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SNUS USE AND MORTALITY

Associations, potential mechanisms, and socioeconomic aspects

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“Snus is really dangerous, don’t you know that?
For example you can choke on it,
and then you might DIE!”

Isak, 7 years old, explains to his little brother the dangers of snus use

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Abstract

Snus is a smokeless tobacco product made of a moist powder of ground tobacco. It is used mainly in the Nordic countries, although increasingly popular internationally. The Swedish snus tradition dates back to the seventeenth century, and it is now used daily by about 23% of the male and 6% of the female population. Snus contains high levels of nicotine as well as carcinogenic substances and microorganisms that could potentially cause adverse health effects. The physiological effects of snus use include acutely raised blood pressure and heart rate, and increased cardiac oxygen demand, while the psychological response results in alertness and anxiety reduction. The high nicotine content causes rapid onset of addiction.

Previous research on snus use and health is largely inconclusive, but indicates increased risks of all-cause, cardiovascular and cancer mortality. This thesis aimed to further investigate the health effects of snus use, with a focus on mortality, potential underlying mechanisms, and the impact of socioeconomic factors.

Four original papers form the base of this thesis. The first study was performed on a pooled dataset of eight Swedish cohorts (The Swedish Collaboration on Health Effects of Snus use), including over 169 000 men. We found an increased risk of all-cause (HR 1.28, 95% CI 1.20; 1.35), cardiovascular, and other cause mortality, and indications of raised cancer mortality. The second study was set within an interventional program in northern Sweden (Västerbotten Intervention Programme) and included 46 000 men and women. It showed increased mortality overall (estimates similar to first study), from cardiovascular diseases, and external causes (e.g., accidents and suicide) that remained after controlling for socioeconomic status. We found these associations in groups of varying socioeconomic background (e.g., both basic education and high-income groups), suggesting that increased mortality risks among snus users are not restricted to certain socioeconomic groups.

Studies three and four investigated potential underlying mechanisms that might contribute to increased mortality among snus users, including established cardiometabolic risk factors in study three (the metabolic syndrome and its components: obesity, hypertension, type 2-diabetes and abnormal blood lipids) and more novel risk factors in study four (low-grade inflammation, low vitamin D-concentrations, and altered

testosterone levels). The analytical samples were drawn from a long-term follow-up study of around 900 16-year-olds in a municipality in northern Sweden (Northern Sweden Cohort, study three) and more than 6 000 participants in another population-based cohort (the Northern Sweden MONICA study, study four). We found no associations between snus use and established cardiometabolic risk factors, but there was evidence of lower concentrations of inflammatory and vitamin D-status biomarkers in both men and women, and higher testosterone concentrations in men who were currently using snus.

We conclude that snus use is associated with increased all-cause and cardiovascular mortality, and to death by other causes, that may be restricted to external causes. Cancer mortality may also be increased among snus users. The associations cannot be fully explained by differences in socioeconomic status among snus users and non-users. Established cardiometabolic risk factors do not seem to be the main mechanisms behind these associations. Lower inflammatory biomarker levels among snus users may serve as a protective factor, while lower vitamin D-concentrations and increased testosterone levels may be part of an underlying mechanism linking snus use to increased mortality.

Future research should focus on the health consequences of snus use among women, on other possible links between snus use and death, and on mortality in different cancers among users of snus. The health consequences of dual use of snus and cigarettes should also be assessed.

Sammanfattning på svenska

Bakgrund

I Sverige finns en lång tradition av att tillverka och använda snus. Efter att ha minskat i popularitet under mitten av 1900-talet har försäljningen återigen ökat, och idag snusar ungefär 23 % av de svenska männen och 6 % av de svenska kvinnorna dagligen. Trots att snus är vanligt och blir alltmer populärt, både i Sverige och i andra länder såsom Norge, Finland, Island och USA, vet vi inte särskilt mycket om vilka effekter snusning kan ha på vår hälsa.

Nikotinet i snus står för många av snusets effekter på kroppen. När man lägger in en prilla stiger nivåerna av nikotin snabbt i blodet, och de fortsätter vara höga även efter att man tagit ut snuset. Nikotinet ger ett stresspåslag med ökat blodtryck och puls, vilket gör att hjärtat och kärlsystemet utsätts för extra påfrestning. Samtidigt är nikotin verksamt på delar av hjärnan som får oss att känna lugn och tillfredsställelse. Tungmetaller, rester av bekämpningsmedel och så kallade tobaksspecifika nitrosaminer är exempel på cancerframkallande ämnen som finns i snus. Eftersom nikotinet är starkt beroendeframkallande är det vanligt att snusare utsätts för dessa ämnen under många år.

Det är svårt att dra entydiga slutsatser utifrån den forskning som har gjorts tidigare avseende snusets effekter på hälsan. Det finns dock enstaka stora studier som visar att snusare har ökad risk för att dö i hjärtinfarkt och stroke, och att snusare har kortare livslängd generellt, jämfört med personer som inte snusar. Med tanke på hur många människor som snusar och att hjärtkärlsjukdomar är vår vanligaste dödsorsak är det särskilt angeläget att ta reda på mer omkring de här frågorna. Denna avhandling har därför som syfte att undersöka huruvida snusning medför en ökad risk för död, och i så fall, vilka mekanismer som kan ligga bakom. Vi ville också studera vilken betydelse som sociala bakgrundsfaktorer, till exempel utbildningsnivå, kan ha för riskerna.

Metoder och resultat

Avhandlingen omfattar fyra vetenskapliga arbeten. I studie ett använde vi en databas som har samlat flera stora svenska studier om snusning och hälsa. Sammanlagt ingick mer än 169 000 män. Resultaten visade att snusarna hade 28 % ökad risk för död oavsett orsak jämfört med icke-snusare. Snusare hade också 27 % ökad risk för död i hjärtkärlsjukdom såsom hjärtinfarkt eller stroke. Dödligheten i cancersjukdomar var också förhöjd, men inte på ett lika övertygande sätt. Även i kategorin ”övriga dödsorsaker”, som omfattade alla orsaker utom hjärtkärlsjukdom och cancer, sågs högre dödlighet bland snusare.

Studie två genomfördes på data från Västerbottens hälso-undersökningar, som omfattar de hälsokontroller och efterföljande hälsosamtal som erbjuds västerbottningar när de fyller 40, 50 respektive 60 år. Analyserna gjordes på ca 46 000 män och kvinnor. Också här var frågeställningen om snusning förkortar livet. Våra resultat visade återigen på ökad total dödlighet och hjärtkärldödlighet bland snusare jämfört med icke-snusare. Även död av ”övriga orsaker” var vanligare bland snusare. I denna studie hade vi möjlighet att titta närmare på vad de övriga orsakerna bestod av, och fann då att det var kategorin ”yttre orsaker” som gav denna ökade risk. Yttre orsaker omfattar bland annat olyckor, förgiftningar och självmord.

Sociala faktorer såsom utbildningsnivå, boendeområde och inkomst spelar roll för vår hälsa och livslängd. Eftersom snusning traditionellt sett har förknippats med mindre gynnsam social bakgrund måste vi ta hänsyn till detta när vi undersöker dödlighet. Vi hade inom studie två möjlighet att belysa detta mer ingående genom att dela upp analyserna och studera hur risken för död såg ut i olika samhällsskikt. Vi såg då ökad risk för död både bland dem som i grunden har högre risk (personer med lägre utbildningsnivå), men också bland grupper med lägre risk för död (höginkomsttagare, stadsbor).

I studie tre och fyra ville vi försöka ta reda på varför snusning leder till förkortad livslängd. Vi undersökte först om det fanns ett samband mellan snusning och etablerade riskfaktorer för hjärtkärlsjukdom såsom övervikt, högt blodtryck, typ 2-diabetes och förhöjda blodfetter. Detta gjordes i studie tre som omfattade ungefär 900 personer i Luleå kommun som följts från 16 till 43 års ålder. Vi hittade inga samband mellan snusning och dessa riskfaktorer. När vi i stället inom ramen för studie fyra undersökte riskmarkörer i blodet upptäckte vi att snusare har lägre nivåer av D-vitamin än icke-snusare. I andra studier har låga D-vitaminnivåer visat sig vara kopplat till exempelvis ökad förekomst av hjärtkärlsjukdom och depression, och försämrad överlevnad. Vi såg också lägre nivåer av en inflammatorisk markör i blodet. Det är något som snarast kan verka skyddande mot sjuklighet. Vi undersökte även testosteronnivåer och såg att de var något högre bland snusande män. Studien genomfördes på drygt 6 000 deltagare från MONICA-undersökningarna i Norr- och Västerbotten.

Slutsatser

Våra slutsatser utifrån denna avhandling är att snusning är associerat med ökad dödlighet, främst drivet av en ökad risk för död i hjärtkärlsjukdom och av yttre orsaker. Risken för död i cancersjukdom kan också vara ökad. Det verkar inte vara de etablerade riskfaktorerna för hjärtkärlsjukdom såsom högt blodtryck som är huvudorsak till den ökade dödligheten, men lägre nivåer av D-vitamin och höjda testosteronnivåer bland män skulle kunna vara en del i förklaringen. Våra resultat tyder också på att de associationer vi ser mellan snusning och förkortad livslängd inte beror på snusarnas ofta socialt mindre fördelaktiga position.

Framtida forskning bör fokusera på andra bakomliggande orsaker till förkortad livslängd bland snusare, och undersöka snusares risk för död i olika typer av cancersjukdomar. Eventuella risker för kvinnliga snusare bör undersökas mer noggrant, liksom hälsoeffekter av att samtidigt både röka och snusa.

List of papers

This thesis is based on the following papers:

I Marja Lisa Byhamre*, Marzieh Araghi*, Lars Alfredsson, Rino Bellocco, Gunnar Engström, Marie Eriksson, Maria Rosaria Galanti, Jan-Håkan Jansson, Anton Lager, Michael Lundberg, Per-Olof Östergren, Nancy L Pedersen, Ylva Trolle Lagerros, Weimin Ye, Patrik Wennberg, Cecilia Magnusson. Swedish snus use is associated with mortality: a pooled analysis of eight prospective studies. Int J Epidemiol. 2021;49(6):2041-2050.

II Marja Lisa Byhamre, Margareta Norberg, Ulf Näslund, Patrik Wennberg. Snus use, mortality, and socioeconomic factors. In manuscript.

III Marja Lisa Byhamre, Per E Gustafsson, Jan-Håkan Jansson, Maria Wennberg, Anne Hammarström, Patrik Wennberg. Snus use during the life-course and risk of the metabolic syndrome and its components. Scand J Public Health. 2017;45(8):733-740.

IV Marja Lisa Byhamre, Stefan Blankenberg, Per Dahlqvist, Marie Eriksson, Viktor Oskarsson, Stefan Söderberg, Tanja Zeller, Patrik Wennberg. Associations between snus use and concentrations of CRP, 25(OH)D and testosterone – a population-based study. In manuscript.

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Abbreviations

25(OH)D	25-hydroxyvitamin D
AMI	Acute myocardial infarction
BiomarCaRE	Biomarker for Cardiovascular Risk Assessment in Europe
BMI	Body mass index
cfT	Calculated free testosterone
CI	Confidence interval
CV	Cardiovascular
CVD	Cardiovascular disease
CWC	The Construction Workers Cohort (Bygghälsokohorten)
DAG	Directed acyclic graph
DM	Diabetes mellitus
HDL-C	High-density lipoprotein cholesterol
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive protein
ICD	International classification of diseases
LDL-C	Low-density lipoprotein cholesterol
MONICA	The Northern Sweden MOnitoring of Trends and Determinants in CArdiovascular Disease study
MRR	Mortality rate ratio
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	*N′*-nitrosonornicotine

NoSCo	The Northern Swedish Cohort
NRT	Nicotine replacement therapy
OR	Odds ratio
RCT	Randomised controlled trial
RR	Risk ratio; relative risk
SES	Socioeconomic status
Snus Collaboration	The Swedish Collaboration on Health Effects of Snus use
T2D	Type 2 diabetes mellitus
TG	Triglycerides
TSNA	Tobacco-specific nitrosamines
VIP	Västerbotten Intervention Programme

Definitions

Amount of snus use, snus dose: Number of snus boxes consumed per week.

Dip: A portion of snus, either a pinch of loose snus or a portion bag.

Duration of snus use: Number of years of regular snus use.

Ever snus user: Individual who is currently or have previously used snus.

Exclusive snus user: Individual who is currently or have previously used snus and have no history of cigarette smoking.

Smokeless tobacco: All forms of tobacco that are not smoked. Includes tobacco that is chewed, sucked or inhaled as a powder. Does not include vaped tobacco.

Snus: The moist powder of smokeless tobacco developed in Sweden. Sometimes referred to as “snuff”, “Swedish snuff”, or “moist snuff”.

Aims

The overall aim of this thesis was to investigate the health effects of snus use, with a focus on mortality.

Specifically, we aimed:

a) to investigate the association between snus use and all-cause, cardiovascular, cancer, and other cause (non-cardiovascular and non-cancer) mortality;

b) to explore whether the associations between snus use and all-cause, cardiovascular, cancer, and other cause mortality are modified by socioeconomic factors;

c) to study the association between life-course exposure to snus and prevalence of the metabolic syndrome and its components in adulthood, and;

d) To evaluate the associations of snus use with serum biomarkers representing low-grade inflammation, vitamin D-status and testosterone concentrations.

