UiT The Arctic University of Norway

Faculty of Health Sciences

Inflammation and Sleep as Risk Factors for Psychological Distress During Adolescence

The influence of low-grade inflammation and sleep duration on psychological distress in girls and boys aged 15-18 years. The Fit Futures study.

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Summary

Background: The onset of depression and psychological distress (symptoms of anxiety and depression) increases dramatically during adolescence. In adults, research has indicated that low-grade inflammation and short sleep duration are risk factors for depression. Less research has been conducted on these risk factors in healthy adolescents, where the findings have been mixed and there has been a lack of adjustment for potential confounders. It is important to study these risk factors to increase knowledge relevant for the prevention of psychological distress and depression.

Methods: This thesis explores associations between two respective exposures, 1) five inflammatory markers and 2) sleep duration and psychological distress as outcome in upper-secondary school students from the Fit Futures, a prospective study with data from two time-points. Cross-sectional and prospective regression analyses were conducted to explore associations between the mentioned exposures and psychological distress. Additionally, we explored the moderating effects of body-fat percentage, physical activity, and sleep duration on the associations between inflammatory markers and psychological distress. We used change scores to explore whether changes in sleep duration were associated with changes in psychological distress.

Results: The overall results showed no cross-sectional associations between inflammatory markers and psychological distress in girls or boys. In prospective analyses, increased levels of CRP and TGF-α at baseline were associated with increased levels of psychological distress at follow-up two years later in boys. Further in boys, there was found interaction effects indicating that body fat percentage and physical activity moderated the effects of CRP on psychological distress, and that sleep duration moderated the effect of TWEAK on psychological distress. Regarding sleep duration as exposure, we found that changes in sleep duration predicted changes in psychological distress in both girls and boys. Increases in sleep duration predicted decreases in psychological distress in both genders.

Conclusion: This thesis explored two risk factors in healthy adolescents and included important potential confounders. In boys, CRP and TGF-α at baseline were significantly associated with psychological distress at follow-up. We found significant effect-modifications in boys indicating that interventions to promote mental health during adolescence should focus on decreasing body fat percentage and increasing physical activity. Further, our results

suggest that decreased sleep duration is a risk factor for increased psychological distress, thus interventions to promote mental health during adolescence should consider aiming to increase sleep duration. The results indicate that low-grade inflammation and short sleep duration are risk factors for psychological distress in adolescence, in a similar way as previous studies have shown in adults. Future studies should examine causality between the risk factors and psychological distress.

Sammendrag

Bakgrunn: Depresjon og psykiske plager (symptomer på angst og depresjon) øker drastisk i ungdomsalderen. Blant voksne er kronisk inflammasjon og kort søvnvarighet risikofaktorer for depresjon. Mindre forskning har blitt gjort på disse risikofaktorene blant frisk ungdom, hvor funnene har variert og det i liten grad vært justert for konfundering. Det er viktig å studere disse risikofaktorene for å få kunnskap relevant for forebygging av psykiske plager og depresjon.

Metoder: Denne doktorgraden utforsker sammenhenger mellom to eksponeringer 1) fem inflammasjonsmarkører og 2) søvnvarighet og utfallet psykiske plager hos elever på videregående skole fra Fit Futures studien, en prospektiv studie med data fra to måletidspunkter. Regresjonsanalyser gjort på tverrsnittsdata og prospektive data ble gjort for å utforske sammenhengene mellom eksponeringene og psykiske plager. I tillegg undersøkte vi om sammenhengen mellom inflammasjonsmarkører og psykiske plager ble moderert av fettprosent, fysisk aktivitet og søvnvarighet. Vi brukte endringsskårer for å utforske om endringer i søvnvarighet hang sammen med endringer i psykiske plager.

Resultater: Resultatene viste ingen tverrsnitt-sammenhenger mellom inflammasjonsmarkører og psykiske plager hos verken jenter eller gutter. I de prospektive analysene fant vi at CRP og TGF-α predikerte psykiske plager hos gutter. Vi fant også interaksjonseffekter som tyder på at fettprosent og fysisk aktivitet modererte effektene fra CRP på psykiske plager, og at søvn modererte effekten fra TWEAK på psykiske plager. Når det gjelder søvnvarighet, så fant vi at endringer i søvnvarighet predikerte endringer i psykiske plager hos både gutter og jenter. Økning i søvnvarighet predikerte reduksjon i psykiske plager for både jenter og gutter.

Konklusjon: Denne doktorgraden utforsket to risikofaktorer hos frisk ungdom, og inkluderte viktige konfundere. CRP og TGF-α predikerte psykiske plager to år senere hos gutter. Vi fant signifikante moderasjonseffekter hos gutter, som tyder på at intervensjoner for å promotere psykisk helse blant ungdom bør ta sikte på å redusere fettprosent og øke fysisk aktivitet. Videre tyder resultatene på at redusert søvnvarighet er en risikofaktor for psykiske plager. Derfor bør intervensjoner for å promotere psykisk helse blant ungdom vurdere å sette søkelys på økt søvnvarighet. Resultatene kan indikere at kronisk inflammasjon og kort søvnvarighet er risikofaktorer for psykiske plager hos ungdom, på samme måte som tidligere studier har

vist blant voksne. Fremtidige studier bør utforske kausaliteten mellom risikofaktorene og psykiske plager.

List of papers

The following papers are part of this thesis:

Paper 1:

Linkas, J., Ahmed, L. A., Csifcsak, G., Emaus, N., Furberg, A. S., Grimnes, G., Pettersen, G., Rognmo, K., & Christoffersen, T. (2022). Are pro-inflammatory markers associated with psychological distress in a cross-sectional study of healthy adolescents 15–17 years of age? The Fit Futures study. BMC Psychol 10, 65. https://doi.org/10.1186/s40359-022-00779-8

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Abbreviations:

ANOVA: Analysis of variance

CI: Confidence interval

CRP: C-reactive protein

HSCL-10: Hopkins Symptom Checklist

ICSD: The International Classification of Sleep Disorders

IL-6: Interleukin 6

PDS: Pubertal Development Scale

SD: Standard deviation

SPSS: Statistical Package for the Social Sciences

TGF-α: Transforming growth factor-alpha

TNF-α: Tumour necrosis factor alpha

TRANCE: Tumour necrosis factor alpha variant 1

TWEAK: Tumour necrosis factor alpha variant 2

Vitamin D: Standardised version of (25-OH)D

UNN: The University Hospital of North Norway

WHO: World Health Organization

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1 Introduction

Psychological distress can be defined as an emotional state characterised by symptoms of anxiety and depression [1]. Psychological distress is not a clinical diagnosis, yet it is viewed as an emotional disturbance with the potential to influence social functions and day-to-day living [2]. Psychological distress increase rapidly during adolescence [3] and has the potential to develop into mental disorders [3]. To prevent onset of psychological distress in adolescence, it is imperative to explore potential risk factors. I have chosen to explore two biological risk factors, namely inflammation and sleep. Historically, it has been debated whether bodily functions have the capacity to influence the mind.

Mind and body dualism is a metaphysical viewpoint that regards mind and body as two separate substances, with different natures. Dating back to ancient times, the most known version of dualism is attributed to Rene Descartes in the 17th century. He claimed that human-beings consist of mind and body, and that these two substances do not exist in unity. According to Descartes, the mind is immaterial, and the body is material [4]. Today, our understanding has changed, and we view the mind and body as interacting with each other [5]. In mind-body medicine, mind and body are seen as a functioning unit, where mind and body affect each other mutually [6]. In my thesis, I have explored whether two bodily mechanisms, inflammation and sleep, influence the mind in the form of psychological distress.

Inflammation comes from Latin “inflammare” (to set on fire). The Roman Celsus is acknowledged for describing the following cardinal signs of inflammation in the year 100 AD: “calor” (warmth), “dolor” (pain), “tumor” (swelling), and “rubor” (redness). In 1871, a fifth cardinal sign, “function laesa” (reduced function), was added by Virchow [7]. This definition of inflammation identifies what we today term “acute inflammation”, which is a response from the immune system to protect the body against threats, such as pathogens, infection, and injuries [8]. This intermittent acute increase in inflammation is critical for survival. Contrastingly, a systemic chronic low-grade inflammation caused by social, environmental and lifestyle factors can lead to several diseases, including cancer, diabetes mellitus, chronic kidney disease, non-alcohol fatty liver disease, neurogenerative disorders, hearth diseases, auto-immune diseases, and diseases of the mind, such as depression [9, 10]. Further, chronic low-grade inflammation has also been found to be associated with psychological distress [11-13].

The other bodily function I have focused on is sleep. Hippocrates (460 to 375 BC) was the first to systematise information about sleep from a medical perspective [14]. Greek physicians viewed sleep as a biological phenomenon, regarding it as essential for the maintenance of human health and considering it in medical prognosis and diagnosis [14]. Today, researchers agree that the human body requires a certain amount of sleep to be healthy [15, 16]. Regarding mental health, short sleep duration has been associated with depression and psychological distress [17-20].

Inflammation and sleep are intertwined, and the literature indicates that they act together as risk factors for mental disorders and psychological distress. It is not a new realisation that inflammation and sleep disturbance are associated. Empirical findings showing associations between sleep and inflammation date to the works of Aristotle (384-322 BC), who recorded that lethargy and fatigue often appear in the course of fever [21].

The theoretical and empirical background that follows starts by giving an overview of mental disorders and mental health, with a focus on the adolescent period. Subsequently, the two respective risk factors, low-grade inflammation and sleep, are presented alongside their associations with psychological distress. Finally, I present the "two-hit" model that explains how sleep disturbance and inflammation intertwines and jointly may result in depressive symptoms and depression.

1.1 Mental disorders and psychological distress

A mental disorder is a behavioural or mental pattern that results in considerable distress or impairment of personal functioning [22]. Mental disorders are highly prevalent, with a lifetime risk of approximately 50% worldwide [23]. Globally, it has been reported that one in three adults experience a common mental disorder (mood disorder, anxiety disorder or substance use disorder) during the course of a lifetime, and that one in five adults has experienced a common mental disorder in the past 12 months [24]. There are numerous mental disorders, with unique symptomology. In general, symptoms include abnormal thoughts, feelings, perceptions, behaviour and difficulties in relations with other people [25]. Mental disorders are diagnosed by a medical practitioner based on patterns of symptoms, and whether daily function is affected [26]. Two international systems are used for classification and diagnoses of mental disorders, namely the World Health Organization (WHO)'s International Classification of Diseases [27] and the Diagnostic and Statistical Manual of Mental Disorder, the latter developed by the American Psychiatric Association [28]. This

thesis focuses on the two most common mental disorders, namely depression and anxiety [29], and subclinical versions of these two disorders, in form of symptoms of depression and anxiety, known as psychological distress.

1.1.1 Depressive disorder

Depressive disorder is a common but serious mood disorder. As opposed to having normal feelings of sadness occasionally, depressive disorder may be present when individuals are sad most of the time, and daily functioning is affected. There are different subtypes of depressive disorders, such as major depressive disorder, dysthymia, bipolar disorder, psychotic depression, postpartum depression, and seasonal affective disorder [28].

Symptoms of depressive disorder include persistent feelings of sadness, hopelessness, fatigue, appetite changes, and irritability [28]. Major depression is a common disorder that WHO projects will be the primary cause of disease worldwide by 2030 [30]. Almost one in five will experience an episode of major depression during the course of a lifetime [31]. Depression is almost twice as common in women compared to men [32], and has an onset early in life, with 40% having their first episode before the age of 20 [32]. Depressive episodes are highly recurrent; 50% of people who have experienced an episode will suffer from one or several episodes later in life. Among those who have experienced two episodes, the risk of having a third episode is 80% [33]. People with depressive disorder have an elevated risk of suffering from anxiety disorder [34]. The correlation between general anxiety disorder and major depression has been reported to be as high as 0.62 [35].

1.1.2 Anxiety disorder

Anxiety disorder is another common mental disorder. Some anxiety, in the form of feeling anxious or nervous in relation to, for example, work problems, a job-interview or taking a test is considered normal. Normal levels of anxiety can help us notice dangers and focus our attention on our own safety. An anxiety disorder, however, entails anxiety beyond regular nervousness and occasionally feeling fear [28]. Anxiety disorders are characterised by excessive worry and/or fears that affect daily functioning. There are several subtypes of anxiety disorders, such as general anxiety disorder, panic disorder, social anxiety, and phobia disorders [36]. Combined, anxiety disorders are reported to be the most common class of mental disorders [36], affecting up to 33.7% of the population during their lifetime [35]. In general, anxiety disorders have an early onset, ranging from childhood to adolescence to early

adulthood depending on the type [37]. Like depression, the prevalence of anxiety disorders is twice as high in women compared to men [35].

1.1.3 Psychological distress

Psychological distress is not a mental disorder but rather a continuous measure of emotional suffering. Psychological distress is commonly used to measure mental health in the general population. As mentioned, psychological distress can be defined as an emotional state characterised by symptoms of anxiety and depression [1]. This is the most widely accepted definition of psychological distress [38]. However, symptoms of anxiety and depression may also be accompanied by somatic symptoms (for example headaches, musculoskeletal pain, and fatigue) that vary across cultures [39]. Other criteria have been suggested for inclusion in the definition of psychological distress, but without consensus [38]. Exposure to stressful events (stressors) has been suggested as a particularly salient part of the definition, because psychological distress commonly occurs when people are unable to cope with stressors [40]. Hence, psychological distress will normally disappear when the stressor disappear or are effectively coped with [41]. However, by including stressors in the definition of psychological distress, the presence of psychological distress without stressors is not recognised [38].

The prevalence of psychological distress ranges between 5% and 27% depending on which scale was used to measure it, the use of different time-windows in the documentation of symptoms, and different cut-offs applied to indicate psychological distress [38]. Three of the most validated and frequently used families of instruments applied to measure psychological distress are the General Health Questionnaire, the Kessler scales, and scales derived from the Hopkins Symptom Checklist. These three families of scales all differ from each other with respect to the time windows used in the documentation of symptoms and cut-offs. Further, although the three families of scales have several items in common, there are also different items across the three scales [38].

It is noteworthy that psychological distress is reported to be more prevalent among women than among men, across different instruments [38].

1.1.4 Mental disorders and psychological distress during adolescence

The word adolescence comes from the Latin “adolescere” (to grow up). Adolescence is the life phase between childhood and adulthood, yet the definition of adolescence has been disputed. Adolescence is characterised by biological changes known as puberty, which in

general starts earlier today compared to previous periods [42]. Further, new understanding of continued biological changes suggests that the endpoint of adolescence is in the mid-20s, in contrast to the past view that the biological changes related to puberty ended before the age of 20 [42]. Additionally, adolescence is characterised by changes in social roles. Completion of education, start of work life, marriage and parenthood all occur later today compared to earlier. Thus, social roles related to adulthood begin later in today's society [42]. Previously, the age span of 10-19 was defined as adolescence, but more recently it has been suggested that the age span of 10-24 years is a more precise definition of adolescence, given a new understanding of the biological development and changes in social roles that occur at a higher age today compared to earlier [42].

Many mental disorders have an onset during adolescence [43]. In particular, depressive disorders and anxiety disorders considerably increase during this age [44], especially among girls [45, 46]. For adolescent girls and boys combined, 6.1% meet the criteria for a depressive disorder and 10.7% meet the criteria for an anxiety disorder [47]. The prevalence of psychological distress in adolescents has been reported to be 25% and 31% on a global basis [48]. Among high school students in Norway, self-reported psychological distress has been reported to be 31% among girls and 12% among boys [49].

1.1.5 Preventing psychological distress during adolescence

Mental health was recognised as a critical component of the global health agenda by the Sustainable Development Goals in 2015 [50]. Correspondingly, WHO has called for increased attention to adolescent health [51], including the promotion of mental health and prevention of mental disorders [52]. Because depression is one of the most important contributors to the burden of disease worldwide [53], it is thus important to study how it can be prevented. Further, mental disorders disable people's ability to work and are thus an economic burden for both the individual and society. It has been estimated that investment in interventions aiming to prevent psychological distress would lead to considerable economic gain [54].

Prevention of mental disorders may be done by preventing psychological distress during adolescence because psychological distress has the potential to develop into mental disorders [3]. By preventing depressive disorder in adolescence, future recurrent episodes will also be prevented. Further, by preventing depressive disorder, the associated increased risk for suicide will also be reduced [55].

Psychological distress is caused by an intricate combination of biological, social and psychological vulnerability risk factors, including influences from events during childhood, psychosocial factors and genetics [56]. In 1977, Engel proposed the biopsychosocial model to explain bodily and mental disorders [57], which explains disorders by biological, psychological, and social predictors. The biopsychosocial model has guided psychiatry and health psychology for over 40 years [58, 59]. Of the three predictors of the biopsychosocial model, we have explored two biological predictors, namely inflammation and sleep.

1.2 Inflammation as a risk factor for depression, anxiety, and psychological distress

One way the body and mind are connected is via the immune system and inflammation [60]. Acute inflammation is initiated by the immune system when the body becomes injured or is exposed to threats such as pathogens, allergens, or toxins [61]. The purpose of the inflammatory process is to protect the body from threats and repair from injury [8]. This acute elevation of inflammation is critical for survival. In strong contrast, a prolonged low-level inflammation, also termed chronic inflammation [10], is the most significant cause of death worldwide [10]. Chronic inflammation has been reported to lead to chronic inflammatory diseases such as diabetes, cardiovascular diseases, arthritis and joint diseases, allergies and chronic obstructive pulmonary disease, cancer, diabetes mellitus and chronic kidney disease [9, 10].

Inflammation's role in depression was first proposed by Smith in 1991 [62]. Alongside acute inflammation comes what has been called sickness behaviour, including fever, nausea, loss of appetite, and loss of interest in the environment. Additionally, sickness behaviour includes feelings of fatigue, changes in sleep, depressed feelings, irritability, and changes in attention [63]. It has been suggested that sickness behaviour associated with acute inflammation has been beneficial through evolution for coping with the healing of wounds, and fighting pathogens and infections [63]. This hypothesis is called the "pathogen host defense" hypothesis of depression [60].

1.2.1 Inflammatory markers, depression, and anxiety

Of those adults with depressive disorder, one in three show elevated levels of inflammatory markers [64]. Further, people with depressive disorder have on average higher levels of inflammatory markers than controls [65-68], and patients with inflammatory diseases have an elevated risk for depressive disorders compared to controls [64]. Additionally, as much as

50% of patients treated with the therapeutic administration of the cytokine interferon-α develop a depressive disorder [69]. Further evidence comes from animal studies indicating that inflammation leads to sickness behaviour. When proinflammatory cytokines is administered to rodents, it results in sickness behaviour, including less exploration, depressed motor activity, less social activity, anorexia and increased sleep [70]. Mechanistically, inflammatory cytokines enter the brain via various paths, and influence domains related to depressive disorder, such as changes in the metabolism of dopamine, serotonin and norepinephrine leading to changes in emotions, psychomotor function, and reward systems [64].

Figure 1. Inflammatory proteins influence domains in the brain related to depressive disorder

Evgeny Atamanenko / mostphotos.com

Additional evidence of inflammation's relation to depression and anxiety comes from meta-analyses on clinical trials indicating that anti-inflammatory medicine has antidepressant effects [71, 72]. In adults, meta-analyses have concluded that there is evidence for associations between inflammatory markers and depressive disorder [73] and preliminary

evidence for associations between inflammatory markers and anxiety disorder [74]. In adolescents, however, meta-analyses show mixed findings on associations between inflammatory markers and depressive disorder and anxiety disorder, respectively [75-77]. Furthermore, the meta-analyses on adolescents have contained few studies.

In line with previous research, we have explored associations between the following inflammatory markers and psychological distress: C-reactive protein (CRP), Interleukin 6 (IL-6), Transforming growth factor-alpha (TGF-α), Tumour necrosis factor alpha variant 1 (TRANCE) and Tumour necrosis factor alpha variant 2 (TWEAK) [73, 78, 79]. CRP is an acute phase reactant synthesised in the liver in reaction to pro-inflammatory cytokines [80]. The circulating concentration of CRP increases quickly and extensively in response to injury, infection and inflammation [81]. IL-6 is a pro-inflammatory cytokine secreted by the activated macrophages response to infection and tissue damage [80], and this leads to increases in acute-phase proteins such as CRP [82]. TGF-α is a member of the epidermal growth factor family and is thus a mitogenic polypeptide [83]. Monocytes and neutrophils, which are important effector cells in inflammatory reactions, store TGF-α in cytoplasmic granules [84], while TRANCE and TWEAK are members of the Tumour necrosis factor superfamily that are involved in inflammation [85].

1.2.2 Inflammatory markers and psychological distress

In addition to having the potential to induce depressive disorder, inflammation may have the potential to influence general well-being. Indeed, inflammation has been associated with poor well-being [86]. Further, inflammatory markers have been linked with psychological distress in adults, both cross-sectionally and prospectively [11-13]. In adolescents, associations between inflammatory markers and symptoms of depression and anxiety have been inconsistent, and the research is limited. [79, 87-92]. Another risk factor of psychological distress with inconsistent findings and limited research during adolescence is sleep.

1.3 Sleep as risk factor for depression, anxiety, and psychological distress

Sleep is a biologically recurring state of mind and body, marked by altered consciousness [93]. Sleep is differentiated from wakefulness by a reduced reactions to external stimuli [93]. During a night of sleep, the human body cycles through four sleep stages that are together called a sleep cycle. A sleep cycle lasts on average 90 minutes [94]. The three first stages are known as non-rapid eye movement (NREM), where sleep becomes progressively deeper with

each stage. Deeper sleep is characterised by decreased muscle tone, pulse and breathing rate, respectively. The fourth stage, where dreams occur, is characterised by and termed rapid-eye-movement (REM) [94]. Later in the night, fewer NREM stages occur, and the duration of the REM sleep increases [95]. Sleep is necessary for numerous reasons, and the mechanisms involved are very complicated [96]. Sleep has recuperative and regulative functions and is important for many aspects of health [97, 98], including mental health [99]. Sleep needs vary with age, and the National Sleep Foundation in the US recommends sleep durations of 14-17 hours for new-borns, 12-15 hours for infants, 11-14 hours for toddlers, 10-13 hours for pre-schoolers, 8-10 hours for teenagers, 7-9 hours for young adults and 7-8 hours for older adults [100]. Regarding adolescents, the National Sleep Foundation specifies that 7-11 hours may be appropriate for some individuals, because there are individual differences in sleep need [100].

Figure 2. Adolescent sleeping

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1.3.1 Sleep-disorders

The International Classification of Sleep Disorders (ICSD) is the most widely used classification system for sleep disorders. The third edition of the ICSD (ICSD-3) includes 60 specific diagnoses, organised in seven major categories of sleep disorders [101].

Insomnia disorder is defined as problems with sleep, including nocturnal symptoms and diurnal symptoms. Nocturnal symptoms are symptoms experienced during the night such as

prolonged sleep onset, difficulties maintaining sleep and early morning awakening. Diurnal symptoms are symptoms experienced during the day such as sleepiness/tiredness (affecting school/work or private life) and dissatisfaction with sleep. To be diagnosed with insomnia, both nocturnal and diurnal symptoms must be present. Further, these symptoms need to be present at least three days a week, and for a duration greater than 3 months [28]. In adults, it has been estimated that between 10% and 30% suffer from chronic insomnia [102], and women have a lifetime risk of insomnia that is 40% higher than men [103].

Sleep apnoea is a sleep disorder where breathing stops and starts several times during the night. More specifically, sleep apnoea has been defined as repeated episodes of obstructive apnoea (cessation of breathing) and hypopnea (shallow breath for 10 seconds or longer), combined with daytime sleepiness or altered functions in the heart and lungs [104]. When using a broad definition of obstructive sleep apnoea, 15-30% of males [105] and 10-30% of women [106] meet the definition.

Restless leg syndrome is characterised by an uncontrollable urge to move the legs, because of an uncomfortable sensation that is alleviated temporarily when moving. Restless leg syndrome typically occurs during evening and night-time when sitting or lying down and disturbs sleep [107]. In adults, the prevalence has been reported to be 10-12% [107]. The prevalence is higher in females than in males [108].

1.3.2 Short sleep duration

Studies on adults vary in their cut-offs for short sleep duration, indicating a lack of consensus concerning the definition of short sleep duration. Common cut-offs in studies on short sleep duration in adults are <6 hours, ≤6 hours or <7 hours per night/ per 24-hour period [109]. The National Sleep Foundation in the US recommends that adolescents sleep 8-10 hours per night and specify that 7 hours may be appropriate for some individuals. With these individual differences in sleep need, it is difficult to define short sleep duration in adolescence. Nonetheless, sleep duration below 7 hours during adolescence is below all recommendations from the National Sleep Foundation. Hence, sleep duration below 7 hours may be defined as short sleep duration for adolescents.

Short sleep duration is prevalent worldwide [110]. For example, approximately 30% of Americans slept less than six hours per night in 2012 [111], and in a study from Norway, 42% of women and 52% of males reported sleeping below 7 hours [112]. Short sleep duration is

also highly prevalent among adolescents. Most adolescents do not meet the recommended 8-10 hour per night [113]. Mean sleep duration in adolescents from Europe, America and Asian countries has been reported to be approximately 7 hours in 15-18 year-olds [114]. In Norwegian adolescents aged 16-18 years, sleep duration has been reported to be 6 hours and 25 minutes [115].

One reason for short sleep duration during adolescence is hormonal shifts during puberty resulting in a phase delay in the circadian timing [116]. Adolescents' body clock is shifted forward approximately one to two hours, resulting in sleepiness occurring one to two hours later. Thus, adolescents fall asleep as much as two hours later, but still need to get up early because of an early school start [113]. Another report on adolescents explains short sleep duration by increased screen time, caffeine intake, and stress [117].

Long sleep duration has been found to be uncommon during adolescence. For example, a study from the US reported that only 2-3% of adolescents aged 15-18 years slept above 10 hours per night [118]. Additionally, long sleep duration is frequently a result of disease in many cases, rather than a risk factor for disease [118].

1.3.3 Sleep, depression, anxiety, and psychological distress

Without enough sleep, mood is disturbed [119]. Participants in an experiment slept in a laboratory and were allowed only 5 hours of sleep per night for a week. The participants showed an increase in emotional disturbance [120]. Further, it has been shown that sleep loss over time leads to stronger negative emotions and unsettling experiences [121]. Correspondingly, an experiment on the effects of sleep loss for one night reported increased levels of stress, anxiety and anger when encountering low-stress situations [122].

Short sleep duration alters activity in the regions of the brain related to emotion-regulation. In humans that are sleep deprived, increased activity in the amygdala, a region in the brain responsible for emotional activity, has been reported [119]. Additionally, sleep deprivation has been found to decrease the connection between the prefrontal cortex and amygdala, thereby impeding the ability of the prefrontal cortex's ability to regulate emotions [119]. Further, sleep loss reduces the restoration of central noradrenergic signalling that occurs during a full night of sleep [119]. In addition, not getting enough REM sleep has been related to increased activity in the areas of the limbic brain that regulate negative mood [123]. Lastly,

sleep deprivation has been linked with a negative emotional memory dominance [99], which may contribute to depressive disorder [99].

The mentioned findings from experimental studies regarding changes in the brain from sleep loss indicate that short sleep duration has the potential to influence mental health. Findings from observational studies suggest that such effects occur in real life outside the laboratory as well. Sleep duration has been found to be associated with depression, anxiety, and psychological distress in adults [17, 19, 20, 124]. In adolescents, sleep disturbance has been reported to be more prevalent in those with depressive disorders compared to controls, and findings show that sleep disturbance predicts depression more than depression predicts sleep disturbance [125]. Further, prospective associations between short sleep duration and anxiety disorders have been reported in adolescents [126]. In healthy adolescents, a recent systematic review and meta-analysis reported cross-sectional and prospective associations between sleep duration and various mood measurements, including depressed mood, anxiety, and anger [127]. Few studies have examined associations between sleep duration and specifically psychological distress as outcome during adolescence. Some studies have reported significant associations [128-130], while others have not [131]. A call has been made for prospective studies examining associations between sleep duration and psychological distress in this age-group [131].

1.4 A “two-hit” model of depression

1.4.1 Inflammatory markers and sleep are associated

Findings indicate that inflammation and sleep are intertwined. In adults, sleep disturbance has been shown to be associated with inflammatory markers (C-reactive protein (CRP) and Interleukin 6 (IL-6)), both cross-sectionally and prospectively [132, 133]. In adolescents, CRP has been found to be elevated in adolescents with variability in sleep duration from night to night [134]. There are mechanisms that can explain how sleep disturbance and inflammation influence each other bidirectionally.

1.4.2 How inflammation and sleep act together in the development of depression

Sleep disturbances, such as short sleep duration, may lead to the dysregulation of inflammatory responses [21]. More specifically, sleep is a psychophysiological process mediated by the central nervous system, which has a role in the regulation of the immune system by changing physiological systems that influence the production of inflammatory

cytokines and immune cell distribution [21]. Thus, when sleep is disturbed, changes in the immune system will follow, including an increase in inflammatory responses. In support, there is evidence showing that when insomnia is treated, there are decreases in inflammatory markers, including CRP, IL-6 and Tumour necrosis factor alpha (TNF-α) [21]. Indeed, insomnia treatment has been shown to reduce CRP levels as much as changing to a healthier diet and exercise [135, 136]. The effect from sleep disturbance on inflammation is comparable or even greater than those of age, race, body mass index and physical activity [21]. The direction may also be in the opposite direction, where inflammation induces sickness behaviour, including impaired sleep [69]. Thus, inflammation and sleep influence each other mutually.

Cho et al. [137] have proposed a "two-hit" model of depression that suggests that inflammation and sleep are two hits that together may result in depression. Sleep disturbance may be the vulnerability factor, and subsequent increased levels of inflammation trigger depressive symptoms [137]. Cho et al. [137] also proposed the reversed order, where inflammation is the vulnerability factor, and subsequent sleep disturbance is the trigger of depressive symptoms. Both alternatives have been supported by clinical and experimental evidence [137, 138]. In females with pre-existing sleep disturbance, an inflammatory challenge (low dose of endotoxin) resulted in higher increase in depressed mood compared to females without pre-existing sleep disturbance [137]. This indicates that sleep disturbance was the vulnerability factor, and that inflammation was the triggering factor that led to increases in depressed mood. There is also experimental evidence showing that inducing sleep disturbance in persons with an inflammatory disorder results in greater increases in depressive symptoms compared to healthy controls [139]. This indicates that inflammation is the vulnerability factor and that sleep disturbance is the triggering factor of increases in depressive symptoms.

Furthermore, evidence has shown that inflammation and sleep disturbance may occur simultaneously, without any of them preceding the other. Experiments indicate that insomnia and inflammation act together to increase depressive symptoms after an inflammatory challenge with endotoxin, which corresponds with clinical observations showing that the possibility of depressive disorder increases when sleep disturbance and inflammation occurs simultaneously during infections and psychological stress [21]. Treatments that intervene in both the body and mind, such as meditation, have been shown to reduce both insomnia and

inflammation, with effects on depression [21]. It is possible that inflammation and sleep increase together and decrease together without any of them preceding the other.

1.5 Summary of knowledge gaps

There is limited research and inconsistent findings on associations between inflammatory markers and psychological distress in healthy adolescents. Likewise, there is a scarcity of research on associations between sleep duration and psychological distress in this age-group. Therefore, there is a need for more research on associations between 1) inflammatory markers and 2) sleep duration and psychological distress in healthy adolescents.

It is important to explore risk factors for psychological distress during adolescence because psychological distress and mental disorders are increasing problems worldwide, and the onset is typically during adolescence. By exploring inflammation and sleep duration as risk factors for psychological distress during adolescence, knowledge relevant for the prevention of psychological distress and mental disorders is extended.

1.6 Aims

The aims of this thesis are to explore whether inflammation and short sleep duration are risk factors for psychological distress during adolescence, with data from the Fit Futures study. The main study aims are as follows:

1) Examine cross-sectional associations between inflammatory markers and psychological distress in adolescent girls and boys.

2) Examine whether five inflammatory markers at baseline are associated with psychological distress at follow-up two years later, separately for girls and boys.

3) Explore associations between changes in sleep duration and changes in psychological distress in adolescent girls and boys.

2 Materials and methods

2.1 The Fit Futures study

This thesis and the included papers are based on the Fit Futures study, which is an extensive study on adolescent health. The participants were from Tromsø and Balsfjord, two municipalities in Northern Norway. Data were collected at two time points (2010-2011 and 2012-2013). The study was conducted in collaboration between UiT – The Arctic University of Norway, Norwegian Institute of Public Health, and the University Hospital of Northern Norway (UNN) [140].

In Norway, all adolescents who finished primary and lower secondary school have the right to three years of upper secondary education. At baseline (2010-2011) all first level upper secondary school students in the two neighbouring municipalities, Tromsø and Balsfjord, were invited to participate in the study. Data was collected from seven schools in Tromsø, which can be considered an urban area, and one school in Balsfjord, which can be considered a rural area. A total of 1,117 students were invited, of which 1,038 participated. Follow-up was conducted two years later (2012-2013), where every attendant from baseline and all students in the third level of the same upper secondary schools were invited to participate. A total of 1,129 were invited, and 870 participated, of which 694 (67%) had participated at baseline. Most of the participants were 15-17 years at baseline, and 17-19 years at follow-up.

Figure 3. Inflammatory markers were measured by blood tests

Praisaeng / mostphotos.com

The participants were driven to the research site at UNN during school hours, and the data collection took approximately 3 hours. Data about lifestyle, health and disease were collected by web-based questionnaires. Qualified research nurses conducted clinical examinations, collected blood samples and did interviews on the use of contraceptives and presence of acute and/or chronic diseases [140]. We applied for and were approved access to data on psychological distress, inflammatory markers, and sleep duration. Additionally, we were given access to data about lifestyle variables, health, disease, contraceptives, medication intake, and serum vitamin D levels.

2.1.1 Compliance with ethical guidelines

All participants provided informed consent. Participants aged 16 years or older gave a written informed consent to participate, and for participants under 16 years a consent from a parent/guardian was given. The Fit Futures study was conducted in accordance with the Declaration of Helsinki and was approved by the Norwegian Data Protection Authority (reference number 2009/1282). The Regional Committee of Medical and Health Research Ethics approved the Fit Futures study (reference number 2011/1702/REK Nord) and the present project (reference number: 2019/60811/REK Nord). Further, the present project was evaluated by the Norwegian Centre for Research Data (reference number 2021/934242).

2.2 Measurements

2.2.1 Hopkins symptoms checklist – 10 (HSCL-10)

Psychological distress was self-reported in the web-based questionnaires at both time-points by HSCL-10. HSCL-10 is not a measure of a clinical diagnosis but rather psychological distress (emotional suffering characterised by symptoms of depression and anxiety). The scale measures symptoms of anxiety and depression over the past week [141]. HSCL-10 has been found to correlate as high as 0.97 with a longer version of Hopkins Symptom Checklist (HSCL-25). HSCL-25 consists of 25 items measuring psychological distress (10 items measure anxiety symptoms, 15 items measure depressive symptoms) and has been shown to be a valid and reliable measure of psychological distress [141-143]. The high correlation between HSCL-10 and HSCL-25 indicates that HSCL-10 is a valid measure of psychological distress. The reliability of HSCL-10 has been shown to be high, with a Cronbach's alpha of 0.88 [141]. HSCL-10 is an economical measure of psychological distress in extensive studies

that includes several other questionnaires [143]. The ten items from HSCL-10 are shown in Table 1.

