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LIFESTYLE MATTERS –
EPIDEMIOLOGICAL STUDIES OF OILY FISH, BMI, LIFE EVENTS, PHYSICAL WORKLOAD AND RISK OF RHEUMATOID ARTHRITIS

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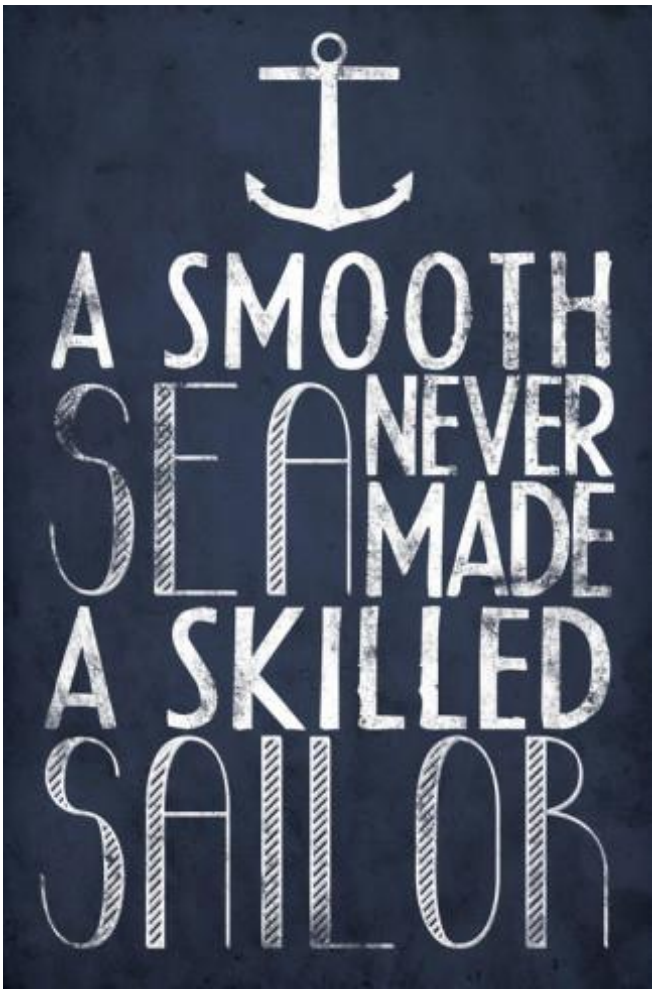

English proverb

Don’t cry because it’s over smile because it happened

Dr. Seuss

ABSTRACT

Rheumatoid arthritis (RA), divided in two major subsets defined by presence or absence of anti-citrullinated protein antibodies (ACPA), is chronic inflammatory disease which lead to joint damage and bone destruction. It is a complex disease where both genetic and environmental factors contribute to its development. The overall aim of my research was to mount greater knowledge of how some environmental and potentially modifiable lifestyle factors are related to the risk of developing rheumatoid arthritis. More specifically the aim was to study the association between body mass index (BMI) at symptom onset, as well as intake of oily fish and fish oil supplements, life events and physical workload five year prior symptom onset and the risk of developing RA.

The thesis is based on information from the EIRA (Epidemiological Investigation of Rheumatoid Arthritis) study. EIRA is a population based case-control study comprised of information from incident cases of RA, diagnosed in accordance with the 1987 American College of Rheumatology criteria, and randomly selected controls aged 18-70 years matched by age, sex and residential area. Cases and controls answered an extensive questionnaire with regard to environmental, lifestyle and occupational exposures and provided a blood sample for genetic and serological analyses. The response proportion was 94% for cases and 78% for controls.

Several lifestyle factors have been raised for the contribution in the pathogenesis of RA e.g. smoking, vitamin D, and alcohol). Consuming oily fish 1-7 times per week five year prior to symptom onset was associated with a decreased risk of developing RA, without major differences according to ACPA status. A BMI>30 was associated with an increased risk of developing ACPA-negative RA in women, while for ACPA-positive RA in men the association was reverse. Experience of life events (impaired economic situation, unemployment, marriage) five year prior symptom onset was associated with and increased risk of developing RA in women, independent of ACPA status. For men an association was observed with an increased risk of developing ACPA-positive RA. Substantial physical workload five year prior to symptom onset was associated with an increased risk of developing ACPA positive and ACPA negative RA in foremost in men.

Key words: Rheumatoid Arthritis, epidemiology, oily fish, BMI, life events, physical work load

LIST OF PUBLICATIONS

This thesis is based on four original papers. They are listed below and will be referred to in Roman numerials.

I. Rosell M, **Wesley** AM, Rydin K, Klareskog L, Alfredsson L.
Dietary fish and fish oil and the risk of rheumatoid arthritis.
Epidemiology. 2009 Nov;20(6):896-901.

II. **Wesley A**, Bengtsson C, Elkan AC, Klareskog L, Alfredsson L, Wedrén S
Association between body mass index and anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis: results from a population-based case-control study.
Arthritis Care Res (Hoboken). 2013 Jan;65(1):107-12. doi: 10.1002/acr.21749.

III. **Wesley A.,** Bengtsson C., Skillgate E., Saevarsdottis.S., Nordmark B., Theorell T., Holmqvist M., Klareskog L., Alfredsson L., Wedrén S.
Association between life events and Rheumatoid Arthritis, results from the EIRA case-control study

IV. **Wesley A.,** Källberg H**.,** Bengtsson C., Skillgate E. Saevarsdottis.S., Mullazehi M., Rönnelid J., Klareskog L., Alfredsson L., Wedrén S.
Association between physical workload and Rheumatoid Arthritis, results from the EIRA case-controls study

CONTENTS

LIST OF ABBREVIATIONS

Anti-Cit C1	Antibodies to citrullinated collagen type II
Anti-CII	Antibodies to collagen type II
ACPA	Anti-citrullinated protein antibodies
ACR	American College of Rheumatology
BMI	Body Mass Index
CI	Confidence Interval
DAS28	Disease activity score 28 joint count
EIRA	Epidemiological Investigation of Rheumatoid Arthritis
HAQ	Health assessment questionnaire disability index
HLA	Human leukocyte region
HPA	Hypothalamic-pituitary-adrenal
HrH	Health related habits
IL-1	Interleuking-1
IL-6	Interleukin-6
NYK	Nordic occupational classification
RA	Rheumatoid arthritis
RF	Rheumatoid factor
SAS	Statistical Analysis System
SEP	Socio economic position
TNF	Tumor necrosis factors
VDR	Vitamin D receptors
WHO	World health organization

PREFACE

Several chronic diseases can today be prevented, cured, or suppressed by effective medication. Still there are many reasons to engage in research to advance the knowledge of how an individual's lifestyle and health-related habits can prevent or decrease the risk of developing chronic diseases. Epidemiology constitutes an instrument for studying how changes in the surrounding environment and modifiable factors can influence the development and course of a disease.

Rheumatoid arthritis is a chronic disease and the most common rheumatic disease. It affects 0.5-0.8% of the population in Sweden. Previously, rheumatoid arthritis conferred severe disability and loss of working ability, and even if there is still no cure for the disease it is today possible, with available treatments to suppress most cases of the disease and achieve a symptom free state. The downsides are that the costs of the new biological treatments are high and the long-term side-effects are not yet fully known.

Most of the world's populations are the aging due to an overall increasing lifespan. By gaining knowledge of the benefits of a more health-prone lifestyle and effects of changes in modifiable habits it might be possible to delay the faltering health which often accompanies advancing age. Preventing the occurrence of disease which otherwise would demand health care and longstanding treatment might offer possibilities for savings in health care expenditure as well as, no less importantly, quality of life in the individual.

With the work of this thesis I have become more humble, there are no longer simple answers to any questions. Instead I am caught by "it depends" and "under these circumstances" in a very complex reality compounded by multi-dimensional human behavior. I know now there is no an easy way out only many interesting paths to follow.

1. INTRODUCTION

1.1 LIFESTYLE

The concept of lifestyle can have different meanings depending on the context in which it's being used. The notion of lifestyle was first initiated by the psychologist Alfred Adler in the beginning of the 20th century where it was referenced to the way an individual, group or culture is living. In this sense, lifestyle refers to a combination of the individual's demographic profile i.e. sex, age, occupation, religion, civil status, residential area, social network etc. as well as the psychological aspects i.e. personality, values and life perspectives (figure 1) [1] .

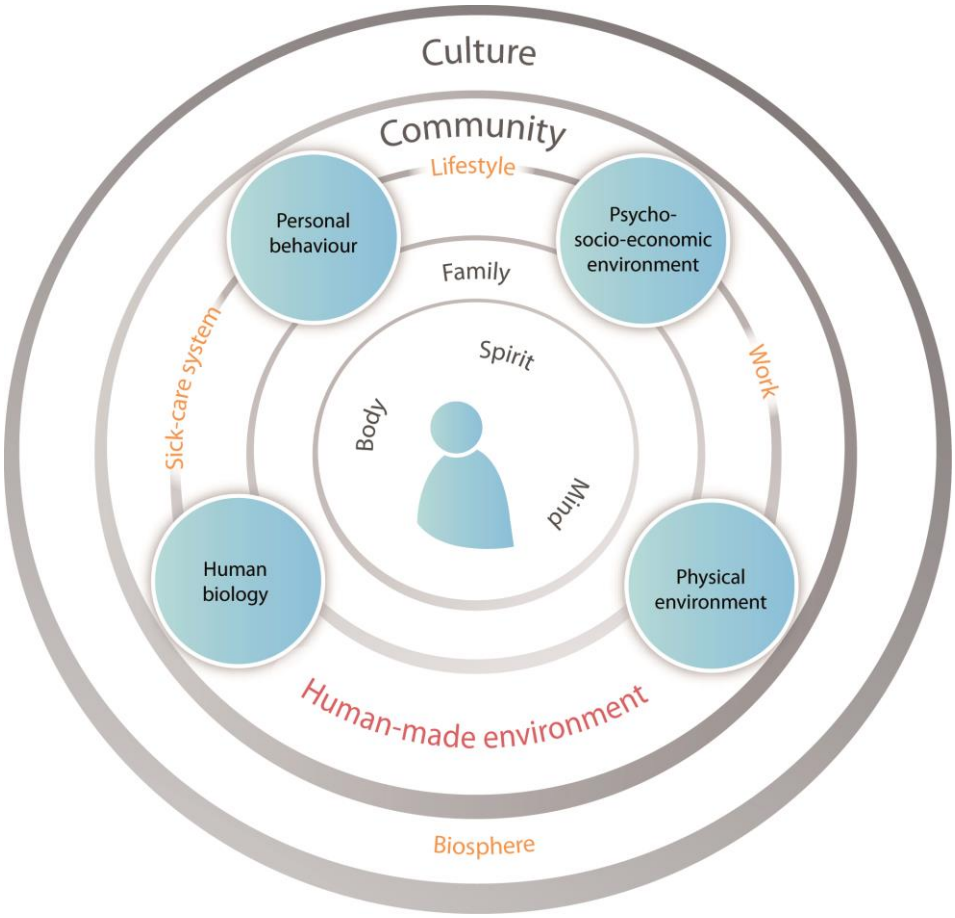

Figure 1. Mandala of Health

A concept closely associated with Lifestyle is Health-related Habits (HrH) - determinants or contributors to health, characterized by different patterns of complex behaviors. The HrH concept can be categorized into two dimensions; "positive health-related habits" – patterns of habits which will increase the lifespan and years without disability, and "negative health-

related habits – habits associated with increased illness and poor health. Exercise, Diet, Vitality/stress, Sleep, Cognition and Substance use are the six dimensions of HrH. An individual's lifestyle consists of both positive and negative HrH patterns as well as behavior e.g. an individual could be a non-smoker, have strategies for handling stress but be physically inactive [2].

1.2 RHEUMATOID ARTHRITIS

Rheumatoid arthritis RA is the most common inflammatory polyarthritis and an autoimmune disease, i.e. a disease caused by recognition of self-molecules and subsequent inflammatory response by the immune system [3]. Rheumatoid arthritis has for long been considered a disease with an uncertain pathogenesis, although recently it has evolved to a disease with specific subsets and with an increasing knowledge of its risk factors [4].The first symptoms are in many cases fatigue, morning stiffness and the symmetrically inflammation of the small joints of hands and feet [3]. Previously, rheumatoid arthritis caused severe disability and permanent joint damage, while nowadays early treatment with new effective drugs can to a large extent prevent disability. Though, despite new treatments and increased knowledge RA is still a chronic disorder with several severe and life expectancy reducing comorbidities e.g. cardiovascular disease, lung cancer and lymphomas [4, 5].

Rheumatoid arthritis is more common in women than in men. The annual incidence have been reported to range between 0.2 to 0.4 cases per 1,000 inhabitants in the population aged 16 years and older [6]. Changes in lifestyle and environmental exposures are suggested to be involved in the indicated trend of increasing incidence of rheumatoid arthritis [7, 8]

Individuals with Rheumatoid arthritis were until recently diagnosed in accordance with the 1987 American college of rheumatology (ACR) criteria, presented in table 1:

Table 1 1987 ACR criteria
Morning stiffness
In an around the joints for more than one hour for maximum improvement
Arthritis in ≥3 joints
Arthritis of joints in hands
Symmetric arthritis
Rheumatoid nodules
Radiographic changes
Presence of serum Rheumatoid factors

To be classified with the disease at least four out of these seven criteria must be fulfilled and criteria 1 through 4 needs to have been present for at least six weeks to be regarded as valid. The 1987 ACR criteria have during the last decade been challenged, since they were difficult to apply in individuals in an early phase of the disease for the reason that e.g. radiographic changes and rheumatic changes are not present in initial stages of rheumatoid arthritis where initiation of treatment is most beneficial [4]. Additionally they have been criticized for focusing on individuals with a potentially more severe or progressive disease, especially since one of the criteria is the presence of rheumatoid factor (RF). In the past the presence of RF have been a marker, dividing Rheumatoid arthritis in two subset; RF positive and RF negative rheumatoid arthritis, but the specificity of rheumatoid arthritis is limited since it is also present in other autoimmune diseases (e.g. Sjögens syndrome) as well as in health elderly individuals (10-30%) [9]. In the late 1990s a marker with 80% sensitivity and a specificity of >90% for Rheumatoid arthritis was established; antibodies to citrullinated protein antigen (ACPA). The presence of ACPA is a significant indicator for a more erosive RA and the antibodies are detectable several years before the clinical onset of RA, ACPA is thus a more informative diagnostic indicator for early RA [9, 10]. Given ACPA and the advantage of detecting rheumatoid arthritis in an earlier stage, new diagnostic criteria for Rheumatoid arthritis was presented in 2010 [11]. The criteria are an evolvement of the 1987 ACR criteria where the criteria for rheumatoid nodules and radiographic changes have been excluded and the presence of ACPA has been added for increased sensitivity (Table 2) [11].