Introduction

What is snus?

Snus is a moist tobacco powder, derived from the leaves of the *Nicotiana tabacum* plant. After the tobacco leaves have dried, they are ground and mixed with water, sodium chloride, sodium carbonate, flavouring agents, and other additives. The mixture is pasteurised through a heating process designed to reduce the number of bacteria. The snus is then stuffed into boxes; either as a loose powder or in portion bags with a cellulose wrapping. The snus boxes are kept refrigerated until sold[1].

Figure 1. Cultivation of tobacco plants[i].

There are multiple varieties of snus with differences in coarseness of grind, strength, moisture levels, and taste. The modern box of loose snus typically holds 42 grams of tobacco, and a box of portion bags between 6 and 24 grams, each portion containing around 0.3—1.1 grams of tobacco.

To use snus, you either form a 'dip' (Swedish "prilla") of loose snus, commonly using your fingers, or take a portion bag and put it between the lip and the gingiva. The snus is kept in the mouth for as long as desired, and replaced according to taste. Time of use averages 13 hours per day[1].

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[i] "Tobacco Plants" by David Hoffman '41 is licensed under CC BY-NC-SA 2.0. To view a copy of this license, visit https://creativecommons.org/licenses/by-nc-sa/2.0.

Figure 2a: Portioned snus[ii], and 2b: Loose snus[iii].

Worldwide, there is an abundance of smokeless tobacco products, some of them similar to snus, others very different. Examples include the American *dipping* and *chewing tobacco*; the Asian *guthka,* where tobacco is mixed with areca nuts; the African *toombak*; the Afghan *naswar;* and the Alaskan *iq'mik*, which combines tobacco with punk ash (derived from a certain fungus)[1]. Snus is most similar to dipping tobacco, but still different in contents and health effects.

History of snus use

Tobacco use in Europe was inspired by Native Americans, and spread to Sweden in the seventeenth century. During this period, it was highly popular among the upper stratum of society to inhale a fine powder of tobacco into one's nose. The so-called "snuff", and its personalized container, was an important marker of status during the time for both men and women, until a rapid decline in its use was initiated by the French revolution. Snuff use was also popular among the Swedish upper classes, while the working class used tobacco for chewing or smoking pipes. To meet the national demand for tobacco, the Swedish king in 1724 decided that tobacco should be grown in-country. The Swedish grown tobacco was not as pleasant as the imported one to chew, which led to experiments with different ways of preparing tobacco and thus the development of snus. Apart from a good taste, it was also cheap and convenient to use during work, as it kept your hands free[1,2].

[ii] Photo by Mostphotos.
[iii] "Lössnus" by Uffe Johansson is licensed under CC BY 3.0. To view a copy of the license, visit https://creativecommons.org/licenses/by/3.0. Additionally licensed under GNU Free Documentation License, https://www.gnu.org/licenses/fdl-1.3.html.

Figure 5a and 5b. Snus dipping then[iv] and now[v].

In the 19th century, there was rapid increase in popularity and production of Swedish snus, accelerated further by improved railway communications and better snus preservation, making delivery to a larger part of Sweden possible. The popularity kept rising until World War One, which led to difficulties obtaining enough tobacco to meet demand. Snus had to be rationed. When the war was over, snus consumption peaked, with 7 000 tonnes of snus consumed in Sweden in 1919. During the Second World War, snus use declined substantially due to the marketing of cigarettes, and reached its all-time low in the 1960's. However, with reports of the harms of smoking and a strong marketing campaign by the Swedish tobacco company (including introduction of portioned snus, more flavours, and rounded boxes), snus again grew in popularity. As more regulations and higher taxes hit the cigarette industry, snus continued to find new users during the late 20th and early 21st century[1,3].

Prevalence of snus use

Today, snus is used mainly in Nordic countries, with the highest number of users in Sweden and Norway. The European Union (of which Sweden is part, but Norway is not) prohibits the sale of snus products in all EU countries. However, Sweden was granted an exemption upon entering the EU in 1995, enabling the sale and marketing of snus within the country. Snus is also available in other EU countries through private

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[iv] Photo by PW Häger, Digitalt Museum, Länsmuseet Gävleborg. Licensed for use under CC BY-NC. View a copy of the license at https://creativecommons.org/licenses/by-nc/4.0/.
[v] Photo by ML Byhamre.

import and illegal sales, and is used regularly by thousands of inhabitants in Finland, Denmark, and Iceland. Transnational tobacco companies have also marketed snus in the United States in recent years[4], which has resulted in quickly increasing prevalence[5].

In Sweden, after the rapid increase in snus use during the 1990's, prevalence decreased at first, but started to increase again in the last decade, especially among women (see Figure 4). In 2021, 23% of Swedish men and 6% of Swedish women used snus daily[6].

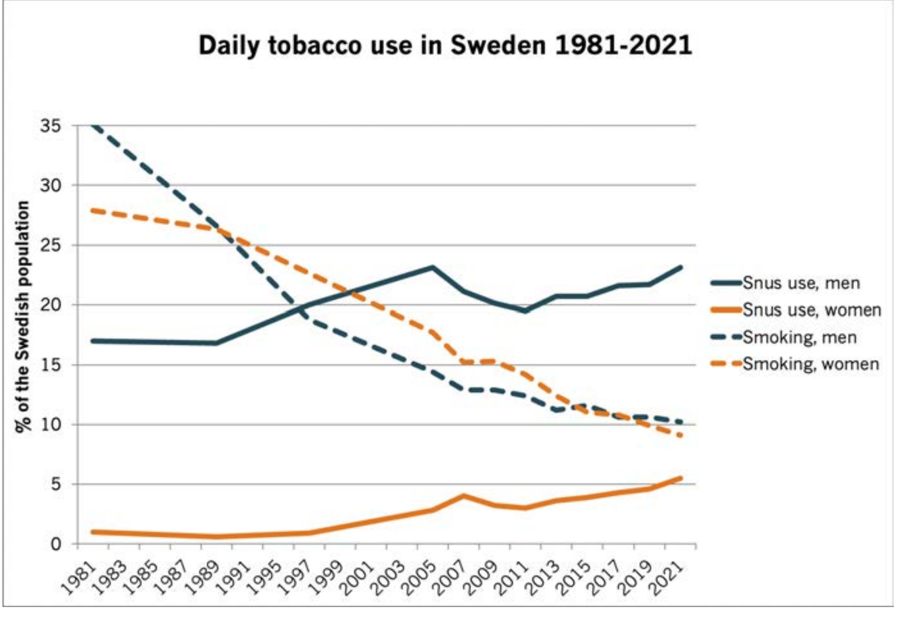

Figure 4. Daily use of cigarettes and snus in Sweden between 1981 and 2021.

Who uses snus?

As time has passed, snus use has shifted away from the sole provision of working-class men, due in large to marketing efforts. It now includes all groups of society, and both sexes. Use of snus increased the most among those with higher education in the 1980's and 1990's, but despite this, reports from 2010 show that snus use, still, is most common among men with less favourable socioeconomic situation[7]. Interestingly, among women—who constitute a relatively new group of users—the connections to socioeconomic disadvantage are much weaker than they are for men. This was demonstrated in a study from 2011[8], where snus use among men was associated with low education, low income and rural living, but among women it was only associated with being single.

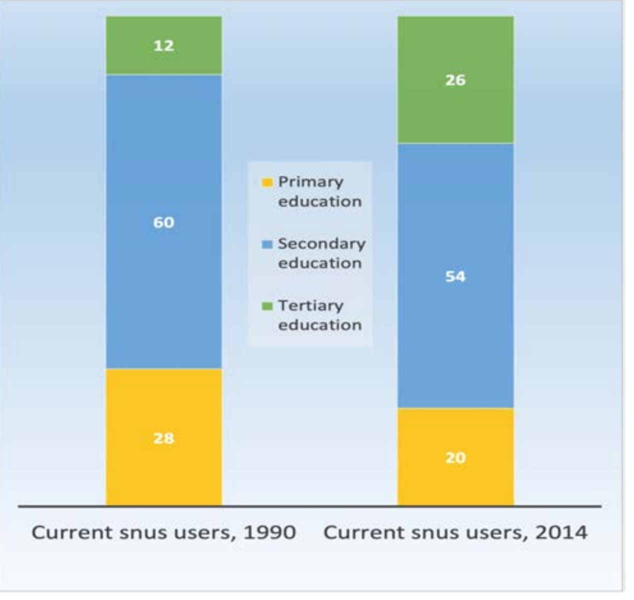

Figure 7. Educational level among daily snus users in 1990 and 2014. Primary education: did not complete high school. Secondary education: completed high school. Tertiary education: college or university degree. Data from MONICA surveys[9].

Snus in sports

The use of snus has become a popular habit in certain sports[3]. In Sweden, ice hockey is the most predominant example, where the sporting environment and norms incorporate snus use as a way of living. Sports practice thus tends to foster foremost young boys into snus use[10,11]. The same pattern is seen in Norway and Finland connected to certain team sports, and in the US among baseball players[12–14]. There is a belief among athletes that snus use might enhance performance[15], and it is also used to relieve anxiety, improve focus, and as a means of weight control[16]. In addition, there is a practical aspect—while smoking takes up time, snus can be used even while sporting.

However, studies reveal that snus use does not increase performance, but rather decreases it due to higher mental fatigue and reduced heart rate variability in subjects exposed to snus versus placebo[17]. It has also been shown that nicotine administration leads to earlier lactate accumulation[18] and reduction of muscle strength[19].

Contents in snus

Snus is made of 40–45% tobacco and 50% water. Sodium carbonate (1.2–2.5%) is added to adjust the pH. Other important ingredients are sodium chloride (1.6–3.3%), humidifiers (1.5–3.5%) and aromas (<1.0%). There are thousands of chemical substances in snus that either originate from the tobacco or other ingredients, or arise during the preparation processes. These substances vary due to place of cultivation, subspecies of tobacco plant, use of fertilisers and pesticides, and ways of curing and otherwise processing the tobacco[31].

Figure 3a. Traditional oval snus box, and 3b. Dog-shaped snus box[vi].

Physiological effects by snus

Three main groups of biologically-active constituents can be identified, namely nicotine, carcinogens, and microbiota.

Nicotine

The nicotine in snus is absorbed into the bloodstream through the oral mucosa. The absorption rate and amount depend on nicotine content, tobacco cut, pH and moisture of the product—higher nicotine levels, smaller cut, alkaloid pH, and higher moisture content all lead to higher absorption. The tobacco cut in snus is generally very fine, although varying. Nicotine content in Swedish products ranges from 0.5–4.5%, and pH and moisture also differ substantially between different snus products. However, individual factors like oral pH and saliva production as well as style of use are also important factors for absorption[20,21,2,22].

[vi] Both photos from Digitalt Museum, Snus- och Tändsticksmusem, licenced under CC BY 4.0. To view a copy of the license, visit https://creativecommons.org/licenses/by/4.0/.

Stimulation of the sympathetic nervous system. Nicotine mediates its effects on the human body through nicotinic cholinergic receptors. These are present in the brain, in autonomic ganglia, in the adrenals and in neuromuscular junctions. Here, it works primarily by stimulating the sympathetic nervous system, including the release of catecholamines[23].

Nicotine exposure cause an immediate rise in heart rate of 10–15 beats per minute, increased blood pressure of 5–10 mmHg, and increased myocardial contractility. This results in a higher cardiac output and demand for oxygen and nutrients. At the same time, nicotine causes constriction of blood flow in vascular beds, including the heart, skin, and kidneys, while blood vessels in, e.g., skeletal muscle are dilated. Hence, we get a lower supply of oxygen and nutrients to the heart. This has been demonstrated in studies on animals and humans after administration of nicotine[23], and upon snus use[24,25]. Catecholamine release also stimulate lipolysis, causing a release of free fatty acids in blood[23].

Studies show that the nicotinic effects from a dip of snus are sustained for a longer period than after smoking a cigarette[26]. However, the sympathetic effects are reversed some time after the snus is removed, and increased blood pressure and pulse during abstinence has not been proven[24]. Still, it should be remembered that given its highly addictive nature, snus is commonly used during a large part of the day, and sometimes even at night, creating prolonged periods of sympathetic activation in the user.

Another interesting phenomenon is the adverse effects caused by snus use during pregnancy. Snus-using mothers-to-be have been shown to suffer increased risk of stillbirth, preterm delivery, of having a baby that is small for gestational age, and—contradictory to the effect of smoking—snus users have a higher risk of preeclampsia[27]. These complications are caused by impaired placental circulation, suggesting that snus may influence blood vessel regulation. The effects of snus use also seem to influence cardiovascular health in utero, as children who were exposed to snus during pregnancy have higher systolic blood pressure and altered heart rate variability[28].

Other biochemical mechanisms. Apart from catecholamines, several other neurotransmitters are released upon nicotine stimulation. These include dopamine, acetylcholine, serotonin, vasopressin, nitric monoxide, and beta-endorphins, and are responsible for many of the psychological effects of nicotine: alertness, pleasure, and reduction of anxiety and stress—positive symptoms that are key factors in the development of nicotine dependence[23].

