The association between smoking behaviour and depressive symptoms in adolescence: the role of biology and of societal influences

Elena Raffetti
THE ASSOCIATION BETWEEN SMOKING BEHAVIOUR AND DEPRESSIVE SYMPTOMS IN ADOLESCENCE: THE ROLE OF BIOLOGY AND OF SOCIETAL INFLUENCES

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THESIS FOR DOCTORAL DEGREE (Ph.D.)

by

Elena Raffetti

The thesis will be defended in public at Inghesalen, Tomtebodavägen 18, Karolinska Institutet, Solna, on Wednesday, December 16, 2020 at 9:00 am

Principal Supervisor:
Professor Maria Rosaria Galanti
Karolinska Institutet
Department of Global Public Health

Opponent:
Professor Paolo Vineis
Imperial College
MRC-PHE Centre for Environment and Health

Co-supervisors:
Professor Francesco Donato
University of Brescia
Department of Medical and Surgical Specialties, Radiological Sciences and Public Health

Examination Board:
Professor Jouko Miettunen
University of Oulu
Center for Life Course Health Research

Professor Yvonne Forsell
Karolinska Institutet
Department of Global Public Health

Professor Curt Hagquist
Karlstad University
Centre for Research on Child and Adolescent Mental Health

Professor Torbjörn Åkerstedt
Karolinska Institutet
Department of Clinical Neuroscience
To my parents, Antonietta Pelamatti and Bortolo Raffetti
ABSTRACT

Tobacco use is common among people who suffer from depressive symptoms. This co-occurrence is associated with several negative health outcomes. The present thesis will contribute to the understanding of if, how, and in which contexts this specific association occurs in early adolescence. We investigated the longitudinal association between tobacco use and depressive symptoms, the potential biological mechanisms behind this association and the extent to which the social context modifies the co-occurrence of these two major health determinants. Data are obtained from the KUPOL study, a population-based cohort of Swedish adolescents. The evaluation of the social context also includes data from the Italian BE-TEEN study.

Study I. The possible association between smoking or snus use and depressive symptoms was examined in 3,195 KUPOL study participants. Smoking behaviour was associated with an increased risk of the onset of depressive symptoms among adolescents.

Study II. We explored the possible involvement of activation of the hypothalamic-pituitary-adrenocortical axis, measured through salivary cortisol concentration, in the pathway from cigarette smoking to depressive symptoms in 409 KUPOL study participants. There was no evidence for an association between cigarette smoking and the hypothalamic-pituitary-adrenocortical axis levels of activity, and the latter did not predict an increased risk of the onset of depressive symptoms.

Study III. Salivary cortisol concentration as a predictor of the onset of tobacco use was investigated in 381 KUPOL study participants. Morning cortisol concentration was associated with an increased risk of smoking and snus initiation as well as duration of use. These findings suggest an association between activation of the hypothalamic-pituitary-adrenocortical axis and tobacco use.

Study IV. We evaluated the presence of the association between smoking and depressive symptoms in a restrictive (Sweden) and in a non-restrictive (Italy) tobacco control environment including 3,283 Swedish and 1,947 Italian participants. Cross-sectional associations between smoking and depressive symptoms were found in both the restrictive and non-restrictive tobacco control environments, with the strongest association in the former.

Conclusions. This thesis indicates that smoking behaviour is a predictor of the development of depressive symptoms in adolescence. The pathways behind this longitudinal association are not clear. Seemingly, the hypothalamic-pituitary-adrenocortical axis is not implicated in the process from smoking to depressive symptoms. However, findings indicate that the initial activation of the hypothalamic-pituitary-adrenocortical axis may be linked to smoking onset. This opens up for new research avenues on dysregulation of the response to stressors, such as adverse life events, and smoking onset at a young age. Finally, translating this emerging knowledge into prevention programmes may help to develop context-specific interventions and to direct efforts towards specific subgroups of adolescents at high risk of tobacco use and depressive problems.

Key words: tobacco use, depressive symptoms, stress, HPA axis, adolescence.
SAMMANFATTNING


Studie I. I denna studie undersökte vi det potentiella kausala sambandet mellan tobaksanvändning, både rökning och snusning, och depressive symtom hos 3195 ungdomar i KUPOL-studien. Vi fann att rökning var associerat med ökad risk för depressiva symtom hos dessa ungdomar.


Studie IV. I denna studie utvärderade vi sambandet mellan rökning och depressive symtom i två olika kontextuella miljöer, en begränsande (Sverige) och en tillåtande (Italien). Data baserades på 3323 svenska och 1947 italienska deltagare. Från denna tvärsnittsstudie fann vi att sambandet mellan rökning och depressive symptom både fanns i den begränsande och den icke-begränsande miljön. Vidare fann vi här att sambandet var starkare i den begränsande miljön än den icke-begränsande.


Nyckelord: tobaksbruk, depressive symtom, stress, HPA-axel, ungdomar.
RIASSUNTO

L’uso del tabacco è comune tra le persone che soffrono di sintomi depressivi, e contribuisce a determinare conseguenze negative sulla salute. L’adolescenza è una fase della vita in cui nascono e si consolidano alcune abitudini, come l’uso del tabacco, che poi si mantengono nell’età adulta. Anche diversi disturbi mentali, come la depressione insorgono spesso in età giovanile, per poi mantenersi o ricomparire nel tempo.

Questa tesi si propone di indagare, mediante uno studio longitudinale prospettico, l’associazione tra uso di tabacco e comparsa successiva di sintomi depressivi, esaminando anche alcuni possibili meccanismi biologici e il contesto sociale in cui vivono i giovani. I dati utilizzati nella ricerca sono stati ottenuti dagli studi KUPOL e BE-TEEN, comprendenti adolescenti svedesi e italiani, rispettivamente.

**Studio I.** Abbiamo studiato la possibile associazione causale tra il fumo di sigaretta, l’uso di snus (tipico tabacco da masticare svedese) e l’insorgenza di sintomi depressivi negli anni successivi in 3195 partecipanti allo studio KUPOL. L’abitudine al fumo, ma non l’uso di snus, è risultata associata all’aumento in un anno del rischio dell’insorgenza di sintomi depressivi tra gli adolescenti.

**Studio II.** Abbiamo esaminato il possibile coinvolgimento dell’attivazione dell’asse ipotalamo-ipofisi-surrene, attraverso la concentrazione di cortisolo salivare, come intermedio dell’associazione tra fumo di sigaretta e sintomi depressivi in 409 partecipanti allo studio KUPOL. Non abbiamo trovato una chiara evidenza di un’associazione tra fumo di sigaretta e l’attivazione dell’asse ipotalamo-ipofisi-surrene e di quest’ultimo fattore sull’insorgenza dei sintomi depressivi.

**Studio III.** Abbiamo indagato la concentrazione di cortisolo salivare come possibile predittore dell’inizio del consumo di tabacco in 381 partecipanti allo studio KUPOL. La concentrazione mattutina di cortisolo è risultata associata con un aumento del 20%-30% del rischio di iniziare e continuare a fumare e usare snus. Questi risultati suggeriscono un’associazione tra l’attivazione dell’asse ipotalamo-ipofisi-surrene e l’uso di tabacco e snus.

**Studio IV.** Abbiamo valutato la presenza dell’associazione tra fumo e sintomi depressivi in un contesto permissivo (Italia) e in uno restrittivo (Svezia) riguardo all’uso del tabacco, includendo 3323 partecipanti allo studio KUPOL e 1947 partecipanti allo studio italiano BE-TEEN, mediante un disegno di studio trasversale. L’associazione tra fumo e sintomi depressivi è stata rilevata sia nel contesto più restrittivo che in quello più permissivo, con una maggiore forza di associazione nell’ambiente sociale più restrittivo sull’uso del tabacco (Svezia).

**Conclusioni.** Questa tesi mostra che il fumo di tabacco è un predittore dello sviluppo successivo di sintomi depressivi nel corso dell’adolescenza. Non è chiaro il meccanismo biologico sottostante a questa associazione. Verosimilmente, l’asse ipotalamo-ipofisi-surrene non è coinvolto nella relazione tra fumo di sigaretta e insorgenza di sintomi depressivi. Al contrario, lo studio mostra che l’attivazione dell’asse ipotalamo-ipofisi-surrene potrebbe essere associata all’inizio dell’uso di tabacco. Questi risultati aprono nuovi spunti di ricerca sul ruolo della disregolazione della risposta dell’organismo allo stress nell’inizio e stabilizzazione dell’abitudine fumatoria in giovane età. Infine, queste informazioni potrebbero essere utili per programmi di prevenzione mirati sulla base del contesto, con particolare riguardo a specifici sottogruppi di adolescenti ad alto rischio di iniziare a usare tabacco e di sviluppare sintomi depressivi.

**Parole chiave:** uso di tabacco, sintomi depressivi, stress, asse ipotalamo-ipofisi-surrene, adolescenza
摘要

吸烟常见于有抑郁症状的人群中。吸烟与抑郁的共同存在可能会引起多种不良的健康结果。这篇论文将有助于我们对吸烟和抑郁症状之间关系是否存在于青少年早期、如何存在的以及在哪些环境下存在的理解。我们研究吸烟和抑郁症状的长期关系及其背后的潜在生物学机制，以及社会环境多大程度上影响这两个重要的健康要素。研究数据来源于一项针对瑞典青少年人群的队列研究（KUPOL），此外评估社会环境的数据还包含一项来自意大利的研究（BE-TEEN）。

研究1：我们基于来自KUPOL的3195名研究对象调查吸烟或鼻烟和抑郁症状的因果关系。结果显示吸烟可能会增加青少年人群抑郁症状的发生风险。

研究2：我们基于来自KUPOL的409名研究对象调查吸烟是否是通过激活下丘脑-垂体-肾上腺皮质轴（通过唾液中皮质醇的浓度判定）来增加抑郁症状的发生风险。结果显示吸烟可能和下丘脑-垂体-肾上腺皮质轴的激活无关，而且也不能预测抑郁症状的发生风险。

研究3：我们基于来自KUPOL的381名研究对象调查唾液中皮质醇浓度是否能够预测吸烟的发生。结果显示早晨唾液皮质醇浓度可能会增加吸烟或鼻烟的发生，以及会影响吸烟或鼻烟的使用时间。这些结果提示下丘脑-垂体-肾上腺皮质轴的激活与吸烟有关联。

研究4：基于瑞典（严格）和意大利（宽松）的不同控烟环境，我们在3323名瑞典和1947名意大利研究对象中调查了吸烟和抑郁症状的关系。横断面调查的数据显示吸烟和抑郁症状之间的关联既存在于严格的控烟环境，也存在于宽松的控烟环境，且前者的关联更强。

结论：这篇论文提示吸烟可以预测青少年抑郁症状的发生。这个关系背后的机制尚不清楚。有可能的是，下丘脑-垂体-肾上腺皮质轴对从吸烟发展成抑郁症的过程没有影响。但数据提示，下丘脑-垂体-肾上腺皮质轴的激活可能引起吸烟行为。这些研究结果打开了一条新的研究思路，比如生命过程中的不良生活事件会引起年轻人群对压力、吸烟和成瘾的反应失调。最后，将这个新的发现应用到预防项目中可能有助于制订环境特异性的干预措施从而直接应用于有吸烟和抑郁症状高风险的青少年人群。

关键词：吸烟、抑郁症状、压力、HPA轴、青少年
LIST OF SCIENTIFIC PAPERS

The present thesis is an original contribution based on four papers stated below and mentioned in the text using Roman numerals.


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<tr>
<td>ACTH</td>
<td>Adrenocorticotropic Hormone</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>$\beta$</td>
<td>Beta</td>
</tr>
<tr>
<td>CES-DC</td>
<td>Centre for Epidemiological Studies Depression Scale for Children</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>dl</td>
<td>Decilitre</td>
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<td>EU</td>
<td>European Union</td>
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<tr>
<td>GDP</td>
<td>Gross Domestic Product</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-Pituitary-Adrenocortical</td>
</tr>
<tr>
<td>$\mu g$</td>
<td>Microgram</td>
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<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<td>RR</td>
<td>Risk Ratio</td>
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<td>SD</td>
<td>Standard Deviation</td>
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<td>SDQ</td>
<td>Strengths and Difficulties Questionnaire</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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1 INTRODUCTION

1.1 ADOLESCENCE AND HEALTH

1.1.1 Adolescence, a sensitive period

Adolescence is normally conceptualised as the period in life between 10 and 19 years of age and represents a time of rapid individual changes (1). As a period of transition from childhood to adulthood, it plays a unique role in human development and is a pivotal time for laying the foundations for a healthy life. Fundamental cognitive, physical and psychosocial developments take place during this age period (2, 3). Socially, young people experience increased pressures from the surrounding environment with regard to relational and intellectual performances; at the same time, profound physiological changes occur, such as the pubertal increase in hormonal levels and growth acceleration (4, 5).