Table 2 Classification criteria for rheumatoid arthritis (score-based algorithm: add score of categories A-D;a score of ≥6/10 is needed for classification of a patient as having definite rheumatoid arhtritis)	
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
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
Acute-phase reactants (at least 1 test result is needed for classification)	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
Duration of symptoms	
<6 weeks	0
≥6 weeks	1

1.3 RISK FACTORS FOR RA

The etiology of RA is not completely understood. Twin studies have estimated the relative contribution of genetic factors to be approximately 50% or less, leaving 50% or more to be contributed from other factors e.g. modifiable- and environmental factors [12, 13]. Recently it has been suggested that ACPA positive RA develops in three phases; The first phase is characterized by predisposition for RA caused by the e.g. the presence of genetic risk factors associated with RA [4]. No signs of inflammation or symptoms are evident and no biomarkers are present. This phase can last for years before a transition from phase one to two occurs. The second phase is suggested to be where the genetic predisposition in the individual begins to consociate with the surrounding context (e.g. environment and lifestyle see section 1.3.1 for more information). Phase two is characterized by an asymptomatic autoimmunity with presence of ACPA and supplementary immunological factors as well as potentially other elevated inflammatory markers. The length of phase two are likely influenced by lifestyle and environmental factors (dose-response) apart from factors like age and sex. The third phase marks the clinical onset with presence of symptom and other signs of RA i.e. ACPA and inflammatory biomarkers [14]. For ACPA negative RA the etiology are less known. In a recent article it was estimated that the heritability for ACPA negative RA is approximately 20%, which

indicates that the major risk factors for this subset of disease is dependent on non-genetic factors [4, 13].

1.3.1 Environmental and modifiable risk factors for RA

Several environmental and potentially modifiable factors have been suggested to contribute to the pathogenesis of RA e.g. dietary factors (vitamin D, protein,caffeine, iron) [15-24], alcohol [25, 26], socioeconomic status [27, 28], Silica [29], mineral oil[30], oral contraceptives [31, 32], parity [33] . The most established HrH associated with RA risk is smoking with an estimated attributable proportion for RA overall of 25% [34]. A dose-response relationship between smoking and RA has been observed [34, 35], and a model for the etiological linkage between smoking, citrinulation and RA has been proposed [36].

The additional environmental and potentially modifiable HrH in focus of this thesis will be more thoroughly presented in section (1.3.2-1.3.6).

1.3.2 Oily fish intake and omega-3 supplement

Dietary intake is a substantial characteristic of an individual's lifestyle and modifiable. The essential omega-6 and omega-3 fatty acids are of particular interest in relation to physiological states involving immunological and inflammatory processes. The long-chain metabolites of these fatty acids are needed for the production of eicosanoids that in turn play key roles in the immune system. Since the omega-6 fatty acids are precursors for proinflammatory eicosanoids that mediate platelet aggregation and vasoconstriction, while the omega-3 fatty acids are precursors for eicosanoids that lack this biological activity, the balance of these fatty acids in the diet may potentially affect the inflammatory activity in the body [37]. The omega-6 fatty acids are abundant in vegetable oils (e.g. sunflower, maize), milk and meat and the intake of these fatty acids is generally relatively high. The intake of the long-chain omega-3 fatty acids on the other hand is low and the most important dietary source is oily fish. Several studies have shown that supplementation of fish oil can improve clinical symptoms and delay the progression of the disease in people with already established RA [38]. In addition a milder form of RA has been

observed on the Faroe Islands which has been suggested to be related to the high fish consumption in this society [39]. Whether oily fish intake affects the risk of developing RA is less clear since the associations observed in previous studies are inconclusive; One of three case-control studies indicate a protective effect of fish intake [40-42] and one cohort study reported that the intake of oily fish was inversely associated with the risk of developing RA whereas a positive association was seen for the intake of medium oily fish [20]. No previous study has investigated possible effects of supplements containing omega-3 fatty acids on the risk of developing RA.

1.3.3 Body Mass Index

A high Body Mass Index (25≤BMI) is associated with several chronic diseases, e.g. cardiovascular diseases (CVD) [43, 44]. Inflammation is involved in the pathogenesis of both RA and CVD and since the two diseases have common risk factors - notably smoking -, and overweight/obesity is associated with an overall increased level of inflammation [34, 45, 46], it has been hypothesize that weight status may influence RA risk.

The estimated association between BMI and RA in previous studies has been inconclusive. With some finding no association [16, 47-51] and others showing obesity to increase the risk of developing RA [52, 53]. In a relatively small case-control study Pedersen found the association of overweight/obesity to be confined to those developing RA without ACPA, a subgroup analysis not carried out in the earlier studies [20]. In another small cohort of 55 individuals at risk (presence of autoantibodies specific for RA) for developing RA an increased risk was found among individuals with a 25≤BMI [54]. A large nested case-control study from Minnesota identified a modest increased risk of RA for individuals with a history of obesity (BMI>30) [55]. Finally, a Swedish study observed a significant decreased association between obesity and ACPA positive RA risk in men [56].

1.3.4 History of life event

Life events are here defined as changes in a person’s life that affect relationships and activities in a way which may be perceived as stressful

[57]. Life events can be acute, i.e. events with a defined onset and duration ("earthquake", "fire"), or chronic associated with undetermined onset and/or duration, i.e. chronic life experiences ("impaired economic situation", "unemployment", "change of workplace") [58]. Several lines of research indicate that protracted exposure to stress such as experiences of chronic life events, is associated with an alteration in the immune system causing increased vulnerability to disease (e.g. depression, cardiovascular disease, diabetes, multiple sclerosis, upper respiratory infections) [59-62]. Life event in the association to RA risk has previously been studied with contradicting results. Several studies with positive findings are based on small samples and/or heterogeneous groups of patients [63-65], while some larger studies have indeed shown associations between life events and RA [66, 67]. Contradictory however, other both large and small studies show no association between certain life events (negative childhood experience, death of a child) and RA [68, 69]. Additionally, work-related stress, defined by high psychological demands and low decision latitude, has previously been shown as associated with risk for RA [70]. Although no one has previously investigated the impact of life events in a more general sense in a sufficiently large cohort that enables the control of potential confounding factors, such as smoking, has not been conducted. Furthermore, no study has investigated the effects of life events on the two major and pathogenetically distinct subsets of RA (ACPA positive and negative).

1.3.5 Physical workload

Physical challenges to the joint with tissue injury promotes an initiation of an inflammatory cascade of pro-inflammatory cytokines (e.g. IL-1, IL-6, TNF), a process suggested to trigger and/or perpetuate systemic activation of the immune system [71-75].

The hypothesis that physical trauma is involved in the pathogenesis of RA was first introduced several decades ago, though these studies are based on case-report without a comparison group [76-78]. In a more recent case control study physical trauma 6 months prior to study inclusion was observed to be significantly associated with an increased risk of RA [79]. In addition an association between occupations with an increased workload

(farmers, quarry workers, construction workers, assistant nurses, freight, and transport workers) and RA has been suggested [80-84]. Finally, a significant association between physically demanding work and an increased risk of ACPA positive RA has been observed in a Danish case-control study [20]. [70]. However, no one has previously investigated the impact of physical work load in the two major subsets of RA, defined by the presence or absence of ACPA in a sufficiently large study.

2. OBJECTIVES

2.1 OVERALL OBJECTIVES

The overall aim of this thesis was to mount greater knowledge of the relationship between some environmental and potentially modifiable lifestyle factors and risk of developing RA.

2.2 SPECIFIC OBJECTIVES

I. To study the association between the development of RA and the intake of oily fish and fish oil supplements in a population based case-control study.

II. To investigate the association between Body Mass Index (BMI) and risk of developing the two main subtypes of RA

III. To study the association between life events and the risk for RA (RA) with and without antibodies to citrullinated peptides (ACPA).

IV. To assess if a history of perceived substantial physical work load is associated with a risk of developing rheumatoid arthritis with and without antibodies to citrullinated peptides (ACPA)

3. MATERIAL AND METHODS

In 1996 the population-based case control study EIRA (Epidemiological Investigation of Rheumatoid Arthritis) was initiated with the objective to study environmental exposures and risk of RA. Since 1996 EIRA has enrolled incident RA cases and population controls, aged 18 to 70 years, living in defined parts in the south and middle of Sweden. The study is continuously ongoing and the observation periods for the four studies in this thesis are: May 1996 to December 2005 (study I) and May 1996 to November 2009 (study II-IV).

3.1 THE EIRA STUDY

3.1.1 Recruitment of subjects

Incident cases with RA were identified at all public as well as most of the very few private rheumatology units in the defined regions. Cases were diagnosed by a rheumatologist in accordance with the 1987 ACR criteria [85]. For every new reported case a control was randomly selected from the study base matched for age, sex, and residential area using the national population register (continuously updated). If a control refused to participate, was not traceable or reported having RA, a new control was selected, using the same procedure. For cases and their matched control(s) an index year was given that indicated the year at which the case got their first symptoms of RA. All participating cases and controls were required to be fluent in Swedish.

During the study period for study I (May 1996 - November 2005) 96% of cases and 78% of controls while in the study period for study II-IV (May 1996- December 2005) 94% of cases and 78% of controls participated in EIRA.

3.1.2 Data collection

A questionnaire containing a wide range of questions about environmental exposures was given to the cases, at the rheumatology clinic shortly after their diagnosis and sent by mail to the controls. The questionnaires were supposed to be answered at home and incomplete questionnaires were completed, by telephone or mail, by purpose-trained persons at the EIRA

secretariat, not connected to the rheumatology units. Non-responders were reminded for at the most four times. Blood sample was drawn from cases at the rheumatology clinic and controls at the local health centre and thereafter sent by post to the study laboratory at Karolinska Institutet. In the initial phase cases not fulfilling the ACR criteria were reported, these subjects were later excluded from the study. Although controls belonging to excluded cases remained in the study in order to increase the power.

3.1.3 Genetic and serological analyses

Rheumatoid factor (positive or negative) status for all cases was determined at the local clinic using nephelometry were the cut-off for positivity was determined by the level in the highest 5% in healthy controls. The presence of ACPA, collagen type II antibodies (anti-CII) and antibodies specifically to citrullinated collagen type II (anti-Cit C1) was determined using the Immunoscan-RA Mark2 enzyme-linked immunosorbent assay (ELISA) (Rönnelid 2005). We considered ACPA as present if the serum antibody level exceeded 25 U/ml, 29 AU/ml for anti-CII and 10 AU/ml for anti-Cit-C1. We extracted DNA from the blood and genotyped HLA-DRB1 to establish presence/absence of "shared epitope" (SE) alleles (DRB1*01, DRB1*04 and DRB1*10) as described in detail in Padyukov et al 2004 [86]

3.2 ENVIORNMENTAL EXPOSURES

3.2.1 Oily fish and fish oil supplements

Fish intake was estimated by: 'How often have you consumed oily fish (e.g. herring, mackerel, salmon) during the latest five years on average?' with the following answer options 'daily', 'sometimes per week', 'sometimes per month' and 'very seldom or never'. Very few participants reported a daily consumption of oily fish and the categories of daily consumption and weekly consumption were therefore collapsed into one category resulting in three exposure categories for oily fish intake.

Data on intake of fish oil supplements were obtained through: 'Have you during the latest five years regularly taken vitamin supplements for at least one month?' (Yes/No) and 'Have you during the last 5 years taken natural remedies for at least one month?' (Yes/No). Participants who answered

'yes' were asked to report what kind of supplement or remedy they had taken and between which years it was taken.

The reported lists of supplements were classified into four groups according to their content of omega-3 fatty acids: i) supplement with no omega-3 fatty acids; ii) supplement with omega-3 fatty acids in the form of fish oil; iii) supplement with omega-3 fatty acids in the form of algae, and iv) supplement with omega-3 fatty acids in the form of plant extracts (e.g. linseed). Only supplements reported to be taken before the index year were considered as exposure since the onset of the disease may affect the participants' use of supplements. Subjects who reported they took supplements with omega-3 fatty acids before index year were accordingly classified as exposed and subjects who reported they did not take any form of omega-3 fatty acids containing supplements before index year or who started to take such supplements after index year were classified as unexposed. In total 130 women and 36 men were excluded due to missing information on index year, leaving 2732 women and 1146 men for the analyses on supplements (study I). Less than 1 % of the subjects reported an intake of supplements containing omega-3 fatty acids in the form of algae or plant extracts and therefore only fish oil supplements were examined in relation to the risk of developing RA.

3.2.2 BMI

Self-reported weight and height at inclusion were used to calculate body mass index (BMI, Kg/m^2). We used the WHO BMI classification of underweight ($BMI<18.5$), normal weight ($18.5 \leq BMI<25$), overweight ($25 \leq BMI<30$) and obese ($30 \leq BMI$). Less than 2% of the observations had a BMI below 18.5 so the categories for underweight and normal weight ($BMI<25$) were combined into one. We also categorized BMI in the three groups using information on weight and height at the age of 20.

3.2.3 Life events

The questionnaire contained a question covering the occurrence of 15 particular life events the past five years. These events have been identified in a previous investigation as having profound impact on a person's life and

have been used in previous epidemiological studies [87, 88]. The events were measured in two sections. One section, with 7 items, covered events that were presumed to foremost have a negative impact where the respondent could grade the impact of the event as strong negative-,tangible-, or not significant impact. The other section, with 8 items, covered events which may have either positive or negative impact on the individual and the respondent could grade the impact of the event as very negative, negative, not significant, positive or very positive. The reason for the division into two sections was that it was felt, at the design of the study, that it was impertinent to imply that; for example, bereavement could be perceived as having a positive impact. The items in section one were: serious conflict with spouse, serious conflict with close relative/friend, disease or accident of spouse or child, death of spouse or child, death of close relative/friend, personal economic decline, conflict at work. The items in section two were: divorce or separation, marriage or common law marriage, change of residence, change of workplace, decreased responsibility at work, increased responsibility at work, unemployment, and other self-reported stressful live event. The respondents were asked to report the year in which each event had occurred. Events which had occurred during or after the index year or more than five years prior to the index year were not considered exposures in this study and were consequently disregarded.

3.2.4 Physical workload

Perceived physical work load 5 years prior to inclusion, were concerned in one item in the questionnaire, where the respondent could grade their work load in; not working, sedentary- very light-, light-, relatively light-, slightly strenuous-, strenuous-, very strenuous- and highly strenuous work. The respondents also reported their occupation during the same time period, this information was used to validate the physical workload. Occupation was classified according to the Nordic Occupational Classification system (NYK "Nordisk Yrkesklassifikation", Nordic Occupational Classification based on International standard Classification of Occupations. Geneva:ILO, 1958). Every other year Swedish Work Authority conducts a work environment survey, including assessment of physical work load, where approximately 16 000 individuals of the working population are interviewed by telephone.

The physical work load is estimated and categorised in 9 occupational sectors (legislators, senior officials and managers/professionals/technicians and associate professionals/clerks), service workers and shop sales works/skilled agricultural and fishery workers/ craft and related trade workers/ plant and machine operators and assemblers/ elementary occupations [84].

3.3 THE SWEDISH RHEUMATOLOGY QUALITY REGISTER

The Swedish Rheumatology Quality register, was established in the mid-nineties and includes RA-patients aged 16 years or older fulfilling the 1987 ACR criteria for RA [89] [90]. The SRQ was initiated as a surveillance register, in order to allow longitudinal follow-up of patients with RA with regard to medical interventions and outcome. In general a patient is included at their first visit to a rheumatologist after symptom onset, when biological treatment is initiated (mandatory) or when the physician aim to follow up all enrolled patients. The register provides a clinical tool to evaluate and monitor patients and to assess the quality of care in the RA population. In the register the physician enters information on diagnosis, symptom onset, ACR criteria, RF status, treatment, information on disease activity parameters (C - reactive protein, erythrocyte sedimentation rate, number of swollen and tender joints, physicians global assessment of disease activity, and current treatment), and by patient estimated general health assessed with a visual analog scale. A disability score – measured with the Health Assessment Questionnaire Disability Index assessing limitations in eight areas of daily living activities -[91], and disease activity – measured with Disease Activity Score (DAS28), calculated by previously mention disease activity parameters [92]**.** Via a unique 10-digit national registration number issued to all Swedish residents [90], clinical information from the SRQ can be linked to environmental exposure information in EIRA.