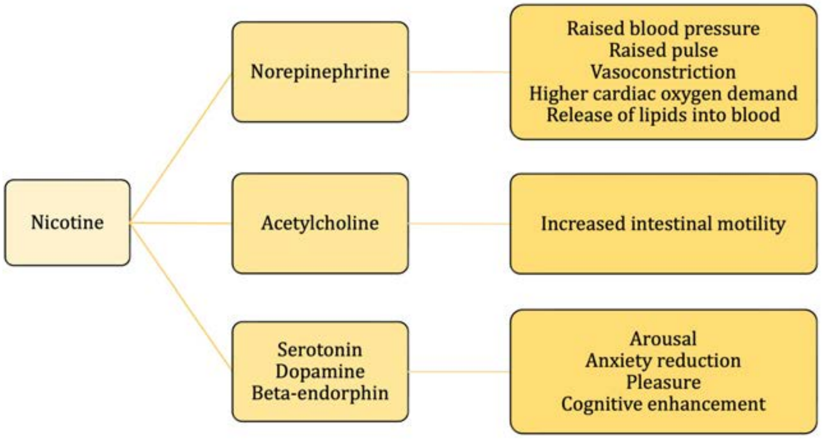

Figure 8. Summary of major physiological effects of nicotine in humans.

Withdrawal and addiction. When nicotine levels in the body decrease, withdrawal symptoms arise; for example cravings, restlessness, anxiety, increased hunger, and difficulties focusing. These symptoms are then relieved upon renewed exposed to nicotine[1,29].

Addiction is created and sustained by a combination of the experience of the positive symptoms and the desire to avoid withdrawal symptoms, together with psychological factors like cues from surroundings and conditioning. The addictiveness of tobacco is associated with nicotine absorption rates, and comparable with that of other abused substances like alcohol and cocaine[30]. How much nicotine exposure is needed to become addicted depends on the properties of the nicotine product, and on individual and environmental factors, but addiction may arise even after a single dose[23,31].

Nicotine receptors become less sensitive after some time of nicotine exposure; a phenomenon known as adaptation[26]. This means that to achieve the same desired effects, the user must increase the dose.

Nicotine and behaviour. Nicotine provides stimulation and pleasure without impairing cognition or acutely affecting behaviour. However, there are indications that use of nicotine products increase the probability of other substance abuse, including smoking, alcohol, and drugs[32–34]. Snus use has also been associated with increased risk-taking behaviour among both Nordic and North American youth[32,33].

Carcinogens

At least 28 different known carcinogens have been identified in snus. The most important are the tobacco-specific nitrosamines (TSNA), specifically *N′*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which are classified as highly carcinogenic to humans[1]. Low concentrations of TSNA are formed from nicotine and other alkaloids in the tobacco plant during growth, but the large production occurs during the curing and preparation processes. The levels of TSNA are therefore dependent on both the type of tobacco and how the tobacco is handled[35]. Due to preparation practices, the levels of TSNA are lower in snus than in many other forms of smokeless tobacco.

Other carcinogens found in snus are N-nitrosoamino acids, volatile N-nitrosamines, volatile aldehydes like formaldehyde, polycyclic aromatic hydrocarbons, heavy metals like nickel, lead, cadmium and arsenic, and radioactive compounds including polonium-210 which decays to radon. Several of these carcinogenic substances originate from air pollution, irrigation, fertilizers, and pesticides that the tobacco plants are exposed to during cultivation. They therefore differ between tobacco sources[1,21,29].

Microbiota

More recent studies have focused on the microbiotic contents of tobacco products, and found a number of bacterial species in snus, including Proteobacteria, Firmicutes and Actinobacteria[36]. The fungal contents of snus have not been investigated, but have been ascertained in other smokeless tobacco products[37]. Although the identified microorganisms include ones that are known pathogens to humans, their implications for oral and systemic health among users is still uncertain[36,37], and represent an interesting focus for future studies.

Previous research on snus and health outcomes

All-cause mortality

All-cause mortality is defined as death by any cause. It can be seen as a way of expressing overall detrimental health consequences for a given exposure. The main causes of death in Sweden are illustrated in Figure 9. They are also representative of other developed countries[38].

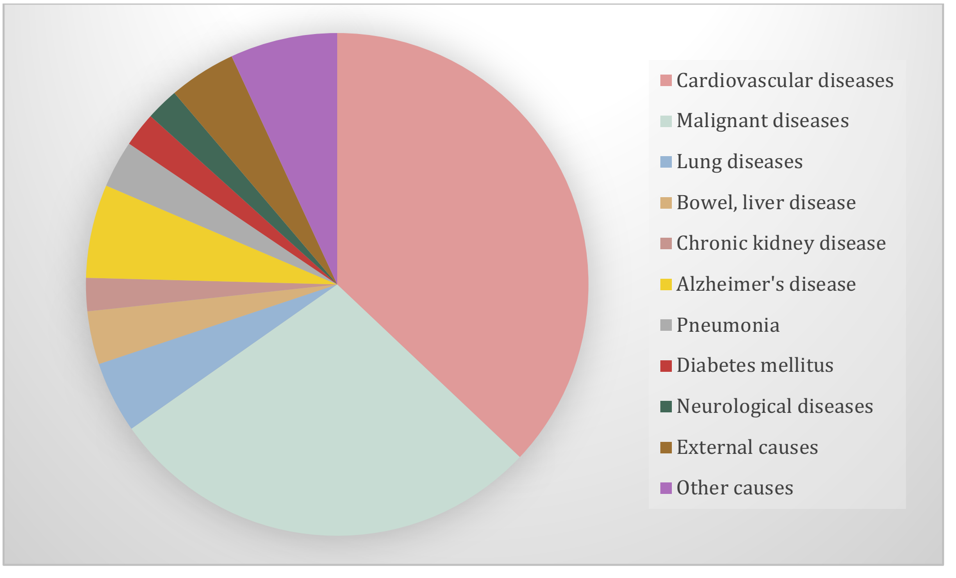

Figure 9. Causes of death in Sweden 2019[38].

Three previous studies performed on two different cohorts have evaluated the relationship between snus use and all-cause mortality[39–41]. The study by Bolinder et al., performed on the Construction Workers Cohort (CWC) in 1994, revealed a 40% increase in overall mortality (relative risk (RR) 1.4, 95% confidence interval (CI) 1.3; 1.8) among current snus users[41]. Within the same cohort, Nordenvall et al. (2013) also found an increased overall mortality among snus users (hazard ratio (HR) 1.13, 95% CI 1.05; 1.20)[39]. The comparison was made between never-users of tobacco and a combined category of current and former snus users, hereafter referred to as “ever snus users”. In 2008, Roosaar et al. found the risk of all-cause mortality for ever snus users to be 23% (HR 1.23, 95% CI 1.09; 1.40) in a cancer cohort[40].

Cardiovascular mortality and morbidity

Cardiovascular diseases (CVDs) include conditions that affect our heart and circulatory system, for example angina pectoris, arrhythmias, myocardial infarction, heart failure, and stroke. CVDs are the leading cause of death in developed countries, including Sweden, causing around one third of deaths every year[38].

Total cardiovascular mortality among snus users have been explored in two previous studies. In 1994, Bolinder et al. reported an excess overall mortality of 40% (RR 1.4, 95% CI 1.2; 1.6) in snus users who had never smoked from a 12-year follow up of the CWC[41], while Roosaar et al. (2008) found a non-significant risk increase of 15% (HR 1.15, 95% CI 0.97; 1.37) for ever snus users[40].

Several studies on snus use and incidence of major cardiovascular outcomes have been performed, although many of them were small-sized and included smokers. Most of these studies found no association between snus use and myocardial infarction[42–52] or stroke[53,44–47,54], the latter with two exceptions[42,44]. Two studies found no increased risk of atrial fibrillation among snus users[42,55], but one study demonstrated that snus users have an increased risk of heart failure[56].

Interestingly, although seemingly not increased in incidence, an increased risk of dying from heart attack[57,58] or total cardiovascular disease[41] has been found in three studies, while that risk did not appear in another five studies[47–49,51] (although one of these showed increased fatality with borderline significance[43]). In analogy with myocardial infarction, snus use has also been associated with increased stroke fatality in two studies[42,46], while the risk was borderline significant in another[53] (Table 1a). Two studies show non-increase of stroke fatality[41,47], but one of these was based on only four cases[47].

Apart from these individual observational studies, recent meta-analyses on snus use and cardiovascular mortality (which included the aforementioned studies) also confirmed that the evidence collected thus far shows increased mortality, but not incidence, in cardiovascular diseases (Table 1b). The risk ratio of coronary heart disease was 0.96 (95% CI 0.86–1.06) among snus users, while it was 1.37 (95% CI 1.14; 1.61) for fatal coronary heart disease[59]. The risk of suffering a stroke was 1.04 (95% CI 0.94; 1.15), and for fatal strokes 1.30 (95% CI 0.96; 1.63)[60].

Table 1a. Overview of studies on cardiovascular mortality.

Author	Year	Type of study	**Participants** (*n* total or *n* cases + *n* controls)	Outcome	**Risk estimate** (95% confidence interval)
Huhtasaari et al.[51]	1999	Case-control	687 + 687 (m)	Fatal AMI	OR 1.50 (0.45; 5.03)
Hergens et al.[49]	2005	Case-control	1 432 + 1 810 (m)	Fatal AMI	OR 1.7 (0.48; 5.5)
Haglund et al.[47]	2007	Cohort	5 002 (m)	Fatal AMI	MRR 1.15 (0.54; 2.41)
Hergens et al.[58]	2007	Cohort	118 395 (m)	**Fatal AMI**	**RR 1.32 (1.08; 1.61)**
Wennberg et al.[48]	2007	Case-control	525 + 1 798 (m)	Fatal AMI	OR 1.18 (0.38; 3.70)
Hansson et al.[43]	2012	Cohort	130 361 (m)	Fatal AMI	OR 1.28, (0.99; 1.68)
Arefalk et al.[57]	2014	Cohort	2 474 (m, w)	**AMI mortality**	**HR 0.51 (0.29; 0.91)**
Bolinder et al.[41]	1994	Cohort	84 781 (m)	Fatal stroke	Younger: RR 1.90 (0.60; 5.70) Older: RR 1.20 (0.70; 1.80)
Haglund et al.[47]	2007	Cohort	5 002 (m)	Fatal stroke	MRR 1.01 (0.35; 2.92)
Hergens et al.[46]	2008	Cohort	118 465 (m)	**Fatal stroke**	**Fatal ischemic stroke: RR 1.72 (1.06; 2.78)** Fatal haemorrhagic stroke: Not significant
Hansson et al.[53]	2014	Cohort	130 485 (m)	Fatal stroke	OR 1.42 (0.99; 2.04)
Titova et al.[42]	2021	Cohort	41 162 (m, w)	**Fatal stroke**	**Total stroke: HR 1.52 (1.01; 2.30)** **Ischemic stroke: HR 1.63 (1.05; 2.54)**
Bolinder et al.[41]	1994	Cohort	84 781 (m)	**CV mortality**	**RR 1.4 (1.2; 1.6)**
Roosaar et al.[40]	2008	Cohort	9 976 (m)	CV mortality	HR 1.15 (0.97; 1.37)
Titova et al.[42]	2021	Cohort	41 162 (m, w)	CV mortality	HR 1.57 (0.93; 2.64)

AMI = acute myocardial infarction, CV = cardiovascular, HR = hazard ratio, M = men, MRR = mortality rate ratio, OR = odds ratio, RR = relative risk or risk ratio, W = women. Significant results marked in **bold**. The same study may appear on several lines if it examined multiple outcomes.

Table 1b. Meta-analyses on cardiovascular mortality.

First author	Year	Outcome	Risk estimate (95% CI)
Gupta et al.[59]	2019	**Fatal coronary heart disease**	**RR 1.37 (1.14; 1.61)**
Gupta et al.[60]	2020	Fatal stroke	OR 1.30 (0.96; 1.63)

CI = confidence interval, OR = odds ratio, RR = risk ratio.

Cardiovascular risk factors

Established risk factors for cardiovascular disease and death include non-modifiable determinants such as age, sex and genetic predisposition, and modifiable risk factors. Some of the most important of the latter are hypertension, obesity, diabetes mellitus type 2 (T2D), abnormal blood lipids (increased triglycerides, total cholesterol and low-density lipoprotein cholesterol (LDL-C); decreased high-density lipoprotein cholesterol (HDL-C)), smoking, unhealthy diet, and low physical activity level[61].

The metabolic syndrome is a concept stating that a combination of several of these risk factors may interact and together cause even higher cardiovascular risk. It is defined by the International Diabetes Federation[62] as:
(a) central obesity (waist circumference ≥80 cm for women and ≥94 cm for men), and
(b) at least two of the following:
(i) low serum HDL-C (<1.29 mmol/L for women and <1.03 mmol/L for men), or specific therapy for that lipid abnormality,
(ii) high serum triglycerides (TG) (≥1.7 mmol/L), or specific treatment for that lipid abnormality,
(iii) high blood pressure (≥130 mm Hg systolic and/or ≥85 mm Hg diastolic) or antihypertensive medication, and
(iv) raised fasting glucose levels (≥5.6 mmol/L) or diagnosed T2D.