At a young age, individuals establish behaviours that will influence their long-term physical and mental health and which may be difficult to modify later on (6). For example, adolescents who are physically active and follow a balanced diet increase their chances of preventing chronic disease. Conversely, a sedentary lifestyle in combination with a high-calorie diet and substance use has a negative influence on health, and increases the long-term risk of chronic diseases. A strategy towards good physical and mental health in adolescence encompasses safe and encouraging social contexts, education programmes, opportunities to develop skills and goals, and access to healthcare and education (1). Efforts to fulfil such goals, along with the promotion of active participation of adolescents in maintaining their health, might encourage healthy ageing and awareness of personal risk.

1.1.2 Public health challenges and the role of preventive medicine

Since the 1970s, the world has faced an exponential increase in the absolute number of the human population. By 2100, the absolute number will reach around 11 billion people (7). Together with a rise in life expectancy and the development of novel treatments, this is contributing to an increase in the older population and poses important challenges to the sustainability of healthcare systems and welfare worldwide (7, 8). Within the EU, average healthcare expenditure amounted to 10% of GDP in 2016 (9). According to projections, the cost will escalate unless cost-effective prevention policies are put in place (8).

While biomedical sciences and public health focused on innovations to improve life expectancy during the 20th century, the main goal to date during the 21st century has been to ensure a long and healthy life, referred to as the concept of “healthy aging”. Promoting a healthy lifestyle and well-being from early adolescence is one of the key contributors to healthy ageing (1). In the US, for example, every dollar invested in health promotion provides a return of 5.3 dollars in decreased healthcare and crime costs within the Communities That Care prevention system (10). Despite knowledge gained about the cost-efficacy of prevention programmes, many national healthcare systems focus on curing rather than preventing disease, while it is clear that substantial benefits would be achieved by allocating more economic resources to preventive medicine.
1.1.3 Smoking behaviour in a life-course perspective

Recent decades have produced extensive literature on the determinants and negative effects of five major public health issues, namely smoking, obesity, physical inactivity, excessive drinking, and insufficient sleep. This has led to two research priorities. The first is to identify individual and contextual factors that facilitate a healthy lifestyle. Secondly, researchers have attempted to distinguish periods in life of increased vulnerability, in which one or more of the above exposures can exert an adverse effect on the development of diseases in line with a life-course approach (11). Finally, given increasing knowledge of both determinants and effects of different health behaviours, resources should also move towards a translation into preventive programmes.

A number of methodological aspects should be carefully considered to ensure the interpretation and the generalisability of findings in this field. Some associations may be spurious and non-reproducible due to reverse causality, confounding, adjustment for the intermediates of the association, or misclassification. Studying an association, when one occurs, reduces the risk of these methodological pitfalls.

Conceptually, different stages of life may be of interest in evaluating factors that determine the onset of a behaviour, as well as those that may contribute to the change in behaviour. Figure 1 illustrates a hypothetical cohort and the prevalence of current smoking in relation to the age of the individuals. In this example, early adolescence is a relevant age period for questions that aim to evaluate factors that facilitate smoking initiation. However, the time span should be broader if we are interested in determinants that influence the transition from experimental to regular smoking. Finally, it is the later periods of life that should be considered when focusing on determinants for smoking cessation.

![Figure 1. The trajectory of current smoking prevalence in a cohort. Estimates of current smoking prevalence from 11 to 18 years of age were derived from the BROMS study (12).](image-url)
1.2 TOBACCO USE AND DEPRESSIVE SYMPTOMS

1.2.1 Tobacco, a major public health issue

Currently, the global population is 7.7 billion, out of which 1.1 billion are smokers (13). Robust evidence exists on the poor health outcomes associated with cigarette smoking, in particular the increased risk for chronic obstructive pulmonary disease, cardiovascular disease and cancer, making smoking one of the major causes of premature mortality (14-16). According to WHO estimates, tobacco kills up to half of its users, with an annual rate of more than eight million people. This figure includes 1.2 million deaths that are attributable to second-hand smoking (17). During the 20th century, smoking resulted in 100 million deaths, mostly in high-income countries (18). According to projections, the corresponding burden during the 21st century is likely to be about one billion deaths, with the majority occurring in low and middle-income countries (19). The worldwide health-related costs attributable to tobacco use are calculated to be within the billion-dollar range between 0.1% and 1.1% of GDP in high-income economies (20).

This high prevalence of tobacco use, along with its important impact on health outcomes, makes tobacco the single most preventable risk factor worldwide (21). Since Doll and Peto’s first studies in the 1950s, several advances in epidemiologic methods have been influenced by epidemiologic research on smoking and health (22). Country-level tobacco policies influence smoking behaviour via three main mechanisms (21, 23, 24): 1) economic instruments (i.e. taxation on tobacco); 2) regulations (i.e. smoke-free air policies); and 3) interventions to facilitate tobacco cessation (i.e. tobacco control funding). Despite the fact that these policy mechanisms are well-known and have been evaluated in different contexts, many countries still resist implementing effective policies (21) and instead put the economic interests of a state-owned tobacco monopoly before the public health interest.

In addition to national tobacco control policies, effective dissemination of public health messages focused particularly on smoking behaviour is also necessary. The negative effects on health caused by smoking do not receive the required attention on traditional and social media platforms. Communication on public health issues is often disproportional and sometimes even false (25). Researchers and media have the responsibility to change from market-influenced messages (26) to communicating clear and non-conflicting messages on the harmful effect of our lifestyle on health.

1.2.2 The usage of different forms of nicotine

The tobacco plant originates from the Americas and was introduced worldwide in the 16th century (27). The high concentration of nicotine - a potent stimulant with addictive properties - in dry tobacco leaves (up to 6% in cured tobacco leaves) (28), along with the development and marketing of cigarettes, facilitated the spread of tobacco use, resulting in a tobacco epidemic in the 20th century. Besides smoking tobacco, smokeless products have been introduced over time, such as chewing tobacco and oral and nasal snuff (29). A particular form of moist oral snuff, “snus”, is traditionally used in Sweden and Norway (30, 31). The presence of alkaline components in snus increases the bioavailability of the nicotine compared to cigarettes (28).
If the cigarette was the prominent engineered device for drug self-administration during the 19th and 20th centuries, the e-cigarette may be its equivalent in the 21st century. Since 2017, the prevalence of e-cigarette use, known as vaping, has tripled among American students (32). As recently as in 2019, there was an outbreak of acute lung injuries in the US (33) connected to e-cigarette use. This is indeed worrisome, given that the long-term health effects related to vaping are not yet known. These alternative tobacco products could help to distinguish the effects of nicotine from the effects of the other component, burning, on health outcomes. Nonetheless, researchers and health professions should be aware of a possible “beyond smoking tobacco epidemic”.

1.2.3 Global burden of depression

A number of countries have recorded an increase in mental health disorders in recent decades. The prevalence of depression in the global population is as high as 4.4%, which implies that about 322 million people are living with depression (34, 35). Interestingly, this proportion is comparable between high-income and low-income countries (36, 37), making the hypothesis that factors specific to individualistic and high-achieving societies are the main causes of depression inadequate to explain the recent growth. The onset of the majority of major depressive disorders occurs between adolescence and the mid-40s, with 40% of individuals experiencing the first episode of clinical depression as early as during adolescence (38, 39).

Today, mental health disorders are responsible for a significant proportion of the global burden of disease, impacting both quality of life and life expectancy (39, 40). It is noteworthy that depression is considered to be among the main causes of years lived with disability, and along with dysthymia accounts for about 10% of global years lived with disability (41). Depression is a dynamic condition characterised by symptoms as diverse as depressive mood and anhedonia to suicidal ideation and psychomotor retardation (39). Individuals with depression experience a reduction in psychosocial functioning and quality of life, and need social and economic support from family members and/or friends (39). The co-occurrence of depressive problems, substance use and an unhealthy lifestyle, along with a reduction in the ability to take care of oneself, undermines everyday functioning and increases the risk of chronic disease as well as earlier mortality with a 2-fold excess mortality risk compared to the general population (42).

Hence, the management of depressive problems remains a leading public health challenge. More than 75% of individuals affected by depressive symptoms in middle and low-income countries do not access healthcare (43), and among those who do, the effect of treatment is limited and the risk of relapse is high. To take an example, the Sequenced Treatment Alternatives to Relieve Depression trial is the largest randomised control trial to have evaluated the management of the major depressive disorder (44). This trial showed that under ideal circumstances only two-thirds of individuals reach the goal of complete remission of the symptoms after up to four different treatment lines (45). This, along with the increasing prevalence of depression in the population and its impact on daily functions, makes depression a global and complex issue that requires specific attention from policymakers and the allocation of economic resources.
1.2.4 Co-occurrence of smoking and depressive symptoms

Epidemiological studies have consistently demonstrated that smoking is more common among individuals with mental health disorders, depression in particular (46-48). Moreover, a significant proportion of the excess risk of morbidity and premature mortality associated with psychiatric disorders is attributable to smoking (49). Beyond the observation that depression increases the odds of smoking, depression is linked to the development of dependence, more severe withdrawal symptoms and, consequently, unsuccessful attempts to stop smoking (50).

The possible mechanisms that link smoking and mental health disorders deserve a deeper understanding and will be discussed later in this thesis. A systematic review published in 2016 examined a possible bi-directional association between smoking and depression, and encompassed as many as 148 studies (51). The results of the studies included were somewhat heterogeneous, showing some support for associations in either direction, as well as null findings. During the last decade, new findings have emerged regarding the underlying mechanism of a possible causal relationship between smoking and mental health disorders (52). Interestingly, a recent Mendelian randomisation study indicated that smoking is a risk factor for both depression and schizophrenia (53). Even though smokers perceive that smoking alleviates emotional problems as well as relieving stress, another meta-analysis pointed out that smoking cessation, compared to continued smoking, was related to reduced feelings of anxiety and depression. The size of the effect appeared to be comparable for individuals both with and without mental health disorders (54).

1.2.5 Smoking and depressive symptoms in adolescence

While earlier studies assessing the smoking-depression association considered adult populations, more recent research focuses on younger populations. There is scant evidence on the association between smoking and depressive symptoms during adolescence. For example, in the above-mentioned review on the smoking-depression association (51), only 24 studies out of 148 evaluated the prospective association between cigarette smoking and the onset of depressive symptoms in adolescents. Sixteen of these studies reported a positive association and eight no association. The length of the follow-up differed among the studies, varying from one to 36 years. Only four studies considered sex differences while three considered lifetime smoking as exposure. In addition, most of these studies were carried out in North America and were based on the same data (US Add Health). Notably, both current smoking at baseline and smoking initiation were associated with increased odds of depressive symptoms over time (55, 56). This result was replicated in another study that included data from the National Teenage Attitudes and Practices Survey, which showed that, compared with never-smokers, current smokers at baseline had an increased risk of developing depressive symptoms at follow-up (57). Among the negative findings, the Children in the Community Study indicated that exposure to smoking in adolescence and young adulthood did not increase the risk of major depressive disorder (58). Finally, in other studies, depressive symptoms have been found to predict subsequent smoking onset, but smoking did not predict later depression (59).

Two main reasons make a study of the association between smoking behaviour and depressive symptoms essential. First, adolescence is a critical period for the onset of both smoking behaviour and depressive symptoms (60), making this the optimal time to study temporal
relationships. Indeed, about 90% of adult daily smokers smoked their first cigarette before turning 18 years of age (61). Also, neuropsychiatric disorders are the most common cause of years lost because of disability in the 10-24 age group (62), and one in five teenagers has experienced mental health problems at least once in their life (63), with ensuing healthcare costs. For depressive symptoms, for example, the median age of onset was 13 years. Twelve-month and lifetime prevalence was 10% and 14.3% in adolescence, respectively (64). However, it should be noted that while there is widespread agreement regarding an actual increase in the prevalence of self-reported depressive symptoms and of care seeking among adolescents during recent decades, how changes in diagnostic criteria and increased awareness have affected this trend is still being debated (65). Second, smoking behaviour at a young age might exert stronger biological effects than it does in other age periods. The influence of gonadic maturation makes puberty a pivotal time for brain development through the remodelling of synaptic circuits and regulation of affectivity (2, 66). Exposure to tobacco-related toxicants, in particular nicotine, within this time window might entail the risk of disruption of stress regulation and of the autonomic nervous system, with a negative impact on anxiety and depressive disorders, as well as on cardiovascular disease (67-71).
1.3 POTENTIAL MECHANISMS LINKING SMOKING AND DEPRESSION

1.3.1 The direction of the association

Several hypotheses have been proposed to explain an elevated prevalence of smoking behaviour among individuals with depressive symptoms: i) self-medication, namely the use of smoking to counterbalance negative affective symptoms; ii) the potential impact of tobacco-related toxicants on the neurocircuitry of depression; and iii) the presence of common liability (72).