3.4 THE SWEDISH INPATIENT REGISTER

Inpatient care in Sweden is public and virtually accessible to all Swedish residents. Data on hospitalization have been computerized and registered by county since 1964 and nationwide since 1987 in the inpatient registered also referred to as Hospital Discharged Register. The register include the national

registration number of hospitalized individuals – defined as at least one overnight stay, date of admission, date of discharge, and diagnosis code – classified according to International Classification of disease (ICD)version 7-10 (one main diagnosis and up to seven contributory medical diagnosis.

3.5 CONFOUNDING FACTORS

In the analysis, matching variables taken into consideration in the selection of controls (age in 10 categories, sex and residential area in 13 categories) were adjusted for. Additionally we also investigated the influence of several potential confounding factors. In study I-IV we adjusted for smoking (never, current, past, non-regular), alcohol were adjusted for in study I-III (no intake/low intake/ high intake in study I, never consumed alcohol during past 12 months/ever consumed alcohol during past 12 months in study II and no alcohol/2.9/2.9-4.53/≥4.53 units of alcohol per week - 1 unit is 20 cl wine/33 cl beer/5 cl liqueur or spirits in study IV), BMI – kg/m² (<25/25-29.9/30<) in study I and finally university education (yes/no) in study II-IV.

3.6 STATISTICAL ANALYSIS

With the binary outcome of interest (RA yes/no) in this thesis we have used logistic regression method to estimate the odd ratios (OR) for developing RA with a 95% confidence interval (95% CI). These calculations have been performed with Statistical Analysis System (SAS) software version 9.1.3 (study I), version 9.2 (study II), version 9.3 (study III-IV) (SAS institute, Cary, NC, USA). The cases were stratified on the presence or absence of ACPA, without taking ACPA into consideration in the controls.

In the analysis we chose to break the matched design and present the results calculated with unconditional logistic regression (study I, III-IV). This enables us to include all subjects with questionnaire information, increase the number of observations in the models and gave better precision in the estimates especially in the analysis stratified by ACPA. In all unconditional models the matching variables (sex, age and residential area) were adjusted for. All displayed estimates calculated with unconditional logistic regression have also been analysed with conditional

logistic regression. There ORs for the two methods (unconditional and conditional) were in close agreement in study I, III-IV.

In study II we chose to present the estimates calculated only with conditioned (age, sex and residential area) logistic regression. In figure 1 the conditioned selection of the study sample is presented.

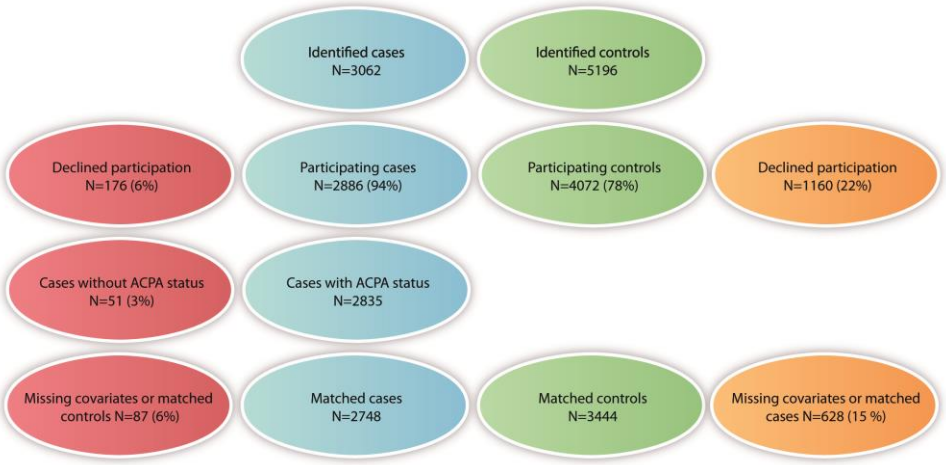

Figure 2. Flow of cases and controls in data analysis of BMI and risk of developing RA

3.7 STUDY I

The exposure of interest in study I was oily fish consumption (never-seldom/1-3 times per month, and 1-7 times per week) where intake of oily fish never or seldom was used as reference, and intake of fish oil supplements (no intake before index year/regular intake before index year), using no intake as reference. Analyses were stratified with reference to status in cases by presence or absence of RF and ACPA,. Men and women were analysed together as well as separately. Adjustments were made for matching variables (age and residential area) and sex (where appropriate) as well as smoking.

3.8 STUDY II

The effect of overweight ($25 \leq$ BMI ≤ 30) and obesity ($30 \leq$ BMI) compared to normal weight (BMI < 25) was the exposure of interest in study II. The final multivariate model was conditioned on matching variables (age, residential area, and sex together) and adjusted for smoking, alcohol

consumption, and university education. The analyses were stratified by ACPA. In a sub-analysis we excluded cases reporting having psoriasis.

3.9 STUDY III

In study III objects with a history of life events five years prior to index year were considered exposed. Any life event, number of events (0/1/2/3+) as well as particular events was analysed. Respondents who reported having experience no life events during the defined period were considered unexposed and used as reference group. In addition we investigated the association with particular events, by impact. History of life events were analysed with adjustments of matching variables (age and residential area) and sex when women and men were analysed together. The final multivariate model was adjusted for alcohol, smoking and university education, in addition we stratified the analyses by ACPA positive and ACPA negative RA and analysed women and men separately.

3.10 STUDY IV

Perceived physical work load was the exposure of interest and dichotomized into "minor physical work load" (sedentary- very light-, fairly light work) and "substantial" physical work load" (slightly strenuous-, strenuous-, very strenuous- and highly strenuous work). As an alternative exposure, nine occupational sectors were divided into "minor work load" and "substantial" physical work load. For both exposures "minor physical work load" was used as reference category. The analyses were adjusted for matching factors (age and residential area) as well as sex where all subjects were analysed together. As potential confounding factors were smoking and university education considered and included in the final model. We used chi-squared test to investigate if substantial physical workload five years prior to study inclusion was associated with presence of anti-CII and Cit-C1 at diagnosis. Also in this study the analysis were stratified by ACPA and women and men were analysed separately. To assess potential trend in participation among objects we stratified the analysis on time-period (1996-2000/2001-2006/2007-2009), age (less than 47 years, 48-57 years, 58 years or more), and SEP measured as blue collar vs. white collar professions. Finally we estimated the potential impact of selection bias by assessing non-responding

subjects as exposed, in accordance with the proportions of non-responding subjects without university education (85% in cases and 82% in controls), based on the proportions of non-responding subjects without university education in the article by Bengtsson et al [93].

In addition we performed a sub-analysis, where we restricted the analyses to subjects who reported having a minor workload five years prior to study inclusion. To study the association between physical activity during leisure time and RA risk, among those with a sedentary work. Physical activity during leisure time was measured in four categories: Sedentary (Sedentary leisure time, you mostly pursue your time with reading, TV or other sedentary interests. You walk/walked, ride a bike or are active in other forms for less than 2 hours/week.), Light leisure time activity (You walk/walked, ride a bike or are active in other way at least 2 hours/week, without sweating. This includes walk and ride a bike to and from work, other walking, heavier house-hold work, gardening, fishing, table tennis and bowling.), Regular moderate leisure time activity (You are running, swimming, playing tennis/badminton or participating in other forms of activity, on a regular basis 1-2 times/week for a least 30 minutes at the time), Regular exercise (You pursue running, swimming, play badminton, work out or other similar activity at least 3 times/week each occasion lasting for at least 30 minutes). We dichotomised the four categories into sedentary (sedentary/light leisure time activity) and regular physical activity (regular moderate physical activity /regular exercise).

All studies included in this thesis are approved by relevant ethical committees all the participants consented to contribute to the study on a voluntary basis.

4. RESULTS

The population-based case-control study EIRA has conveyed data to the results in this thesis. We have also used some additional material to preclude bias or misclassification. In study I the recruitment period was May 1996 to December 2005 with a participation proportion of 96% in cases and 82% controls, study II-IV are based on a longer recruitment period (May 1996 to November 2009) and have a slightly lower participation proportion (94% and 78% in cases and controls respectively).

In study I the recruitment period was May 1996 to December 2005 with a participation proportion of 96% in cases and 82% controls, study II-IV are based on a longer recruitment period (May 1996 to November 2009) and have a slightly lower participation proportion (94% and 78% in cases and controls respectively).

4.1 STUDY I

In study I we analyzed 1899 (1349 women and 550 men) cases and 2145 (1514 women and 631 men) controls.

Compared with subjects who never or seldom consumed oily fish the OR for developing RA was OR 0.8 (95% CI 0.6-1.0) for subjects who consumed oily fish 1-7 times/week. The results did not change when stratifying by ACPA or RF. Similar results were seen for subjects consuming oily fish 1-3 times a month. Cases and controls did not differ in their consumption of fish oil supplements.

4.2 STUDY II

In this study we analyzed 2748 (1962 women and 786 men) cases and 3444 (2468 women and 976 men).

There was no overall association between BMI and RA risk, when stratifying for ACPA presence and sex there was a significantly increased risk for ACPA negative RA in women (OR 1.6, 95% CI 1.0-3.3). For ACPA

positive RA there was an inversed association with BMI in men (OR 0.6, 95% CI 0.3-0.9).

When stratifying the analysis by HLA-DRB1 SE alleles, women who were non-carriers of the HLA-DRB1 SE alleles and had a BMI equal or above 30 had an OR for developing ACPA negative RA of 3.6 but with a wide confidence interval, 1.5-8.4. Although there were no other convincing changes to the overall results (Table 3).

Table 3 Odds ratios of developing RA overweight and obese compared to normal weight according to the WHO classification, stratified by ACPA status, sex and HLA-DR SE alleles

	Women		Men	
ACPA positive	**[1]n ca/ n co**	**[2]OR (95%CI)**	**[1]n ca/ n co**	**[2]OR (95%CI)**
No HLA-DRB1 SE alleles				
25<BMI	82/1344	REF	20/333	REF
25≤BMI<30	36/626	1.1 (0.5-2.3)	19/439	0.9(0.3-2.6)
30≤BMI	19/262	2.2 (0.7-6.5)	6/125	0.7(0.1-3.5)
Any HLA-DRB1 SE alleles				
25<BMI	444/1339	REF	43/337	REF
25≤BMI<30	202/657	0.9(0.6-1.1)	60/421	0.6(0.4-0.9)
30≤BMI	78/274	0.7(0.4-1.9	31/125	0 4(0.2-0.8)
ACPA negative				
No HLA-DRB1 SE alleles				
25<BMI	125/1344	REF	33/333	REF
25≤BMI<30	71/626	1.6(0.9-2.8)	37/439	0.9(0.4-1.9
30≤BMI	40/262	3.6(1.5-8.4)	15/125	0.8(0.3-2.2)
Any HLA-DRB1 SE alleles				
25<BMI	149/13 9	REF	43/337	REF
25≤BMI<30	78/657	1.2(0.7-1.8)	60/421	0.9(0.5-1.8)
30≤BMI	43/274	1.5(0.8-2.7)	15/125	0.8(0.3-2.2)

[1]Number of exposed cases

[2]Logistic regression model conditioned on age and residential area and adjusted for smoking (ever/never), alcohol consumption (ever/never during past 12 months), and education (university education: yes/no).

A proportion of the RA cases have psoriasis, a disease which is well known to be associated with BMI [94-96]. In order to determine whether concomitant co-morbidity with psoriasis would explain our results, we removed 215 (8%) cases who reported psoriasis in the questionnaire or who appeared in the national in-patient or out-patient registries with a main or contributory diagnosis of psoriasis, from the analysis. The association between obesity and ACPA negative RA in women was weakened (adjusted OR 1.3 95%CI 0.9-1.8).

4.3 STUDY III

In this study, 2774 cases (1981 women and 793 men) and 3911 controls (2803 women and 1108 men) were included in the analyses.

We found a weak significant association between having experienced any life event and ACPA positive RA (OR 1.1, 95%CI 1.0-1.2) and ACPA negative RA (OR 1.2 95%CI 1.0-1.4). There was a significant trend of increased risk for developing ACPA negative RA overall with increasing number of events experience during the five year period prior to study inclusion. Further there was no overall indication that induction/latency would be longer in ACPA positive RA than in ACPA negative RA, as would have been indicated by stronger associations for events that occurred in the interval 3-5 years prior to onset in ACPA positive then in the interval 0-2 years in ACPA negative (table 4).

Table 4 Odds ratios (OR) and 95% confidence intervals (CI) of incident rheumatoid arthritis associated with stressful life events. By total number of events and time interval.

		Women		Men	
		Cases (%)/Controls(%)	OR (95%CI)[1]	Cases (%)/Controls(%)	OR (95%CI)[1]
ACPA positive					
No event		274/685	REF	168/337	REF
Any event		993(78)/2118(76)	1.2(1.0-1.4)	342(67)/771(70)	0.9(0.7-1.2)
Number of events					
1		321(54)/675(50)	1.2(1.0-1.5)	131(44)/305(48)	0.9(0.7-1.2)
2		246(47)/573(46)	1.1(0.9-1.4)	90(35)/212(39)	0.9(0.6-1.2)
3+		426(61)/870(56)	1.3(1.0-1.6)	121(42)/254(43)	0.9(0.7-1.3)
	Interval[2]				
1	0-2	182(40)/391(36)	1.2(1.0-1.5)	66(28)/158(32)	0.9(0.6-1.3)
	3-5	139(34)/284(29)	1.3(1.0-1.6)	65(28)/147(30)	1.0(0.7-1.4)
2	0-2	89(25)/213(24)	1.1(0.8-1.6)	32(16)/84(20)	0.9(0.5-1.4)
	3-5	70(20)/144(17)	1.2(0.9-1.7)	27(14)/54(14)	1.0(0.6-1.6)
3+	0-2	77(22)/167(20)	1.2(0.9-1.7)	20(11)/52(13)	0.8(0.4-1.5)
	3-5	56(17)/121(15)	1.2(0.9-1.8)	24(13)/40(11)	1.1(0.6-1.9)
ACPA negative					
No event		151/685	REF	84/337	REF
Any event		563(79)/2118(76)	1.2(1.0-1.5)	199(70)/771(70)	1.2(0.9-1.6)
Number of events					
1		169(53)/675(50)	1.1(0.9-1.5)	72(46)/305(48)	1.0(0.7-1.5)
2		150(50)/573(46)	1.3(1.0-1.6)	50(37)/212(39)	1.1(0.8-1.7)
3+		244(62)/870(56)	1.4(1.1-1.8)	77(48)/254(43)	1.4(1.0-2.1)
	Interval[2]				
1	0-2	97(39)/391(36)	1.2(0.9-1.6)	40(32)/158(32)	1.2(0.7-1.8)
	3-5	72(32)/284(29)	1.1(0.8-1.6)	32(28)/147(30)	0.8(0.5-1.4)
2	0-2	59(28)/213(24)	1.4(1.0-2.0)	20(19)/84(20)	1.2(0.7-2.1)[3]
	3-5	37(20)/144(17)	1.2(0.8-1.8)	16(16)/54(14)	1.2(0.6-2.3)[3]
3+	0-2	45(23)/167(20)	1.3(0.8-2.0)	19(18)/52(13)	2.0(1.0-3.8)
	3-5	40(21)/121(15)	1.6(1.0-2.4)	9(10)/40(11)	0.9(0.4-2.1)[3]

[1]Adjusted for matching factors (age and area of residence), alcohol consumption, smoking, and education.
[2]Time interval in years before symptom onset in cases or index year in controls, respectively. Respondents with events in both time periods are excluded in the period stratified analysis.
[3] Model stability questionable

For particular events, 8 events were associated with ACPA positive RA in women and 8 events (partly different ones) with ACPA negative RA in women. Events associated with both ACPA positive and negative RA were

"Conflict at work", "Change of residence", "Change of workplace", and "Increased responsibility at work". Having experienced the "Death of a child or spouse" was associated with ACPA positive and ACPA negative RA in women if the event had occurred with a more distant time interval to onset/index year. Marriage was associated with RA in men, regardless of ACPA status, if it had happened between 3 and 5 years before onset (table 5). Among the items where the impact could be graded as positive-not significant -negative, significant associations tended to be the strongest where impact was graded as negative (table 6). Beyond that, there was no discernible pattern of association with regard to the perceived impact of events. Overall, among men the analyses were hampered by small numbers of cases and events and results seemed to be most convincingly blank for ACPA positive RA.

