Physical and psychological characteristics in adolescence and risk of gastrointestinal disease in adulthood
This book is dedicated to Risper Odero, Jeconia Odongo, Jared Odhiambo and George Otieno.
I will always cherish your unconditional love and belief in me.
I am the person I am today because of you.
Physical and psychological characteristics in adolescence and risk of gastrointestinal disease in adulthood
Abstract


Background and objectives: Physical fitness and stress resilience may influence the risk of gastrointestinal (GI) disease. High physical fitness level may reduce levels of systemic inflammation while psychosocial stress exposure can increase inflammation levels and intestinal permeability. The main objectives are to evaluate if poorer physical fitness and stress resilience in adolescence are associated with a raised risk of inflammatory bowel disease (IBD), peptic ulcer disease (PUD) and GI infections in adulthood and to assess evidence of causality.

Materials and methods: Swedish registers provided information on a cohort of approximately 250,000 men who underwent military conscription assessments in late adolescence (1969–1976) with follow-up until December 2009 (up to age 57 years). Cox regression evaluated the associations of physical fitness and stress resilience in adolescence with subsequent GI disease risk in adulthood.

Results and conclusions: IBD: Poor physical fitness was associated with an increased risk of IBD. The association may be explained (in part) by prodromal disease activity reducing exercise capacity and therefore fitness. Low stress resilience was associated with an increased risk of receiving an IBD diagnosis. Stress may not be an important cause of IBD but may increase the likelihood of conversion from subclinical to symptomatic disease. PUD: Low stress resilience was associated with an increased risk of PUD. This may be explained by a combination of physiological and behavioural mechanisms that increase susceptibility to H. pylori infections and other risk factors. GI infections: Low stress resilience was associated with a reduced risk of GI infections, including enteric infections rather than the hypothesised increased risk.

Keywords: Physical fitness, stress resilience, adolescence, inflammatory bowel disease, peptic ulcer disease, gastrointestinal infections.

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# Table of Contents

LIST OF PUBLICATIONS ........................................................................................................... 9  
ABBREVIATIONS ..................................................................................................................... 10  
RATIONALE ........................................................................................................................... 11  
The gastrointestinal tract ........................................................................................................ 11  
INTRODUCTION ..................................................................................................................... 12  
Life course approach ............................................................................................................. 12  
Adolescence – transition from childhood to adulthood ....................................................... 14  
Physical and psychological characteristics in adolescence .................................................. 15  
  Physical fitness .................................................................................................................... 15  
  Psychological stress and stress resilience ......................................................................... 16  
Gastrointestinal diseases in adulthood .................................................................................. 17  
  Inflammatory bowel disease ............................................................................................. 17  
  Genetic factors ................................................................................................................... 18  
  Environmental factors ....................................................................................................... 19  
  Peptic ulcer disease .......................................................................................................... 22  
  Risk factors ....................................................................................................................... 23  
  Gastrointestinal infections ............................................................................................... 25  
    Common enteric infections ............................................................................................. 26  
AIMS ........................................................................................................................................ 28  
MATERIALS AND METHODS .............................................................................................. 29  
Study population ................................................................................................................... 29  
Registers and measures ........................................................................................................ 31  
  Total Population Register ............................................................................................... 31  
  Population and Housing Censuses .................................................................................. 31  
  The Swedish Military Service Conscription Register ..................................................... 31  
  The National Patient Register ......................................................................................... 34  
Statistical analysis .................................................................................................................. 36  
  Sensitivity analyses ......................................................................................................... 37  
Statistical software ............................................................................................................... 38  
Ethical approval .................................................................................................................... 38  
RESULTS .................................................................................................................................. 38  
PAPER 1: Physical fitness and inflammatory bowel disease risk ........................................... 39  
  Crohn’s disease ............................................................................................................... 39
List of publications

The thesis is based on the following papers, referred to in the text by the corresponding roman numerals.


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## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>CD</td>
<td>Crohn’s disease</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>CSN</td>
<td>Central nervous system</td>
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<tr>
<td>ENS</td>
<td>Enteric nervous system</td>
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<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
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<tr>
<td>EVR</td>
<td>Erythrocyte volume fraction</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<tr>
<td><em>H. pylori</em></td>
<td>Helicobacter pylori</td>
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<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
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<td>HR</td>
<td>Hazard ratio</td>
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<td>IBD</td>
<td>Inflammatory bowel disease</td>
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<td>N(n)</td>
<td>Number</td>
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<tr>
<td>NOD2</td>
<td>Nucleotide binding oligomerization domain containing protein 2</td>
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<tr>
<td>NPR</td>
<td>National Patient Register</td>
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<tr>
<td>PPI</td>
<td>Proton pump inhibitor</td>
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<td>PUD</td>
<td>Peptic ulcer disease</td>
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<tr>
<td>SEI</td>
<td>Socioeconomic index</td>
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<tr>
<td>TJ</td>
<td>Tight junctions</td>
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<td>UC</td>
<td>Ulcerative colitis</td>
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Rationale

The gastrointestinal tract

The thesis is mostly concerned with examining behavioural and psychological influences on gastrointestinal disease. In addition to its digestive function, the gut is the largest endocrine organ in the body. A number of peptides including serotonin and insulin are synthesised and released from the endocrine cells and neurones in the stomach and intestines. There exists an interplay between the gut and the brain, which is bidirectional whereby the brain regulates gut’s activity and gut microbes may influence the brain. The gut is home to $10^{13} - 10^{14}$ micro-organisms. This represents 10 times the number of human cells in the body and largely consists of anaerobes but includes also virus, fungi, protozoa and archaea.

The gut-brain communication operates via several parallel pathways including the central nervous system (CNS), autonomic nervous system (ANS), enteric nervous system (ENS) and the hypothalamic–pituitary–adrenal (HPA) axis. For example, the ENS regulates the motility, endocrine functions and microcirculation of the gastrointestinal (GI) tract. Evidence is also accumulating that the ANS, HPA axis, and ENS have direct interactions with the immune system. This interaction has been suggested as the pathways through which CNS function and behavioural factors can influence inflammation and the immune system at the local tissue and systemic levels. All these factors provide evidence of a close connection between the gut and brain and the importance of maintaining the existence and quality of the interplay. Alterations can have consequences not only in the GI function and related disorders (like inflammatory bowel disease) but also other suggested influences including mood and affect as well as related disorders like anxiety and depression.

This thesis uses a life course approach as a theoretical framework to examine physical fitness and stress resilience in adolescence, which reflects inherited and acquired elements from childhood, and their association with subsequent GI disease risk (inflammatory bowel disease, peptic ulcer disease and GI infections). Physical fitness and stress resilience are both associated with inflammation and barrier function and therefore may influence aspects of these gastrointestinal diseases.
Introduction

Life course approach
The life course approach (life course epidemiology) refers to the study of physical/social exposures across life (during prenatal and gestation periods, childhood, adolescence, young adulthood and later adulthood) and their long-term effects on health or disease risk in later life.\textsuperscript{6,7} One focus of life course epidemiology has been on chronic disease but has increasingly expanded to include other aspects of health, development and ageing.\textsuperscript{8} Below are some descriptions of the models that have been proposed in medical, biological and social research with examples of how they may be relevant to the outcomes investigated in this thesis.

Critical/sensitive period model
During a critical period, some exposures may have lifelong effects on the structure or function of body organs, tissues and body system during a specific time or developmental window. Such changes are not easily modified by later exposures.\textsuperscript{6,7} A sensitive period is where an exposure has a stronger impact on the development and therefore disease risk than it would have had at other times. Meaning that the same exposure can still be associated with an increased risk of an outcome (after the sensitive period) but the association will be weaker than during the sensitive period.\textsuperscript{6,7} The Postnatal period has a limited time window for a stable development of the infant gut microbiome (microorganisms like bacteria that are living in or on the human body).\textsuperscript{9} During the first three years of life, the infant’s gut needs to be exposed to appropriate microbes for the development of the gut and to educate the immune system.\textsuperscript{10} Bacterial colonisation of the gut during this period will thus play a major role in the development and maturation of the key systems (including immune and endocrine systems) that may be relevant to influences on CNS programming and signalling.\textsuperscript{10} This adaptation in host immune responses and intestinal microbiota is also crucial for other reasons. It potentially enables the host to survive constant exposure to malnutrition or undernutrition and diarrhoea throughout the life course.\textsuperscript{11} The adaptation also provides protection when the immune system declines in later life.\textsuperscript{11} The microbiome is also very sensitive during the first three years of life for influences of diet, infection and pharmacological interventions (such as antibiotic exposures).\textsuperscript{12} For example, it is currently known that antibiot-
ics-mediated clearance of the gut leads to susceptibility to infections by various gut pathogens in animal models but also in clinical settings where antibiotic treatment is associated with increased risk of *Clostridium difficile*\textsuperscript{10}. Directed alterations of the gut flora can therefore potentially result in intestinal disorders like IBD or GI infections,\textsuperscript{10} making the critical/sensitive period model relevant to these outcomes. In IBD, even as an adult onset disease (typical onset age is 20-40 years),\textsuperscript{13} the strongest evidence for influence on risk is early life exposures in infancy and childhood. The patterns of bowel colonisation may be disrupted due to influences of antibiotics or infections, such that the homoeostasis of the immune response to gut microbiota does not properly develop.\textsuperscript{14,15}

**Accumulation of risk model**

Unlike the previous model where the specific exposure time points are crucial, what matters in this model is the total accumulation of relevant exposures (risk factors) and duration of the exposures.\textsuperscript{7} This is because health risks may be explained by accumulated risk exposures over the life course of an individual. Furthermore, risk factors at different life stages can accumulate independently over time and may cluster with other exposures (risk clustering).\textsuperscript{6,7} For example, exposure to poor socioeconomic conditions in childhood is associated with many aspects of impaired physical, psychological, and cognitive function which will increase adult disease risk.\textsuperscript{16} These include low birth weight, impaired postnatal growth, poor/inadequate diet and lower respiratory tract infections.\textsuperscript{7,16}

**Chains of risk model**

This suggests a sequence of linked exposures “chains of risk” whereby one exposure has the tendency to lead to another.\textsuperscript{6} These chains of risks (social, biological or psychological) can either increase or decrease disease risk and also have addictive or trigger effects.\textsuperscript{6,7} An experience (risk factor) that increases the disease risk in a cumulative way is said to have an additive effect. Sometimes earlier exposures have no effect on the disease risk without the final link (exposure) in the chain that triggers disease onset (trigger effects).\textsuperscript{6}

It is noteworthy that life course models are not mutually exclusive, they can be interconnected or operate simultaneously.\textsuperscript{7,8} Therefore, both accumulation of risk and chains of risk models may be relevant in PUD. The aetiology of PUD involves *helicobacter pylori* (*H. pylori*) infection.\textsuperscript{17,18} The infection is normally acquired in childhood and will lay latent for decades
until some episode occurs to disturb this host-bacteria equilibrium.\textsuperscript{19} The episode can be health-risk behaviours established in adolescence\textsuperscript{20} acting as the trigger leading to the development of PUD. \textit{H. pylori} will, therefore, increase the susceptibility of PUD but may not result in symptomatic disease without the final exposure(s) in the chain that triggers PUD onset. Further, an individual who is a smoker is likely to be a drinker (chains of risk); behaviours that are associated with increased PUD risk.\textsuperscript{21}

