the training and the control group, just switching places. Not exactly the same subjects were in both training and control group, and the groups did not have equal numbers participants. There were also some drop-outs after the study commenced. The linear mixed model used, took all this into account, making it possible to compare the effect of training to the control group.

3. Results

3.1 Characteristics and fitness of the participants

Baseline characteristic of all the participants are shown in Table 3.1. Nearly all of them were treated for their disease with medication, but only 38.9% of them used TNF-α inhibitors as medication. Disease modifying anti-rheumatic drugs (DMARD) was used by 72.2% of the participants.

Table 3.1: Baseline characteristics for the 18 participants in the study.

Variables	Value
Age, yr	34±8
Height, cm	167.3±5.6
RA diagnoses	7
JIA diagnoses	11
ACPA positive	7
RFIgM positive	6
ANA positive	1
Erosions in hands/feet, positive	7
Uses DMARD	13
Uses antiTNF-α medication	7
Smokes	3

Values for age and height are presented as mean ± SD, RA=Rheumatoid Arthritis, JIA=Juvenile Idiopathic Arthritis, ACPA= Anti Cyclic Citrullinated Peptide Antibodies, RFIgM= Rheumatoid Factor for immunoglobulin M, ANA= Anti-Nuclear Antibodies, DMARD= Disease Modifying Anti-rheumatic Drugs, TNF-α= Tumor Necrosis Factor-alpha

3.1.1 Maximal oxygen uptake and heart rate

VO_{2max} increased with 12.2% ±0.4 during the exercise period ($p < 0.001$). There was no significant difference from pre to post in the control period (Figure 3.1). The group difference between the HIIT and the control periods was significantly different ($p < 0.001$). The 1-minHRR decreased 3.7% ±0.2 after HIIT ($p = 0.02$), whereas no changes were observed during the control period (Table 3.2.B).

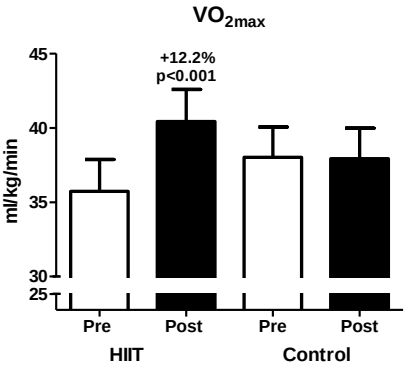


Figure 3.1: VO_{2max} pre (white) and post (black) in HIIT and control group.
VO_{2max}: Maximal oxygen uptake

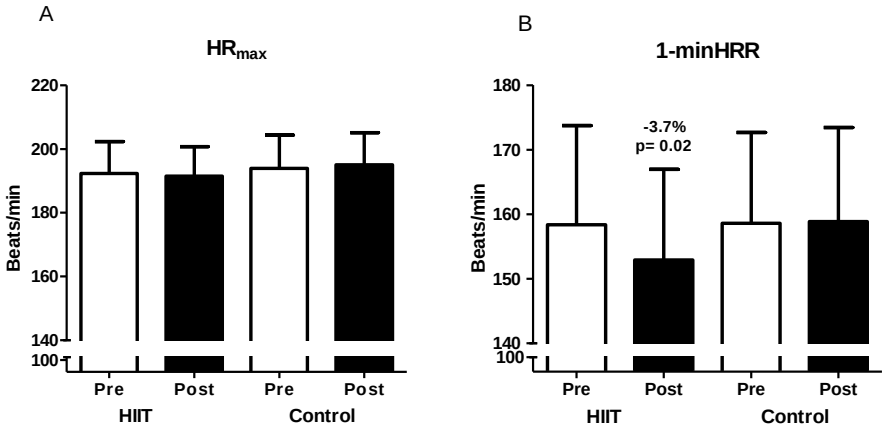


Figure 3.2: HR_{max} (A) and 1-minHRR (B) in HIIT and control group, pre-tests in white, post-tests in black.
HR_{max}: maximal heart rate, 1-minHRR: one minute heart rate recovery, HIIT: high intensity interval training.