Table 5 Separate events by time interval from event to onset/index year: Odds ratios (OR) and 95% confidence intervals (CI) of rheumatoid arthritis associated with life events.

	[2]	All		Women		Men	
		ca (%)/co(%)	[1]OR (95%CI)	ca (%)/co (%)	[1]OR (95%CI)	ca (%)/co (%)	[1]OR (95%CI)
ACPA positive							
No event	442/1022	REF	274/685 REF	168/337)	REF		
Conflict with spouse	0-5	171(28)/417(30)	0.9(0.7-1.2)	130(32)/307(31)	1.0(0.8-1.3)	41(20)/110(25)	0.7(0.5-1.2)
	0-2	84(14)/218(15)	0.9 (0.7-1.2	64(16)/154(16)	1.0(0.7-1.4)	20(10)/64(10)	0.6(0.4-1.6)
	3-5	87(14)/199(14)	1.0(0.7-1.3)	66(16)/153(15)	1.0(0.7-1.4	21(10)/46(10)	0.9(0.5-1.6
Conflict with relative	0-5	190(30)/393(28)	1.1(0.9-1.4)	159(37)/318(32)	1.0(0.8-1.3)	31(16)/75(18)	0.7(0.5-1.2)
	0-2	111(18)/218(15)	1.1(0.9-1.5)	94(22)/166(17)	1.4(1.0-1.9)	17(9)/52(13)	0.7(0.3-1.2)
	3-5	79(13)/175(12)	1.0(0.7-1.4)	65(15)/152(15)	1.1(0.8-1.5)	14(7)/23(6)	1.3(0.6-2.7)
Disease/ accident of close relative /friend	0-5	215(33)/487(32)	1.0(0.9-1.3)	176(39)/383(36)	1.2(1.0-1.6)	39(19)/104(24)	0.8(0.5-1.4)
	0-2	115(18)/246(16)	1.1(0.9-1.4)	92(20)/188(18)	1.3(0.9-1.7)	23(11)/58(13)	1.0(0.6-1.7)
	3-5	100(15)/241(16)	1.0(0.7-1.3)	84(19)/195(18)	1.1(0.8-1.5)	16(8)/46(10)	0.6(0.4-1.3)
Death of child/ spouse	0-5	34(7)/63(6)	1.1(0.7-1.8)	30(10)/48(7)	1.3(0.8-2.2)	4(2)/15(4)	0.5(0.2-1.8)
	0-2	18(4)/46(4)	0.8(0.4-1.4)	15(5)/33(5)	0.9(0.5-1.8)	3(2)13(4)	0.5(0.1-1.9)
	3-5	16(3)/17(2)	2.0(1.0-4.2)	15(5)/15(2)	2.2(1.0-4.8)	1(0.6)/2(0.6)	0.8(0.1-1.5)
Death of relative/ friend	0-5	494(53)/1088(52)	1.1(0.9-1.3)	351(56)/798(54)	1.2(0.9-1.4)	143(46)/290(46)	1.0(0.8-1.4)
	0-2	262(28)/578(27)	1.1(0.9-1.3)	190(30)/430(29)	1.2(0.9-1.5))	72(23)/148(24)	1.0(0.7-1.4)
	3-5	232(25)/510(24)	1.1(0.9-1.3)	161(26)/368(25)	1.1(0.9-1.5)	71(23)/142(23)	1.0(0.7-1.5)
Impaired economy	0-5	256(37)/483(32)	1.1(0.9-1.4)	196(42)/363(35)	1.3(1.0-1.6)	60(26)/120(26)	0.8(0.5-1.2)
	0-2	135(19)/243(16)	1.2(0.9-1.6)	107(23)/183(17)	1.4(1.0-1.9)	28(12)/60(13)	0.8(0.5-1.4)
	3-5	121(17)/240(16)	1.0(0.8-1.3)	89(19)/180(17)	1.1(0.8-1.5)	32(14)/60(13)	0.8(0.5-1.3)
Conflict at work	0-5	237(35)/475(32)	1.3(1.0-1.6)	190(41)/354(34)	1.5(1.2-2.0)	47(22)/121(26)	0.8(0.5-1.3)
	0-2	140(21)/261(17)	1.4(1.1-1.8)	115(25)/199(19)	1.8(1.3-2.3)	25(12)/62)14)	0.9(0.5-1.5)
	3-5	97(14)/214(14)	1.1(0.9-1.5)	75(16)/155(15)	1.3(0.9-1.8)	22(10)/59(13)	0.7(0.4-1.3)

[1]Adjusted for matching factors (age and area of residence), alcohol consumption, smoking, and education.
[2]Time interval in years from event to symptom onset/index year.

Table 5 continued Separate events by time interval from event to onset/index year: Odds ratios (OR) and 95% confidence intervals (CI) of rheumatoid arthritis associated with life events.

	[2]	All		Women		Men	
		ca *(%)*/co(%)	[1]OR (95%CI)	ca *(%)*/co (%)	[1]OR (95%CI)	ca *(%)*/co (%)	[1]OR (95%CI)
ACPA negative							
No event		235/1022	REF	151/685	REF	84/337	REF
Conflict with spouse	0-5	117(33)/417(29)	1.5(1.1-2.0)	86(36)/307(31)	1.5(1.1-2.2)	31(27)/110(25)	1.6(0.9-2.7)
	0-2	58(16)/218(15)	1.4(1.0-2.0)	43(18)/154(16)	1.5(1.0-2.3)	15(13)/64(14)	1.4(0.7-2.7)
	3-5	59(17)/199(14)	1,6(1.1-2.3)	43(18)/153(15)	1.6(1.0-2.4)	16(14)/46(10)	1.8(0.9-3.6)
Conflict with relative	0-5	114(33)/393(28)	1.3(1.0-1.7)	92(38)/318(32)	1.5(1.1-2.2)	22(21)/75(18)	1.6(0.9-2.7)
	0-2	68(19)/218(15)	1.4(1.0-2.0)	54(22)/166(17)	1.4(1.0-2.1)	14(13)/52(13)	1.2(0.6-2.4)
	3-5	46(13)/175(12)	1.2(0.8-1.7)	38(16)/152(15)	1.1(0.7-1.7)	8(8)/23(6)	1.7(0.7-4.3)
Disease/ accident of close relative /friend	0-5	149(39)/487(32)	1.3(1.0-1.7)	114(43)/383(36)	1.3(0.9-1.8)	35(29)/104(24)	1.4(0.8-2.5)
	0-2	74(19)/246(16)	1.3(1.0-1.7)	59(22)/188(18)	1.4(1.0-2.0)	15(13)/58(13)	1.2(0.6-2.2)
	3-5	75(20)/241(16)	1.3(1.0-1.8)	55(21)/195(18)	1.2(0.9-1.8)	20(17)/46(10)	1.6(0.8-2.9)
Death of child/ spouse	0-5	26(10)/63(6)	1.5(0.9-2.4)	20(12)/48(7)	1.5(0.9-2.7)	6(7)/15(4)	1.1(0.4-3.3)
	0-2	10(4)/46(4)	0.7(0.4-1.5)	7(4)/33(5)	0.7(0.3-1.7)	3(3)/13(4)	0.7(0.2-2.6)
	3-5	16(6)/17(2)	3.5(1.7-7.3)	13(8)/15(2)	3.5(1.6-7.9)	3(3)/2(0,6)	4.9(0.6-39.1)
Death of relative/ friend	0-5	286(55)/1088(52)	1.2(1.0-1.5)	199(57)/798(54)	1.2(0.9-1.5)	87(51)/290(46)	1.3(0.9-1.8)
	0-2	163(31)/578(27)	1.3(1.0-1.6)	113(32)/430(29)	1.3(1.0-1.7)	50(29)/148(24)	1.4(0.9-2.2)
	3-5	123(24)/510(24)	1.1(0.8-1.4)	86(25)/368(25)	1.1(0.8-1.5)	37(22)/142(23)	1.1(0.7-1.8)
Impaired economy	0-5	140(37)/483(32)	1.3(1.0-1.6)	103(41)/363(35)	1.2(0.9-1.7)	37(31)/120(26)	1.4(0.8-2.2)
	0-2	73(19)/243(16)	1.3(0.9-1.8)	52(20)/183(17)	1.2(0.8-1.7)	21(17)/60(13)	1.8(1.0-3.4)
	3-5	67(18)/240(16)	1.2(0.9-1.7)	51(20)/180(17)	1.3(0.9-1.9)	16(13)/60(13)	1.0-(0.5-1.9)
Conflict at work	0-5	47(13)/214(14)	1.4(1.1-1.9)	104(41)/354(34)	1.5(1.1-2.1)	31(27)/121(26)	1.3(0.8-2.2)
	0-2	66(23)/204(19)	1.7(1.3-2.4)	66(26)/199(19)	1.8(1.2-2.6)	22(19)/62(14)	2.1(1.1-3.9)
	3-5	38(13)/158(15)	1.1(0.7-1.6)	38(15)/155(15)	1.3(0.8-1.9)	9(8)/59(13)	0.6(0.3-1.4)

[1]Adjusted for matching factors (age and area of residence), alcohol consumption, smoking, and education.
[2]Time interval in years from event to symptom onset/index year.

Table 6 (item 8-15) Separate events by time interval from event to onset/index year: Odds ratios (OR) and 95% confidence intervals (CI) of rheumatoid arthritis associated with life events.

		All		Women		Men	
		Ca (%)/Co (%)	OR (95%CI)[1]	Ca (%)/Co (%)	OR (95%CI)[1]	Ca(%)/Co(%)	OR (95%CI)[1]
ACPA positive	Interval[2]						
No event		**442/1022**	**REF**	**386/837**	**REF**	**168/337**	**REF**
Divorce	All	111(20)/266)	1.0(0.8-1.3)	81(23)/198(22)	1.1(0.7-1.5)	30(15)/68(17)	0.9(0.5-1.5)
	0-2	48(9)/136(11)	0.9(0.6-1.2)	36(10)/95(11)	1.0(0.6-1.6)	12(6)/41(10)	0.5(0.2-1.1)
	3-5	63(11)/130(10)	1.2(0.8-1.7)	45(13)/103(12)	1,1(0.7-1.7)	18(9)/27(7)	1.6(0.8-3.1)
Marriage	All	149(25)/360(26)	1.1(0.9-1.5)	105(28)/273(29)	1.4(0.8-1.6)	44(21)/87(21)	1.3(0.8-2.1)
	0-2	80(14)/209(15)	1.0(0.8-1.5)	60(16)/154(16)	1.1(0.8-1.7)	20(9)/55(13)	0.8(0.5-1.7)
	3-5	69(12)/151(11)	1.3(0.9-1.8)	45(12)/119(12)	1.1(0.7-1.8)	24(11)/32(8)	1.9(1.0-3.7)
Change of residence	All	395(47)/834(45)	1.2(1.0-1.4)	305(53)/644(48)	1.2(1.0-1.6)	90(35)/190(36)	1.1(0.7-1.5)
	0-2	196(23)/444(24)	1.1(0.9-1.4)	147(25)/332(25)	1.2(0.9-1.6)	49(19)/112(21)	1.0(0.6-1.5)
	3-5	199(24)/390(21)	1.2(1.0-1.5)	158(27)/312(23)	1.3(1.0-1.7)	41(16)/78(15)	1.2(0.7-1.8)
Change of work place	All	369(46)/778(43)	1.2(1.0-1.4)	286(51)/580(46)	1.4(1.1-1.7)	83(33)/198(37)	0.8(0.6-1.2)
	0-2	207(26)/452(25)	1.1(0.9-1.4)	159(28)/347(27)	1.3(1.0-1.7)	48(19)/105(20)	0.9(0.6-1.5)
	3-5	162(20)/326(18)	1.2(0.9-1.5)	127(23)/233(18)	1.5(1.1-2.0)	35(14)/93(17)	0.7(0.4-1.1)
Decreased responsibility at work	All	72(21)/129(16)	1.6(1.1-2.2)	72(21)/129(16)	1.6(1.1-2.2)	26(13)/45(12)	1.3(0.7-2.2)
	0-2	41(12)/81(10)	1.4(0.9-2.2)	41(12)/81(10)	1.4(0.9-2.2)	15(8)/26(7)	1.2(0.6-2.4)
	3-5	31(9)/48(6)	1.8(1.1-3.0)	31(9)/48(6)	1.8(1.1-3.0)	11(6)/19(5)	1.4(0.6-3.1)
Increased responsibility at work	All	374(46)/746(42)	1.2(1.0-1.5)	279(50)/560(45)	1.3(1.0-1.7)	95(36)/186(36)	1.0(0.7-1.5)
	0-2	202(25)/387(22)	1.3(1.0-1.6)	156(28)/298(24)	1.4(1.1-1.8)	46(17)/89(17)	1.0(0.6-1.6)
	3-5	172(21)/359(20)	1.1(0.9-1.4)	123(22)/262(21)	1.2(0.9-1.6)	49(19)/97(19)	1.1(0.7-1.6)
Unemployment	All	140(24)/265(21)	1.2(0.9-1.5)	112(29)/203(23)	1.4(1.0-1.9)	28(14)/62(16)	0.9(0.5-1.5)
	0-2	65(11)/145(11)	1.0(0.7-1.3)	53(14)/109(12)	1.1(0.8-1.7)	12(6)/36(9)	0.6(0.3-1.2)
	3-5	75(13)/120(9)	1.5(1.1-2.1)	59(15)/94(11)	1.7(1.2-2.5)	16(8)/26(7)	1.3(0.6-2.5)
Other	All	172(28)/348(25)	1.2(0.9-1.5)	130(32)/280(29)	1.2(0.9-1.6)	42(20)/68(17)	1.5(0.9-2.3)
	0-2	84(14)/186(14)	1.1(0.8-1.5)	64(16)/157(16)	1.1(0.8-1.5)	20(10)/29(7)	1.7(0.9-3.2)
	3-5	88(14)/162(12)	1.3(1.0-1.7)	66(16)/123(13)	1.4(1.0-2.0)	22(10)/39(10)	1.3(0.7-2.4)

[1]Adjusted for matching factors (age and area of residence), alcohol consumption, smoking, and education.
[2]Time interval in years from event to symptom onset/index year.