\textbf{Adolescence – transition from childhood to adulthood}

The World Health Organization (WHO) has defined adolescence as ages between 10-19 years, where late adolescence is defined as ages 15-19 years.\textsuperscript{20}

Adolescence, being the interface between childhood and adulthood, is a critical period of transition that lays the foundation for health and influences health trajectories across the life course, including the establishment of the emotional, physical, cognitive, social, cultural, educational and economic resources that will lay the foundations for the future.\textsuperscript{20,22} From a life course perspective, aspects of adolescence can be influenced by adverse childhood experiences.\textsuperscript{23} This can include exposures such as early maltreatment, conflicts in familial relationships, stressful life events, and adverse physical and social conditions due to lower socioeconomic circumstances that can influence the neuroplasticity. Alterations in neuroplasticity have consequences for mental and physical health.\textsuperscript{24}

Adolescence symbolises a dynamic period of brain development as well as maturation of cognitive and behavioural systems occurring at different rates and under the control of both common and independent biological processes.\textsuperscript{22} During early adolescence, a remodelling of the brain’s reward system takes place. Psychological characteristics during this phase include susceptibility to peer influences and low-risk perception which may result in increased risk-taking behaviour and poor self-regulation. Late adolescence is characterised by pubertal maturation (especially in boys) and a later phase of brain development of executive and self-regulatory skills. Maturation of the neural systems during late adolescence shapes the emotional development and capacities that adolescents bring into adulthood. Likewise, cognitive reserves between adolescence and the mid-
20s is a strong independent predictor of midlife cognitive capacity.\textsuperscript{20} Adolescence is a time of adaptation to social and cultural complexity.\textsuperscript{20} Development of social competence and other personal characteristics and resources are influenced by family characteristics, neighbourhood, and school. However, family influences are less important while social contexts beyond the family become more important.\textsuperscript{16} Adolescence is also an important life stage for health-related behavioural development and some behaviour persists into adulthood. Health risk behaviours including smoking, alcohol drinking, sedentary lifestyles as well as overweight and obesity emerge in adolescence\textsuperscript{20} and these behaviours tend to cluster.\textsuperscript{25} Thus, adolescence is not only a critical period but is also characterised by the accumulation of risks (risk clustering) as well as “chains” of risks.

**Physical and psychological characteristics in adolescence**

The thesis focuses on physical fitness and stress resilience measured in late adolescence and their relevance to later gastrointestinal disease risk.

**Physical fitness**

Physical fitness is mainly determined by physical activity patterns over a prolonged period but genetic factors also contribute to individual variations in physical fitness.\textsuperscript{26,27} The health-related components of physical fitness include cardiopulmonary fitness, body composition (such as body mass index) and musculoskeletal function (including muscular strength, endurance and flexibility).\textsuperscript{28,29}

From a life course perspective, life-long patterns of physical fitness are being set during adolescence following influential experiences in childhood.\textsuperscript{20,30,31} Studies of adolescents have shown that participation in sports is strongly associated with parental socioeconomic circumstances,\textsuperscript{32,33} individuals from economically disadvantaged families reporting lower physical fitness levels.\textsuperscript{32} While physical activity is regarded as a behaviour and physical fitness as the performance; the stability of these two measures from adolescence to adulthood seems to differ.\textsuperscript{31} Physical fitness has demonstrated higher levels of stability from adolescence to middle adulthood than physical activity. Physical inactivity from adolescence tended to continue into adulthood in studies tracking the stability of the two measures (from adolescence to adulthood).\textsuperscript{31}
Physical fitness has been suggested to have an inverse association with inflammatory markers. Exercise training has been demonstrated to reduce C-reactive protein (CRP) levels by influencing inflammatory processes in obese people. Apart from adipose tissue influences, exercise reduces circulating levels of cytokines in older people and has been shown to protect the bowel by reducing inflammatory cytokine and apoptotic protein expression in animal models. Also, in vivo studies indicate that exercise can induce autophagy, a lysosomal degradation pathway known to protect against diseases like cancer, neurodegeneration, diabetes, liver disease, autoimmune diseases and inflammatory disorders including IBD and also infections. Physical fitness in adolescence persists into adulthood and may, therefore, influence subsequent inflammatory GI disease risk due to its anti-inflammatory influences.

**Psychological stress and stress resilience**

Psychological or emotional stress is a transactional process arising when a situation is considered threatening or demanding and influenced by the availability of adaptive coping resources. A stressor can be physical (trauma, pain etc.) or psychological (fear, insecurity etc.). The physiological stress response is designed to help an individual respond appropriately to an acute situation involving physical or psychological threats. Being chronically stressed is potentially damaging to health, as this is associated with changes in lifestyle or health-related behaviours and some increased disease risks. Chronic stress has been suggested to increase levels of CRP, interleukin (IL)–6 and other inflammatory markers and some inflammatory pathways may be influenced by the reaction to the stress of the sympathetic nervous system. Psychological stress has been shown to influence the integrity of the intestinal barrier. Loss of intestinal barrier integrity results in increased intestinal permeability and this may facilitate passage of unwanted pathogens into the gut. Stress resilience in adolescence may, therefore, influence subsequent GI disease risk due to its influence on chronic stress susceptibility and therefore its impact on inflammation and intestinal barrier function.

Due to variation in an individual’s susceptibility to stress, measuring stressful exposures is challenging. Therefore, this thesis focuses on stress resilience rather than stressful exposures. Resilience has been described as "the process of effectively negotiating, adapting to, or managing significant
sources of stress or trauma. Assets and resources within the individual, their life and environment facilitate this capacity for adaptation and ‘bouncing back’ in the face of adversity’.47 Thus, stress resilience relates to how a person copes with stressful experiences in day-to-day life. Early research in this area on biological mechanisms has been conducted using animals. Animal experiments have shown that early life exposures shape the stress response through influences on regulation of the HPA axis.46,48 One of the experiments involved separation of rat pups from their mother (dam). This stressful maternal separation led to a decrease of glucocorticoid receptor (stress hormone receptor) expression, in the hippocampus and hypothalamus.48 In general; having a larger number of stress hormone receptors means the stress response is more effectively regulated and through negative feedback mechanisms. Fewer receptors result in physiological stress arousal at a higher level for longer. The above experiments provide evidence that early life is a critical period with consequences for stress resilience. The persistence of stress resilience in animals indicates that the same characteristic can remain across the life course in humans as well: poorer control (less rapid down-regulation) of the stress response, due to earlier life exposures or genetic characteristics, results in greater susceptibility to stress as the physiological response is more pronounced and prolonged, and hence potentially more damaging to health.41,49

Gastrointestinal diseases in adulthood

Inflammatory bowel disease
The causes of major IBD including Crohn’s disease (CD) and ulcerative colitis (UC) are incompletely understood. The commonly accepted theory is that IBD results from a failure to develop or maintain immunological tolerance towards normal gut microbiota, and this is thought to be a consequence of environmental exposures among genetically susceptible individuals.14,50,51

IBD is characterised by chronically relapsing and remitting inflammation in the gastrointestinal tract. CD has skip lesions and can involve any part of the GI tract usually terminal ileum or the perianal region, while in UC the intestinal inflammation is continuous and affects only the colon.50,51 Symptoms in CD vary depending on the location of the intestinal inflammation. Symptoms like weight loss and fever are common in CD patients than in
UC patients. UC patients usually suffer from symptoms like bloody diarrhoea. The diagnoses are based on combinations of clinical presentation, macroscopic appearance (endoscopy and radiology) and microscopic findings. Figure 1, illustrates genetic and environmental factors involved in the aetiology of IBD from a life course perspective.

**Figure 1. Risk factors for IBD**

*Genetic factors*

Genetic predisposition in IBD has been illustrated in twin studies especially in CD. Concordance rates in CD have been reported to be 38% and 2% in monozygotic and dizygotic twins, respectively. Similarly, concordance rates in UC were 15% and 8% in monozygotic and dizygotic twins, respectively. Genome-wide association studies (GWAS) have also provided insights into the pathogenesis of IBD. Nucleotide binding oligomerization domain containing protein 2 (NOD2) was the first gene to be associated convincingly with IBD. Approximately more than 100 non-overlapping genetic risk loci have been identified currently and some of these are shared between Crohn’s disease and ulcerative colitis. Further evidence of familial associations in IBD has been reported among relatives of CD and UC patients. First degree relatives, especially siblings, are at the greatest risk but
distant relatives have also displayed increased IBD risk.\textsuperscript{54} Despite the importance of genetic involvement in IBD aetiology, environmental factors seem also to be important as indicated by temporal trends in increasing IBD incidence.\textsuperscript{53}

**Environmental factors**

IBD incidence and prevalence in adults vary across geographical regions.\textsuperscript{55} Historically IBD incidence was high in Northern Europe, the United Kingdom and North America.\textsuperscript{14,55} Incidence of UC has been increasing in regions previously regarded as low incidence areas in Asian countries (Including Japan and Singapore) and Latin America, while the incidence of CD remains rare in most of these regions.\textsuperscript{14} Data from migration studies show that migration from a low-incidence to a higher incidence region increases IBD risk, especially in the first generation children.\textsuperscript{55,56} The children take on the risk factors of the new environment but their parents maintain original risk pattern, indicating that environmental influence in childhood is crucial.\textsuperscript{55} Migration studies further suggest that ethnic and racial differences are not due to true genetic differences but are consequences of lifestyle and environmental influences.\textsuperscript{14} The incidence of IBD has stabilised in most formerly high incidence regions (like Scandinavian countries) but is now rising in regions where incidences have been rare due to rapid socioeconomic development (urbanisation of societies). Urbanisation and industrialisation are accompanied by lifestyle changes.\textsuperscript{14,55,56} The changing epidemiology of IBD incidence may be associated with changes including antibiotic use, diet and hygiene.\textsuperscript{14,55,56}

**Early life exposures and natural history**

There is persuasive evidence that early life exposures are important. Influences in early bowel colonisation including infections and antibiotic exposure have been associated with IBD.\textsuperscript{14,15,56} These factors alter intestinal microbial composition (gut flora) and may trigger inappropriate immune responses (promoting inflammation).\textsuperscript{15,56} Infections and antibiotic therapy in early childhood but not later in childhood has been associated with increased CD risk in adulthood.\textsuperscript{56,57} The role of breastfeeding in infancy and IBD risk remains unresolved. Some studies have reported a protective effect for IBD, whereby the associations with CD is of higher magnitudes than for UC,\textsuperscript{14,58} others found no association.\textsuperscript{14}
There is also good evidence of long silent natural history with inflammation and subclinical disease activity but not at a level where symptoms result in a diagnosis. This has been illustrated in a twin study where one of the twins had IBD diagnosis but not the other one, even though there was inflammation consistent with an IBD diagnosis in the apparently unaffected twins.59

**Exposures in adolescence and adulthood**

Very few relevant exposures in adolescence and adulthood have been established conclusively in the aetiology of IBD. Smoking is associated with increased risk of onset and course of CD and is protective in UC.14,55,56 Similarly, acute appendicitis, especially before age 20 years is associated with lower UC risk60,61 and possibly increased CD risk (although the association with CD is less well established).14,56 Even though environmental exposures may explain the inverse association with UC, it may also be due to genetic factors, since having a first degree relative with a history of appendicitis is associated with reduced UC risk.62 The role of appendicectomy and acute appendicitis in CD has been debated. However, a meta-analysis showed an elevated CD risk in the first year after the operation but not after five years after the operation.63 Results from studies on diet and IBD are inconclusive, even though dietary habits may be among the factors involved in the changing epidemiology of IBD incidence over time.14,55 Western diets characterised by high fat and carbohydrate and low fibre content have been suggested to increase IBD risk.56 Also, sugar intake is consistently associated with IBD, especially CD. However, most studies on dietary factors in IBD are affected by methodological limitations like the poor recall of diet.14 There may also be an influence of reverse causation, where subclinical disease has consequences for dietary preferences. Vitamin D has been implicated in the pathogenesis and therapy of IBD.56 IBD is associated with vitamin D deficiency. Lower plasma vitamin D levels were reported to lead to increased risk of hospital admission and surgery for both CD and UC patients. In contrast, high Vitamin D intake was associated with a reduced risk of CD development. This may be because vitamin D is involved in innate and adaptive immunity and also influences autophagy participation in the pathogenesis of IBD.56
Physical fitness and IBD
Fitness may protect against IBD due to anti-inflammatory effects\textsuperscript{34,64} and also because fitness can induce autophagy,\textsuperscript{37} thereby providing protection against inflammatory disorders.