Which of the established cardiovascular risk factors that are most important depend on the setting and the number of risk factors in an individual[63]. On a population level, high waist-to-hip ratio and abnormal blood lipids are the most important risk factors for myocardial infarction[64], while hypertension and high waist-to-hip ratio are the major determinants of stroke risk[65].

There have been several studies on different risk factors for cardiovascular disease evaluating a possible impact by snus use (Table 2). The results speak in favour of increased risk of overweight/obesity and T2D among snus users, while the other evaluated risk factors have inconclusive overall results (hypertension, metabolic syndrome, impaired glucose regulation, abnormal lipid profile). The effects of snus on different aspects of thrombosis formation and the atherosclerotic process have also been investigated as potential mechanisms for raised disease risk. The studies on pro-thrombotic factors such as thrombocyte aggregation[66,67] and fibrinogen levels[66,68] found non-increased levels among snus users, with one exception[69]. Nicotine causes endothelial dysfunction[70–72], the first step in the atherosclerotic process, and a study from 2004 identified endothelial dysfunction among snus users[25]. However, two other studies could not demonstrate increased atherosclerosis among snus users[68,73]. It should be noted that several of the above-mentioned studies are small or have methodological issues, limiting possible conclusions.

Possible mechanisms

Potential mechanisms behind increased cardiovascular mortality among snus users may include the catecholamine release causing acute rise in blood pressure, heart rate, vasoconstriction and higher cardiac oxygen demand. Animal studies have shown that nicotine may induce cardiac arrhythmia, including ventricular fibrillation[74–76]. While atrial fibrillation is not increased among snus users[42,55], no studies have examined life-threatening arrhythmias such as ventricular fibrillation, which are common causes of death related to myocardial infarction[77].

Table 2. Overview of previous research on cardiovascular risk factors and snus use.

First author, year	Obesity	Hyper-tension	T2D	Other impairment of glucose metabolism	Abnormal blood lipids	Other
Eliasson 1991[69]		↔		↑ Insulin levels	↔ Tot cholesterol, TG	↑ Fibrinogen levels*
Wennmalm 1991[67]		↔				↔ Thromboxane A2*
Bolinder 1992[78]		↑				
Hirsch 1992[79]		↔				
Attvall 1993[80]				↔		
Bolinder 1994[41]		↑				
Eliasson 1995[66]				↔ Insulin levels, glucose tolerance		↔ Fibrinogen levels*, tPA*, PAI-1*
Bolinder 1997[68]					↔ Serum lipids	↔ Carotid intima media thickness° ↔ Fibrinogen*
Persson 2000[81]			↑	↓ Insulin production		
Wallenfeldt 2001[73]	↑	↔		↔	↑ TG	↔ Subclinical atherosclerosis°
Eliasson 2004[82]			↔	↓ Glucose tolerance		
Rodu 2004[83]	↑					
Rohani 2004[25]						↓ Arterial elasticity°
Hergens 2005[49]	↑	↑				
Norberg 2006[84]	↑	↔		↔	↑ TG ↔ HDL-C	↑ Metabolic syndrome
Nafziger 2007[85]	↑					
Angman 2008[86]		↔				
Hergens 2008[46]		↑				

Wändell 2008[87]			↔			↔ Metabolic syndrome
Janzon 2009[45]	↑	↔				
Sundbeck 2009[88]	↔					
Engström 2010[7]	↔					
Hansson 2011[89]	↑					
Östenson 2012[90]			↑			
Øverland 2013[91]	↑	↑		↔ Non-fasting glucose	↑ HDL-C ↔ TG	
Varga 2013[92]	↑					
Skaug 2016[93]						↓ Flow-mediated dilation°
Carlsson 2017[94]			↑			
Rasouli 2017[95]			↔			
Antoniewicz 2018[96]		↑ in women				↔ Arterial stiffness°
Carlsson 2019[97]			↑			
Söderström 2021[98]						↔ Homocysteine concentrations
Antoniewicz 2022[99]						↑ Arterial stiffness° ↓ Endothelial vasodilation°
Byhamre 2022[100]					↔ TG, non-HDL-C ↑ HDL-C	
Edstorp 2022[101]				↑ LADA, T2D		

HDL-C = high-density lipoprotein cholesterol, LADA = latent autoimmune diabetes in adults, T2D = diabetes mellitus type 2, TG = triglycerides.
↑ = statistically significant increased risk compared to reference category; ↓ = significantly decreased risk; ↔ = no significant difference.
* Indicates increased risk of thrombosis formation.
° Pre-atherosclerotic changes.

Cancer mortality and morbidity

Cancer is the second most common cause of death in Sweden, but the evidence on snus use and cancer mortality is inconclusive (Table 3). One large study found cancer to be associated with snus use[39], while another did not[41]. In 2008, Roosaar et al. found an increased risk of cancer deaths among ever snus users (HR 1.28, 95% CI 0.96; 1.69)[40]. The result was non-significant, but likely diluted as it encompassed both current and former users, of which the latter would reasonably have a lower risk. Regarding mortality in specific malignant diseases, one study showed an increased fatality rate from oropharyngeal cancers, but this increased risk was non-significant, and based only on five cases (odds ratio (OR) 2.3, 95% CI 0.7; 8.3)[40]. Another study found increased mortality in prostate cancer for snus users (HR 1.24, 95% CI 1.03; 1.49)[102]. Mortality in colorectal cancer was not increased in a study performed on the Snus Collaboration[103].

Table 3. Studies on cancer mortality among snus users.

First author	**Year**	**Outcome**	**Risk estimate** (95% CI)	**Participants** (all men)	**Type of study** (all Swedish)
Bolinder[41]	1994	Overall cancer mortality	RR 1.1 (0.9; 1.4)	135 036	Cohort
Roosaar[40]	2008	Overall cancer mortality	HR 1.28 (0.96; 1.69)	9 976	Cohort
Nordenvall[39]	2013	**Overall cancer mortality**	**HR 1.15 (1.05; 1.26)**	336 381	Cohort
Roosaar[40]	2008	Oropharyngeal cancer mortality	OR 2.3 (0.7; 8.3)	9 976	Cohort
Wilson[102]	2016	**Prostate cancer mortality**	**HR 1.24 (1.03; 1.49)**	9 582	Cohort
Araghi[103]	2017	Colorectal cancer mortality	HR 1.00 (0.66; 1.53)	417 872	Pooled cohort

CI = confidence interval, HR = hazard ratio, OR = odds ratio, RR = risk ratio. Significant results indicated in bold.

Studies on incidence of cancer among snus users are summarised in Table 4. Despite the number of studies, overall conclusions are difficult to draw due to methodological issues such as low power, inclusion of smokers, or analysing current and former snus users together. The results so far, however, suggest that while oral, oropharyngeal[40,104–109] and gastric cancers[110–112,108,113] are not associated with snus use, oesophageal[111,108,113], pancreatic [106,108,114] and rectal cancers[115,103] may be.

Risk factors for malignant diseases

Risk factors for cancer include genetic factors, exposure to carcinogens such as radiation, arsenic, and silica, and infection by certain viruses and bacteria. Modifiable risk factors include smoking, sun exposure, dietary factors, alcohol drinking and obesity, and are estimated to account for one third of all cancer cases[118].

In recent years, it has been discovered that many cardiovascular risk factors are also fact risk factors for certain cancers. This can be traced back to changes at the molecular level, where inflammatory response has been described as a bridge between atherosclerotic and malignant processes, as suggested in the 'common soil hypothesis'[119]. We therefore regard cardiovascular risk factors to also be risk factors for cancer.

Snus thus has the potential to cause cancer through both direct carcinogenic contents and possibly by nicotinic effects leading to increased risk of common risk factors such as diabetes mellitus, obesity, and metabolic syndrome.

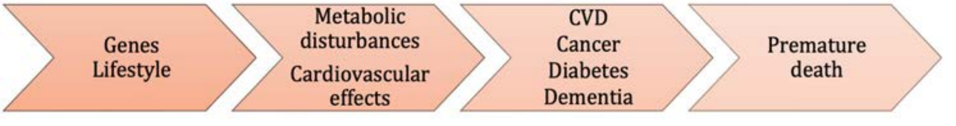

Figure 10. Common soil hypothesis.

Possible mechanisms

Carcinogens in snus, such as the nitrosamines NNN and NNK, heavy metals, and polycyclic aromatic hydrocarbons, may cause mutations in different regulatory genes, enabling unregulated proliferation, and induce local inflammation and oxidative stress that promotes tumour growth. They have also been shown to interact with antitumor treatments; for example by stimulating angiogenesis[120,121]. These mechanisms could be plausible reasons behind raised cancer incidence and mortality among snus users.

Furthermore, certain TSNAs, present in smokeless tobacco, can cause epigenetic changes[35,122]. These are modifications that do not alter the DNA itself, but affect how DNA is expressed. Some epigenetic changes are permanent and can be inherited. As gene expression is altered, diseases may occur, including different cancers[35,123].

Table 4. Studies on cancer incidence among snus users.

Author	Year	Cancer type	Result	Risk estimate (95% CI)	Participants *n* in cohort, or *n* of cases + *n* of controls	Type of study, country
Schildt et al.[104]	1998	Oral*		OR 0.7 (0.4; 1.2)	410 + 410	Case-control, Swe
Lewin et al.[105]	1998	Head and neck*		RR 3.3 (0.8; 12.0)	605 + 756	Case-control, Swe
Luo et al.[106]	2007	Oral*		RR 0.9 (0.4; 1.8)	279 897	Cohort, Swe
Asthana et al.[109]	2019	Oral		OR 0.86 (0.58; 1.29)	See individual studies	Meta-analysis
Rosenquist et al.[107]	2005	Oropharyngeal		OR 1.1 (0.5; 2.5)	132 + 320	Case-control, Swe
Bofetta et al.[108]	2005	Oropharyngeal		RR 1.13 (0.45; 2.83)	10 136	Cohort, No
Roosaar et al.[40]	2008	Oropharyngeal		HR 2.3 (0.7; 8.3)	9 976	Cohort, Swe
Lagergren et al.[111]	2000	Oesophageal		SCC: OR 1.4 (0.9; 2.3) AC: OR 1.2 (0.7; 2.0)	618 + 820	Case-control, Swe
Boffetta et al.[108]	2005	Oesophageal		RR 1.06 (0.35; 3.23)	10 136	Cohort, No
Zendehdel et al.[113]	2008	**Oesophageal**	↑	**SCC: RR 3.5 (1.6; 7.6)** AC: RR 0.2 (0.0; 1.9)	336 381	Cohort, Swe
Hansson et al.[110]	1994	Gastric		OR 0.70 (0.47; 1.06)	338 + 679	Case-control, Swe
Ye et al.[112]	1999	Gastric		Cardia: OR 0.5 (0.2; 1.1) Distal stomach: OR 0.6 (0.3; 1.2)	514 + 1 164	Case-control, Swe
Lagergren et al.[111]	2000	Gastric		OR 1.2 (0.8; 1.8)	618 + 820	Case-control, Swe
Boffetta et al.[108]	2005	Gastric		RR 1.00 (0.71; 1.42)	10 136	Cohort, No
Zendehdel et al.[113]	2008	**Gastric**	↑	Cardia: RR 0.9 (0.4; 2.0) **Non-cardia: RR 1.4 (1.1; 1.9)**	336 381	Cohort, Swe
Boffetta et al.[108]	2005	**Pancreatic**	↑	**RR 1.60 (1.00; 2.55)**	10 136	Cohort, No
Luo et al.[106]	2007	**Pancreatic**	↑	**RR 2.1 (1.2; 3.6)**	279 897	Cohort, Swe

Araghi et al.[114]	2017	Pancreatic		HR 0.96 (0.83; 1.11)	424 152	Pooled cohort, Swe
Nordenvall et al.[115]	2011	Colorectal		Colon: HR 1.08 (0.91; 1.29) Rectum: HR 1.05 (0.85; 1.31)	336 381	Cohort, Swe
Araghi et al.[103]	2017	Colorectal	↑	Colon: HR 1.02 (0.81; 1.29) **Rectum: HR 1.38 (1.07; 1.77)**	417 872	Pooled cohort, Swe
Boffetta et al.[108]	2005	**Kidney**	↓	**RR 0.47 (0.23; 0.94)**	10 136	Cohort, No
Boffetta et al.[108]	2005	Bladder		RR 0.72 (0.52; 1.06)	10 136	Cohort, No
Boffetta et al.[108]	2005	Lung		RR 0.80 (0.58; 1.11)	10 136	Cohort, No
Luo et al.[106]	2007	Lung		RR 0.8 (0.4; 1.3)	279 897	Cohort, Swe
Odenbro et al.[116]	2005	**Skin**	↓	**IRR 0.64 (0.44; 0.95)**	337 311	Cohort, Swe
Fernberg et al.[117]	2007	Haematological		ALL: IRR 1.24 (0.39; 4.01) AML: IRR 0.81 (0.41; 1.60) CML: IRR 1.17 (0.60; 2.28) MM: 0.92 (0.61; 1.40)	330 000	Cohort, Swe

CI = confidence interval, No = Norway, Swe = Sweden, AC = Adenocarcinoma, ALL = Acute lymphocytic leukaemia, AML = Acute myelogenous leukaemia, CML = Chronic myelogenous leukaemia, MM = Multiple myeloma, SCC = Squamous cell carcinoma.
Significant results indicated in **bold** and with arrow marking the direction of the association. The same study may appear on several lines if it investigated more than one type of cancer.