Table 6 (item 8-15) Separate events by time interval from event to onset/index year: Odds ratios (OR) and 95% confidence intervals (CI) of rheumatoid arthritis associated with life events.

		All		Women		Men	
		ca *(%)*/co(%)	[1]OR (95%CI)	ca *(%)*/co (%)	[1]OR (95%CI)	ca *(%)*/co (%)	[1]OR (95%CI)
ACPA negative	Interval[2]						
No event		235/1022	REF	151/685	REF	84/337	REF
Divorce	All	89(27)/266(21)	1.8(1.3-2.5)	70(32)/198(22)	1.9(1.3-2.8)	19(18)/68(17)	1.7(0.9-3.2)
	0-2	43(13)/136(1)	1.7(1.1-2.5)	35(16)/95(11)	2.0(1.2-3.3)	8(8)/41(10)	1.2(0.5-2.9)
	3-5	46(114)/130(10)	1.9(1.3-2.9)	35(16)/103(12)	1.9(1.2-3.0)	11(11)/27(7)	2.4(1.0-5.4)
Marriage	All	87(27)/360(26)	1.4(1.0-2.0)	64(30)/273(29)	1.4(0.9-2.1)	23(22)/87(21)	1.5(0.8-2.8)
	0-2	47(15)/209(15)	1.3(0.9-2.0)	37(17)/154(16)	1.4(0.9-2.4)	10(9)/55(13)	1.1(0.5-2.4)
	3-5	40(12)/151(11)	1.5(1.0-2.4)	27(13)/119(12)	1.3(0.8-2.2)	13(12)/32(8)	2.2(1.0-4.9)
Change of residence	All	249(51)/834(45)	1.5(1.2-1.8)	191(56)/644(48)	1.5(1.2-2.0)	58(41)/190(36)	1.5(1.0-2.4)
	0-2	129(27)/444(24)	1.4(1.1-1.9)	101(30)/332(25)	1.5(1.1-2.1)	28(20)/112(21)	1.3(0.8-2.2)
	3-5	120(25)/390(21)	1.5(1.1-2.0)	90(26)/312(23)	1.5(1.1-2.0)	30(21)/78(15)	1.8(1.1-3.1)
Change of work place	All	193(45)/778(43)	1.2(1.0-1.6)	147(49)/580(46)	1.3(1.0-1.8)	46(35)/198(37)	1.2(0.8-2.0)
	0-2	111(26)/452(25)	1.2(0.9-1.7)	87(29)/347(27)	1.3(0.9-1.8)	24(18)/105(20)	1.3(0.7-2.2)
	3-5	82(19)/326(18)	1.2(0.9-1.7)	60(20)/233(18)	1.3(0.9-1.9)	22(17)/93(17)	1.2(0.7-2.2)
Decreased responsibility at work	All	48(17)/174(15)	1.4(0.9-2.0)	33(18)/129(16)	1.3(0.8-2.0)	15(15)/45(12)	1.6(0.8-3.2)
	0-2	27(10)/107(9)	1.4(0.8-2.2)	16(9)/81(10)	1.1(0.6-2.0)	11(11)/26(7)	2.6(1.1-6.1)#
	3-5	21(7)/67(6)	1.3(0.8-2.3)	17(9)/48(6)	1.5(0.8-2.9)	4(4)/19(5)	0.7(0.2-2.4)#
Increased responsibility at work	All	193(45)/746(42)	1.3(1.0-1.7)	153(50)/560(46)	1.5(1.1-1.9)	40(32)/186(36)	1.2(0.7-1.9)
	0-2	86(20)/387(22)	1.2(0.9-1.6)	67(22)/298(23)	1.2-0.8-1.7)	19(15)/89(17)	1.3(0.7-2.5)
	3-5	107(25)/359(20)	1.5(1.1-2.0)	86(28)/262(21)	1.7(1.2-2.4)	21(17)/97(19)	1.1(0.6-2.0)
Unemployment	All	59(20)/265(21)	1.0(0.7-1.4)	42(22)/203(23)	1.0(0.6-1.5)	17(17)/62(16)	1.2(0.6-2.3)
	0-2	28(10)/145(11)	0.9(0.6-1.4)	20(10)/109(12)	0.9(0.5-1.5)	8(8)/36(9)	1.0(0.4-2.4)
	3-5	31(11)/120(9)	1.1(0.7-1.7)	22(11)/94(11)	1.1(0.6-1.8)	9(9)/26(7)	1.5(0.6-3.6)
Other	All	106(31)/348(25)	1.3(1.0-1.7)	87(37)/280(29)	1.4(1.0-1.9)	19(18)/68(17)	1.1(0.6-2.1)
	0-2	66(19)/186(14)	1.6(1.1-2.2)	52(22)/157(16)	1.5(1.0-2.2)	14(14)/29(7)	2.1(1.0-4.5)
	3-5	40(12)/162(12)	1.0(0.7-1.5)	35(15)/123(13)/	1.2(0.8-1.8)	5(5)/39(10)	0.4(0.2-1.2)

[1]Adjusted for matching factors (age and area of residence), alcohol consumption, smoking, and education.
[2]Time interval in years from event to symptom onset/index year.

Stratifying the analysis by the impact of the most influential life event (Table 7 and 8), there appeared to be an association with ACPA positive RA in women, especially if the most influential event was perceived as having tangible or negative impact. This association was slightly stronger if the most influential event had occurred more distantly (in the interval 3-5 five years prior to onset/index year as opposed to immediately before onset/index year). The association was not weakened by adjusting for the total number of events experienced. Among ACPA negative, however, there was a similar association among women and men (although mostly non-significant among men) and this association was visibly weakened by adjusting for total number of events.

There was no obvious tendency of a stronger association for events in the more distant time interval among ACPA negative. In summary, having experienced at least one stressful life event was associated with both ACPA positive and ACPA negative RA, if the most influential event was perceived as tangible or negative. In this analysis, the association with ACPA positive RA seemed to depend somewhat on time interval since event while the association with ACPA negative RA seemed to depend more strongly on the total number of events experienced.

There were some significant correlations between the variables in our data. Conflicts with different parties (spouse, relative, friend, at work) were mutually correlated, as were conflicts with personal economic decline. Accident/disease in loved ones were correlated with deaths but otherwise death and accident/disease variables were not correlated with any other variables. Events concerning marriage, moving house, and work related events were weakly and mutually correlated. Since the numbers of exposed cases for analysis of separate events was small, we did not attempt to perform any adjustments as a consequence of these correlations.

Table 7a (item 1-7) Separate events by impact: Odds ratios (OR) and 95% confidence intervals (CI) of rheumatoid arthritis associated with life events.

	All		Women		Men	
	cases *(%)*/controls (%)	OR(95%CI)[1]	cases *(%)*/controls (%)	OR(95%CI)[1]	cases *(%)*/controls (%)	OR(95%CI)[1]
ACPA positive						
No event	442/1022	REF	274/685	REF	168/337	REF
Conflict with spouse						
Not significant	4(0.7)/9(0.6)	1.0(0.3-3.2)	1(0.3)/6(0.6)	0.4(0.0-3.6)	3(1)/3(0.7)	2.4(0.4-11)
Tangible	46(8)/88(6)	1.1(0.8-1.7)	30(7)/51(5)	1.4(0.9-2.3)	16(8)/37(8)	0.9(0.5-1.7)
Strong	121(20)/320(22)	0.8(0.6-1.1)	99(25)/250(25)	0.9(0.7-1.3)	22(11)/70(16)	0.6(0.4-1.1)
Conflict with relative						
Not significant	10(2)/21(1)	1.0(0.4-2.2)	3(0.7)/13(1)	0.7(0.2-2.4)	7(4)/8(2)	1.3(0.4-4.0)
Tangible	50(8)/120(8)	1.0(0.7-1.4)	36(8)/88(9)	1.0(0.7-1.6)	14(7)/32(8)	0.9(0.5-1.9)
Strong	128(20)/252(18)	1.2(0.9-1.5)	118(27)/217(22)	1.3(1.0-1.8)	10(5)/35(9)	0.6(0.3-1.3)
Disease/accident relative /friend						
Not significant	5(0.8)/12(0.8)	1.3(0.4-3.8)	2(0.4)/11(1)	0.6(0.1-3.1)	3(1)/1(0.2)	4.9(0.5-52)
Tangible	56(9)/111(7)	1.3(0.9-1.8)	40(9)/72)	1.5(1.0-2.3)	16(8)/39(9)	0.9(0.5-1.8)
Strong	153(23)/363(24)	1.0(0.8-1.3)	134(30)/299(28)	1.1(0.9-1.4)	19(9)/64(15)	0.7(0.4-1.2)
Death of child/spouse						
Tangible	1(0.2)/3(0.3)	0.6(0.0-6.7)	1(0.3)/2(0.3)	0.9(0.1-11)	0(-)/1(0.3)	-
Strong	33(7)/57(5)	1.3(0.8-2.0)	29(10)/43(6)	1.5(0.9-2.5)	4(2)/14(4)	0.6(0.2-1.9)
Death of relative/friend						
Not significant	13(1)/49(2)	0.6(0.3-1.1)	6(1)/22(1)	0.7(0.3-1.8)	7(2)/27(4)	0.5(0.2-1.2)
Tangible	179(19)/371(18)	1.2(1.0-1.5)	102(16)/235(16)	1.2(0.9-1.5)	77(25)/136(22)	1.3(0.9-1.9)
Strong	298(32)/659(31)	1.1(0.9-1.3)	240(39)/534(36)	1.2(0.9-1.5)	58(19)/125(20)	0.8(0.6-1.2)
Impaired economy						
Not significant	29(4)/71(5)	0.9(0.6-1.5)	22(5)/50(5)	1.0(0.6-1.7)	7(3)/21(5)	0.7(0.3-1.9)
Tangible	131(19)/257(17)	1.1(0.8-1.4)	99(21)/200(19)	1.2(0.9-1.6)	32(14)/57(13)	0.8(0.5-1.4)
Strong	92(13)/152(10)	1.3(0.9-1.7)	73(16)/111(11)	1.5(1.1-2.1)	19(8)/41(9)	0.8(0.4-1.4)
Conflict at work						
Not significant	24(4)/43(3)	1.5(0.9-2.5)	17(4)/18(2)	2.7(1.3-5.4)	7(3)/25(5)	0.6(0.2-1.5)
Tangible	90(13)/187(13)	1.3(1.0-1.8)	65(14)/140(14)	1.4(1.0-2.0)	25(12)/47(10)	1.2(0.7-2.2)
Strong	121(18)/242(16)	1.2(0.9-1.6)	106(23)/194(19)	1.5(1.1-2.1)	15(7)/48(11)	0.6(0.3-1.1)

[1]Adjusted for matching factors (age and area of residence), alcohol consumption, smoking, and education.

Table 7a continued (item 1-7) Separate events by impact: Odds ratios (OR) and 95% confidence intervals (CI) of rheumatoid arthritis associated with life events.

	All		Women		Men	
	cases *(%)*/controls (%)	OR(95%CI)[1]	cases *(%)*/controls (%)	OR(95%CI)[1]	cases *(%)*/controls (%)	OR(95%CI)[1]
ACPA negative						
No event	235/1022	REF	151/685	REF	84/337	REF
Conflict with spouse						
Not significant	3(0.9)/9(0.6)	1.6(0.4-6.1)	1(0.4)/6(0.6)	0.8(0.1-7.1)	2(2)/3(0.7)	3.2(0.5-22)
Tangible	31(9)/88(6)	1.7(1.1-2.7)	16(7)/51(5)	1.6(0.9-3.1)	15(13)/37(8)	2.2(1.1-4.5)
Strong	83(24)/320(22)	1.3(1.0-1.8)	69(29)/250(25)	1.6(1.1-2.3)	14(12)/70(16)	1.1(0.6-2.3)
Conflict with relative						
Not significant	4(1)/21(1)	0.8(0.3-2.4)	2(0.8)/13(1)	0.6(0.1-2.9)	2(2)/8(2)	1.0(0.2-5.3)
Tangible	35(10)/120(8)	1.4(0.9-2.1)	28(12)/88(9)	1.4(0.9-2.3)	7(7)/32(8)	1.1(0.4-2.7)
Strong	73(21)/252(18)	1.3(1.0-1.9)	61(25)/217(22)	1.3(0.9-1.8)	12(11)/35(9)	1.6(0.7-3.5)
Disease/accident relative /friend						
Not significant	4(1)/12(0.8	1.4(0.4-4.6)	2(0.8)/11(1)	0.7(0.1-3.4)	2(2)/1(0.2)	11(0.9-143)
Tangible	35(9)/111(7)	1.4(0.9-2.1)	22(8)/72(7)	1.5(0.9-2.5)	13(11)/39(9)	1.2(0.6-2.4)
Strong	109(28)/363(24)	1.3(1.0-1.7)	89(34)/299(28)	1.3(0.9-1.7)	20(17)/64(15)	1.4(0.8-2.5)
Death of child/spouse						
Tangible	2(0.8)/3(0.3)	3.4(0.5-21)	1(0.6)/2(0.3)	2.5(0.2-31)	1(1)/1(0.3)	2.9(0.2-49)
Strong	24(9)/57(5)	1.5(0.9-2.5)	19(11)/43(6)	1.6(0.9-2.8)	5(6)/14(4)	1.0(0.3-3.2)
Death of relative/friend						
Not significant	12(2)/49(2)	1.1(0.6-2.2)	7(2)/22(1)	1.5(0.6-3.6)	5(3)/27(4)	0.8(0.3-2.3)
Tangible	108(21)/371(18)	1.3(1.0-1.7)	63(18)/235(16)	1.3(0.9-1.8)	45(26)/136(22)	1.5(0.9-2.3)
Strong	165(32)/659(31)	1.1(0.9-1.4)	129(37)/534)	1.2(0.9-1.5)	36(21)/125(20)	1.2(0.8-1.9)
Impaired economy						
Not significant	16(4)/71(5)	0.9(0.5-1.5)	11(4)/50(5)	0.8(0.4-1.6)	5(4)/21(5)	1.1(0.4-3.2)
Tangible	70(19)/257(17)	1.2(0.9-1.7)	48(19)/200(19)	1.1(0.7-1.6)	22(18)/57(13)	1.8(1.0-3.2)
Strong	54(14)/152(10)	1.6(1.1-2.3)	44(17)/111(11)	1.8(1.2-2.8)	10(8)/41(9)	1.0(0.5-2.2)
Conflict at work						
Not significant	12(3)/43(3)	1.5(0.8-3.0)	6(2)/18(2)	1.7(0.6-4.4)	6(5)/25(5)	1.6(0.6-4.5)
Tangible	53(14)/187(13)	1.5(1.0-2.1)	36(14)/140(14)	1.4(0.9-2.1)	17(15)/47(10)	1.9(1.0-3.9)
Strong	70(19)/242(16)	1.4(1.0-2.0)	62(24)/194(19)	1.6(1.1-2.4)	8(7)/48(11)	0.8(0.3-1.8)

[1]Adjusted for matching factors (age and area of residence), alcohol consumption, smoking, and education.