However, the role of physical fitness for risk of onset of IBD is not fully established. Earlier studies to report a protective association between better fitness and IBD onset include a study by Sonnenberg.\textsuperscript{65} Physically demanding jobs were associated with reduced IBD risk while sedentary and jobs performed indoors were associated with increased risk.\textsuperscript{65} Since then, prospective and retrospective cohort and case-control studies have reported a protective association of physical activity, thus fitness, with IBD onset.\textsuperscript{66-69} In recent research, a nested case-control study\textsuperscript{68} found that being physically active was associated with a reduced risk of both CD and UC risk among 102 CD and 678 UC cases. Other studies have not shown an association between fitness and IBD including a case-control study,\textsuperscript{58} a cohort study\textsuperscript{70} and a twin study\textsuperscript{15} that reported no significant differences between the twin who was physically active before IBD diagnosis and the co-twin. The inconsistencies in the results may be due to methodological limitations. Physical activity level is usually self-reported (a subjective measure) and it is generally known that people have a tendency to overestimate their fitness levels in questionnaire surveys. In some cases, individuals are asked to recall (retrospective) fitness levels at certain time points leading to possible recall bias. Also, if fitness levels are recorded at a later age after typical IBD onset age, it is difficult to know whether the exposure really pre-dated the outcome (increasing the likelihood of reverse causation).

Stress resilience and IBD
As IBD can have a subclinical manifestation over many years, it is possible that exposure to psychosocial stress may promote conversion to symptomatic clinical disease through pro-inflammatory influences or barrier function effects.

The majority of studies have looked at IBD exacerbations\textsuperscript{71-75} but very few have looked at the association of stressful exposures before IBD onset with later disease risk. Lerebours et al.\textsuperscript{76} reported an increased frequency of major stressful life events before the diagnosis of CD but not UC in a general population-based case-control study, but none of the associations remained
statistically significant in the multivariate analyses. In another study, Li et al. did not observe any association with IBD for previous major stressful events, defined as loss of a child, in a Danish general population-based case-control study. In contrast, the Nurses’ Health Study reported a two-fold increased risk of CD in women with depressive symptoms, but no association with UC was observed. The results were consistent after taking into account that depressed mood prior to diagnosis could be due to subclinical IBD. Depressive symptoms and psychological distress prior to IBD were assessed prospectively through questionnaires. Studies with retrospective collection of data on exposures prior to IBD diagnosis, including psychological stress are likely to suffer from information bias. Also, as there is variation in an individual’s susceptibility to stress, looking at exposure alone may be inadequate to characterise stress.

**Peptic ulcer disease**

Peptic ulcer disease (PUD) is a consequence of mucosal damage usually due to atypical patterns of exposure to pepsin and gastric acid, affecting mainly the stomach or duodenum. Historically, psychological stress was regarded as the main cause of PUD but this changed following the discovery of *H. pylori* infection in 1982 by Warren and Marshall. It has been established that *H. pylori* infection and use of nonsteroidal anti-inflammatory drugs (NSAIDs) are the major causes of duodenal ulcers and gastric ulcers.

Duodenal ulcers are found in the duodenal bulb, where the gastric contents enter the small intestine and this is the area most exposed to gastric acid. In western countries, duodenal ulcers are more common than gastric ulcers, while elsewhere gastric ulcer is the most common type. Gastric ulcers can occur anywhere from the pylorus to the cardia but typical locations are along the lesser curvature of the stomach, at the transition from corpus to antrum mucosa. The reported onset age for PUD is typically 20-50 years for duodenal ulcers and above age 40 years for gastric ulcers. The most common features for uncomplicated PUD include a history of an epigastric pain sometimes accompanied by dyspeptic symptoms like bloating, early satiety and nausea. A PUD diagnosis is usually based on clinical features and specific tests. There are a number of test-and-treat strategies aimed at eradicating *H. pylori* infection. Depending on the clinical setting, these include endoscopic/invasive methods (based on gastric specimens for histol-
ogy, culture) and non-endoscopic/non-invasive methods (based on peripheral samples, such as blood, stools, urine, or saliva). Primary treatments for *H. pylori* infection is usually acid-inhibiting therapy with proton pump inhibitors (PPI) in combination with antibiotics.

**Risk factors**

*H. pylori* infection and NSAID use are established risk factors associated with increased risk of PUD onset and bleeding. More than 50% of the world’s population has a chronic *H. pylori* infection yet only 5–10% of infected individuals develop PUD, suggesting the involvement of other factors. Evidence that PUD occurs independent of *H. pylori* infection and NSAID use has been elucidated in some studies. Figure 2, provides an overview of factors that may be implicated in PUD risk from a life course perspective.

**Figure 2. Risk factors for PUD**

![Risk factors for PUD diagram]

**Exposures in childhood**

*H. pylori* infection has a social gradient; the rates of infection decrease steeply as socioeconomic circumstances increase. This is probably because lower socioeconomic circumstances are often accompanied by poorer hygienic living conditions. *H. pylori* infections normally cluster in families,
and children living in crowded conditions are the most susceptible.\textsuperscript{85} Therefore, socioeconomic circumstances and living conditions in childhood are important factors that can determine infections patterns from a life course perspective.

\textit{Exposures in adolescence and adulthood}

Health risk behaviours including smoking and alcohol consumption are established in adolescence and normally persist into adulthood.\textsuperscript{20} Cigarette smoking is a major risk factor for PUD,\textsuperscript{18,86–88} but it is not an independent ulcerogenic.\textsuperscript{89} Smoking deteriorates the gastroduodenal mucosal defence thus increases \textit{H. pylori} infection by allowing the reflux of harmful duodenal contents back to the stomach.\textsuperscript{89} Smokers have an elevated risk of being infected with \textit{H. pylori}. This may be because of smoking’s effect on the reduction of antioxidants or the defensive immune system that are locally present in the gastroduodenal mucosa.\textsuperscript{89} Research on alcohol consumption and PUD is inconsistent.\textsuperscript{21,86,90} Heavy alcohol consumption has been associated with increased PUD risk and mortality.\textsuperscript{21} Also, intake of spirits is associated with increased risk among \textit{H. pylori} infected individuals.\textsuperscript{86} Other studies did not show an association between alcohol consumption and PUD.\textsuperscript{18,90} However, the study by Levenstein et. al\textsuperscript{18} reported associations between use of NSAID and socioeconomic disadvantage in adulthood with PUD. Low socioeconomic position is an accepted indicator of unhygienic conditions and job roles.\textsuperscript{91,92} Thus, the association with socioeconomic disadvantage may be due to circumstances rendering individuals more susceptible to \textit{H. pylori} infection. PUD is usually chronic and curable but in some instances, it can be life-threatening due to perforated ulcers. This risk can be increased by prolonged use of NSAIDs (like aspirin), particularly among older patients.\textsuperscript{17}

\textit{Stress resilience and PUD}

Psychological stress has been implicated in the pathogenesis of PUD since the 1940s.\textsuperscript{40} Even after the discovery of \textit{H. pylori} infection, studies have reported an association between psychosocial stress and PUD,\textsuperscript{88,93} sometimes independent of \textit{H. pylori} infection and NSAID use.\textsuperscript{18,94} One of the most recent studies is a prospective cohort study by Levenstein et. al\textsuperscript{18} which reported that high levels of stress doubled the risk of developing PUD. This association of stress with PUD was independent of risk factors including, \textit{H. pylori}, NSAID, smoking and socioeconomic status. Another population-based study assessed the risk of PUD among health care workers in Taiwan. Physicians and nurses working under higher job stress and rotating shifts
had increased PUD risk than other professions in the health care. Other studies have not found an association between stressful exposures and PUD. In a case-control study, stressful life events were not associated with duodenal ulcer and two cohort studies reported that PUD was not associated with psychosocial stress.

Earlier studies on psychosocial stress have not clearly identified that stressful exposures pre-dated disease onset. There is also inconsistency in study results probably due to methodological limitations. Some of these earlier studies have utilised retrospectively collected data, potentially affecting the interpretation of the results. None of the studies has taken into account variation in stress susceptibility among individuals.

**Gastrointestinal infections**

The intestinal mucosa is constantly exposed to the antigenic load resulting from ingested food and resident bacteria. The mucosa is protected by a barrier formed by a lining of epithelial cells connected by tight junctions (TJ), secreted mucus and immunological cells including tissue macrophages, which are all critical components of its innate defence. The barrier regulates the uptake of nutrients and simultaneously prohibits harmful substances (antigens and pathogens) from entering from the gut lumen. However, enteric pathogens have developed strategies to degrade TJ through influences on paracellular and transcellular pathways, provoking inflammation in the gastrointestinal mucosa and potentially causing disease. Intestinal permeability can also be influenced by psychological stress exposure: increased intestinal permeability is well documented in animal models. Studies have shown that psychological stress activates a mucosal immune response via enhanced penetration of luminal antigens. Evidence is emerging that psychological stress induces intestinal permeability in humans as acute psychological stress increased intestinal permeability in healthy people who had to give a public speech. This effect was suggested to be mediated by activation of the HPA axis, reflected by increased cortisol levels. The mechanisms by which chronic psychological stress may induce intestinal barrier dysfunction in healthy individuals may involve activation of HPA axis and dysregulation of the immune system. Chronic stress influences cytokine profiles (including Th1 and Th2) which become dysregulated leading to suppressed immunity (both humoral and cellular immunity). Chronic stress will, therefore, lead to low-
grade inflammation due to failure to downregulate inflammatory responses\textsuperscript{98} and inflammation can increase intestinal permeability allowing commensal bacteria and their toxins to translocate and increase disease risk through transcellular or paracellular pathways.\textsuperscript{98}

GI infections are classified into four broad categories; bacterial (including toxin-induced), parasitic and viral diseases, as well as those where an infectious aetiology is assumed but not fully characterised\textsuperscript{102,103} but the majority, are enteric bacterial infections.\textsuperscript{102-105} The most common symptom of GI infections is diarrhoea. Several laboratory tests are available for diagnosing the infectious pathogen that causes gastroenteritis.\textsuperscript{102} However, most diarrhoea is self-limiting but in severe cases, diagnostic tests include faecal leukocyte determination (microscopic examination of stool samples); stool cultures for enteric pathogens; \textit{Clostridium difficile} (\textit{C. difficile}) toxin testing and flexible sigmoidoscopy or colonoscopy with biopsy.\textsuperscript{102}

\textbf{Common enteric infections}

GI infections were combined in the thesis but some of the more common enteric infections in developed (high income) countries were also identified separately:

\textit{Salmonella}

\textit{Salmonella} species are gram-negative and include several serotypes.\textsuperscript{106} In high-income countries, the infection is usually acquired when travelling to areas of where the infection is endemic or when food preparers are carriers of \textit{Salmonella} strains.\textsuperscript{106}

\textit{Campylobacter}

\textit{Campylobacter} is gram-negative bacillus found in the GI tract of the majority of birds and mammals. Transmission to humans is commonly associated with the handling and consumption of poultry.\textsuperscript{107} Survival and infections mechanisms are poorly understood. After ingestion by humans, the bacteria colonise the lower GI tract (ileum, jejunum and colon), and the infection is sometimes asymptomatic.\textsuperscript{107}

\textit{Clostridium difficile}

\textit{C. difficile} is an anaerobic gram-positive bacterium that colonises the large intestine and releases two protein exotoxins (TcdA and TcdB) and causes
colitis in susceptible persons. Colonisation is prevented by effective intestinal barrier function and weakening of this resistance by antibiotics is the major risk factor.