* = included in meta-analysis by Asthana et al.

Snus-induced oral lesions

Snus users develop specific oral lesions at the site where the dip is placed[124]. The exact pathology behind the lesions is not fully known, but involves local inflammatory responses[125]. Lesions are classified on a four-grade scale, where grade I lesions include wrinkling of the mucus but no colour change or thickening, while grade IV exhibit severe colour changes, furrows and heavy thickening of the mucosa[125]. The degree of severity is strongly associated with the amount of snus used and appears more pronounced among users of loose snus than portioned snus[126]. They are not related to smoking or alcohol use[124]. Snus-induced lesions are reversible following snus cessation, but some users develop dysplasia and there has been an interest in snus lesions as a potential pre-malignant condition[127]. However, the studies on this subject have not proven any association between snus use and increased risk of oral cancer[124].

Emerging risk factors

New risk factors for non-communicable diseases have been discovered during the last few decades. Low-grade inflammation has turned out to be a crucial part of the pathology behind a vast spectrum of conditions, such as malignancies, cardiovascular diseases, dementia, and obesity. An association with snus use could be a part of the mechanism linking snus use to death. A common way of evaluating low-grade inflammation is to measure hs-CRP (high-sensitivity C-reactive Protein) in serum[128]. There is one previous study on CRP-concentrations and snus use that shows no associations between the two[73]. However, smoking has been linked to increased CRP levels, constituting a pathway to higher risk of CVD and cancer among smokers[129].

Another emerging risk factor is low vitamin D-concentration. Vitamin D is well-known for its impact on musculoskeletal health, but more recent research has found that low levels of vitamin D are also associated with CVDs, cancer, and increased overall mortality[130–132]. There is no previous research in this area with regard to snus, but an association between smoking and lower vitamin D-levels is evident[133]. Vitamin D-status is commonly evaluated using 25-hydroxyvitamin D (25(OH)D)-concentrations in blood[133].

Testosterone also has many roles in the human body, in both men and women. Both too-high and too-low levels of this hormone may have negative health consequences on cardiovascular health in a manner that is not fully understood[134]. Previous research has shown an association between smoking and raised testosterone levels[135]. Also, a study on smokeless tobacco (but not snus in particular) has shown an association with increased testosterone levels[136]. The levels of bioavailable, unbound testosterone determine its effects on the body, therefore calculated free testosterone (cfT) is commonly used for evaluation of testosterone concentrations.

The tobacco industry

The tobacco industry producing Swedish snus has a long trading history and a turnover of millions of Swedish crowns each year (for example, Swedish Match reported a turnover of 18 489 000 Swedish crowns in 2021[137]). Products are continuously marketed and developed to fit a wider audience. Tobacco habits are generally established in youth and early adult life; therefore marketing efforts are strongest for these groups. Examples include providing free samples of snus at concerts and online, and collaborations with social media influencers. In the US, marketing efforts have specifically targeted smokers in situations where smoking is prohibited[138].

Examples of successful product development include nicotine pouches (also called white snus). They are tobacco-free, but high in nicotine content; sometimes even higher than regular snus. As they are not a tobacco product, they have escaped Swedish legislation and was for example allowed for sale to minors up until August 2022 (although traders agreed on a voluntary age limit of 18 years). Other examples are colourful boxes, ever new flavourings and “do it yourself”-kits for making your own snus, attractive to new categories of users.

In addition to marketing, the tobacco industry also works politically and legally, trying to relieve taxes and restrictions, opposing new regulations and court verdicts, and through collaborations with researchers working to highlight either health positive or null-findings about snus, and arguing against studies showing negative health effects[139,140]. The tobacco companies have also been active in the debate on harm reduction of smoking, by promoting snus as a healthier form of tobacco. In these ways, the tobacco industry is trying to influence the views of policymakers, public health workers, and the general public regarding the health aspects of snus use.

Why is evidence so sparse?

Despite the high prevalence and long history of snus use in Sweden, the well-known negative health consequences of other nicotine products, and the considerable number of studies that have been performed on snus use and health, the scientific evidence in this field is considerably weaker than one might expect. This lack of evidence is caused by several factors.

Snus only

First, research possibilities are limited to snus, as this product differs substantially from other types of smokeless tobacco[1]. This means that until new cohorts on snus use and health effects can be initiated in countries with recent increase in snus use, studies can only be performed in Swedish and Norwegian populations. Secondly, studies are limited to men. As the number of non-smoking female snus users in both countries has historically been very low, investigating health outcomes among snus-using women separately is difficult— they are simply too few women using snus. It is, however, important to identify potential differences in risk between the sexes[141].

Only snus

When undertaking research on the effects of snus use it is important to avoid residual effects from current and previous smoking, as these represent another common form of tobacco exposure, and the effects of smoking and snus use may therefore overlap. Another risk of including smokers is the possibility of interactions caused by dual use of snus and cigarettes[142,143]. Therefore, we need to examine never-smoking snus users (hereafter referred to as “exclusive snus users”) and compare them to people that have never used any tobacco product regularly. This is another reason for evidence limitations, because many snus users are, in fact, current or previous smokers[8], leaving few to be included in analyses. Also, as earlier snus research has often included smokers, the conclusions we may draw from this research are limited.

Use, not user

Low socioeconomic status (SES) is associated to adverse health outcomes including increased mortality[144]. The socioeconomic differences between snus users and non-users therefore risk to confound the results[8], and require careful consideration. There may also be differences in health-related behaviour among snus users and non-users that must be accounted for. In short, there are a several factors to take into consideration to be able to conduct a high-quality study where one can measure the effect of snus use, instead of the effect of being a snus user.

Knowledge gaps

Evidently, large population-based studies on exclusive snus users are needed so that we can reliably evaluate the potential health effects by snus use. Neither all-cause nor cause-specific mortality among snus users has been thoroughly investigated, nor has the possible mechanisms underlying such potential associations. Considering the large and increasing number of daily snus users, the health consequences of snus use are of vast importance, both from the individual and a public health perspective.

Material and methods

Overview of methods

This thesis includes four original studies based on prospectively collected data from different Swedish cohorts. An overview is given in Table 5.

Cohort settings and participants

Snus Collaboration—Study I

The Swedish Collaboration on Health Effects of Snus use (Snus Collaboration) was established to clarify the impact of snus use on health across time and geographical regions, with adequate statistical power and control for confounding factors[43]. It includes pooled individual data from nine Swedish prospective studies, of which eight were included in Study I[145–148,58,149,9,150]. The dataset comprises several major potential confounders, for example body mass index and educational level, and is linked to the National Cause of Death Register[151]. Due to few female snus users at the time of data collection, the database includes only men.

Data was collected at baseline through questionnaires or interviews, and health measurements and blood sampling were performed by medical personnel. The principal investigator of each cohort provided individual participant data, which was then harmonised centrally in the database. Further details on the included cohorts can be found in Table 6 and in previously published work[145–148,58,149,9,150].

The eight cohorts comprised 383 015 participants. To minimise residual effects of cigarette use, we excluded all participants with a history of smoking. We also excluded individuals under the age of 18, and those with incomplete data, leaving 169 103 men for inclusion in our first study.

Analyses on non-participants have been performed in some of the included cohorts[9,145,146,148,150], but have not been feasible in all[58,147,149]. Generally, non-responders were more likely to be older, of male sex, less educated, immigrated from non-Nordic countries, smokers, and have higher morbidity and mortality compared to responders.

Table 5. Overview of design and methods for Studies I–IV.

Study	I	II	III	IV
Topic	Snus use and mortality	Snus use, mortality and SES	Snus use and metabolic syndrome	Snus use and biomarkers
Study design	Pooled cohort	Cohort	Cohort	Cross-sectional
Participants	169 103 men from 8 Swedish cohorts in the Snus Collaboration	51 894 men and women from VIP	880 men and women from the northern Sweden Cohort	6 158 men and women from the northern Sweden MONICA study
Primary outcome	Mortality (all-cause, cardiovascular, cancer, other cause)	Mortality (all-cause, cardiovascular, cancer, external cause, other cause)	Metabolic syndrome and its individual components	Concentrations of hs-CRP, 25(OH)D and cfT
Exposure	Snus use (never, current, or former; amount and duration of use)			
Data collection	Baseline data from 1973–2010, and data from the National Cause of Death Register	Baseline data from VIP 1990–2014, and linkages to national registers	Baseline data from 1981 and follow-ups in 1986, 1995 and 2008	MONICA surveys from 1990–2014; biomarker analyses
Analysis	Cox regression	Cox regression	Logistic regression	Linear and logistic regression
Covariates	Age, calendar year, education, physical activity, BMI, alcohol consumption	Age, sex, year of examination, education, area of residence, co-habiting, income, alcohol, DM, physical activity, BMI, blood pressure, cholesterol	Age, sex, smoking, SES, family history of DM, BMI at baseline, physical activity, alcohol consumption	Age, sex, year of examination, education, DM, physical activity, BMI, blood pressure, alcohol consumption, current medication

25(OH)D = 25-hydroxyvitamin D, BMI = body mass index, cfT = calculated free testosterone, DM = Diabetes mellitus, hs-CRP = high-sensitivity C-reactive protein, SES = socioeconomic status, VIP = Västerbotten Intervention Programme.

Table 6. Characteristics of included cohorts in the Swedish Collaboration on Health Effects of Snus Use.

Cohort	Study population	Period of recruitment	Participants (*n*, men)	Deaths (*n*)	Mean age at baseline	Current snus users (%)
Construction Workers Cohort[58]	All blue- and white-collar workers in the Swedish construction industry	1978–1993	279 897	31 429	34	27
Malmö diet and Cancer Study[145]	Population-based, Malmö	1991–1996	12 120	4 372	59	7
Northern Sweden MONICA study[9]	Population-based, Norrbotten and Västerbotten Counties	1986–2004	4 563	643	48	24
National March Cohort[149]	Participants in a national charity walk	1997	15 318	2 531	52	9
Scania Public Health Cohort[148]	Population-based, Scania County	1999–2000	6 201	231	48	20
Screening Across the Lifespan Twin Study[147]	Twins born in Sweden between 1926–1958, national	1998–2002	18 331	2 522	56	16
Stockholm Public Health Cohort[150]	Population-based, Stockholm County	2002–2010	39 406	1 465	50	18
Work, Lipids, and Fibrinogen Study[146]	Employees, Västernorrland, Jämtland, and Stockholm Counties	1992–1997	7 189	265	42	23
Total		**1978–2010**	**383 025**	**43 458**	**39**	**24**

VIP—Study II

Västerbotten Intervention Programme (VIP) is a community- and individual-level intervention program[152]. In VIP, men and women in Västerbotten county, upon reaching 40, 50 and 60 years of age, are invited to participate in a health screening at their primary health care centre. The participants undergo a health examination with a focus on risk factors for cardiovascular diseases and diabetes and answer a comprehensive questionnaire on lifestyle habits and health[152]. The participant is then invited to a health dialogue with a nurse, where the results are communicated and a discussion with focus on preventing CVD is held. VIP questionnaire and health examination data is combined with records from the administrative registers provided by Statistics Sweden and the National Board of Health and Welfare, including data on socioeconomic conditions (the LISA-database[153]), hospitalisation (the National Patient Register[154]), and causes of death (the National Cause of Death Register[151]).

The participation rate has been around 67% during 1995-2014[152,155]. Comparisons between participants and non-participants show that the differences in age, education, and area of residency were <5%. Low-income groups and single individuals had 10% lower participation rates[156].

A total of over 100 000 individuals participated in the yearly cross-sectional VIP surveys during 1990–2014. Of these, 46 258 were never-smoking men and women with complete data on tobacco use, who were thus eligible for inclusion in Study II.

NoSCo – Study III

The Northern Swedish Cohort (NoSCo) is a 27-year prospective study. The cohort consists of all students in the municipality of Luleå in northern Sweden, who in 1981 were in 9th grade (age 16 years) in Swedish compulsory school (*n* =1 083). Surveys have been performed in 1981, 1983, 1986, 1995 and 2008 (at ages 18, 21, 30 and 43), with consistently very high response rates (94% in 2008). The sample has been found to be representative of the corresponding age cohort in Sweden in various demographic comparisons[157].

At baseline and follow-ups, participants completed self-administered questionnaires. Health exams were performed by school nurses at age 16 and by medical professionals at local health care centres in 2008. The latter included venous blood sampling after fasting overnight. Results from the 2008 health examination were used to define the presence of metabolic syndrome in each participant. All other covariates were derived from the questionnaire data from the surveys in 1981, 1986, 1995 and 2008.

After exclusions of participants missing information on metabolic syndrome or tobacco habits, our study sample included 880 participants (88% of those still participating in 2008). We excluded current and former smokers from the sample for most analyses, but in one statistical model the exclusion of smokers left us with a sample that was too small for meaningful analyses. We therefore kept the smokers in this model and adjusted for smoking.