Table 1. The ten items from HSCL-10

Item	Answer options
Have you experienced sudden fear without apparent reason during the last week?	□ None □ Slightly □ Much □ Very much
Have you felt afraid or worried during the last week?	□ None □ Slightly □ Much □ Very much
Have you experienced faintness or dizziness during the last week?	□ None □ Slightly □ Much □ Very much
Have you been tense or upset during the last week?	□ None □ Slightly □ Much □ Very much
Have you easily blamed yourself during the last week?	□ None □ Slightly □ Much □ Very much
Have you experienced sleeplessness during the last week?	□ None □ Slightly □ Much □ Very much
Have you felt depressed or sad during the last week?	□ None □ Slightly □ Much □ Very much
Have you felt useless, worthless during the last week?	□ None □ Slightly □ Much □ Very much

Have you felt that life is a struggle during the last week?	□ None □ Slightly □ Much □ Very much
Have you felt hopelessness with regard to the future during the last week?	□ None □ Slightly □ Much □ Very much

The first four items of HSCL-10 measure anxiety symptoms, and the last six items measure depressive symptoms. The items are answered on a four-point Likert Scale, where response categories are scored from 1 to 4 where the lowest, "none", is scored as 1 and the highest "very much" is scored as 4. The scores of the 10 items are averaged to get a HSCL-10 score ranging from 1 to 4. In adolescents, an average score of 1.85 on HSCL-10 is used as a cut-off, with the interpretation that a score of 1.85 or higher indicates psychological distress [141]. The 1.85 cut-off has shown a sensitivity of 0.89 and a specificity of 0.98 when using HSCL-25 with a 1.75 cut-off as criterion [141].

All papers included in this thesis have HSCL-10 as outcome, however with different coding. In paper 1, a dichotomised version was used with a cut-off of 1.85. In paper 2, a continuous HSCL-10 score from follow-up was used, while in paper 3, a change score of HSCL-10 (follow-up – baseline) was used.

2.2.2 Inflammatory markers

At baseline, our participants provided non-fasting blood samples, obtained from the antecubital vein in the University Hospital of Northern Norway. Serum was transported to Supelco glass vials (Sigma-Aldrich Norway AS, Oslo, Norway) by Pasteur glass pipettes. Sera was separated and stored at -70°C and thawed one time for biomarker analyses. High-sensitive C-reactive protein (CRP) was analysed at UNN, Norway and assessed by a particle-enhanced immunoturbidimetric assay on a Modular P autoanalyser (Roche Diagnostics, Mannheim, Germany) with a detection limit of 0.12 mg/L (high-sensitive). Proximity extension assay (PEA) technology (Olink® Target 96 Inflammation panel; Olink Proteomics, Uppsala, Sweden) was applied for the relative quantification of proteins related to inflammation in serum samples from baseline. Further details concerning the analysis are

presented elsewhere [96]. CRP, IL-6, TGF-α, TRANCE, and TWEAK served as exposures in paper 1 and 2, and as potential confounders in paper 3.

2.2.3 Sleep duration

Sleep duration was measured at both time points by one question: "How many hours sleep do you normally get per night?" The response categories started with "4 hours or less" and increased by 30 minutes per category ("4.5 hours", "5 hours", "5.5 hours", etc.), and ended with "12 hours or more". We coded the lowest category as 4 hours, and the highest as 12 hours. Sleep duration from baseline served as a potential confounder and was examined as a moderator in paper 1 and 2. In paper 3, the change score of sleep duration (follow-up – baseline) served as exposure. Further in paper 3, we created categorical variables at baseline and follow-up to describe short and long sleep duration with the following three categories "≤6 hours", ">6 to 9 hours" and ">9 hours".

2.2.4 Covariates

The web-based questionnaire incorporated data about lifestyle, health, and disease, that were considered as potential confounders. Further, the participants were interviewed by qualified research nurses regarding use of contraceptives and presence of acute and chronic diseases. Data from these interviews were also considered as potential confounders. More details about assessment and adjustment for potential confounders are described in the papers.

2.3 Statistical analyses

Statistical Package of Social Science (SPSS) versions 26 and 28 were used for all analyses, and two-sided p-values <0.05 were chosen as an indication of statistical significance. All analyses were gender stratified. Descriptive statistics of the cohort were presented as mean (SD) or frequency (*n* and %) for most variables, and median (IQR) for variables with skewness. Group differences were examined using independent samples t-test, Paired samples t-test, Fisher's exact test, Pearson's Chi-squared test, ANOVA and Mann Whitney U test. Residual analyses were conducted to assess linearity, distribution, variance homogeneity and explore outliers. Regression analyses were used in all three papers. In all papers, the regression analyses were gender stratified based on previous research [90, 144]. Potential confounders were assessed in simple regressions. After testing crude associations, relevant and available potential confounders were added in multiple regressions. Testing for interactions was limited to physical activity levels, body fat percentage and sleep duration.

When participants had missing values in exposure, outcome, or potential confounders, they were excluded from the analyses.

In paper 1, we used binary logistic regression to estimate the odds ratio (OR) and 95% confidence intervals (CIs) between quartiles of the five respective inflammatory markers and psychological distress. This was a cross-sectional study with data from baseline. The clinical cut-off of 1.85 on HSCL-10 was used to indicate psychological distress [141]. To make the results more robust, we adjusted for potential confounders by forward stepwise logistic regressions. Interaction was tested between quartiles of inflammatory markers and physical activity levels, body fat percentage and sleep duration. Significant interactions were found in boys but could not be included in the adjusted analysis because there were too few events. In supplementary analyses, we conducted forward logistic regressions with a dichotomous version of the six items from HSCL-10 that measure depressive symptoms as outcome. This was done to examine associations between the respective inflammatory markers and psychological distress without confounding from symptoms of anxiety. Further supplementary analyses were done by doing logistic regression with continuous inflammatory markers as exposure for both the respective outcomes (dichotomous HSCL-10 and a dichotomous version of the six items measuring depressive symptoms). As a final supplementary analysis, we did linear regressions with mean HSCL-10 as outcome.

In paper 2, we used linear regressions to estimate the unstandardised beta regression coefficients and 95% CI between the five respective inflammatory markers at baseline and psychological distress at follow-up (the latter serving as outcome variable). After estimating crude associations (Model 1), adjustment for psychological distress at baseline was conducted (Model 2). Subsequently, potential confounders were added to the model (Model 3). Lastly, interaction terms were tested in simple linear regressions and added to Model 3 when the p-value < 0.05 and significant interaction-terms were further investigated in stratified analyses. In supplementary analyses, linear regressions with the mean score of the six items of depressive symptoms from HSCL-10 at follow-up were used as outcome. This was done to assess prospective associations between the respective inflammatory markers and depressive symptoms without the confounding from the anxiety items.

In paper 3, we used linear regression to estimate the unstandardised beta regression coefficients and 95% CI between sleep duration and HSCL-10 at both time points. Further, prospective associations between changes in sleep duration and changes in HSCL-10 were

estimated. In the prospective analyses, crude associations between the change score of sleep duration and change score of HSCL-10 were first estimated. Secondly, we adjusted for potential confounders. When variables were available from both time-points, we used the change scores (FF2-FF1), and for variables without data from FF2, we used variables from FF1. We chose to adjust for the change scores of the potential confounders because they fluctuate over time. Because the item "Have you experienced sleeplessness during the last week?" is included in HSCL-10, we did supplementary analyses without the item to avoid our findings being confounded by this item. We calculated the mean of the remaining 9 items and used this mean as the outcome in cross-sectional analyses at baseline and follow-up, respectively. Further, in supplementary analyses, we calculated the change score for HSCL-10 without the sleep item and used it as outcome to explore associations between changes in sleep duration and HSCL-10 without the sleep item. Finally, we did supplementary analyses where we adjusted for the baseline values of potential confounders, instead of adjusting for change scores of the potential confounders in the prospective analysis of associations between change in sleep duration and change in psychological distress.

3 Results - summary of papers

3.1 Paper 1

Inflammatory markers have been associated with depression and anxiety disorder in both adults and adolescents. In adults, there is also evidence for associations between inflammatory markers and subclinical symptoms in the form of psychological distress. Less is known about the association between inflammation and psychological distress during adolescence. The aims of paper 1 were to 1) describe the prevalence of psychological distress in adolescent girls and 2) examine the associations between inflammatory markers and psychological distress in adolescent girls and boys.

The mean and median values of HSCL-10 were higher in girls compared to boys. Girls reported a mean HSCL-10 (SD) of 1.63 (0.59) and boys 1.35 (0.41). Corresponding median (IQR) in girls and boys were 1.40 (0.70), and 1.20 (2.20), respectively.

Further, a higher percentage of girls scored above the clinical cut-off compared to boys. In total, 26.9% of the girls and 10.8% of the boys scored above the 1.85 cut-off of HSCL-10 at baseline. The prevalence of psychological distress was significantly higher in girls compared to boys, χ^2 (1, N = 931) = 39.6, $p < 0.01$. No significant associations were found between quartiles of inflammatory markers and HSCL-10 in crude or adjusted analyses.

We concluded that, according to this study, the prevalence of psychological distress is higher in girls than boys during adolescence. The prevalence we found corresponds with previous studies in this age-group. We found no support for cross-sectional associations between inflammatory markers and psychological distress in healthy adolescents. Our recommendation was to conduct studies to examine prospective associations between inflammatory markers and psychological distress.

3.2 Paper 2

Inflammatory markers have been associated with psychological distress in adults. In adolescents, there is a scarcity of research on associations between inflammatory markers and psychological distress. The studies conducted have yielded mixed results, and there has been a lack on adjustment for potential confounders. The literature has indicated a need for gender-stratified analyses. Therefore, we aimed to examine whether five respective inflammatory markers at baseline were associated with prevalence of psychological distress at follow-up two years later, separately for girls and boys.

The results showed no significant prospective associations between inflammatory markers and HSCL-10 in girls in crude or adjusted analyses. In boys, CRP and TGF-α at baseline showed significant associations with HSCL-10 in crude and adjusted analyses. In addition, we found moderators in boys. CRP was associated with HSCL-10 in those with high body fat percentage and those physically inactive, and the association between TWEAK and HSCL-10 was dependent upon sleep duration. Surprisingly, the association was stronger in boys sleeping ≥ 7 hours compared to boys sleeping < 7 hours.

In conclusion, our study indicated that increased levels of CRP and TGF-α at baseline were associated with increased levels of psychological distress at follow-up two years later in adolescent boys. Our results further suggest that boys who were physically inactive and/ or had a higher body fat percentage were more vulnerable to higher CRP levels, and surprisingly, boys who slept longer were more vulnerable to higher TWEAK levels. Future studies examining associations between inflammatory markers and psychological distress in adolescents should consider the moderating roles of body fat percentage, physical activity, and sleep duration.

3.3 Paper 3

Depressive disorders, anxiety disorders and psychological distress increase dramatically during adolescence, particularly among girls. Therefore, it is central to explore potential predictors of psychological distress during this age. Studies have indicated an association between sleep duration and psychological distress. Hence, our aim was to explore associations between changes in sleep duration and changes in psychological distress in adolescent girls and boys.

In girls and boys, sleep duration was approximately 7 hours at both time-points. About 25% of girls and boys reported a sleep duration ≤ 6 hours. At both time-points, cross-sectional associations showed that a 30-minute increase in sleep duration (one unit) was associated with a significantly lower level of HSCL-10 in girls and boys. Prospectively, an increase of 30 minutes in sleep duration was associated with a significant decrease in HSCL-10 score from baseline to follow-up in girls and boys. These prospective associations were significant in both crude and adjusted analyses.

We concluded that short sleep duration was highly prevalent in healthy adolescents. Increased sleep duration from baseline to follow-up two years later was associated with decreased

psychological distress from baseline to follow-up. Due to inconsistent findings in the field, more studies examining prospective associations between sleep duration and psychological distress during adolescence are warranted.

4 Discussion

4.1 Methodological considerations

4.1.1 Study design

Observational studies are those that observe without intervening and utilise the natural variation in exposures that occur in populations [145]. The three papers in this thesis are all observational studies.

The design of paper 1 was cross-sectional. A cross-sectional design measures exposure and outcome at the same time-point [146]. The cross-sectional design is suitable for hypothesis-generation, yet it cannot provide evidence on causality because the time order of cause and effect cannot be resolved [147]. An additional advantage with the cross-sectional design is that it provides the opportunity to measure prevalence, as we did with psychological distress in paper 1 [148], and further cross-sectional studies can be done relatively quickly; moreover, they have low costs and are relatively simple to administer [149]. The design of paper 2 and 3 was longitudinal. The longitudinal study design provides the opportunity to examine the development of health over time [150]. In paper 2, we examined the time order between inflammation at baseline as a potential cause and psychological distress at follow up as an effect. In paper 3, we examined whether sleep duration and psychological distress changed together from baseline to follow-up.

Neither longitudinal observational studies can provide evidence on causality [151]. Experimental studies, on the other hand, allow for control over the exposure by assigning it to a random sample of the participants and are therefore more suited to study causality than observational studies [145]. However, experimental studies often take place in an artificial setting and/or restrict the participants included and are thus less generalisable than observational studies [152].

4.1.2 Internal validity

Internal validity is about how certain we can be about the association between the exposure and the outcome [153]. Statistically significant associations between exposure and outcome may be true, or alternatively be explained by bias (systematic errors), chance or confounders [148].

4.1.3 Selection bias

Selection bias is a systematic error related to how the participants were selected, and from other factors that may have influenced study participation [154]. The question about potential selection bias is whether the participants were systematically different from those who did not attend the study, with respect to inflammatory markers, sleep duration, psychological distress, and potential confounders. Differences on those characteristics could potentially lead to different associations between exposure and outcome between participants and non-participants [154]. For example, because those with high levels of psychological distress may have been more inclined to not participate, non-attendants might have had higher levels of psychological distress compared to those who attended. Thus, it may be that the association between the exposures and outcome was stronger in non-participants compared to participants. This is a potential threat to the internal validity, because our participants would not be representative of the population.

However, in cohort studies, when the participation rate is above 80%, there is a low chance of a substantial selection bias [155]. At the baseline of the Fit Futures study, all participants were students in first-year upper secondary school. Of the 1,301 individuals registered in school, 70 were missing. This may be due to drop-out from school before data collection from Fit Futures at baseline. Further, 114 students did not attend school because of persistent disease, or because they did not respond when contacted. Of the remaining 1,117 students that were invited, 1038 participated at baseline in the Fit Futures study (92.9% attendance rate). Those 70 dropping out of school, and those 114 that did not attend school because of persistent disease or did not respond when contacted, may in theory have had higher levels of psychological distress, inflammation, and shorter sleep duration compared to the participants. Nonetheless, Fit Futures had an attendance rate as high as 92.9%, thus selection bias would probably be of minor consequence to our results.

In paper 1, 30 participants were excluded because of missing values on HSCL-10 at baseline, and in paper 2 and 3, respectively, 16 participants were excluded because of missing values on HSCL-10 at either baseline or follow-up. Theoretically, those with missing values may have had higher levels of psychological distress compared to participants with complete data. Thus, the associations between exposures and outcome may be different between participants and non-participants. However, only 2-3% of participants were excluded because of missing values on HSCL-10 in the respective studies. Thus, exclusion of participants probably had

minor effects on selection bias. In sum, there is a low probability that selection bias influenced our results.

4.1.4 Self-reporting of HSCL-10

Reporting bias can be defined as participants reporting incorrect information, either consciously or unconsciously [153]. HSCL-10 was self-reported in the web-based questionnaire. Thus, reporting bias may have occurred. It is possible that some participants consciously reported incorrect information to give a more favourable impression of themselves. This is known as social-desirability bias [156], which can be viewed as a sub-category of reporting bias. In this way, some participants may have underreported symptoms of anxiety and depression. This is a systematic error than may have occurred.

Additionally, participants may have reported incorrect or imprecise information by having difficulties interpreting the response categories and choosing the appropriate one. This kind of incorrect information reported is unsystematic (random) and would not matter because we had relatively large sample sizes. Following the central limit theorem, large sample-sizes decrease the effects of random error [157].

Overall, even though reporting bias may have occurred to a certain extent, HSCL-10 has been shown to have good validity [141]. Thus, substantial reporting bias of psychological distress is unlikely.

4.1.5 Self-reporting of sleep duration

Like HSCL-10, sleep duration was self-reported in the web-based questionnaire. Sleep duration was self-reported by one question: “How many hours sleep do you normally get per night?” A similar item from National Health and Nutritional Survey, “How much sleep do you usually get at night on weekdays or workdays (hours)?”, was recently validated against a wrist worn accelerometer (actigraphy) by Lee [158]. Actigraphy has previously been validated by comparing it to polysomnography, the gold standard for sleep assessment [159, 160]. In the study by Lee, participants over-reported their sleep duration from 0.72 hours to 1.13 hours depending on sub-group. Further, the correlation between self-reported sleep duration from the single-item and the objective measure from the accelerometer was low (ranging from 0.02 to 0.19 based on subgroup) [158]. When the self-report of sleep duration by a single item like the one we used correlates weakly to actigraphy, the validity of our measure of sleep duration should be questioned.

When aiming to measure sleep duration by self-report in children and adolescents, it has previously been recommended to use more than one item [161]. Better validity for sleep duration measured by questionnaires compared to single items has been supported by the following validation studies. The correlation between self-reported sleep duration measured by a questionnaire and actigraphy has been reported to be moderate in children and adolescents [162]. A meta-analysis on children and adolescents reported a strong correlation between questionnaires and actigraphy for weeknights and moderate correlation for weekend nights [163].

In contrast to objective measures of sleep duration such as actigraphy and polysomnography, self-reported sleep duration is subjective and retrospective and is thus potentially less precise. In contrast to the over-reporting found in the mentioned validation study [158], some participants in our study may have subjectively felt that their sleep was poor and exaggerated how little they sleep. It may also be difficult for participants to quantify exactly when sleep onset occurred and the total duration of waking periods during the night. Additionally, there may be recall bias, as our participants may not have paid attention to and remembered their average sleep duration.

Another weakness with the item we used is that it does not discriminate between sleep duration on weekdays and weekends, respectively. Thus, we do not know whether our participants reported average sleep duration for weekdays, weekends, or a weekly average. These differences are important because it has been shown that adolescents sleep 1-2 hours longer on weekends compared to weekdays [113]. Further, we did not have data on whether the participants slept during daytime (napping). Adolescents with short sleep duration during the night have been reported to nap during the day [164].

Ideally, we should have used objective measures of sleep duration. However, polysomnography requires a controlled environment, in a clinical laboratory condition [163]. Therefore, wrist actigraphy has been called the gold standard in a free-living context [163]. Unfortunately, the logistics for this technology is high, and we did not have data from such measurements available in the Fit Futures study. With findings showing moderate correlations between wrist actigraphy and self-reported sleep duration in questionnaires, self-reported sleep duration by questionnaires seems to be a feasible strategy. As mentioned, the low correlation between actigraphy and sleep duration measured with one item warrants questioning of the validity of our measure of sleep duration. However, other studies [18, 165]

have used the following single item from the Pittsburgh Sleep Quality Index to measure sleep duration: “During the past month, on average, how many hours of actual sleep did you get per night?” This item is comparable to the item we used: “How many hours sleep do you normally get per night?”.

In further support of the validity of our measurement of sleep duration, our finding that participants slept approximately 7 hours per night corresponds to findings in a review and meta-analysis on adolescents from Europe, America and Asian countries [114]. In this study, sleep duration was measured by actigraphy and questionnaires. Our finding on sleep duration also supports a study from Norway, who reported a sleep duration of 6 hours and 43 minutes on school nights [166]. In this study, sleep duration was measured by questionnaire. Our finding that approximately 25% of the participants slept ≤ 6 hours per night support a study from America where 20% of 11–17-year-olds slept ≤ 6 hours per night [167]. In this study, sleep duration was measured by interviews. The slightly higher percentage that slept ≤ 6 hours in our study may be explained by the fact that our participants were older. Overall, our descriptive findings regarding sleep duration corresponds to measures done by questionnaires, actigraphy and interviews. This correspondence with other measures may suggest that our measure of sleep duration is valid.

In sum, there are reasons for questioning the validity of self-reported sleep duration. Self-report of sleep duration is inferior to objective measures of sleep duration, and self-report of sleep duration by one item is inferior to self-report by a questionnaire. The validity of sleep duration is especially important for paper 3, where sleep duration was the main exposure, whereas in paper 1 and 2 sleep duration served as confounder-and effect-modifier. Nonetheless, other studies have used a similar item to the one we used as the main exposure. Additionally, our descriptive findings regarding sleep duration correspond to similar populations of healthy adolescents. Further, our aim in paper 3 was merely to explore (not examine) associations between sleep duration and psychological distress. Based on our significant findings, future studies should use more valid measures of sleep duration to examine the association with psychological distress further. Ideally, sleep actigraphy should be used for the best validity. Alternatively, sleep diary should be considered as it has been shown to have better validity than sleep questionnaires [158].

4.1.6 Chance

By setting the p-value < 0.05 and 95% CI in the statistical analyses, there is a low probability that the associations occurred by chance. However, this could not be completely ruled out. With a p-value of < 0.05, the association between exposure and outcome would occur by chance five times out of a hundred. The probability of the associations occurred by chance can also be expressed by CI. By setting the CI at 95% it will contain the actual population value in 95% of samples. Thus, the 95% CI correspond to p-values of < 0.05. However, CI additionally shows the largest and smallest effects that are probable [168].

4.1.7 Confounding

Confounding variables that are measured can be accounted for. Residual confounding is confounding from variables that have not been measured and thus cannot be adjusted for [169]. Confounders are variables that are associated with both the exposure and outcome, and not in the causal pathway between them [149]. In this way, confounders distort the association between exposure and outcome [149]. In other words, by adjusting for confounders, the effect from exposure on the outcome may be more precise [170].

There are several strategies to control confounding. For example, in experiments, participants are randomly assigned to the research group and control group, thus the confounding factors will be equal in both groups. A disadvantage with observational studies is that it is impossible to control for all other factors (third variables). Our strategies to control for cofounders were stratification and adjustment for potential confounders. Because previous studies have showed different associations between our exposures and outcome dependent on gender, we performed all regressions stratified by gender. Additional stratification for other confounders was not possible because of sample size. Further stratification would have led to small subgroups with insufficient statistical power [171]. Potential confounders were selected based on literature and assessment by simple regressions. Even though we had access to several potential confounders from the Fit Futures study, we cannot rule out residual confounding. Nonetheless, we were able to adjust for several confounders in all three papers. In paper 1, we found no crude associations. Therefore, we conducted forward stepwise regressions to explore whether the associations were suppressed by confounders. After adjustment in this manner, all associations remained non-significant, making our results more robust. In paper 2 and paper 3, all associations that were statistically significant in crude analyses remained significant after adjustment of potential confounders.

4.1.8 External validity (generalisability)

External validity relates to the degree to which findings can be generalised from the sample (study population) to other populations and measures [172]. The question about external validity is about the extent to which the results from a study can be applied in a broader context.

External validity is dependent upon internal validity. In other words, if there is uncertainty about the association between the exposure and outcome, this association cannot be generalised to other populations [172]. In the discussion of internal validity above, we could not exclude the possibility that different biases threatened internal validity. However, my interpretation is that there were no major biases that clearly distort the associations.

Our sample from Northern Norway may be systematically different from adolescents living in other places in Norway and other countries. For example, it may be that the sun-conditions in Northern Norway (with little sun exposure during autumn and winter, and very much sun exposure during spring and summer) affect sleep duration and psychological distress. A study on the general population of Tromsø showed increased insomnia problems and fatigue during the winter compared to summer [173]. However, the seasonal variation in insomnia was only weak to moderate. On the other hand, another large population study in Norway found no seasonal variation in either insomnia or time in bed, despite seasonal variations in daylight from 4 to 21 hours [174]. Thus, the special daylight conditions in Northern Norway are probably not a threat to external validity.

Our participants were mostly between 15 and 19 years old. Thus, our results may not be generalisable to younger adolescents who in general may show lower levels of psychological distress, as previous literature has shown that depressive symptoms increase with age during adolescence [175, 176].

The data used in the three papers were collected from 2010 to 2013. Since then, the use of smartphones has increased, and this use has been associated with mental health problems and sleep deficiencies in adolescents [177, 178]. This development of smartphone-use and its associations with mental health and sleep in adolescents is a threat to the external validity of our studies. For example, it is possible that adolescents sleep less today compared to in 2010-2013 because of the increased use of smartphones. However, a study with data collected in 2019 from Norwegian adolescents found a similar sleep duration as we did in our study [166],

indicating that sleep duration has not changed since our data was collected. Thus, it is unlikely that the development in smartphone use is a major threat to external validity.

The external validity of the inflammatory markers in our study is probably decent as physiological measures tend to have high external validity [172].

Overall, there are some minor threats to external validity in the three papers, but these minor threats do not make generalisation unreasonable.

4.1.9 Causality

David Hume characterised a causal relationship in the 18th century. Hume suggested several rules to infer cause and effect. Some of the most important rules were as follows: 1) The cause and effect occur close together in time, known as contiguity; 2) the cause has to occur before the effect; and 3) the effect should never occur without the presence of the cause [179]. Later, several researchers have described other criteria for deciding whether an association is causal. John Stuart Mill developed "Mills canons" in the 19th century, which consists of five canons [180]. More recently, Bhopal suggested guidelines for epidemiological reasoning on cause and effect [148]. According to Bhopal, these guidelines are especially useful for revealing a lack of causality. The guidelines from Bhopal are the following: 1) Temporality, 2) Strength and dose-response, 3) Specificity, 4) Consistency, 5) Experiment, and 6) Biological plausibility. The use of criteria and guidelines for establishing causality have, however, been criticised as too vague and not suitable to all associations [148, 154, 181]. Perhaps such criteria and guidelines are best suited to reveal a lack of causality, as Bhopal suggested [148]. As Bhopal asserts, caution is warranted before concluding about causality, and conclusions about causality should not be finite [148].

As mentioned, psychological distress is influenced by many different factors, including biological, social and psychological factors [56]. Because factors may individually and/or synergistically cause psychological distress, it is difficult to determine individual causal factors. We found significant prospective associations between low-grade inflammation and sleep duration respectively and psychological distress. The prospective findings are in line with the guideline of temporality and biological plausibility. The latter will be discussed under main findings. However, we cannot conclude about causality based on these findings from our observational studies. According to Bhopal [148], most association observed in epidemiological studies are not causal.

As discussed under the internal validity section, we cannot rule out that bias, chance, and confounding may have affected the significant associations found in the studies. Even though it is unlikely, we cannot be sure that our results have been affected by selection bias. There is also a risk of reporting bias for HSCL-10; however, the scale has been shown to have good validity. The measure of the inflammatory markers is objective and probably also has a decent validity. However, sleep duration was only measured with one item, and there is a risk for low validity for our measure of sleep duration. Perhaps the most important reason for why we cannot infer causality is confounding. Even though we adjusted for potential confounders, we cannot rule out third variables (residual confounding) as an explanation for the associations. For example, there may be health variables that we did not measure that influenced both inflammatory markers and psychological distress, and there may be psychological variables that influenced both sleep duration and psychological distress. Neither change-scores can be used to conclude about causality [182]. Even though changes in sleep duration were associated with changes in psychological distress in paper 3, this does not imply causation because it is possible that third variables influenced both changes in sleep duration and changes in psychological distress.

In sum, causality should be interpreted with caution. Based on Bhopal's guidelines for epidemiological reasoning on cause on effect, there do not seem to be any obvious reasons to dismiss causality. However, there are uncertainties related to the associations found because of potential bias and residual confounding. Associations/correlations may in themselves point to causality, but associations are in themselves not enough to conclude about causality. Other methods, such as experiments and clinical trials are more suited to examine causality compared to the observational methods conducted in the papers included in this thesis.

4.2 Main findings

In this section, our main findings, clinical relevance, possible explanations, and consistency with other studies will be discussed. Overall, the aims of the thesis are to examine low-grade inflammation and sleep duration as risk factors for psychological distress during adolescence. Paper 1 and 2 examined cross-sectional and prospective associations between inflammatory markers and psychological distress, while paper 3 explored prospective associations between changes in sleep duration and changes in psychological distress.

4.2.1 Strength of associations and clinical relevance

Findings within psychological research have traditionally been evaluated by statistical significance. More recently, there has been an increased focus on effect size [183]. Effect size is a measure of the strength of an association, and even though a finding is highly significant, the corresponding effect size may be small [184]. The p-value, which is a measure of statistical significance, gives information about the probability that the association occurred by chance. The effect-size, however, is a measure of clinical relevance [185]. Thus, the clinical relevance of the significant associations in the three papers should be discussed. This discussion concerns the practical importance of our significant findings.

The associations we found in the three papers were not strong, as indicated by low and non-significant odds ratios in paper 1 and small beta coefficients in paper 2 and 3. This indicates small effect-sizes. However, effect-sizes are dependent upon the research field. The three papers may be considered as psychological research, a research field where small effect-sizes are common and expected [184]. Psychology is inherently complex, and the studied variables share their predictive validity with a plethora of non-measured variables [184]. Thus, small effect-sizes from exposures predicting psychological distress are expected. Regarding effect sizes from inflammatory markers on psychological distress, weak associations/effect sizes were additionally expected because inflammatory markers are generally more strongly associated with clinical depression and severe depressive symptoms compared to lower levels of depressive symptoms [73].

In paper 2 and 3, we examined prospective associations between exposures and outcome over two years. The weak effects over this time frame may, however, be important in the long run [184]. It is possible that low-grade inflammation will have a cumulative effect on psychological distress when inflammation is sustained for a time longer than two years. Similarly, it is possible that the cumulative effects from short sleep duration could lead to further increases in psychological distress in the long run. Even if the effect from the exposures themselves does not accumulate over time, it is possible it will affect other factors (mediators) that in the next step have an effect on the outcome [184]. For example, inflammation may increase fatigue [186], which in the next step will increase psychological distress [187]. Similarly, the effect from short sleep duration may, for example, affect self-control negatively [188], which will further increase psychological distress [189]. In sum, our small effect sizes may have potential consequences in the long run and should therefore not be

dismissed as irrelevant and without clinical significance. Additionally, small effect sizes are expected and common in psychological research.

4.2.2 Biological plausibility

In the introduction, biological mechanisms explaining associations between 1) low-grade inflammation and 2) sleep duration and psychological distress were presented. In short, inflammatory proteins access the brain, where they affect regions associated with emotion and depression. Sleep has regulative function that affects health, including regions in the brain regulating emotion. Thus, lack of sleep may result in emotional disturbances in the form of depressive symptoms and psychological distress. Our results cannot confirm or refute these biological mechanisms. However, these mechanisms offer explanations for our observed associations. Biological plausibility is one of Bhopal's guidelines for reasoning about cause and effect in epidemiological studies [148]. Thus, the biological mechanisms presented in the introduction may be indicative of causal mechanisms.

In paper 1, we found no significant cross-sectional associations between inflammatory markers and psychological distress. A disadvantage with the cross-sectional design in this paper is a lack of knowledge about for how long the inflammatory markers had been elevated. It has been suggested that an inflammatory state must endure over a longer period to influence the brain and its circuits associated with emotion, which in turn influence depressive symptoms [63, 79, 89, 190]. Thus, it is possible that inflammatory proteins need to affect regions of the brain that regulate emotion for a certain duration before resulting in increased psychological distress. Empirical evidence supports that inflammation needs to be sustained for a certain time before affecting mental health. The strongest associations between inflammatory markers and depressive symptoms in healthy adolescents have been reported in prospective studies where time to follow up is 13 months or longer [90]. In paper 2, we found prospective associations between inflammatory markers (CRP and TGF-α) and psychological distress in boys. The reason we found prospective associations in paper 2, and no cross-sectional associations in paper 1, may be that the inflammatory markers had been elevated for a longer time, and thus influenced levels of psychological distress more compared to the cross-sectional associations in paper 1, where inflammatory markers and psychological distress were measured at the same time-point.

Studies on healthy adults have found cross-sectional associations between inflammatory markers and psychological distress. The discrepancy in results from these studies on adults

and our null-findings may be explained by consistent findings showing that adolescents in general have lower levels of inflammation compared to adults [78]. Thus, it is more difficult to detect associations between inflammatory markers and psychological distress in adolescents compared to adults. Another explanation is that the inflammation levels in healthy adolescents are too low in healthy adolescents to influence or be associated with psychological distress cross-sectionally.

We found evidence indicating effect-modification in paper 1 and 2. The significant interactions in paper 1 in boys (between sleep duration and CRP and TGF-α, respectively) could not be further examined because of too few events. In paper 2, our findings showed that boys that were sedentary and/or had a high body fat percentage (≥ 25%) were more vulnerable to the effects of CRP compared to boys that were physically active and/or had a low body fat percentage (< 25%). It seems reasonable that physical activity and a healthy body fat percentage protect against the adverse effects from increased inflammation on psychological distress. Physical activity has been shown to be cross-sectionally and prospectively associated with better mental health in adolescents [191, 192]. Further, obesity has been shown to associate with higher levels of psychological distress in adolescents [193]. Further, there is evidence showing that both higher body fat percentage and low levels of physical activity are associated with increased systemic inflammation in adolescence [194, 195]. A possible mechanism is that low body fat percentage and physical activity defend against the effects of CRP on psychological distress in the long run, in adolescent boys. In this sense, body fat percentage and physical activity moderates the effect of CRP on psychological distress. Alternatively, high body fat percentage and physical inactivity increase inflammatory levels, which in the next step increase psychological distress. In the latter alternative, the associations between high body fat percentage/physical activity and psychological distress are mediated by inflammation.

Additionally, in boys, effect-modification indicated that the association between TWEAK and psychological distress was dependent upon sleep duration. However, this was not in the direction we expected. An increase in TWEAK predicted increased psychological distress in boys with a long sleep duration (slept ≥ 7 hours). To my knowledge, there is no biological explanation for this. This finding further contrasts a study by Cho et al. that showed stronger associations between inflammatory markers and depressed mood in young females with sleep disturbance compared to controls [137]. In the same study, there was no moderating effect from sleep disturbance on the association between inflammatory markers and depressed mood

in young males. It is possible that our finding indicating effect-modification by sleep duration occurred by chance. Another possible explanation is that the cut-off of 7 hours in sleep duration in paper 2 (based on median sleep duration in our sample) is below the recommended sleep duration of 8-10 hours in adolescents. Thus, it is possible that many of the boys in our sample with sleep duration above the cut-off of 7 hours still needed more sleep. Thus, it is possible that effect-modification from sleep duration on the association between TWEAK and psychological distress in boys would not have been significant with a higher cut-off for sleep duration.

In paper 3, we found significant associations between changes in sleep duration and changes in psychological distress. The associations were in the expected direction. Increased sleep duration was associated with decreased psychological distress, and vice versa decreased sleep duration was associated with increased psychological distress. These associations are in line with recommendations of sleep duration during adolescence. When adolescents sleep less than the recommended duration of 8 to 10 hours, it can be viewed as a sleep disturbance, and may thus adversely influence psychological distress. It is further plausible that increasing sleep duration by 30 minutes has the potential to influence psychological distress to a small degree. Given a linear association, increasing sleep duration by more than 30 minutes will probably decrease psychological distress more, especially for adolescents that had especially short sleep duration to begin with. We found cross-sectional associations between sleep duration and psychological distress at both time points. These cross-sectional findings correspond to associations between changes in sleep duration and changes in psychological distress, indicating that sleep duration and psychological distress change together over time.

In paper 2, we found prospective associations between inflammatory markers (CRP and TGF-α) in boys only. This was surprising as girls in general show higher levels of psychological distress than boys. Results showing different associations dependent on gender are inconsistent. (See more about gender differences under “Consistency with other studies” below). Therefore, it is premature to speculate about biological mechanisms explaining gender differences concerning associations between inflammatory markers and psychological distress. It is possible that inflammation affects psychological distress differently in girls and boys. However, to my knowledge there is no biological process explaining why we only found significant findings in boys.