Figure 3. Risk factors for common enteric infections

In developed countries, the more common enteric infections are mainly foodborne, though the main risk factors vary by species and strain. The immune status of the host and microbial strain virulence can also influence risk and severity of infections such as Salmonella, Campylobacter and Clostridium. Both Campylobacter and Salmonella infections are among the most commonly identified bacterial causes of infectious gastroenteritis worldwide. Gastroenteritis represents one of the most common infectious diseases in humans. Individuals with low or high BMI have been reported to be at increased risk of hospital admission due to infectious gastroenteritis especially the elderly population. Evidence further suggests that treatment with proton pump inhibitors (PPI) reduces gastric acidity and thereby increases susceptibility to enteric infections leading to gastroenteritis. Higher socioeconomic circumstances in adulthood have been suggested as a risk factor for both Salmonella and Campylobacter infections in developed countries. Even smoking has been suggested as a risk factor for GI infection but the evidence is very limited. Smoking has been associated with increased risks of C. difficile infection and also Salmonella infection in patients with Crohn’s disease. This may be because smoking promotes bacterial and viral infections through alterations in immune responses.
Psychosocial stress is associated with an increased risk of bacterial infections (such as pneumonia) and viral infections (such as the common cold).\textsuperscript{113,114} Psychosocial factors (including stressful life events, and inadequate coping strategies) have been found to accelerate viral load increase rate\textsuperscript{115,116} and CD4 cell decline in human immunodeficiency virus (HIV)\textsuperscript{115} and human papillomavirus (HPV) infection,\textsuperscript{116} and this may result from the influence of psychological stress on the HPA axis and in turn, immunological regulation.\textsuperscript{113,114} Evidence is lacking on the influence of stress resilience for other types of GI infections, including enteric infections.

**Aims**

The overall aims of this thesis are to evaluate if physical and psychological characteristics in adolescence are associated with subsequent risk of gastrointestinal disease (IBD, PUD and GI infections) in adulthood and to assess evidence of causal associations. This involves assessing independence from potential confounding factors, identifying aspects of the underlying mechanisms and examining whether subclinical disease activity, particularly in IBD, could have influenced the exposure measures, resulting in ‘reverse causation’. The four papers addressing these issues have the following specific aims:

I. To assess whether better physical fitness during adolescence is associated with a reduced risk of CD and UC in adulthood.

II. To investigate if stress resilience in adolescence influences the risk of CD and UC diagnoses in adulthood.

III. To examine if stress resilience in adolescence is associated with risk of PUD in adulthood.

IV. To investigate if low stress resilience in adolescence is associated with increased risk of GI infections in adulthood.
Materials and methods

Study population
The study population comprises a representative cohort of males all resident in Sweden, born 1952-1956 who were assessed for compulsory Swedish military conscription between 1969 and 1976. Most of the males were 18-19 years old at the conscription assessment. The follow-up was from conscription, until the first recorded GI disease, death, emigration or study end on 31st December 2009, whichever occurred first.

A total of 284,198 men were identified. Exclusions were made due to; uncertain vital status or errors in the personal identification number, female sex (conscription was limited to men at the time), if emigration or death occurred before the follow-up period, or if the man was not assessed for conscription during the time period. Men were also excluded due to missing data for the variables used in each paper. Missing data at conscription assessment was due to unsuitability to proceed to military training. Participants who had GI diseases (except appendicitis) or GI surgery before the study period, identified through medical review and examination at the conscription assessment or through the National Patient Register (NPR) were also excluded as appropriate. Excluded GI diseases were identified using codes from the Swedish International Classification of Diseases (ICD) revision 8 (000-009, 530-539, 543, 555-558, 560-577). Men who underwent ulcer-related surgery identified through the NPR were excluded in paper III. Surgical procedure codes for exclusions were 4401-4403, 4431, 4438-4439, 4449-4450 for ICD-9 and 4450, 4589 for ICD-8. Papers I-III did not exclude GI infections (ICD-8 codes 000-009). In paper IV, men who had GI infections and appendicitis prior to follow-up were also excluded. Appendicitis diagnoses were identified through ICD-8 codes 540-542 in the Conscription Register and NPR. Surgical procedure codes for appendicectomy in the NPR were 4510 and 4511 (ICD revision 8). After all exclusions, the study population varied slightly in papers I-IV, as illustrated in Figure 4.
Figure 4. Flow chart - exclusion, follow-up periods and final study populations

Total population of Swedish men born 1952-1956 (n=284,198)

Excluded (n=2,564), due to:
Unreliable personal numbers or vital status and females

Papers I-IV, (n=281,634)

Additional exclusion due to:
Conscription assessment before age 17 years, did not undertake all or part of the conscription assessments (during 1969-1976) resulting in missing data for variables used in the analyses, death or emigration or GI disease/surgery had occurred before the follow-up periods

Papers I and II:
Followed from at least 4 years after conscription assessment until 31st December 2009
Study populations = 240,984 men (paper I)
239,591 men (paper II)

Paper III:
Followed from 1st January 1985 until 31st December 2009
Study population = 233,093 men

Paper IV:
Followed from conscription assessment until 31st December 2009
Study population = 237,577 men
Registers and measures

Total Population Register
Information on birth date, sex, region of residence, vital status and migration was provided by this register held by the governmental organisation Statistics Sweden.

Population and Housing Censuses
Parental socioeconomic index (SEI) is a Swedish measure characterised by the occupation of the head of household. Parental SEI, household crowding and number of siblings of the cohort members came from the 1960 census, also held by Statistics Sweden. Parental SEI was grouped as office workers, business owners/managers, manual workers, agricultural workers, farm owner/managers and other. Household crowding was divided into two categories to indicate a ratio of less than two persons per room or more than two persons per room. The number of siblings was collapsed into four categories (no siblings, 1 sibling, 2-3 siblings and 4 or more siblings).

The Swedish Military Service Conscription Register
Swedish military service was mandatory for all men born in this birth cohort and resident in Sweden. Exemptions only applied to men with documented severe medical conditions,\textsuperscript{117} (2-3\% were exempted\textsuperscript{118}). This register provided information on a number of indicators of health and development based on physical and psychological characteristics in adolescence, as outlined below.

Physical fitness
A physical working capacity test with good reliability was performed on eligible men, using a bicycle ergometer.\textsuperscript{119} The men performed a maximal test using increased loads until exhaustion, starting load depended on the history of physical activity, physical stature and medical history. For those unable to perform the standard maximal test for medical reasons, a sub-maximal test was conducted. Some men were unable to perform the test (due to current infections or other reasons) so their physical fitness was estimated according to physical stature, history of physical activity and medical history. The fitness test provided scores, ranging from 0 (lowest) to 9 (highest performance) which was collapsed into five categories.
**Stress resilience**

A psychologist evaluated psychological function through a semi-structured interview with potential conscripts.\(^{117,118,120}\) An evaluation of psychological function testing from 1972/1973 reported the inter-rater reliability to be high (r = 0.85).\(^{118}\) The psychologist followed a manual where certain topics were discussed. For the interview, the psychologist had access to cognitive and physical fitness evaluation results, school grades and questionnaire survey results (the conscripts had responded in advance to questions regarding friends, family, hobbies).\(^{120}\) The interview which usually took 20-30 minutes,\(^{117,118,120}\) covered school experience and adaptation to the school environment as well as work experience and the ability to function in a workplace (potential conflicts with employers or co-workers). Conscripts with limited or no work experience were interviewed about career plans (if they had one and how realistic the plan was). Leisure time activities (did the conscript participate in team sport, where the activities introvert/extrovert in nature, potential leadership roles etc.) were also covered as well as home environment and upbringing (how were relations with siblings and parents, did the conscript adopt to difficult circumstances or is he dependent on parents etc.).\(^{120}\)

The main purpose of the interview was to assess the conscripts’ ability to cope in war situations and produced a stress resilience assessment that included evaluation of emotional control, social maturity and psychological energy.\(^{117,118,120}\) Emotional control assessed the emotional maturity, mental stability and the ability to tolerate psychological stress in general. Social maturity evaluated to what extent a person would be responsible for group activities, independent and socially extrovert. Psychological energy provided an indication of an individual’s ability to solve problems, engage in various activities and perform the chosen activities even when faced with difficulties.\(^{118}\)

Stress resilience is an ordinal measure that was summarised using a 9-grade stanine scale, with a normal distribution, ranging from 1 (lowest) to 9 (highest).\(^{117}\) In accordance with earlier studies,\(^{121-124}\) the score was categorised as low (1-3), medium (4-6) or high (7-9).

**Cognitive function**

The assessment of cognitive function included four subtests measuring logical (general intelligence), verbal (synonym detection), spatial (geometry perception) and technical abilities (mathematics or physics problems). This
evaluation was translated into a summary score (stanine scale) with a normal distribution ranging between 1 (lowest) – 9 (highest).125,126 The scores were grouped into low (1-3), medium (4-6) and high (7-9).

**Height, weight and body mass index**
Measures of height were grouped into five categories. BMI was calculated from height and weight measures. We excluded invalid or implausible values for height, weight and BMI: height < 144 cm, weight > 178 kg and BMI < 15 kg/m². The three categories of BMI included underweight (15 to 18.49), normal weight (18.50 to 24.99) and overweight/obese (25 to 60). The overweight and obese categories (≥ BMI 25) were combined because there were few obese men at the conscription assessment.

**Erythrocyte sedimentation rate**
Erythrocyte sedimentation rate (ESR), a measure of systemic inflammation, is measured based on the distance that a column of anticoagulated blood drops within one hour.127 Blood samples were collected and analysed for ESR and erythrocyte volume fraction (EVF). EVF is the proportion of whole blood that is made up of red blood cells. ESR was standardised for EVF by adjustment since the volume of red blood cells influences ESR independently of inflammation.126,127 ESR < 1 or > 98 mm/h and EVF < 0.20 or > 0.75 were treated as non-valid values,127 grouping ESR into five categories: 1 mm/h, 2-6 mm/h, 7-10 mm/h, 11-14 mm/h and >= 15 mm/h.