MONICA and BiomarCaRE—Study IV

The Northern Sweden MOnitoring of Trends and Determinants in CArdiovascular Disease study (MONICA)[9,158] comprises several cross-sectional population-based surveys in Norrbotten and Västerbotten. Surveys have been performed in 1986, 1990, 1994, 1999, 2004, 2009 and 2014. For each round, 2 500 randomly-selected inhabitants between 25 and 74 years of age were invited to participate (2 000 inhabitants between 25–64 years of age in 1986 and 1990). Data in the MONICA study was obtained by self-administered questionnaires and professional health examinations. Blood samples were frozen to −80 degrees Celsius and saved at Biobank Norr in Umeå.

The participation rate has decreased over the years from 81% in 1986 to 63% in 2014. A comparison between participants and non-participants performed in 2009 showed that non-participants were younger, more likely to smoke, and less likely to have a university degree or be married[9].

The blood samples of around 12 000 MONICA participants were analysed at the BiomarCaRE (Biomarker for Cardiovascular Risk Assessment in Europe) laboratory in Germany between 2016 and 2018. Study IV was based on these participants. After exclusion of ever smokers and subjects lacking information on exposure or outcome, the resulting study sample was 6 158 men and women.

Measurement of exposure and outcome

Exposure

Snus use was reported in questionnaires in most cohorts, and via telephone interview in one cohort included in the Snus Collaboration. The questions on snus use included current and former use of snus and often weekly amounts as measured by pre-formed categories, and duration of snus use reported either as number of years or in categories (see Figure 11).

Have you ever used snus?

1: No
2: Yes, previously, but not any more
3: Yes, I use less than 2 boxes/week
4: Yes, I use 2–4 boxes/week
5: Yes, I use more than 4 but less than 7 boxes/week
6: Yes, I use 7 or more boxes/week

Figure 11. Example of question on snus use, here from VIP.

We classified each participant as either never-user, current user, or former user of snus. Current users were further grouped according to number of boxes per week (amount) and duration of snus use in years for the purpose of dose–response analyses. Exposure was measured at baseline only, except in Study III, where it was evaluated at several follow-ups.

Outcomes

Outcomes, their definitions and measurements are specified in Table 7.

Table 7. Details on outcome variables.

Outcome	Variable operationalisation	Definition/measurement	
Mortality	Time from study start to death	Death date registered in the National Cause of Death Register run by the Swedish National Board of Health and Welfare. Main cause of death grouped using the International classification of diseases (ICD):	
		ICD-9	*ICD-10*
All-cause		All deaths regardless of cause, including unknown and unregistered causes	
Cardiovascular		3900-4599	I00-I99
Cancer		1400-2089	C00-C97
External causes (included in "other causes" in Study I)		8000-9999 E000-E999	S00-T98 V01-Y98
Other causes		All other specified causes of death	
Established risk factors			
Metabolic syndrome	Dichotomised: yes or no	Defined according to International Diabetes Federation[62]: a) Central obesity and b) two of i) low HDL, ii) high TG, iii) hypertension or anti-hypertensive medication iv) impaired fasting glucose or T2D.	
Central obesity	Dichotomised: yes or no	Measured in light clothing. Defined as waist circumference ≥80 cm (women) or ≥94 cm (men).	
Hypertension	Dichotomised: yes or no	Measured according to WHO MONICA standards[159]. Defined as SBP≥130 mm Hg and/or DBP ≥85 mm Hg and/or antihypertensive medication.	
Lipid abnormalities	Dichotomised: yes or no	Defined as serum HDL-C <1.29 mmol/L (women) or <1.03 mmol/L (men), and/or serum TG ≥1.7 mmol/L	Blood sampling after overnight's fast and analysed by Department of Clinical Chemistry, Umeå University Hospital.
T2D/impaired fasting glucose	Dichotomised: yes or no	Defined as fasting glucose ≥5.6 mmol/L and/or self-reported T2D.	

Emerging risk factors		Blood samples drawn after ≥4 hours of fasting, frozen to –80 degrees and saved in biobank. Analyses performed at the BiomarCaRE laboratory in Germany 2016-18 using immunoassay methods.
Low-grade inflammation	Continuous and in quartiles	Serum-hs-CRP-concentrations, first quartile hs-CRP
Low vitamin D-concentrations	Continuous and in quartiles	Serum-25(OH)D-concentrations, first quartile 25(OH)D
Altered testosterone concentrations	Continuous and in quartiles	Serum-cfT-concentrations, first and fourth quartile cfT

25(OH)D = 25-hydroxyvitamin D, cfT = calculated free testosterone, DBP = diastolic blood pressure, HDL-C = high density lipoprotein cholesterol, hs-CRP = high-sensitivity C-reactive protein, SBP = systolic blood pressure, T2D = type 2 diabetes mellitus, TG = triglycerides.

Covariates

Covariates are factors other than exposure and outcome that may affect the results of the analyses. In our studies we looked for mediators, colliders and confounders of associations. Table 8 shows examples of covariates considered in the four studies.

Knowledge-based selection of confounders and mediators

Potential confounding factors, mediators and colliders were identified through existing literature and availability in the specific cohort. In Studies II and IV there were large numbers of potential covariates. In those studies, we drew directed acyclic graphs (DAGs) to illustrate the complex relationships between covariates, and to facilitate the discussion of their classification as possible confounders, mediators, colliders, or non-related factors.

Important confounders of the associations between snus use and health outcomes are, for example, age, sex, socioeconomic factors and physical activity, given that these are associated to both snus use (exposure) and health measures (outcome). While BMI, alcohol use, blood pressure and T2D are also essential for health, they have in previous studies been shown to be influenced by snus use, rather than affecting the use of snus, implicating that they may be a part of a causal chain that results in adverse health outcomes—that is, they may be regarded as mediators.

Table 8. Examples of covariates included in the different studies.

Covariate	Study	Variable operationalisation	Definition and measurements
Alcohol consumption	I	Categorised as never, low, medium, or high	Continuous variable of self-reported grams/week used. We split the variable into terciles to define three levels of use
Educational level	I	Categorised as primary, secondary, or tertiary	Self-reported years of schooling grouped as ≤9, 10–12 and ≥13 years, respectively
Area of residence	II	Dichotomised: Rural or urban	Classified according to health care centre listing
Physical activity	II	Categorised as inactive, moderately active, or highly active	Self-reports on activity in leisure time and way of getting to and from work[160]
Body mass index	III	Continuous	Defined as weight/height squared. Measured at age 16 by school nurses.
Socioeconomic status in adolescence	III	Dichotomised: socially favourable or socially disadvantaged	Self-reported parental occupation reported at age 16 used for classification as defined by Statistics Sweden[161]. Disadvantaged group consisted of the group "manual workers".
Current medication	IV	Categorised as anti-inflammatory medication, D-vitamin supplementation, and hormonal or opioid medication	Self-reports of drugs used during the last 14 days, converted into ATC-code and then grouped into relevant category
Risky drinking	IV	Dichotomised: yes or no	Yes: ≥14 (men) or ≥9 (women) standard drinks per week[162], approximated using self-reported drinking frequency

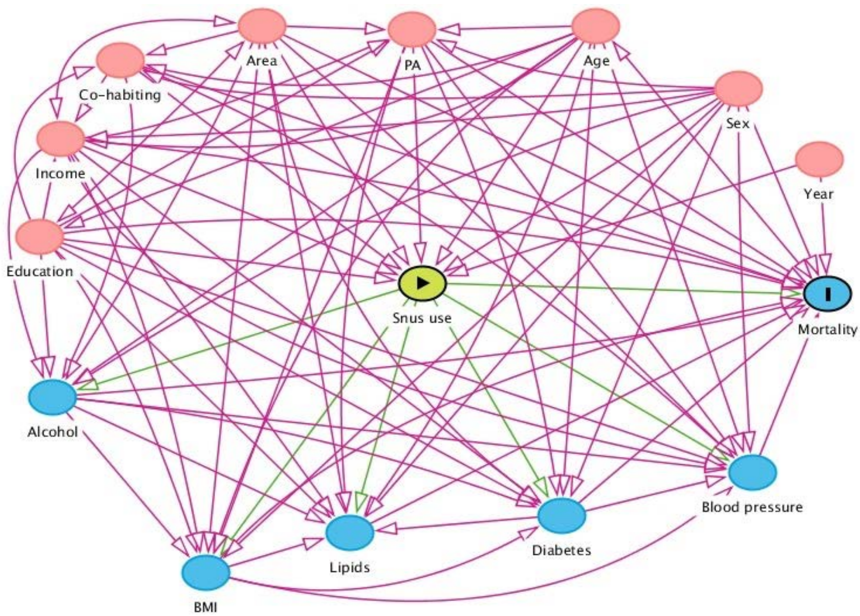

Figure 12. Directed acyclic graph from Study II drawn for separation of confounders (associated with both exposure and outcome, purple lines) and mediators (factors that may be on the causal pathway between exposure and outcome, green lines). Created using dagitty.net[163].

Area = area of residence, BMI = body mass index, PA = physical activity, year = year of examination.

Statistical modelling

The choice of statistical method was guided by the research question and study design and is described below. After knowledge-based identification of potential confounders, mediators and colliders, these variables were further evaluated using statistical methods. For example, we used stepwise-forward selection in Study IV. Interactions were tested by adding relevant interaction terms to the models.

In all studies, we first created crude analytical models, and then used the identified relevant confounders and mediators to construct multivariable models. We often started by adding basic confounders such as age and year of examination into a first multivariable model, after which we incorporated all identified confounders into a second multivariable model. Stratification was performed if considered necessary. In Study II we also added potential mediators to the fully adjusted model to evaluate their influence on the results.

Baseline characteristics

We reported characteristics at baseline using means and standard deviations for continuous variables, and numbers and/or percentages for categorical variables. In Study III, the *p*-values for differences in characteristics between never-users, current users, and former snus users were calculated using Kruskal-Wallis test for continuous variables and the chi-square test for categorical variables. A *p*-value of <0.05 was considered statistically significant.

Primary outcome analyses

Cox regression—Studies I and II

We used Cox proportional hazards regression, a form of survival analysis, to model time to event for our mortality outcomes[164]. Participants were followed from study entry until they either developed the outcome event, dropped out of the study, or until study end. The results were presented as hazard ratios (HR) of obtaining the outcome for snus users compared to the reference category of never-users. In Study I, a shared frailty model was employed to compensate for repeated measurements within each cohort.

Cox regression assumes that the hazards among different groups are proportional. This assumption was assessed by a chi-square test based on scaled Schoenfeld residuals in Study I, and graphical methods using survival curves in Study II. There were no major violations of the assumption in Study I, however in Study II the age variable did not meet the proportionality assumption. Therefore, we stratified the analyses by age group. We also added another variable that adjusted for age deviation within each age group. In this way, the proportionality assumption was fulfilled, while still adjusting for age.

Logistic regression—Studies III and IV

Logistic regression was used in Study III to estimate the odds of metabolic syndrome and its components for different categories of tobacco use; and in study IV to find potential associations between snus use and biomarkers representing low-grade inflammation, low vitamin D-concentrations and altered testosterone levels. The results were presented as odds ratios of obtaining the outcome compared to the reference category.

Linear regression—Study IV

We used linear regression in Study IV to evaluate the relationship between biomarker concentrations and snus use. Results were given as beta-coefficients, which represent the change in outcome value between snus use categories. We evaluated the normality assumption through graphical and mathematical methods, for example histograms and test of skew. Several of our outcome variables were non-normally distributed and therefore log-transformed to meet the normality assumption.

Evaluation of statistical methods

Outliers

Some individuals had extreme values in certain covariates, which may give these observations an unreasonably high influence on the regression results. We therefore screened our data for such influential points, mainly by graphical methods. Some of these outliers were excluded from analyses. Other times we could categorise the variable, reducing extreme impact while still keeping the observation in the analyses. This was for example done in Study II, where there were individuals with extremely high income, and we consequently grouped the income variable into terciles.

Collinearity

Collinearity, resulting from high correlation between two or more covariates in a statistical model, was evaluated by calculations of variance inflation factors[165]. They were in all cases satisfactorily low.

Interaction

Interaction occur when the effects of an exposure on the studied outcome differ with another variable[166]. For example, the effect of snus use on mortality may be different depending on age, sex or education. Interaction between exposure and covariates was evaluated in all studies, either by stratification by categorical variables such as sex, or by adding interaction terms into the statistical models. If the *p*-value for the interaction term was <0.05, the interaction was regarded as significant.

Secondary outcome analyses

Sensitivity analyses were performed in all studies in order to evaluate the robustness of our results. For example, in Study I we limited follow-up time to a maximum of 10 years to reduce the risk of misclassifying tobacco habits that may change over time; added additional covariates to the analytical models in the cohorts where these were available; and repeated the analyses excluding the CWC (which was by far the largest cohort). Study IV included analyses using alternative cut-off points for vitamin D-concentrations, and analyses using a complete case-variable instead of an imputed one on risky alcohol consumption.

When both sexes were included in the datasets, we performed sex-stratified analyses to evaluate whether there were differences in outcomes between men and women. In Study II we repeated the analyses by five socioeconomic variables: education, income, area of residency, co-habiting status, and overall SES. In all studies, we performed dose–response analyses on snus amount categories (in boxes per week) and/or duration of snus use in years, all reported at baseline.