3.1.2 Blood pressure

No significant differences were observed from pre to post-tests in either the HIIT or the control period, and no significant differences were observed between the groups (Table 3.2).

Table 3.2: Systolic and diastolic blood pressure in the HIIT and control group, and calculation for the group difference between HIIT and control.

	HIIT group		*Control group*		*Group differences*
	pre	post	pre	post	p-value
Systolic BP (mmHg)	120.7±9.6	118.8±11.4	120.0±14.0	116.6±9.4	0.57
Diastolic BP (mmHg)	73.7±7.5	73.1±7.0	73.5±10.2	73.8±10.2	0.82

Values presented as mean ± SD, HIIT=high intensity interval training, BP=blood pressure.

3.1.3 Body composition

In the HIIT group, BMI, body fat, visceral fat and waist circumference decreased by 1.2% (p= 0.04), 1.0% (p= 0.05), 0.2% (p= 0.08) and 1.6% (p= 0.004) respectively, after the exercise period. HIIT resulted in an increase in percent muscle mass from 26.4 to 27.0% (p= 0.03) within the same group. No significant differences were found in the control period (Table 3.3).

Table 3.3: Physiological parameters in the HIIT and control group, and calculation for the group difference between HIIT and control.

	HIIT group		*Control group*		*Group differences*
	pre	post	pre	post	p-value
Weight (kg)	70.5±14.6	69.8±14.0	67.7±15.0	67.4±13.7	0.56
BMI (kg/m^2)	24.8±4.9	24.5±4.6*	24.0±4.3	24.2±4.9	0.05*
Body fat (%)	36.9±9.1	35.9±9.5*	35.5±8.5	35.1±8.1	0.18
Visceral fat (%)	5.5±2.6	5.3±2.6$^{\#}$	5.0±2.4	5.1±2.5	0.02*
Muscle mass (%)	26.4±3.9	27.0±4.2*	26.8±3.7	27.0±3.4	0.08$^{\#}$
Waist (cm)	89.5±12.0	88.1±11.2*	86.2±12.9	85.9±12.2	0.07$^{\#}$

Values presented as mean ± SD, HIIT=high intensity interval training, BMI=body mass index,
* $p \leq 0.05$, $^{\#}$ $0.05<p<0.15$.

3.2 Blood analysis

In the HIIT group, glucose increased by 6.3% (p= 0.05), haemoglobin decreased by 2.2% (p= 0.03), and ferritin decreased by 24.0% (p= 0.006) after the exercise period. The hsCRP level decreased by 41.9% (p= 0.08) from pre to post in the HIIT period. No significant differences were found for insulin C-peptide, IGF-1, HDL-cholesterol, cholesterol, triglyceride, leucocytes, neutrophile granulocytes, thrombocytes, eosinophiles, or white blood cell count, either in the HIIT or the control period (Table 3.4).

Table 3.4: Blood variables in the HIIT and control group, and calculation for the group difference between the HIIT and control group.