In paper 3, significant associations between changes in sleep duration and changes in psychological distress were found in both girls and boys. The beta-values in the adjusted analyses were almost identical (-0.068 in girls and -0.078 in boys). This was unexpected given that associations between insomnia and psychological distress have been found to be stronger in girls compared to boys [144]. Thus, it would be expected that the association between changes in sleep duration and changes in psychological distress would be stronger in girls compared to boys. Additionally, one might have expected to find stronger associations in girls because their psychological distress was higher at both time-points.

One the other hand, there were also gender- similarities in our data, which may explain the findings of prospective associations between sleep duration and psychological distress in both genders. There was a significant increase in psychological distress from baseline to follow-up in both genders. Further, even though the reduction in sleep duration from baseline to follow-up in girls was non-significant, it showed the same direction as the significant reduction in sleep duration in boys. Given these similarities regarding the exposure and outcome across gender, the similar prospective associations found in girls and boys are plausible. In sum, even though girls showed higher levels of psychological distress, the biological mechanisms behind the association between changes in sleep duration and changes in psychological distress may be the same in both genders. Increasing sleep duration seems to be beneficial for mental health in both girls and boys. This is plausible in a sample with a mean sleep duration of approximately 7 hours, and approximately 25% of the participants slept ≤ 6 hours, which is below the recommended sleep duration of 8 to 10 hours during adolescence.

4.2.3 Consistency with other studies

Our findings regarding the distribution of psychological distress correspond with other studies showing that psychological distress is highly prevalent during adolescence, and higher in girls compared to boys [3, 49]. Our results also correspond with studies showing that short sleep duration is common during adolescence [114, 166].

In paper 1, we found no cross-sectional associations between inflammatory markers and psychological distress. There is a scarcity of studies on such associations in healthy adolescents. Our non-significant findings in healthy adolescents correspond to the results in the few studies that have been done [196, 197]. In these studies, depressive symptoms were used as outcome, in contrast to our outcome of psychological distress, which consists of both depressive and anxiety symptoms. However, both depressive symptoms and psychological

distress measure subclinical burdens, and are thus probably highly correlated as it has been shown that measures of anxiety and depression correlate highly during adolescence [198]. When we removed the anxiety items, and only used the depressive items as outcome in our supplementary analyses, the results remained non-significant. Thus, it is not likely that the anxiety items included in HSCL-10 are the reason for the lack of significant findings in paper 1.

In contrast to our null-findings, a study found a significant cross-sectional association between CRP and depressive symptoms in adolescent girls [87]. However, this was a case-control study, where girls with depressive symptoms were matched with controls. It was found that CRP had the strongest association with CRP in girls with severe depressive symptoms. This corresponds to research showing that inflammatory markers are strongest associated with severe symptoms of depressive symptoms [199]. Thus, it may be that the levels of psychological distress in paper 1 were too low to be able to detect associations with CRP. In other words, our participants were healthy, and thus not directly comparable with adolescents with clinical depression or severe depressive symptoms.

In paper 2, we examined prospective associations between inflammatory markers and psychological distress. Our finding of a prospective association between CRP and psychological distress in boys supports the study by Moriarity et al. [90], who found that CRP predicted depressive symptoms in healthy adolescents. However, most studies that have examined the prospective association between CRP and depressive symptoms in healthy adolescents have not reported significant associations [89, 91, 190, 200, 201]. Thus, with significant findings only in our study and the study of Moriarity et al. [90], with the majority of studies finding no association between CRP and depressive symptoms, there is a need for more studies to explore this association.

Our finding that TGF-α was prospectively associated with psychological distress supports the finding of Walss-Bass et al. [79], who reported a significant prospective association between TGF-α and depressive symptoms. However, to my knowledge there is no other study supporting this finding. Therefore, further research is warranted on the prospective association between TGF-α and psychological distress.

Our non-significant cross-sectional and prospective associations between IL-6, TRANCE, TWEAK and psychological distress are not unexpected given findings showing that

inflammatory markers are in general more strongly associated with clinical depression than subclinical outcomes, such as depressive symptoms. However, there are studies showing significant prospective associations between IL-6 and TNF-α, respectively, and depressive symptoms in healthy adolescents, depending on gender and time-to follow-up [89, 90, 201]. Thus, there are mixed findings concerning IL-6 and TNF-α (TRANCE and TWEAK are variants of TNF-α), respectively, and psychological distress, and further study of these associations are needed.

Our results indicated prospective associations between inflammatory markers (CRP and TGF-α) in boys but not in girls. This was unexpected because adolescent girls exhibit higher levels of psychological distress than adolescent boys. However, another study found different associations between inflammatory markers and depressive symptoms, dependent on gender and time to follow-up [90]. Thus, other studies have also found associations between inflammatory markers and subclinical outcomes, in boys only. On the other hand, most studies on healthy, non-clinical adolescents have not found prospective associations between CRP and depressive symptoms [89, 91, 190, 200, 201]. Thus, it is possible that some of the findings on prospective associations between CRP and depressive symptoms/psychological distress, in either girls or boys, dependent on time to follow-up, may be out of chance. Further, in paper 2, we found significant associations between TGF-α and psychological distress in boys only. Another study on adolescents (n=254) conducted by Walss-Bass et al. [79] found associations between TGF-α and depressive symptoms. However, this study did not examine if the associations between TGF-α and depressive symptoms were different in girls and boys.

In sum, regarding associations between inflammatory markers and psychological distress, there is little evidence indicating cross-sectional associations between inflammatory markers and subclinical outcomes in healthy adolescents. There is more evidence indicating prospective associations; however, the findings are mixed, and there is a scarcity of studies conducted. The significant prospective associations that have been reported in these studies have been dependent on inflammatory marker, gender, and time to follow-up.

In paper 3, we found significant associations between changes in sleep duration and changes in psychological distress. Our findings support Fuligni et al. [129], who reported that short sleep duration was correlated with higher psychological distress the following day in adolescents. Our significant findings also support Orchard et al. [130] who found that sleep

duration at 15 years of age was associated with psychological distress at 21 years of age. Hence, our findings over two years are in line with both shorter and longer time lags in other studies.

We found that an increase of 30 minutes of sleep duration was associated with a decrease in psychological distress. Our finding supports two studies that evaluated the effect from delayed school start [202, 203] and a sleep education programme for adolescents [204], both of which found that an increase of approximately 30 minutes was associated with decreased psychological distress/ depressed mood.

In contrast to our significant findings, some studies have not found prospective associations between sleep duration and symptoms of anxiety and depression. A study by Doane et al. on participants aged 18 years at baseline found no significant associations between sleep duration at baseline and symptoms of anxiety and depression at follow-up [205]. However, the sample was small (n=71), and the study may thus have been underpowered to detect significant associations. Nonetheless, the non-significant finding by Doane et al. is supported by a larger study conducted by Fan et al. [206], who did not find any significant prospective association between sleep duration at baseline and depressive symptoms at follow-up one year later when adjusting for baseline depressive symptoms.

A reason for conflicting results between our results in paper 3 and the results of Fan et al. [206] may be different research questions and methods. Fan et al. used sleep duration at baseline as exposure, depressive symptoms at follow up as outcome, and adjusted for depressive symptoms at baseline. By this approach, the direct effect from sleep duration at baseline on depressive symptoms at follow-up is examined. By using change scores, as we did in paper 3, another research question is explored, namely whether changes in sleep duration are associated with changes in psychological distress. This approach examines whether sleep duration and psychological distress varies together across time. A rational for using change scores is that it has been suggested that the effect from insufficient sleep on depressed mood is relatively short [207]. Thus, it may seem unlikely that sleep duration from baseline would have a direct effect on psychological distress at follow-up two years later. Because the use of change scores explores whether sleep duration and psychological distress change together over time, the associations may be interpreted as short time effects from sleep duration on psychological distress. Thus, our findings in paper 3 may not be directly comparable to

studies like the one by Fan et al., which examined the direct effect from sleep duration at baseline on psychological distress/depressive symptoms at follow up one year later.

Somewhat related, Short et al. [127] claimed that the inconsistent findings on associations between sleep duration and mood may be caused by different operationalisations of sleep duration, such as different cut-offs for short sleep duration and the use of continuous sleep duration. By using change-scores of sleep duration as exposure, we may thus expect different results compared to studies that operationalised sleep duration differently. Perhaps the most important difference between our study and other studies is that we explored another research question by our method compared to studies examining if sleep duration at baseline predicts mental health at follow up. Therefore, caution is warranted before comparing our results directly with such studies. Nonetheless, there are similarities between our methods in paper 3 and the studies I have discussed above. They all examined prospective associations between sleep duration and psychological distress/depressive symptoms. Another similarity is that other studies have found significant effects from increasing sleep duration by 30 minutes on psychological distress/depressive symptoms, corresponding to our finding showing that increasing sleep duration by 30 minutes was associated with decreased psychological distress. Thus, our results are consistent with other studies showing that increases in sleep duration by 30 minutes in healthy adolescents are associated with statistically significant decreases in psychological distress.

5 Conclusions, implications, and future research

5.1 Overall conclusions

We found no cross-sectional associations between inflammatory markers and psychological distress in girls or boys. However, we found prospective associations between inflammatory markers (CRP and TGF-α) and psychological distress in adolescent boys. Higher levels of CRP and TGF-α at baseline were associated with higher levels of psychological distress at follow-up two years later. Further, results from analyses of effect modification suggest that boys who are sedentary and/or have a higher body fat percentage are more vulnerable to the effects of CRP on psychological distress. Additionally, and surprisingly, boys who slept above 7 hours were more vulnerable to the effects from TWEAK on psychological distress. Our studies regarding associations between inflammatory markers and psychological distress contribute to a research field with few studies and inconsistent results. Overall, research on associations between inflammatory markers and depressive symptoms/psychological distress can still be characterised as inconsistent. In this research field, there are more results pointing towards prospective associations compared to cross-sectional associations.

We found cross-sectional associations between sleep duration and psychological distress in girls and boys. A 30-min increase (one unit) in sleep duration was associated with lower levels of psychological distress at both time-points. Prospectively, we found that changes in sleep duration were associated with changes in psychological distress in girls and boys. Increased sleep duration by 30 minutes was associated with decreased psychological distress. Experimental studies have found corresponding effects by increasing sleep duration by 30 minutes.

There has previously been a call for adjustment for potential confounders when examining associations between inflammatory markers and psychological distress. Our results from crude analyses on associations between inflammatory markers and psychological distress remained the same after adjustment for potential confounders. Similarly, adjustment for potential confounders did not change our crude associations between changes in sleep duration and changes in psychological distress.

5.2 Implications for public health

The studies in this thesis were observational, thus we cannot conclude about causality. Further, there are also mixed findings in the literature regarding the studied associations.

Therefore, it is premature to recommend interventions aiming to reduce inflammation to reduce psychological distress in healthy adolescents.

Our results support previous findings showing positive effects from a healthy lifestyle during adolescence. Health behaviour such as physical activity, getting enough sleep, and a having a healthy body fat percentage promotes physical and mental health.

Adolescents do, in general, sleep below recommended hours. Research has shown that short sleep duration affects both physical and mental health. Our results in paper 3 support previous studies indicating that short sleep duration adversely affect mental health. Thus, our results are in line with studies that have indicated that increasing sleep duration during adolescence prevents psychological distress and depression.

5.3 Future research

The results from studies on associations between inflammatory markers and subclinical mental outcomes are inconsistent, and it is difficult to explain the inconsistency across studies. A promising approach may be to examine how individual depressive symptoms are associated with inflammation. Some researchers have already examined such individual depressive symptoms, with significant findings [208, 209]. Inflammation has been associated with symptoms such as sleeping problems and low energy [210].

Based on our significant findings concerning effect-modification, future research should explore these moderators and other moderators. Exploration of moderators may elucidate the inconstancies in findings across studies. Future studies could also explore whether the effects from high body fat percentage and physical inactivity on psychological distress are mediated through inflammation.

Because adolescents sleep below the recommended hours, future studies should continue to examine the effect that short sleep duration has on mental health. Future studies may aim to replicate our findings using change scores. Future studies should use more validated measures of sleep duration compared to our measure with a single item. For example, adolescents frequently take naps during the day. Therefore, napping should also be accounted for in future research. Sleep is a complex phenomenon, and other factors beyond sleep duration are important when studying sleep. Thus, other aspects of sleep, such as sleep quality should be examined in relation to mental health.

There is a need for studies exploring how inflammation and sleep affect each other, and in the next step, influence mental health. Our finding that sleep duration moderated the association between TWEAK and psychological distress in paper 2 was in the opposite direction of what we expected based on previous findings. However, previous research has supported the hypothesis by Cho et al., suggesting that inflammation and sleep disturbance act together and influence depression. Therefore, studies should aim to elucidate these mechanisms.

The overall theme in this thesis was whether the body (in the form of inflammation and sleep duration) influence the mind. Future studies should include the examination of the opposite direction and examine whether the mind influences the body. More specifically, longitudinal studies could examine how psychological distress affect sleep duration and inflammation. This should be examined to uncover whether bidirectional associations between bodily functions (inflammation and sleep) and psychological distress/depressive symptoms result in a negative spiral in the long run.

6 References

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PAPER 1

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Are pro-inflammatory markers associated with psychological distress in a cross-sectional study of healthy adolescents 15–17 years of age? The Fit Futures study

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Abstract

Background: Inflammatory markers have been associated with depression and anxiety disorder in adolescents. Less is known about the association between inflammation and subclinical symptoms in the form of psychological distress. We investigated prevalence of psychological distress and examined the associations between common pro-inflammatory markers and psychological distress in an adolescent population sample.

Methods: The study was based on data from 458 girls and 473 boys aged 15–17 years from the Fit Futures Study, a large-scale study on adolescent health, conducted in Northern Norway. Psychological distress was measured with the Hopkins Symptom Checklist (HSCL-10). Serum-levels of the following low-grade inflammatory markers were measured: C-reactive protein (CRP), interleukin 6 (IL-6), transforming growth factor-alpha (TGF-α), tumor necrosis factor alpha variant 1 (TRANCE) and tumor necrosis factor alpha variant 2 (TWEAK). Associations between quartiles of inflammatory markers and HSCL-10 were examined by logistic regression and adjusted for potential confounders in sex-stratified analyses.

Results: The proportion of psychological distress above cutoff were 26.9% and 10.8% among girls and boys, respectively. In both girls and boys, crude analysis showed positive associations between all inflammatory markers and HSCL-10, except for TWEAK and TRANCE in boys. However, none of these associations were statistically significant. Further, there were no significant findings in the adjusted analyses.

Conclusion: There was a higher prevalence of psychological distress in girls compared to boys. Pro-inflammatory markers were not significantly associated with psychological distress in data from healthy adolescents aged 15–17 years.

Keywords: Psychological distress, Inflammatory markers, Depressive symptoms, Anxiety symptoms, Adolescence

Background

Mental disorders are a leading cause of disability globally. Many mental disorders have age-of-onset in childhood or adolescence [1] with a worldwide-pooled prevalence of 13.4% in 2015 [2]. In most countries, the median age-of-onset of having any mental disorder is during teenage years [1]. Subclinical mental burdens are even more

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prevalent [3]. In a recent survey among high school students in Norway, self-reported psychological distress was 31% and 12% among girls and boys respectively [4]. 'Psychological distress' can be defined as "a state of emotional suffering characterized by symptoms of depression and anxiety" [5]. The term refers to subclinical symptoms of anxiety and depression, but may also be an indication of mental disorder onset [5]. For some adolescents, psychological distress during growth leads to psychiatric conditions later in life [6, 7]. In order to promote good mental health among adolescents and reduce the burden of mental disorders and reduce the risk of suicidal ideation [8], more detailed investigations into the development of mental disorders in this age group are warranted.

There has been a growing interest in research on biological mechanisms that may increase our understanding of the aetiology of mental disorders and psychological distress. In the last two decades, the possible role of inflammation as a triggering factor for depression has been studied [9, 10]. Reports indicate that about one in three adults with depression have elevated levels of inflammatory cytokines [11]. Furthermore, patients with inflammatory conditions have higher risk for depressive disorders, and treatments based on cytokines may induce depressive symptoms [11]. In a meta-analysis of inflammatory markers in patients with major depressive disorder [12], it was concluded that interleukin 6 (IL-6), C-reactive protein (CRP), interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNF-α) were positively associated with major depressive disorder, yet the latter two were dependent on age. In addition, the same inflammatory markers and white blood cell count have been associated with anxiety disorders in adults [13–18]. Psychological distress is widely used in population-based studies of mental health among adults [19], and has been found to be associated with CRP in large population studies [20, 21].

Studies investigating associations between inflammatory markers and depressive disorders in adolescent samples have produced conflicting results [22–27].

A meta-analysis from 2019 on children and adolescents with a diagnosis of depressive disorder included five studies and reported a trend for higher levels of TNF-α in participants with depressive disorders compared to controls [28]. Other inflammatory markers were not significantly different between healthy and diagnosed subjects [28]. A more recent meta-analysis on children and adolescents identified nine studies on inflammatory markers and anxiety-based disorders and concluded that there were no significant associations [29]. However, both meta-analyses included few studies with small sample sizes, and the authors reviewed the results as provisional and concluded that more studies are warranted [28, 29].

Regarding investigations on the association between inflammatory markers and subclinical depressive symptoms in adolescents, Mills et al. [30] conducted a systematic review with 18 studies including both subclinical depressive symptoms and clinical depression as study outcomes. The review reported that the associations between inflammatory markers and depression have many similarities with adult findings, but some noticeable differences appeared. Especially, a further broad exploration of differential roles from specific markers (e.g., IL-6, CRP and TNF-α) during growth were requested. A later cross-sectional study confirmed a positive association between high sensitive serum CRP (hs-CRP) and depression-score, controlling for anthropometric and lifestyle factors [31]. The study included solely girls with a range from 12 to 18 years of age. In contrast, a large population-based study with data from 1535 participants 13 and 16 years of age did not support an association between elevated hs-CRP and depressive symptoms [32].

One study of US adolescents has looked at the association between CRP and symptoms of generalized anxiety disorder GAD [33]. GAD includes a range of symptoms highly comorbid with depression symptoms. Indeed, increased levels of CRP were found to be associated with symptoms of GAD in bivariate cross-sectional analyses. However, all associations were attenuated when controlling for other health-related covariates, demographics, and substance use.

In summary, there is limited literature and inconsistent findings on the associations between inflammatory markers and subclinical symptoms of depression and anxiety in healthy adolescents. Furthermore, in the vast majority of descriptive reports, adolescent girls show higher levels of psychological distress than boys, and the reported associations between inflammatory markers and psychological distress seem to be dependent on sex [4, 23, 34, 35].

With the scarcity of research, and inconsistent findings on the associations between inflammatory markers and psychological distress in healthy adolescents, further research is warranted. The aims of this study were to a) describe the prevalence of psychological distress in girls and boys 15–17 years of age and b) examine the associations between inflammatory markers and psychological distress in girls and boys 15–17 years of age.

Methods

Study population and design

In 2010–2011, all first-year upper secondary school students in two municipalities in Northern Norway were invited to participate in a broad health study, namely the Fit Futures, an expansion of the population-based Tromsø Study. Fit Futures has previously been

comprehensively described [36]. In brief, the study was conducted during school hours at the Clinical Research Unit, at the University Hospital of North Norway, Tromsø. In total, 1117 students were invited to participate, and 1038 (92.9%) attended the study (Fig. 1).

All participants provided informed consent. Participants younger than 16 years provided written informed consent from a guardian. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Norwegian Data Protection Authority (reference number 2009/1282). The Regional Committee of Medical and Health Research Ethics has also approved the study (reference number 2011/1702/REK Nord), and the present project (reference number: 2019/60811/REK Nord).

Data about lifestyle, health and disease was collected with a web-based battery of questionnaires. Dedicated and trained research nurses performed clinical examinations, collected and administered blood samples, and conducted interviews on medication including use of hormonal contraceptives, and acute and chronic diseases. Height and weight were measured following standard procedures [36]. An automatic electronic scale, the Jenix DS 102 stadiometer (Dong Sahn Jenix, Seoul, Korea) was used to measure weight. Total body fat mass was measured by dual X-ray absorptiometry (DXA; GE Lunar prodigy, Lunar Corporation, Madison, WI, USA).

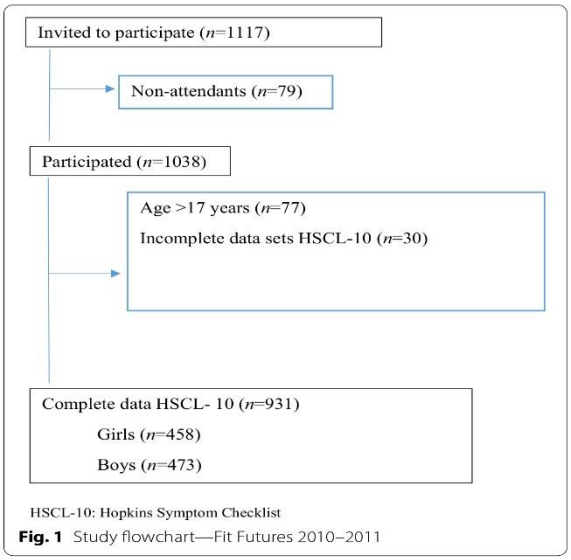

Fig. 1 Study flowchart—Fit Futures 2010–2011

Measurements and questionnaires

Hopkins Symptom Check List (HSCL-10)

HSCL-10 was included in the web-based questionnaire. HSCL-10 is a valid and reliable instrument [37] measuring symptoms of anxiety (4 items) and depression (6 items) during the last seven days [37]. The response categories are "none" (1), "slightly" (2), "much" (3), and "very much" (4). Cronbach's alpha in this sample was 0.87 (for girls 0.88, for boys 0.83). To quantify psychological distress, the average score of the 10 items was calculated. A dichotomized version of HSCL-10 was created, with 1.85 as cutoff, since values at or above that threshold indicate psychological distress of clinical relevance in community samples of adolescents [37]. This cutoff has been found to have a sensitivity of 89% and a specificity of 98% when the using HSCL-25 (cutoff 1.75) as criterion [37]. There were 458 girls and 473 boys with complete data on HSCL-10.

Main exposure variable: pro-inflammatory markers

The participants provided non-fasting blood samples, which was collected from the antecubital vein. Serum samples were transferred to Supelco glass vials (Sigma-Aldrich Norway AS, Oslo, Norway), and stored at − 70 °C. Serum levels of inflammatory proteins were analysed by Protein Extension Array Technology (Proseek Multiplex Inflammation panel; Olink Bioscience, Uppsala, Sweden). More details about the process of analysis are described elsewhere [38]. Based on current knowledge, the following inflammatory markers were selected for analyses: CRP, IL-6, transforming growth factor-alpha (TGF-α), TNF-α variant 1 (TRANCE) and TNF-α variant 2 (TWEAK) [12, 30, 39]. The number of girls and boys respectively with data on the inflammatory markers were as following: CRP (394 and 429), IL-6 (398 and 445), TGF-α (398 and 445), TRANCE (398 and 445), and TWEAK (398 and 445).

Covariates

Several variables are associated with both inflammation and psychological distress and were therefore included as covariates. Demographic and anthropometric variables included were: age, age at menarche (girls), pubertal status (boys), high school program as a proxy for socioeconomic status, body fat percentage and serum vitamin D levels. Lifestyle variables included were smoking, snuffing tobacco, alcohol use, physical activity, and sleep. Health variables included were hormonal contraceptives (girls), chronic disease, current infection, and medications, use of analgesics and antibiotics that potentially can influence systemic inflammation. In addition, all analyses were sex-stratified because girls

show higher levels of depressive symptoms than boys during adolescence, and there are reports that the associations between inflammatory markers and depressive symptoms are sex-dependent.

For smoking and snuffing tobacco, there were three alternative answers: "daily", "sometimes" and "never". Smoking and snuffing tobacco were recoded into a dichotomous variable, with "never" as the first category, while "sometimes" and "yes" were collapsed together as the second category. This was done because a low frequency of participants reported daily smoking and a low frequency reported that they snuffed sometimes. Frequency of alcohol-consumption was measured from 1 (never) to 5 (four or more times per week) and was recoded into three categories: "never", "once per month" and "twice or more per month".

Physical activity was measured by the Saltin-Grimby physical activity level scale [40] which addresses leisure time physical activity, asking about the type of activity and intensity in an average week during the last year. The four alternatives were: 1 (reading, watching TV, or other sedentary activity), 2 (walking, cycling or exercises at least 4 h a week), 3 (participation in recreational sports, heavy outdoor activities, snow clearing etc. at least 4 h a week) and 4 (participation in hard training or sports competitions several times each week). Physical activity was recoded into a dichotomous variable, with sedentary activities coded as zero and moderate and higher levels of activity coded as one.

For girls, pubertal status was estimated through the question: "When did you have your first menstruation". We created a dichotomized variable, "early" (at mean 12.68 years or below) or "late" (above mean). The reliability of self-reported menarche age is established [41]. In boys, pubertal status was measured with the Pubertal Development Scale (PDS) [41, 42]. Participating boys answered four questions: growth spurt, pubic hair growth, changes in voice and facial hair growth. The four alternatives were 1 (have not begun), 2 (barely started), 3 (underway), and 4 (completed). We summarized the total score on the four items and divided by four to create a mean score. Further, we used the mean score to categorize into four categories: "not begun" (mean score below 2) "barely started" (mean score from 2 to 2.99), "underway (mean score from 3 to 3.99) and "completed" (mean score of 4). For sleep, participants reported how many whole hours they normally slept every night, with the lowest category being "four hours or less" and the highest category being "12 h or more". The lowest category was coded as four hours, and the highest category was coded as 12 h. We created a dichotomous sleep variable divided by mean hours of sleep (6.95 h for girls and 7.09 h boys respectively). High school program consisted of three categories: "general studies", "sports and physical" and "vocational".

Body fat percentage was calculated as total fat mass (kg) divided by weight (kg). We created a dichotomous body fat percentage variable with cutoffs on 30 and 25% in girls and boys respectively [43]. Participants answered "yes" or "no" on questions about current infection, chronic disease and oral contraceptives, and dichotomized variables were created. Participants self-reported on their use of different types of medication. To assess for intake of medications that potentially influence systemic inflammation a dichotomized variable was created (medication intake). Vitamin D status was assessed by serum 25-hydroxyvitamin D (25-OH)D, analysed by high pressure liquid chromatography mass spectroscopy (LC–MS/MS) in stored sera (− 80 °C) at Haukeland University Hospital, Norway [44]. To standardize the results according to the Vitamin D Standardization program (VSDP), stored samples were re-analysed at the Cork Centre for Vitamin D and Nutrition Research, Ireland [45]. More details are described elsewhere [46]. The standardized version of (25-OH)D (nmol/L) was used as a continuous variable.

Statistical analysis

We excluded participants aged 18 years or above, and with incomplete data on the outcome variable (psychological distress as measured with HSCL-10) (Fig. 1). Data was inspected for outliers and normal distribution, using QQ plots, means and trimmed means. Exposure variables and potential confounders were tested for multicollinearity. All analyses conducted were stratified by sex.

Chi-square test was used to compare the number of girls and boys scoring above the cutoff of HSCL-10. Furthermore, variables were compared between those above and below the cutoff (with- and without psychological distress). Categorical variables were compared using Chi-Square test. Continuous variables with normally distributed data were compared with independent sample *t*-tests. Continuous variables with skewness were compared with Mann–Whitney U-test.

Binary logistic regression was conducted to estimate the odds ratio (OR) and 95% confidence intervals (CIs) between pro-inflammatory markers and psychological distress. Quartiles of inflammatory marker variables were created, and the crude associations between the quartiles of inflammatory markers and psychological distress were estimated. Subsequently, adjustment for potential confounding with stepwise regressions was conducted. Potential confounders were first tested in simple logistic regressions with the categorical version of HSCL-10 as outcome, and included when the *p*-value was below 0.05. Forward selection was stopped

when the added confounder had a p-value above 0.2 when added to the model.

Interaction was tested between the quartiles of the inflammatory markers and physical activity, body fat percentage and sleep [47–52]. In boys, there were significant interactions between CRP and sleep ($p = 0.03$) and between TGF-α and sleep ($p = 0.04$). However, these interaction terms could not be included in the adjusted analysis because of too few events (13 events in boys with psychological distress and high sleep), per covariate in the models [53].

As supplementary analyses, the mean score for the six depressive symptoms and four anxiety symptoms respectively were calculated and compared with the mean of HSCL-10. Additionally, stepwise forward logistic regressions with a dichotomous version of the six items of depressive symptoms as outcome were conducted. Also, the same stepwise regressions with continuous inflammatory markers as exposure for both the respective outcomes were conducted. Lastly, linear regressions with mean HSCL-10 as outcome were conducted.

A significance level of $p < 0.05$ as an indication of statistical significance was chosen. All statistical analyses were conducted with the Statistical Package of Social Science (SPSS v. 26).

Results

Prevalence of psychological distress in girls and boys

Girls reported a mean HSCL-10 (SD) of 1.63 (0.59), and boys reported 1.35 (0.41). Corresponding median (IQR) in girls and boys were 1.40 (0.70), and 1.20 (2.20), respectively (Fig. 2). In girls, 26.9% ($n = 123$) scored above the 1.85 cutoff value of HSCL-10. In boys, 10.8% ($n = 51$) scored above the cutoff value. The prevalence of psychological distress was significantly different between girls and boys, χ^2 (1, $N = 931$) = 39.6, $p < 0.01$.

Characteristics by psychological distress for girls and boys are shown in Tables 1 and 2 respectively.

In girls with psychological distress, a higher percentage scored above the cutoff for body fat percentage, slept below 7 h, smoked, snuffed tobacco, drank alcohol, were less physically active, had chronic diseases, used oral contraceptives and used medication compared to girls without psychological distress. The distribution across high school programs was significantly different for girls with- and without psychological distress (Table 1).

In boys with psychological distress, a higher percentage slept below 7 h, were less physically active and had chronic diseases compared to boys without psychological distress.

Associations between inflammatory markers and psychological distress

In girls, crude analyses showed positive associations between all inflammatory markers (in quartiles), and

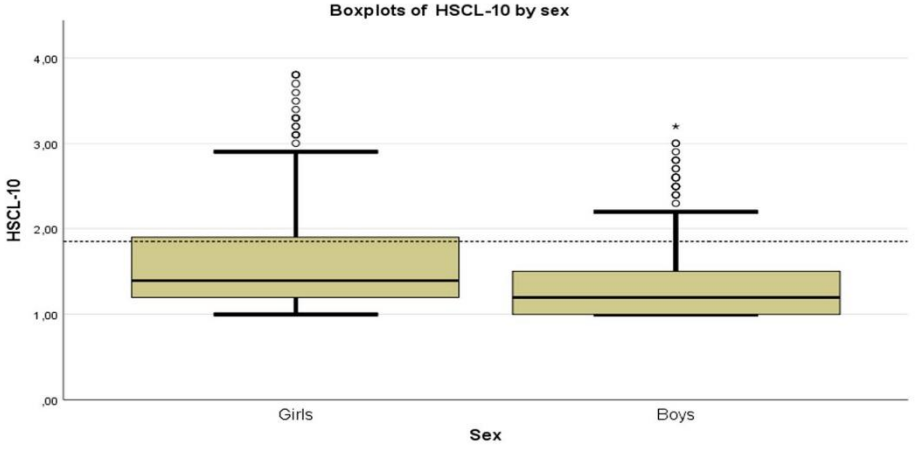

Fig. 2 Median and IQR of HSCL-10, for girls and boys respectively. Fit Futures 2010–2011

Table 1 Characteristics by psychological distress for girls

	With psychological distress		Without psychological distress		*p*-value*
	n	Mean (SD)	*n*	Mean (SD)	
Age in years Mean (SD)	123	16.15 (0.44)	335	16.13 (0.41)	0.23
Body fat percentage, dichotomous	123		332		0.03
< 30		33.3%		44.9%	
≥ 30		66.7%		55.1%	
Age menarche (years)	121		331		0.88
Early (≤ 12.68)		39.7%		40.5%	
Late (> 12.68)		60.3%		59.5%	
Smoking	123		334		< 0.01
No, never		62.6%		85.9%	
Yes		37.4%		14.1%	
Snuffing	123		334		< 0.01
No, never		53.7%		71.3%	
Yes		46.3%		28.7%	
Alcohol	123		335		0.01
Never		14.6%		26.6%	
Once per month or less		46.3%		46.6%	
Twice or more per month		39.0%		26.9%	
Physical activity	123		335		< 0.01
Sedentary		22.0%		11.3%	
Active		78.0%		88.7%	
Sleep (h)	123		334		< 0.01
Low (≤ 7)		51.2%		34.7%	
High (> 7)		48.8%		65.3%	
Current infection	122		334		0.20
No		82.8%		87.4%	
Yes		17.2%		12.6%	
Chronic disease	122		334		0.01
No		59.9%		72.2%	
Yes		41.0%		27.8%	
Hormonal contraceptives	122		333		< 0.01
No		50.8%		65.8%	
Yes		49.2%		34.2%	
Intake of medication	122		334		0.05
No		59.8%		69.8%	
Yes		40.2%		30.2%	
High school program	123		335		0.02
General studies		45.5%		54.3%	
Sports and physical		4.9%		9.6%	
Vocational		49.6%		36.1%	
CRP mg/ L median and IQR	97	0.72 (1.39)	297	0.47 (1.08)	0.11
IL-6 NPX median and IQR	98	2.79 (0.59)	300	2.68 (0.57)	0.05
TGF- α NPX median and IQR	98	3.90 (0.80)	300	3.90 (0.71)	0.91
TRANCE NPX median and IQR	98	5.62 (0.77)	300	5.53 (0.76)	1.00
TWEAK NPX MEDIAN and IQR	98	8.94 (0.51)	300	8.88 (0.42)	0.87
Vitamin D nmol/L median and IQR	98	39.36 (25.94)	300	41.90 (24.07)	0.65

Fit futures 2010–2011 (n = 458)

With psychological distress: a score above 1.85 on HSCL-10

Without psychological distress: a score below 1.85 on HSCL-10

Mean (SD) of continuous variable and percentages of categorical variables are reported

Table 1 (continued)

Median and IQR are reported for inflammatory markers and Vitamin D

Intake of medication: Intake of medications, analgetics or antibiotics in the last 24 h

Vitamin D: Standardized version of (25-OH)D

CRP C-reactive protein, *IL6-α* Interleukin 6 alpha, *TGF-α* Transforming growth factor alpha, *TRANCE* tumor necrosis factor-related activation-induced cytokine (O14788: TNF-related activation-induced cytokine within limits of detection), *TWEAK* Tumor necrosis factor-like weak inducer of apoptosis (O43508: TNF-like weak inducer of apoptosis within limits of detection), *NPX* Normalized protein expression

*Chi-Square for categorical variables and t-test or Mann Whitney U for continuous variables

HSCL-10. The highest OR (95% CI) was found for IL-6, 1.21 (0.98, 1.48). In boys, all crude associations were positive, except for those between TWEAK, TRANCE and HSCL-10. The highest OR (95% CI) was found for TGF-α, 1.20 (0.91, 1.58). However, none of these associations were statistically significant. Further, there were no significant findings in the adjusted analyses (Table 3). Body fat percentage did not confound the associations in any of the adjusted analyses.

Supplementary analysis

Using the six items measuring depressive symptoms as outcome did not alter the non-significant associations. All p-values were above 0.06 (Additional file 1). Neither using continuous inflammatory markers as exposure did alter the non-significant outcomes. All p-values were above 0.14 with HSCL-10 as outcome (Additional file 2) and all p-values were above 0.10 with the six depressive symptoms items as outcome (Additional file 3). Lastly, there were no significant crude nor adjusted associations in the linear regressions with mean HSCL-10 as outcome. All p-values were above 0.14 (Additional file 4).