1. The self-medication hypothesis suggests that individuals turn to smoking to cope with depressive symptoms (depressive symptoms lead to smoking) (72). Smokers report that they feel better after the first experience of smoking. Such a misperception may increase over time with the onset of tobacco dependence. With the consolidation of the behaviour, the withdrawal syndrome after acute abstinence from smoking accompanies anxiety and depressive symptoms and is alleviated by smoking. In other words, smoking seems to alleviate withdrawal symptoms in the short term (72).

2. Alteration of the synaptic circuits may stem from exposure to tobacco-related toxicants mainly between the prenatal stage and late adolescence (smoking leads to depressive symptoms). Animal studies indicate that nicotine exposure dysregulates stress and monoamine neurotransmitter systems (73). For example, acute nicotine administration is associated with an overall activation of the autonomic nervous system and HPA axis with increased secretion of catecholamine, corticotrophin-releasing hormone, ACTH and corticosterone (74, 75). This pathway is supported by a higher cortisol concentration among adult smokers compared to non-smokers (76-79). The HPA axis appears to normalise after nicotine withdrawal, supporting the hypothesis of a short-term effect of nicotine. For example, lower cortisol concentration was found among abstinent smokers compared to non-abstinent smokers (80).

3. The co-occurrence of smoking behaviour and depressive symptoms may stem from shared risk factors (72, 81). For example, a shared genetic liability to both smoking behaviour and mental disorders may lead to the development of both smoking behaviour and depression (82).

1.3.2 HPA axis activation in the pathway between smoking behaviour and depression

Cortisol concentration, as the main output of the HPA axis activity, has been associated with several mental and physical health conditions in adolescents, including anxiety, depression and sleeping problems (83). As mentioned above, the HPA axis activation may be involved in the pathway between smoking behaviour and depressive symptoms. This hypothesis relies mainly on indirect findings. On the one hand, cortisol secretion is more elevated among smokers than non-smokers; on the other, the HPA axis is activated in depressed individuals. However, while the association between the HPA axis activation and severe depression is well-established (84, 85), the association between smoking behaviour and the HPA axis activation is not well-understood. Unfortunately, no evidence has been provided thus far on the complete pathway from smoking to the HPA axis activation, and depression.

The relationship between smoking behaviour and consequent dysregulation of the HPA axis among adolescents is still a matter of speculation (76-78, 86, 87). Although several studies have
linked stress with smoking behaviour in both adolescents and adults, a number of weaknesses limit the possibility of drawing firm conclusions about the causal nature of the relationship. First, these studies have measured stress mainly using self-reported scales, and have thus considered psychological and perceived dimensions of stress (88). Second, other important limitations arise from the cross-sectional design of these studies and the study population included, the majority being adult individuals (76, 77).

Several studies in recent decades have shown clearly that changes in the HPA axis activity are involved in the onset of severe depression (39). The impairment stems from the joint effect of a disproportionate release of cortisol in stressful situations and an alteration of the glucocorticoid receptor-negative feedback. Despite this pathway being recognised as one of the biological bases of severe depression, mainly among hospitalised patients, the possible involvement of the HPA axis activation in the onset of depressive symptoms among adolescents is far from being fully understood (89). Longitudinal studies examining the association during its onset are required to improve the understanding of the presence and the direction of the association in youth populations.

1.3.3 Activation of the HPA axis and smoking

A possible effect of the HPA axis activation on the onset of smoking behaviour may also be hypothesised. In other words, early HPA axis dysregulation may be not only an effect but also a cause of smoking behaviour. Insight into the underlying association between dysregulation of the HPA axis and the onset of smoking behaviour in adolescence has the potential to contribute to effective preventive strategies. However, as mentioned earlier, there is a lack of longitudinal studies on this pathway.

Dysregulation of the HPA axis could indicate early exposure to environmental stressors (90, 91). For example, early life adversities may disrupt the HPA axis, in turn influencing the reward system and potentially leading to substance use. An upregulated stress response may enhance the perceived pleasure during early episodes of smoking behaviour through sensitisation of dopaminergic pathways. Notably, stress hormones released in the central nervous system may reduce excitatory input to the nucleus accumbens, and thus may diminish its inhibitory output to dopamine-releasing neurons in the ventral tegmental area (92, 93). Such an impairment of the reward system may increase the risk of smoking behaviour and of the use of other substances.

1.3.4 Sex differences

Previous studies have indicated sex differences in the prevalence of mental health disorders. For example, externalising symptoms are more common among males, while internalising symptoms, such as depressive symptoms, are more common among females (64, 94-96). Interestingly, sex differences have been found to vary according to age. Studies among early adolescents have found either no sex differences in the prevalence of depression or even a higher prevalence among males (97), while the risk of depression during late adolescence has consistently been found to be greater among females than males (98, 99). In addition, the increase in the prevalence of mental health problems among young adults appears to be particularly elevated among females. For example, during the 1980s and 1990s, the incidence of mental health disorders
remained relatively stable among males, while it increased among females (100). However, sex differences in the prevalence of depression may also be explained through the higher scores that the diagnostic instruments commonly used attribute to symptoms commonly reported by females (such as feelings of sadness or apathy). The phenotype of male depression may manifest itself through symptoms such as aggression, anger, irritability or risk-taking behaviour, as well as substance abuse, that are typically not included in the current diagnostic criteria (65, 101).

Concerning smoking behaviour, females appear to be more vulnerable than males to the addictive properties of cigarettes, revealing higher rates of nicotine dependence and withdrawal symptoms, more frequent unsuccessful efforts to cut down and persistent health problems (102). Nicotine activates neural reward centres, resulting in a positive reinforcement, and relieves withdrawal symptoms during abstinence, resulting in a negative reinforcement (103, 104). It appears that females experience greater rewarding effects of nicotine than males. This effect seems to be mediated, to a certain extent, by the impact that oestrogen has on dopamine release in the mesocorticolimbic system after nicotine administration (105). In addition, females, when compared to males, experience higher levels of stress, anxiety and depression during nicotine withdrawal. This would contribute to the higher incidence of relapse after smoking cessation observed in females (106).

There are also reasons to hypothesise that sex hormones play a role in smoking-depressive symptoms pathways. In particular, the effect of nicotine on the HPA axis appears to differ by sex. In rodent models, single-dose nicotine induces a higher HPA axis response among females than among males, with higher ACTH and corticosterone secretion (107).

### 1.3.5 A complex scenario

The association between smoking and depressive symptoms and the possible role of the activation of the HPA axis in this association is complex. As described in Figure 2, such a complexity arises both from possible bi-directional associations and from the roles of intrinsic and environmental factors that cannot be easily identified and measured. First, the associations between smoking and depressive symptoms, between smoking and activation of HPA axis, and between the HPA axis activation and subsequent depressive symptoms may be present in either direction. Second, the role of distal risk factors such as upbringing, parental psychiatric morbidity and parental socio-economic status, as well as unknown factors such as a genetic liability, should be considered. In order to examine such a multidimensional phenomenon, one option would be to isolate and focus on each distinct pathway. In other words, we should examine how any two pieces of the puzzle fit together without forgetting the global picture.
Figure 2. A framework of the association between smoking and depressive symptoms, ancestors and intermediate factors.
1.4 THE MEANING OF THE SOCIAL CONTEXT

Every country has its own legislation, norms, societal values and different levels of economic development. Some of these factors are likely to have a profound impact on smoking behaviour in the population. For example, country-level tobacco policies (i.e. taxes on tobacco products, smoke-free air policies and tobacco control funding) may have an impact on cigarette smoking prevalence, smoking acceptance and normalisation of the behaviour (108-110). Similarly, country-level factors (i.e. economic stagnation, political instability, healthcare availability and lack of exposure to natural light) may also influence the occurrence or the diagnosis of depression in a population (111).

It is, therefore, possible to hypothesise that the association of smoking behaviour with depressive symptoms in a given population can be moderated by these contextual factors. A stronger co-occurrence of smoking behaviour and depressive symptoms may exist in restrictive tobacco control and low smoking prevalence environments compared to permissive and high prevalence environments. Restrictive environments may reduce the uptake of smoking among individuals with low liability to substance use and mental health problems, but less so among individuals with strong liability. In permissive environments, a larger proportion of smoking initiation would be attributable to pro-smoking social norms, thus attenuating the association with mental distress. Figure 3 illustrates the decrease of current smoking prevalence in a restrictive tobacco control environment and the possible concurrent reduction of social smoking share, but not of smoking behaviour among dependence-vulnerable individuals (hypothetical scenario). No studies have evaluated how contextual factors might affect the association between smoking and depression.

![Figure 3](image)

**Figure 3.** The trajectory of current smoking prevalence and of its two main components: social smoking and smoking among dependence-vulnerable groups after the implementation of tobacco control measures. Estimates of current smoking prevalence were derived from the Swedish general population (112).
1.4.1 Tobacco control in Sweden and in Italy

Sweden and Italy are two member states of the EU characterised by different tobacco control environments (113, 114), the former being more restrictive than the latter. Accordingly, the prevalence of adult daily smoking is considerably lower in Sweden (7%) than in Italy (25%) (112, 115). Some tobacco control measures do not appear to differ between the two countries. For example, smoking was banned in all indoor public places in January 2005 in Italy (116) and in June 2005 in Sweden. The Italian tobacco taxation on most brands is higher than in Sweden (75.9% vs 68.5%) (13). A striking feature concerns funding for tobacco control activities: the Italian government allocates only 10 million euros per year to tobacco control, to be compared with 40 million euros allocated in Sweden (13), a country with a 6-fold smaller population.

Concerning indicators of mental ill-health, rates of suicide are higher in Sweden than in Italy (12.7 vs 5.4 per 100,000 population in 2015) (35), but the prevalence of depression and the number of age-standardised years lost attributable to depression (1,113-1,180.7 per 100,000) are similar between the two countries (41).

Therefore, these two countries offer a suitable opportunity to study the role of a tobacco control environment in the association between smoking and depressive symptoms.
1.5 KNOWLEDGE GAPS MOTIVATING THIS THESIS

During the last two decades, several studies have reported a strong association between smoking behaviour and depressive symptoms, with smoking prevalence increasing with the severity of depressive symptoms (51). Notably, individuals with depression disorders, compared to those without, tend to report smoking more heavily, being more dependent on smoking and starting to smoke at an earlier age (50).

There are several reasons why studies that further investigate this association are needed. First, only a handful of studies have examined the longitudinal association between smoking behaviour, a possible dysregulation of the HPA axis and the subsequent onset of depressive symptoms, and none of them has focused on adolescents. Second, the possible role of the Swedish moist smokeless tobacco, “snus”, in the onset of depressive symptoms needs to be explored. This product can be seen as a nicotine delivery device without toxicants related to burned tobacco (117). The analysis of snus may be particularly important in clarifying the underlying biological mechanisms of nicotine-depressive symptoms association. Third, no studies have explored the modifying role of country-level contextual factors on the association between smoking behaviour and depressive symptoms. Finally, sex differences deserve further investigation, given the higher vulnerability to tobacco, the higher incidence of depressive symptoms and the potential effect of oestrogen as an enhancer of nicotine effect on the HPA axis among females (98, 99, 102, 107).

These studies would help to shed light on possible determinants and pathways of juvenile depression and also provide important cues for tobacco prevention strategies. In fact, if tobacco use were to be causally linked to the onset of adolescent depression, depression should be added to the list of tobacco-related health problems and incorporated in universal prevention. If, on the other hand, tobacco use were to represent a response to depression, through the accumulation of stressors during the life course, the screening and early treatment of adolescent mental distress symptoms might offer a way to prevent, or encourage cessation of, tobacco use.
2 AIMS

2.1 OVERALL AIM

This thesis aimed to assess the potential causal relationship between tobacco use and depressive symptoms among early adolescents, to untangle the role of hypothalamic-pituitary-adrenocortical axis activation in this association and to investigate the impact that a restrictive and non-restrictive tobacco control environment respectively exert on this association.

2.2 SPECIFIC AIMS

The specific aims are listed below.

1. To examine the association between tobacco use (smoking behaviour and snus use) and depressive symptoms.

2. To assess whether levels of activity of the hypothalamic-pituitary-adrenocortical axis (assessed through salivary cortisol) play a role in the association between smoking behaviour and depressive symptoms.

3. To clarify whether cortisol, as an indicator of stress and of levels of activity of the hypothalamic-pituitary-adrenocortical axis, predicts the onset of smoking behaviour and snus use among adolescents.