	HIIT group		*Control group*		*Group differences*
	pre	post	pre	post	p-value
hsCRP (mg/l)	2.89±2.77	1.68±1.19#	2.75±2.31	2.79±1.84	0.37
Insuline C-peptides (nmol/l)	0.59±0.24	0.62±0.29	0.66±0.29	0.62±0.22	0.54
IGF-1 (nmol/l)	27.2±9.5	23.4±7.6	26.7±9.9	26.0±10.1	0.22
Glucose (mmol/l)	4.75±0.44	5.12±0.55*	4.86±0.44	4.53±0.47*	0.05*
HDL-cholesterol (mmol/l)	1.52±0.47	1.50±0.42	1.62±0.52	1.59±0.53	0.88
Cholesterol (mmol/l)	4.78±1.11	4.79±1.08	4.83±1.09	4.71±0.96	0.86
Haemoglobin (g/dl)	13.9±0.9	13.6±1.1*	13.6±0.9	13.6±1.0	0.22
Ferritin (µg/l)	78.8±69.6	59.9±54.3*	63.6±41.6	66.8±52.5	0.06#
Triglyceride (mmol/l)	0.91±0.42	0.95±0.52	1.04±0.56	1.01±0.64	0.96
Leucocytes (10^9/l)	6.33±2.07	6.58±1.66	6.16±1.42	6.42±1.98	0.94
Neutrophile granulocytes (10^9/l)	3.88±1.69	3.88±1.21	4.10±1.05	4.98±1.79	0.58
Thrombocytes (10^9/l)	235.2±27.9	223.4±24.7	242.8±30.2	233.3±29.1	0.70
Eosinophiles (10^6/l)	200.0±185.9	185.6±114.6	240.0±240.8	186.7±146.7	0.63

Values presented as mean ± SD, HIIT=high intensity interval training, hsCRP=high sensitive CRP, IGF-1=insulin growth factor 1, HDL=high-density lipoprotein, * p≤0.05, # 0.15<p<0.05

3.3 Disease activity

MHAQ score decreased by 24.6% ±1.6% during HIIT (p=0.15), and the change tends to be different from the change in the control period (p=0.13). Self reported global health VAS-score decreased by 33.8% ±1.5% (p=0.134) during the HIIT, with no

difference between the groups. Total pain score did show a decreasing trend when comparing the groups (p=0.12). The fatigue score did not change during HIIT, but decreased significantly during the control period (p=0.03) (Table 3.5).

Table 3.5: Disease activity in the HIIT and control group, and calculation for the group difference between HIIT and control.

	HIIT group		*Control group*		*Group differences*
	pre	post	pre	post	p-value
DAS28	2.56±0.94	2.29±0.58	2.48±0.93	2.48±0.64	0.44
MHAQ	0.65±0.43	0.49±0.45#	0.42±0.41	0.50±0.49	0.13#
Self reported health score	32.0±20.7	21.2±18.6#	26.0±20.4	25.2±17.3	0.26
Self reported joint pain	2.45±2.34	2.36±1.86	3.08±2.29	3.46±2.26	0.56
Pain score	30.2±23.1	19.7±20.0	24.3±20.6	28.0±19.6	0.12#
Fatigue score	39.2±30.2	39.3±28.2	40.1±31.2	26.2±26.3*	0.21

Values presented as mean ± SD, HIIT=high intensity interval training, DAS28=Disease Activity Score from 28 joints, MHAQ=Modified Health Assessment Questionnaire, * $p \leq 0.05$, # $0.05 < p < 0.15$

SF-36

According to the questionnaire SF-36, there was a trend towards that the participants felt less pain and they had better emotional status after HIIT (Figure 3.3). No such difference was seen in other of the categories or in the control period.