Discussion

In this sample of 15–17 years old adolescents, girls reported a statistically significantly higher prevalence of psychological distress than boys. There were no statistically significant associations between any of the pro-inflammatory markers and psychological distress, neither for girls nor boys. After adjustment for potential confounders, the associations remained statistically non-significant.

Prevalence of psychological distress

The sex difference in prevalence of psychological distress is consistent with previous studies on this age group [32, 54]. Kleppang et al. found a corresponding sex difference in a Norwegian sample of 15–16 year olds, measuring psychological distress with HSCL-10 in 2009 [34]. Similar sex differences were also found in a study when a subset of HSCL-10 was used (the six items that measure depressive symptoms were included, whilst anxiety items were excluded). This was a study from 2015 on a Norwegian sample aged 13–16 years [55]. The proportions of psychological distress in girls and boys in this age group seems to be consistent [56, 57].

The finding that psychological distress differs according to several lifestyle factors is consistent with another study on Norwegian adolescents aged 13–18 years [58]. Differences in psychological distress according to socioeconomic status (high school as a proxy in the present study) [59], body fat percentage [60] oral contraceptives use [61], medication use [62–65], and the prevalence of chronic diseases [66] are also reported in other studies.

Associations between pro-inflammatory markers and psychological distress

This study investigated five inflammatory markers, separately for girls and boys, and could not show any significant associations with psychological distress in crude or adjusted analyses. Thus, this study does not provide any indications for cross-sectional associations between inflammatory markers and psychological distress in adolescents.

In line with the results in this study, there are several studies that report no associations between inflammatory markers and depressive symptoms. Chaiton et al. [32] found no crude or adjusted associations between CRP and depressive symptoms in a sample of 1532 healthy adolescents aged 13–16. They used the Center for Epidemiologic Studies Depression Scale (CES-D) to measure depressive symptoms. CES-D consists of 20 items measuring depressive symptoms in the general population. This outcome is somewhat different from HSCL-10, which measures psychological distress more generally, including 4 items about anxiety symptoms. However, since most of the HSCL-10 items measure depressive symptoms, and both scales measure primarily subclinical burdens and can be used to identify individuals with risk of clinical burdens, they probably have a high degree of concordance. Supporting this, measures of anxiety and depression generally have high correlations during adolescence [67]. In the supplementary analyses, conducting the regressions with the 6 depressive symptoms as outcome did not alter the findings, indicating that including anxiety items to the outcome variable was not the reason why we did not find any significant associations.

Table 2 Characteristics by psychological distress for boys

	With psychological distress		Without psychological distress		*p*-value*
	n	Mean (SD)	*n*	Mean (SD)	
Age in years mean (SD)	51	16.14 (0.49)	422	16.05 (0.45)	0.09
Body fat percentage, dichotomous	50		422		0.41
< 25		68%		73.5%	
≥ 25		32%		26.5%	
PDS status	42		334		0.52
Completed		23.8%		16.8%	
Underway		69.0%		74.6%	
Barely started		7.1%		8.7%	
Not begun		0%		0%	
Smoking	51		422		0.07
No, never		66.7%		78.0%	
Yes		33.3%		22.0%	
Snuffing	51		421		0.50
No, never		54.9%		59.9%	
Yes		45.1%		40.1%	
Alcohol	51		420		0.32
Never		31.4%		32.9%	
Once per month or less		29.4%		37.6%	
Twice or more per month		39.2%		29.5%	
Physical activity	51		422		< 0.01
Sedentary		49.0%		27.0%	
Active		51.0%		73.0%	
Sleep (h)	51		415		0.01
Low (≤ 7)		74.5%		54.9%	
High (> 7)		25.5%		45.1%	
Current infection	51		421		0.86
No		86.3%		87.2%	
Yes		13.7%		12.8%	
Chronic disease	51		420		0.02
No		58.8%		74.8%	
Yes		41.2%		25.2%	
Intake of medication	51		421		0.57
No		78.4%		81.7%	
Yes		21.6%		18.3%	
High school program	51		422		0.12
General studies		39.2%		28.7%	
Sports and physical		5.9%		14.7%	
Vocational		54.9%		56.6%	
CRP mg/ L median and IQR	44	0.47 (1.22)			
	385	0.49 (0.78)	0.64		
IL-6 NPX median and IQR	47	2.76 (0.61)	398	2.72 (0.62)	0.82
TGF- α NPX median and IQR	47	3.75 (0.68)	398	3.61 (0.74)	0.09
TRANCE NPX median and IQR	47	6.01 (0.77)	398	6.04 (0.65)	0.99
TWEAK NPX median and IQR	47	9.00 (0.30)	398	9.02 (0.37)	0.15
Vitamin D nmol/L median and IQR	47	25.91 (15.81)	399	30.44 (21.43)	0.01

Fit futures 2010–2011, (n = 473)

With psychological distress: a score above 1.85 on HSCL-10

Without psychological distress: a score below 1.85 on HSCL-10

Mean (SD) of continuous variable and percentages of categorical variables are reported

Table 2 (continued)

Median and IQR are reported for inflammatory markers and Vitamin D

PDS status: Pubertal Development Scale status

Intake of medication: Intake of medications, analgetics or antibiotics in the last 24 h

Vitamin D: Standardized version of (25-OH)D

CRP C-reactive protein, *IL6-α* Interleukin 6 alpha, *TGF-α* Transforming growth factor alpha, *TRANCE* Tumor Necrosis Factor-related activation-induced cytokine (O14788: TNF-related activation-induced cytokine within limits of detection), *TWEAK* Tumor necrosis factor-like weak inducer of apoptosis (O43508: TNF-like weak inducer of apoptosis within limits of detection), *NPX* Normalized protein expression

*Chi-square for categorical variables and *t*-test or Mann Whitney U for continuous variables

Table 3 Crude and adjusted associations between quartiles of inflammatory proteins and HSCL-10

	Crude analysis			Adjusted analysis		
	n	**Odds ratio (95% CI)**	***p*-value**	***n***	**Odds ratio (95% CI)**	***p*-value**
Girls						
CRP quartiles	394	1.18 (0.96, 1.45)	0.11	389	1.11 (0.90, 1.39)	0.33
IL-6 quartiles	398	1.21 (0.98, 1.48)	0.08	393	1.15 (0.92, 1.42)	0.22
TGF-α quartiles	398	1.01 (0.82, 1.24)	0.92	393	1.03 (0.83, 1.28)	0.80
TRANCE quartiles	398	1.07 (0.87, 1.31)	0.53	393	1.13 (0.90, 1.40)	0.29
TWEAK quartiles	398	1.02 (0.83, 1.25)	0.84	393	1.11 (0.89, 1.38)	0.35
Boys						
CRP quartiles	429	1.04 (0.79, 1.37)	0.79	420	1.00 (0.75, 1.33)	0.98
IL-6 quartiles	444	1.05 (0.80, 1.38)	0.73	435	0.99 (0.75, 1.32)	0.95
TGF-α quartiles	444	1.20 (0.91, 1.58)	0.19	435	1.190 (0.89, 1.59)	0.23
TRANCE quartiles	445	0.99 (0.76, 1.30)	0.95	436	0.99 (0.75, 1.30)	0.91
TWEAK quartiles	445	0.87 (0.66, 1.14)	0.31	436	0.88 (0.62, 1.16)	0.35

For girls, the adjusted models for CRP, IL-6, TGF-α and TRANCE included the following covariates: smoking, physical activity and chronic disease

The adjusted TWEAK model included the following covariates: smoking, snuffing tobacco, physical activity and chronic disease

For boys, adjusted model for all inflammatory markers included the following covariates: physical activity, sleep and chronic disease

Correspondingly, another study found no cross-sectional associations between IL-6 and Children's Depression Inventory (CDI), as a measure of depressive symptoms [68]. As in the present study, this study was also conducted in a community sample, with 288 participants, 51.4% girls and a mean age of 16.33 years. In sum, across samples of different ages, and with the use of different measures of psychological distress, there is generally a lack of associations in healthy adolescents. This suggests that the null-findings are neither related to age nor to the use of HSCL-10 as a measure of psychological distress.

In contrast to the present null findings, there are studies that found associations between inflammatory markers and depressive symptoms in adolescents. Tabatabaeizadeh et al. [31] found an association between CRP and depressive symptoms in 563 girls aged 12–18 years. This was a cross-sectional case–control study, which used the Beck depression Inventory-II (BDI-II). The study included 244 cases with mild to severe depression, and 319 age matched controls without depressive symptoms. Severe depressive symptoms had the strongest association with CRP, followed by moderate and mild symptoms. This is in line with previous research showing that the strongest associations with inflammatory markers are found with more severe symptoms and in clinical samples [69]. In contrast, the sample in the present study had low levels of psychological distress and is therefore not directly comparable with case-studies with higher proportions of participants having depressive symptoms.

Interpretations

When using cross-sectional data, the duration of the elevation of inflammatory markers is unknown. It is possible that inflammation needs to persist over a certain time period to influence the brain enough to result in increased levels of depressive symptoms [39, 70–72]. Indeed, it has been suggested that the strongest associations between inflammatory markers and depressive symptoms in adolescents are found in prospective studies where time to follow-up is at least 13 months [35]. Findings indicate that the same mechanism is present at older age (aged > 60 years), with corresponding findings of prospective associations and lack of cross-sectional associations [73]. Further, the elevated risk for depressive

disorder found in patients with inflammatory conditions [11] supports that enduring inflammation is associated with increased levels of depressive symptoms. It is possible that the duration of elevated inflammatory markers may have been short, or even acute, for many of the participants in the present study.

Another possibility mentioned by Chaiton et al. [32] is that the pathophysiology of depression is different in adolescents and adults. Therefore, the association between inflammation and depressive symptoms found in healthy adults will not necessarily be present among healthy adolescents. The association may also be weaker during adolescence since adolescents in general have lower levels of inflammation compared to adults [30]. Lower levels of inflammation make it more difficult to detect associations with depressive symptoms in adolescents. Furthermore, it is possible that the positive associations we found in this study might have been significant with a larger sample size.

The lack of associations may be caused by using HSCL-10, which is a combination of depressive and anxiety symptoms, as outcome. Depression consists of different symptoms, which seem to associate differently with inflammation [74, 75]. For example, inflammation has been found to associate specifically with sleeping problems and lack of energy [76]. The trend in psychoneuroimmunology research is therefore to investigate associations with different types of symptoms. Investigating different symptoms also increases power compared to using total symptoms [77].

Strengths and limitations

This study has several strengths. Firstly, the study was conducted on a sample of healthy adolescents and the attendance rate was very high. Adolescents represent an understudied population in general and with respect to the association between inflammation and depressive symptoms literature is scarce. One advantage of studying this age group is less noise from confounders, such as chronic diseases and obesity, typically more common in older subjects [30]. Secondly, all analyses were sex-stratified, since earlier studies have shown sex-differences in prevalence of both psychological distress and associations between inflammatory markers and depressive symptoms. Finally, we included an array of inflammatory markers that have been recommended by previous systematic reviews [30], that allowed us to explore the associations of different inflammatory markers with psychological distress.

This study has some limitations. The cross-sectional design restrict interpretations and we cannot infer causality. Further, the design measures outcome and exposure at the same time and cannot assess persistent inflammation. Secondly, we used self-report of psychological distress. This may not be as clinically relevant as diagnostic interviews. However, HSCL-10 is a reliable and valid instrument to measure psychological distress, with high sensitivity and specificity with the applied cutoff of 1.85 [37]. Thirdly, the sample size of this study may be too small to detect weak associations as previously found in large adult population samples, with approximately 70,000 participants [20, 21]. Even though larger sample sizes permit the detection of statistically significant associations between inflammatory markers and depressive symptoms, these associations may not be clinically relevant. Thus, smaller sample sizes, like the one used in this study, are still useful to investigate potential associations of clinical significance. Fourthly, we used dichotomous variables for current infection and chronic disease respectively. Thus, when assessing potential confounding, we did not examine different types of infection and chronic disease. Future studies, with bigger samples may benefit from discriminating between different kinds of infections and chronic diseases. However, the relatively few cases with psychological distress in our study (especially in boys) justify our adjustment for dichotomous versions of infection and chronic disease. Finally, since blood samples used in this study were collected from non-fasting participants, they may have been affected by diurnal effects [78]. Nonetheless, measurement errors are probably random in association with the outcome [79].

Conclusion

According to this study, the prevalence of psychological distress is higher in girls than in boys aged 15–17 years of age. The prevalence found in both girls and boys corroborates with previous findings in this age group. No evidence was found for associations between pro-inflammatory markers and psychological distress in healthy adolescents aged 15–17 years.

It is recommended to conduct prospective studies to elucidate possible longitudinal mechanisms and directionality. Future studies should also consider using larger sample sizes to detect possible significant positive associations.

Abbreviations

(25-OH)D: Serum 25-hydroxyvitamin D; BDI-II: Beck depression Inventory-II; CDI: Children's Depression Inventory; CES-D: Center for Epidemiologic Studies Depression Scale; CRP: C-reactive protein (CRP); GAD: Generalized anxiety disorder; HSCL-10: Hopkins Symptom Checklist; IL-6: Interleukin 6 (IL-6); PDS: Pubertal Development Scale; TGF-α: Transforming growth factor-alpha; TNF-α: Tumor necrosis factor alpha; TRANCE: Tumor necrosis factor alpha variant 1; TWEAK: Tumor necrosis factor alpha variant 2; VSDP: Vitamin D Standardization program.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40359-022-00779-8.

Additional file 1. Associations between quartiles of inflammatory-proteins and six depression-items from HSCL-10, by logistic forward stepwise regression.

Additional file 2. Crude and adjusted associations between continuous inflammatory-proteins and HSCL-10, by logistic forward stepwise regression.

Additional file 3. Associations between continuous inflammatory-proteins and six depression-items from HSCL-10, by logistic forward stepwise regression.

Additional file 4. Crude and adjusted associations quartiles of inflammatory proteins and HSCL-10, by linear regressions.

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Authors' contributions
N.A., A.S.F, G.G, and L.A.A. contributed to the study conception and design. Material preparation, data collection and analysis were performed by J.L., T.C., L.A.A, and G.C. The first draft of the manuscript was written by J.L. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The dataset supporting the conclusions of this article is available in the Tromsø-study repository, https://uit.no/research/tromsostudy.

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Norwegian Data Protection Authority (Reference Number 2009/1282) and the Regional Committee of Medical and Health Research Ethics has also approved the study (Reference Number 2011/1702/REK Nord), and the present project (Reference Number: 2019/60811/REK Nord). Participants younger than 16 years also provided written informed consent from a guardian.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Publisher's Note

Supplementary

Additional file 1: *Associations between quartiles of inflammatory-proteins and six depression-items from HSCL-10, by logistic forward stepwise regression.*

	Crude analysis			Adjusted analysis		
	Girls					
	n	Odds ratio (95 % CI)	*p*-value	*n*	Odds ratio (95 % CI)	*p*-value
CRP quartiles	394	1.07 (0.88, 1.31)	0.50	391	1.00 (0.81, 1.24)	0.99
IL-6 quartiles	398	1.22 (0.99, 1.50)	0.06	395	1.17 (0.95, 1.45)	0.14
TGF-α quartiles	398	1.02, (0.83, 1.24)	0.88	395	1.01 (0.82, 1.25)	0.93
TRANCE quartiles	398	1.11 (0.90, 1.36)	0.33	395	1.17 (0.94, 1.45)	0.15
TWEAK quartiles	398	0.98 (0.80, 1.21)	0.88	395	1.02 (0.82, 1.26)	0.87
	Boys					
	n	Odds ratio (95% CI)	*p*-value	*n*	Odds ratio (95 % CI)	*p*-value
CRP quartiles	429	1.11 (0.88, 1.41)	0.38	418	1.06 (0.83, 1.36)	0.64
IL-6 quartiles	444	1.19 (0.94, 1.50)	0.15	435	1.15 (0.90, 1.48)	0.26
TGF-α quartiles	444	1,19 (0.94, 1.50)	0.15	435	1.16 (0.91, 1.49)	0.24
TRANCE quartiles	445	0.92 (0.73, 1.16)	0.48	436	0.91 (0.71, 1.16)	0.44
TWEAK quartiles	445	0.84 (0.67, 1.07)	0.16	436	0.86 (0.67, 1.09)	0.21

For girls, adjusted models for CRP and TRANCE included the following covariates: smoking, physical activity and chronic disease
Adjusted model for IL-6 included the following covariates: smoking and physical activity
Adjusted models for TGF-α and TWEAK: smoking, physical activity and sleep

For boys, adjusted model for CRP included the following covariates: smoking, physical activity and sleep
Adjusted model for IL-6, TGF-α, TRANCE and TWEAK included the following covariates: smoking, physical activity, sleep and chronic disease.

Additional file 2: *Crude and adjusted associations between continuous inflammatory-proteins and HSCL-10, by logistic forward stepwise regression.*

	Crude analysis			Adjusted analysis		
	Girls					
	n	Odds ratio (95 % CI)	*p*-value	*n*	Odds ratio (95 % CI)	*p*-value
CRP	394	1.04 (0.97, 1.13)	0.29	389	1.03 (0.95, 1.12)	0.52
IL-6	398	1.25 (0.88, 1.77)	0.22	393	1.06 (0.73, 1.54)	0.75
TGF-α	398	1.04 (0.70, 1.53)	0.86	393	1.02 (0.67, 1.55)	0.926
TRANCE	398	0.89 (0.62, 1.30)	0.56	393	0.97 (0.66, 1.44)	0.89
TWEAK	398	0.94 (0.47, 1.87)	0.85	393	1.22 (0.59, 2.53)	0.59
	Boys					
	n	Odds ratio (95% CI)	*p*-value	*n*	Odds ratio (95 % CI)	*p*-value
CRP	429	0.99 (0.89, 1.09)	0.77	420	0.97 (0.87, 1.08)	0.59
IL-6	445	0.81 (0.46, 1.42)	0.47	436	0.73 (0.39, 1.39)	0.34
TGF-α	445	1.45 (0.88, 2.40)	0.15	436	1.40 (0.83, 2.35)	0.21
TRANCE	445	0.94 (0.53, 1.67)	0.84	436	0.95 (0.54, 1.69)	0.87
TWEAK	445	0.47 (0.17, 1.30)	0.14	436	0.48 (0.17, 1.36)	0.17

For girls, all adjusted models included the following covariates: smoking, physical activity and chronic disease.

For boys, all adjusted models included the following covariates: physical activity, sleep and chronic disease

Additional file 3: *Associations between continuous inflammatory-proteins and six depression-items from HSCL-10, by logistic forward stepwise regression.*

	Crude analysis			Adjusted analysis		
	Girls					
	n	Odds ratio (95 % CI)	*p*-value	*n*	Odds ratio (95 % CI)	*p*-value
CRP	394	1.02 (0.94, 1.10)	0.62	391	1.00 (0.92, 1.09)	0.93
IL-6	398	1.28 (0.90, 1.81)	0.17	395	1.09 (0.76, 1.58)	0.63
TGF-α	398	1.06 (0.72, 1.55)	0.78	395	1.01 (0.67, 1.51)	0.96
TRANCE	398	0.96 (0.67, 1.40)	0.85	395	1.00 (0.68, 1.48)	1.00
TWEAK	398	0.86 (0.43, 1.70)	0.66	395	0.99 (0.48, 2.03)	0.98
	Boys					
	n	Odds ratio (95% CI)	*p*-value	*n*	Odds ratio (95 % CI)	*p*-value
CRP	429	0.97 (0.89, 1.07)	0.58	418	0.95 (0.85, 1.06)	0.38
IL-6	445	0.97 (0.63, 1.49)	0.88	436	0.94 (0.57, 1.56)	0.82
TGF-α	445	1.42 (0.92, 2.20)	0.12	436	1.32 (0.84, 2.09)	0.23
TRANCE	445	0.85 (0.52, 1.39)	0.51	436	0.85 (0.52, 1.40)	0.53
TWEAK	445	0.46 (0.19, 1.12)	0.09	436	0.46 (0.18, 1.15)	0.10

For girls, the adjusted CRP model included the following covariates: smoking, physical activity and chronic disease.
The adjusted models for IL-6, TGF-α, TRANCE and TWEAK included the following covariates: smoking, physical activity and sleep.

For boys, all adjusted models included the following covariates: smoking, physical activity, sleep and chronic disease

Additional file 4: *Crude and adjusted associations quartiles of inflammatory proteins and HSCL-10, by linear regressions.*

	Crude analysis					Adjusted analysis				
Girls										
			95 % CI					95 % CI		
Inflammatory proteins	*n*	B	Lower	Upper	*p*-value	*n*	B	Lower	Upper	*p*-value
CRP quartiles	394	0.04	-0.01	0.09	0.14	393	0.02	-0.03	0.06	0.51
IL-6 quartiles	398	0.03	-0.02	0.08	0.26	397	0.01	-0.04	0.06	0.69
TGF-α quartiles	398	<-0.01	-0.05	0.05	0.88	397	<-0.01	-0.05	0.05	0.95
TRANCE quartiles (TNF)	398	<-0.01	-0.06	0.04	0.76	397	<0.01	-0.05	0.05	0.90
TWEAK quartiles (TNF)	398	-0.02	-0.07	0.03	0.51	397	0.01	-0.04	0.05	0.84
Boys										
			95 % CI					95 % CI		
	n	*B*	Lower	Upper	*p*-value	*n*	*B*	Lower	Upper	*p*-value
CRP quartiles	429	<0.01	-0.03	0.03	0.99	420	0.02	-0.04	0.03	0.70
IL-6 quartiles	444	<0.01	-0.03	0.04	0.90	435	-0.01	-0.04	0.03	0.65

TGF-α quartiles	444	0.02	-0.01	0.06	0.16	435	0.02	-0.02	0.05	0.32
TRANCE quartiles (TNF)	445	-0.03	-0.06	0.01	0.15	436	-0.02	-0.06	0.01	0.19
TWEAK quartiles (TNF)	445	<-0.01	-0.04	0.03	0.80	436	<-0.01	-0.04	0.03	0.94

For girls, the adjusted models for CRP, IL-6, TGF-α and TRANCE included the following covariates: smoking, physical activity and chronic disease.
The adjusted TWEAK model included the following covariates: smoking, snuffing tobacco, physical activity and chronic disease

For boys, adjusted model for all inflammatory markers included the following covariates: physical activity, sleep and chronic disease

PAPER 2

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C-Reactive Protein and TGF-α Predict Psychological Distress at Two Years of Follow-Up in Healthy Adolescent Boys: The Fit Futures Study

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Objective: The scarcity of research on associations between inflammatory markers and symptoms of depression and anxiety during adolescence has yielded inconsistent results. Further, not all studies have controlled for potential confounders. We explored the associations between baseline inflammatory markers and psychological distress including moderators at follow-up in a Norwegian adolescent population sample.

Methods: Data was derived from 373 girls and 294 boys aged 15–18 years at baseline, in the Fit Futures Study, a large-scale 2-year follow-up study on adolescent health. Baseline data was gathered from 2010 to 2011 and follow-up data from 2012 to 2013. Psychological distress was measured with Hopkins Symptom Checklist (HSCL-10). Serum levels of the following inflammatory markers were measured: C-reactive protein (CRP), Interleukin 6 (IL-6), Transforming growth factor alpha (TGF-α), Tumor necrosis factor alpha variant 1 (TRANCE), and variant 2 (TWEAK). Independent associations between baseline inflammatory markers and HSCL-10 at follow-up were explored by linear regressions, in sex-stratified analyses.

Results: In girls, analyses showed positive associations between all inflammatory markers and HSCL-10, except for TRANCE. However, all associations were non-significant in crude as well as in adjusted analyses. In boys, CRP ($p = 0.03$) and TGF-α ($p < 0.01$) showed significant associations with HSCL-10, that remained significant after adjustment. Additionally, moderators were found. In boys, CRP was associated with HSCL-10 in those with high body fat and those being physical inactive, and the association between TWEAK and HSCL-10 was dependent upon sleep duration.

Conclusion: There were significant prospective associations between CRP, TFG-α, and HSCL-10 in boys aged 15–18 years at baseline.

Keywords: psychological distress, inflammatory markers, depressive symptoms, anxiety symptoms, adolescence

INTRODUCTION

Adolescents who experience psychological distress have increased risk of developing mental disorders later in life (Silva et al., 2020). "Psychological distress," defined as "state of emotional suffering characterized by symptoms of depression and anxiety" (Mirowsky and Ross, 2002), is commonly reported in younger age groups. In Norway, self-reported psychological distress during high school years was recently reported to be 31 and 12% among girls and boys, respectively (Bakken, 2019). To improve prevention of psychological distress, better knowledge about biological risk factors acting during adolescence is needed. One potential risk factor is low-grade chronic inflammation, which has been linked to mental illness in adulthood.

Interestingly, one in three adults with major depressive disorder (MDD) have elevated levels of inflammatory markers (Leighton et al., 2018), and the average levels of inflammatory markers in MDD patients are higher than in controls (Raison et al., 2006; Howren et al., 2009; Dowlati et al., 2010; Liu et al., 2012; Osimo et al., 2020). Patients with inflammatory conditions have higher risk for MDD compared to controls (Leighton et al., 2018), and up to 50% of patients receiving therapeutic administration of the cytokine interferon-α develop MDD (Raison et al., 2006). There is also preliminary evidence suggesting that patients with general anxiety disorders show increased levels of inflammatory markers compared to controls (Vogelzangs et al., 2013; Michopoulos et al., 2017; Costello et al., 2019). Inflammatory cytokines access the brain through several routes and interact with pathophysiologic domains relevant to depression, including alterations in the metabolism of dopamine, serotonin, and norepinephrine in brain regions that regulate emotion, psychomotor function, and reward [8]. With respect to psychological distress, studies have investigated associations between inflammatory markers and psychological distress in adults, and reported cross-sectional associations with C-reactive protein (CRP), in large population studies (Wium-Andersen et al., 2013; Baek et al., 2019), and prospective associations with Interleukin 6 (IL-6; Virtanen et al., 2015).

There are few studies of inflammatory markers in relation to psychological distress and mental disorders (depression and anxiety) during adolescence. In a recent systematic review including studies from both children and adolescents with clinical depression, five studies were found eligible for meta-analysis (D'Acunto et al., 2019). On average, individuals with clinical depression showed a non-significant trend for higher Tumor necrosis factor alpha (TNF-α) levels compared to controls. No other inflammatory markers differed between the groups. However, no conclusions should be drawn based on only five studies with small sample sizes ($n = 17–31$). In contrast, another recent systematic review which included 22 studies on children and adolescents (Colasanto et al., 2020), reported both cross-sectional and prospective associations between CRP, IL-6, and clinical depression. However, studies are generally hampered by lack of information on potential confounding factors or effect modifiers, that is, sex, obesity, physical activity, and sleep. CRP has been found to be associated with depression in obese men only (Ladwig et al., 2003). Body fat measured *via* the proxy BMI has indeed been associated with psychological distress during adolescence (Kubzansky et al., 2012). Physical activity has been reported to protect against the effect of IL-6 on depressive symptoms in primary care patients (Rethorst et al., 2011), and mild sleep disturbance has been found to moderate the associations between inflammatory markers (IL-6 and TNF-α) and depressed mood in young females (Cho et al., 2016). Additionally, some studies have investigated associations between inflammatory markers and clinical anxiety in adolescents, where CRP was reported to be cross-sectionally associated with anxiety (Copeland et al., 2012b; Khandaker et al., 2016), yet not prospectively (Copeland et al., 2012b).

C-reactive protein has been associated with depressive symptoms among adolescents in cross-sectional studies (Guan et al., 2016; Tabatabaeizadeh et al., 2018). Prospectively, inflammatory markers [transforming growth factor alpha (TGF-α), CRP, and TNF-α] have been found to predict depressive symptoms in adolescents (Moriarity et al., 2018, 2019; Walss-Bass et al., 2018). However, in the latter associations between inflammatory markers and depressive symptoms have been found to be dependent upon sex and time to follow-up, with the strongest associations when time to follow-up was above 13 months (Moriarity et al., 2018). On the other hand, null-findings have also been reported, such as non-significant prospective associations between CRP, IL-6, TNF-α, and depressive symptoms (Copeland et al., 2012a; Moriarity et al., 2018, 2019; Walss-Bass et al., 2018). As it pertains to prospective associations between inflammatory markers and anxiety symptoms during adolescence, a study investigated 39 inflammatory markers (including TGF-α, IL-6, and TNF-α) without any significant findings (Walss-Bass et al., 2018). Psychological distress is commonly used to screen mental health in healthy populations. However, few studies have explored prospective associations between inflammatory markers and psychological distress during adolescence. Increased knowledge about this association is important for prevention of psychological distress.

To summarize, limited research has examined prospective associations between inflammatory markers and psychological distress in a general adolescent population, including information on potential confounders or effect modifiers. Literature indicates a need for sex stratification when examining such associations. Thus, this study aims to test whether circulating levels of five inflammatory markers at baseline are associated with prevalence of psychological distress at follow-up 2 years later, separately for girls and boys.

MATERIALS AND METHODS

Study Population and Design

In 2010–2011, all first-year upper secondary school students in two municipalities in Northern Norway were invited to participate in a broad health study, the Fit Futures Study. Participants were invited to a follow-up in 2012–2013. Fit Futures is thoroughly described elsewhere (Winther et al., 2014). Briefly, data were collected at the Clinical Research Unit, at the University Hospital of North Norway, Tromsø (UNN). At baseline in 2010, 1,117 students from first year in upper

secondary school were invited to participate, of which 1,038 (92.9%) attended the study (FF1). In 2012–2013, all participants in FF1 and all third-year upper secondary school students were invited to participate in the follow-up study (FF2), and 868 attained. The final sample consisted of 667 participants with complete data on the outcome variable Hopkins Symptom Check List (HSCL-10) at both time-points. Participants lost to follow-up did not differ on baseline data for the inflammatory markers and HSCL-10 items (data not shown; see **Figure 1**).

All participants provided informed consent. Participants below 16 years additionally provided written informed consent from a parent/guardian. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Norwegian Data Protection Authority (reference number 2009/1282). The Regional Committee of Medical and Health Research Ethics has also approved the study (reference number 2011/1702/REK Nord), and the present project (reference number: 2019/60811/REK Nord).

Measurements and Questionnaires

Data about lifestyle, health and disease was collected with a web-based battery of questionnaires. Qualified research nurses conducted clinical examinations and collected blood samples. They also did interviews on medication, use of hormonal contraceptives, and acute and chronic diseases.

Outcome: Hopkins Symptom Check List

We used HSCL-10 to measure psychological distress. HSCL-10 was a part of the web-based questionnaire at both time-points. HSCL-10 has been found to have good psychometric properties, with both high reliability and validity (Strand et al., 2003). The scale contains four items measuring symptoms of anxiety and six items measuring symptoms of depression during the last week (Strand et al., 2003). Symptoms are reported as "none" (1), "slightly" (2), "much" (3), and "very much" (4). Cronbach's alpha at baseline and follow-up were 0.87 and 0.90 for girls, and 0.82 and 0.87 for boys. The individual mean score of the

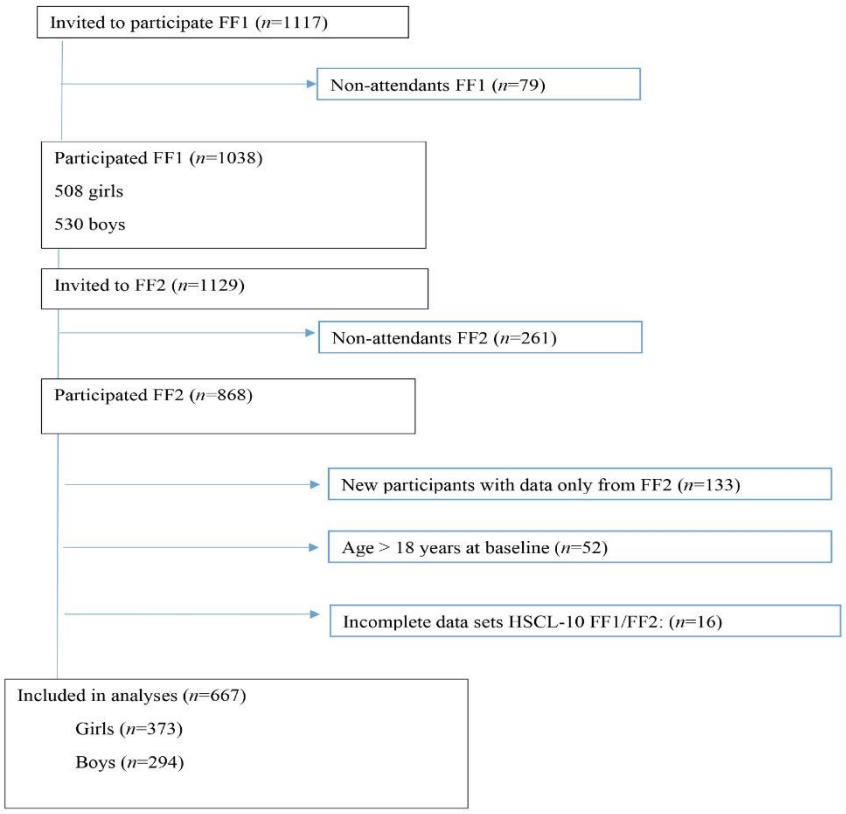

FIGURE 1 | Study flowchart, Fit Futures (FF1) 2010–2011 and Fit Futures 2 (FF2) 2012–2013. HSCL-10, Hopkins Symptom Checklist.

10 items was calculated. There were 373 girls and 294 boys with complete data on HSCL-10 at both time-points.

Main Exposure Variable: Pro-inflammatory Markers

Non-fasting blood samples were collected from the antecubital veins of the participants at baseline. Serum was separated and stored at −70°C. Protein extension array technology (Proseek Multiplex Inflammation panel; Olink Bioscience, Uppsala, Sweden) was used to analyze serum levels of inflammatory proteins. Details about the analysis are available elsewhere (Schistad et al., 2020). Based on previous research, the following inflammatory markers were further explored in the statistical analyses: CRP, IL-6, TGF-α, TNF-α variant 1 (TRANCE), and TNF-α variant 2 (TWEAK; Mills et al., 2013; Haapakoski et al., 2015; Walss-Bass et al., 2018). For CRP, there were 330 girls and 266 boys with data. For the four remaining inflammatory markers, there were 331 girls and 279 boys with data.

Baseline Covariates

Smoking and snuff use were answered by three alternatives: "daily," "sometimes," and "never." Because there were few participants who smoked daily and few participants that snuffed sometimes, smoking and snuff use were recoded into dichotomous variables, with never coded as zero, and "sometimes" and "yes" were combined and both coded as one.

Alcohol consumption was measured in frequency from "never" (1) to "four or more times per week" (5). Alcohol consumption was recoded into three categories: "never," "once per month or less," and "twice or more per month."

Physical activity was measured by Saltin–Grimby physical activity level scale. The scale measures physical activity during leisure time and includes type of activity and intensity during an average week in the last year (Grimby et al., 2015). The four categories were: "reading, watching TV, or other sedentary activity" (1), "walking, cycling or exercises at least 4 h a week" (2), "participation in recreational sports, heavy outdoor activities, snow clearing, etc. at least 4 h a week" (3), and "participation in hard training or sports competitions several times each week" (4). A dichotomous variable was created, with sedentary activities (the first category) coded as zero and the three other categories coded as one.