4. To compare the association between smoking behaviour and depressive symptoms among adolescents in a restrictive and in a less restrictive tobacco control environment, i.e. the modifying role of tobacco control on this association.
3 MATERIALS AND METHODS

3.1 STUDY POPULATION

3.1.1 The Swedish KUPOL study

All four studies included in the present project use data from the Swedish KUPOL study (118), a longitudinal population-based study of students aged 13-14 years old. All schools located in eight regions of southern and central Sweden, Gävleborg, Jönköping län, Örebro län, Stockholm, Södermanland, Uppsala, Värmland and Västmanland with at least 20 students in the seventh, eighth and ninth grade were eligible to participate. In the schools that accepted (n=101, Figure 4), student enrolment took place during two academic years: 2013-2014 and 2014-2015. In agreement with ethical obligations for research including minors, parents gave informed consent to participate for 3,959 students. 3,671 students answered the baseline questionnaire (Figure 5). The participation rate among students was 92.7% at baseline (seventh grade), 88.5% in the first (eighth grade), 84.6% in the second (ninth grade) and 65.2% in the third follow-up (non-compulsory education).

Data were collected at the child, parent and school level using multiple types of data collection. A questionnaire was given to the seventh-grade students (13-14 years of age) when baseline data collection began. They were followed up once a year in the following three years (Figure 6). The parents or legal guardians filled out a questionnaire about their children’s mental health, school life, family relationships, and their smoking and alcohol behaviour. Additionally, a sample of students gave a saliva sample that was used to analyse morning and afternoon cortisol concentration and epigenetic markers. At the school level, the school climate was assessed using the teacher and ninth-grade student anonymous questionnaires. Registries were linked to retrieve medical and socio-economic information on students and their parents.

3.1.2 The Italian BE-TEEN study

Study IV included a sample from the Italian BE-TEEN study. The BE-TEEN study was initiated to allow cross-country comparisons between the Italian and Swedish context concerning lifestyles and mental health problems. 39 public schools in the Brescia Province, an area located in Northern Italy, were invited to participate and 15 agreed to join the study. In the academic year 2017-2018, a total of 2,316 eligible students aged 15-16 years old were invited to participate in an anonymous survey, of whom 2,166 participated. At the same time, a longitudinal study was initiated including students in the first grade of the Italian high school (14-15 years of age). The longitudinal sample has been followed up every year for four years. Thus, different individuals are included in the longitudinal and cross-sectional samples of the BE-TEEN study. The BE-TEEN study adapted questionnaires from the KUPOL study translated into Italian according to a back-translation approach. Questionnaires were administrated at school using a paper or a web-based version.
Figure 4. Schools included in the KUPOL study. Data source: BMC Psychiatry. 2016 July 16;16:243.

Figure 5. Flow chart of participation in the KUPOL study.

Figure 6. KUPOL study (2013–2018). Green boxes represent saliva sample collection and orange boxes represent non-compulsory education years.
3.1.3 Study designs and selection criteria

Study I and III have a longitudinal design, Study II includes both a cross-sectional and longitudinal design, and Study IV consists of a cross-country cross-sectional comparison. Four different study samples were considered from the KUPO study to address the research questions. Study IV also included a sample from the Italian BE-TEEN study. In Study I, participants with information on tobacco use and depressive symptoms at baseline and one-year follow-up were included (n=3,195). In Study II, participants who had a cortisol measurement at baseline or at the two-year follow-up, as well as valid measures for exposures and outcomes, were selected (n=409). In Study III, participants with information on cortisol concentration, without a history of tobacco use at baseline, and with information on tobacco use during follow-up were included (n=381). Finally, in Study IV, participants aged 15-16 years old with valid measures for cigarette smoking and depressive symptoms from the KUPO study and BE-TEEN study were included (n=5,230).
3.2 STUDY VARIABLES

3.2.1 Tobacco use

For three studies included in this project (Study I, II and IV), tobacco use was considered as exposure, while tobacco use was included as the outcome in only one study (Study III). Three main phenotypes of tobacco use were of interest: cigarette smoking, snus use and either type of tobacco use. Cigarette smoking and snus use were self-reported and assessed once a year for the duration of the study. Specifically, current use was defined as past-30 day use and initiation was defined as first time self-reported current use in the past 30 days among students without a history of tobacco use at baseline. Also, perceived tobacco dependence among tobacco users was explored. With the aim of exploring the role of the activity levels of the HPA axis in the risk of tobacco maintenance (Study III), we also included the duration of use defined as the number of years of reported use from the first to the third year of follow-up.

3.2.2 Depressive symptoms

In the present thesis, two different scales were considered to evaluate depressive symptoms: the CES-DC and the internalising score of the SDQ.

The CES-DC is a 20-item internationally validated scale used in epidemiological studies of children and adolescents (six-17 years of age), with a total score ranging from zero to 60 (119). The items refer to the past week and encompass four alternatives: “not at all”, “a little”, “some”, and “a lot”. In the present project, a cut-off score of ≥ 30 was applied to define the presence of depressive symptoms (120). At baseline, the Cronbach’s Alpha was 0.90 in the KUPOL study.

Self-reported and parental-reported SDQ internalising scores were also included to evaluate internalising problems (121). These scores are obtained by adding up a total of ten items from the emotional and peer problem SDQ subscales. The items refer to the past six months and include three options: “not true”, “somewhat true”, and “certainly true”. The score ranges from zero to 20, where a cut-off score of ≥ nine was considered suggestive of high internalising problems for the self-reported score, and a cut-off score of ≥ seven for the parental-reported score in this project. At baseline, the Cronbach’s Alpha for the self-reported SDQ internalising score was 0.71 in the KUPOL study.

Depressive symptoms were the main outcome of Study I, II and IV and were evaluated in terms of change and incidence over time (Study I and II), as well as presence of depressive symptoms (Study I, II and IV). The scores were considered as continuous (Study I and II) or binary (Study I, II and IV) variables.

3.2.3 Cortisol measurements

Study II and III considered salivary cortisol concentration as an indicator of levels of activity of the HPA axis. Saliva samples were collected by trained staff at school before the first lesson in the morning and the early afternoon. The protocol followed for collecting, storing and quantifying cortisol concentration is reported in papers II and III.
To compare cortisol concentration among students, we standardised morning and afternoon cortisol values at two and eight hours from awakening, respectively. To this end, cortisol-predicted values at each time point were modelled through mixed models with random intercepts for individuals, including two repeated measures at student level (morning and afternoon concentrations), and a linear and a quadratic term for time. Mixed models were stratified for grade and sex. We then calculated the cortisol AUC from two to eight hours after awakening as the sum of standardised morning cortisol and standardised afternoon cortisol multiplied by six (number of hours between the two cortisol measures), and then divided by two (122). Finally, three standardised measures for cortisol concentration were considered: morning cortisol (two hours from awakening), afternoon cortisol (eight hours from awakening) and cortisol AUC. Morning and afternoon salivary cortisol concentration and cortisol AUC served as proxies for morning and basal levels, as well as for total hormonal output between two and eight hours from awakening, respectively. Cortisol concentration was measured in microgram per decilitre (µg/dl). Cortisol concentration was grouped according to the quartiles of the distribution when included as exposure, or continuous when considered as outcome.

3.2.4 Social context

To examine the role of social context as a possible effect modifier of the association between cigarette smoking and depressive symptoms (Study IV), the Swedish and Italian contexts were used as proxies of a restrictive and permissive tobacco control environment, respectively.

3.2.5 Covariates

Information on sex, alcohol consumption, parental education, parental birthplace and school was also collected to describe the analytical samples. Sex at birth was considered and examined as a possible effect modifier. Alcohol consumption was dichotomised as < vs ≥ once a month. Parental education was measured according to the number of schooling years and categorised in < vs ≥ university level. Parental birthplace was considered as a binary variable as at least one parent born outside Sweden vs both parents born in Sweden. Parental education and birthplace were also included to adjust for possible confounding. The school was included to examine possible clustering effects.
3.3 STATISTICAL ANALYSIS

Characteristics of participants were expressed in terms of percentages and absolute numbers. Continuous variables were reported as means and SDs or grouped according to the quartiles of the distribution. All analyses were stratified by sex. In Study IV, characteristics of participants were also stratified by country. Three main measures of association were presented: β coefficient, OR and RR. β coefficient should be interpreted as mean difference comparing exposed and unexposed group considering zero as the null value. OR and RR have a similar meaning and represent the increased odds or risk of an event in the exposed compared to the unexposed group. All three measures were also calculated conditioning on a set of covariates. Stata Statistical Software: Release 14 (StataCorp. 2014.College Station, TX: StataCorp LP) were used for all analyses. Specific analytical strategies for each study are described in Table 1.

3.3.1 Study I

Different analytical strategies were employed to analyse the association between tobacco use and depressive symptoms. Cigarette smoking, snus use or either type of tobacco served as the exposure variables, while CES-DC, self-reported and parent SDQ internalising scores were the outcome variables.

First, linear regression models were used to evaluate the role of exposure to tobacco use in a one-year change in depressive symptoms. Results were expressed in terms of β coefficient and 95% CI. In a second analysis, individuals with depressive symptoms at baseline, according to the specific cut-off for each score, were excluded and a possible association between tobacco exposure and incidence of depressive symptoms was tested in logistic regression models. Depressive symptoms were considered as a binary variable. Finally, we analysed the one-year risk of depressive symptoms according to tobacco initiation using logistic regression models. This analysis was limited to individuals without a history of tobacco use or depressive symptoms at baseline. Results were expressed in terms of OR and 95% CI.

Regression models were presented unadjusted or adjusted for potential confounding, namely depressive symptoms at baseline, sex, alcohol consumption, parental education, and parental birthplace. A possible interaction between tobacco use and sex was tested in all three steps.

3.3.2 Study II

Different strategies were applied to evaluate the association between smoking behaviours and cortisol concentration, and the association between cortisol concentration and depressive symptoms. We examined current smoking, smoking intensity and perceived dependence from cigarettes (among ever-smokers) in relation to differences in cortisol mean concentration.

In the cross-sectional analysis, linear mixed-effects models with a random intercept on individual level were performed to test the association between cigarette smoking and cortisol concentration. The models included repeated measures for the same individuals at 13-14 and 15-16 years of age. Mixed-effects linear models were adjusted for school grade and time after awakening. Final estimates were expressed as β coefficient and 95% CI. We then explored the possible association between cortisol concentrations and depressive symptoms. Cortisol mean values were compared
3.3.3 Study III

Differences in mean cortisol concentration at baseline for tobacco initiation and duration were explored. Poisson regression models were used to test the association of morning and afternoon cortisol levels as well as cortisol AUC with three years cumulative risk of tobacco initiation and duration. Cortisol salivary concentrations were categorised according to the quartiles of the distributions. Risk Ratio (RR), as increased risk for each quartile increase, and 95% CI was presented as the measure of association. To account for nonlinearity, morning cortisol concentration was fitted using restricted cubic spline with four knots at the 10th, 40th, 60th and 90th percentile of morning cortisol distribution. The same statistical approach was used to analyse perceived tobacco dependence as outcome among tobacco users.

A series of sensitivity analyses were performed. First, the analyses were repeated considering only the first and second year of follow-up to evaluate the possible selection bias that resulted from dropouts in the first year after compulsory education (third year of follow-up). Second, we weighted the models using an inverse probability technique to accommodate the possible selection from the main cohort. Finally, we performed multilevel models including a school level to account for possible clustering within schools to take into account a possible selection process from the whole cohort.

3.3.4 Study IV

Logistic regression analyses were used to test for an association between smoking behaviour and depressive symptoms, both unadjusted and adjusted for parental education as a possible confounder, in the Swedish and Italian contexts.

We tested effect modification by country and smoking behaviour using stratified analyses and estimating conventional multiplicative and additive interaction terms (123). Non-smoker Swedish students served as the reference group. Multiplicative interaction was defined as ORs: OR11 /
(OR10 × OR01). OR11 defined the odds for being an Italian smoker, OR10 the odds for being Italian and non-smoker, OR01 for being a Swedish smoker. Additive interactions were tested using the Relative Excess Risk Due to Interaction (RERI) according to the following formula, \( \text{RERI} = \text{OR11} - \text{OR10} - \text{OR01} + 1 \) and the delta method for CI calculation (124). A possible role of sex as effect modifier was evaluated using stratifying analyses.

A sensitive analysis including data from the longitudinal sample of the Italian BE-TEEN study was conducted. This allowed us to investigate whether differences in the recruitment process impacted the selection of participants and therefore the interaction estimates.
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<td>Depressive symptoms</td>
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<td>Linear and logistic regression models</td>
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**Table 1.** An overview of research questions, study designs and analytical approaches.
3.4 ETHICAL CONSIDERATIONS

All four studies were based on data collected in the Swedish KUPOL study, while one study also included data derived from the Italian BE-TEEN study.