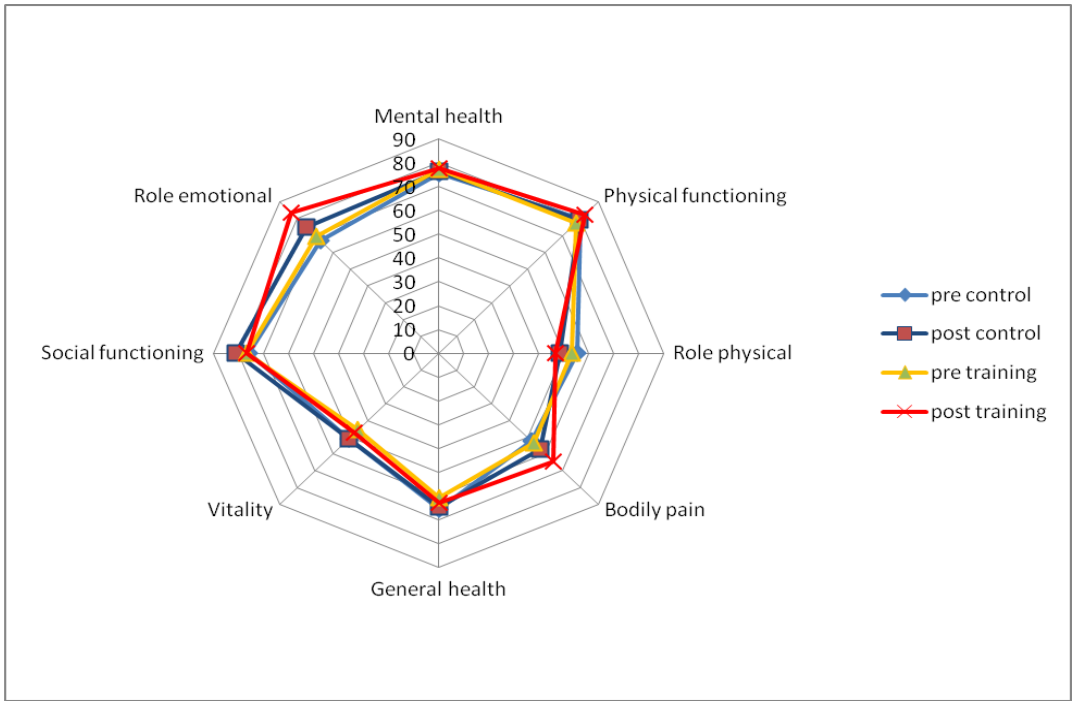


Figure 3.3: A radar-plot for the questionare SF-36. Totally 36 questions were answered and categorisied into 8 groups. Higher score is equal with better health.

3.4 Molecular analysis

3.4.1 IL-6

Real-time qPCR showed that expression of IL-6 mRNA increased with 32.6% (p=0.02) after HIIT (Figure 3.4).

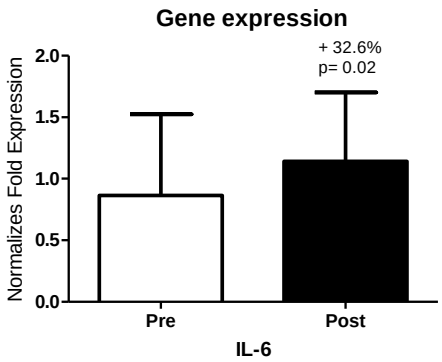


Figure 3.4: Quantitative gene-expression of IL-6 pre (white) and post (black) HIIT. Gene-expression was performed with RT-qPCR from whole-blood taken from participants pre and post 10 weeks of HIIT. The IL-6 expression increased significantly with 32.6% (p=0.02).

IL-6: Interleukin-6.

3.4.1 ELISA results

The ELISA analysis of COMP and Pentraxin-3 did not show any significant differences for either HIIT or control period, but the group differences had a trend to increase for COMP, and decrease for Pentraxin-3 (Table 3.6). Most of the TNF-α values were below the detection limit of the ELISA kit, making it impossible to calculate group differences (data not shown).

Table 3.6: ELISA results in the HIIT and control group, and calculation for the group difference between the HIIT and control group.

	HIIT group		*Control group*		*Group differences*
	pre	post	pre	post	p-value
COMP (ng/ml)	114.5±55.3	128.9±52.1	122.6±62.8	110.4±54.9	0.06[#]
PTX-3 (ng/ml)	0.22±0.20	0.16±0.16	0.18±0.12	0.21±0.22	0.14[#]

Values presented as mean ± SD, HIIT=high intensity interval training, COPM=Human Cartilage Oligometric Matrix Protein, PTX-3=Pentraxin-3, * $p \leq 0.05$, [#] $0.05 < p < 0.15$

4. Discussion

The main findings of the present study were that the participants had less pain, felt better, and had reduced several risk factors for CVD. In addition, 10 weeks of high intensity interval training was well tolerated and there was no increase in disease activity or inflammation.