Table 8 (item 8-15) Separate events by impact: Odds ratios (OR) and 95% confidence intervals (CI) of rheumatoid arthritis associated with life events.

	All		Women		Men	
	Cases *(%)*/Controls (%)	OR (95%CI)[1]	Cases *(%)*/Controls (%)	OR(95%CI)[1]	Cases *(%)*/Controls (%)	OR (95%CI)[1]
ACPA positive						
No event	442/1022)	REF	274/685	REF	168/337	REF
Divorce						
Positive	19(3)/62(5)	0.8(0.4-1.4)	14(4)/52(6)	0.6(0.3-1.3)	5(3)/10(2)	1.4(0.4-4.3)
Not significant	15(3)/17(1)	2.0(0.9-4.1)	9(3)/13(1)	1.7(0.7-4.3)	6(3)/4(1)	3.9(1.0-15)
Negative	76(14)/187(15)	1.0(0.7-1.4)	58(16)/133(15)	1.1(0.8-1.7)	18(9)/54(13)	0.6(0.3-1.2)
Marriage						
Positive	81(25)/338(25)	1.4(1.0-2.0)	59(27)/257(27)	1.3(0.9-2.1)	22(21)/81(19)	1.5(0.8-2.9)
Not significant	1(0.3)/9(0.7)	0.6(0.1-4.6)	1(0.5)/5(0.5)	0.9(0.1-8.2)	0(-)/4(1)	
Negative	5(2)/10(0.7)	3.4(1.1-40)	4(2)/9(1.0)	2.6(0.7-9.3)	1(1)/1(0.2)	6.5(0.3-129)
Change of residence						
Positive	318(38)/677(37)	1.2(1.0-1.4)	253(44)/526(40)	1.3(1.0-1.6)	65(25)/151(29)	1.0(0.7-1.5)
Not significant	26(3)/56(3)	1.0(0.6-1.7)	15(3)/34(3)	1.1(0.6-2.1)	11(4)/22(4)	1.0(0.4-2.3)
Negative	50(6)/96(5)	1.3(0.9-1.9)	36(6)/80(6)	1.2(0.8-1.9)	14(5)/16(3)	1.8(0.8-3.9)
Change of work place						
Positive	247(31)/598(33)	1.0(0.8-1.3)	193(35)/452(36)	1.2(0.9-1.5)	54(22)/146(27)	0.7(0.5-1.1)
Not significant	32(4)/59(3)	1.2(0.8-2.0)	22(4)/37(3)	1.4(0.8-1.4)	10(4)/22(4)	1.0(0.4-2.2)
Negative	86(11)/115(6)	1.7(1.2-2.4)	67(12)/86(7)	2.1(1.5-3.1)	19(8)/29(5)	1.2(0.6-2.3)
Decreased responsibility at work						
Positive	267(33)/599(34)	1.1(0.9-1.3)	191(35)/451(36)	1.1(0.9-1.4)	76(29)/148(28)	1.1(0.7-1.6)
Not significant	34(4)/67(4)	1.3(0.8-2.0)	24(4)/43(3)	1.5(0.9-2.6)	10(4)/24(5)	0.9(0.4-1.9)
Negative	69(9)/72(4)	2.3(1.6-3.3)	60(11)/60(5)	2.5(1.7-3.9)	9(3)/12(2)	1.3(0.5-3.3)
Increased responsibility at work						
Positive	28(5)/59(5)	1.2(0.8-2.0)	19(6)/40(5)	1.4(0.8-2.6)	9(5)/19(5)	1.0(0.4-2.5)
Not significant	14(3)/18(2)	1.8(0.9-3,9)	7(2)/11(1)	1.6(0.6-4.4)	7(4)/7(2)	2.0(0.6-6.5)
Negative	55(10)/96(8)	1.5(1.0-2.2)	45(13)/77(9)	1.7(1.1-2.5)	10(5)/19(5)	1.2(0.5-2.8)
Unemployment						
Positive	13(2)/28(2)	1.0(0.6-2.2)	10(3)/22(2)	1.2(0.5-2.6)	3(2)/6(2)	1.3(0.3-6.0)
Not significant	18(3)/41(3)	1.0(0.6-1.9)	15(4)/34(4)	1.1(0.5-2.1)	3(2)/7(2)	1.1(0.3-4.6)
Negative	106(18)/191(15)	1.3(0.9-1.7)	85(22)/142(16)	1.5(1.1-2.1)	21(11)/49(12)	0.7(0.4-1.3)
Other						
Positive	62(10)/176(13)	1.2(1.0-1.6)	41(10)/141(15)	0.8(0.5-1.2)	21(10)/35(9)	1.4(0.7-2.7)
Not significant	9(1)/16(1)	0.8(0.4-1.5)	6(1)/7(0.7)	2.6(0.8-8.5)	3(1)/9(2)	1.0(0.2-4.1)
Negative	69(21)/187(15)	2.0(1.4-2.8)	53(24)/133(15)	2.1(1.4-3.3)	16(16)/54(13)	1.9(0.9-3.9)

[1]Adjusted for matching factors (age, and area of residence), alcohol consumption, smoking, and education.

Table 8 continued (item 8-15) Separate events by impact: Odds ratios (OR) and 95% confidence intervals (CI) of rheumatoid arthritis associated with life events

	All		Women		Men	
	Cases *(%)*/Controls (%)	OR (95%CI)[1]	Cases *(%)*/Controls (%)	OR(95%CI)[1]	Cases *(%)*/Controls (%)	OR (95%CI)[1]
ACPA negative						
No event	235(1022)	REF	151/685	REF	84/337	REF
Divorce						
Positive	14(4)/62(5)	1.3(0.7-2.4)	12(5)/52(6)	1.3(0.6-2.6)	2(2)/10(2)	1.1(0.2-5.3)
Not significant	6(2)/17(1)	2.1(0.8-5.5)	5(2)/13(1)	2.4(0.8-7.2)	1(1)/4(1)	1.3(0.1-13)
Negative	69(21)/187(15)	2.0(1.4-2.8)	53(24)/133(15)	2.1(1.4-3.3)	16(16)/54(13)	1.9(0.9-3.9)
Marriage						
Positive	144(24)/338(25)	1.2(0.9-1.6)	102(27)/257(27)	1.2(0.8-1.7)	42(20)/81(19)	1.3(0.8-2.2)
Not significant	3(0.5)/9(0.7)	0.7(0.2-2.9)	1(0.3-5(0.5)	0.4(0.0-4.0)	2(1)/4(1)	1.5(0.2-10)
Negative	2(0.3)/10(0.7)	0.5(0.1-2.4)	2(0.5)/9(1.0)	0.6(0.1-3.1)	0(-)/1(0.2)	
Change of residence						
Positive	192(40)/677(37)	1.4(1.1-1.8)	149(44)/526(40)	1.4(1.1-1.9)	43(31)/151(29)	1.4(0.9-2.3)
Not significant	21(4)/56(3)	1.9(1.1-3.0)	14(4)/34(3)	2.3(1.2-4.6)	7(5)/22(4)	1.5(0.6-3.9)
Negative	35(7)/96(5)	1.9(1.3-3.0)	28(8)/80(6)	1.9(1.2-3.1)	7(5)/16(3)	2.5(0.9-6.9)
Change of work place						
Positive	132(31)/598(33)	1.1(0.8-1.4)	101(34)/452(36)	1.2(0.8-1.6)	31(24)/146(27)	1.2(0.7-1.9)
Not significant	20(5)/59(3)	1.6(0.9-2.7)	14(5)/37(3)	1.9(1.0-3.6)	6(5)/22(4)	1.2(0.5-3.3)
Negative	41(10)/115(6)	1.7(1.2-2.6)	32(11)/86(7)	1.8(1.2-3.0)	9(7)/29(5)	1.6(0.7-3.8)
Decreased responsibility at work						
Positive	138(32)/599(34)	1.2(0.9-1.5)	112(37)/451(36)	1.3(1.0-1.8)	26(21)/148(28)	1.0(0.6-1.7)
Not significant	20(5)/67(4)	1.5(0.9-2.6)	12(4)/43(3)	1.4(0.7-2.9)	8(6)/24(5)	1.7(0.7-4.3)
Negative	33(8)/72(4)	2.4(1.5-3.8)	27(9)/60(5)	2.4(1.5-4.1)	6(5)/12(2)	2.3(0.8-6.9)
Increased responsibility at work						
Positive	18(6)/59(5)	1.6(0.9-2.8)	11(6)/40(5)	1.4(0.6-2.9)	7(7)/19(5)	1.8(0.7-4.9)
Not significant	5(2)/18(2)	1.2(0.4-3.4)	3(2)/11(1)	1.2(0.3-4.4)	2(2)/7(2)	1.2(0.2-6.9)
Negative	24(9)/96(8)	1.3(0.8-2.1)	19(10)/77(9)	1.3(0.7-2.3)	5(5)/19(5)	1.3(0.4-3.9)
Unemployment						
Positive	10(28(2)	1.7(0.8-3.7)	8(4)/22(2)	1.9(0.8-4.6)	2(2)/6(2)	1.1(0.2-6.6)
Not significant	11(4)/41(3)	1.3(0.6-2.7)	9(5)/34(4)	1.4(0.6-3.1)	2(2)/7(2)	1.4(0.2-7.4)
Negative	38(13)/191(15)	0.9(0.6-1.3)	25(13)/142(16)	0.8(0.5-1.3)	13(13)/49(12)	1.2(0.6-2.5)
Other						
Positive	45(13)/176(13)	0.9(0.5-1.4)	35(15)/141(15)	1.0(0.7-1.6)	10(10)/35(9)	1.3(0.6-2.9)
Not significant	7(2)/16(1)	1.0(0.4-2.2)	5(2)/7(0.7)	3.4(1.0-11)	2(2)/9(2)	0.8(0.1-4.0)
Negative	53(16)/153(11)	2.2(0.8-5.9)	46(19)/131(14)	1.6(1.1-2.4)	7(7)/22(5)	1.2(0.5-3.1)

[1]Adjusted for matching factors (age, and area of residence), alcohol consumption, smoking, and education.

4.4 STUDY IV

In total, 2272 cases (1602 women and 670 men) and 3183 controls (2235 women and 948 men), answered the question of self-reported physical workload, were working and did not have any missing on the potential confounding factors (smoking and educational level).

Being exposed to substantial perceived physical workload five years prior to inclusion in the study was significantly associated with ACPA positive as well as ACPA negative RA (OR 1.3, 95%CI 1.1-1.4 for ACPA positive RA and OR 1.5, 95%CI 1.3-1.8 for ACPA negative RA). The associations were stronger in men (OR 1.7, 95% CI 1.3-2.2 for ACPA positive and OR 1.8, 95% CI 1.3-2.5 for APCA negative RA, respectively).

To have a sedentary work and be regular physical active during leisure time five years before study inclusion was associated with an approximately 50% decreased risk for developing ACPA positive RA in both women and men (OR 0.6, 95% CI 0.4-0.9 in women and OR 0.5, 95% CI 0.3-1.2 in men) compared to having a sedentary leisure time five years prior to disease onset. In ACPA negative RA, regular physical activity during leisure time was associated with a decreased risk in men (OR 0.4 95% CI 0.2-1.0) while the association for women was converse (OR 1.9 95% CI 1.3-3.3) (Table 9).

Table 9. Odds ratios (OR) and 95% confidence intervals (CI) of incident RA associated with physical activity in the group of no physical workload 5 years prior to disease onset stratified by ACPA and sex adjusted for smoking EIRA2

	All		Women		Men	
	ca(%)/co(%)	[1]OR CI(95%)	ca(%)/co(%)	[1]OR CI(95%)	ca(%)/co(%)	[1]OR CI(95%)
ACPA Positive						
Sedantary/ low physical activity	118(57)/350(45)	REF	92(57)/240(46)	REF	26(59)/110(44)	REF
Regular physical activity	88(43)/422(55)	0.6(0.4-0.9)	70(43)/282(54)	0.6(0.4-0.9)	18(41)/140(56)	0.5(0.3-1.2)
ACPA Negative						
Sedantary/ low physical activity	45(45)/350(45)	REF	26(36)/240(46)	REF	19(68)/110(44)	REF
Regular Physical activity	55(55)/422(55)	1.2(0.8-1.8)	46(64)/282(54)	1.9(1.1-3.3)	9(33)/140(56)	0.4(0.2-1.0)

[1]Adjusted for matching factors (sex, age, and area of residence) and smoking (never smoking, current smoking, ex-smoker, non-regular smoking).

5. DISCUSSION

With this thesis I aim to contribute to a better understanding of the impact of lifestyle—and potentially modifiable factors—on the risk of developing RA in the two major subsets defined by the presence or absence of ACPA. In this section the main findings of each study are discussed in relation to previous research, more recent findings, and methodological aspects. Finally, the findings are interpreted in the context of public health.

5.1 EXPOSURE AND RA RISK – MAIN FINDINGS

5.1.1 Oily Fish and risk of developing RA (Study I)

In the present study we found that regular consumption of oily fish was associated with a decreased risk of developing RA. The protective effect of oily fish consumption was seen irrespective of the presence or absence of the RA factor or ACPA.

The association between oily fish consumption — its contents of omega-3 fatty acids — and omega-3's bearing in the pathogenesis of RA are still inconclusive and based on few studies [40-42]. In recent years the interest in omega-3 has partly decreased in favor of an interest in vitamin D [15, 97-100].

Cells from both the innate and adaptive immune system express vitamin D receptors (VDR), and cells in the innate immune system are able to convert $25(OH)D_3$ (the major circulating form of vitamin D) to an active form of vitamin D ($1,25(OH)_2D_3$) [99]. The active form of vitamin D can induce response in the cells by binding to their VDRs, promote transcriptional regulation, and suppress inflammatory T-cells [99]. This function of vitamin D is hypothesized to act as a preventive factor against RA. One of the most important dietary sources of vitamin D is fish such as salmon, mackerel, and sardines, that is, oily fish also rich in omega-3 [101]; therefore, it might be true that the inverse association between oily fish consumption and RA risk we observed in study I could be because of vitamin D and not only omega-3, as proposed in the study.

5.1.2 BMI and risk of developing RA (Study II)

The principal finding in this study was an association differentiated in women and men with an increased risk for women with a BMI≥ 30 to develop ACPA negative RA, an association not seen in men. On the contrary, there was an inverse association with a reduced risk for men with a BMI≥ 30 to develop ACPA positive RA, and this association was not seen in women.

Previous studies have been inconclusive in estimating an association between BMI and RA [16, 20, 47, 49-51, 53]. Since the present study was conducted, the association between high BMI and RA risk has been confirmed in several studies. In a small cohort of 55 individuals at risk (presence of autoantibodies specific for RA) for developing RA, they found an increased risk among individuals with a BMI ≥ 25, although this association was seen mostly in smokers and not stratified by sex [54]. A nested case-control study from Minnesota identified a modest increased risk for individuals with a history of obesity (BMI > 30) to develop RA [55]. Finally, a Swedish study confirmed the significantly decreased association between obesity and ACPA positive RA risk we saw in men, even if these analyses were without stratification by ACPA [56].