To compare and combine our results with previous findings, we conducted a meta-analysis on snus use and all-cause mortality using a random effects model.

Handling of missing data

All studies were based on complete case data, excepting the alcohol variable in Study IV, which was imputed using median values within the same sex and age groups. In study II, non-responders in the diabetes and risky drinking variables were included in an “unknown” category.

Analyses were made to compare characteristics such as sex, age, tobacco habits and outcome variables between responders and non-responders. These included those who were excluded from our analytical samples, or individuals who did not respond to certain questions. While we did not have data to conduct analyses on individuals who chose not to participate, such analyses had been performed previously in several of the cohorts[145,148,9,157,156,150].

Ethical considerations

The different projects were all approved by ethical committees. Study participants of the individual cohorts in the Snus Collaboration gave informed consent before taking part, or were informed that their data would be used for research purposes. All participants in NoSCo and MONICA gave written consent upon participation. Participants in VIP were informed that their data was collected in a database that may be used for research purposes, and had the option to be excluded from the database. Participants also gave written consent for blood sample donation for future research.

When our studies started, all data and blood samples had already been collected, and plasma analyses had been conducted within previous research projects. Pseudonymized data were used, meaning we did not have access to individual-level data within the projects, and code lists were securely kept at the medical biobanks. The results of individual analysis were not distributed to participants.

The studies may, to some extent, be perceived as a violation of the participant's personal integrity, but the risk and potential harm for participants of this thesis is likely to be low, while the potential benefit for society at large must be regarded as substantial.

Results

Baseline findings

Baseline characteristics and presence of outcome in the four studies are summarised in Table 9. Snus users were younger than non-users (Study I), particularly among women (Studies II, III, IV), which is consistent with increasing prevalence of snus use during the last few decades, and low use in the 1960’s. In all mixed-sex studies, snus use was significantly more common among men. Among never-smokers (that is, the studied population), approximately 3% of women and 17-23% of men were current snus users. In NoSCo the rate of current snus use for men and women combined was 12% at the age of 16.

Snus-using men were less educated compared to non-using participants (Studies I, II, IV). Where sex-stratified analyses were performed, it was evident that female snus users were not bound by the same socio-economic patterns as men: they were more well-educated (Studies II and IV) and had higher income (Study II) than non-users. Alcohol intake or risky drinking was more prevalent among snus users than among non-users in Studies I–III.

Another finding in NoSCo was that snus use increased steadily over the life-course, and that a large proportion of snus users also smoke at some point during their lives.

Table 9. Baseline characteristics and presence of outcome in the four studies.

	Study I	Study II	Study III	Study IV
Analytical sample (*n*)	169 103	46 258	880	6 158
Mean age at baseline (years)	36	47	16	49
Male sex (%)	100	48	52	47
Current snus use (%)	23	M: 17 W: 3.0	1981: 12 2008: 9.3	M: 21 W: 3.3
Recruitment period	1978-2010	1990-2014	1981-2008	1990-2014
Outcome (cases)	Deaths: 10 928 (6.5%)	Deaths: 2 678 (5.3%)	Metabolic syndrome: 237 (27%)	1st Q hs-CRP: 1 357 1st Q 25(OH)D: 1 431 4th Q cfT: M: 689, W: 627

25(OH)D = 25-hydroxyvitamin D, cfT = calculated free testosterone, hs-CRP = high-sensitivity C-reactive Protein, M = men, metsy = metabolic syndrome, Q = quartile, W = women.

Findings from primary and secondary outcome analyses

Mortality—Studies I and II

Study I found an increased risk of all-cause mortality of 28% for current exclusive snus users compared to non-users (HR 1.28, 95% CI 1.20; 1.35). The excess risk was seemingly most attributable to cardiovascular (HR 1.27, 95% CI 1.15; 1.41) and "other" causes (non-cardiovascular, non-cancer) (HR 1.37, 95% CI 1.24; 1.52), although there were also indications of increased cancer mortality (HR 1.12, 95% CI 1.00; 1.26). The risk increased in a dose-dependent manner with the baseline reports of duration, but not with amount of snus use. Former users generally displayed lower risk increases. The main analyses were adjusted for BMI and age. Several sensitivity analyses were performed that added further credibility to the results; for example restricting follow-up time to a maximum of ten years to avoid misclassification bias, excluding cases of major cardiovascular events or cancer within a year from baseline, and further adjusting for additional covariates (calendar year, education, physical activity and alcohol consumption). The results of these sensitivity analyses supported the main findings with the exception of an attenuated association between snus use and cardiovascular mortality when limiting follow-up time to ten years (HR 1.13, 95% CI 0.93; 1.38).

Study II—which also evaluated mortality risks but did so with comprehensive adjustment for several socioeconomic factors—also demonstrated excess mortality for current snus users, with all-cause mortality in the mixed-sex group increased by 23% (HR 1.23, 95% CI 1.04; 1.46) and cardiovascular mortality by 45% (HR 1.45, 95% CI 1.08; 1.92) compared to never-users of tobacco. External cause mortality displayed raised hazard ratios, but was only significant in basic adjustment models (HR 1.75, 95% CI 1.07; 2.85 in basic model, attenuated to HR 1.59, 95% CI 0.97; 2.61 in the fully adjusted model). Snus use was associated to cancer mortality in stratified models. Other cause mortality showed no differences between snus users and non-users.

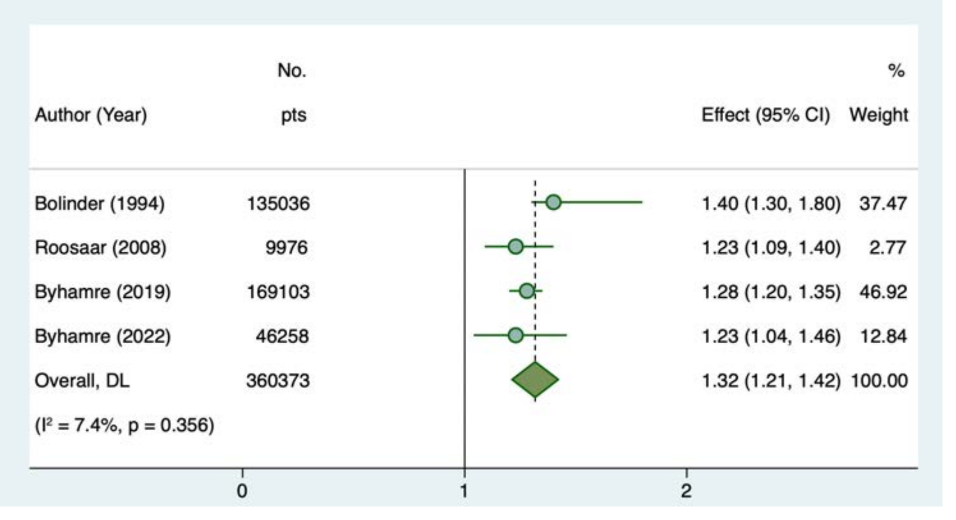

Figure 13. Meta-analysis of studies on snus use and all-cause mortality. Effect estimates are hazard ratios with corresponding 95% confidence intervals.

Our meta-analysis showed an overall association between snus use and all-cause mortality with HR 1.32 (95% CI 1.21; 1.42). Heterogeneity, measured as I^2, was 7.4% (Figure 13).

Impact of socioeconomic factors

In study II, we performed additional analyses to explore if the associations between snus use and mortality were modified by socioeconomic factors. All analyses were based on never-smoking men. First, we tested for interaction between snus use and our socioeconomic variables: education (primary and secondary versus tertiary), individual income (in terciles), area of residence (urban or rural), co-habiting status (living alone or co-habiting), and overall SES-variable based on the previous four variables and family income. We found a significant interaction between snus use and area of residence on the association of all-cause and cardiovascular mortality.

Secondly, we analysed all-cause, cardiovascular, cancer and external cause mortality stratified by our five socioeconomic variables. The results for all-cause mortality are shown in Figure 14. We found that the risk of all-cause and cardiovascular mortality was increased among participants with basic education (borderline significance for cardiovascular mortality), but not with higher education, and in the highest income tercile, but not in the lower terciles. Snus users living in urban areas had increased all-cause and cardiovascular mortality risk, while snus users in rural areas did not. Co-habiting snus users, but not single, had increased risk of dying from cardiovascular disease. Snus users living alone had increased risk of external cause mortality.

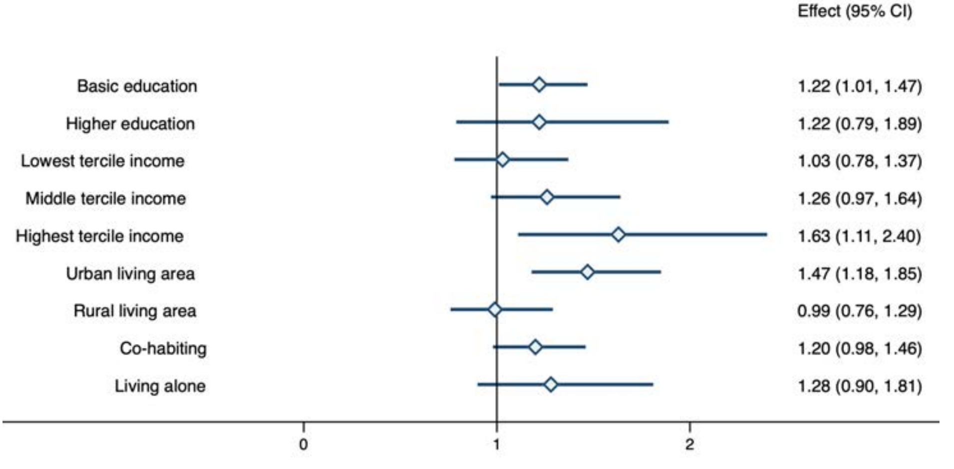

Figure 14. All-cause mortality among snus users compared to non-users stratified by socioeconomic variables. Presented effect estimates are hazard ratios, followed by corresponding 95 % confidence intervals.

Mortality analyses stratified by the overall SES variable (categorised as low, medium or high SES) showed significantly increased risk of all-cause and cause-specific mortality (all tested outcomes) in the medium SES group (Table 10).

Table 10. Cox regression evaluating the mortality risks among current snus users compared to never-users, stratified by overall SES.

	Low SES	**Medium SES**	**High SES**
	HR (95% CI)	HR (95% CI)	HR (95% CI)
All-cause mortality	0.93 (0.69; 1.26)	**1.44 (1.14; 1.83)**	1.50 (0.99; 2.28)
Cardiovascular mortality	0.98 (0.58; 1.63)	**1.71 (1.12; 2.59)**	1.99 (0.96; 4.12)
Cancer mortality	0.80 (0.44; 1.46)	**1.60 (1.08; 2.38)**	1.34 (0.69; 2.61)
External cause mortality	0.68 (0.20; 2.33)	**1.97 (1.08; 3.60)**	1.84 (0.38; 8.98)

Male-only models. Stratified by age group and socioeconomic status (SES) group, and adjusted for age, year of examination and physical activity.

Established risk factors—Study III

Our findings in Study III showed that snus exposure at ages 16, 21, 30 and 43 years was not associated with development of the metabolic syndrome nor any of its components at age 43. In crude analyses, snus use was associated with raised triglycerides and high blood pressure, but the results were attenuated upon adjustments for confounders. Table 11 presents parts of these results.

Table 11. Metabolic risk for current snus users who have never smoked, evaluated at different ages.

	Metabolic syndrome OR, 95% CI		**Central obesity** OR, 95% CI		**Raised triglycerides** OR, 95% CI		**Hypertension or anti-hypertensive treatment** OR, 95% CI	
	Crude	Multi-variate*	Crude	Multi-variate*	Crude	Multi-variate*	Crude	Multi-variate*
Age 16	1.17	0.95	1.28	1.40	**1.83**	1.38	1.45	1.08
n =81	0.70; 1.96	0.54; 1.65	0.79; 2.07	0.83; 2.35	**1.11; 3.01**	0.81; 2.37	0.91; 2.32	0.66; 1.77
Age 21	1.68	1.15	1.18	1.24	**2.05**	1.27	**2.08**	1.31
n =53	0.91; 3.08	0.60; 2.21	0.66; 2.13	0.65; 2.34	**1.11; 3.79**	0.66; 2.45	**1.16; 3.72**	0.71; 2.42
Age 30	1.51	1.01	1.20	1.15	**2.21**	1.37	**2.37**	1.61
n =57	0.82; 2.80	0.52; 1.99	0.68; 2.13	0.61; 2.15	**1.20; 4.06**	0.71; 2.63	**1.33; 4.19**	0.88; 2.96
Age 43	1.60	1.15	1.76	1.65	1.78	1.10	**2.06**	1.41
n =37	0.78; 3.35	0.52; 2.51	0.85; 3.62	0.76; 3.58	0.83; 3.80	0.49; 2.45	**1.04; 4.09**	0.69; 2.89

CI = confidence interval, OR = odds ratio. Never tobacco-users form the reference category.

* Adjusted for sex, BMI at 16 years, socioeconomic status at 16 years, family history of diabetes, alcohol consumption at 43 years and physical activity level at 43 years.