4.1 Characteristics and fitness of the participants

4.1.1 Maximal oxygen uptake and heart rate

As expected, the VO_{2max} increased during the exercise period. It has previously been shown that HIIT is effective in improving VO_{2max} (Helgerud et al., 2007). Cardiorespiratory fitness measured as VO_{2max} or peak oxygen uptake (VO_{2peak}) may be the single best predictor of cardiovascular morbidity and premature cardiovascular mortality (Aspenes et al., 2011), and low VO_{2max} is clearly associated with a clustering of cardiovascular risk factors (Aspenes et al., 2011). Keteyian and colleges even found that 1 ml/kg/min increase in VO_{2peak} was associated with an approximate 15% decrease in risk for all-cause and CVD-specific mortality (Keteyian et al., 2008). Since aerobic training causes the heart's stroke volume to increase during both exercise and rest, it also reduces the heart beats per minute in rest (McArdle, 2010). Slow heart rate recovery (HRR) is associated with an increased risk of all-cause death in individuals without CVD, and slow HRR may therefore be regarded as an independent risk factor for mortality (Wandell et al., 2010). Slow and abnormal HRR is also associated with inflammatory markers, which could contribute to the high incidence of cardiovascular disease in these subjects (Jae et al., 2007). The 1-minHRR in this study decreased

significantly during HIIT by 3.7%. Since VO_{2max} increased it was the stroke volume that must have changed in response to training, since HR_{max} remained the same. Our results might indicate that the participants, since their stroke volume increased, decreased the risk for CVD.

4.1.2 Blood pressure

According to reference values for blood pressure (BP) set by the American Heart Association, normal values for systolic BP are < 130 mmHg and diastolic BP < 85 mmHg. At pre tests both the HIIT and the control groups had normal blood pressure. High BP is associated with risk for CVD, and exercise has been shown to lower BP in individuals with high BP (McArdle, 2010). Since the participants in this study had normal BP initially, no improvements were expected or shown.

4.1.3 Body composition

In the present study there was no focus on dieting or food intake, and no significant decrease regarding weight was found. Significant changes were seen in the BMI, total body fat, visceral fat, muscle percent, and waist circumference (WC). According to the standards set by the National Institutes of Health, healthy weight is indicated by a BMI between 19 and 25 (NIH, 2010). However, recent studies show that the lowest death rates occurred in women with BMI in the range of 22.0 to 23.4 (McArdle, 2010, Fox, 2011). In this study mean values for BMI were measured to be in the range 24.0 to 24.8, also under the definition of overweight (BMI>25), but over the range for the lowest death rates. BMI over 25 is a risk factor for CVD, but the distribution of fat in the body is also important. There is a greater risk of CVD when a high amount of visceral fat is present (Fox, 2011). Visceral fat is the fat in the mesenteries and greater

omentum. While the BMI is a measurement for total body fat, WC is a measurement of visceral fat, since visceral fat is stored in the abdominal cavity. Both BMI and WC are predictors of CVD, and they may independently contribute to the prediction of total, non-abdominal, abdominal subcutaneous, and visceral fat (Janssen et al., 2002). Recent studies have suggested that WC might be an even better predictor of mortality risk than BMI, since people with normal BMI might have an increased WC (Koster et al., 2008, Leitzmann et al., 2011). The participants in this study decreased both total body fat and visceral fat after the exercise period, indicating a lowered risk for CVD. An increase in muscle mass percentage will result in higher energy consumption, meaning that the participants in this study might used more energy daily after the HIIT than before. Increased daily energy consumption may help keep the total body fat, and also BMI and WC, within healthy range.

4.2 Blood analysis

The blood variables measured in the present study were either variables affecting disease activity for RA/JIA, or variables indirectly associated with the risk of developing CVD. At the beginning of the study, mean values for all variables were within the reference values set for the specific variables. The results indicated that for some of the blood variables, the HIIT intervention was sufficient to start a regulation towards more preferable values, while the HIIT intervention was insufficient to have clinical effect on other variables.