In girls, early or late menarche was measured with one question: "When did you have your first menstruation?." From this, it was created a dichotomous ("early" vs. "late") menarche variable based on the mean of 12.68 years of age. Self-reported menarche has good reliability (Koo and Rohan, 1997). In boys, Pubertal Development Scale (PDS; Petersen et al., 1988; Koo and Rohan, 1997) was used to measure pubertal status. The scale consisted of four questions about growth spurt, pubic hair growth, changes in voice, and facial hair growth. The four alternatives were: "have not begun" (1), "barely started" (2), 3 "underway" (3), and "completed" (4). An individual mean score was created. Further, the mean score was categorized into four categories of pubertal development: "not begun" (mean score below 2) "barely started" (mean score from $2 < 3$), "underway" (mean score from $3 < 4$) and "completed" (mean score of 4).

Sleep duration was measured with one question: "How many hours sleep do you normally get per night?," with alternatives from "four or less hours" to "12 h or more." The lowest and highest category were coded as four and 12, respectively. Further, a dichotomous variable was created based on the median hours of sleep, 7:00 h. High school program was self-reported, with the three following alternatives: "general studies," "sports and physical," and "vocational."

Height and weight were measured following standard procedures, that is, in light clothing without shoes [39]. The Jenix DS 102 stadiometer (Dong Sahn Jenix, Seoul, Korea), an automatic electronic scale, was used to measure weight. Dual X-ray absorptiometry was used to measure total body fat mass (DXA; GE Lunar prodigy, Lunar Corporation, Madison, WI, United States). Body fat percentage was calculated from total fat mass in kilogram divided by weight in kilogram. Dichotomous variables for each sex were created, with cutoffs for body fat percentages of 30 and 25% in girls and boys, respectively (Marques-Vidal et al., 2008). For current infection, chronic disease and current use of hormonal contraceptives dichotomous variables were created. Self-reported medication for daily or regular use were ATC-coded (Anatomical Therapeutic Chemical code). A dichotomized variable (medication intake) was created.

Vitamin D status was measured by serum 25-hydroxyvitamin D (25(OH)D), analyzed by high pressure liquid chromatography-mass spectroscopy (LC-MS/MS) in sera stored at −80°C at Haukeland University Hospital, Norway (Grimnes et al., 2010). Stored samples were re-analyzed at the Cork Centre for Vitamin D and Nutrition Research, Ireland (Cashman et al., 2016) to be able to standardize the results according to the Vitamin D Standardization program (VSDP). More details are elaborated elsewhere (Teigmo et al., 2018). The standardized version of 25(OH)D (nmol/L) was used as a continuous variable.

Self-rated health was reported with one question: "How do you in general consider your own health to be?" The five categories were: "very bad" (1), "bad" (2), "neither bad nor god" (3), "good" (4), and "excellent" (5). This question has been found to predict mortality (Idler and Benyamini, 1997), and morbidity in the general population (Latham and Peek, 2013). Further, a change score was calculated between follow-up and baseline so that positive and negative scores indicated better and worse self-rated health condition at follow-up versus at baseline, respectively.

Statistical Analysis

All statistical analyses were conducted with the Statistical Package of Social Science (SPSS v. 26). A significance level of $p < 0.05$ as an indication of statistical significance was chosen. We excluded participants aged 19 years or older at baseline, and with incomplete data on HSCL-10 at baseline and/or follow-up (**Figure 1**). Residual analysis was conducted to assess linearity, distribution, variance homogeneity, and to detect outliers. Exposure variables and potential confounders were tested for multicollinearity.

Because girls exhibit higher levels of depressive symptoms than boys in adolescence (Bakken, 2019) and because the associations between inflammatory markers and depressive symptoms have been sex-dependent in former studies (Moriarity et al., 2018), all analyses were done separately for girls and boys. Paired-samples *t*-tests were conducted to compare HSCL-10 at baseline and HSCL-10 at follow-up. Independent samples *t*-tests were conducted to compare HSCL-10 levels between girls and boys at baseline and at follow-up, respectively.

Variables assessed at baseline are presented for girls and boys separately, with means and standard deviations for continuous variables with normal distribution, median and IQR for continuous variables with skewness, and proportions for categorical variables. Data from girls and boys were compared in the following way: continuous data with normal distribution with independent sample *t*-tests, continuous data with skewness with Mann–Whitney *U*-tests, and categorical data with chi-square tests.

Linear regressions were conducted to estimate the unstandardized beta regression coefficients and 95% confidence intervals (CI) between pro-inflammatory markers at baseline and psychological distress at follow-up (the latter serving as outcome variable). Firstly, crude associations between the respective inflammatory markers and psychological distress were estimated (Model 1). Secondly, adjustment for baseline psychological distress was conducted (Model 2). Thirdly, potential confounders were added (Model 3). Potential confounders, coded as presented in **Table 1** were first tested in simple linear regressions with the mean of HSCL-10 score at follow-up as outcome and were included in the multivariable regression analysis (Model 3) when the value of p was below 0.10. For self-rated health, both the baseline score and the change score (follow-up minus baseline) were added simultaneously. This was done to adjust for baseline self-rated health in addition to change in self-rated health from FF1 to FF2 (**Supplementary Table 1**).

As supplementary analysis, we did linear regressions with the mean score of the six items of depressive symptoms from HSCL-10 at follow-up as outcome. This was done to assess the association between depressive symptoms and inflammatory markers more directly, without confounding the findings with the severity of anxiety.

RESULTS

Characteristics at Baseline and Outcome Distribution

Characteristics at baseline for girls and boys are presented in **Table 1**. In girls and boys, 57.4 and 26.9%, respectively, were measured above the sex-specific cutoffs for body fat percentage of 30 and 25. Sedentary behavior was more prevalent in boys (27.9%) than in girls (12.6%). A higher proportion of girls (30.7%) used medication compared to boys (18.4%). Among girls, the most common high school program was general studies (54.4%), while among boys, vocational studies were most common (49.0%). Drinking alcohol was more common in girls than in boys. TGF-α and 25(OH)D were significantly higher in girls than in boys, whereas TRANCE and TWEAK were lower (**Table 1**).

Girls reported a mean (SD) HSCL-10 of 1.59 (0.56) at baseline and 1.69 (0.64) at follow-up, a statistically significant increase in HSCL-10 from baseline to follow-up, $p < 0.001$. Girls scored significantly higher compared to boys at baseline, $p < 0.001$, and follow-up, $p < 0.001$. Boys reported a mean (SD) HSCL-10 of 1.36 (0.41) at baseline, and 1.41 (0.48) at follow-up. Also, in boys, there was a statistically significant increase in HSCL-10 from baseline to follow-up, $p < 0.001$ (**Figure 2**).

Prospective Associations Between Inflammatory Markers and Psychological Distress

In girls, all inflammatory markers were positively associated with HSCL-10 in crude analyses, except for TRANCE, which showed a negative association. However, none of these associations were statistically significant (**Table 2**). None of the adjusted analyses (models 2 and 3) showed statistically significant associations. In boys, both CRP [$b = 0.021$, 95% CI (0.003 and 0.039)] and TGF-α [$b = 0.134$, 95% CI (0.037 and 0.230)] at baseline showed significant associations with HSCL-10 at follow-up. Both associations remained statistically significant after adjustments (**Table 2**).

Effect Modification by Body Fat Percentage, Physical Activity, and Sleep

Interaction terms were tested to examine if associations between baseline inflammatory markers and psychological distress at follow-up differed by dichotomous versions of physical activity levels, body fat percentage, and sleep duration. Interaction terms were tested in simple linear regressions and included in further analysis when the value of p was below 0.05. In girls, there was a significant interaction between CRP and sleep duration ($p = 0.007$). In boys, there were significant interactions between CRP and both body fat percentage ($p = 0.009$) and physical activity ($p = 0.025$). Further, in boys, there were significant interactions between TGF-α and body fat percentage ($p = 0.028$), TRANCE and physical activity ($p = 0.037$), and TWEAK and sleep duration ($p = 0.038$). The significant interaction terms concerning these covariates were added to the fully adjusted models for the respective inflammatory markers. For girls, an interaction term between CRP and sleep duration was added to the fully adjusted model. For boys, interaction terms between CRP and body fat percentage and physical activity were added separately in two models. Further, in boys, interaction terms between TGF-α and body fat percentage, TRANCE and physical activity, and TWEAK and sleep duration were added in the three respective models. The interaction terms were interpreted as statistically significant when their value of p was below 0.05, and when there was a significant increase in R^2 change from the respective versions of Model 3.

In boys, body fat percentage [$b = 0.042$, 95% CI (0.008 and 0.075)] and physical activity [$b = -0.045$, 95% CI (−0.076 and

TABLE 1 | Characteristics at baseline for girls and boys. Fit Futures 2010-2011.

	Girls		Boys		*p*-Value*
	n	Mean (SD)	*n*	Mean (SD)	
Age in years, mean (SD)	373	16.15 (0.44)	294	16.11 (0.52)	0.230
Self-rated health	370	3.93 (0.76)	293	3.94 (0.87)	0.923
Body fat percentage, dichotomous[a]	373		294		N/A
<Cutoff	159	42.6%	215	73.1%	
≥Cutoff	214	57.4%	79	26.9%	
Age menarche (years)	367				
Early (≤12.70)	141	38.4%			
Late (>12.70)	226	61.6%			
PDS status			239		
Completed			23	9.6%	
Underway			172	72.0%	
Barely started			44	18.4%	
Not begun				0%	
Smoking	373		294		0.771
No, never	304	81.5%	237	80.6%	
Yes	69	18.5%	57	19.4%	
Snuffing	373		293		0.407
No, never	257	68.9%	193	65.9%	
Yes	116	31.1%	100	34.1%	
Alcohol	373		293		**0.039**
Never	95	25.5%	101	34.5%	
Once per month or less	172	46.1%	116	39.6%	
Twice or more per month	106	28.4%	76	25.9%	
Physical activity	373		294		**<0.001**
Sedentary	47	12.6%	82	27.9%	
Active	326	87.4%	212	72.1%	
Sleep duration (hours)[b]	373		291		0.793
<Cutoff	146	39.1%	111	38.1%	
≥Cutoff	227	60.9%	180	61.9%	
Current infection	371		293		0.387
No	318	85.7%	244	83.3%	
Yes	58	14.3%	49	16.7%	
Chronic disease	371		292		0.247
No	254	68.5%	212	72.6%	
Yes	117	31.5%	80	27.4%	
Hormonal contraceptives	370				
No	229	61.9%			
Yes	141	38.1%			
Intake of medication	371		293		**<0.001**
No	257	69.3%	239	81.6%	
Yes	114	30.7%	54	18.4%	
High school program	373		294		**<0.001**
General studies	203	54.4%	107	36.4%	
Sports and physical	35	9.4%	43	14.6%	
Vocational	135	36.2%	144	49.0%	
		Median (IQR)		Median (IQR)	
CRP, mg/L Median and IQR	330	0.54 (1.11)	266	0.53 (0.87)	0.542
IL-6, NPX Median and IQR	331	2.70 (0.58)	279	2.72 (0.61)	0.994
TGF-α, NPX Median and IQR	331	3.89 (0.75)	279	3.61 (0.70)	**<0.001**
TRANCE, NPX Median and IQR	331	5.54 (0.79)	279	6.03 (0.70)	**<0.001**
TWEAK, NPX Median and IQR	331	8.88 (0.44)	279	9.02 (0.37)	**<0.001**
25(OH)D, nmol/L Median and IQR	331	41.0 (24.6)	280	30.9 (21.3)	**<0.001**

*Mean (SD) of continuous variable and percentages of categorical variables are reported. Median and IQR are reported for inflammatory markers and Vitamin D. PDS, Pubertal Development Scale; Intake of medication, Intake of medications, analgesics, or antibiotics in the last 24h; CRP, C-reactive protein; IL6-α, Interleukin 6 alpha; TGF-α, Transforming growth factor alpha; TRANCE, Tumor Necrosis Factor-related activation-induced cytokine (O14788: TNF-related activation-induced cytokine within limits of detection); TWEAK, Tumor necrosis factor-like weak inducer of apoptosis (O43508: TNF-like weak inducer of apoptosis within limits of detection); NPX, Normalized protein expression; 25(OH)D, Standardized version of (25-OH)D; N/A, Not applicable. Bold, Statistically significant with a value of p of 0.05. [a]Cutoff body fat percentage girls (30) and boys (25). [b]Cutoff sleep duration 7.00h. *Chi-square for categorical variables and t-test or Mann–Whitney U for continuous variables.*

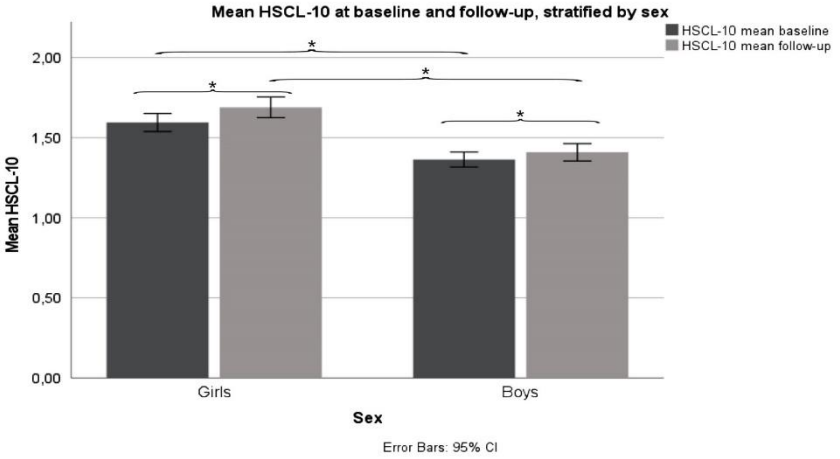

FIGURE 2 | Mean HSCL-10 levels, stratified by sex. Fit Futures (FF1) 2010–2011 and Fit Futures 2 (FF2) 2012–2013. *$p < 0.001$. HSCL-10, Hopkins Symptom Checklist.

−0.013)] moderated the association between CRP and HSCL-10. Sleep duration [$b = -0.046$, 95% CI (−0.750 and −0.062)] moderated the association between TWEAK and HSCL-10. The significant interaction terms were further investigated with stratified analyses. The results showed a positive association between CRP and HSCL-10 in boys with body fat percentage ≥25 [$b = 0.041$, 95% CI (0.013 and 0.069)] and in sedentary boys [$b = 0.049$, 95% CI (0.019 and 0.080)], and that TWEAK predicted HSCL-10 in boys that slept ≥7 h per night [$b = 0.212$, 95% CI (0.011 and 0.412); **Table 3**]. For more details about winning models, see **Supplementary Table 2**.

Supplementary Analysis Using the Six Items Depressive Symptoms as Outcome

Supplementary analysis restricted to the six HSCL-10 items measuring depressive symptoms as outcome and covariate did not alter the significant associations with CRP and TGF-α in boys (**Supplementary Table 3**).

DISCUSSION

In this population-based longitudinal study among adolescents, boys with higher levels of CRP and TGF-α at baseline reported more psychological distress at two-year follow-up. The findings remained significant after adjustment for confounding factors. The associations were not found in girls. Levels of TRANCE, TWEAK, and IL-6 were not associated with psychological distress.

In boys, we found a prospective association between CRP at baseline and psychological distress at follow-up where an increase of 0.01 mg/L in CRP indicated an increase of 0.021 units in HSCL-10 at follow-up. The positive association is in line with findings from Moriarity et al. (2018). Their study was conducted in a community sample ($n = 201$, 109 females) aged 12.3–20 years, with a mean age of 16.8 years at first blood draw (Moriarity et al., 2018). Moriarity et al. found a small effect from CRP on depressive symptoms measured by Children's Depression Inventory (CDI). In contrast, we found a positive effect of CRP only in boys. At the same time, girls in our sample had higher levels of psychological distress at baseline and follow-up, yet we found no differences in CRP levels between girls and boys at baseline. Regardless of the differences between our study and the study of Moriarity et al. (2018), both studies indicate that CRP is positively associated with the respective outcomes; depressive symptoms and psychological distress.

Most studies among healthy adolescents, have not found significant prospective associations between CRP and depressive symptoms (Miller and Cole, 2012; Copeland et al., 2012a; Khandaker et al., 2014; Khandaker et al., 2018; Moriarity et al., 2019). A study by Moriarity et al. (2019) found no association between CRP and depressive symptoms measured with CDI. This study included 140 adolescents (54% girls) with a mean age of 16.1 years. Our study resembles this sample as it pertains to age, health of participants, and time to follow-up above 13 months as recommended by Moriarity et al. (2018). A crucial difference is that we stratified on sex. To our knowledge, our study is the first study to find an effect of CRP on psychological distress across adolescence among healthy boys. Clearly, more studies are warranted to explore the association between CRP and psychological distress during adolescence and investigate potential sex differences.

We found that higher levels of TGF-α at baseline increased psychological distress 2 years later in boys. To our knowledge,

TABLE 2 | Crude and adjusted associations between baseline inflammatory proteins and HSCL-10 at follow-up, assessed by linear regressions.

	n	*B*	95% CI		*p*-Value	R^2 change
			Lower	Upper		
Girls						
CRP						
Model 1	330	0.017	−0.004	0.039	0.118	0.008
Model 2	330	0.013	−0.005	0.030	0.152	**0.363**
Model 3	324	0.005	−0.013	0.023	0.575	**0.057**
IL-6						
Model 1	331	0.064	−0.044	0.171	0.247	0.004
Model 2	331	0.035	−0.051	0.122	0.419	**0.362**
Model 3	325	0.002	−0.086	0.090	0.964	**0.059**
TGF-α						
Model 1	331	0.046	−0.070	0.161	0.437	0.002
Model 2	331	0.057	−0.035	0.150	0.221	**0.366**
Model 3	325	0.039	−0.051	0.128	0.397	**0.059**
TRANCE (TNF)						
Model 1	331	−0.016	−0.129	0.097	0.776	<0.001
Model 2	331	0.039	−0.051	0.130	0.393	**0.366**
Model 3	325	0.006	−0.086	0.098	0.895	**0.059**
TWEAK (TNF)						
Model 1	331	0.058	−0.153	0.270	0.588	0.001
Model 2	331	0.108	−0.061	0.276	0.209	**0.367**
Model 3	325	0.123	−0.047	0.293	0.155	**0.061**
Boys						
CRP						
Model 1	266	0.021	0.003	0.039	0.022*	**0.020**
Model 2	266	0.027	0.011	0.043	0.001*	**0.255**
Model 3	262	0.026	0.010	0.042	0.002*	0.011
IL-6						
Model 1	279	0.035	−0.054	0.124	0.443	0.002
Model 2	279	0.062	−0.013	0.138	0.105	**0.286**
Model 3	274	0.064	−0.011	0.139	0.096	0.016
TGF-α						
Model 1	279	0.134	0.037	0.230	0.004*	**0.030**
Model 2	279	0.123	0.042	0.203	0.003*	**0.274**
Model 3	274	0.122	0.040	0.203	0.004*	0.014
TRANCE (TNF)						
Model 1	279	−0.022	−0.130	0.086	0.688	0.001
Model 2	279	−0.004	−0.096	0.087	0.929	**0.281**
Model 3	274	0.001	−0.091	0.093	0.982	0.015
TWEAK (TNF)						
Model 1	279	0.012	−0.189	0.214	0.903	<0.001
Model 2	279	0.039	−0.132	0.210	0.652	**0.282**
Model 3	274	0.042	−0.129	0.212	0.631	0.015

*The results are presented for girls and boys, respectively. Fit futures 2010–2011 and 2012–2013. B, Unstandardized beta; CRP, C-reactive protein; IL6-α, Interleukin 6 alpha; TGF-α, Transforming growth factor alpha; TRANCE, Tumor Necrosis Factor-related activation-induced cytokine (O14788: TNF-related activation-induced cytokine within limits of detection); TWEAK, Tumor necrosis factor-like weak inducer of apoptosis (O43508: TNF-like weak inducer of apoptosis within limits of detection); Model 1, Crude analysis with the respective inflammatory marker; Model 2, Model 1+ baseline HSCL-10; Model 3 girls, Model 2+ current infection, medication intake, sleep duration, hormonal contraceptives, smoking, self-rated health, and change score self-rated health (inclusion criteria 0.1 from simple regressions); Model 3 boys, Model 2+ physical activity, sleep duration, self-rated health, and change score self-rated health (inclusion criteria 0.1 from simple regressions). Bold, Significant R^2 change. *Statistically significant with a value of p of 0.05.*

this is the first study to find an effect of TGF-α on psychological distress during adolescence. Our result corresponds with a study from Walss-Bass et al. (2018) indicating that TGF-α predicted depressive symptoms measured with Mood-Feelings Questionnaire-Child (MFQC), in a sample of 254 adolescents aged 12–15 years (54% female at baseline), with follow-ups 1 and 2 years later.

Contrary to the study by Walss-Bass et al., our study found associations between TGF-α and outcome in boys only. In our sample, girls had significantly higher levels of TGF-α at baseline ($p < 0.01$) compared to boys. We are not aware of other studies, except from the aforementioned (Walss-Bass et al., 2018) that have investigated prospective associations between TGF-α and depressive symptoms or psychological distress during adolescence. Thus, the finding in our study warrants further study of the prospective associations between TGF-α and psychological distress during adolescence, including investigations of sex differences.

The null-findings from the present study for IL-6, TRANCE, and TWEAK are in line with previous findings showing that inflammatory markers are more strongly prospectively associated with clinical depression than with depressive symptoms. Indeed,

TABLE 3 | Stratified analyses of the associations between baseline inflammatory proteins and HSCL-10 at follow-up, assessed by linear regressions.

	n	*B*	95% CI		*p*-Value
			Lower	Upper	
Boys					
CRP Model A					
<25% body fat	195	0.002	−0.022	0.026	0.877
≥25% body fat	67	0.041	0.013	0.069	0.005*
CRP Model B					
Sedentary	68	0.049	0.019	0.080	0.002*
Active	194	0.006	−0.014	0.025	0.573
TWEAK Model					
<7.00 h sleep duration	101	−0.188	−0.501	0.124	0.234
≥7.00 h sleep duration	173	0.212	0.011	0.412	0.038*

*Boys in Fit futures 2010–2011 and 2012–2013. B, Unstandardized beta; CRP, C-reactive protein; TWEAK, Tumor necrosis factor-like weak inducer of apoptosis (O43508: TNF-like weak inducer of apoptosis within limits of detection); CRP Model A, CRP, baseline HSCL-10, physical activity, sleep duration, self-rated health, and change score self-rated health; CRP Model B, CRP, baseline HSCL-10, sleep duration, self-rated health, and change score self-rated health; TWEAK Model, TWEAK, baseline HSCL-10, physical activity, self-rated health, and change score self-rated health. *Statistically significant with a value of p of 0.05.*

in a cumulative meta-analysis in adult patients with clinical depression, conducted on 58 observational studies, it was concluded that IL-6, CRP, and TNF-α were more strongly associated with severe depression than with moderate depression (Haapakoski et al., 2015). Because the associations between inflammatory markers and depression get stronger with more severe depression, weaker and more inconsistent findings are expected when using depressive symptoms. It may also be that TGF-α is only associated with psychological distress and depressive symptoms, whereas IL-6 and TNF-α are more sensitive predictors of clinical depression, and that CRP is a sensitive predictor for both clinical depression and depressive symptoms/psychological distress.

The inconsistent findings across studies may be explained by use of different outcomes. In our study, we used HSCL-10, while other studies have used measures, such as CDI and MFQC. The respective measurements of depressive symptoms aim to measure the same latent construct and are all validated and reliable measures of depressive symptoms. On the other hand, Moriarity et al. (2018) reported that many of their participants were nearing the upper end of the age range for which CDI has been validated. Different measurement scales of depressive symptoms may vary in what kind of symptoms their items are covering. Indeed, some studies have reported that inflammatory markers are associated with only specific depressive symptoms (Moriarity et al., 2018). Some scales might have more items about dysphoria and anhedonia, while other include more items about bodily symptoms, such as fatigue, pain, and sleeplessness. Whether there is an association between inflammatory markers and total depressive symptoms may be dependent on which symptoms that are included and weighted in the respective measurement scale.

In boys, there were statistically significant interactions between CRP, body fat percentage, and physical activity. This indicates that the effect of CRP on psychological distress is dependent upon body fat percentage and physical activity. These interaction effects are in line with studies on adolescents showing that body fat percentage and less physical activity are associated with increased systemic inflammation (Platat et al., 2006; Warnberg et al., 2007).

The significant interaction for body fat percentage and physical activity indicates that CRP has stronger association with psychological distress at 2 years of follow-up (after controlling for confounders, most importantly, for distress at baseline, and for self-assessed health condition) in boys that have high body fat percentage and/or are less physically active. One might speculate that low body fat percentage and physical activity among boys protects against the deleterious effects of CRP-associated inflammation on psychological distress in the long-run. Alternatively, high body fat percentage and physical inactivity, respectively, may increase inflammation which in the next step increases psychological distress. Otherwise, it may be that inflammation *per se* is not the driving factor for increased psychological distress, but rather a marker of unhealthy lifestyle (such as high body fat and low levels of physical activity) that are the actual drivers. Regardless of the mechanisms, the results in our study suggest that interventions should be designed to promote lower body fat percentage and more physical activity in boys to prevent increases in inflammation and psychological distress.

In boys, we also found a statistically significant interaction between TWEAK and sleep, indicating that the prospective association between TWEAK and psychological distress is dependent upon sleep duration. Surprisingly, an increase in TWEAK predicted increased psychological distress in boys that slept ≥7 h. This contrasts a study by Cho et al. (2016) that found stronger correlations between inflammatory markers (IL-6 and TNF-α) and depressed mood in young females with mild sleep disturbance compared to controls. In males, no moderating effect from sleep disturbance was found (Cho et al., 2016). Our finding may be spurious and could be explained by a median sleep duration of 7 h in our sample, which is below the recommendation of 8–10 h per night for adolescents (Paruthi et al., 2016). Nevertheless, shorter sleep has been associated with increased inflammation (Fernandez-Mendoza et al., 2017) and increased depressive symptoms (Gangwisch et al., 2010) in adolescents. Hence, further research on the association between sleep and depressive symptoms is warranted.

This study has several strengths. We explored the prospective associations between inflammatory markers and psychological distress as outcome in healthy adolescents, a population that have been understudied regarding such associations. Second, the sample size in our study was large enough to allow sex stratification. Third, the study explored several inflammatory markers that previously have been found to be prospectively associated with depressive symptoms during adolescence. This is in line with a call for a novel exploration of different markers (Mills et al., 2013). Finally, the present study examined several confounders recommended in literature (Haapakoski et al., 2015).

This study has some limitations. We used self-reported psychological distress as outcome. Inflammatory markers have been reported to show stronger associations with depressive symptoms measured through clinical interviews than in self-report. For CRP, the effect size has been reported to be twice as strong when using structured interviews compared to questionnaires (Howren et al., 2009). Nevertheless, HSCL-10 is considered a reliable and valid instrument for measurement of psychological distress (Finbråten et al., 2021). Secondly, because the prospective associations between inflammatory markers and psychological distress are relatively weak, the sample size may have been too small to detect associations. Large population studies that have detected statistically significant associations between CRP and psychological distress in adults used sample sizes of approximately 70,000 participants (Wium-Andersen et al., 2013; Baek et al., 2019). On the other hand, weak associations may not be clinically relevant. Thus, smaller sample sizes, as the one applied in our study, are justified. Although we found statistically significant associations in our study, the changes in HSCL-10 were small. It is unclear whether these associations are clinically significant. Future studies should examine the clinical significance of such associations.

According to our study, increased levels of CRP and TGF-α are associated with the development of more psychological distress across adolescence in boys. Other inflammatory markers that have been associated with clinical depression or anxiety (IL-6, TRANCE, and TWEAK) seem to be less relevant for psychological distress in adolescence. Our study suggests that boys who are physically inactive or have a higher body fat mass are particularly vulnerable to higher CRP, while surprisingly, boys who sleep longer are more vulnerable to higher TWEAK levels. Thus, investigation of the moderating role of body fat percentage, physical activity, and sleep duration are warranted in future prospective studies examining associations between inflammatory markers and psychological distress during adolescence.

DATA AVAILABILITY STATEMENT

The data analyzed in this study is subject to the following licenses/restrictions: Data may be obtained from a third party, UiT—The Artic University of Norway. Restrictions apply to the availability of these data, which were used under license for the current study, and are thus not publicly available. Requests to access these datasets should be directed to Elin Kristin Evensen, elin.k.evensen@uit.no.

ETHICS STATEMENT

The Regional Committee of Medical and Health Research Ethics has also approved the study (reference number 2011/1702/REK Nord), and the present project (reference number: 2019/60811/REK Nord). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

NE, A-SF, GG, and LA contributed to the study conception and design. Material preparation, data collection, and analysis were performed by JL, TC, LA, and GC. The first draft of the manuscript was written by JL. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2022.823420/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary

Supplementary table 1: Assessing self-rated health and change score of self-rated health as confounders. *Associations between self-rated health and the HSCL-10 at follow-up, assessed by linear regressions. The results are presented for girls and boys, respectively. Fit futures 2010-2011 and 2012-2013.*

		95 % CI			
Girls	*B*	Lower	Upper	p-value	R square change
Model 1					**0.021**
Change score self-rated health	-0.111	-0.190	-0.033	0.006*	
Model 2					**0.104**
Change score self-rated health	-0.252	-0.337	-0.216	<0.001*	
Self-rated health baseline	-0.308	-0.401	-0.216	<0.001*	
Model 3					0.003
Change score self-rated health	-0.092	-0.410	0.225	0.567	
Self-rated health baseline	-0.306	-0.399	-0.214	<0.001*	
Interaction[a]	-0.043	-0.124	0.039	0.305	
Boys					
Model 1					0.001
Change score self-rated health	-0.015	-0.081	0.052	0.663	
Model 2					**0.076**
Change score self-rated health	-0.109	-0.184	-0.035	0.004*	
Self-rated health baseline	-0.176	-0.247	-0.105	<0.001*	
Model 3					<0.001
Change score self-rated health	-0.082	-0.317	0.153	0.492	
Self-rated health baseline	-0.175	-0.246	-0.103	<0.001*	
Interaction[a]	-0.007	-0.068	0.053	0.812	

B: Unstandardized beta
*Statistically significant with a p-value of 0.05
Bold: Significant R^2 change
a: Change score self-rated health* self-rated health baseline

Supplementary table 2: *Winning models. Crude and adjusted associations between baseline inflammatory proteins and HSCL-10 at follow-up, assessed by linear regressions. Boys in Fit futures 2010-2011 and 2012-2013.*

Boys

			95 % CI		
	N	*B*	Lower	Upper	*p*-value
CRP Model A	262				
CRP		0.001	-0.025	0.027	0.914
Baseline HSCL-10		0.610	0.466	0.753	<0.001*
Physical activity		0.014	-0.103	0.131	0.809
Sleep duration		-0.014	-0.117	0.088	0.781
Self-rated health		-0.016	-0.095	0.064	0.702
Change score self-rated health		-0.059	-0.130	0.011	0.098
Body fat percentage		-0.043	-0.172	0.085	0.508
CRP*body fat percentage		0.042	0.008	0.075	0.014*
CRP Model B	262				
CRP		0.049	0.026	0.072	<0.001*
Baseline HSCL-10		0.615	0.472	0.757	<0.001*
Physical activity		0.066	-0.056	0.189	0.286
Sleep duration		-0.019	-0.120	0.083	0.720
Self-rated health		-0.023	-0.100	0.053	0.550
Change score self-rated health		-0.065	-0.134	0.005	0.069
CRP*physical activity		-0.045	-0.076	-0.013	0.006*
TGF-α Model 2	279				
TGF-α		0.123	0.042	0.205	0.004*
Baseline HSCL-10		0.621	0.502	0.739	<0.001*
TWEAK Model	274				
TWEAK		0.210	-0.011	0.432	0.062
Baseline HSCL-10		0.598	0.464	0.733	<0.001*
Physical activity		-0.030	-0.143	0.083	0.600
Sleep duration		3.679	0.574	6.785	0.020*
Self-rated health		0.043	-0.115	0.030	0.251
Change score self-rated health		0.070	-0.140	-0.001	0.047*
TWEAK*Sleep duration		-0.046	-0.750	-0.062	0.021*

B: Unstandardized beta
*Statistically significant with a p-value cutoff of 0.05
CRP: C-reactive protein
TGF-α: Transforming growth factor alpha
TWEAK: Tumor necrosis factor-like weak inducer of apoptosis (O43508: TNF-like weak inducer of apoptosis within limits of detection)

Supplementary table 3: *Crude and adjusted associations between baseline inflammatory proteins and the six depressive symptoms from HSCL-10 at follow-up, assessed by linear regressions. The results are presented for girls and boys, respectively. Fit futures 2010-2011 and 2012-2013.*

Girls						
			95 % CI			
	N	*B*	Lower	Upper	*p*-value	R^2 change
CRP						
Model 1	330	0.017	-0.008	0.043	0.174	0.006
Model 2	330	0.016	-0.005	0.036	0.127	**0.342**
Model 3	324	0.004	-0.017	0.024	0.722	**0.070**
IL-6						
Model 1	331	0.086	-0.039	0.211	0.179	0.006
Model 2	331	0.048	-0.054	0.151	0.353	**0.336**
Model 3	325	0.009	-0.094	0.112	0.866	**0.074**
TGF-α						
Model 1	331	0.083	-0.052	0.217	0.227	0.005
Model 2	331	0.104	-0.005	0.213	0.060	**0.343**
Model 3	325	0.076	-0.029	0.181	0.156	**0.072**
TRANCE (TNF)						
Model 1	331	0.007	-0.125	0.138	0.922	<0.001
Model 2	331	0.048	-0.059	0.155	0.374	**0.341**
Model 3	325	0.027	-0.081	0.136	0.620	**0.075**
TWEAK (TNF)						
Model 1	331	0.118	-0.128	0.363	0.345	0.003
Model 2	331	0.149	-0.050	0.349	0.141	**0.342**
Model 3	325	0.184	-0.015	0.384	0.070	**0.078**
Boys						
			95 % CI			
	N	*B*	Lower	Upper	*p*-value	R^2 change
CRP						
Model 1	266	0.025	0.002	0.047	0.030*	**0.018**
Model 2	266	0.031	0.012	0.050	0.002*	**0.249**
Model 3	262	0.029	0.010	0.049	0.004*	0.011
IL-6						
Model 1	279	0.060	-0.049	0.169	0.278	0.004
Model 2	279	0.074	-0.019	0.168	0.118	**0.263**
Model 3	274	0.077	-0.016	0.170	0.105	0.015
TGF-α						
Model 1	279	0.154	0.036	0.272	0.010*	**0.024**
Model 2	279	0.127	0.026	0.229	0.014*	**0.253**
Model 3	274	0.125	0.023	0.226	0.017*	0.014
TRANCE (TNF)						
Model 1	279	-0.017	-0.148	0.115	0.802	<0.001
Model 2	279	0.002	-0.112	0.115	0.979	**0.261**
Model 3	274	0.008	-0.106	0.122	0.890	0.015

TWEAK (TNF)						
Model 1	279	0.049	-0.196	0.295	0.693	0.001
Model 2	279	0.102	-0.110	0.313	0.345	**0.263**
Model 3	274	0.104	-0.107	0.316	0.334	0.015

B: Unstandardized beta
*Statistically significant with a p-value of 0.05
Bold: significant R2 change
CRP: C-reactive protein
IL6-α: Interleukin 6 alpha
TGF-α: Transforming growth factor alpha
TRANCE: Tumor Necrosis Factor-related activation-induced cytokine (O14788: TNF-related activation-induced cytokine within limits of detection)
TWEAK: Tumor necrosis factor-like weak inducer of apoptosis (O43508: TNF-like weak inducer of apoptosis within limits of detection)
Model 1: Crude analysis with the respective inflammatory marker
Model 2: Model 1 + baseline 6 depressive items from HSCL-10
Model 3 girls: Model 2 + current infection, medication intake, sleep duration, hormonal contraceptives, smoking, self-rated health, and change score self-rated health (inclusion criteria 0.1 from simple regressions)
Model 3 boys: Model 2 + physical activity, sleep duration, self-rated health, and change score self-rated health (inclusion criteria 0.1 from simple regressions)

PAPER 3

Title: Two-year changes in sleep duration are associated with changes in psychological distress in adolescent girls and boys. The Fit Futures study.