The biological mechanism by which an increased BMI may affect RA is not fully understood. Several mechanisms have been postulated that pursue the association, including the low-grade inflammation observed in individuals with an excess of body fat (white adipose tissue) [102, 103]. The white adipose tissue produces adipocytokines and inflammatory cytokine — including adiponectin, leptin, tumor necrosis factor, interleukin-6, c-reactive protein. Increased white adipose tissue, caused by weight gain, induces an increase of these inflammatory cytokines and an initiation of a low-grade inflammation [102, 103]. More specifically, leptin may be relevant for the results in women, because the leptin levels are higher in women as compared to those in men, and this difference may possibly partly explain the sex differences we observed in study II [104]. An additional explanation for the association between increasing BMI and RA risk may be related to vitamin D, because obese individuals often have a vitamin D deficiency

[105], and as previously mentioned, vitamin D deficiency is suggested to increase the risk of RA [100].

A final remark in study II worth mentioning is that this study gives additional evidence for the hypothesis that ACPA negative RA is an apparently etiologically distinct subgroup with different etiology [106]. Analysis stratified by the presence or absence of the HLA-DRB1 SE alleles, where we see a more pronounced association between BMI and ACPA negative RA in women lacking the HLA-DRB1 SE alleles, further confirms this hypothesis.

5.1.3 Life events and risk of developing RA (Study III)

In this study, we found that having experienced at least one life event was weakly associated with an increased risk of developing ACPA positive RA in women and ACPA negative RA in women and men. In ACPA negative RA, the association increased with the increasing number of experienced events.

In previous literature, there are findings supporting an association between life events and RA, although these studies are based mainly on case reports without comparison groups [63, 66, 107-109], although other studies show no association [68, 69, 110]. In the NOAR cohort, no significant association was observed between stressful life events preceding inflammatory polyarthritis, although a non-significant association was observed for life events preceding the development of RA [68]. In two other studies no association was identified between exposure to critical life events (independent of impact) and RA overall [69, 110], although in the study by Li and colleagues, the analysis was not stratified by ACPA [69], while Conway and colleagues studied life events 12 months prior to disease onset [110]. In the present study, we saw an increased RA risk foremost in ACPA negative RA and an association restricted to women for ACPA positive RA.

We assessed a history of life events occurring in the five years leading up to the study inclusion. Because autoantibodies specific for ACPA positive RA have been identified in patients at least five years prior to development of

the disease [10, 111, 112], it is plausible that events occurring earlier in the life span are of greater importance for the risk of ACPA positive RA. This hypothesis is supported by an article by Dube and colleagues observing an increased risk for RA within individuals with a history of childhood stress [66]. In our analysis, we saw a weak tendency of a more distant latency/induction interval (three-to-five years) increasingly associated with ACPA positive RA.

Another aspect is the fact that many life events are interrelated (conflicts->divorce, unemployment->economic decline, etc.). This dimension supports the hypothesis that it is events more distant than five years before study inclusion (not measured in our study) that could be of importance because they contribute to the aggregate load of stress.

The smaller number of subjects and events in men may explain the lower precision in the estimates for men. The discrepancy between women and men is in line with previous literature also identifying sex differences in the perception of stressful events [113, 114]. A previous finding, that women report experience from past events as having a more intense impact than do men [113], may explain partly the association in men with ACPA negative RA, where life events perceived as "tangible" (conflict at work, economic decline) have a more increased association than "strong," whereas for women, events associated with a "strong" impact seem to be more important.

For women, the strongest association with RA was among those who had experienced a loss of a child or spouse. In the previously mentioned study by Li and colleagues, the researchers did not find any association with RA in parents who had lost a child in the previous 12 months [69]. This discrepancy in results may be that although losing a child is intensely traumatic, the loss of a spouse induces to a higher extent a whole series of life changes (e.g., economic decline, isolation, change of residence, sole responsibility of bringing up children, etc.) that may cause an accumulation of stress and, as a consequence, an increased allostatic load, which in turn has an increased bearing on the risk for disease.

The biological mechanism driving the association observed between history of life events and RA could be implicated by the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis is part of the physiological response to stress, regulating the immune system [115]. In chronic (long-term) stress, the HPA axis is constantly activated with sustained elevated plasma cortisol levels [115]. A constant activation of the stress-response system may eventually set the immune system off track and trigger an autoimmune disease such as RA. In addition do patients with RA have an altered HPA axis characterized by a normal HPA function despite ongoing inflammation? Furthermore, it is not known if the alteration of the HPA axis predates disease onset and contributes to predisposition to autoimmune development [116, 117].

The present study was the first large-population-based case-control study investigating the impact of life events on RA. We found that a person's having experienced a stressful life event was weakly associated with RA independent of ACPA subgroup. The findings were statistically significant among women, became non-significant for ACPA negative RA in men, while no association remained between life events and ACPA positive RA in men. Further, it seemed as if an increasing number of events may be associated with the risk of developing ACPA negative RA, independent of sex.

5.1.4 Perceived workload and RA risk (Study IV)

In the final study, we observed an association between a substantial physical workload five years prior to inclusion in the study and risk of RA independent of the presence or absence of ACPA. There was a more pronounced association in men compared to women, and this association was seen irrespective of whether the exposure was self-reported or an occupation-based classification. Further, we found no positive association between substantial workload five years prior to the inclusion and presence of anti-CII or anti-Cit-C1 antibodies at the time of diagnosis.

In previous studies investigating the association between occupational exposures and RA, the focus has been on chemical agents [69, 80, 81] and

not primarily on physical workload, although a Danish case-control study observed a significant association between physically demanding work and an increased risk of RA [20]. The hypothesis that physical trauma was involved in the pathogenesis of RA has been present for several decades, although these studies are based on case reports without a comparison group [76-78]. In a more recent case-control study, physical trauma six months prior to study inclusion was observed to be associated significantly with an increased risk of RA, although these analyses were not stratified by ACPA or by sex [79]. Further, do patients to some extent consider physical trauma and joint strain as presumable explanations for the development of their disease [118] ?.

There is strong support for the presumption that the immunity and inflammation in ACPA positive RA may emerge from exposures in the lung [4, 34]. It is therefore conceivable that individuals with a high physical workload are more likely to be exposed to inhaled particles or substances, in turn suggesting that the association with workload that we observe in ACPA positive RA may be caused by airborne exposure instead of the actual physical workload. This potential confounding cannot explain the association with ACPA negative RA, however.
Collagen type II is the predominant cartilage collagen, and autoantibodies to CII and Cit-C1 are thought to contribute to the disease development and to be present in an early stage of the development of RA [119, 120]. Whether physically demanding work contributes to the presence of autoantibodies to CII and Cit-C1 and catalyzes the onset of RA in predisposed individuals is still not known. We found no observed increased prevalence of autoantibodies to CII and Cit-C1 at the diagnosis in patients with a substantial physical workload five years prior to study inclusion. Therefore, our data do not support the hypothesis that an association between workload and RA would act through production of collagen autoantibodies.

Physical activity is an established protective factor for chronic diseases (including cardiovascular diseases, depression, cancer) [121-123], and the association between physical activity and RA risk has been investigated in two previous studies [16, 20] with inconclusive results. Given the lack of

studies within the area of physical activity and RA risk, more studies are needed to validate the association between ACPA positive RA and regular physical activity five years prior to study inclusion we identified in EIRA.

To summarize, an association was observed between substantial physical workload five years prior to study inclusion and increased risk of developing ACPA positive and ACPA negative RA. A similar pattern was observed when we defined exposure using an occupation-based classification of physical workload with perceived physical workload. The association was similar in both blue-collar and white-collar workers. If this association is causal, it would be of interest from a preventive point of view to know whether the mechanism is through joint trauma or through some other mechanism.

5.2 METHODOLOGICAL CONSIDERATIONS

In this section a number of interesting issues and epidemiological concepts will be discussed. I have chosen to restrict the discussion to a few of them that I find are the most important ones for this thesis.

5.2.1 Strengths

EIRA provides a unique opportunity to study the influence from a plethora of lifestyle/environmental and genetic factors, including their interaction, on the risk of developing RA. This richness of information also implies an excellent possibility to take important potential confounding factors into consideration when studying the association between specific exposures and RA risk. The study has been collecting information from cases and controls for almost two decades, and is by now the largest case-control study in the world with both environmental and biological information within the area of RA. Other strengths of the study are the population-based design with randomly selected controls—matched for sex, age, and area of residence—and the stringent case definition, where all cases are diagnosed by a rheumatologist in accordance with the 1987 ACR criteria for RA. In addition, most cases are included within 12 months of symptom onset (85%), which to some extent reduces the magnitude with recall of exposure prior to disease onset.

Even so, there are some methodological issues that need to be considered.

5.2.2 Limitations

Selection bias can arise from the procedure by which the study participants are chosen from the source population [124]. Even if there is universal access to publicly funded health care in Sweden, including inpatient care for all residents, and almost all patients with suspected RA are referred to a rheumatology clinic and diagnosed by a rheumatologist in accordance with the 1987 ACR criteria [85], there is still a small risk that eligible cases are missed because of workload and administrative issues. In accordance with an article by Bengtsson and colleagues, between May 1996 to December 2005 there were 359 cases with incident RA (fulfilling the ACR criteria with fewer than 12 months' disease duration) in the EIRA catchment area that were included in SRQ but not reported to the EIRA secretariat [93]; this constitutes an actual participation rate among cases of 82%. Although it was also reported that the missing cases were older, this might indicate a selection of cases in accordance with age. Since this study was conducted, the monitoring of patients has progressed because of new biological treatments and the obligatory surveillance that goes with them. With advances in the monitoring systems, the detection of eligible cases for inclusion is facilitated, although an increasing workload in the health-care system might still lead to a potential increase in the proportion of missing cases. Missing eligible cases are a threat to the external validity and to the generalizability and are difficult to estimate; therefore, if the proportion of missing cases has increased, we can hope only that their absence is random and not caused by any individual decision at the participating rheumatology clinic. The controls are population-based and selected at random, which gives a stronger indicator that any selection bias introduced in the recruitment process of the control is minor.

Another increasing matter in epidemiological studies is declining participation proportions [125]. Declining participation may increase the scope of selection bias in case-control studies. The EIRA study has had for a long time exceptionally high participation rates (96% in cases and 82% in controls included in EIRA between 1996 and 2005). In recent years the

participation rates have declined in EIRA (92% in cases and 73% in controls between 2006 and 2009); this means that the aspect of selection bias nowadays needs to be more thoroughly considered. The non-participation in EIRA has previously been investigated by Bengtsson and colleagues. [93], with the conclusion that non-participating cases compared to participating cases were in general older while non-participating controls were younger, unmarried, and living in urban areas. In addition, low income, not being born in Sweden, and being less educated were associated with non-participation in both cases and controls. Considering that individuals with a low socioeconomic position (SEP) (limited economic resources, low decision latitude and locus of control) also have a poorer diet, higher BMI, and occupations with increased physical workload [126] — that is, all exposures focused on in this thesis — we cannot disregard that the associations seen in the studies included in this thesis might be biased because of selective non-participation. Although because the characteristics for non-participation were conformed between cases and controls (low income, not being born in Sweden, and less education), the selection bias due to non-participation is likely to have only a marginal impact on the estimated odds ratios. Even so, in study IV, where we investigated the association between physical workload and RA risk, we wanted to preclude an overestimation of the association caused by bias from non-participation. We therefore performed a sensitivity analysis, where we assigned non-responding men, as exposed and distributed in accordance with the findings in the previously mentioned article by Bengtsson and colleagues, to a proportion of non-responders without a university education [93]. This sensitivity analysis did change marginally the estimates toward the null value.

Even if we in EIRA have used a stringent case definition defined by fulfillment of the 1987 ACR criteria [85] as diagnosed by a rheumatologist, we still need to ponder the limitations of these criteria. The presence of RF or ACPA is a strong predisposing factor for later development of RA; that is, it is likely that subjects diagnosed with ACPA negative RA need to have more indicators to fulfill the ACR criteria, which might mean that some early cases with ACPA negative RA are misdiagnosed as having other

diseases and are therefore missed, or the reverse: subjects are diagnosed in the initial phase with ACPA negative RA by mistake. If there is misclassification of outcome in EIRA, this is more likely to affect the association for ACPA negative RA. In study II, we hypothesized that the association between BMI and ACPA negative RA risk actually is driven by subjects having inflammatory osteoarthritis or psoriatic arthritis, two diagnoses that may resemble RA in the absence of ACPA, especially because inflammatory osteoarthritis and psoriatic arthritis have been shown to be associated with obesity [127-130]. In an attempt to explore the magnitude of this potential problem, we used the Swedish inpatient register to exclude cases diagnosed with psoriasis or who had reported having psoriasis in the questionnaire (89 observations). This changed the odds ratio for women with a BMI≥30 to develop ACPA negative RA, which could be an indication of some degree of misclassification of diagnosis for ACPA negative RA. We had no means of investigating whether there were individuals with inflammatory osteoarthritis among our ACPA negative RA cases.

Another important aspect in epidemiological research is that the exposure of interest precedes the outcome. Because RA is a disease that in some cases develops over years, it is difficult to know exactly which time period is of etiologic relevance for different exposures. As previously mentioned, ACPA can be present years before the first physical symptom of disease [10]; considering that consenting cases are given the questionnaire at their first visit to the rheumatology unit, it is possible that some of the exposure in the studies could have occurred after the onset of symptoms or in a prodromal phase of the RA (when the patients had not yet recognized their health symptoms as part of a developing health condition)—for example, study II pointed out that weight loss might appear as an early symptom of RA [131, 132]. If individuals with RA lose weight before they are diagnosed, this would introduce a differential misclassification of exposure and cause an underestimation of the results, although we believe that it is unlikely that the weight loss in the beginning of disease is of the extent that a high proportion of the cases would be classified as normal weight instead of overweight or obese; thus a differential misclassification because of weight loss may be

uncommon and have a small impact on the results. The reverse situation was considered as well because a side effect of the first-line medication (cortisone) for patients with RA is associated with weight gain. If the patients start taking medication for their disease and gain weight before they are included in EIRA, this would also bias the results and introduce reverse causality, meaning that the exposure (increased BMI) is introduced after the outcome. If the estimates in study II would have been affected by reverse causality, this would most probably have had an impact on both women and men, independent of ACPA status. Therefore, could reverse causality not explain the difference we see between women and men as well as between ACPA positive and ACPA negative RA. Also in study III, some of the particular life events associated with RA —"conflict at work", "change of workplace," and "increased responsibility at work" — might be affected by reverse causality. When an individual is not feeling well, it is surely possible that this could lead to changes in attitudes about work and in effect "cause" these life events. To preclude this situation, we reanalyzed the data in study III, of the association between history of life events and risk of RA, and excluded all cases and controls with an event the last year before the index year. This exclusion did not significantly change the estimates, and the association remained, although with a wider confidence interval.