Low-grade chronic inflammation is reflected by increased CRP concentrations and increased systemic levels of some cytokines (Ross, 1999). Regular exercise has been

proven to protect against diseases associated with low-grade systemic inflammation. This long-term effect of exercise may be ascribed to the anti-inflammatory response elicited by an acute bout of exercise, which is partly mediated by muscle-derived IL-6 (Petersen and Pedersen, 2005). In the present study the decrease in hsCRP was not significant, but it was a positive trend that the mean values for hsCRP decreased during HIIT. People with rheumatic diseases most often have elevated values of hsCRP because of joint inflammation (Dundar et al., 2008). Since inflammation plays a pivotal role in the pathogenesis of atherosclerosis, elevated levels of hsCPR are also associated with higher risk of cardiovascular morbidity and mortality (Ross, 1999). Decrease in hsCRP levels signifies less inflammation and hence lower risk for developing CVD.

In the present study both serum ferritin and haemoglobin levels decreased significantly after the HIIT. Measurement of serum ferritin is representative of the total body iron stores. Reduction of iron status in athletes is well documented (Newhouse and Clement, 1988). Both low haemoglobin values and decreased serum ferritin values are reported in endurance athletes and even non-endurance athletes exhibit low serum ferritin values. One explanation for this might be exercise induced plasma volume expansion, making a dilution of the iron components in the serum (Davidson et al., 1987). Increased iron loss in urine and sweat from athletes is also well documented (Newhouse and Clement, 1988, Carlson and Mawdsley, 1986). Measurements of fecal, urinary or sweat iron loss were not done in this study. However, this might partially explain the loss of iron stores observed after a period of repeated bouts of HIIT. High levels of stored iron are associated with inflammatory processes and CVD (MacDonald, 1993). During exercise serum ferritin levels, and also iron stores,

decrease, and could be one mechanism for reducing inflammation and risk for CVD. The participants in this study did not have exceptionally high iron levels.

Glucose levels increased during HIIT, and decreased during the control period. These results were not expected, since glucose levels normally decrease during exercise (Ivy, 1997). Exercise improves the insulin action and as a consequence of this, the glucose concentrations in blood decreases (Ivy, 1997). One explanation might be that some of the participants changed their diet or eating habits. Shift to a higher carbohydrate diet, after prolonged adaptation to fat diet, during an exercise period has been shown to cause increased resting muscle glycogen levels (Helge et al., 2002). Another explanation for the unexpected results might be that some of the participants did eat or drink before blood collection, which was to be taken after 12 hours fasting.

4.3 Disease activity

None of the parameters for disease activity, as DAS28, MHAQ and VAS-scores, showed significant changes, meaning that the participants neither got much worse nor much better after 10 weeks of HIIT. However, there were trends reporting that they felt better after the exercise period. Both the MHAQ questionnaire and the self reported global health VAS-score showed promising improvements for the disease activity. The VAS-score for total body pain showed a positive trend, with decreased pain, after the HIIT period, compared to the control period. The questionnaire SF-36, outlined in the radar-plot (Figure 3.3), also showed an improvement for bodily pain. Results indicate that HIIT improves comfort and none of the participants reported any worsening of the disease. The SF-36 also indicated better emotional state after the exercise period. It

seemed that better fitness gave better life quality. The fatigue VAS-score indicated decreased fatigue during the control period. This finding was unexpected. There was no difference between pre and post values in the HIIT period, meaning that the hard physical exercise did not influence the feeling of fatigue in the participants. One possible explanation for the observed decrease in the control group might be that half of the subjects had the control period post-test in June, the brightest period of the year, making them feel less tired.