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Abstract

Objective: Studies indicate an association between sleep duration and psychological distress. We aimed to explore associations between changes in sleep duration and changes in psychological distress in girls and boys.

Methods: The Fit Futures Study is a broad adolescent study providing data from 373 girls and 294 boys aged 15-18 years collected in 2010/2011 (FF1) and 2012/2013 (FF2). Psychological distress was measured by the Hopkins Symptom Checklist (HSCL-10) and sleep duration was self-reported. Change score variables were calculated as the change between baseline and follow-up for sleep duration and HSCL-10, respectively. Associations between changes in sleep duration and changes in HSCL-10 were explored by linear regressions, in gender-stratified analyses.

Results: At FF1, girls and boys slept on average 6.93 (SD=1.08) and 7.05 (SD= 1.20) hours per night respectively, and correspondingly, 6.83 (SD=1.19) and 6.85 (SD= 1.21) at FF2. At FF1, 22.8 % of the girls and 25.8 % of the boys slept ≤ 6 hour per night, and correspondingly 28.0 % and 28.2 % at FF2. Change in sleep duration was associated with a change in HSCL-10 score in girls, *B* [95% *CI*] = -0.090 [-0.131, -0.048], $p < 0.001$, and in boys -0.054 [-0.091, -0.017], $p < 0.001$. The associations remained significant after adjusting for confounders.

Conclusion:
Our findings indicate that sleep duration has an effect on measured psychological distress during adolescence. Future studies should examine the causality between sleep duration and psychological distress.

Introduction

Depressive disorders and anxiety disorders increase dramatically during adolescence [1], especially among girls [2, 3]. Subclinical symptoms of anxiety and depression are even more prevalent in this age group [4]. Such symptoms are often termed "psychological distress", defined as "a state of emotional suffering characterized by symptoms of depression and anxiety" [5]. Self-reported levels of psychological distress in Norway was recently reported to be 31 % and 12 % among girls and boys, respectively [4]. Adolescents with psychological distress are at risk of developing mental disorders [6]. Therefore, exploring potential predictors of psychological distress during adolescence is central for development of effective prevention measures.

According to a review on the sleep-deprived human brain, sleep loss has been associated with negative emotional processing, including irritability, emotional volatility, and anxiety [7]. Neuroimaging studies have shown that these emotional changes are associated with increased activity in the amygdala and with decreased connection between amygdala and the prefrontal cortex [7]. The change in brain activity indicates that short sleep duration hampers the prefrontal cortex's ability to execute regulatory control of emotions [7]. Sleep loss seems to reduce the restoration of central noradrenergic signalling that occurs during a full night of sleep [7], and in this respect, it might play a pivotal role in mood and anxiety disorders [8]. Interestingly, sleep loss has been shown to reduce positive mood more than to increase negative mood, and anhedonia has been associated with reductions in non-rapid eye movement (NREM) slow-wave sleep [9, 10]. Sufficient amount of rapid eye movement (REM) sleep has been linked with the overnight dissipation of emotional sensitivity, and too little REM sleep has been associated with increased activity in areas in the limbic brain involved in negative mood [11]. Furthermore, sleep loss has been associated with a negative emotional memory dominance [12]. This memory bias may in the long run contribute to depression [12]. Additionally, inadequate sleep has been shown to lead to higher-order-emotional-dysfunction, including interpersonal conflict, social withdrawal and loneliness, [11], which in turn have been associated with depression and anxiety [13]. A meta-analysis on prospective associations between short or long sleep and depression in adults, support the mechanistic linkage between sleep duration and clinical depression [14].

To our knowledge, only a few studies have investigated the relation between sleep duration and non-clinical mental health outcomes. Two studies in adults have reported both cross-sectional and prospective associations between sleep duration and psychological distress [15, 16]. In young adults 17-24 years of age, short sleep duration was associated with psychological distress cross-sectionally [17]. Yet only individuals sleeping below 5 hours per night had increased symptoms 12-18 months later. Nevertheless, the literature on non-clinical outcomes important for mental health prevention is limited in this context.

The American Academy of Sleep Medicine and the National Sleep Foundations recommend that young people aged 13-18 years should sleep 8-10 hours every night [18, 19]. However, most adolescents do not meet this recommendation [20]. In a large study on Norwegian

adolescents aged 16-18 years, mean sleep duration on weekdays was 6 hours and 25 minutes [21]. This finding is consistent with a report concluding that adolescent sleep loss and sleepiness are serious public health issues [22]. A meta-analysis including adolescents 12-20 years of age found that sleep disturbances are more common among adolescents with depressive disorders compared to age-matched healthy controls. Furthermore, the same study claimed that sleep disturbance was more likely to be prospectively associated with depression rather than in the opposite direction [23]. In healthy adolescents, a systematic review and meta-analysis concluded that sleep duration was both cross-sectionally and prospectively associated with mood (depressed mood, anxiety, anger, negative affect and positive affect) [24].

Due to feasibility, psychological distress measurements are commonly used to screen mental health in healthy populations [25]. Yet, few studies have investigated associations between sleep duration and psychological distress in the important adolescent transition phase. One cross-sectional study on Swedish adolescents aged 13-18 years reported a negative correlation between sleep duration and psychological distress [26]. Similar, a study on 419 youths 15 years of age from the US found associations between both short and long sleep duration and psychological distress the following day [27]. In contrast, no association between sleep duration and psychological distress was reported in a study on 12 year-olds [28]. Based on this inconsistency, more prospective studies to investigate age-related changes in sleep and psychological distress are warranted [28]. Indeed, there are indications that sleep duration at the age of 15 is prospectively associated with both anxiety and depression symptoms as long as six years later [29]. In addition, the association between insomnia and depressive symptoms have been reported to be stronger in girls than in boys [30], suggesting that investigations on the association between sleep duration and psychological distress should be gender stratified.

Overall, there is a scarcity of studies on prospective associations between sleep duration and psychological distress in healthy adolescents. Exploring this association is central for prevention of psychological distress and mental disorders. Previous findings suggest that such examinations should be gender stratified. The aim of this study is to explore associations between change in sleep duration and change in psychological distress in adolescent girls and boys.

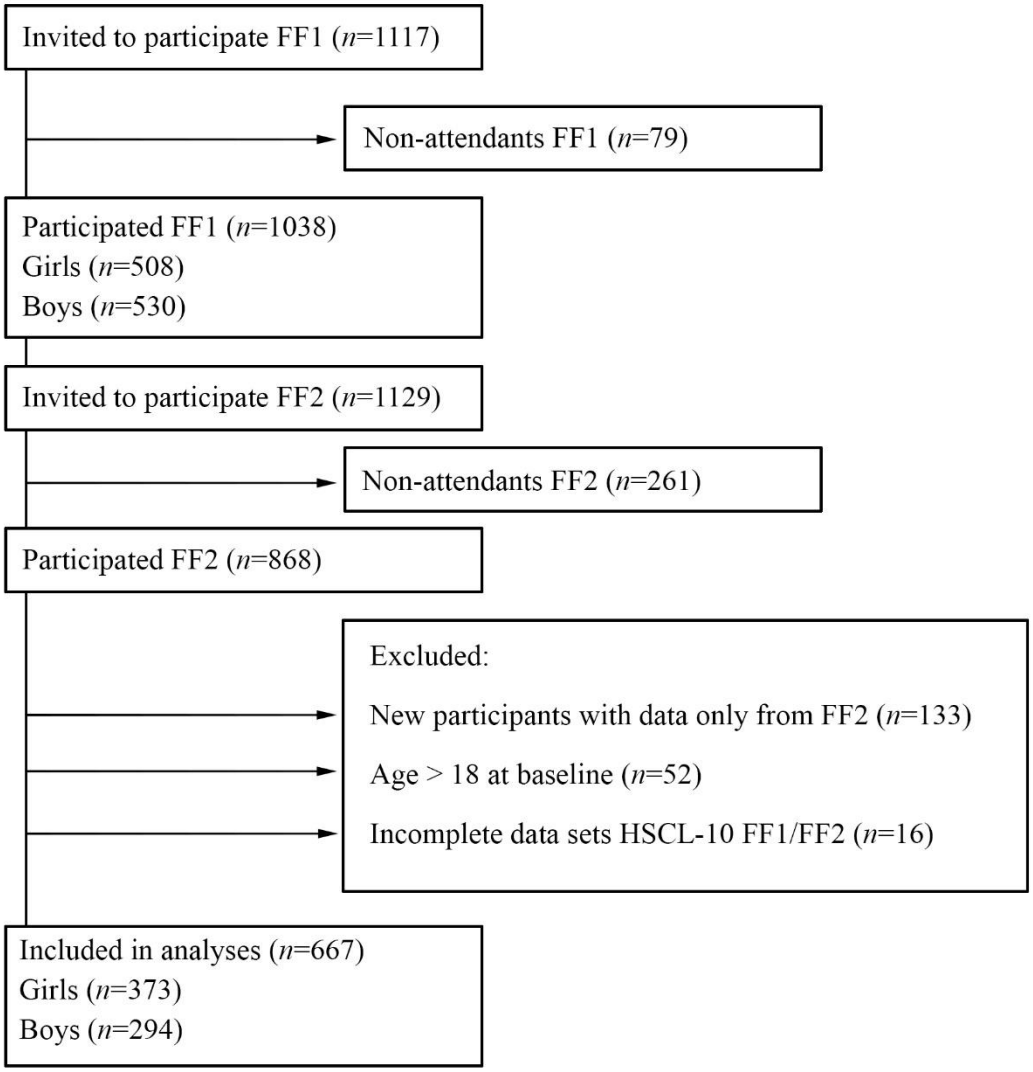
Materials and methods

Study population and design

All first-year upper secondary school students from two municipalities in Northern Norway in 2010-2011 were invited to join the Fit Futures Study (FF1), a comprehensive health study. Two years later, in 2012-2013, a follow-up study was conducted (FF2). Further details about Fit Futures is available elsewhere [31]. Data was collected at the Clinical Research Unit, the University Hospital of North Norway (UNN) in Tromsø. At FF1, 1117 students were invited to participate, of which 1038 (92.9%) attended (FF1). At FF2, all participants that attended FF1 and all third-year upper secondary school students were invited, where a total of 868 attained. After exclusion of participants aged 19 years or older at baseline, our final sample

consisted of 667 participants with complete data on the outcome variable, Hopkins Symptom Check List (HSCL-10) at both FF1 and FF2 (Figure 1). Participants lost to follow-up did not differ on baseline data for neither sleep duration nor HSCL-10 items (data not shown).

Figure 1. *Study flowchart, Fit Futures (FF1) 2010-2011 and Fit Futures 2 (FF2) 2012-2013.*



HSCL-10: Hopkins Symptom Checklist

All participants provided written informed consent. For participants below 16 years of age, written informed consent was also provided from a parent/guardian. Our study was conducted according to the Declaration of Helsinki. The Norwegian Data Protection Authority (reference number 2009/1282) and The Regional Committee of Medical and Health Research Ethics (reference number: 2019/60811/REK Nord) approved the study.

Measurements and questionnaires

A web-based general questionnaire was used to collect data about lifestyle, health, and disease. Clinical examinations, collection of blood samples and interviews (on use of contraceptives, presence of acute or chronic diseases) were conducted by qualified research nurses.

Outcome: Hopkins Symptom Checklist (HSCL-10)

Psychological distress was measured with HSCL-10, which was included in the web-based questionnaire at both time-points. HSCL-10 has been reported to have high reliability and validity [32]. The scale contains six items measuring symptoms of depression and four items measuring symptoms of anxiety during the last week [32]. Severity of symptoms are reported as "none" (1), "slightly" (2), "much" (3), and "very much" (4). Cronbach's alpha for girls were 0.87 and 0.93 at baseline and follow up, and correspondingly 0.82 and 0.87 for boys. The individual score of the 10 items was calculated for baseline and follow-up respectively and used in cross-sectional models. A change score variable (FF2-FF1) was created and used as outcome variable in the prospective models. There were 373 girls and 294 boys with complete data on HSCL-10 at both time-points.

Main exposure variable: Sleep duration

Sleep duration was measured with one question in the web-based questionnaire: "How many hours sleep do you normally get per night?" at both time-points. The lowest category was "four hours or less". The successive categories increased 0.5 hours per category ("4.5 hours", "5 hours", "5.5 hours" etc.), except for the highest category that was "12 hours or more". The lowest category was coded as 4 hours, and the highest as 12 hours.

These numerical variables were used in cross-sectional models. A categorical variable was created to describe short and long sleep duration at baseline and follow, up respectively. The three categories were: "≤6 hours", ">6 to 9 hours" and ">9 hours". A change score variable for sleep duration (FF2-FF1) was calculated and served as exposure in the prospective models. There was 372 girls and 291 boys with data on sleep duration at both time-points.

Potential confounders

Based on literature we assessed potential confounders [14, 33]. For variables with data from both time-points, we assessed the change scores (FF2-FF1).

High school program was collected from the school administration system with the alternatives: "general studies", "sports and physical" and "vocational".

Lifestyle and health variables from questionnaire:

Alcohol-intake was reported at both time points from "never" (1) to "four or more times per week" (5), and was recoded in three categories "never", "once per month or less" and "twice or more per month". At baseline, smoking and snuff use were reported as "no, never" (1), "sometimes" (2) and "daily" (3). At follow-up, smoking and snuff use were reported as "no,

never" (1), "in the past, but not now" (2) "sometimes" (3) and "never" (4), and "no, never" (1) and "in the past, but not now" (2) were collapsed into one category: "no, never" (1). Physical activity was reported by the Saltin-Grimsby physical activity level scale at both time points, which measures physical activity in leisure time, and specifies type of activity and intensity in a typical week in the last year [34], by the four labels "physically inactive" (1), "some light physical activity" (2), "regular physical activity and training" (3), "regular hard physical activity (for competitive sports)" (4).

Menarche was reported by one question at baseline "When did you have your first menstruation?" and answered in whole years. Self-reported menarche in girls has been shown to be reliable [35]. We used Pubertal Development Scale (PDS) [35, 36] to measure pubertal status in boys at baseline, with four questions about growth spurt, pubic hair growth, changes in voice, and facial hair growth. All four response alternatives were: "have not begun" (1), "barely started" (2), "underway" (3), and "completed" (4). We created an individual mean score and categorized into four categories describing pubertal development: "not begun" (mean score below 2) "barely started" (mean score from 2 to < 3), "underway" (mean score from 3 to < 4) and "completed" (mean score of 4).

Self-rated health was reported with the question at both time-points: "How do you in general consider your own health to be?", answered by five: "very bad" (1), "bad" (2), "neither bad nor god" (3), "good" (4), and "excellent" (5). This measure has been shown to predict mortality [37], and morbidity in a general population [38].

Variables on health and medication from clinical measurements and interview:
Height and weight were measured without shoes and in light clothing at both time points. An automatic electronic scale, The Jenix DS 102 stadiometer (Dong Sahn Jenix, Seoul, Korea) was used to measure weight. Body Mass Index (BMI) was calculated as weight (kg) divided by height in meters squared (m^2).

Chronic disease was reported with one question at baseline: "Do you have any chronic or persistent disease?", and a dichotomous variable was created. Current infection was reported by one question at both time-points: "Do you have any kind of infection (e.g., respiratory, urinary tract, skin)?", and a dichotomous variable was created. Use of contraceptives was reported by one question at baseline: "If you have started menstruating; do you use any kind of contraceptives?", and a dichotomous variable was created.

Biomarker variables from laboratory measurements:
Non-fasting blood samples were drawn from an ante-cubital vein, and serum separated and stored at −70 °C for measurements of Vitamin D and inflammatory biomarkers. We measured Vitamin D status by serum 25-hydroxyvitamin D (25(OH)D) at both time-points using high pressure liquid chromatography mass spectroscopy (LC-MS/MS) at the Hormone laboratory, Haukeland University Hospital, Norway [39]. The analysis was rerun on a subsample (n=168) from FF1 at the Cork Centre for Vitamin D and Nutrition Research, Ireland [40] to standardize the results in accordance with the Vitamin D Standardization program (VSDP).

We used the standardized version of 25(OH)D (nmol/L). High-sensitive C-reactive protein (hs-CRP) was analyzed at UNN, Norway and assessed by a particle-enhanced immunoturbidimetric assay, on a Modular P autoanalyser (Roche Diagnostics, Mannheim, Germany) with a detection limit of 0.12 mg/L.

We used Protein extension array technology (Proseek Multiplex Inflammation panel; Olink Bioscience, Uppsala, Sweden) for relative quantification of proteins associated to inflammation in serum samples in FF1. Further details concerning the analysis are presented elsewhere [41]. Interleukin-6 (IL-6), (Transforming growth factor-alpha (TGF-α) Tumor necrosis factor alpha (TNF-α) variant 1 (TRANCE), and TNF-α variant 2 (TWEAK) were explored as potential confounders.

Statistical analysis

The statistical analyses were conducted with the Statistical Package of Social Science (SPSS v. 28). We chose a significance level of $p < 0.05$ as an indication of statistical significance. We conducted residual analyses to assess linearity, distribution, variance homogeneity and to explore outliers. We tested exposure variables and potential confounders for multicollinearity. Since girls have been shown to exhibit higher levels of psychological distress than boys in adolescence [4] and because female gender is an independent risk factor for insomnia [42], we did all analyses separately for girls and boys.

We conducted independent samples t-tests to compare sleep duration in girls and boys. This was done at both baseline and follow-up. We conducted paired samples t-tests to compare sleep duration at baseline and follow-up, stratified on gender. Baseline characteristics are presented for three categories of sleep duration and gender-stratified, with means and standard deviations for continuous variables, and proportions for categorical variables. One-way ANOVA was used to compare continuous demographic data across the three categories of sleep duration. When Levene's test indicated unequal variances, Welch's corrected F- and p-values are reported. Fisher's exact test was used to compare categorical data across sleep duration.

Linear regressions were conducted to estimate the unstandardized beta regression coefficients and 95% confidence intervals (CI) between sleep duration and HSCL-10 (outcome variable), cross-sectionally, at baseline and follow-up separately. To explore prospective associations, similar linear regressions were conducted to estimate the effect of change in sleep duration on change in HSCL-10. Crude associations between the change score of sleep duration and change score of HSCL-10 were estimated (Model 1). Potential confounders were tested: For variables with data from both time-points, we assessed the change scores (FF2-FF1), and for variables without data from FF2, we assessed variables from FF1. We adjusted for change scores of the potential confounders because they are not stationary, but rather fluctuate over time. Confounders were included in multivariable regression analysis (Model 2) when the p-value was below 0.10.

Due to the item “Have you experienced sleeplessness during the last week?” was present in HSCL-10, we did supplementary analyses without it to ensure that our findings are not confounded by this item. The mean of the remaining 9 items was calculated and used as outcome in cross-sectional analyses at baseline and follow-up, and the change score for HSCL-10 without the sleep item was calculated and used as outcome in the prospective analyses. Finally, we did supplementary analyses adjusting for baseline values of potential confounders, instead of using the change scores.

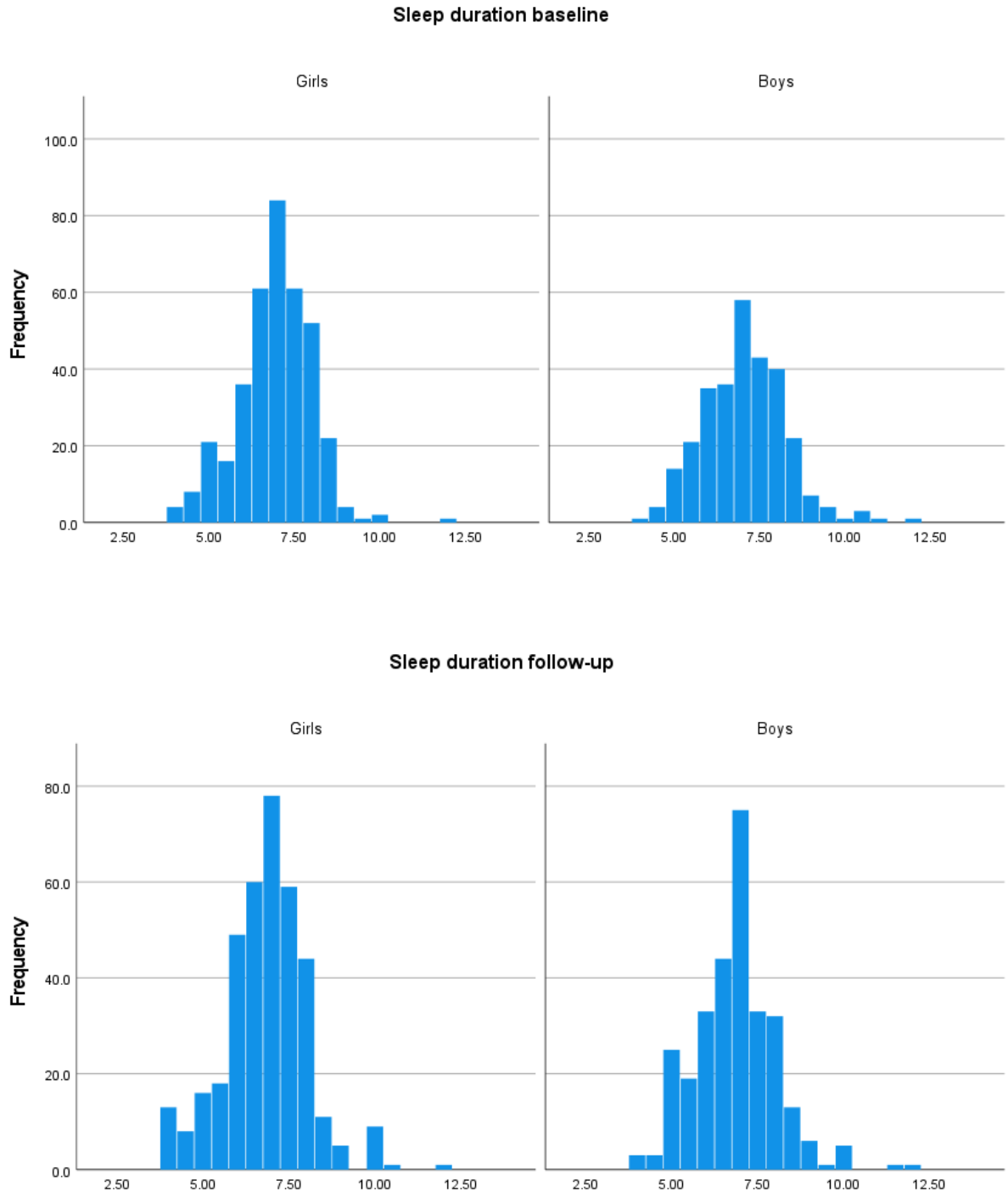
Results

Description of sleep duration and psychological distress

In both genders, sleep duration was close to normally distributed at both time-points (Figure 2). At baseline, 22.8% of the girls and 25.8% of the boys reported sleep duration of ≤ 6 hours. At follow-up, the corresponding percentages were 28.0% and 28.2%. At baseline, 1.1% of the girls and 3.4% of the boys reported sleep duration of > 9 hours. At follow-up, the corresponding percentages were 3.0% and 2.7% respectively. In girls, the mean (SD) sleep duration of 6.93 (1.08) hours at baseline was not significantly different from a mean sleep duration of 6.83 (1.21) hours at follow-up, $p = 0.170$. Boys reported a mean (SD) sleep duration of 7.05 (1.20) hours at baseline and 6.85 (1.19) hours at follow-up, a decrease from baseline to follow-up which was statistically significant, $p = 0.007$. Sleep duration among girls was not significantly different from sleep duration among boys at baseline, $p = 0.154$. Nor was there a gender-difference in sleep duration at follow-up, $p = 0.839$.

In girls and boys, mean (SD) of HSCL-10 was 1.59 (0.56) and 1.36 (0.41) at baseline and 1.69 (0.64) and 1.41 (0.48) at follow-up, respectively.

Figure 2: *Sleep duration in hours at baseline and follow up, in girls and boys respectively. Fit Futures 2010-2011 and 2012-2013.*



Baseline characteristics

In girls, HSCL-10 scores differed across categories of sleep duration. The highest level was seen in those who slept ≤6 hours. Further, girls that slept ≤6 hours smoked more, were less physically active and drank more alcohol compared to girls that slept longer. Also, in boys, those who slept ≤6 hours had higher levels of HSCL-10 compared to those who slept longer. A higher proportion of boys who slept ≤6 hours smoked, snuffed, and drank alcohol compared to those who slept longer. A higher proportion of boys that attended a vocational high school slept ≤6 hours compared to boys that attended general studies.

Table 1: *Baseline characteristics for girls and boys, stratified on sleep duration. Fit Futures 2010-2011.*

	Girls							**Boys**						
	≤6 hours		>6 hours to >9 hours		≥9 hours			≤6 hours		>6 hours to >9 hours		≥9 hours		
	n	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD)	*p*-value*	*n*	Mean (SD)	*n*	Mean (SD)	*n*	Mean (SD	*p*-value*
Age (years)	85	16.11 (0.38)	280	16.16 (0.44)	8	16.13 (0.84)	0.513	75	16.17 (0.55)	199	16.08 (0.48)	17	16.12 (0.78)	0.414
Self-rated health	85	3.58 (0.84)	277	4.05 (0.71)	8	3.50 (0.54)	0.087	74	3.96 (0.86)	199	4.24 (0.86)	17	3.94 (0.90)	0.210
HSCL-10	85	1.86 (0.69)	280	1.51 (0.48)	8	1.71 (0.67)	**<0.001**	75	1.50 (0.48)	199	1.31 (0.36)	17	1.32 (0.47)	**0.014**
Body height (cm)	85	1.64 (6.22)	279	165.24 (6.73)	8	165.59 (7.34)	0.463	75	176.49 (6.85)	199	177.69 (6.26)	17	179.36 (6.46)	0.184
Body weight (kg)	85	59.56 (11.21)	279	60.75 (9.99)	8	62.95 (21.08)	0.659	75	70.99 (16.36)	199	69.50 (13.10)	17	74.98 (16.64)	0.271
BMI (kg/m^2)	85	22.13 (4.38)	279	22.24 (3.38)	8	22.82 (6.90)	0.948	75	22.73 (4.68)	199	21.97 (3.71)	17	23.28 (5.02)	0.214
Age menarche (years)	85	12.58 (1.31)	274	12.73 (1.08)	8	12.88 (0.99)	0.501							
hs-CRP (mg/ L)	69	1.88 (3.00)	253	1.41 (3.24)	8	1.07 (1.44)	0.512	64	1.72 (4.60)	183	1.25 (2.37)	16	1.33 (1.46)	0.740
IL-6 (NPX)	69	3.08 (9.67)	254	2.79 (0.49)	8	2.80 (0.49)	0.086	68	2.80 (0.50)	191	2.88 (0.67)	17	2.54 (0.55)	0.082
TGF- α (NPX)	69	3.94 (0.54)	254	3.88 (0.60)	8	3.98 (0.70)	0.711	68	3.64 (0.62)	191	3.57 (0.57)	17	3.40 (0.44)	0.281
TRANCE (NPX)	69	5.52 (0.63)	254	5.50 (0.62)	8	5.81 (0.35)	0.351	68	5.99 (0.52)	191	6.02 (0.51)	17	6.05 (0.61)	0.901
TWEAK (NPX)	69	8.89 (0.34)	254	8.89 (0.32)	8	8.80 (0.31)	0.726	68	9.02 (0.31)	191	9.03 (0.27)	17	8.95 (0.25)	0.500

Vitamin D (nmol/L)	69	41.51 (18.66)	254	45.03 (16.91)	8	39.56 (12.47)	0.242	69	33.44 (16.48)	191	34.53 (14.81)	17	38.38 (18.34)	0.499
Smoking	85		280		8		**0.001**	75		199		17		**0.002**
No, never	56	65.9%	240	85.7%	8	100.0 %		51	68.0%	171	85.9%	14	82.4%	
Sometimes	24	28.2%	34	12.1%	0	0.0 %		18	24.0%	27	13.6%	3	17.6%	
Daily	5	5.9%	6	2,1%	0	0.0%		6	8.0%	1	0.5%	0	0%	
Snuffing	85		280		8		0.298	75		198		17		**0.010**
No, never	52	61.1%	198	70.7%	7	87.5%		38	50.7%	143	72.2%	11	64.7%	
Sometimes	14	16.5%	40	14.3%	1	12.5%		11	14.7%	21	10.6%	1	5.9%	
Daily	19	22.4%	42	15.0%	0	0%		26	34.7%	34	17.2%	5	29.4%	
Alcohol intake	85		280		8		**0.024**	74		199		17		**<0.001**
Never	14	16.5%	77	27.5%	4	50.0%		16	21.6%	76	38.2%	9	52.9%	
Once per month or less	37	43.5%	132	47.1%	3	37.5%		27	36.5%	84	42.2%	3	17.6%	
Twice or more per month	34	40.0%	71	25.4%	1	12.5%		34	45.9%	39	19.6%	5	29.4%	
Physical activity	85		280		8		**0.015**	75		199		17		0.331
Physically inactive	15	17.6%	32	11.4%	0	0.0%		24	32.0%	52	26.1%	4	23.5%	
Some light physical activity	42	49.4%	103	36.8%	4	50.0%		16	21.3%	51	25.6%	3	17.6%	
Regular physical activity and training	22	25.9%	86	30.1%	2	25.0%		21	28.0%	48	24.1%	2	11.8%	
Regular hard physical activity (for competitive sports)	6	7.1%	59	21.1%	2	25.0%		14	18.7%	48	24.1%	8	47.1%	

Current infection	85		278		8		0.667	75		198		17		0.330
No	74	87.1%	236	84.9%	8	100.0%		64	85.3%	165	83.3%	12	70.6%	
Yes	11	12.9%	42	15.1%	0	0.0%		11	14.7%	33	16.7%	5	29.4%	
Chronic disease	85		278		8		0.725	75		197		17		
No	61	71.8%	187	67.3%	6	75.0%		59	78.7%	141	71.6%	9	52.9%	
Yes	24	28.2%	91	32.7%	2	25.0%		16	21.3%	56	28.4%	8	47.1%	
Hormonal contraceptives	85		277		8		0.083							
No	44	51.8%	179	64.6%	6	75.0%								
Yes	41	48.2%	98	35.4%	2	25.0%								
PDS status								63		158		16		0.358
Not begun								0	0.0%	0	0.0%	0	0.0%	
Barely started								7	11.1%	16	10.1%	0	0.0%	
Underway								48	76.2%	111	70.3%	11	68.8%	
Completed								8	12.7%	31	19.6%	5	31.3%	
High School Program	85		280		8		0.219	75		199		17		**0.006**
General Studies	44	51.8%	155	55.4%	4	50.0%		24	32.0%	81	40.7%	2	11.8%	
Sports and Physical	4	4.7%	31	11.1%	0	0.0%		6	8.0%	31	15.6%	6	35.3%	
Vocational	37	43.5%	94	33.6%	4	50.0%		45	60.0%	87	43.7%	9	52.9%	

* One-way ANOVA was used to compare continuous data across the sleep duration categories. When Levene's test indicated unequal variances, Welch's corrected F- and p-values are reported. Fisher's exact test was used to compare categorical data across sleep duration.
Bold: Statistically significant with a p-value of 0.05
Mean (SD) of continuous variable and percentages of categorical variables are reported
BMI: Body Mass Index
hs-CRP: high-sensitive C-reactive protein
IL6-α: Interleukin 6 alpha
TGF-α: Transforming growth factor alpha
TRANCE: Tumor Necrosis Factor-related activation-induced cytokine (O14788: TNF-related activation-induced cytokine within limits of detection)
TWEAK: Tumor necrosis factor-like weak inducer of apoptosis (O43508: TNF-like weak inducer of apoptosis within limits of detection)

NPX: Normalized protein expression
Vitamin D: Standardized version of (25-OH)D
PDS status: Pubertal Development Scale status

Cross-sectional associations between sleep duration and HSCL-10

At baseline, a 30-minute increase in sleep duration (one unit) was associated with a significant lower level of HSCL-10 in girls B [95% CI] = -0.132 [-0.183, -0.081] and boys, B [95% CI] = -0.089 [-0.127, -0.051]. At follow-up, the corresponding crude associations were significant among girls, *B* [95% CI] = -0.134 [-0.186, -0.082] and boys, *B* [95% CI] = -0.087 [-0.132, -0.042] (Table 2).

Associations between change in sleep duration and change in HSCL-10

In girls, increasing sleep duration by 30 minutes from baseline to follow-up (one unit) was associated with a significant decrease in HSCL-10 from baseline to follow-up *B* [95% CI] = -0.090 [-0.131, -0.048]. In boys, the corresponding association was also significant, *B* [95% CI] = -0.054 [-0.091, -0.017]. The associations remained significant after adjustment for potential confounders in both girls, *B* [95% CI] = -0.068 [-0.109, -0.027] and boys, *B* [95% CI] = -0.078 [-0.125, -0.031] (Table 2).

Table 2: *Crude and adjusted associations between sleep duration and psychological distress (Hopkins Symptom Check List HSCL-10), assessed by linear regressions, 1 unit sleep duration equals 30 minutes. The results are presented for girls and boys, respectively. Fit Futures from baseline 2010-2011 and follow-up in 2012-2013.*

Girls

			95 % CI			
	n	*B*	Lower	Upper	*p*-value	R^2
Baseline univariate	373	-0.132	-0.183	-0.081	<0.001*	0.066
Follow-up univariate	372	-0.134	-0.186	-0.082	<0.001*	0.065
Model 1	372	-0.090	-0.131	-0.048	<0.001*	0.047
Model 2	361	-0.068	-0.109	-0.027	0.001*	0.137

Boys

			95 % CI			
	n	*B*	Lower	Upper	*p*-value	R^2
Baseline univariate	291	-0.089	-0.127	-0.051	<0.001*	0.069
Follow-up univariate	294	-0.087	-0.132	-0.042	<0.001*	0.048
Model 1	291	-0.054	-0.091	-0.017	0.005*	0.027
Model 2	117	-0.078	-0.125	-0.031	0.001*	0.376

B: Unstandardized beta
*Statistically significant with a p-value cutoff of 0.05

Baseline univariate: Crude analysis baseline
Follow-up univariate: Crude analysis follow-up
Model 1: Crude analysis with change score sleep duration as exposure and change score Hopkins Symptom Checklist (HSCL-10) as outcome
Model 2 for girls: Model 1 + use of contraceptives, change score smoking, change score snuffing, change score physical activity and change score self-rated health
Model 2 for boys: Model 1 + chronic disease, change score current infection, change score self-rated health, change score smoking, high-sensitive C-reactive protein, interleukin 6 alpha and transforming growth factor alpha

Supplementary analysis
Removing the sleep item from HSCL-10, did not change the significant cross-sectional findings from neither baseline nor follow-up. Similarly, the significant associations between change in sleep duration and change in HSCL-10 remained significant when excluding the sleep item (Supplementary table 1)**.**

Adjusting for potential confounders from baseline only, did not change the results (data not shown).