The Swedish KUPOL was approved by the Ethical Review Board of Stockholm Region, Sweden (reference number: 2012/1904-31/1 and supplementary application 2016/1280-32). Since the participants were minors, permission from parents or legal guardians was required by law, but the children could themselves decide to withdraw from the study at any time. Parents or legal guardians were allowed to give informed consent separately for the questionnaire, the registers and the saliva samples. If any test results were abnormal for CES-DC or SDQ internalising scores, the school nurse was contacted. The questionnaires were scanned and entered digitally, and the original questionnaires were archived. The electronic database is stored on a KI server and only authorised researchers have access to the data. The database is de-identified and encrypted; participants cannot be mixed up with one another in the database.

The Italian BE-TEEN study was approved by the Ethical Review Board of the Brescia Province, Italy (reference number: 2761-07-06-2017). The Italian anonymous survey at 15-16 years of age did not require an informed consent from legal guardians. The longitudinal part applied a similar procedure as the KUPOL study for informed consent, confidentiality and protection of integrity. If any test results were abnormal for CES-DC or SDQ internalising scores, parents were contacted and, upon request, were directed to specific services. The questionnaires were administered both online and on paper.

Results of research conducted on KUPOL and BE-TEEN data were presented aggregated and disseminated through seminars to schools and scientific audiences, as well as through scientific and popular science publications.
4 RESULTS

4.1 CHARACTERISTICS OF THE STUDY POPULATION

The KUPOL cohort included 3,671 students (13-14 years of age) who provided information at baseline (51.8 % females). Generally, the retention rate was quite high, with the exception of the last year of follow-up (included only in Study III). Study IV also included a sample of Italian students (n=1,947, 53.9% females) recruited within the Italian BE-TEEN study. As stated above, different inclusion criteria were employed for the individual studies. Sample characteristics and additional statistical analyses are described in the corresponding papers.

4.2 ASSOCIATION BETWEEN TOBACCO USE AND THE ONSET OF DEPRESSIVE SYMPTOMS

The analytical sample of Study I consisted of 3,195 students (51.2% females) with valid measures for tobacco use and depressive symptoms. Only 2.0% were current smokers and 0.8% current snus users at baseline (13-14 years of age, seventh grade).

The mean depressive score (CES-DC) at one-year follow-up was greater among smokers as compared to non-smokers (26.4 vs. 15.5). According to the results presented in Table 2, cigarette smoking, but not snus use, was associated with a greater change in the one-year depressive symptom score ($\beta= 11.4$, 95% CI 8.8, 14.0). Adjusting for potential confounding resulted in attenuated estimates ($\beta= 3.4$, 95% CI 1.0, 5.7). Importantly, stratifying by sex highlighted that the association was mainly driven by males.

When looking only at students without depressive symptoms at baseline (considering CES-DC $\geq$ 30 as the threshold for depressive symptoms), the one-year incidence of depressive symptoms was 8.2% among non-smokers and 17.6% among smokers (Table 2). Thus, being a smoker was associated with an elevated one-year risk of the onset of depressive symptoms. The additional analysis separated for sex showed that the association was mostly confined to males, with 20.0% incidence of depressive symptoms among male smokers compared to 2.9% among male non-smokers. Yet, when considering snus use as exposure, these association patterns were not observed.

Examining those who reported no tobacco use and no depressive symptoms at baseline allowed us to evaluate the one-year risk of depressive symptoms according to the onset of smoking (Table 2). Similar to the previous analyses, the onset of smoking behaviour was strongly associated with the risk of developing depressive symptoms after one year. Interestingly, the onset of snus use was associated with increased odds of development of depressive symptoms (OR = 3.6, 95% CI 2.0-6.4).

In additional analyses, using different depressive scales as the outcome, namely the student and parent SDQ internalising scores, did not affect the pattern of associations. Separated analyses for sex showed elevated odds of depressive symptoms according to smoking behaviour in both males and females.
Table 2. Longitudinal associations between smoking and depressive symptoms applying three separate analytical approaches.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Smoking</th>
<th>Depressive symptoms</th>
<th>Measure of association</th>
<th>Unadjusted models</th>
<th>Adjusted models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in depressive symptoms</td>
<td>All students</td>
<td>Mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>15.5 (10.6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>26.4 (15.3)</td>
<td>$\beta$ (95% CI)</td>
<td>11.4 (8.8,14.0)</td>
<td>3.4 (1.0,5.7)*</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>19.2 (11.3)</td>
<td>$\beta$ (95% CI)</td>
<td>10.7 (7.4,13.9)</td>
<td>3.0 (−0.3,6.2)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>29.8 (14.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>11.5 (7.9)</td>
<td>$\beta$ (95% CI)</td>
<td>7.2 (3.4,10.9)</td>
<td>4.4 (0.5,8.3)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>16.9 (12.9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-year risk of depressive symptoms</td>
<td>All students</td>
<td>Incidence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>8.2%</td>
<td>OR (95% CI)</td>
<td>2.4 (1.0,5.9)</td>
<td>2.0 (0.7,5.8)*</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>17.6%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>13.7%</td>
<td>OR (95% CI)</td>
<td>1.2 (0.3,4.1)</td>
<td>1.0 (0.3,3.9)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>15.8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2.9%</td>
<td>OR (95% CI)</td>
<td>8.3 (2.3,30.5)</td>
<td>12.7 (2.5,63.9)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>20.0%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-year risk of depressive symptoms according to tobacco initiation</td>
<td>All students</td>
<td>Incidence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>7.6%</td>
<td>OR (95% CI)</td>
<td>4.3 (2.6–7.2)</td>
<td>3.9 (2.1–7.2)*</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>26.3%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>13.0%</td>
<td>OR (95% CI)</td>
<td>3.9 (2.0–7.4)</td>
<td>3.1 (1.5–6.4)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>36.6%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2.5%</td>
<td>OR (95% CI)</td>
<td>7.1 (2.8–18.1)</td>
<td>6.7 (2.5–18.2)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>15.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\beta$ = beta coefficient, CI = confidence interval, OR = odds ratio and SD = standard deviation. Models adjusted for depressive symptoms at baseline, alcohol consumption, parental education, parental birthplace and *sex. Analyses limited to non-smokers without depressive symptoms at baseline.
4.3 SALIVARY CORTISOL CONCENTRATION, SMOKING BEHAVIOUR AND DEPRESSIVE SYMPTOMS

The analytical sample of Study II consisted of 409 students (53.5% females) with valid measures for salivary cortisol concentration at 13-14 or 15-16 years of age.

Figure 7 shows the change in cortisol concentration over time at age 13-14 (seventh grade) and 15-16 (ninth grade). Generally, moving from the seventh to the ninth grade led to an overall increase in average cortisol levels and brought about cortisol diurnal rhythm.

![Figure 7. Cortisol diurnal in the 13-14 and 15-16 years of age groups. µg/dl = microgram per decilitre. Black lines = estimated values, dashed line = 95% confidence intervals.](image)

In cross-sectional analyses, being a smoker was not associated with increased cortisol concentration (mean [SD] morning cortisol 0.10 [0.07] vs 0.11 [0.11] µg/dl in smokers and non-smokers in the seventh grade). Additionally, the intensity of smoking (more than 15 cigarettes per day) and dependence (among ever-smokers) was also not related to cortisol levels. Using mixed-effects models confirmed no clear evidence of associations. For example, the mean difference in morning cortisol concentration for smokers was $\beta = 0.003$ (95% CI -0.050 to 0.056).

When looking at the other part of the association, from cortisol concentration to depressive symptoms, elevated cortisol concentration was not associated with depressive symptoms when considering either CES-DC or student SDQ internalising score at baseline and at two-year follow-up (Table 3). Mixed logistic models supported the pattern of no association between cortisol concentration and depressive symptoms.

Focusing exclusively on the longitudinal associations, being a current smoker in the seventh or eighth grade was not associated with changes in cortisol concentration between the seventh and ninth grade (for morning cortisol concentration as outcome $\beta = 0.064$ [95% CI -0.029 to 0.157]). In the same direction, having a higher baseline cortisol concentration did not predict the onset of depressive symptoms two years later (for morning cortisol concentration as the exposure OR = 0.87 [95% CI 0.69 to 1.10]).
**Table 3.** Association between morning cortisol concentration at baseline and depressive symptoms.

<table>
<thead>
<tr>
<th>Depressive symptoms at baseline</th>
<th>n</th>
<th>Morning cortisol concentration at baseline, µg/dl mean (SD)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CES-DC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36</td>
<td>0.12 (0.13)</td>
<td>1.31 (0.83, 2.07)</td>
</tr>
<tr>
<td>No</td>
<td>359</td>
<td>0.11 (0.11)</td>
<td></td>
</tr>
<tr>
<td>SDQ internalising score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>40</td>
<td>0.12 (0.14)</td>
<td>1.11 (0.75, 1.65)</td>
</tr>
<tr>
<td>No</td>
<td>354</td>
<td>0.11 (0.11)</td>
<td></td>
</tr>
</tbody>
</table>

CES-DC = Centre for Epidemiological Studies Depression Scale for Children, CI = confidence interval, µg/dl = microgram per decilitre, OR = odds ratio and SDQ = Strengths and Difficulties Questionnaire. Logistic mixed regression models included measures at baseline and two-year follow-up.

In additional analyses, we re-estimated all models and found similar results stratifying by sex, including parent SDQ internalising score, calculating 95% CI estimates with the bootstrap method, accounting for school clusters as well as weighting models for the sampling selection.
4.4 CORTISOL CONCENTRATION AS PREDICTOR OF SMOKING BEHAVIOUR AND SNUS USE

The analytical sample of Study III consisted of 381 students (13-14 years of age, 52.2% females) with valid measures for salivary cortisol concentration and no history of tobacco use at baseline. At baseline, morning cortisol, afternoon cortisol and cortisol AUC means were 0.11, 0.06 and 0.51 µg/dl respectively, with slightly higher levels among females.

Individuals who started to use snus and reported tobacco dependence had higher morning and AUC cortisol means at baseline compared to those who did not, while such a difference was not present for smokers.

Table 4 shows the RRs for three-year tobacco initiation in relation to the quartiles of cortisol concentration. Students with a greater morning cortisol concentration had a 1.2 to 1.3-fold increased risk of cigarette smoking or snus use initiation. The adjusted RRs were somewhat attenuated, but the same pattern was observed. While considering AUC cortisol as exposure yielded a similar pattern, afternoon cortisol concentration was not associated with any outcomes.

Table 4. Association between cortisol concentration (for each quartile increase) and three-year risk of smoking as well as snus use initiation.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Exposure</th>
<th>Outcome</th>
<th>Unadjusted RR (95% CI)</th>
<th>Adjusted RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation</td>
<td>Morning</td>
<td>Smoking</td>
<td>1.28 (1.06,1.55)</td>
<td>1.27 (1.03,1.56)</td>
</tr>
<tr>
<td></td>
<td>Afternoon</td>
<td>1.16 (0.97,1.40)</td>
<td>1.07 (0.87,1.31)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>1.29 (1.07,1.56)</td>
<td>1.19 (0.97,1.46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morning</td>
<td>Snus use</td>
<td>1.32 (1.05,1.66)</td>
<td>1.33 (1.03,1.70)</td>
</tr>
<tr>
<td></td>
<td>Afternoon</td>
<td>1.06 (0.85,1.32)</td>
<td>1.30 (1.01,1.67)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>1.25 (1.00,1.57)</td>
<td>1.31 (1.02,1.68)</td>
<td></td>
</tr>
<tr>
<td>Duration of use</td>
<td>Morning</td>
<td>Smoking</td>
<td>1.29 (1.06,1.58)</td>
<td>1.26 (1.02,1.57)</td>
</tr>
<tr>
<td></td>
<td>Afternoon</td>
<td>1.13 (0.93,1.39)</td>
<td>1.10 (0.89,1.37)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>1.35 (1.10,1.65)</td>
<td>1.24 (1.00,1.54)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Morning</td>
<td>Snus use</td>
<td>1.39 (1.04,1.86)</td>
<td>1.40 (1.03,1.90)</td>
</tr>
<tr>
<td></td>
<td>Afternoon</td>
<td>1.17 (0.88,1.55)</td>
<td>1.30 (0.96,1.77)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AUC</td>
<td>1.30 (0.98,1.74)</td>
<td>1.33 (0.98,1.80)</td>
<td></td>
</tr>
</tbody>
</table>

RR = risk ratio, CI = confidence interval, AUC = area under the curve. Models adjusted for parental education.

These findings remained consistent in separate analyses by sex. Similar results were found when considering duration of tobacco use as the outcome. A possible dose-response relationship between morning cortisol concentration and the risk of tobacco initiation was confirmed in the dose-response analysis. The higher the morning cortisol concentration was, the higher the risk of tobacco initiation (Figure 8).