4.4 Molecular analysis

4.4.1 IL-6

In the present study IL-6 mRNA levels increased after HIIT. It has previously been shown that IL-6 has both pro- and anti-inflammatory activity (Petersen and Pedersen, 2005). IL-6 is a multifunctional cytokine. It regulates humoral and cellular responses and plays a central role in inflammation and tissue injury. Unlike other pro-inflammatory cytokines, IL-6 appears to be the primary inducer of acute-phase proteins, many of which have anti-inflammatory properties, as well as inhibiting TNF-α and IL-1 (Pedersen et al., 2001). During recent years, it has been demonstrated that IL-6 is produced locally in contracting skeletal muscles and that the net release from the muscle can account for the exercise-induced increase in arterial IL-6 concentration. Physiological concentrations of IL-6 stimulate the appearance in the circulation of the anti-inflammatory cytokines IL-1ra and IL-10. This indicates that IL-6 may represent an important link between contracting skeletal muscles and exercise-related metabolic changes (Pedersen and Febbraio, 2008). In our study, blood

samples were taken within 48-96 hours after the last exercise. Most research on exercise and cytokines released from muscle, myokines, show an acute elevated level of IL-6 and IL-6 mRNA, not elaborating on the long-term duration of the phenomenon. In our case the IL-6 mRNA level was increased several days after the last exercise session, compared to pre-exercise levels. Chronic elevated levels of IL-6 could exert pro-inflammatory effects and have detrimental effects on metabolism (Pedersen and Fischer, 2007). The IL-6 mRNA in this study was isolated from white blood cells. Maximal exercise, with large muscle groups involved, provokes higher immune responses during repetitive bouts (Nielsen et al., 1996). IL-6 is produced in larger amounts than other cytokine in relation to exercise, and also plasma levels of cytokines increase in response to exercise. The cytokine response has been linked to muscle damage in earlier studies (Bruunsgaard et al., 1997), but recent studies have shown IL-6 to be produced without muscle damage (Pedersen, 2000). Given that IL-6 is produced locally in skeletal muscle in response to exercise, and IL-6 is known to have growth factor abilities, it is likely that IL-6 plays a beneficial role and may be involved in mediating exercise-related metabolic changes (Pedersen, 2000). Since the immune system during recovery from repeated bouts of maximal exercise has been shown not to be normalized within seven days after the last exercise (Bruunsgaard et al., 1997), it is likely to assume that the increased levels of IL-6 mRNA in this study are mediated by muscle contractions. Other inflammations markers, such as hs-CRP and PTX3, did decrease after HIIT, pointing to a possible anti-inflammatory role of IL-6.

4.4.2 ELISA results

COMP

RA patients are able to increase their functional ability and physical capacity by long-term intensive exercise (de Jong et al., 2003). It has been hypothesized that an increase in damage to the large joints might be a consequence of cartilage wearing, a potentially negative effect of high-intensity exercise. De Jong *et al*. found that intensive exercise does not increase radiographic damage of the large joints, except possibly in patients with considerable baseline damage of the joints. In recent years, many studies have aimed to identify a role for glycoproteins, as COMP, in autoimmune diseases and in other diseases that damage cartilaginous tissue (Morozzi et al., 2007). COMP is secreted first into synovial fluid and subsequently into serum, making it relatively simple to monitor in evaluation of the extent of cartilage damage. However, serum levels are significantly affected by physical exercise (Andersson et al., 2006). Intense physical exercise induces acute-phase inflammatory reactions, such as an increase in hs-CRP, CPK (creatine phosphokinase) and IL-6 levels, as a result of muscle contraction, muscle damage and changes in the liver (Petersen and Pedersen, 2005). Cartilage damage and/or metabolic changes also increase during physical exercise, making COMP levels increase (Kim et al., 2009). Recovery to pre-exercise level might take several days. Kim *et al*. found that increased levels of COMP after an ultra-marathon remained high for 3 days after the race, not returning to pre-race level before day 6 of recovery. Another study found that increased COMP levels after intensive exercise could not predict longitudinal progression of damage of the large joints in RA patients (de Jong et al., 2008). In the present study the COMP levels increased in the HIIT period compared to the control. Whether this increase is a result of acute or permanent cartilage destruction induced by high intensity exercise is not

clear. Blood was collected 48-96 hours after the last exercise session, making it difficult to say how long of a recovery period was necessary to get levels back to baseline. However, since other studies have shown that COMP levels are elevated some days after exercise, and exercise induced high levels do not predict longitudinal damage, it is likely to believe that this also is the case in this study.