Discussion
In this population-based prospective study on adolescents between 15-18 years of age, we found significant prospective associations between sleep duration and psychological distress in both girls and boys. Reduced sleep duration was associated with increased psychological distress. Inverse cross-sectional associations between sleep duration and psychological distress were also apparent at baseline as well as at follow-up. With an average sleep duration of 7 hours per night, this sample slept less than the recommended 8-10 hours. In both genders, a large proportion slept less or equal to 6 hours at both time-points, and only a few slept over 9 hours per night.

After adjusting for confounders, we found that one unit (30 minutes) increase in sleep duration from baseline to follow-up gave a decrease in HSCL-10 score from baseline to follow-up of 0.068 in girls and 0.078 in boys. Previous reports indicate that the association between insomnia and depressive symptoms is stronger in girls compared to boys [30]. However, in the present study the associations were significant in both genders. Our findings on prospective associations between change in sleep duration and change in psychological distress support results of Fuligni et al. [27]. In a study including approximately 750 healthy adolescents 14 to 15 years of age, they found that short sleep duration was correlated with increased psychological distress the following day. To consider individual differences in the association between sleep duration and psychological distress, Fuligni et al. [27] used daily measurements for a 2-week period. In our study, we applied change scores over two years as an approach to assess such individual differences in sleep need. Both approaches indicate significant relations between sleep duration and psychological distress. Frequent sleep and distress measurements as conducted by Fuligni et al. [27], and the use of change scores as in our study indicate significant relations between sleep duration and psychological distress. Studies have reported prospective associations between sleep duration and psychological distress with longer time lags. A study by Orchard et al. [29] reported that sleep duration on school nights among adolescents 15 years of age predicted psychological distress at the age of 21 [29]. Thus, our findings of prospective associations between change in sleep duration and change in psychological distress over two years are consistent with studies both with shorter and longer time lags.

Our finding that an increase of 30 minutes in sleep from baseline to follow-up was associated with a decrease in psychological distress from baseline to follow-up may be clinically relevant in adolescents that are sleep deprived. In support, two studies that evaluated the effect from delayed school start [43, 44], and a study that evaluated a sleep education program for adolescents [45] reported that an increase of approximately 30 minutes of sleep duration was associated with decreased psychological distress and decreased depressed mood. In their randomized controlled trial (RCT), Bonnar et al. [45] found that an increase of 27 minutes of sleep duration in school nights in the intervention group, resulted in a decline in depressed mood compared to the control group.

On the other hand, there are findings pointing towards no prospective associations between sleep duration and symptoms of depression and anxiety. Doane et al. [46] found no significant prospective associations between sleep duration and symptoms of anxiety and depression, in adolescents 18 years of age at baseline. Nevertheless, their sample size was small (n=71), and the association might have been significant with a bigger sample size. A larger study by Fan et al. [47] found no association between sleep duration at baseline and depressive symptoms at follow-up one year later in adolescents 15 years of age at baseline. A difference from our study is that Fan et al. [47] adjusted for baseline depressive symptoms, whereas we in our study used change scores of sleep duration and psychological distress as exposure and outcome respectively. Adjusting for baseline depressive symptoms examines whether sleep duration at baseline is associated with depressive symptoms at follow-up, whereas using change scores examines whether a change in sleep duration from baseline to follow-up is associated with a change in psychological distress from baseline to follow-up. Lovato et al. [48] suggested that effect from insufficient sleep on depressed mood may be relatively short. Our approach with change scores may be said to consider such short effects from sleep duration by including sleep duration at follow-up in the exposure. Our cross-sectional associations between sleep duration and psychological distress support short effects from sleep duration.

Overall, literature shows mixed findings on prospective associations between sleep duration and psychological distress during adolescence. Several studies have reported significant associations, both with shorter and longer time lags than ours. Our results support results indicating that an increase of 30 minutes of sleep duration is clinically relevant for reductions in psychological distress. According to Short et al. [24], the mixed findings on associations between sleep duration and mood may be due to different operationalization of sleep duration.

Our findings on mean sleep duration of approximately 7 hours are in line with a review and meta-analysis on adolescents from Europe, America and Asian countries [49], where mean sleep duration was found to be approximately 7 hours in participants 15-18 years of age. Our findings also correspond with a study from Norway, on 4,010 first-years high school students, aged 16-17 years, who reported a mean sleep duration of 6 hours and 43 min on school nights [50]. A study on 4,175 American adolescents aged 11-17 years at baseline reported that 20% slept $\leq$ 6 hours at baseline and 17% slept below 6 hours at follow-up [51]. These percentages

are somewhat below the percentages that slept ≤6 hours in our study, however the sample in the referenced study is younger than the sample in our study.

This study has several strengths. The participants were healthy adolescents, and the attendance rate was very high. Adolescents are understudied in general and with respect to the prospective association between sleep duration and psychological distress. All analyses were performed gender-stratified since earlier studies have shown gender-differences in prevalence of both psychological distress and associations between sleep duration and depressive symptoms. Our study examined several potential confounders. Finally, we explored associations between change in sleep duration and change in psychological distress. This approach considers individual differences in sleep need, which has been recommended [27], and takes into account the possibility that the effect from sleep duration may be short [48].

This is an observational study, and we cannot exclude residual confounding. There may be other factors that have caused changes in both sleep duration and psychological distress. We did not use polysomnography which is regarded as the gold standard for sleep assessment [52]. Neither did we have data on whether our participants slept during daytime nor differentiated data on sleep duration on weekdays and weekends. Other studies [15, 53] have used a question about sleep duration from the Pittsburgh Sleep Quality Index that is like the item we used in the present study. Stratification on gender reduces statistical power. However, our sample sizes were big enough to detect significant associations in both genders, in crude and adjusted analyses. The beta-values are relatively small, and the clinical relevance may be questioned. On the other side, small effect-sized are common in psychological research [54]. Our assumption that changes in sleep duration represents the same exposure across the scale may be questioned. For example, a change in sleep duration from 6.0 to 6.5 hours, may be different from a change from 8.0 to 8.5 hours. The association between change in sleep duration and change in psychological distress may be dependent upon sleep duration level at baseline. There will probably be an upper threshold at which additional sleep will not yield lower levels in psychological distress. Most of the adolescents slept below the recommended hours, and thus it is likely that increasing sleep duration with 30 minutes may be beneficial for most of the participants. There are individual differences in sleep need [55], hence it may be that change in sleep duration is the most precise predictor of psychological distress.

Conclusion

In this study, short sleep duration was highly prevalent in healthy adolescent girls and boys. Increased sleep duration from baseline to follow-up was associated with decreased psychological distress from baseline to follow-up, in both genders, and vice versa, decreased sleep duration was associated with increased psychological distress. Due to variability in results of the existing research, there is a need for more studies examining associations between sleep duration and psychological distress.

Declarations

Acknowledgments
The authors are grateful to the study participants, the Centre for Clinical Research and Education, The University Hospital of North Norway, and the Fit Futures and the Tromsø Study administration.

Ethics approval and informed consent
All participants provided informed consent. Participants below 16 years additionally provided written informed consent from a parent/guardian. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Norwegian Data Protection Authority (reference number 2009/1282). The Regional Committee of Medical and Health Research Ethics has also approved the study (reference number 2011/1702/REK Nord), and the present project (reference number: 2019/60811/REK Nord).

Disclosure of interest
The authors report no conflict of interest

Author Contributions
N.E., A.S.F, and L.A.A. contributed to the study conception and design. Material preparation, data collection and analysis were performed by J.L., T.C., L.A.A, and G.C. The first draft of the manuscript was written by J.L. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement
Data may be obtained from a third party, UiT – The Artic University of Norway. Restrictions apply to the availability of these data, which were used under license for the current study, and are thus not publicly available.

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Supplementary

Supplementary Table 1: *Crude and adjusted associations between sleep duration (1 unit = 30 minutes) and Hopkins Symptom Check List (HSCL-10) without the sleep item, assessed by linear regression. The results are presented for girls and boys, respectively. Fit Futures baseline in 2010-2011 and follow-up in 2012-2013.*

Girls

			95 % CI			
	n	*B*	Lower	Upper	*p*-value	R^2
Baseline univariate	373	-0.122	-0.173	-0.071	<0.001*	0.057
Follow-up univariate	372	-0.120	-0.173	-0.067	<0.001*	0.051
Model 1	372	-0.062	-0.099	-0.024	0.001*	0.028
Model 2	361	-0.043	-0.080	-0.006	0.025*	0.120

Boys

			95 % CI			
	n	*B*	Lower	Upper	*p*-value	R^2
Baseline univariate	291	-0.075	-0.113	-0.038	<0.001*	0.053
Follow-up univariate	294	-0.076	-0.121	-0.031	0.001*	0.036
Model 1	291	-0.042	-0.074	-0.010	0.010*	0.023
Model 2	117	-0.067	-0.109	-0.025	0.002*	0.184

B: Unstandardized beta
*Statistically significant with a p-value of 0.05

Baseline univariate: Crude analysis baseline
Follow-up univariate: Crude analysis follow-up
Model 1: Crude analysis with change score sleep duration as exposure and change score Hopkins Symptom Checklist (HSCL-10) without the sleep item as outcome
Model 2 for girls: Model 1 + use of contraceptives, change score smoking, change score snuffing, change score physical activity and change score self-rated health
Model 2 for boys: Model 1 + chronic disease, change score current infection, change score self-rated health, change score smoking, high-sensitive C-reactive protein, interleukin 6 alpha and transforming growth factor alpha

Appendix A

Ethical Approval 2019/60811/REK nord

Approval from Norwegian Social Science Data Service

Norwegian Versions

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK nord	Lill Martinsen		18.12.2019	60811
			Deres referanse:	

Tore Christoffersen

60811 Innflytelsen av inflammasjon og døgnrytme på symptomer av psykologisk stress blant ungdommer

Forskningsansvarlig: UiT Norges arktiske universitet

Søker: Tore Christoffersen

Søkers beskrivelse av formål:

Psykiske lidelser blant ungdom er et vesentlig helseproblem. Beregninger viser at ca en av fem norske ungdommer har symptomer på angst og depresjon og forekomsten øker, spesielt blant jenter. Dette prosjektet søker ny og utvidet kunnskap om forebygging av psykiske helseplager rundt alderen der begynnelsen og utviklingen av psykiske lidelser ofte oppstår. Ved hjelp av tidligere innsamlet data blant friske ungdom (alderen 15-19 år), søker prosjektet å undersøke sammenhengen mellom infeksjons-markører i blod, selvrapportert søvn og symptomer på angst og depresjon i løpet av ungdomstiden. Prosjektet søker å bidra med kunnskap om forekomst, sårbarhet og risiko for symptomer og utvikling av sykdom i en alder preget av overgangen fra barn til voksen. I tillegg til å fokusere på livsstilsfaktorer som påvirker psykisk helse, søker prosjektet å få og formidle informasjon og kunnskap om relevante områder innenfor folkehelse, samt å bidra til mer presise kunnskapsbaserte forebyggende strategier.

REKs vurdering

Vi viser til søknad om forhåndsgodkjenning av ovennevnte forskningsprosjekt. Søknaden ble behandlet av Regional komité for medisinsk og helsefaglig forskningsetikk REK nord i møtet 28.11.2019. Vurderingen er gjort med hjemmel i helseforskningsloven § 10.

Formål
Prosjektets hovedhypoteser framgår av prosjektbeskrivelsen:

«1. Low-grade inflammation markers, primarily Interleukin (IL)-6 and 1, transforming growth factor-alpha (TGF-), tumor necrosis factor (TNF) and C-reactive protein (CRP), are associated with symptoms of psychological distress in girls and boys at ages 15-17 years. 2. Low-grade inflammation markers, primarily IL-6, IL-1, TGF-, TNF- and CRP are associated with persistent symptoms of psychological distress over 2 years in girls and boys 15-19 years of age.
3. Insomnia is associated with the onset of psychological distress symptoms in girls and boys 15- 17 years of age.
4. All these associations may be modified by sex, physical activity levels and body composition measured at ages 15-19 years of age."

Om prosjektet
Prosjektet skal bruke data fra Fit Futures. I dette inngår også analyser fra humant biologisk materiale som ble innhentet i Fit Futures.

Data/materiale
Det framgår av søknaden at: *«Variabelkategoriene inkluderer antropometriske målinger, blodprøver (inkludert inflammasjonspanel), medisinbruk, pubertets-status, spørreskjemadata (inkludert utfallsmål og relevante kovaraiter). Totalt antall spesifikke variabler anslås til 250»*

Data utleveres og behandles avidentifisert.

Forespørsel/informasjon/samtykkeerklæring
Det er innhentet samtykke for deltakerne i Fit Futures-undersøkelsene.

Samtykkene er vurdert å være dekkende for prosjektets formål.

Vedtak

Godkjent

REK har gjort en helhetlig forskningsetisk vurdering av alle prosjektets sider og godkjenner det med hjemmel i helseforskningsloven § 10.

Prosjektet er godkjent frem til omsøkt sluttdato 30.11.2023. Data skal oppbevares for kontrollhensyn i 5 år etter prosjektslutt. Etter dette skal data anonymiseres eller slettes.

Vi gjør oppmerksom på at etter personopplysningsloven må det foreligge et

behandlingsgrunnlag etter personvernforordningen. Dette må forankres i egen institusjon.

Med vennlig hilsen

May Britt Rossvoll
sekretariatsleder

Sluttmelding
Søker skal sende sluttmelding til REK nord på eget skjema senest seks måneder etter godkjenningsperioden er utløpt, jf. hfl. § 12.

Søknad om å foreta vesentlige endringer
Dersom man ønsker å foreta vesentlige endringer i forhold til formål, metode, tidsløp eller organisering, skal søknad sendes til den regionale komiteen for medisinsk og helsefaglig forskningsetikk som har gitt forhåndsgodkjenning. Søknaden skal beskrive hvilke endringer som ønskes foretatt og begrunnelsen for disse, jf. hfl. § 11.

NSD NORSK SENTER FOR FORSKNINGSDATA

NSD sin vurdering

Prosjekttittel

The influence of circadian and inflammatory variations in onset and course of adolescent psychological distress. The Tromsø Study – Fit Futures.

Referansenummer

934242

Registrert

07.10.2021 av Tore Christoffersen - tore.christoffersen@uit.no

Behandlingsansvarlig institusjon

UiT Norges Arktiske Universitet / Det helsevitenskapelige fakultet / Institutt for helse- og omsorgsfag

Prosjektansvarlig (vitenskapelig ansatt/veileder eller stipendiat)

Tore Christoffersen, tore.christoffersen@uit.no, tlf: 90082718

Type prosjekt

Forskerprosjekt

Prosjektperiode

01.07.2021 - 30.11.2023

Status

18.11.2021 - Vurdert

Vurdering (1)

18.11.2021 - Vurdert

Det er vår vurdering at behandlingen av personopplysninger i prosjektet vil være i samsvar med personvernlovgivningen så fremt den gjennomføres i tråd med det som er dokumentert i meldeskjemaet 18.11.2021 med vedlegg, samt i meldingsdialogen mellom innmelder og NSD. Behandlingen kan starte.

Prosjektet er vurdert og godkjent av Regionale komiteer for medisinsk og helsefaglig forskningsetikk (REK) etter helseforskningsloven (hfl.) § 10 (REK sin ref: 60811/REK nord).

MELD VESENTLIGE ENDRINGER
Dersom det skjer vesentlige endringer i behandlingen av personopplysninger, kan det være nødvendig å melde dette til NSD ved å oppdatere meldeskjemaet. Før du melder inn en endring, oppfordrer vi deg til å lese om hvilke type endringer det er nødvendig å melde:
https://nsd.no/personvernombud/meld_prosjekt/meld_endringer.html

Du må vente på svar fra NSD før endringen gjennomføres.

TYPE OPPLYSNINGER OG VARIGHET
Prosjektet vil behandle særlige kategorier av personopplysninger om helse og alminnelige kategorier av personopplysninger frem til 30.11.2023. Opplysningene oppbevares av kontrollhensyn i 5 år etter prosjektslutt etter vilkår fra REK.

LOVLIG GRUNNLAG
Prosjektet vil innhente samtykke fra de registrerte til behandlingen av personopplysninger. Vår vurdering er at prosjektet legger opp til et samtykke i samsvar med kravene i art. 4 nr. 11 og art. 7, ved at det er en frivillig, spesifikk, informert og utvetydig bekreftelse, som kan dokumenteres, og som den registrerte kan trekke tilbake.
Lovlig grunnlag for behandlingen vil dermed være den registrertes uttrykkelige samtykke, jf. personvernforordningen art. 6 nr. 1 bokstav a, jf. art. 9 nr. 2 bokstav a, jf. personopplysningsloven § 10, jf. § 9 (2).

PERSONVERNPRINSIPPER
NSD vurderer at den planlagte behandlingen av personopplysninger vil følge prinsippene i personvernforordningen om:
- lovlighet, rettferdighet og åpenhet (art. 5.1 a), ved at de registrerte får tilfredsstillende informasjon om og samtykker til behandlingen
- formålsbegrensning (art. 5.1 b), ved at personopplysninger samles inn for spesifikke, uttrykkelig angitte og berettigede formål, og ikke viderebehandles til nye uforenlige formål
- dataminimering (art. 5.1 c), ved at det kun behandles opplysninger som er adekvate, relevante og nødvendige for formålet med prosjektet
- lagringsbegrensning (art. 5.1 e), ved at personopplysningene ikke lagres lengre enn nødvendig for å oppfylle formålet

DE REGISTRERTES RETTIGHETER
Så lenge de registrerte kan identifiseres i datamaterialet vil de ha følgende rettigheter: åpenhet (art. 12), informasjon (art. 13), innsyn (art. 15), retting (art. 16), sletting (art. 17), begrensning (art. 18), underretning (art. 19), dataportabilitet (art. 20).

Det er utgangspunktet at alle som registreres i forskningsprosjektet har rett til å få slettet opplysninger som er registrert om dem. Etter helseforskningsloven § 16 tredje ledd vil imidlertid adgangen til å kreve sletting av sine helseopplysninger ikke gjelde dersom materialet eller opplysningene er anonymisert, dersom materialet etter bearbeidelse inngår i et annet biologisk produkt, eller dersom opplysningene allerede er inngått i utførte analyser. Regelen henviser til at sletting i slike situasjoner vil være svært vanskelig og/eller ødeleggende for forskningen, og dermed forhindre at formålet med forskningen oppnås.

Etter personvernforordningen art 17 nr. 3 d kan man unnta fra retten til sletting dersom behandlingen er nødvendig for formål knyttet til vitenskapelig eller historisk forskning eller for statistiske formål i samsvar med artikkel 89 nr. 1 i den grad sletting sannsynligvis vil gjøre det umulig eller i alvorlig grad vil hindre at målene med nevnte behandling nås.

NSD vurderer dermed at det kan gjøres unntak fra retten til sletting av helseopplysninger etter helseforskningslovens § 16 tredje ledd og personvernforordningen art 17 nr. 3 d, når materialet er bearbeidet slik at det inngår i et annet biologisk produkt, eller dersom opplysningene allerede er inngått i utførte analyser.

Vi presiserer at helseopplysninger inngår i utførte analyser dersom de er sammenstilt eller koblet med andre opplysninger eller prøvesvar. Vi gjør oppmerksom på at øvrige opplysninger må slettes og det kan ikke innhentes ytterligere opplysninger fra deltakeren.

De registrerte har tidligere mottatt informasjon om at deres opplysninger vil bli brukt i nye forskningsprosjekter og at informasjonen om nye prosjekter vil gjøres offentlig tilgjengelig på https://uit.no/research/fitfutures. Det er også en forutsetning at den enkelte forsker ikke får tilgang til

opplysninger som gjør det mulig å identifisere enkeltpersoner og at prosjektet er godkjent av REK. De registrerte har således avgitt brede samtykker til videre behandling av av-identifiserte data til godkjente forskningsformål. REK vurderer at herværende prosjekt faller innenfor kriteriene for dette samtykke. Det gis således ikke ny personlig informasjon om dette spesifikke prosjektet, men Informasjon om prosjektet gjøres offentlig tilgjengelig på www.tromsoundersokelsen.no.

På bakgrunn av dette vurderer NSD at det kan unntas fra informasjonsplikten etter personvernforordningens art. 14 nr. 5 bokstav b. Ettersom opplysningene som skal behandles vil være av-identifiserte og behandlingen oppfyller kriteriene som de registrerte har samtykket til, vurderes det som uforholdsmessig vanskelig å informere hver enkelt registrert sammenlignet med personvernulempen de registrerte påføres som følge av at de ikke blir informert.

Vi minner om at hvis en registrert tar kontakt om sine rettigheter, har behandlingsansvarlig institusjon plikt til å svare innen en måned.

FØLG DIN INSTITUSJONS RETNINGSLINJER
NSD legger til grunn at behandlingen oppfyller kravene i personvernforordningen om riktighet (art. 5.1 d), integritet og konfidensialitet (art. 5.1. f) og sikkerhet (art. 32).

For å forsikre dere om at kravene oppfylles, må dere følge interne retningslinjer og eventuelt rådføre dere med behandlingsansvarlig institusjon.

OPPFØLGING AV PROSJEKTET
NSD vil følge opp ved planlagt avslutning for å avklare om behandlingen av personopplysningene er avsluttet.

Lykke til med prosjektet!

Kontaktperson hos NSD: Lisa Lie Bjordal
Tlf. Personverntjenester: 53 21 15 00 (tast 1)

Appendix B

Pamphlet of information, The Fit Futures

Consent of participation, The Fit Futures

Norwegian Versions

PERSONVERN OG SIKKERHET

Alle medarbeidere som jobber med undersøkelsen, har taushetsplikt. Opplysningene som samles inn, vil bare bli brukt til godkjente forskningsformål, som beskrevet over.

Opplysningene og prøvene vil bli behandlet uten navn og fødselsnummer eller andre direkte gjenkjennende opplysninger. En kode knytter deg til dine opplysninger og prøver. Koden oppbevares separat ved Universitetet i Tromsø, og kun noen få autoriserte personer har tilgang. Den enkelte forsker får ikke tilgang til opplysninger som gjør det mulig å identifisere enkeltpersoner. Det vil ikke være mulig å identifisere deg i resultatene av studien når disse publiseres.

I noen tilfeller kan det være aktuelt å gjøre analyser av blodprøver eller genetiske analyser ved forskningsinstitusjoner i utlandet. Hvis dette gjøres, vil våre utenlandske samarbeidspartnere ikke få opplysninger som kan knytte prøvene opp mot deg som person.

Tromsøundersøkelsen gjennomfører Fit futures i samarbeid med Universitetssykehuset Nord-Norge og Nasjonalt folkehelseinstitutt. Data som samles inn på sykehuset, overføres til Universitetet i Tromsø når datainnsamlingen er avsluttet. Ingen av opplysningene som framkommer i undersøkelsen, lagres i journalsystemet på sykehuset. Databehandlingsansvarlig er Universitetet i Tromsø. Tromsøundersøkelsen administrerer utlevering av data til forskningsprosjekter. Hvem som er ansvarlig for forskningsprosjektene, finner du her http://www.tromsoundersokelsen.no. Fit futures er godkjent av Datatilsynet og Regional komité for medisinsk og helsefaglig forskningsetikk, Nord-Norge. Deltakere er forsikret gjennom Norsk Pasientskadeerstatningsordning.

FRIVILLIG DELTAKELSE

Det er frivillig å delta i studien. Du kan når som helst og uten å oppgi noen grunn trekke ditt samtykke til å delta i undersøkelsen, og dette vil ikke få noen konsekvenser for deg. Dersom du senere ønsker å trekke deg eller har spørsmål til studien, kan du kontakte Tromsøundersøkelsen, Institutt for samfunnsmedisin, Universitetet i Tromsø, 9037 Tromsø, telefon 77644816, e-post: tromsous@uit.no.

RETT TIL INNSYN OG SLETTING AV PRØVER OG OPPLYSNINGER OM DEG

Hvis du sier ja til å delta i studien, har du rett til å få innsyn i hvilke opplysninger som er registrert om deg. Du har også rett til å få korrigert eventuelle feil i de opplysningene vi har registrert. Dersom du trekker deg fra studien, kan du kreve å få slettet innsamlede prøver og opplysninger, med mindre opplysningene allerede er inngått i analyser eller brukt i vitenskapelige publikasjoner.

VIL DU DELTA?

Hvis du er fylt 16 år, gir du selv ditt samtykke til å delta. Du kan da signere vedlagte skjema (hvitt ark) og ta det med til undersøkelsen. Det er også mulig å undertegne skjemaet når du kommer til Forskningsposten.

Hvis du ikke er fylt 16 år, må du be dine foreldre/foresatte om lov til å delta. Da må både du og dine foreldre/foresatte signere vedlagte skjema (hvitt ark) som du tar med deg til undersøkelsen.

ANSVARLIGE FOR GJENNOMFØRING AV FIT FUTURES UNDERSØKELSEN

Fit futures ledes av en styringsgruppe, og følgende forskere er ansvarlige for gjennomføringen:

Anne-Sofie Furberg
prosjektleder, lege, Universitetssykehuset Nord-Norge
e-post: anne-sofie.furberg@unn.no, telefon 77 75 58 24

Christopher Sivert Nielsen
psykolog, Nasjonalt folkehelseinstitutt
e-post: Christopher.Sivert.Nielsen@fhi.no, telefon 21 07 82 77

Guri Grimnes
lege, Universitetssykehuset Nord-Norge og Universitetet i Tromsø
e-post: guri.grimnes@unn.no, telefon 77 66 94 83

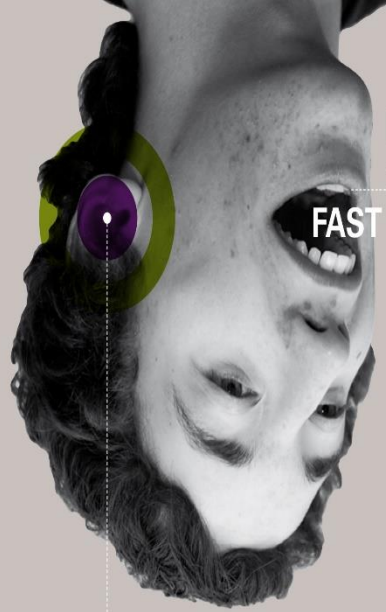
SPØRSMÅL?

Dersom du/dere har spørsmål om undersøkelsen, kontakt Forskningsposten UNN på telefon 77 62 69 09 eller prosjektadministrator for Fit futures på telefon 930 03 925.

ENERGI

www.fitfutures.no

VI BRYR OSS · SAMMEN TAR VI ANSVAR
UNN
UNIVERSITETET I TROMSØ
folkehelseinstituttet



FAST FOOD

SOSIALT NETTVERK

FitFutures
EN DEL AV TROMSØUNDERSØKELSEN

DIN HELSE DIN FREMTID

INVITASJON TIL Å DELTA I HELSEUNDERSØKELSE BLANT UNGDOM

HVA ER FIT FUTURES?

Fit futures er et forskningsprosjekt der vi undersøker ungdommers fysiske helse og livsstil.

HVORFOR ER DETTE VIKTIG?

Voksnes helse undersøkes i mange studier, men man har mindre kunnskap om helse blant ungdom. Selv om få ungdommer har alvorlige sykdommer, legges mye av grunnlaget for fremtidig helse i ungdomsårene. Denne undersøkelsen kan bidra til at vi får økt kunnskap om hvordan man kan forebygge sykdom og om hvordan diagnoser kan stilles på et tidligere tidspunkt.

HVA FORSKES DET PÅ?

Hovedområdene det forskes på er:

- Eksem og kviser
- Infeksjoner
- Fysisk aktivitet og overvekt
- D-vitamin
- Jernmangel
- Genmodifisert mat
- Miljøgifter
- Smerte
- Beintetthet
- Diabetes
- Øresus
- Medisinbruk
- Frafall fra skole
- Tannhelse

Informasjonen fra undersøkelsen vil også bli brukt til forskning om de store folkehelseproblemene generelt, slik som hjerte-karsykdommer, lungesykdommer, kreft, nedsatt fruktbarhet og smerte. Det vil også bli forsket på arbeidsforhet i skole og yrke i forhold til sykdom, helse og livsstil. En del av prosjektene vil studere samspillet mellom arv, miljø og sykdom og helse; til slike prosjekter vil det bli hentet ut genetisk arvestoff fra blodprøvene. I framtiden kan data bli brukt i forskningsprosjekter som i dag ikke er planlagt. For alle slike nye prosjekter kreves det at prosjektet er godkjent av Regional komité for medisinsk og helsefaglig forskningsetikk. En oversikt over godkjente prosjekter finner du her (www.tromsoundersokelsen.no). Nettsiden holdes løpende oppdatert. Her kan du også lese om våre forskningsresultater.

HVEM KAN DELTA?

Alle ungdommer på VG1 blir invitert til å delta. Hvis du er 16 år eller mer, kan du selv bestemme om du vil delta. Er du under 16 år, må du ha samtykke fra dine foreldre eller foresatte.

AKTIVITET

SLIK FOREGÅR UNDERSØKELSEN

Undersøkelsen gjennomføres i skoletiden. Selve undersøkelsen tar 2-3 timer, og du må påregne å være borte fra skolen en halv dag. Skolen anser dette som gyldig skolefravær. Du blir undersøkt på Forskningsposten, Universitetssykehuset Nord-Norge, av erfarne forskningssykepleiere og tannleger/tannhelsesekretærer. Undersøkelsen består av følgende deler:

- *Spørreskjema* der vi spør om livsstil, trivsel, sykdommer og helseplager gjennom livet, og familieforhold.
- *Intervju* der vi spør om hvilke medisiner du bruker, om du har noen sykdom i dag og litt om sosialt nettverk. Kvinner spørres også om menstruasjon og graviditet.
- *Generell helseundersøkelse* der vi måler høyde, vekt, livvidde og hoftevidde, blodtrykk og puls, samt tar blodprøve, en hårprøve fra nakken, og en bakterieprøve fra nesebor og hals med en fuktet vattpinne.
- Måling av *smertefølsomhet* der vi måler følsomhet for trykk, kulde og varme. Smerten kommer gradvis, og du kan selv avbryte når som helst.
- *Kroppsscan* (DEXA) der vi måler beintetthet og forholdet mellom fett- og muskelvev. Dette skjer ved at du ligger rolig i ca. 10 minutter mens kroppen scannes.
- *Tannundersøkelse* som blir din årlige undersøkelse ved den offentlige tannhelsetjenesten og omfatter klinisk undersøkelse, tannrøntgen, kliniske foto og avtrykk for studiemodeller.

Etter undersøkelsen vil du få utlevert en liten *aktivitetsmåler* som er festet i et smalt strikkbelte til å ha under klærne. Denne måler hvor mye du beveger deg i løpet av dagen. Apparatet leveres på skolen etter en ukes bruk. Da vil det samtidig tas ny bakterieprøve fra nesebor og hals.

Noen deltakere vil bli forespurt om å undersøkes en gang til. Det vil da være aktuelt å gjenta noen av undersøkelsene og gjøre enkelte utvidede undersøkelser.

HVA SKJER MED DE BIOLOGISKE PRØVENE?

Med blodprøven gjøres analyser av bl.a. hormonnivåer, fettstoffer, blodsukker, vitaminer, miljøgifter og markører på betennelse og sykdommer. Det blir også hentet ut arvestoff (DNA og RNA) for genetiske analyser. Bakterieprøvene brukes til å måle forekomst av gule stafylokokker. Hårprøven analyseres for å se på nivå av kvikksølv. Prøvene lagres i Forskningsbiobanken for Tromsoundersøkelsen ved Universitetet i Tromsø. Hvis du sier ja til å delta, gir du også samtykke til at de biologiske prøvene og analyseresultatene inngår i biobanken.

INFORMASJON FRA ANDRE KILDER OG BRUK AV DATA I FRAMTIDEN

TEKNOLOGI

Opplysninger og prøver som du gir, blir oppbevart på ubestemt tid til bruk i forskning omkring helse og sykdom som omtalt i denne brosjyren. Det kan også hende at vi tar kontakt med deg igjen for å spørre om du vil være med på en ny undersøkelse. For spesielle forskningsprosjekter kan det være aktuelt å sammenstille informasjon fra Fit futures med nasjonale helseregistre som Reseptregisteret, Medisinsk fødselsregistrer, Kreftregisteret, Norsk pasientregister, Dødsårsaksregisteret og andre nasjonale registre over sykdommer som det forskes på i Tromsoundersøkelsen. I tillegg kan det være aktuelt å innhente helseopplysninger fra spesialist- og primærhelsetjenesten, for eksempel informasjon om beinbrudd og høyde- og vektdata fra helsestasjon, til bruk i forskning på sykdommer og helseproblemer som det forskes på i Tromsøundersøkelsen. Det kan også bli innehentet data fra registre i Statistisk sentralbyrå slik som miljø, befolkning, utdanning, inntekt, offentlige ytelser, arbeidsdeltakelse og andre forhold som kan ha betydning for helsa. For å undersøke om sykdommer går i arv, kan opplysninger om deg sammenstilles med opplysninger om dine slektninger, dersom disse har deltatt i deler av Tromsoundersøkelsen. Dette blir gjort ved å innhente opplysinger om slektskap fra Familieregisteret. Fra skolen vil vi innhente dine opplysninger om studieprogram, klasse, kjønn, antall fraværsdager, om du fullfører skoleåret og om karakterer i fagene norsk, matematikk og engelsk.

Sammenstillinger av informasjon krever noen ganger nytt samtykke og/eller annen type godkjenning slik som dispensasjon fra taushetsplikten eller godkjenning av offentlige instanser, for eksempel Regional komité for medisinsk og helsefaglig forskningsetikk, Datatilsynet eller NAV.

MULIGE ULEMPER OG FORDELER

Deltakelse innebærer at du må bruke noe tid. Deler av undersøkelsen kan også innebære ubehag. Dette gjelder særlig blodprøven. Dersom du vet at du har problemer med å ta blodprøve, kan du kontakte Forskningsposten på telefon 77 62 69 09 eller snakke med sykepleier når du kommer til undersøkelsen for å finne en løsning på dette.

Dersom resultatet av prøvene dine viser at det er nødvendig med oppfølging av tannlege, lege eller henvisning til spesialist, vil du bli orientert om det. Ved behov for henvisning til spesialist, vil vi sørge for henvisning og tilbud om oppfølging ved sykehuset.

Deltakere får et gavekort til en verdi av kr. 200 ved oppmøte som kan brukes i de fleste butikker i Tromsø.

SMERTE

MILJØGIFTER

RØYK OG SNUS

FitFutures

EN DEL AV TROMSØUNDERSØKELSEN

VIL DU DELTA?

Samtykke til å delta i studien Fit futures

Jeg er villig til å delta i studien

____

(DITT FULLE NAVN I BLOKKBOKSTAVER)

Sted ______________________ Dato ______________________

____

(DIN SIGNATUR)

VIL DU DELTA OG ER UNDER 16 ÅR?

Foreldre/foresatte sitt samtykke til deltakelse i Fit futures

Jeg samtykker herved i at mitt/vårt barn kan delta i undersøkelsen

____

(BARNETS FULLE NAVN I BLOKKBOKSTAVER)

Sted ______________________ Dato ______________________

____

(SIGNATUR FORELDER/FORESATT 1)

____

(SIGNATUR FORELDER/FORESATT 2)

Appendix C

Interview guide, The Fit Futures

Printout of Electronic Questionnaire, The Fit Futures

Norwegian Versions

Fit futures

- en del av Tromsøundersøkelsen

Intervju og Spørreskjema

Versjon: 12.04.2010

Intervju

Skriftlig samtykke:

☐ Ja ☐ Nei

Hvis nei, avbrytes undersøkelsen.

Foreldresamtykke (for de som er under 16 år)

☐ Ja ☐ Nei

Dersom de har glemt å ta med dette ber man om lov til å tas kontakt med foreldre for å innhente samtykke per telefon. To teknikere signerer på at dette er gjort.
Dersom det mangler samtykke for de under 16 år, avbrytes undersøkelsen.