When the focus was exclusively on tobacco users, cortisol concentrations were not associated with a greater risk of perceived tobacco dependence. Taking into account the potential selection bias
due to attrition rate, and including the first and second follow-ups only, the pattern of the associations remained unchanged.

Figure 8. Dose-response association between morning cortisol level and three-year risk of smoking and snus use initiation, and the distribution of morning cortisol levels among the individuals included in the study. RR = risk ratio, µg/dl = microgram per decilitre.
4.5 MODIFYING ROLE OF SOCIAL CONTEXT

The analytical sample of Study IV consisted of 3,283 Swedish (52.1% females) and 1,947 Italian students (53.9% females), with valid measures for both cigarette smoking and depressive symptoms (15-16 years of age). Overall, smoking behaviour (32.3% vs 7.3%), as well as depressive symptoms (CES-DC scale 18.4% vs 15.2%), were more common among Italian than Swedish students.

Focusing on the smoking-depressive symptoms association, current smokers, compared to non-smokers, were more likely to report depressive symptoms in both the Italian and Swedish context. In particular, while the prevalence of depressive symptoms was about 14.0% among both Italian and Swedish non-smokers, this proportion increased to 26.3% among Italian smokers and to 33.9% among Swedish smokers.

Quantifying the magnitude of the association revealed that smoking phenotypes were associated with 2-3 times higher odds of depressive symptoms in both countries (Table 5, within-country comparison). Despite some minor attenuations when adjusting for parental education, the pattern remained consistent.

**Table 5.** The role of social context in the association between cigarette smoking and depressive symptoms

<table>
<thead>
<tr>
<th>Country</th>
<th>Smoking</th>
<th>Depressive symptoms cases/n (%)</th>
<th>Within-country OR (95% CI)</th>
<th>Cross-country OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>No</td>
<td>414/3,027 (13.7)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>81/239 (33.9)</td>
<td>3.24 (2.43-4.31)</td>
<td>3.24 (2.43-4.31)</td>
</tr>
<tr>
<td>Italy</td>
<td>No</td>
<td>171/1,175 (14.6)</td>
<td>1.0</td>
<td>1.07 (0.89-1.30)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>152/579 (26.3)</td>
<td>2.09 (1.63-2.67)</td>
<td>2.25 (1.82-2.78)</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>No</td>
<td>350/1,552 (22.5)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>69/154 (44.8)</td>
<td>2.79 (1.99-3.91)</td>
<td>2.79 (1.99-3.91)</td>
</tr>
<tr>
<td>Italy</td>
<td>No</td>
<td>126/629 (20.0)</td>
<td>1.0</td>
<td>0.86 (0.68-1.08)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>127/326 (39.0)</td>
<td>2.55 (1.89-3.43)</td>
<td>2.19 (1.70-2.82)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>No</td>
<td>63/1,474 (4.3)</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>12/85 (14.1)</td>
<td>3.68 (1.90-7.13)</td>
<td>3.68 (1.90-7.13)</td>
</tr>
<tr>
<td>Italy</td>
<td>No</td>
<td>45/546 (8.2)</td>
<td>1.0</td>
<td>2.01 (1.35-2.99)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>25/253 (9.9)</td>
<td>1.22 (0.73-2.04)</td>
<td>2.46 (1.51-3.98)</td>
</tr>
</tbody>
</table>

OR=odds ratio, CI = confidence interval.

An interaction analysis supports the role of the context for the association between current smoking and depressive symptoms (cross-country comparison). In particular, according to a formal interaction analysis on both additive and multiplicative scales, the association was significantly weaker in the Italian compared to the Swedish context (additive scale, RERI: -1.06 [-2.06,-0.06] and multiplicative scale, 0.65 [0.44-0.94]). Interestingly, when stratifying the analysis for
sex, the interaction effect was mainly driven by males (additive scale [RERI]: -2.24 [-4.91,0.43] and the multiplicative scale: 0.33 [0.14-0.77])

Applying an alternative scale for depressive symptoms (SDQ internalising score) yielded a similar pattern. In additional analyses, in which the Swedish KUPOL study was compared with the Italian BE-TEEN longitudinal study, the interaction effect was confirmed on both the additive and multiplicative scale, demonstrating the robustness of the main findings.
5 DISCUSSION

5.1 MAIN FINDINGS

This doctoral thesis aimed to contribute to a better understanding of the association between tobacco use (in particular, smoking) and the onset of depressive symptoms among adolescents.

The main findings are summarised below.

1. Tobacco use (in particular, smoking) was associated with an increased risk of the short-term onset of depressive symptoms in adolescents, particularly among males. Of the current smokers, at 13-14 years of age about 18% developed depressive symptoms after one year compared to 8% of non-smokers (Study I).

2. A possible involvement of the hypothalamic-pituitary-adrenocortical axis as a biological pathway from smoking to depressive symptoms was not supported by the findings. Over two years, smoking behaviour was not related to levels of activity of the hypothalamic-pituitary-adrenocortical axis (salivary cortisol), and this latter did not predict an increased risk of developing depressive symptoms (Study II).

3. Levels of activity of the hypothalamic-pituitary-adrenocortical axis (salivary cortisol) predicted smoking and snus initiation (Study III).

4. In a cross-country comparison, the association between smoking behaviour and depressive symptoms emerged in both a highly restrictive and a less-restrictive tobacco control environment. However, the association was stronger in the restrictive tobacco control environment (Study IV).

5.1.1 Explaining the co-occurrence of smoking behaviour and depressive symptoms in adolescence

Despite a general agreement about the common co-occurrence of smoking behaviour and depressive symptoms, and about smoking as a self-medication approach to cope with depressive symptoms, there is less unanimity regarding the effect of smoking on the onset of depressive symptoms (51). This thesis contributes to the body of literature and supports the hypothesis that smoking behaviour may arise as a response to stress and may be involved in the onset of depressive symptoms among adolescents. In particular, activation of the HPA axis predicted the onset of smoking behaviour and, in turn, smoking behaviour was associated with an elevated risk for the onset of depressive symptoms.

The positive associations between snus, the Swedish smokeless tobacco, and depressive symptoms support the hypothesis of nicotine as the main driver for the possible biologic effect of smoking on depressive symptoms (Study I). However, this association was limited to the concurrent onset of snus use and depressive symptoms within one year. Therefore, this finding may still be compatible with a possible reverse effect, namely the use of snus as self-medication. Another possible explanation may refer to a very short latency between snus uptake and the onset of depressive symptoms. This, in turn, would be in line with a higher cumulative exposure to nicotine when using snus as compared to smoking. For example, the presence of alkaline compounds such as sodium carbonate in snus raises the pH and the levels of uncharged nicotine in the mouth, leading to an increased nicotine bioavailability and concentration in the blood and brain (28, 125).
The sections that follow start by discussing possible biological mechanisms involved in this association pattern. Next, the role of context in the smoking-depressive symptoms association is considered. Finally, the role of sex as modifier of this association is discussed.

5.1.2 Activation of the HPA axis: cause or effect of smoking behaviour?

In Study II, we evaluated the association between smoking and the HPA axis activity, using cortisol concentration as a proxy. The main novelties of this study are represented by the young populations and the longitudinal study design. Interestingly, smoking behaviour was not a predictor of cortisol concentration, i.e. of the HPA axis levels of activity. This contrasts with the a priori hypothesis and with prior evidence of an association between smoking and cortisol concentration. Four main reasons may explain this negative finding and the discrepancies with previous studies.

First, the experimental smoking typical of adolescence entails exposure to intermittent and low doses of nicotine that may be insufficient to affect the HPA axis, as may be the case among adult populations (76-78). Second, previous studies have examined individuals outside the sensitive period window of adolescence, when smoking behaviour and the regulation of the HPA axis have already been stabilised (11). Third, the use of cross-sectional study design in former studies did not allow for the evaluation of the direction of the association (76, 77). Finally, random error in cortisol measurements may have prevented the detection of an association between tobacco use and cortisol levels in the present study. Generally, negative findings from this study and the limitations of previous studies raise fundamental questions: can we rule out that smoking plays a role in the activation of the HPA axis or does this process go undetected? Alternatively, can the activation of the HPA axis actually precede and increase the risk of smoking, which would therefore constitute a response to perceived stressful situations? The precautionary principle calls for testing for alternative hypotheses before rejecting a possible causal relationship between cigarette smoking, the HPA axis activation and depression in adolescence.

Previous studies have attributed the greater cortisol concentration among smokers as compared to non-smokers to an effect of smoking behaviour on the HPA axis. However, another hypothesis might also be in agreement with these findings, namely the HPA axis activation as a risk factor for smoking behaviour. In Study III, we found that the risk of initiation and maintenance of smoking and snus use over three years was predicted by morning cortisol concentration. This supports the hypothesis that the HPA axis activation (due to external factors) would proceed and possibly mediate the uptake and maintenance of tobacco behaviour. To date, only one other study has investigated this pathway in adolescence, presenting results that were in general agreement with ours, namely an increased risk of smoking as a result of rising basal cortisol levels (126). However, this study included older adolescents and a high prevalence of students with depressive symptoms, and used different analytical approaches, which hampers the comparison. Given that the underlying biological mechanisms between the HPA axis activity and tobacco use remain inadequately understood and widely under-researched, these findings need to be confirmed and other aspects of this association should be explored in logical models. For example, it may be hypothesised that early life adversities - possibly through epigenetic changes - may initiate responses that alter the HPA axis and the reward-signalling pathways. As a result, certain individuals may be predisposed to substance use.
5.1.3 The hypothesis of HPA axis activation as a determinant of depressive symptoms

In Study II, the HPA axis activation was not predictive of the onset of depressive symptoms. While research has overwhelmingly focused on the role of the HPA axis dysregulation as a biological basis of severe depression among adult populations (39, 85), a handful of studies have examined this pattern among adolescents looking at depressive symptoms as the outcome (83). Findings from these studies are inconsistent. For example, findings from the British “Avon Longitudinal Study of Parents and Children” show, in line with our findings, that the HPA axis activation, using cortisol concentration as a proxy, was not predictive of an increased risk of depressive symptoms among adolescents (127). This apparent discrepancy between studies in adult and young populations may have different explanations. First, a dysregulation of the HPA axis may not be the main underlying biological mechanism involved in the onset of depressive symptoms during adolescence. Second, as mentioned before, random error in cortisol measurements cannot be completely excluded.

Along with an unclear association of smoking with the HPA axis activation, the findings in Study II do not support the hypothesis that HPA plays a role in the pathway between smoking behaviour and the onset of depressive symptoms. As we are aware that a lack of evidence of association is not equal to evidence of no association, further epidemiological longitudinal studies should bridge the gap and test alternative hypotheses, such as a possible detrimental effect of nicotine exposure on monoamine neurotransmitter systems, that may explain the role of smoking as a risk factor for depressive symptoms.

5.1.4 Moving from individual to country-level factors

The present thesis examines the impact of country-level factors, in particular of restrictive and non-restrictive tobacco control environments, on the association between smoking behaviour and depressive symptoms. Norms and cultural beliefs, along with political, commercial and financial interests, may affect association patterns across places or different contexts. Most of the approaches previously used to study the impact of context-level factors on this pattern have not been within this scope. Firstly, many studies have only focused on individual factors such as gender and ethnicity (128-133). Secondly, the methodological heterogeneity, such as the use of different psychometric scales and measures of association, hampers comparisons between studies (51). In other words, the results of previous studies do not allow us to evaluate how the presence and the magnitude of the association between smoking/tobacco use and depression varies in relation to the characteristics of the context. As mentioned in the Introduction, the Swedish context is an example of a restrictive tobacco control environment. In recent decades, policies and legislations have been enforced at several points in time, leading to an overall drop in the prevalence of daily smoking, but also to an unequal distribution of the behaviour across socio-economic groups (112). During the past two decades, Sweden has also faced an increase in diagnoses of anxiety and depression disorders among adolescents.

Findings from Study I support the hypothesis of a low proportion of social smokers in a restrictive tobacco control environment, as in the Swedish context, where smokers may represent a marginalised group at high risk of negative effects of tobacco. In particular, Study I showed that differences in depressive symptoms (mean CES-DC score) between current smokers and non-
current smokers were notably greater among Swedish adolescents (27.1 vs 14.1 mean CES-DC score, KUPOl sample) compared to American adolescents (13.0 vs 9.6, US Add Health sample) (134), a sample with a 2-fold higher prevalence of smoking.