**Summary disease score**
This measure indicates if an individual suffered from a chronic disease or disability at conscription assessment and how significantly this limited their daily life activities. The five categories represent 1) very significant problems, 2) significant problem, 3) fairly significant problems, 4) no serious problems or 5) no diagnosis.

**Geographical regions**
The counties in Sweden were grouped into three regions; northern, central and southern.
The National Patient Register
GI diseases were identified through the National Patient Register (NPR). The government agency, the National Board of Health and Welfare is responsible for the register and it has recorded information on inpatient diagnoses since 1964. The register achieved full coverage in 1987 and has also included data on outpatient visits since 2001. The thesis used first recorded diagnoses among inpatients (from 1970 onwards) and outpatients (from 2001).

**IBD diagnoses**
Papers I and II included primary and secondary diagnoses, at least four years after the conscription assessment - 31st December 2009. ICD, versions 8, 9 or 10 were used to identify CD and UC diagnoses: The diagnostic codes for CD are 563.00 for ICD-8; 555.x for ICD-9; ICD-10 K50.x, and for UC they are 563.10 for ICD-8; 556.x for ICD-9; ICD-10 K51.x. Some men had records of both CD and UC. The most recent diagnosis was used to define disease phenotype, but the time of the first diagnosis defined disease onset.

**PUD diagnoses**
These diagnoses included duodenal ulcer, gastric ulcer, gastrojejunal ulcer and peptic ulcer (site unspecified). Diagnoses were identified through ICD, versions 8, 9 or 10: gastric ulcer (531 for ICD-8 and ICD-9, ICD-10 K25); duodenal ulcer (532 for ICD-8 and ICD-9, ICD-10 K26); peptic ulcer, site unspecified (533 for ICD-8 and ICD-9, ICD-10 K27) and gastrojejunal ulcer (534 for ICD-8 and ICD-9, ICD-10 K28). In paper III, PUD was the outcome and included only primary diagnoses from 1st January 1985 - 31st December 2009. In paper IV, PUD was a potential confounder, modelled as a time-dependent covariate using primary and secondary diagnoses from conscription assessment - 31st December 2009.

**GI infections**
GI infections included primary and secondary diagnoses from conscription assessment - 31st December 2009. ICD, versions 8, 9 or 10 were: 000-009 for ICD 8 and 9, A00-A09 for ICD-10. These include bacterial, parasitic and viral infections as well as undetermined/presumed infectious disease. Some commoner identifiable enteric bacterial infections were characterised separately to examine if the associations differ by infection type: *Salmonella* included diagnoses that were identified from conscription assessment - 31st December 2009. ICD 8 and 9 were 003, A02 for ICD-10.
Due to a lack of specific codes in earlier ICD revisions, *Campylobacter* (A045 for ICD-10) and *Clostridium difficile* (A047 for ICD-10) infections included diagnoses that were identified from 1st January 1997 - 31st December 2009.

**Appendicitis**

Appendicitis before age 20 years was identified in the Conscription Register and the NPR. ICD-8 codes were 540-542. Appendicectomy due to appendicitis prior to age 20 years was also identified in the NPR through surgical procedure codes (4510 and 4511 for ICD-8). This measure was combined with appendicitis from the conscription register into a single variable, referred to as appendicitis in adolescence. This variable was adjusted for in IBD papers (I and II) as a marker for precision (smoking), as smoking is one of the risk factors of appendicitis.\(^{129,130}\) Furthermore, appendicitis may represent a marker of exposures that are relevant to IBD risk beyond appendicitis itself. Acute appendicitis exposure before age 20 years is associated with a reduced risk of UC\(^{50,62}\) and may involve both environment and genetic components.\(^{62}\)

In the paper on peptic ulcer disease (III), the diagnosis of acute appendicitis was exclusively selected and used as a control diagnosis. Acute appendicitis is not known to be related to psychosocial stress or stress resilience. As smoking may represent a raised risk for appendicitis and perforation of the appendix,\(^{129,130}\) this could provide some evidence of whether associations with PUD are due only to smoking. The diagnosis (from 1st January 1985 - 31st December 2009) were limited to primary diagnosis of appendicitis with inflammation or perforation and ICD codes for identification in the NPR was: ICD-8 (540:00-03), ICD-9 (540A-B) and ICD-10 (K35.0-1)

**Chronic obstructive pulmonary disease (COPD)**

Due to lack of smoking data, COPD diagnosis was used as a control diagnosis in paper I, as a marker of smoking to evaluate if men who were less physically fit in adolescence were more likely to be smokers. Both primary and secondary diagnoses (at least four years after the conscription assessment - 31st December 2009) were included using ICD, versions 8, 9 or 10. The diagnostic codes for COPD were as follows; 490-492 for ICD-8 and ICD-9; 494 and 496 for ICD-9; J40-44 and J47 for ICD-10.
Statistical analysis

Cox regression
The association between physical fitness and stress resilience in adolescence and subsequent GI disease risk in adulthood were evaluated by Cox regression (papers I-IV). Initially, the unadjusted associations were tested separately and then further adjusted for potential confounding factors and markers of potential prodromal disease activity in adolescence (ESR, height and BMI) as appropriate.

The proportional hazard assumption between physical fitness or stress resilience and each outcome were assessed (papers I-IV). This was performed both graphically by using Kaplan-Meier plots (papers I-III) and by plotting Schoenfeld residuals (paper IV). There was no evidence of violation. In addition, Log-rank tests were used to test equality across strata. In papers I-II and IV, age was the underlying time scale. In paper III, birth year was adjusted for. All measures were modelled as categorical variables (papers 1-IV). Hazard ratios (HRs) were estimated with 95% confidence intervals (CIs). P-values <0.05 and not including 1.00 were considered statistically significant.

Stratification and interaction analyses
In paper I, the results indicated that some men had subclinical disease activity in adolescence (systemic inflammation indicated by ESR, lower BMI and reduced height growth). Thus, in paper II, to assist the understanding of mechanisms of associations, stratification analyses were performed to examine if the associations of markers of subclinical disease activity (ESR, BMI and height) differ for each stress resilience level. Included in Cox models were interaction terms for ESR, BMI and height with stress resilience, with adjustment for these variables.

Time-dependent covariate
Paper III provided evidence that stress resilience is related to PUD risk and possibly greater gastric acid production. After a PUD diagnosis, there is likely to be a reduction in acid production due to treatment with proton pump inhibitors (PPI).131 Lower acid production increases susceptibility to enteric infections.110 Therefore, we investigated the possible role of gastric acid in paper IV, by modelling PUD as a time-dependent covariate. The exposure began at the time of PUD diagnosis.
Sensitivity analyses

*Papers I and II*

a) In paper I, potential selection and surveillance bias were investigated by limiting the analysis to men with a primary diagnosis of IBD. Main analyses included both primary and secondary diagnoses. People whose physical fitness tests were approximated (rather than measured) at conscription assessment were also excluded to improve the precision of physical fitness measure.

b) An analysis was conducted to investigate the association between physical fitness and COPD (paper I). COPD was used as a marker of smoking to evaluate if men who were less physically fit in adolescence were more likely to be smokers.

c) Analyses were performed to minimise the possibility that undiagnosed disease in adolescence influenced physical fitness or stress resilience (reverse causation). We excluded men who were more likely to have undiagnosed disease activity in adolescence, defined as low EVF ($\leq 39$) and elevated ESR ($\geq 15$), in paper I. Exclusion of subclinical disease activity also included underweight (BMI 15-18.49 kg/m$^2$), in paper II.

d) Analyses with delayed follow-up entry beginning 15 years after the conscription assessment were conducted in both papers. This was to ensure diagnostic accuracy which increased at the end of the 1970s$^{132}$ and to evaluate whether physical fitness (paper I) or stress resilience (paper II) in adolescence were associated with a first IBD diagnosis several years later, after excluding men who had a diagnosis prior to this follow-up period.

*Paper III*

a) Subgroups of PUD were analysed separately. The subgroups included gastric ulcer, duodenal ulcer, peptic ulcer (site unspecified) and gastrojejunual ulcer.
b) During the study period (1985 - 2009) diagnostics and treatment of PUD underwent changes including the introduction of PPI use and other test-and-treat strategies for *H pylori* infection. Some individuals may have been diagnosed and treated in primary care or as outpatients. Therefore analyses were conducted for calendar period of diagnosis. The first period was 1985-2000, the second period was 2001-2009. The periods were selected because NPR information on outpatients has been available from 2001.

c) Acute appendicitis was used as a control diagnosis to rule out possible surveillance effects. The association of stress resilience and acute appendicitis was analysed to evaluate if the observed association between stress resilience and PUD was specific and not an artefact of the study design, such that there is increased risk for diseases unrelated to stress.

**Statistical software**
The analyses were conducted using SPSS software versions 22 or 23 and Stata version 13 or 14.

**Ethical approval**
The project was approved for by Uppsala Regional Ethics Committee. Diary numbers (Dnr) were: Dnr 2009/306 (paper III) and Dnr 2014/324 (papers I, II and IV).

**Results**

*General characteristics of the study population (papers I-IV)*
In general, men with lower physical fitness in adolescence were more likely to have low BMI, shorter stature and elevated inflammation levels (indicated by erythrocyte sedimentation rate (ESR)), compared with those who had highest physical fitness. Also, men with lower stress resilience in adolescence were more likely to have low BMI and cognition as well as elevated inflammation levels and poor health (indicated by summary disease score), compared with men with high stress resilience. In addition, a higher proportion of men with lower stress resilience had lower parental SEI and more
crowded households in childhood compared with men with high stress res-

**Paper 1: Physical fitness and inflammatory bowel disease risk**

We identified 986 men diagnosed with CD and 1,878 men diagnosed with UC during the median follow-up duration of 32.73 years.

**Crohn’s disease**
Men with the lowest fitness in adolescence showed a 62% higher risk of being diagnosed with CD in adulthood, compared with men with the highest fitness. After adjustment for BMI, ESR, EVF, height, parental SEI, appendicitis before age 20 years, and region of residence, the risk increase was attenuated to 32% (Figure 5). The attenuation was due largely to adjustment for markers of potential prodromal disease activity in adolescence (height, BMI, ESR). A higher inflammation level (indicated by ESR) was associated with raised CD risk (Figure 6). The results for all sensitivity analyses were similar to the main analysis.

**Ulcerative colitis**
The results indicate a dose-dependent association between physical fitness and UC risk, both in the unadjusted and adjusted models (Figure 7). The adjusted results indicate that individuals with the poorest fitness in adolescence have 25% increased risk of being diagnosed with UC in adulthood, in comparison to those with the highest fitness. The magnitude of the association between ESR in adolescence and UC risk was smaller than for CD (Figure 6). All sensitivity analyses results were consistent with the main analysis.
Control diagnosis - physical fitness and COPD risk
The COPD diagnosis was used to evaluate whether men who were less physically fit in adolescence were more likely to be smokers. A total of 1,881 men with a COPD diagnosis were identified in the study population. Poorer physical fitness in adolescence was associated with increased COPD risk in adulthood. HR (and 95% CI) is 2.76 (2.30-3.31) after adjustment in the lowest fitness group compared with highest fitness group.
Figure 6. ESR and IBD risk

* Adjusted for physical fitness, BMI, height, EVF, parental SEI, appendicitis before age 20 years and region of residence.