Dagens dato registreres automatisk. Genererer:

[Alder i hele år]

Føler du deg frisk i dag?

☐ Ja ☐ Nei

Hvis nei:

Hva er det som feiler deg?

☐ Feber ☐ Forkjølet ☐ Hodepine ☐ Magesmerter ☐ Andre smerter
☐ Kvalme ☐ Annet
Tekstfelt for annet: _____

Har du noen form for infeksjon?

☐ Ja ☐ Nei

Hvis ja:
Beskriv: _____

Har du noen form for kroniske eller vedvarende sykdommer?
Hvor gammel var du da du fikk denne sykdommen første gang?

Diagnose 1: [ICD10 kode] Alder sykdom 1:
Diagnose 2: [ICD10 kode] Alder sykdom 2:
Diagnose 3: [ICD10 kode] Alder sykdom 3:
Diagnose 4: [ICD10 kode] Alder sykdom 4:
Diagnose 5: [ICD10 kode] Alder sykdom 5:

Tekstfelt for annet: _____

Tar du noen form for medisiner fast?

☐ Ja ☐ Nei

Hvis ja:
Medisin 1: [ATC kode]
Medisin 2: [ATC kode]
Medisin 3: [ATC kode]
Medisin 4: [ATC kode]
Medisin 5: [ATC kode]

Har du tatt noen form for smertestillende medisiner i løpet av de siste 24 timene, for eksempel Paracet, Ibux, Parlagin forte?

☐ Ja ☐ Nei

Hvis ja:

Medisin 1:	[ATC kode]	[Timer siden]	[Antall tabletter]
Medisin 2:	[ATC kode]	[Timer siden]	[Antall tabletter]
Medisin 3:	[ATC kode]	[Timer siden]	[Antall tabletter]

Har du tatt noen form for antibiotika i løpet av de siste 24 timene, for eksempel Penicillin, mot infeksjon eller kviser?

☐ Ja ☐ Nei

Hvis ja:

Medisin 1: [ATC kode]
Medisin 2: [ATC kode]
Medisin 3: [ATC kode]

Når spiste du sist?

[ ] klokkeslett – omkodes automatisk til timer siden siste måltid

Sosialt nettverkskartlegging (se redegjørelse i protokoll)

[Løpenummer venn 1]
[Løpenummer venn 2]
[Løpenummer venn 3]
[Løpenummer venn 4]
[Løpenummer venn 5]

Jenter

Har du fått menstruasjon?

☐ Ja ☐ Nei

Hvis ja (har fått menstruasjon):

Hvor regelmessig er menstruasjonene dine?

☐ Alltid regelmessig ☐ Oftest regelmessig ☐ Uregelmessig

Hvor mange dager er det mellom start av hver menstruasjon?

[Antall dager]

Hvilken dag startet siste menstruasjon? ***Dato registreres, genererer:***

[Dager siden siste menstruasjon]

Bruker du noen form for hormonell prevensjon, for eksempel p-piller?

(følges eventuelt opp med spørsmål om type prevensjon om dette ikke sies spontant)

☐ Nei ☐ P-piller ☐ P-sprøyte ☐ Annet

Er det noen mulighet for at du kan være gravid nå?
□ Ja □ Nei

Hvis ja:
Er det greit for deg at vi tar en gravitest?
□ Ja □ Nei
(resultat av prøven formidles ikke til foreldre)

Hvis ja:
Resultat av gravitest:
□ Negativ □ Positiv □ Ikke utført

Klarert for DEXA (genereres automatisk)
□ Ja □ Nei

Følgende personer er ikke klarert:
Kvinner som sier det er mulighet for at de er gravide som ikke vil gjøre gravitest
Kvinner som har positiv gravitest.

Alle: ved innsamling av aktigraf

Hvor mange timer totalt var du utendørs i dagslys i løpet av de siste 7 dagene?
[ ] [ ] timer

FF - Generelt spørreskjema - Uke 1

Vi ønsker å vite mer om livsstil og helse.

Bruk den tiden du trenger til å svare så presist du kan.

Alle svarene dine blir behandlet med taushetsplikt.

Bruk "neste >>" og "<< tilbake" - knappene i skjema for å bla deg fremover og bakover.

Lykke til og tusen takk for hjelpen!

DEG OG DIN FAMILIE

1) Er du:

- ○ Jente
- ○ Gutt

2) Hvem bor du sammen med nå? (sett ett eller flere kryss)

- [ ] Mor
- [ ] Far
- [ ] 1-2 søsken
- [ ] 3 eller flere søsken
- [ ] Mors nye mann/samboer
- [ ] Fars nye kone/samboer
- [ ] Fosterforeldre
- [ ] Adoptivforeldre
- [ ] Besteforeldre
- [ ] Venner
- [ ] Alene/på hybel
- [ ] Institusjon
- [ ] Annet

3) Hvor lenge er det siden du flyttet hjemmefra?

- ( ) Mindre enn 6 måneder
- ( ) 6 - 11 måneder
- ( ) 1 - 2 år
- ( ) Mer enn 2 år

4) Er moren din i arbeid? (sett ett eller flere kryss)

- [ ] Ja, heltid
- [ ] Ja, deltid
- [ ] Arbeidsledig
- [ ] Uførerygdet
- [ ] Hjemmeværende
- [ ] Går på skole, kurs, e.l.
- [ ] Pensjonist
- [ ] Mor er død
- [ ] Vet ikke
- [ ] Annet

5) Er faren din i arbeid? (sett ett eller flere kryss)

- [ ] Ja, heltid
- [ ] Ja, deltid
- [ ] Arbeidsledig
- [ ] Uførerygdet
- [ ] Hjemmeværende
- [ ] Går på skole, kurs, e.l.
- [ ] Pensjonist
- [ ] Far er død
- [ ] Vet ikke
- [ ] Annet

6) Hva er den høyeste fullførte utdanningen til dine foreldre? (sett kryss for alle utdanningene du vet om for mor og far)

	Grunnskole	Yrkesfaglig videregående, yrkesskole	Allmennfaglig videregående skole eller gymnas	Høyskole eller universitet, mindre enn 4 år	Høyskole eller universitet, 4 år eller mer	Vet ikke
Mors utdanning	○	○	○	○	○	○
Fars utdanning	○	○	○	○	○	○

7) Hva regner du deg selv som: (kryss av for ett eller flere alternativ)

- ☐ Norsk
- ☐ Samisk
- ☐ Kvensk/Finsk
- ☐ Annet, spesifiser her

8) I hvilken kommune bodde du da du var 5-6 år (førskolealder/1.klasse)?

Velg kommune ▼

9) Er du født i Norge?

- ○ Ja
- ○ Nei, spesifiser hvilket land

10) Er din biologiske mor født i Norge?

- ○ Ja
- ○ Nei, spesifiser hvilket land

11) Er din biologiske far født i Norge?

- ○ Ja
- ○ Nei, spesifiser hvilket land

12) Har du noen gang oppholdt deg 4 uker eller mer sammenhengende i Australia, USA, Argentina eller Sør-Afrika?

- Ja
- Nei

Hvis det har vært flere opphold, oppgi varighet av siste opphold.

13) Hvor lenge varte oppholdet?

- Mindre enn 2 måneder
- 2-6 måneder
- Mer enn 6 måneder

Hvis det har vært flere opphold, oppgi når du hadde siste opphold.

14) Når var oppholdet? (Oppgi årstall når oppholdet sluttet - 4 siffer)

VENNER OG SKOLE

15) Har du vurdert å avbryte eller ta pause fra den videregående opplæringen du er i gang med?

- Ja
- Nei

16) Hvor sannsynlig er det at du fullfører den utdanningen du er i gang med?

- Liten - kommer til å slutte
- God - kommer sannsynligvis til å fullføre
- Stor - Kommer helt sikkert til å fullføre
- Vet ikke

17) Hvor mange tekstmeldinger (SMS/MMS) sendte du med mobiltelefon i går?

- Ingen
- 1-5 meldinger
- 6-10 meldinger
- 11-20 meldinger
- 21-50 meldinger
- Mer enn 50 meldinger

18) Nedenfor er det noen spørsmål om hvordan du synes du selv er. Kryss av for det som passer best for deg.

	Stemmer svært godt	Stemmer nokså godt	Stemmer nokså dårlig	Stemmer svært dårlig
Jeg synes det er ganske vanskelig å få venner				
Jeg har mange venner				
Andre ungdommer har vanskelig for å like meg				
Jeg er populær blant jevnaldrende				
Jeg føler at jevnaldrende godtar meg				

19) Hvilke avgangskarakterer fikk du fra ungdomsskolen? (sett ett kryss for hvert fag)

	1	2	3	4	5	6	Husker ikke
Norsk skriftlig							
Matematikk							
Engelsk							

HELSE

20) Hvordan vurderer du din egen helse sånn i alminnelighet?

- Meget god
- God
- Verken god eller dårlig
- Dårlig
- Meget dårlig

21) Hvor ofte har du i løpet av de siste 4 ukene brukt følgende medisiner?

	Ikke brukt siste 4 uker	Sjeldnere enn hver uke	Hver uke, men ikke daglig	Daglig
Smertestillende på resept (f. eks. Paralgin forte, Pinex forte)	○	○	○	○
Smertestillende uten resept (f. eks. Paracet, Pinex, Ibux)	○	○	○	○
Sovemidler	○	○	○	○
Medisin mot depresjon	○	○	○	○
Medisiner mot ADHD	○	○	○	○
Beroligende medisiner	○	○	○	○

22) Har du diabetes?

○ Ja ○ Nei

23) Har din biologiske mor diabetes?

○ Ja ○ Nei ○ Vet ikke

24) Har din biologiske far diabetes?

○ Ja ○ Nei ○ Vet ikke

25) Bruker mor insulin? (Penn eller pumpe)

○ Ja ○ Nei ○ Vet ikke

26) Hvor gammel var mor da hun fikk diabetes?

○ < 20 år ○ 20 - 40 år ○ > 40 år

27) Bruker far insulin? (Penn eller pumpe)

○ Ja ○ Nei ○ Vet ikke

28) Hvor gammel var far da han fikk diabetes?

○ < 20 år ○ 20 - 40 år ○ > 40 år

PSYKISKE VANSKER

29) Har du gått i behandling hos psykolog, psykiater eller PP-tjenesten det siste året?

○ Ja ○ Nei

30) Under finner du en liste over ulike problemer. Har du opplevd noe av dette den siste uken (til og med i dag)?

	Ikke plaget	Litt plaget	Ganske mye	Veldig mye
Plutselig frykt uten grunn	○	○	○	○
Føler deg redd eller engstelig	○	○	○	○
Matthet eller svimmelhet	○	○	○	○
Føler deg anspent eller oppjaget	○	○	○	○
Lett for å klandre deg selv	○	○	○	○
Søvnproblemer	○	○	○	○
Nedtrykt, tungsindig	○	○	○	○
Følelse av å være unyttig, lite verdt	○	○	○	○
Følelse av at alt er et slit	○	○	○	○
Følelse av håpløshet med hensyn til framtida	○	○	○	○

31) De følgende spørsmålene handler om hva du følte og gjorde de siste to ukene.

	Riktig	Noen ganger riktig	Ikke riktig
Jeg var lei meg eller ulykkelig	○	○	○
Jeg følte meg så trøtt at jeg bare ble sittende uten å gjøre noen ting	○	○	○
Jeg var veldig rastløs	○	○	○
Jeg var ikke glad for noe	○	○	○
Jeg følte meg lite verdt	○	○	○
Jeg gråt mye	○	○	○
Jeg hatet meg selv	○	○	○
Jeg tenkte at jeg aldri kunne bli så god som andre ungdommer	○	○	○
Jeg følte meg ensom	○	○	○
Jeg tenkte at ingen egentlig var glad i meg	○	○	○
Jeg følte meg som et dårlig menneske	○	○	○
Jeg gjorde alt galt	○	○	○
Jeg syntes det var vanskelig å tenke klart eller å konsentrere meg	○	○	○

PUBERTET

Her har vi noen spørsmål om kroppslige forandringer som skjer gjennom ungdomstiden:

32) Har du fått menstruasjon?

○ Ja ○ Nei

Hvor gammel var du da du fikk menstruasjon første gang?

33) År

Velg...

34) Måneder

Velg...

35) Har du fått eller begynt å få kjønnshår?

○ Ja ○ Nei

36) Har du fått eller begynt å få bryster?

○ Ja ○ Nei

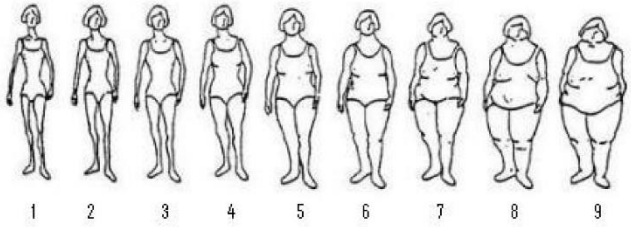
37) Har du fått eller begynt å få kjønnshår?

○ Ja ○ Nei

38) Hvor gammel var du da du begynte å få kjønnshår?

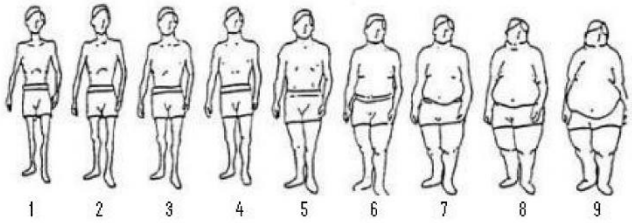
Velg...

KROPP OG VEKT



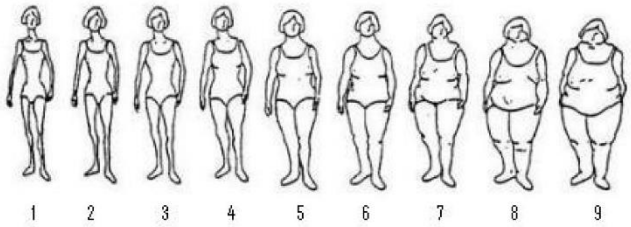
39) Hvilken av disse kroppsfasongene likner mest på kroppen til moren din?

○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9



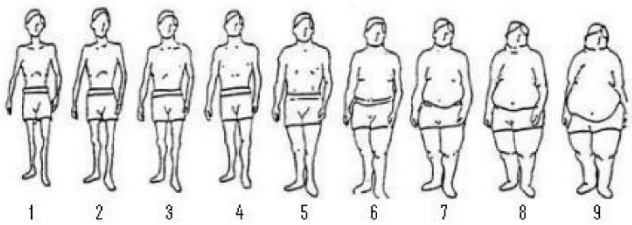
40) Hvilken av disse kroppsfasongene likner mest på kroppen til faren din?

○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9



41) Hvilken av disse kroppsfasongene likner mest på din kropp slik du er i dag?

○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9



42) Hvilken av disse kroppsfasongene likner mest på din kropp slik du er i dag?

○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9

RØYK, SNUS OG ALKOHOL

43) Røyker du?

○ Nei, aldri ○ Av og til ○ Daglig

44) Bruker du snus eller skrå?

○ Nei, aldri ○ Av og til ○ Daglig

45) Hvor mange sigaretter røyker du vanligvis i løpet av en uke?

○ 1 eller færre
○ 2-3
○ 4-6
○ 7-10
○ Mer enn 10

46) Hvor mange sigaretter røyker du vanligvis per dag?

- 1
- 2-3
- 4-6
- 7-10
- Mer enn 10

47) Hvor mange priser snus/skrå bruker du vanligvis i løpet av en uke?

- 1 eller færre
- 2-3
- 4-6
- 7-10
- Mer enn 10

48) Hvor mange priser snus/skrå bruker du per dag?

- 1
- 2-3
- 4-6
- 7-10
- Mer enn 10

49) Hvor ofte drikker du alkohol?

- Aldri
- 1 gang per måned eller sjeldnere
- 2-4 ganger per måned
- 2-3 ganger per uke
- 4 eller flere ganger per uke

50) Hvor mange enheter alkohol (en øl, ett glass vin eller en drink) tar du vanligvis når du drikker?

- 1-2
- 3-4
- 5-6
- 7-9
- 10 eller flere

51) Hvor ofte drikker du 6 eller flere enheter alkohol ved en anledning?

- Aldri
- Sjeldnere enn 1 gang per måned
- 1 gang per måned
- 1 gang per uke
- Daglig eller nesten daglig

FYSISK AKTIVITET

52) Hvilken beskrivelse passer best når det gjelder din fysiske aktivitet på fritiden det siste året?

- Sitter ved PC/TV, leser eller annen stillesittende aktivitet.
- Går, sykler eller beveger deg på annen måte minst 4 timer i uken (her skal du også regne med tur til/fra skolen, shopping, søndagsturer med mer).
- Driver med idrett/trening, tyngre utearbeid, snømåking eller liknende minst 4 timer i uka.
- Trener hardt eller driver konkurranseidrett regelmessig og flere ganger i uka.

53) Hvordan kommer du deg vanligvis til og fra skolen i sommerhalvåret?

- Med bil, motorsykkel/moped
- Med buss
- Med sykkel
- Går

54) Hvor lang tid bruker du vanligvis til og fra skolen (en vei) i sommerhalvåret?

- Mindre enn 5 minutter
- 6 til 15 minutter
- 16 til 30 minutter
- 1/2 til 1 time
- Mer enn 1 time

55) Hvordan kommer du deg vanligvis til og fra skolen i vinterhalvåret?

- Med bil, motorsykkel/moped
- Med buss
- Med sykkel
- Går

56) Hvor lang tid bruker du vanligvis til og fra skolen (en vei) i vinterhalvåret?

- Mindre enn 5 minutter
- 6 til 15 minutter
- 16 til 30 minutter
- 1/2 til 1 time
- Mer enn 1 time

57) Driver du med idrett eller fysisk aktivitet (f.eks. skateboard, fotball, dans, løping) utenom skoletid?

- Ja
- Nei

58) Hvor mange dager i uken driver du med idrett/fysisk aktivitet utenom skoletid?

- Aldri
- Sjeldnere enn 1 dag i uka
- 1 dag i uka
- 2-3 dager i uka
- 4-6 dager i uka
- Omtrent hver dag

59) Omtrent hvor mange timer per uke bruker du til sammen på idrett/fysisk aktivitet utenom skoletid?

- Ingen
- Omtrent 1/2 time
- Omtrent 1 - 1 1/2 time
- Omtrent 2 - 3 timer
- Omtrent 4 - 6 timer
- 7 timer eller mer

60) Hvor slitsom er vanligvis idretten/aktiviteten du driver med utenom skoletid?

- Ikke anstrengende
- Litt anstrengende
- Ganske anstrengende
- Meget anstrengende
- Svært anstrengende

Utenom skoletid: Hvor mange timer per dag ser du på PC, TV, DVD og liknende?

61) Hverdager, antall timer per dag:

- Ingen
- Omtrent 1/2 time
- Omtrent 1 - 1 1/2 time
- Omtrent 2 - 3 timer
- Omtrent 4 - 6 timer
- Omtrent 7 - 9 timer
- 10 timer eller mer

62) Fridager (helg, helligdager, ferie), antall timer per dag:

- Ingen
- Omtrent 1/2 time
- Omtrent 1 - 1 1/2 time
- Omtrent 2 - 3 timer
- Omtrent 4 - 6 timer
- Omtrent 7 - 9 timer
- 10 timer eller mer

Svar på en skala fra 1 til 5, der 1 tilsvarer svært sjelden eller aldri og 5 tilsvarer svært ofte.

63) I hvilken grad har andre oppmuntret deg til å være fysisk aktiv

	1	2	3	4	5
Foreldre/foresatte	○	○	○	○	○
Søsken	○	○	○	○	○
Venner	○	○	○	○	○
Trenere	○	○	○	○	○
Gymlærere	○	○	○	○	○
Nabolaget	○	○	○	○	○

Svar på en skala fra 1 til 5, der 1 tilsvarer helt enig og 5 tilsvarer helt uenig.

64) Hvordan passer disse utsagnene for deg?

	1	2	3	4	5
Det er morsommere å drive med trening eller fysisk aktivitet enn å gjøre andre ting...	○	○	○	○	○
Jeg skulle ønske jeg kunne drive mer med trening eller fysisk aktivitet enn det jeg har anledning til å gjøre...	○	○	○	○	○
Jeg føler at jeg er bedre enn de fleste på min alder i idrett/fysisk aktivitet...	○	○	○	○	○
Jeg føler at jeg lett kan holde følge med de andre på min alder når vi driver med idrett/fysisk aktivitet...	○	○	○	○	○

Svar på en skala fra 1 til 5, der 1 tilsvarer helt enig og 5 tilsvarer helt uenig.

65) Hvordan passer disse utsagnene for deg?

	1	2	3	4	5
Jeg liker ikke å trene mens noen står å ser på...	○	○	○	○	○
Tilgang til egen garderobe hadde gjort det lettere å trene...	○	○	○	○	○
Jeg blir ubehagelig andpusten, svett eller får vondt i kroppen ved trening...	○	○	○	○	○
Gymtimene er organisert slik at jeg ikke henger med...	○	○	○	○	○
Jeg har ingen å trene sammen med...	○	○	○	○	○
Jeg mangler utstyr for å drive med den aktiviteten jeg har lyst til...	○	○	○	○	○
Jeg har for mange andre oppgaver som gjør at jeg ikke får tid til å trene (f.eks lekser, hjemmeoppgaver)...	○	○	○	○	○
Det mangler egnede haller eller gode uteområder for å drive fysisk aktivitet der jeg bor...	○	○	○	○	○

MATVANER OG KOSTHOLD

66) Hvor ofte pleier du å spise følgende i løpet av en uke?

	Hver dag	4-6 dager i uka	1-3 dager i uka	Sjelden eller aldri
Frokost	○	○	○	○
Middag	○	○	○	○

67) Hvor ofte spiser du matpakke hjemmefra på skolen?

- ○ Hver dag
- ○ 3-4 ganger per uke
- ○ 1-2 ganger per uke
- ○ Sjelden eller aldri

68) Hvor ofte spiser du vanligvis disse matvarene?

	Sjelden/ aldri	1-3 ganger per måned	1-3 ganger per uke	4-6 ganger per uke	Hver dag
Ost (alle typer)	○	○	○	○	○
Fet fisk (f.eks. laks, ørret, makrell, sild)	○	○	○	○	○
Mager fisk (f.eks. torsk, sei, hyse)	○	○	○	○	○
Pizza, hamburger eller pølser	○	○	○	○	○
Hermetisert mat (fra metallbokser)	○	○	○	○	○
Godteri (f.eks. sjokolade, drops)	○	○	○	○	○
Snacks og søtsaker (f.eks. potetgull, kake, kjeks, bolle)	○	○	○	○	○
Sukkerfri tyggegummi	○	○	○	○	○

69) Hvor ofte spiser du vanligvis

	Sjelden/ aldri	1-3 ganger per mnd	1-3 ganger per uke	4-6 ganger per uke	1-2 ganger per dag	3-4 ganger per dag	5 eller flere ganger per dag
Frukt	○	○	○	○	○	○	○
Grønnsaker	○	○	○	○	○	○	○

70) Hvor mange ganger i året spiser du vanligvis disse matvarene?

	0	1-3	4-5	6-9	10 eller flere
Mølje med fiskelever	○	○	○	○	○
Måsegg	○	○	○	○	○
Reinsdyrkjøtt	○	○	○	○	○
Selvplukket sopp	○	○	○	○	○

71) Hvor mye drikker du vanligvis av følgende?

	Sjelden/ aldri	1-6 glass per uke	1 glass per dag	2-3 glass per dag	4 glass eller mer per dag
Helmelk, kefir, yoghurt	○	○	○	○	○
Lettmelk, cultura, lettyoghurt	○	○	○	○	○
Skummet melk (sur/søt)	○	○	○	○	○
Ekstra lett melk	○	○	○	○	○
Juice	○	○	○	○	○
Saft med sukker	○	○	○	○	○
Lettsaft, kunstig søtet	○	○	○	○	○
Brus med sukker (1/2 liters flaske = 2 glass)	○	○	○	○	○
Lettbrus, kunstig søtet (1/2 liters flaske = 2 glass)	○	○	○	○	○
Vann	○	○	○	○	○

72) Bruker du følgende kosttilskudd?

	Ja, daglig	Iblant	Nei
Tran, trankapsler, fiskeoljekapsler	○	○	○
Vitamin- og/eller mineraltilskudd	○	○	○

SØVN OG SØVNVANER

73) Når pleier du å legge deg for å sove på ukedagene?

Velg...

74) Når pleier du å legge deg for å sove i helgen?

Velg...

75) Hvor lenge pleier du å ligge våken før du får sove på ukedagene?

Velg...

76) Hvor lenge pleier du å ligge våken før du får sove i helgen?

Velg...

77) Når pleier du å våkne på ukedagene (endelig oppvåkning)?

Velg...

78) Når pleier du å våkne i helgen (endelig oppvåkning)?

Velg...

79) Hvor mange timer sover du vanligvis pr. natt?

Velg...

80) Hvor mange timer søvn trenger du pr. natt for å føle deg uthvilt?

Velg...

81) Synes du at du får tilstrekkelig med søvn?

- Ja, absolutt tilstrekkelig
- Ja, stort sett tilstrekkelig
- Nei, noe utilstrekkelig
- Nei, klart utilstrekkelig
- Nei, langt fra tilstrekkelig

HUD

Her har vi noen spørsmål om vanlige hudplager/hudsykdommer.

82) Har du hatt kløende utslett i løpet av de siste 12 månedene?

Ja Nei Vet ikke

83) Har dette utslettet sittet på noen av de følgende stedene: rundt hals, ører eller øyne, i albuebøyene (på innsiden), under baken, bak knærne eller foran på anklene?

Ja Nei

84) Hvor gammel var du første gang du fikk denne typen utslett?

Velg...

Hvor mye plaget er du av dette utslettet i dag?
Svar på en skala fra 0-10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

0 1 2 3 4 5 6 7 8 9 10

86) Har du hatt håndeksem flere ganger?

○ Ja ○ Nei ○ Vet ikke

Hvor mye plaget er du av håndeksem i dag?
Svar på en skala fra 0-10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

88) Har du noen gang vært plaget av kviser?

○ Ja ○ Nei ○ Vet ikke

Hvor mye plaget er du av kviser i dag?
Svar på en skala fra 0-10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

90) Har du noen gang oppsøkt lege på grunn av kviser?

○ Ja ○ Nei

91) Har du fått noen av disse behandlingene av lege?

	Ja	Nei	Vet ikke
Lokalbehandling (f.eks. kremer eller oppløsninger)	○	○	○
Antibiotika tabletter (f.eks. Tetracyclin)	○	○	○
Roaccutan tabletter	○	○	○

92) Har du eller har du noen gang hatt psoriasis?

○ Ja ○ Nei ○ Vet ikke

Hvor mye plaget er du av psoriasis i dag?
Svar på en skala fra 0-10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

Verkebyller er svært store kviser som er ømme/smertefulle og som ofte gir
arr.

94) Har du noen gang hatt verkebyller under armene/armhulene?

○ Ja
○ Nei
○ Vet ikke

95) Har du noen gang oppsøkt lege pga verkebyllene?

○ Ja ○ Nei

96) Har du noen gang hatt verkebyller i lyskene/nært skrittet?

○ Ja
○ Nei
○ Vet ikke

97) Har du noen gang oppsøkt lege på grunn av verkebyllene?

- Ja
- Nei

98) Har en lege noen gang sagt at du har...

	Ja	Nei	Vet ikke
høysnue eller neseallergi?			
astma?			
barneeksem eller atopisk eksem?			

SMERTER

99) Har du langvarige eller stadig tilbakevendende smerter som har vart i 3 måneder eller mer?

- Ja
- Nei

100) Hvor lenge har du hatt disse smertene? (Dersom du har flere typer smerte, svar for den som har vart lengst)

- 3 - 6 måneder
- 6 - 12 måneder
- 1-2 år
- 3-6 år
- Mer enn 6 år

101) Hvor ofte har du vanligvis disse smertene?

- Hele tiden, uten opphør
- Hver dag, men ikke hele tiden
- Hver uke, men ikke hver dag
- Sjeldnere enn hver uke

Hvor er det vondt?
(kryss av på alle aktuelle steder)

	Venstre side	Høyre side
Skulder	☐	☐
Arm/albue	☐	☐
Hånd	☐	☐
Hofte	☐	☐
Lår/kne/legg	☐	☐
Ankel/fot	☐	☐

	Midten
Hode/ansikt	☐
Kjeve/kjeveledd	☐
Nakke	☐
Øvre del av ryggen	☐
Korsryggen	☐
Bryst	☐
Mage	☐
Underliv/kjønnsorganer	☐

104) Hva mener du er årsaken til smertene? (flere svar mulig)

- [ ] PC-bruk, dataspill og lignende
- [ ] Idrettsskade
- [ ] Ulykke/skade
- [ ] Kirurgisk inngrep/operasjon
- [ ] Migrene/hodepine
- [ ] Medfødt sykdom
- [ ] Tannproblemer
- [ ] Whiplash
- [ ] Prolaps (skiveutglidning i ryggen)
- [ ] Annet ryggproblem
- [ ] Nerveskade
- [ ] Mage- eller tarmsykdom
- [ ] Annet, spesifiser her
- [ ] Vet ikke

Hvis du har langvarige smerter flere steder i kroppen, gjelder de 4 neste spørsmålene smerten som plager deg mest.

Hvor sterke vil du si at smertene vanligvis er?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

Hvor sterke er smertene når de er på sitt sterkeste?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

I hvor stor grad påvirker smertene søvnen din?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

I hvor stor grad hindrer smertene deg i å utføre vanlige aktiviteter hjemme og på skolen?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

MAGE- OG TARMPROBLEMER

109) I løpet av de siste 2 månedene: Hvor ofte har du hatt smerte eller ubehag i magen?

- ○ Aldri
- ○ 1-3 ganger i måneden
- ○ En gang i uka
- ○ Flere ganger i uka
- ○ Hver dag

110) Hvor lenge har du vært plaget av smerte eller ubehag i magen?

- ○ Mindre enn 1 måned
- ○ 2 måneder
- ○ 3 måneder
- ○ 4-11 måneder
- ○ Ett år eller mer

111) I hvilken del av magen er det du har hatt smerte eller ubehag? (kryss av for alt som passer)

- [ ] Over navlen
- [ ] Rundt navlen
- [ ] Nedenfor navlen

112) Når du har smerter eller ubehag i magen, hvor lenge varer det vanligvis?

- ○ Mindre enn 1 time
- ○ 1-2 timer
- ○ 3-4 timer
- ○ Mesteparten av dagen
- ○ Hele døgnet

Når du har smerte eller ubehag i magen, hvor sterke smerter har du vanligvis?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

Dersom du har flere typer smerte, svar den som plager deg mest.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

114) Når du har smerter eller ubehag i magen, hvor ofte blir det bedre etter at du har hatt avføring?

- ○ Sjelden eller aldri
- ○ En del ganger
- ○ For det meste/hver gang

115) Når du har smerter eller ubehag i magen, hvor ofte skjer det i forbindelse med at du..

	Sjelden eller aldri	En del ganger	For det meste
har fastere eller mer klumpete avføring enn vanlig?	○	○	○
har løsere eller mer vannaktig avføring enn vanlig?	○	○	○
hadde avføring oftere enn vanlig?	○	○	○
hadde avføring sjeldnere enn vanlig?	○	○	○

HODEPINE

116) Har du vært plaget av hodepine det siste året?

○ Ja ○ Nei

117) Hva slags hodepine er du plaget av? (Du kan sette flere kryss)

☐ Migrene ☐ Annen hodepine ☐ Vet ikke

118) Omtrent hvor mange dager per måned har du hodepine?

○ Mindre enn 1 dag
○ 1-6 dager
○ 7-14 dager
○ Mer enn 14 dager

119) Er hodepinen vanligvis:

	Ja	Nei
Bankende/dunkende smerte	○	○
Pressende smerte	○	○
Ensidig smerte (høyre eller venstre)	○	○

120) Hvor lenge varer hodepinen vanligvis?

- Mindre enn 4 timer
- 4 timer - 1 døgn
- 1-3 døgn
- Mer enn 3 døgn

121) Før eller under hodepinen, kan du da ha forbigående:

	Ja	Nei
Synsforstyrrelse? (takkede linjer, flimring, tåkesyn, lysglimt)		
Nummenhet i halve ansiktet eller i hånden?		
Forverring ved moderat fysisk aktivitet?		
Kvalme og/eller oppkast?		

122) Hvor ofte pusser du vanligvis tennene dine? (sett ett kryss)

- Sjeldnere enn 1 gang per uke
- 1 gang per uke
- 2-3 ganger per uke
- 4-6 ganger per uke
- 1 gang daglig
- 2 eller flere ganger daglig

Hvor smertefullt, jevnt over, synes du det er å gå til tannlegen?
Svar på en skala fra 0-10, der 0 tilsvarer ingen smerte og 10 tilsvarer verst tenkelig smerte.

0 1 2 3 4 5 6 7 8 9 10

Nedenfor er det fire spørsmål om hvordan du opplever det er å gå til tannlege. Les hvert spørsmål og velg det svaralternativet som du synes passer best for deg.

124) Dersom du skulle gå til tannlegen i morgen, hva ville du føle?

- Jeg ville se frem til det som en ganske hyggelig opplevelse
- Det ville være det samme for meg, ikke bety noe
- Det ville gjøre meg litt urolig
- Jeg ville bli redd for at det skulle bli ubehagelig og vondt
- Jeg ville bli svært redd med tanke på hva tannlegen kanskje skulle gjøre

125) Når du venter på tannlegens venteværelse, hvordan føler du deg da?

- Avslappet
- Litt urolig
- Anspent, nervøs
- Redd, engstelig
- Så redd at jeg av og til begynner å svette eller nesten føler meg syk

126) Når du sitter i tannlegestolen og venter på at tannlegen skal begynne behandlingen, hvordan føler du deg da?

- Avslappet
- Litt urolig
- Anspent, nervøs
- Redd, engstelig
- Så redd at jeg av og til begynner å svette eller nesten føler meg syk

Tenk at du sitter i tannlegestolen og skal få tennene renset og pusset. Mens du sitter og venter på at tannlege skal finne frem instrumentene som brukes til å skrape og pusse med,

127) hvordan føler du deg da?

- Avslappet
- Litt urolig
- Anspent, nervøs
- Redd, engstelig
- Så redd at jeg av og til begynner å svette eller nesten føler meg syk

128) Har du øresus?

○ Aldri ○ Sjelden ○ Ofte

129) Hvor ofte har du øresus?

○ Hele tiden, uten opphør
○ Hver dag, men ikke hele tiden
○ Hver uke, men ikke hver dag
○ Sjeldnere enn hver uke

130) Hvor lenge varer vanligvis periodene med øresus?

○ Mindre enn 10 minutter ○ 10 minutter - 1 time ○ Mer enn 1 time

131) Når får du vanligvis øresus?

☐ Etter sterke lyder ☐ Når det er stille ☐ Vet aldri når

Noen bryr seg ikke om lyden, for andre oppleves det svært plagsomt å ha øresus. Angi hvor plaget du er av øresusen.
Svar på en skala fra 0 til 10, der 0 tilsvarer ingen plager og 10 tilsvarer verst tenkelige plager.

○ 0 ○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10

133) På hvilket øre har du vanligvis øresus?

○ Bare høyre
○ Bare venstre
○ Begge, men mest høyre
○ Begge, men mest venstre
○ Like mye på begge

134) Omtrent hvor gammel var du når du begynte å ha øresus ofte?

Velg...