This line of investigation was expanded in Study IV by comparing the association between smoking and depressive symptoms in two different settings characterised by a restrictive tobacco control environment (the Swedish context) and a permissive tobacco control environment (the Italian context). We hypothesised that the social reward of smoking (e.g. peer pressure and a sense of glamour) becomes weaker in a restrictive tobacco control environment, contributing to a segregation of the behaviour among individuals with high dependence liability, and therefore to an increased share of depressed smokers. This hypothesis was supported by the results obtained in this study. A further mechanism, in addition to subgroup selection, may contribute to stronger cross-sectional association between smoking and depressive symptoms in a restrictive and permissive tobacco control environment respectively. Societal disapproval of smoking behaviour may increase feelings of social exclusion and inadequacy. This is consistent with the on-time and off-time theory widely adopted in psychology (135). This theory states that an event or a behaviour exerts a greater impact on our mental ill health in the period when it is less expected. While smoking a cigarette is a normalised behaviour among Italian adolescents, smoking is off time in Sweden, where the tobacco epidemic is at a more advanced stage and the acceptability of smoking is lower (112). To the best of our knowledge, no comparison with other studies is possible since this study is the first to investigate the role of country level-factors in the smoking-depressive symptoms association. Against the backdrop of community-level factors, social stratification influences who is affected by different processes and how, given that societies are not homogeneous. Such consideration and its implications for tobacco control environments remains to be tested in further longitudinal studies.

5.1.5 Sex differences

As mentioned in the Introduction, females seem to be more vulnerable to nicotine reinforcing effects, and consequently to nicotine dependence, as well as to negative outcomes associated with smoking (102-104). On the other hand, a higher prevalence of depressive symptoms and of severe major depressive disorder has been observed in females, compared to males (64, 94-96). Despite the expected overall higher incidence of depressive symptoms in females, Study I found a strong association of smoking behaviour with depressive symptoms in males.

Two explanations can be put forward to clarify this discrepancy. First, possible misclassification of depressive symptoms among females may have biased the estimates of an association towards the null. For example, the CES-DC scale covers psychosomatic symptoms that largely overlap with premenstrual and menstrual dysphoria (119). Second, differences between males and females may reflect different sensitive periods. Smoking behaviour may associate to depressive symptoms differently during prepuberal (when depression is uncommon) and puberal (when depression become more common) development. The observed sex difference may then result from a lower proportion of males than females having entered puberty before enrolment in the cohort (13-14 years of age). Interestingly, in Study IV the association between smoking and depressive symptoms was stronger among males than among females in the Swedish context, while the reverse was true in the Italian context. This finding suggests that the focus should be moved towards the role of gender, rather than of sex differences, i.e. towards the social norms surrounding smoking
behaviour among females and males. In a less restrictive tobacco control environment, such as the Italian context, the social norms discourage smoking less among boys than among girls. Therefore, among girls, relieving mental distress may be the preponderant determinant of use of tobacco. In a highly restrictive tobacco control environment, such as the Swedish context, social smoking becomes marginal, as reflected in a stronger association with mental distress among boys. Overall, these country-level differences corroborate the hypothesis of increasing segregation of smoking behaviour to vulnerable groups in restrictive tobacco control and low-prevalence environments, and support the need for context-centred knowledge to develop effective preventive programmes.
5.2 METHODOLOGICAL CONSIDERATIONS

5.2.1 Study population

While all four studies of this thesis included students participating in the KUPOL study, Study IV also encompassed a sample of Italian students from the Italian BE-TEEN study. The KUPOL cohort applied a few inclusion criteria: schools with at least 20 students from the seventh to ninth grade located in eight regions of southern/central Sweden and students with an understanding of the Swedish language and without severe learning disabilities. Participants with parents who had a university education and a high socio-economic status were overrepresented compared to the general Swedish student population. The Italian participants were recruited through an anonymous survey and similar inclusion criteria at student levels were applied, resulting in a sample that was reasonably representative of the student population of Brescia Province.

5.2.2 Random error

Randomness may impact both population sampling and measurements (136). In general, epidemiological studies aim to include a random sample from the target population, since including the whole population may be unachievable and not useful in terms of allocation of resources. However, sample selection always entails the risk of obtaining estimates that differ from the true value. Increasing the sample size and using different populations may help reduce estimate variability. Also, randomness can affect measurement precision. For example, in the KUPOL study, salivary cortisol assessment determination was based on two cortisol assessments in one day (137). Generally, salivary cortisol stability over time is a matter of concern with regard to adolescents. However, the measurement of cortisol concentration in duplicate for each sample, the adherence to a strict protocol for cortisol collection, and the inclusion of individuals of the same age should have minimised random error in cortisol assessment.

5.2.3 Systematic error

Bias in the estimates of association due to systematic errors is a common problem, especially in observational studies (136). More specifically, bias is defined as a systematic error that occurs in the design, conduct or analysis of a study and diverts the final estimate from the true value. In epidemiology, it is customary to summarise possible biases in three main groups (136).

5.2.3.1 Selection bias

Selection bias of study participants can arise from the different steps that contribute to the definition of the analytical sample, namely participation, response and attrition. All these factors may potentially affect the results if the selection is a function of both the predictor and the outcome. Although aiming to recruit a random sample of the student Swedish population, the KUPOL study was faced with a selection of participants due to a low initial participation rate. In particular, among 541 schools invited to study, six were not eligible and only 101 agreed to participate in the study (19%). Out of 12,512 eligible students, 3,671 agreed to participate and answered the first questionnaire (response rate 29.3%). A low participation rate in child and young adult cohort studies is a common problem in epidemiological research. In this project, private schools, schools sensitive to mental health problems, students with parents with a university
education and students without a foreign background were overrepresented. This may have resulted in a low prevalence of both smoking behaviour and depressive symptoms, compared to the Swedish student population as a whole.

Other forms of selection bias are attributable to missing data - students not answering specific questions - or attrition – e.g. students leaving high school without completing their education, moving to another school or withdrawing their participation. First, the proportion of missing data for the exposure and outcome variables was low and is unlikely to have impacted the final estimates. Moreover, in the KUPOL study, the attrition rate was low for the first (8.9% in the eighth grade) and second (12.7% in the ninth grade, compulsory education) years of follow-up, although it did increase in the last year of follow-up (32.6%, non-compulsory education). Thus, in Study III, the magnitude of the association between cortisol levels and tobacco initiation could have been biased if students had withdrawn from the study before the onset of tobacco use. To explore the presence of this possible bias, we included a sensitivity analysis restricted to the first and second follow-up.

5.2.3.2 Information bias

Measurement errors can bias estimates. Two main types of misclassification can hypothetically affect all variables included in a study: non-differential and differential misclassification. Non-differential misclassification occurs when a classification error is homogeneously distributed across subgroups. In the case of a dichotomous variable, non-differential misclassification may bias estimates towards the null. Conversely, differential misclassification occurs when a classification error is unevenly distributed among exposed vs unexposed groups (or in relation to the outcome) and may bias final estimates away from and toward the null.

In this project, the use of a strict protocol for data collection and the use of more than one measure to assess both exposures and outcomes, namely two different psychometric scales for depressive symptoms and questions tackling both snus use and smoking behaviour, should have minimised the effect of possible misclassification on the findings. However, we cannot completely exclude a certain level of misclassification, particularly due to self-reporting. For example, a proportion of smokers or snus users may have incorrectly classified themselves as non-tobacco users, because of non-disclosure of a disapproved behaviour. This misclassification should be considered non-differential, i.e. not dependent on depressive symptoms or cortisol concentration and therefore likely to attenuate the estimates.

5.2.3.3 Confounding

Associations from observational studies, if conceived as causal, may be biased by the presence of unmeasured factors that act as ancestors of both exposure and outcome, generating a spurious exposure-outcome association.

In the present thesis, we accounted for possible confounding by adjusting and stratifying for putative confounders (e.g. depressive symptoms and parental education). However, genetic factors or parental psychiatric comorbidities, factors that can simultaneously affect smoking behaviour, depressive symptoms and the HPA axis activation, could not be ruled out.
5.2.4 Generalisability

Research aims at generating evidence that has the potential to be transferred to other populations and settings. However, it is not always possible to include the whole population or a random sample of it, since the recruitment process, inclusion criteria, missing data and number of dropouts contribute to the selection of the final analytical sample.

For example, as mentioned above, in the KUPOI study there was an overrepresentation of students with highly educated parents, indicative of high socio-economic status, compared to the general Swedish student population. Caution is warranted when generalising findings from this study to other student populations, in particular when we consider measures that can be strongly affected by sampling, namely prevalence and incidence estimates. However, measures of association are less prone to this problem when testing a causal and/or biological hypothesis. In addition, measures of association should be first and foremost interpreted in terms of their direction rather than of their magnitude.

To understand whether biological mechanisms underpin a given association, it is of the utmost importance to use different study designs affected by different sources of bias. Other important cues to causal inference include a priori hypotheses on, or explanatory models of, possible pathways that link the exposure and outcome, taking into account confounding and other bias. These approaches are arguably more important than only including a representative sample (138, 139).
6 CONCLUSIONS

1. Smoking behaviour increases the short-term risk for the development of depressive symptoms among young individuals. Studying the development of the association between smoking and depressive symptoms in early adolescence may provide important information on trajectories of these determinants of health as well as hints on underlying biological mechanisms.

2. The studies in this thesis do not support a possible role of the hypothalamic-pituitary-adrenocortical axis activation as an intermediate of the pathway from smoking to depressive symptoms in young individuals. The focus of future research should be shifted to other possible psychobiological pathways underlying smoking-depressive symptoms association.

3. Conversely, activation of the hypothalamic-pituitary-adrenocortical axis may predict initiation and maintenance of tobacco use (smoking and snus) in adolescence. Investigating if this activation indicates the effect of dysregulation of responses to stress and examining the potential role of adverse life events in the life course may inform the development of future public health interventions.

4. The co-occurrence of smoking behaviour and depressive symptoms in adolescents is more common in a highly restrictive tobacco control environment compared to a less restrictive one. A better understanding of the context-level factors may help healthcare providers and policymakers to develop interventions in the field of selective prevention.
7 IMPLICATIONS

Today’s adolescents will be the adults and elderly of the next decades. Substance use and mental health problems during this age period are among the leading determinants of individual health and well-being, influencing both duration and quality of life. They also play a role as determinants of future generations’ behaviours and well-being due to intergenerational influence. For example, having close contact or living with an adult who smokes is the main contributing factor to smoking among adolescents. Research in epidemiology and public health has a major role to play. First, untangling the underlying causal pathways and biological mechanisms that link behaviours and health outcomes helps us to understand complex phenomena. Second, this knowledge may contribute to the development and implementation of effective context-centred preventive programmes. The present thesis contributed to both these aspects.

We believe that we contributed to a better understanding of the causal pathway from smoking to depressive symptoms, pointing towards the need for causal explanations not related to the HPA-axis activation as an intermediary step. At the same time, the studies in this thesis open up new research avenues in support of the possible role of HPA axis activation as a determinant, rather than an effect, of tobacco use. If other pieces of evidence support this hypothesis, future public health interventions can direct efforts towards specific subgroups of children and adolescents characterised by a high liability to tobacco use initiation and maintenance.

Finally, with the present thesis, we shed light on the modifying role of contextual factors on the smoking-depressive symptoms association. This is of utmost importance not only to better understand the relationship but also to target prevention programmes. For example, in countries in a mature stage of the tobacco epidemic, such as Sweden, the primary focus of intervention efforts should be on the early detection of mental health distress among children and young people. Conversely, in countries in an earlier stage of the epidemic, efforts should be directed to adhering to regulations, changing social norms towards social unacceptability of smoking, and reinforcing smoking cessation programmes.
8 FUTURE DIRECTIONS

This thesis contributes to a better understanding of the interplay between tobacco use and depressive symptoms among adolescents, assessing both the biological mechanisms and the role of social context. Framing these findings in a more complex scenario, involving both the gene-environment interaction, as well as public health policies, can help us to understand the underlying mechanisms that influence the development of a healthy lifestyle during adolescence.

First, using cortisol concentration as a proxy of the HPA axis activation allows us to draw only preliminary conclusions, due to cortisol’s high variability. Further epidemiological studies considering different settings, applying other biomarkers of stress, and including possible epigenetic mechanisms are needed to confirm these findings. Second, the focus of the studies in this thesis is on a limited period of the human lifespan. Life course studies would shed light on the possible role that negative life events have on the dysregulation of stress systems, the subsequent onset of smoking behaviours and psychiatric disorders. Third, the cross-sectional design of cross-country comparison limits the possibility of drawing firm conclusions on the role of the tobacco control environment in the presence of the smoking-depressive symptoms association. While future studies should include contexts in other stages of the tobacco epidemic and use a longitudinal design, preventive medicine should assess and evaluate possible intervention programmes that take into account contextual factors as well as country-level characteristics such as variability in norms, values and lifestyles. Finally, to draw solid conclusions on such complex processes, research in public health epidemiology would benefit from using different study designs to tackle identical questions according to the triangulation of evidence approach to overcome biases arising from a single study (138, 139).