Table 8 Odds ratios (OR) and 95% confidence intervals (CI) of incident rheumatoid arthritis associated with life events. By total number of events (excluding subjects with event the last year prior to inclusion in study)

	Women		**Men**	
	Cases (%)/Controls (%)	OR (95%CI)[1]	Cases (%)/Controls (%)	OR (95%CI)[1]
ACPA positive				
No event	274/685	REF	168/337	REF
Any event	849(76)/1,778(72)	1.2(1.0-1.4)	342(63)/627(44)	0.9(0.7-1.2)
Number of events[2]				
1	231(46)/453(40)	1.3(1.1-1.6)	91(35)/210(38)	0.9(0.7-1.3)
2	209(43)/500(42)	1.1(0.8-1.3)	78(32)/180(35)	0.9(0.6-1.3)
3+	409(60)/825(55)	1.3(1.1-1.6)	118(41)/237(41)	1.0(0.7-1.3)
ACPA negative				
No event	151/685	REF	84/337	REF
Any event	481(76)/1,778(72)	1.3(1.0-1.6)	160(66)/627(52)	1.1(0.8-1.5)
Number of events[2]				
1	118(53)/453(40)	1.2(0.9-1.6)	51(38)/210(38)	1.0(0.7-1.5)
2	130(46)/500(42)	1.2(0.9-1.6)	38(31)/180(35)	1.0(0.6-1.6)
3+	233(61)/825(56)	1.4(1.1-1.8)	71(46)/237(41)	1.4(0.9-2.1)

Individuals who are diagnosed with a chronic disease such as RA are known to search for the meaning of their disease [87]. This could entail

remembering or interpreting previous events differently as compared to the recollections of non-diseased individuals, which could potentially give rise to a systematic error: recall bias. For example, in study III, the way the impact of an event in retrospect was perceived could be influenced plausibly by whether a person is sick or healthy. If persons diagnosed with RA were to remember events as having a more negative impact, a spurious association would arise. The trend of increasing ORs from positive to negative impact in Tables 7 and 8 might be influenced by such a bias, although it is hard to explain why the trend is the strongest for the period three-to-five years prior to onset/index, when it is conceivable that recall bias would be either uniform over time or increase as time approaches onset (based on the assumption that cases are generally not aware of the concept of induction time in disease pathogenesis). Conclusively coming to terms with recall bias in a study of life events in relation to RA incidence would require prospective collection of exposure information. A possible approach would be to use register data with information about employment, family events, and the like, but because many other stressful life events are not registered, it is not possible to determine who is unexposed, which consequently places exposed people in the reference group, attenuating any underlying association. In study IV the presence of recall also needs to be considered, because it is likely that cases estimated their previous physical workload in the light of their disease. To overcome the issue with recall bias, we assessed physical workload using an objective measure and found similar patterns of association.

Matching in case-control studies increases the efficiency when the matching variable is a confounder, although it also induces some disadvantages — for example, it is not possible to investigate the relationship between the matching variable and outcome. The matched design needs to be acknowledged in the analysis by using a conditional logistic regression model where the matched pairs are analyzed, which has the disadvantage that if case or control miss information on the covariates, the whole strata are eliminated from the analysis. In EIRA II, where cases are matched to two controls and there is missing information on a covariate for the case, the information for both controls is lost. This leads to a considerable loss of

precision in the estimates, something that occurred when stratifying by ACPA when investigating the association between specific life event (e.g. divorce, impaired economy, change of workplace) and RA risk in study IV. The problem with loss of individual information can be partly solved through using unconditional logistic regression where we can use the information from all the controls irrespective of the ACPA status for their matched case. Additionally, deletion of all strata with missing covariates in case or control would not bias the estimates if the stratum with missing information is unassociated with exposure or outcome; unfortunately, this is very seldom the case, so in practice this can induce an bias in the estimates. Furthermore, is the conditional logistic regression with matched-pair analysis considered to perform better when the sample size is small [133]. EIRA is a large population-based case-control study; therefore, we considered the unconditional multiple logistic regression model adjusted for the matching variable (sex, age and residential area) to be superior, although we have also performed conditional logistic regression analysis to confirm unanimity in the models. Finally, when EIRA was initiated, cases with undifferentiated arthritis not fulfilling the ACR criteria were reported from some clinics. These subjects were eventually excluded from the study, but their corresponding controls were kept in the analysis because their participation strengthens the precision in the unconditional logistic regression analysis. In addition, it can be viewed as unethical to collect extensive information from control individuals and thereafter not use this information.

Problems that may arise when performing a large number of analyses is the increased probability that observed findings may be due to chance. This problem is evident when there is no prior information or prior probability concerning the studied association/associations (e.g. in genome wide association studies).To avoid reporting a false positive finding it is important to evaluate the finding with respect to the a priori information regarding the investigated hypothesis. When the EIRA questionnaire was constructed one of the requirements were that hypotheses behind the questions should have some prior probability, i.e. they were based on prior observations among humans or in animal experiments. Even so, we have

performed many analyses, especially in study III, where we investigated the association between life events and RA risk. However, we found a higher number of statistically significant results than would have been expected merely due to chance, e.g. 10 out of 21 comparisons (i.e .48% vs. 5 % expected by chance) among ACPA negative women. It is therefore unlikely that the main finding in paper III is explained by chance.

5.3 FINAL REMARKS AND PUBLIC HEALTH IMPLICATIONS

We observed an association between a regular intake of oily fish and a decreased RA risk in both women and men independent of the presence e of ACPA, while a BMI ≥ 30 was associated with an increased risk for women to develop ACPA negative RA and a decreased risk for men to develop ACPA positive RA. A history of life events was associated with an increased risk of developing RA in women, whereas physical workload was associated foremost with an increased risk of RA in men. The biological mechanism of how these factors might be associated with RA is at present not fully understood, although even without the full knowledge of how genes and factors investigated in this thesis interact, the results lend additional support for the general practitioner to motivate risk-modifying interventions in individuals.

As previously mentioned, socioeconomic position (SEP) is important to consider with regard to the estimates produced in this thesis, especially because it has been shown previously to be associated with RA both in EIRA and other studies [27, 28, 49]. In addition, SEP is associated with multiple factors (including smoking, physical inactivity, occupation, stress) [134]. The concept of SEP can therefore be considered as a distal exposure operating in different dimensions (figure 2), and its full impact may not be captured in logistic regression analysis methods, where distal (SEP or educational level) and more proximal (oily fish, BMI, life events, and workload) as well as biological factors are included. To fully understand the causal web and determine more appropriate estimates of the interaction among these factors, more complex multilevel models most probably are needed.

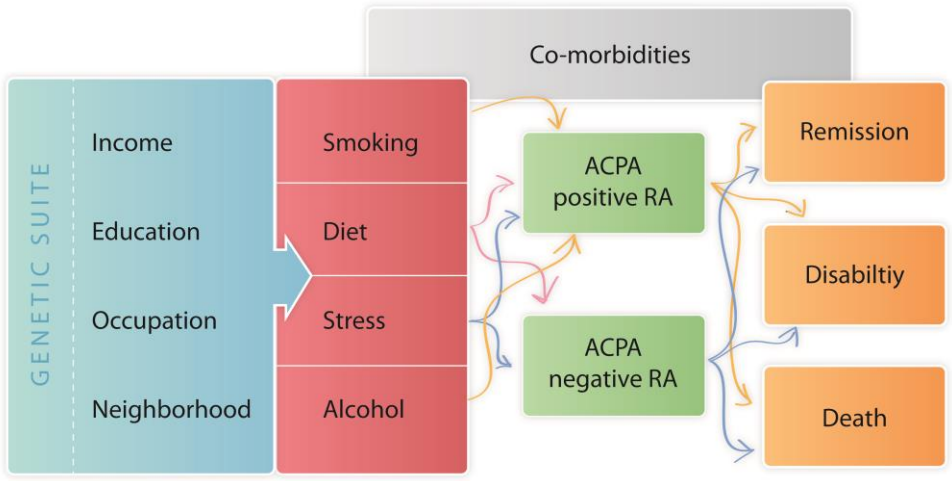

Figure 3. Causal chain of exposure leading to RA

A fundamental component in public health is that a large number of people exposed to a small risk may generate more individuals with disease than a small number of people exposed to a high risk [135]. Therefore, a preventive strategy focusing on high-risk individuals will only influence the incidence marginally, whereas prevention with a focus on exposure common in the society aiming at individuals who are at moderate risk will have a more notable impact on the incidence of a disease [135]. Therefore, even though the associations we have observed in a sense are modest, the exposures of interest are common, yielding a finding that may have a large impact for the proportion of individuals who develop RA.

In summary, this thesis has added additional support to the area of public health by showing that lifestyle matters. However, the results need to be confirmed in future studies allowing further investigation of the biological mechanism linking oily fish, BMI, history of life events, and physical workload to RA. With increased knowledge within this area, promotion of lifestyle modifications in the society in general as well as in primary care may have a beneficial effect on the incidence of RA.

5.4 FUTURE RESEARCH

With the findings in this thesis in mind several questions have been raised, some of importance for the etiological and some concerning the prognosis of RA.

ACPA positive RA, is the most prominent subgroup of RA with a growing body of evidence of the inherited genetic factors and environmental triggers involved in the etiology. Smoking is so far the most prominent modifiable risk factor identified for the etiology of ACPA positive RA, but what other environmental or modifiable factors involved in the pathogenesis of RA are not known. In the EIRA study there is information of a number of environmental factors potentially involved in the pathogenesis of ACPA positive RA e.g. physical activity, dietary factors as well as occupational history. In study II we identified a decreased risk for ACPA positive RA in men with a BMI>30, an association seen also in later studies [56] BMI is often questioned as an indicator for health therefore it would be interesting to investigate the association between overweight/obesity and RA risk measured with hip-waist ratio.

The etiology for ACPA negative RA is much less known, a recent article estimated that approximately 20% of the risk for developing ACPA negative RA caused by genetic factors. In this thesis we have identified potential risk factors for this subset, but how these factors are involved in the etiology needs further investigation.

Physical demanding work was hypothesized to contribute to the presence of autoantibodies towards CII and Cit-C1 antibodies, and that these antibodies catalyse the onset of RA in predisposed individuals. Given the results in study IV it seems that other components are initiating CII and Cit-C1 antibodies, what these components are needs to be assessed in future epidemiological studies.

In a recent article Orellana and collegues found an increased risk of ACPA-negative RA in women giving birth at a young age [33]. In addition obesity in adolescence was associated with and increased risk of developing multiple sclerosis in adulthood [24]. This indicates that there might be a "window-of exposure" where environmental factors have a greater impact of the pathogenesis of autoimmune diseases such as RA. It would be of interest to investigate if the timing is of importance for the exposures in present thesis.

6. CONCLUSION

- We found a reduced risk associated with the intake of oily fish but not with the intake of fish oil supplements. Further research is needed to elucidate whether there is an effect from fish oil per se or if other factors associated with fish oil intake play a role.
- Our results show that obesity is associated with increased risk for developing ACPA negative RA in women, and indicates an inverse association between BMI and ACPA positive RA in men. Based on current knowledge of the pathogenesis of RA, we cannot further explain these findings. We know that ACPA status defines two etiologically different RA subsets that need to be investigated separately, but an RA risk factor having opposing effects in women and men was unexpected and needs corroboration.
- In this large population-based case-control study, we found that having experienced life events was associated with increased risk of both ACPA positive and ACPA negative RA. The association with ACPA positive RA was conferred to women only, while the association with ACPA negative RA was observed in both women and men.
- We observed an association between substantial physical workload five years prior to study inclusion and an increased risk of developing ACPA positive and ACPA negative RA among men. A similar pattern was observed when we defined exposure using an occupation-based classification of physical workload rather than perceived physical workload. We saw similar association in both blue-collar and white-collar workers. If this association is causal it would be of interest from a preventive point of view to know whether the mechanism is via joint trauma or via some other workplace exposure.

7. SAMMANFATTNING PÅ SVENSKA

Reumatoid artrit (ledgångsreumatism) är en kronisk inflammatorisk ledsjukdom, som kännetecknas av svullna och ömma leder. Ledgångsreumatism orsakas av en kombination av kända och okända genetiska-, miljö- och livsstilsfaktorer. Det är en så kallad autoimmun sjukdom, vilket betyder att kroppens egna immunförsvar angriper kroppen istället för främmande ämnen. Sjukdomen delas ofta in i två undergrupper, ACPA/RF-positiva och ACPA/RF negativa, dessa två grupper har visats sig ha skilda riskfaktorer och har olika sjukdomsförlopp. Den ACPA/RF-positiva gruppen kännetecknas av en immunologisk reaktion som bildar antikroppar och resulterar allt som oftast i en svårare sjukdom jämfört med de som insjuknar i ACPA/RF-negativ ledgångsreumatism.

Den här avhandlingen syftar till att öka kunskapen om varför ledgångsreumatism uppkommer, mer specifikt undersöka sambandet mellan intaget av fet fisk via kosten och omega-3 tillskott, övervikt och fetma, livshändelser samt kroppsligt ansträngade arbete.

Avhandlingen är baserad på data från EIRA-studien (Epidemiological Investigation of Reumatoid Arthritis). EIRA är en stor befolkningsbaserad fall-kontrollstudie, där 2886 individer med ledgångsreumatism diagnostiserade i enlighet med 1987 års ACR (American College of Rheumatology) kriterier för reumatoid artrit, och 4072 slumpmässigt utvalda friska individer matchade till fallen i bemärkelsen av kön, ålder och bostadsort, har inkluderats mellan maj 1996 och november 2009. Inkluderade individer fick på frivillig basis fylla i ett omfattande frågeformulär med frågor angående levandsvanor och yrkesliv, därutöver fick de frivilligt lämna ett blodprov för genetisk och serologisk analys. Svarsfrekvensen i EIRA var för fallen 94% och 78% för kontrollerna.

Fet fisk såsom lax, makrill och sill är en av de viktigaste källorna till omega-3 via kosten. I den första studien undersökte vi om intag av omega-3 via kosten i form av fet fisk alternativ via kosttillskott innehållande omega-3 fem år före insjuknande påverkade risken att insjukna i ledgångsreumatism.

Vi fann en minskad risk att insjukna i ledgångsreumatism för de som äter fet fisk 1-7gånger/vecka jämfört med de som sällan eller aldrig äter fisk. Vi fann liknande resultat för de som åt fisk 1-3 gånger/månad och den skyddande effekten fanns för ACPA-positiv såväl som för ACPA-negativ ledgångsreumatism. Kosttillskott innehållande omega-3 påverkade inte risken att insjukna i ledgångsreumatism. I nästa studie fann vi att ett BMI>30 ökar risk för att insjukna i ACPA-negativ ledgångsreumatism för kvinnor, medan det minskade risken att insjukna i ACPA positiv ledgångsreumatism för män. Vi studerade även om livshändelser så som konflikter med anhöriga, skilsmässa, byte av arbete, flytt etc. fem år före inkludering i EIRA-studien påverkar risken att insjukna i ledgångsreumatism. Oavsett om händelsen hade påverkat individen positivt eller negativt, så finns det ett samband mellan livshändelse fem år tidigare och risk att insjukna i ACPA-positiv såväl som ACPA-negativ ledgångreumatism för kvinnor, medan för män verkar livshändelser endast ha betydelse för att insjukna i ACPA-negativ sjukdom. I den sista studien undersökte vi om kroppsligt ansträngande arbete fem år tidigare påverkade risken att insjukna i ledgångsreumatism. Det visade sig att ansträngade arbete fem år tidigare ökade risken för både kvinnor och män att insjukna oberoende av ACPA-status, och att effekten var något starkare för män.

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