Figure 7. Physical fitness and ulcerative colitis risk

* Adjusted for BMI, ESR, EVF, height, parental SEI, appendicitis before age 20 and region of residence.
Paper II: Stress resilience and inflammatory bowel disease risk

A total of 938 and 1,799 men were diagnosed with CD and UC, respectively during the follow-up (median follow-up duration was 32.73 years).

Crohn’s disease

Adjusted results in Table 1, indicate that low and moderate stress resilience in adolescence is associated with increased risk of CD in adulthood, compared with high stress resilience. The association of low and moderate stress resilience with CD risk was also observed in the sensitivity analyses that excluded men who were more likely to have subclinical disease activity in adolescence and during the follow-up beginning at least 15 years after the conscription assessment in adolescence (Table 1).

Table 1. Stress resilience and Crohn’s disease risk

<table>
<thead>
<tr>
<th>Stress resilience</th>
<th>Events/n</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted* HR (95% CI)</th>
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<tr>
<td><strong>Main analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>225/50965</td>
<td>1.54 (1.26-1.88)</td>
<td>1.39 (1.13-1.71)</td>
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<tr>
<td>Moderate</td>
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<td>1.36 (1.14-1.62)</td>
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<tr>
<td>High</td>
<td>166/57632</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td><strong>Sensitivity analysis – excluding those with elevated ESR, low EVF and underweight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
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<td>1.53 (1.23-1.91)</td>
<td>1.45 (1.16-1.81)</td>
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<td>1.34 (1.11-1.62)</td>
<td>1.32 (1.09-1.59)</td>
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<tr>
<td>High</td>
<td>101/53007</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td><strong>Sensitivity analysis – follow-up at least 15 years after the conscription assessment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
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<td>1.37 (1.07-1.75)</td>
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<tr>
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<td>114/55644</td>
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</tr>
</tbody>
</table>

* Adjusted for BMI, ESR, EVF, height, parental SEI, appendicitis before age 20 and region of residence.
Stratification and interaction analyses
The association of BMI, height and ESR with CD risk did not differ by stress resilience level. Thus, there was no evidence of effect modification by stress resilience for the association of prodromal disease activity markers in adolescence with CD risk in adulthood (P>0.05 for all).

Ulcerative colitis
After adjustment, the risk of being diagnosed with UC in adulthood remained statistically significant for men in low stress resilience group, while the association was less notable in those who had moderate stress resilience in adolescence (Table 2). A sensitivity analysis that assessed the potential influence of subclinical disease activity indicate that both low and moderate stress resilience were associated with increased UC risk in both models. The analysis with delayed follow-up entry was similar to the main analysis (Table 2).

Table 2. Stress resilience and ulcerative colitis risk

<table>
<thead>
<tr>
<th>Stress resilience</th>
<th>Events/n</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted* HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>423/50965</td>
<td>1.24 (1.08-1.42)</td>
<td>1.19 (1.03-1.37)</td>
</tr>
<tr>
<td>Moderate</td>
<td>990/130994</td>
<td>1.11 (0.98-1.24)</td>
<td>1.08 (0.96-1.22)</td>
</tr>
<tr>
<td>High</td>
<td>386/57632</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td><strong>Sensitivity analysis – excluding those with elevated ESR, low EVF and underweight</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>339/41735</td>
<td>1.28 (1.10-1.49)</td>
<td>1.26 (1.08-1.47)</td>
</tr>
<tr>
<td>Moderate</td>
<td>843/113417</td>
<td>1.15 (1.02-1.31)</td>
<td>1.14 (1.01-1.30)</td>
</tr>
<tr>
<td>High</td>
<td>335/53007</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td><strong>Sensitivity analysis – follow-up at least 15 years after the conscription assessment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>345/49278</td>
<td>1.26 (1.08-1.46)</td>
<td>1.22 (1.04-1.42)</td>
</tr>
<tr>
<td>Moderate</td>
<td>813/127750</td>
<td>1.12 (0.99-1.28)</td>
<td>1.11 (0.97-1.26)</td>
</tr>
<tr>
<td>High</td>
<td>311/55644</td>
<td>Reference</td>
<td>Reference</td>
</tr>
</tbody>
</table>

* Adjusted for BMI, ESR, EVF, height, parental SEI, appendicitis before age 20 and region of residence.
Stratification and interaction analyses

The only evidence of effect modification by stress resilience level for the association of prodromal disease activity markers for UC was for BMI (Table 3). Underweight was only associated with a raised risk of UC in those with high stress resilience (p-value for interaction <0.05), suggesting that more aggressive disease in adolescence reduces the apparent protective influence of high stress resilience.

Table 3. BMI and ulcerative colitis risk, stratified by stress resilience level

<table>
<thead>
<tr>
<th>Stress resilience level</th>
<th>Events/n</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted* HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>423/50965</td>
<td>1.00 (0.77-1.29)</td>
<td>0.99 (0.76-1.28)</td>
</tr>
<tr>
<td>Underweight</td>
<td></td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Normal weight</td>
<td></td>
<td>0.79 (0.53-1.16)</td>
<td>0.80 (0.54-1.17)</td>
</tr>
<tr>
<td>Obese/overweight</td>
<td></td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Moderate</td>
<td>990/130994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td></td>
<td>0.98 (0.81-1.19)</td>
<td>0.98 (0.81-1.19)</td>
</tr>
<tr>
<td>Normal weight</td>
<td></td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Obese/overweight</td>
<td></td>
<td>0.62 (0.46-0.84)</td>
<td>0.61 (0.45-0.82)</td>
</tr>
<tr>
<td>High</td>
<td>386/57632</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td></td>
<td>1.71 (1.24-2.36)</td>
<td>1.72 (1.25-2.38)</td>
</tr>
<tr>
<td>Normal weight</td>
<td></td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Obese/overweight</td>
<td></td>
<td>1.02 (0.69-1.53)</td>
<td>1.00 (0.67-1.50)</td>
</tr>
</tbody>
</table>

* Adjusted for ESR, EVF, height, parental SEI, appendicitis before age 20 years and region of residence.

Paper III: Stress resilience and peptic ulcer disease risk

A total of 2,259 men were identified with a diagnosis of first PUD during the follow-up period (mean follow-up duration was 24.03 ± 3.80 years). Figure 8, illustrates the association of stress resilience in adolescence with PUD risk in adulthood. The results indicate a dose-dependent association within each all three tested models. After adjustment for both characteristics in early life and adolescence, we observed elevated risks of 84% and 23% for low and moderate stress resilience groups, respectively, compared with high stress resilience.
A sensitivity analysis was conducted to examine two calendar periods separately; 1985-2000 and 2001-2009 due to changes in diagnostics and treatment of PUD during the study period. Incidence rates for PUD were 0.18/1000 person-years (0.17-0.20) and 0.22/1000 person-years (0.21-0.23) for 1985-2000 (inpatients only) and 2001-2009 (inpatients and outpatients), respectively. The association between stress resilience in adolescence and PUD was consistent with the results of the main analysis.

The analyses by PUD diagnostic subgroup indicated results that were similar to results for all PUD. Associations with gastric ulcer showed the highest risk magnitude with adjusted HRs (95% CIs) of 2.07 (1.72-2.50) and 1.25 (1.05-1.48) for low and moderate stress resilience, respectively, compared with high resilience. The results for gastrojejunal ulcer were statistically non-significant; we observed adjusted HRs of 2.27 (0.66-7.78) and 0.96 (0.27-2.94) for low and moderate stress resilience, respectively, in comparison with high resilience. This may be due to the small number of events.
Control diagnosis - stress resilience and acute appendicitis risk
The diagnosis of acute appendicitis (n=1413) was analysed to evaluate if the observed association between stress resilience and PUD was specific and not due to surveillance effects. The association was assessed in a similar manner to the analysis of PUD. There was no association between stress resilience and acute appendicitis; adjusted HRs (95% CIs) were 1.03 (0.88-1.21) and 1.01 (0.88-1.15) for low and moderate stress resilience, respectively, compared with high resilience.

Paper IV: Resilience to stress and gastrointestinal infections risk
For the main analysis, we identified 5,532 men diagnosed with GI infections, during the follow-up period (median follow-up time was 36.7 years).

Both low and moderate stress resilience are statistically significantly associated with a reduced risk of GI infections, after adjustment (Table 4). Adjusted results indicate a 12% and 17% reduced risk in low and moderate stress resilience groups, respectively, in comparison to high stress resilience group.

Salmonella
A total of 662 men had first diagnoses of Salmonella among those diagnosed with GI infections. Adjusted results indicate that low and moderate stress resilience in adolescence is associated with a statistically significant reduced risk of Salmonella infection in adulthood (Table 4).

Campylobacter
We identified 284 men with incidences of Campylobacter infection among GI infections. In the adjusted model, lower stress resilience was associated with non-statistically significant reduced risk of Campylobacter infection compared with the high stress resilience group (Table 4).

Clostridium difficile
Among GI infections, 242 men with diagnoses of C. difficile infections were identified. Low and moderate stress resilience was not associated with C. difficile infections risk compared with high stress resilience, in the adjusted model (Table 4).
Table 4 Stress resilience and gastrointestinal infections risk

<table>
<thead>
<tr>
<th>Stress resilience</th>
<th>Events/n</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted* HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Main analysis – GI infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1283/50825</td>
<td>0.98 (0.91-1.06)</td>
<td>0.88 (0.81-0.97)</td>
</tr>
<tr>
<td>Moderate</td>
<td>2785/129835</td>
<td>0.82 (0.77-0.87)</td>
<td>0.83 (0.77-0.88)</td>
</tr>
<tr>
<td>High</td>
<td>1464/56917</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td><strong>Common enteric infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>135/50825</td>
<td>0.86 (0.68-1.07)</td>
<td>0.70 (0.53-0.91)</td>
</tr>
<tr>
<td>Moderate</td>
<td>350/129835</td>
<td>0.85 (0.71-1.02)</td>
<td>0.83 (0.69-1.00)</td>
</tr>
<tr>
<td>High</td>
<td>177/56917</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>58/46841</td>
<td>0.89 (0.63-1.25)</td>
<td>0.95 (0.64-1.39)</td>
</tr>
<tr>
<td>Moderate</td>
<td>152/122341</td>
<td>0.88 (0.67-1.16)</td>
<td>0.89 (0.67-1.18)</td>
</tr>
<tr>
<td>High</td>
<td>177/52647</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>67/46841</td>
<td>1.76 (1.20-2.58)</td>
<td>0.96 (0.62-1.51)</td>
</tr>
<tr>
<td>Moderate</td>
<td>132/122341</td>
<td>1.31 (0.93-1.84)</td>
<td>1.11 (0.78-1.58)</td>
</tr>
<tr>
<td>High</td>
<td>43/52647</td>
<td>Reference</td>
<td>Reference</td>
</tr>
</tbody>
</table>

* Adjusted for BMI, cognitive function, ESR, EVF, summary disease score, peptic ulcer disease, parental SEI, household crowding in 1960 and region of residence.