In the coming decades, modern democracies will strive to sustain public healthcare systems under the pressure of an ageing population. With a growing knowledge of the biological mechanisms associated with determinants of health, translating this into effective policies and community interventions should be the next step. Promoting healthy lifestyles and well-being, as well as facilitating healthy ageing, are among the greatest challenges for public health in the near future.

If, on the one hand, adolescence is a period of experimentation and risk-taking behaviour, teenagers are, on the other hand, more sensitive than adults to injustice and global problems. Since a fundamental cognitive development takes place during this age period, in turn influencing the lack of risk awareness (2) and vulnerability to tobacco dependence, adolescents are the most desirable target group for tobacco companies (140). However, looking for a sense of belonging as human beings enables adolescents to develop self-awareness on an individual level, and sometimes leads to the emergence of international movements. Health professionals and researchers should seize this moment in history, and inspire as well as empower the young generation to share their concern and strive for health at the individual, global and planetary level.
9 ACKNOWLEDGEMENTS

This thesis is part of a long journey, for me as a woman and researcher, and is coming to an end in a time of uncertainty for our societies. As a public health medical doctor, I strive for universal access to health care. Exponential economic and population growth along with no war, the growth of welfare states and the rolling back of dictatorships during the 1990s, have contributed to increasing our vulnerability to rare events. Modern democracies rely on an independent judiciary system, free media, freedom of thought, speech and organisation. Individuals have a duty to make the greatest contribution they can to the collective well-being and exercise the right to choose their representatives. During the current pandemic, increasing inequalities and enforcing measures that limit personal freedom may lead to the emergence of conflicts and non-adherence to imposed measures. As citizens, researchers, and healthcare staff, we should encourage community involvement and make communities the centre of the response. In such a complex world, this, along with allowing a certain degree of variability within our systems, may decrease the impact of uncertainty and rare events, and may support the foundation of modern democracies, such as universal access to healthcare. This may be seen as a modern interpretation of the 19th century “social chain” concept.

A number of people who are part of my “social chain” have supported my development as a citizen, doctor and researcher during this journey and deserve to be acknowledged.

My deepest gratitude goes to Professor Maria Rosaria Galanti, who is bright, with an endlessly curious mind, and attentive to detail. You are an inspiring woman, a doctor of preventive medicine, researcher and actress (and soon-to-be-writer). This rare multidisciplinary approach to science allows you to apply the mindset from one field to another, contributing to the progress of science and encouraging young generations. Thank you for showing me the way of beauty in research, for always challenging my mind, for a four-year-long stimulating discussion. Tibi gratias agio!

Thanks to Professor Francesco Donato, my co-supervisor during this PhD thesis and the person who understood my aptitude for epidemiology before I did (I took more than two years to reach this awareness). Thank you for ten-year-long scientific and philosophical discussions and for being the professor that every student deserves.

Thanks to Professor Yvonne Forsell, my co-supervisor. Thanks for involving me in your research group, for introducing me to the field of mental health, for contributing to a good work climate, and for your encouraging words and positive comments on my work.

I would really like to thank the students, families, teachers and schools that participated in the studies included in this PhD project and made all of this possible.

A big thank you to my mentor, Associate Professor Francesco Barone-Adesi, who was always available with wise advice and positive energy.

Many colleagues and friends at Karolinska Institutet and at the Department of Global Public Health deserve my thanks. I have striven to contribute to a collaborative spirit and transparency as a breeding ground for new research ideas, high-quality teaching and research. I am so grateful to have joined such a productive team with motivated people who share ideas and create collaborations.

From the Epidemiology and Public Health Intervention Research Group, Dorien Becres, one of the most brilliant and free-thinking minds I have ever met, who always reminds me to face my cognitive bias; Filip Andersson, for his genuine friendship and pockets full of tips on statistical methods (and cars, cat and dog behaviour, as well as the Italian language); Associate Professor Jette Möller, for stimulating discussions; and Louise Ehrenberg, where there is a problem she sees ten solutions. Also Carla Nooijen, Cecilía Söderberg, Christine Takami Lageborn, Eleonor Sáfsten, Fen Yang, Fouad Jabri, Gisela Nyberg, Hanna Hultin, Israa Ali, Jasmin Luco Castro, Johanna Hoffsten, Associate Professor Karin Engström, Krisztina Laszló, Marie Skyving, Susanne Andermo, Yajun Liang, Yang Zhao.

I would like to thank the past KUPOL and TOPAS teams: Associate Professor Catharina Lavebratt, Emma Carlsson, Elin Arnö, Fai Wenneberg, Fanny Engman, Hanna Hultin, Jia Zhou, Johanna Lindman, Laura Ferrer-Wreder, Martin Karlberg, My Riseid, Nina Klang, Shahram Mansoory, Sofia Murad, Tharshini Thangavelu, Zangin Zeebari.
From the Department of Global Public Health and from Karolinska Institutet, Professor Marie Hasselberg, Associate Professor Nicola Orsini for involving me in teaching biostatistics, Chen Chu, Emilie Agardh Kyriaki Kosidou, Lisa Harber-Aschan, Professor Lucie Laflamme, Professor Marie Reilly, Professor Rino Bellocco.

A big thank you to all friends and employees from Centrum för Epidemiologi och Samhälls medicin (CES), for stimulating lunches, tea (jika)-time discussions and smiles (along with the last apple mystery which taught me about Swedish customs) that have made Torsplan my home during the last four years. I really want to thank Professor Cecilia Magnusson for contributing to high-quality research and such a good work climate.

Thanks to my fellow PhD students for their support and collaboration throughout the years: Jad Shedrawy, a very fast mind, skilled researcher and amazing Lebanese chef; Megan Doheny, not only a skilled researcher but also an outstanding Irish dancer; Diego Vacaman Mendez, my fellow PhD medical doctor (in the worst-case scenario there is Mexcal); Anton Landgren, for always giving prompt and constructive feedback on our papers; Björg Helgadóttir, Charisse Johnson, Christian Rausch (meeting him in the corridors stimulates natural happiness), Constance Boissin, Dang Wei (the best dumpling chef and secret keeper), Hua Chen, Isidora Stark, Katalin Gémes, Maki Morinaga, Melody Almroth (a rock star), Menghan Gao, Patricia Eustachio Colombo, Rynaz Rabiee, Therese Wirback, Viktor Ahlqvist, Zakir Hossin.

Thanks to the Climate Change group at the Department of Global Public Health. A special thanks to the group coordinator, Ester Gubi, for striving for a better future, for being a natural leader and a person with high integrity.

Thanks to my fellow PhD representatives in the Doctoral Programme in Epidemiology, Alicia Nevriana, Anna Meyer, Alva Wallis, Marios Rossides and the Programme Coordinator, Anita Berglund.

A special thanks to The Tobacco and Alcohol Research Group (TARG) at the University of Bristol, Hannah Sallis, Professor Marcus Munafó, Robyn Wootton. This collaboration has inspired me and boosted my motivation, provided me with a broader insight in the field and helped to deepen my understanding in the triangulation of evidence and reproducibility of science.

Many (present and former) colleagues and friends from the BE-TEEN team, the University of Brescia and the Italian Health Care System deserve acknowledgement. I am impressed by your strength and resilience during the current pandemic. Associate Professor Eugenia Quiros Roldan for her long-standing friendship; her office has always been open for laughs, scientific discussions and wise advice. Elia Croce, the best example of doctor, organiser, friend, politician (and gastronome); I’m very proud of you. Sara Mentasti, for blossoming as a person and public health doctor. Chiara Baiguera, Daria Gott, Francesca Brogno, Ementus Professor Giampiero Carosi, Laura Albini, Professor Pietro Nicolai. Thanks for being my rocks, for contributing to the feeling that my family and I will always have a home and a job to return to. Marco Bortazzoli, Marta Zangrandi, Michele Magoni (il capitan), Rita Bertuetti, Sara Simoncini, Silvia Amadasi, Susanna Facchetti, Valentina Pasqualin and my former fellow tutors for university freshmen during medical school, Alberto Paderno (Professor Paderno, excellent thinker and surgeon), Luca Facchetti (il capo), Sarah Molfin.

Despite my tendency to work long hours (my working computer is specifically set on Greenwich Mean Time to fool myself on time), I have learned that I need to secure some time off. I would like to thank my (present and former) international crew for amazing dinners, philosophical discussions, unconditional support and reminding me to keep fit. Alessandro Pini, for being the most adorable person on earth, Federico Triolo, an incredible thinker, attentive to details (lilli forever), Giulia Grande, an inspiring woman, for her friendship, memorable dinners and stimulating discussions, Davide Vetrano, Professor Vetrano, an inspiring man and researcher (good taste in truffles), Caterina Trevisan, one of those rare people who make people around her shine (and a natural talent as an idol translator even in Mandarin and Taiwanese), Federica Prinelli, for your energy and positiveness, Alberto Zucchelli, great climber who made me face my fear of heights, Thomas Escriva Izquierdo, for creating beauty, Kuan Yu Pan (forever Claudio), Eleonora Bianchini, for beautiful discussions, Alessandra Grotta, for inspiring me, Stefania Manetti, for being my first dear friend in Stockholm and always being so genuine, Jonathan and Alessandro Taglialetela, Jesús Prego Domínguez, for always being so caring, my former flatmates Marco Trevisan and Alessio Crippa.

I really want to thank my extended Swedish family; you make me feel at home in the North. I would like to start with my dear friend Joakim Lindqvist, a great philosopher and a solution-oriented driven medical doctor (also for cars) and his family Malin, Nathan, Sidner, Edwin and Henry. A special thanks to Björn Nilsson and Charlotte Holst who welcomed me in Lekvattner, one of the most beautiful places in Sweden. My friends in Stockholm: Sarah Klaréus,
Sven, Maria, Sam and Edgar Blume Dorrian, Sewit and Semay Mogos (my little challenger), Malin, Hanna and Evan Berggren Griffiths, Linn, Henrik, Paul and Jan Söderström Nordén, Joacim, Eva and Emilia Hagström, Pontus and Lotta Hagström Lindley. A special thanks to Jan Hagström and Josefin Losada Hagström, my dear family in Spain.

As researchers, I believe we should aspire for active citizenship (viva activa) through volunteering and political engagement. With current challenges such as increasing migration, climate denial, and the growth of extremist parties, I believe well-informed translation of scientific knowledge is highly needed. In June 2019 I was elected the representative for the Italian Social Democratic Party in the Nordic Countries, and together with The Brothers Rosselli Association, of which I am a board member, we promote a space for debate on political innovation, sustainable development and access to welfare. I would like to thank Lara Olivetti, for long talks and laughs along with dinners and weekend adventures, Silvano Garnerone, for contributing to and caring for the Italian Community in Sweden, Antonella Dolci, Camilla Di Battista, Elisa Baroni, Elisa Martini, Giuliano Di Baldassarre, Laura Parducci, Manlio Palacci, Thomas Lo Monaco, and my fellow companions in Europe, among whom I would like to thank especially my dear trustworthy friends Federico Quadrilli, tireless and visionary politician (next MP), and Nicoletta Leo, always available for brainstorming or advice. I am currently volunteering as a medical doctor in a summer camp for children with heart transplantation and neuropsychiatric disorders (Il trampolino). It has been a fulfilling experience and every year I discover something new about myself. Thanks to Franco Tovagliari, for contributing to making all this happiness in my life possible, Annalisa Tovaglari, a very dear friend with a wonderful life in front of her, Matteo Filippini, for always being a dear friend, a natural leader, and a great doctor, and Duilio Magri.

To my dear friends Silvia Benassai and Elisa Ottanelli, a beautiful Italian married couple, Marta Fedriga and Marta Tamagna (squared Marta), for long philosophical discussions. Agnese Roversi, Federica, Pier and Emma Taboni, Elias Allara, Sara Pestelli, Serena Corti.

All the teachers and fellow students who have challenged my logical and critical thinking during my long academic journey. A special thanks to my high-school friend Fabio Giorgi (Befeldo), who summarised my PhD journey in a marvelous comic.

To give our best and go beyond our limits, all of us need an antagonist sometimes. I would really like to thank everybody who has been strong enough to fit that role and contribute to my development.

To my best friend in life, Demis Menolfi and my dearest friend Andrea Mattera. Despite eight time zones between us, I always have you both with me, your support, intelligent thoughts and laughs.

To my sister, Anna Raffetti and Sergio Marchioni for always supporting me and showing me a pathway to a more sustainable society.

To my family, my “feelow” traveller who brings happiness, adventure, home feeling and unconditional support in my life ("så har du satt en nylonstrumpa på min svartvita TV"), Ana Hagström, and to Ed Raffetti-Hagström, for being so funny and transforming me into a morning person.

To my parents, Antonietta Pelamatti and Bortolo Raffetti, along with Godot, for their unconditional trust and support, and for making me an antifragile citizen.
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