PTX3

The difference in PTX3 levels in this study was not statistically different, but manifested a decreasing trend when HIIT was compared to control. Like hs-CRP, PTX3 plays a role in inflammatory circuits of RA, and is a marker of atherosclerosis and CVD (Luchetti et al., 2000, Norata et al., 2010). Regular exercise is known to induce anti-inflammatory effects and protect against the risk of CVD (Petersen and Pedersen, 2005). For the present study, decrease, although not statistically significant in PTX3, could signify lower inflammation and hence lower CVD-risk.

4.5 Limitations

The number of participants included in this study was low. The reason for this was that this was considered a pilot study because high intensity physical training has not previously been tested in arthritis patients. Several trends were evident and inclusion of more patients would have enhanced the statistical power and could have produced statistical significance.

The TNF-α ELISA kit was not highly sensitive. Well treated or healthy persons have values below detection limits for the kit used. Most of the participants were treated for

their disease, and 7 were treated with TNF-α inhibitors. Only two of the participants had levels high enough for detection, so a high sensitive kit had been more appropriate in this case.

Further, the patients suffered from RA or adult JIA, which are diseases with very unstable disease activity. Persons suffering from these diseases may consider themselves healthy one day, while the next day they may experience joint pain and general illness. It is therefore difficult to have these conditions stabilized and subjects have to resort to use anti-inflammatory drugs on demand. This should be taken into account when reading the results, since some of the results have very high ranges and high standard deviations.

Blood analyses were taken 4 times, pre and post HIIT, and pre and post control. The result reflects the status of the patient at that exact moment when the sample was taken, and it only gives a story of one temporal moment while offering no information about the underlying molecular mechanisms of the disease.

At last, the cross-over-design may have resulted in the patients first included into HIIT to have extended benefit from the training that also translated into their control period. As a result they could have had better results than the patients first included as controls. We have tried to reduce this effect by using a wash out period of two months.

5. Conclusion and practical applications

Females with RA and JIA, with stabile disease, tolerate high intensity interval training. The results showed tendency towards less pain, better global health, better function, and decreased inflammation. Even though not all of the results were statically significant in response to exercise period, they showed that high intensity exercise was well tolerated by these patient groups. Inflammation markers were decreased during exercise, and inflammation and CVD risk factors are tightly connected. Other factors associated with decreased cardiovascular risk, such as increased VO_{2max}, decreased BMI, decreased waist circumference, and decreased total and visceral fat, showed that women with RA and JIA decreased the risk of cardiovascular diseases with HIIT. Questionnaires used in this study showed that the HIIT improved the score for performing activities of daily living, achieved better score for general health, reduced pain, and gave fewer stressors with work or daily activities caused by emotional problems. In general, HIIT improved the quality of life in persons with RA and JIA.

In conclusion, ten weeks of high intensity interval training was well tolerated by females with RA and JIA, and improved the disease activity, quality of life, and traditionally risk factors for cardiovascular diseases. This study suggests that high intensity interval training can be safely recommended for persons with RA and JIA.

Future directions

The present study examined the effect of HIIT on premenopausal females with RA or JIA over a period of 10 weeks. Several questions raises for future studies, as the effect of the HIIT intervention on similar groups with longer duration, effect on other rheumatic diseases, effect on both genders, and effect on other elderly or younger patients. Further investigations are needed for HIIT and rheumatic diseases. IL-6

levels also need more investigation, since it is still unclear whether increased levels are a good or a bad thing for joint inflammation and rheumatic diseases.

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