Discussion

Main findings
This thesis evaluated the association of physical fitness and stress resilience in adolescence and subsequent risk of inflammatory bowel disease (IBD), peptic ulcer diseases (PUD) and gastrointestinal (GI) infections in adulthood. It also assessed evidence of causal associations. This involved evaluating independence from potential confounding factors, identifying aspects of the underlying mechanisms and examining whether subclinical disease activity, particularly in IBD, could have influenced the exposure measures,
resulting in ‘reverse causation’. Specific aims and main findings were as follow:

**Paper I**, evaluated whether better physical fitness is associated with a lower risk of Crohn’s disease (CD) and ulcerative colitis (UC). Poorer fitness in adolescence was associated with a raised risk of IBD in adulthood. **Paper II**, investigated if stress resilience influences the risk of being diagnosed with CD or UC. Low stress resilience in adolescence was associated with an increased risk of receiving an IBD diagnosis in adulthood. Evidence of effect modification by stress resilience for the association of prodromal disease activity markers in adolescence with subsequent IBD was only observed for the association of BMI with UC. Underweight was associated with an increased risk of UC in men who had high stress resilience in adolescence. This may indicate that the presence of more aggressive disease in adolescence is a risk for a UC diagnosis, irrespective of high stress resilience. **Paper III**, evaluated the association between stress resilience and peptic ulcer disease (PUD) risk. Lower stress resilience in adolescence was associated with a higher risk of PUD in subsequent adulthood. **Paper IV**, assessed if low stress resilience was associated with an increased risk of gastrointestinal (GI) infections. In contrast with the *a priori* hypothesis, low stress resilience in adolescence was associated with a reduced risk of GI infections in adulthood and there was no evidence of increased risk for selected bacterial enteric infections, *Salmonella*, *Campylobacter* and *Clostridium difficile*.

**Potential mechanisms**

**Paper 1: Physical fitness and inflammatory bowel disease risk**

The observed inverse association of physical fitness with IBD risk is consistent with a protective role for exercise as physical fitness reduces inflammation levels.34,64 Another potential pathway influenced by physical fitness may involve autophagy as this is important in innate immune response towards intracellular bacteria.133 Exercise can induce autophagy and provide protection against inflammatory disorders like IBD.37 Genetic variants coding for proteins involved in autophagy have been associated with IBD as autophagy genes (ATG16L1 and IRGM) have been identified as risk factors for Crohn’s disease.133 Therefore, low levels of physical fitness might exacerbate disease risk in genetically susceptible individuals.
There was, however, already evidence of disease activity in adolescence signalling subclinical IBD. ESR, signalling inflammation, was raised in adolescents who would subsequently develop IBD, particularly Crohn’s disease (CD). BMI was on average lower in those who would develop CD and there was evidence of shorter stature associated with subsequent ulcerative colitis (UC). The pro-inflammatory state signalled by ESR does not identify the source of inflammation but is consistent with the GI inflammation of IBD. Lower BMI is associated with malabsorption, especially in CD and reduced growth rate indicated by shorter stature suggests the influence of malabsorption on growth. The associations seen between markers of disease in adolescence and IBD risk indicating subclinical disease activity provide evidence of the long silent natural history of IBD. It is possible that at least some, if not all, of the association between poor physical fitness and raised IBD risk is explained by the influence of subclinical disease activity on exercise capacity and therefore fitness: this interpretation is supported by the attenuation of the estimates for the association of fitness with IBD risk following adjustment for the markers of prodromal disease.

**Paper II: Stress resilience and inflammatory bowel disease risk**

Chronic stress is associated with increased inflammation signalled by inflammatory markers and also activates the sympathetic nervous system (SNS) and HPA axis to release catecholamines and glucocorticoids. Chronic stimulation of HPA axis can result in glucocorticoid resistance making the immune system become insensitive to its inhibitory and modulatory actions. Thus significant psychological stress and stress resilience, while unlikely to initiate pathogenesis, could contribute to the conversion of subclinical inflammation to symptomatic IBD through its pro-inflammatory influence and mucosal immune response, in individuals at risk of the disease. Lower stress resilience may make individuals adopt behaviours like heavier and prolonged smoking, which is a risk for CD. Impaired barrier function is another potential mechanism as stress may increase permeability, but the two potential mechanisms are not mutually exclusive. Chronic exposure to systemic inflammation appears to increase the permeability and may compromise the integrity of the gastrointestinal mucosal barrier.
Sensitivity analyses
We took into account markers of prodromal disease activity (as in paper I) as this might influence contemporaneous stress resilience and excluded men with a prior IBD diagnosis and any gastrointestinal diagnoses in adolescence to minimise the possibility of including men with symptomatic but undiagnosed IBD. Adjustment for potential disease activity markers had only a modest effect on the magnitude of the associations, thus it makes it less likely that the associations were driven by early disease activity. Further evidence that the direction of the association is from stress resilience to IBD, is that the associations remained even after 15 years of follow-up, even though this reduced statistical power. Therefore, it is plausible that those with low stress resilience and who are susceptible to IBD are more likely to develop symptoms and receive a diagnosis.

Paper III: Stress resilience and peptic ulcer disease risk
Plausible mechanisms include physiological (including acid secretion) and or behavioural pathways (like smoking). Psychological stress activates the HPA axis\textsuperscript{19,98,100,101} and causes mast cells to release histamine and other chemical mediators like proteases\textsuperscript{21,94,100} which triggers acid production. Acid damage is among the mechanisms associated with PUD\textsuperscript{19,21,94} impairing mucosal defences in the stomach and duodenum.\textsuperscript{19} Psychological stress may also suppress immune function and decrease upper gastro-duodenal blood flow thereby increasing susceptibility to \textit{H. pylori} infection as well as altering gastric motility and the stomach emptying rate.\textsuperscript{19,21} Stress influences not only the physiological but also the behavioural responses in individuals.\textsuperscript{41} The behavioural pathway in PUD includes smoking which is a major risk factor.\textsuperscript{18,86-88} Lower stress resilience may increase the likelihood of smoking or minimise the chance of giving up smoking.\textsuperscript{137} Psychological stress may make individuals adopt behaviours like smoking, heavy alcohol consumption, or increased use of NSAIDs.\textsuperscript{21} Psychological stress and poor stress resilience may increase behaviour that increases susceptibility to PUD, but more direct physiological mechanisms may also be implicated.

Sensitivity analyses
Psychological stress seems to be involved in the aetiology of PUD as this is also observed in the analysis of peptic ulcer location. A higher magnitude
association of stress resilience and gastric ulcer was observed than for duodenal ulcer. This finding is consistent with other studies and potential mechanisms may involve the influence of HPA axis on mediators that are capable of triggering acid production thus increasing gastric ulcer risk.\textsuperscript{137} Acute appendicitis was analysed as a control diagnosis to rule out surveillance effects. There was no association between acute appendicitis and stress resilience as expected. Suggesting that the association is not an artefact of the study design such that there is raised risk for diseases unrelated to stress.

**Paper IV: Resilience to stress and gastrointestinal infections risk**

Chronic psychological stress may induce intestinal barrier dysfunction by activating the HPA axis and dysregulating the immune system, resulting in low-grade inflammation as a result of failure to downregulate inflammation and inflammation, in turn, increases intestinal permeability.\textsuperscript{98} We hypothesised that this would increase the risk of GI infections, but there was an unexpected inverse association between stress resilience and subsequent GI infections in adulthood. We did not identify the underlying mechanism but speculate that factors such as increased gastric acid production in those with low stress resilience may play a role or childhood infectious exposures associated with the disadvantage that lowers stress resilience may improve the ability of the immune system to fight infections in later life.\textsuperscript{138}

Evidence for the putative role of gastric acid comes from analysis of PUD, which was modelled as a time-dependent covariate, included in the analysis as a potential confounding factor. As previously identified, low stress resilience is associated with an increased risk of receiving a PUD diagnosis (paper III). After diagnosis, a high proportion of patients will be treated using proton pump inhibitors (PPI) reducing gastric acid production; and this is a well-recognised risk factor for GI infections.\textsuperscript{110} Therefore, PUD was modelled as a time-dependent covariate, such that the models only applied this exposure after PUD diagnosis. We speculate that the majority of men with low stress resilience have greater acid production resulting in lower gastric pH without receiving a PUD diagnosis and are not treated with PPI. This would potentially reduce their enteric infection risk as lower gastric pH is a protective factor,\textsuperscript{110} and this may help explain the unexpected results.
Potential confounding factors

A range of potential confounding factors was considered in this thesis. From a life course perspective, childhood exposures can influence disease risk (and risk accumulation in adulthood) due to their potential persistence. Socioeconomic circumstances in childhood (parental socioeconomic index (SEI), household crowding and number of siblings) were taken into account due to potential influences on the main exposures, stress resilience and fitness. Lower parental socioeconomic circumstances are associated with increased exposure to infectious agents and decreased resistance against infection as well as greater risk of poorer diet. Dietary factors can suppress the immune system’s ability to fight off infections and have also been identified as a pathway linking poorer children to raised disease risk. Studies have also shown that cumulative low social class from birth to midlife is associated with increased levels of biomarkers that signal raised levels of inflammation, such as CRP. Consequences of lower parental socioeconomic circumstances on stress resilience and fitness across life may also be mediated by behaviour; lower socioeconomic conditions have been associated with lower levels of physical activity, poorer diet, alcohol consumption and obesity. In turn, these behavioural factors may lead to poorer immune functioning and higher levels of inflammation signalled by raised levels of peripheral inflammatory markers including CRP and IL-6.

Characteristics in adolescence accounted for in the thesis included measures of cognitive function, BMI, height, ESR - an inflammation marker, disease summary score as well as a history of acute appendicitis. Individual responses to stressful situations are determined by how a person perceives situations and the physical health of a person. Physical health is not only determined by genetic but also behavioural and lifestyle choices. Thus, responses to psychosocial stress may involve cognitive, emotional, behavioural, and physiological processes that are interconnected. Some people may cope with challenges by consuming alcohol or smoking, making unhealthy dietary choices or adopting to sedentary lifestyles. All these forms of behaviour can influence the risk of gastrointestinal diseases. For example, smoking is a risk factor for the development of inflammation-related diseases (CD and PUD) and GI infections involving mechanisms like a change of blood flow, increased viral or bacterial infections and the dysfunction of
the immune system in the GI mucosa. Cognition in adolescence has been included in the analyses, as this is relevant to behaviours like smoking but may also be a consequence of stress. Chronic stress exposure influences brain function, including the hippocampus which has high concentrations of cortisol receptors. Impairment of hippocampal function decreases the ability to store and recall memories. The presence of some chronic mental disorders (like anxiety and depression) may impair the ability to control - downregulate - the stress response. Chronic disease indicator (summary disease score) was also included in the analyses as this may have consequences for stress resilience, physical fitness and possibly for the risk of the outcome diseases. The study population for the IBD papers comprised ostensibly healthy adolescents as individuals who were symptomatic were excluded in the sensitivity analyses.

**Methodological considerations**

**Study design and strengths**

The thesis utilises longitudinal data on a representative cohort of men from the general population in Sweden. The data were collected prospectively thereby minimising potential methodological limitations such as recall bias, which is often an issue when using retrospectively collected data.

Strengths include: prospectively measured potential confounding factors such as socioeconomic index of the family of origin; prospectively collected measures of physical fitness and stress resilience before typical IBD onset age; the ability to take into account potential effects of prodromal disease activity in adolescence. Another advantage is the use of stress resilience rather than stressful events as a measure of psychosocial stress, due to pronounced inter-individual differences in susceptibility to stress.

**Potential limitations**

**Lack of direct information on coping**

We examined stress resilience in adolescence but we had no direct measure of coping to capture the extent to which situations were perceived as stressful, as captured by instruments such as the Perceived Stress Scale (PSS). Neither did we have direct information on coping strategies which are an
important component of stressful experiences. We may have underestimated the magnitude of associations between stress resilience and IBD or PUD or GI infections.

**Lack of smoking data**
Smoking is an established risk factor for CD and is associated with lower levels of physical fitness\textsuperscript{142} and psychological stress,\textsuperscript{143} thus is a potential confounder. Smoking is also a risk factor for PUD.\textsuperscript{89} The observed associations between stress resilience and CD risk or and PUD risk might also be consequences of behaviour, as lower stress resilience may increase the likelihood of smoking or reduce the chances of giving up. We considered smoking as a potential mediating rather than confounding factor, in the PUD paper. The influence of *H. pylori* infection in PUD seems to be mediated by smoking.\textsuperscript{89} Further evidence that smoking is a mediator in PUD was indicated by the study by Rosenstock et. al.\textsuperscript{86} which showed significant effect modification between *H. pylori* and tobacco smoking. The effect of smoking in PUD was only seen in patients infected with *H. pylori*. The Inflammation indicator (ESR) in adolescence was included in the analysis; raised ESR has been linked with smoking although not always consistently.\textsuperscript{127,144,145} There was no association between ESR and PUD.

Smoking has also been associated with infections like *Salmonella* and *C. difficile* infections\textsuperscript{\textsuperscript{111,112}} but the literature is very limited. ESR was not associated with GI infections. As there is an inverse or null association with the infections, confounding by smoking should not be a concern.

Smoking was more common in the 1970s, so the majority of men were probably smokers in adolescence. Therefore, we examined if longer duration and heavier smoking are associated with poorer physical fitness in adolescence by examining associations with subsequent diagnoses of COPD. The relationship between smoking and COPD has a genetic component as approximately 50% of smokers develop the disease to some extent.\textsuperscript{146} COPD diagnoses only identify a small subset of smokers but it may provide information on the pattern of association with physical fitness in adolescence. Poorer physical fitness was associated with COPD indicating these men are more likely to be heavier and persistent smokers. Therefore, smoking is likely to account for at least some of the association between physical fitness or stress resilience and CD or PUD risk. However, smoking cannot explain the associations with UC in papers I and II, as smoking is inversely associated with UC risk. This suggests that smoking is not the sole reason
for the observed associations of physical fitness, and particularly stress resilience, with IBD. If the association of stress resilience with PUD was entirely due to confounding by cigarette smoking, we might expect to see an association with other diseases linked to smoking, such as acute appendicitis,\textsuperscript{147,148} where smoking is also associated with a raised risk of perforation.\textsuperscript{130} Even though we reduced the diagnostic heterogeneity\textsuperscript{60} by identifying appendicitis with inflammation or perforation, there was no association with stress resilience.

**Residual confounding**

We were unable to take into account diet, early life infections, exposure to antibiotics and other personal characteristics that may be associated with both stress resilience or physical fitness and the investigated gastrointestinal diseases (including ‘triggering events,’ stressful or otherwise, that result in frank disease onset), thus residual confounding is possible.

**Error, bias and validity**

**a) Selection bias**

This can occur if the chance of being included in the study sample differs for individuals depending on the exposures and outcomes of interest.\textsuperscript{149}

**Use of the Military Conscription Register**

The thesis utilises exposures that were assessed during military conscription assessment. Military conscription was limited to only men at the time the exposures were assessed in the 1970s (only 2-3% of the male population were exempted\textsuperscript{118}). The observed results in all of the papers may be at least partially invalid for the female population.

**Inpatient and outpatient diagnoses**

The Patient Register does not include information from primary care, so only more severe disease is likely to be detected. Outpatient diagnoses were only recorded in the later years of the register, but no notable differences in the association were found when inpatient or outpatient outcome diagnoses were examined. However, the lack of primary care data means that the majority of diagnoses such as gastrointestinal infections will not be identified as most are not treated in a hospital setting. Therefore, it is possible that the
associations observed with infections may not be representative of more common less severe episodes.

**b) Information error and bias**

Individuals that are selected in the study population can be incorrectly given exposure or outcome status, leading to misclassification.\(^\text{149}\)

**Diagnostic misclassification**

Some men received apparently contradictory IBD diagnoses at different times, usually beginning with UC then revised to CD. We used the later diagnosis to define disease type but the first date to define the first diagnosis, as this pattern of change is often reported in IBD.\(^\text{150,151}\) The distinction between CD and UC is not always possible, especially during early disease stages. Some features of CD are not easily detected and histopathological details have to be available to make the distinction between the diseases.\(^\text{150,151}\) Another issue is variation in the rate of misclassification over time, due to changes in diagnostic methods. This issue was addressed by conducting sensitivity analyses beginning at least 15 years after the conscription assessment, a period when endoscopy had become the main diagnostic method for IBD, which offers greater reliability. From this period, the majority of IBD diagnoses would have involved colonoscopy with the use of histological and radiological criteria.\(^\text{152,153}\) The results of these sensitivity analyses were in line with the results of the main analyses. In addition, a recent validation of IBD diagnoses in the NPR reported a positive predictive value (PPV) of 90% for UC and 81% for CD for patients who did not subsequently change diagnosis between UC and CD.\(^\text{154}\) Only 8% of UC and 6% CD diagnoses were classified as non-IBD where there was a change of diagnosis.\(^\text{154}\) Even though some inaccuracy cannot be ruled out, diagnostic inaccuracy is unlikely to account for the results in our studies of IBD.

Misclassification of PUD is possible. We believe that misclassification is not a major issue for PUD diagnoses, especially in paper III where the diagnoses were based on the *primary* reason for hospital admission or attendance as an outpatient therefore, they are most likely to be consistent with the level of accuracy of over 85% in the NPR.\(^\text{128}\) In addition, the incidence rate for PUD reported in the thesis (0.40/1000 person-years) is in line with incidence rates from other studies during the same study period.\(^\text{137}\)
c) Surveillance bias and use of health care services

Surveillance bias can occur when information is obtained through sources such as healthcare records, where health care associated with one diagnosis makes it more likely that other diagnoses are also recorded. Here, it is theoretically possible that the associations between characteristics in adolescence and the outcome diseases are due to an association with diseases other than the outcome: the findings would be due to associations with a different diagnosis while the target diseases are identified coincidentally. To tackle this issue, sensitivity analyses were performed where the target diseases were defined only where it was the primary reason for hospital admission, reducing the risk of this form of surveillance bias.

Could characteristics in adolescence make it more likely that the cohort members seek medical care, resulting in a greater likelihood of diagnosis? It has been reported that patients with depression or anxiety, which are associated with low stress resilience, are likely to seek medical care frequently, increasing likelihood of a diagnosis being made at an earlier time. On the other hand, individuals with low stress resilience may be reluctant to seek medical care resulting into a greater diagnostic delay, which is a relatively common phenomenon among adults. It is, therefore, difficult to estimate the effect on the studies reported here. However, as the outcome diseases are all sufficiently to require a hospital, particularly inpatient, treatment this potential source of bias may be limited.
Conclusions

There is evidence that characteristics already present in adolescence are associated with the subsequent risk of gastrointestinal disease. Poorer physical fitness in adolescence is associated with an increased risk of IBD, but this association seems to be explained – at least in part – by prodromal disease activity reducing exercise capacity and therefore fitness. In contrast, the association of low stress resilience in adolescence with later IBD does not appear to be explained by prodromal disease activity. However, we do not believe that stress is an important cause of IBD, but that it may increase the likelihood of conversion from subclinical to symptomatic disease. Poor stress resilience is also associated with a greater risk of PUD: this may be explained by a combination of physiological and behavioural mechanisms that increase susceptibility to \textit{H. pylori} infections and other risk factors. Low stress resilience does not increase the risk of gastrointestinal infections and the unexpected inverse association is worthy of further investigation. Early influences on health and development are relevant to gastrointestinal health in adulthood.
Future perspectives

While stress management approaches and psychotherapy are effective in functional gastrointestinal disorders, the approaches have been disappointing when applied to the management of IBD and PUD\textsuperscript{19,158} What this thesis reveals is the association of stress resilience in ostensibly healthy young men with a range of gastrointestinal diseases, suggesting a general influence relevant to the general population before onset of a chronic disease. If stress resilience is indeed relevant to gut health then research is required to ascertain more specific pathways and exposures before this information can be used to promote better health. This can only be achieved using a life course perspective and longitudinal research as some health trajectories begin in early life, tracking through to an increasing disease burden in later adulthood. Is poorer ability to cope with stress really an important influence or an epiphenomenon? Examination of the combination of susceptibility and stressful exposures may provide more conclusive evidence. What are the specific exposures and mechanisms that could be used as intervention targets? These may be a complex combination of behaviour and physiological changes that influence characteristics such as inflammation and gastrointestinal permeability, possibly involving the microbiota. Early childhood may be the critical starting point in identifying the exposures that influence the development of stress resilience and related exposures relevant to the gastrointestinal tract, even if some pathological processes are the result of the exposures that are accumulated across life for those whose origins lead to an unhealthier life trajectory.
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Svensk sammanfattning

Fysiska och psykologiska egenskaper under tonåren och risk för mag-tarmsjukdomar i vuxen ålder

Forskningsrön tyder på att fysisk kondition och stresståthet kan påverka risken för mag-tarmsjukdomar. Hög fysisk kondition kan minska systemisk inflammation medan exponering för psykosocial stress kan öka inflammationsutveckling och tarmpermeabilitet. Det övergripande syftet med avhandlingsprojektet var att utvärdera om det finns ett samband med låg fysisk kondition och låg stresstolerans i tonåren och en förhöjd risk för inflammatorisk tarmsjukdom (IBD), magsår och mag-tarminfektioner i vuxen ålder, samt att undersöka belägg för orsakssamband (kausalitet).


Inflammatorisk tarmsjukdom: Låg fysisk kondition i tonåren var associerat med en ökad risk för IBD. Sambandet verkar delvis kunna förklaras av att subklinisk sjukdomsaktivitet i tonåren minskar fysisk kapacitet och därmed fitness. Låg stresstolerans i tonåren var associerat med en ökad risk för att erhålla en IBD diagnos. Stresstolerans är inte en viktig orsaksfaktor till IBD, men kan öka sannolikheten för övergång från subklinisk till symtomatisk sjukdom.

Magsår: Låg stresstolerans i tonåren var associerat med en ökad risk för magsår. Sambandet kan förklaras av en kombination av fysiologiska och beteendemässiga mekanismer som öka mottaglighet/risken för H. pylori infektioner och andra riskfaktorer.

Mag-tarminfektioner: Låg stresstolerans var associerat med minskad risk för mag-tarminfektioner (i motsats till vår a priori hypotes) och verkar inte öka risken för enteriska infektioner.
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71


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