Emerging risk factors—Study IV

Snus users had lower 25(OH)D-concentrations, lower hs-CRP-concentrations and, among men, raised cfT-concentrations, when compared to never-users of snus (Table 12). In dichotomised analyses we tested the risk of having a biomarker concentration in the lowest or highest quartile versus not, and found that snus users compared to non-users in fully-adjusted models had higher odds ratios of being in the lowest quartile of hs-CRP (OR 1.51, 95% CI 1.22; 1.86) and 25(OH)D (OR 1.29, 95% CI 1.05; 1.59), and in the highest quartile of cfT for men (OR 1.31, 95% CI 1.04; 1.64). There was no significant difference among female snus users and non-users regarding cfT levels. The results were partly supported by dose–response trends found for duration for 25(OH)D-levels.

When stratifying the results on hs-CRP and 25(OH)D by sex, we found that lower hs-CRP-concentrations seemed to be restricted to snus-using men, while low 25(OH)D was more pronounced in women, although non-significant (possibly due to few female snus users).

Table 12. Results on biomarker concentrations among snus users.

	n	Never snus use	Current snus use
hs-CRP-concentration*[a] (β_{ln}, 95% CI)	5 062	ref.	**–0.13 (–0.21; –0.04)**
25(OH)D-concentration[b] (β, 95% CI)	5 240	ref.	**–0.95 (–1.63; –0.26)**
cfT-concentration, men[c] (β_{ln}, 95% CI)	2 455	ref.	**0.04 (0.01; 0.07)**
cfT-concentration, women[c] (β_{ln}, 95% CI)	2 449	ref.	0.03 (–0.07; 0.13)

hs-CRP = High-sensitivity C-Reactive Protein, 25(OH)D = 25-hydroxyvitamin D, cfT = calculated free testosterone, CI = confidence interval. Bold indicates statistically significant results.

* Cut off for values indicating inflammatory process at 99th percentile.
[a] Adjusted for age, sex, year of examination, education, physical activity, body mass index, diabetes mellitus, systolic blood pressure and risky drinking. Users of anti-inflammatory medication were excluded from analyses.
[b] Adjusted for age, sex, year of examination, Nordic origin, sampling month, body mass index, education and physical activity. Participants reporting use of D-vitamin supplements were excluded from analyses.
[c] Adjusted for age, year of examination, body mass index, education, physical activity, and risky drinking. Users of hormonal and opioid medication were excluded from analyses.

Analyses of non-responders

Comparisons of characteristics on participants with incomplete data (non-responders) and those with complete data (responders) were performed when possible. In study II, non-responders were younger, more often of male sex, with less education and more likely to have a risky alcohol consumption. Non-responders in Study III had higher alcohol consumption at age 43 and were more likely to smoke at ages 21 and 30, but were similar to responders regarding exposure and outcome variables. In Study IV, no significant differences were found among responders and non-responders with regard to age, snus habits, sex, or average concentrations of outcome biomarkers.

Discussion

Discussion of material and methods

Study design, validity, and precision

The aim of a public health study is to be able to draw conclusions that are true for a certain population. The type of study generally considered to most closely mimic a test of the population as a whole is the randomised controlled trial (RCT). However, long-term effects of snus use cannot be investigated within the framework of an RCT. It is ethically not possible to randomise participants to snus use or non-use; and the need for long follow-up times makes randomisation virtually impossible. We therefore choose the second-best option for our studies on snus and health: cohort studies.

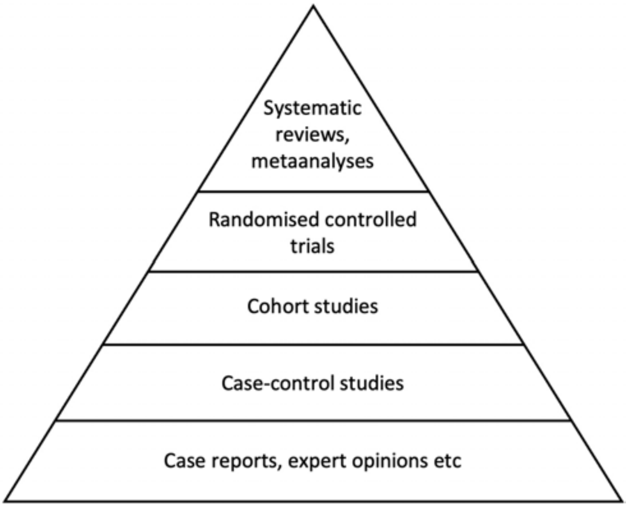

Figure 15. The hierarchy of evidence, showing studies of highest probability of establishing causality closer to the top. Adapted from internetmedicin.se[167].

Common measures of study quality are external and internal validity, and precision. Internally-valid studies produce results that are true for the studied population, and not likely to be due to bias, confounding or chance. This requires the design and execution of the study to be correctly performed. External validity means that the results are applicable outside the study, to the population that the analytical sample is meant to represent. This can only be achieved if the studied sample and the represented population are similar enough regarding important characteristics. For clinical studies, the relevance of the outcome is also fundamental[168], meaning that it should be important in a clinical or public health setting. Precision indicates the study's ability to correctly measure the outcome.

External validity—generalizability

The four studies in this thesis were all conducted on population-based Swedish cohorts, often with a large number of participants. There was representation of all geographical areas of Sweden, with a focus on the northern parts, which have the highest rates of snus use. The studies also encompassed a wide range of age groups. All these factors considerably strengthen the external validity of our results, as they become representable for a larger group of people. However, the validity of the results in other countries and for people of ethnicities other than the mainly Caucasian Swedish population cannot be ascertained.

Exclusively snus-using women were often too few to allow for sex-stratified analyses, making the results less applicable to women, especially since some risk factors for CVD and cancer, among them smoking, differ between the sexes[141]. To make the results more valid for women, we modelled analyses including both men and women in Studies II–IV. We also made sex-specific analyses whenever possible, and found that the point estimates were generally similar for men and women (although often insignificant for women, potentially due to few users). However, to fully determine the risk of snus use among women, future studies are needed.

Data was collected during different time periods. This offers an advantage in the form of increased representability, but can also cause problems in our analyses, as living conditions, health care standards, tobacco-preventive efforts, and snus products have changed during the study periods. The prerequisites are not the same now as they were in the 1970s when data collection for Study I began, which must be remembered when interpreting the results. To ensure our results were also valid in more recent times, Study II was set within VIP with recruitment period between 1990 and 2014.

Internal validity

An internally-valid study is achieved when the outcome is attributed to the exposure and not to other causes, such as chance or systematic errors (bias)[169]. There are three main groups of bias: misclassification bias, selection bias, and confounding bias[168].

Misclassification bias

Information or misclassification bias is caused by errors in collecting data on exposure and outcome. In observer bias, the researcher's own prior hypotheses or knowledge influences the collection or interpretation of data. In our studies, we minimised this risk by using data that had already been collected, and by interpreting the data together within the research groups (most of which included snus users).

Snus use was measured through self-reports, which may be unreliable and a source of another type of misclassification bias called recall bias, where participants wrongly recall past exposure. This might influence our results, particularly regarding duration and amount of snus, which can typically differ between time periods. Social desirability bias arises if participants answer questions in a way that is perceived as more favourable and may lead to underestimation of our results. However, snus use in Sweden is not stigmatised in the same way as smoking is, and previous studies have identified self-reports of snus use as reliable[66], also when comparing them to cotinine levels[170].

In most studies, exposure was measured only at baseline, creating a risk of misclassification of tobacco habits during follow-up. This effect may be especially pronounced in study II, due to the interventional nature of VIP that could trigger behaviour change shortly after baseline. This increases the risk of misclassifying former snus users as current users, which in turn could lead to dilution of the associations for current snus users. However, few individuals take up smoking in adult age[171], and snus use is a stable habit, with continued use among 77–80% after 5–13 years[172–174]. The same pattern has been seen within VIP[34]. In the Snus Collaboration study, we performed sensitivity analyses where we restricted follow-up time to a maximum of ten years, to evaluate misclassification bias. Only the association with cardiovascular mortality changed significantly after this restriction, giving further credit to the conception of snus use as a persisting habit, and for our results to be valid.

The health examinations in the various studies were carried out by experienced medical staff using regularly-calibrated devices, to minimise instrument bias. Blood samples were analysed at large laboratories using well-known, validated methods and instruments, but are still potential sources of error. Indeed, in Study IV we found that the absolute values obtained from the BiomarCaRE laboratory were lower than the values we got when we repeated the analyses using other analytical methods.

However, the correlation between the methods was high, and thus the relative values were still valid. Consequently, we chose data-driven cut points for extreme values of the biomarkers rather than absolute ones. The absolute biomarker concentrations should be interpreted carefully; on the other hand, there is nothing to suggest that the correlation between the methods should be distributed unequally over groups of tobacco use. The potential error would, therefore, likely not lead to biased results.

Death date and cause of death was defined by linking to the National Cause of Death Register of Sweden[175]. Linkage is made possible through the system of a unique personal identification number assigned to every Swedish resident. The register data is based on the death certification as reported by medical doctors in connection to each death. Even though this register includes 98–99% of all deaths and thus gives a chance of unique precision in defining cause and time of death, there are still a number of potential error sources, the most important being that the majority of death certificates are based on clinical evaluations and less than 20% are based on autopsy reports[151,176]. However, the most recent evaluation of the register showed correct underlying cause of death in 77% of cases, highest for malignant tumours (90%) and ischemic heart disease (87%), which are some of the most important causes of death in our studies[176]. Also, there is nothing to suggest that users and non-users of snus would differ in autopsy rates or registration completeness, which suggests that the probability of a correct diagnosis can be expected to be similar in the two groups.

Selection bias

Selection bias is caused by differences in characteristic between studied groups, and should be considered when interpreting our results. Health survey participation is, for example, more likely among non-smokers and people with high SES[177]. Analyses performed in the included cohorts showed similar patterns: non-participants were more likely to be younger and of male sex, less educated, and to have a risky alcohol consumption compared to participants. Hence, our findings may be less representative for these populations. However, the response rates were high throughout (ranging from 94% in NoSCo to 63% in the 2014 MONICA survey), and analyses on non-responders in our studies showed that there were no major differences in exposure or outcome variables among those who stayed in the study and those who did not.

Another example of selection bias is the healthy worker effect: you need to be relatively healthy to work, and therefore the risk of disease or death caused by a risk factor may be underestimated among the working group. The CWC, which is a part of the Snus Collaboration, has been criticised for this, however, we performed sensitivity analyses excluding this cohort and still received the same results as in our main analyses.

Confounding bias

Confounding bias arises when a factor that is independently associated with both exposure and outcome, but is not on the causal pathway between them, simulates a causal relationship where there is none. If a potential confounder is unevenly distributed among the studied groups, confounding bias occurs, resulting in both over- and underestimations of an association. Confounding can be controlled in the analytical phase by adding appropriate variables to create multivariable statistical models, by stratification of the results based on group characteristics, or by standardisation using a reference population to nullify differences in characteristics between studied groups.

In studies on snus' effects on health, confounding is one of the biggest challenges, given that snus use is traditionally linked to several factors that also influence risk of death: sex, physical activity, living area, education, income, and other socioeconomic factors. Research has also indicated, although not clearly proven, that a number of other factors important for health may be associated to snus use: diabetes mellitus[94,97], risky drinking behaviour[155], BMI[89,92], blood pressure[46,91] and altered blood lipids[84]. To account for this, we first evaluated all covariates using both knowledge-based (e.g. DAGs) and data driven approaches. We then included all relevant and available confounders in the analytical models, and performed stratified analyses. To attempt to further capture of residual confounding, we included four major socioeconomic variables together with other common confounders in Study II.

Overall, we had access to a large number of socioeconomic, health and lifestyle factors, both through comprehensive questionnaire and health examination data, and by linkage to population registers run by the Swedish state. Still, as exposure cannot be randomised, there is always a risk of unmeasured confounding that may bias our results and decrease the internal validity of our studies.

Precision

Precision refers to the study's ability to accurately estimate risk, and requires sufficient sample size, meticulous measurement of exposure, and a large enough number of exposed[169]. Precision in our studies was mostly high, as we had access to large datasets with high exposure rates. This gave us enough participants to detect even small differences in risk in mixed-sex groups and among men, despite exclusion of smokers, stratifications, and additional analyses on smaller groups. However, exclusively snus-using women were few, resulting in low precision in analyses of female snus users, and hence these results must be interpreted carefully. The precision of detecting small differences in the incidence of the metabolic syndrome was also less than desired. Using biomarker verification of tobacco exposure instead of relying on self-reports could have increased the precision in our studies.

The Bradford Hill criteria

The critical difference between an RCT and an observational study is the risk of bias due to confounding. As participants in an RCT are randomised, with sufficient sample size, all background factors are equally represented in both the exposed and unexposed group, and we can therefore measure the "true" effect of an exposure—in other words, we can test causality. In observational studies, there is risk of systematic differences in background factors between two studied groups, and therefore the associations found in these studies are not regarded as causal[178].

However, much can be done to strengthen the associations of observational studies. Sir Austin Bradford Hill developed a set of guidelines in 1965 for this purpose[179]. These have later been revised and the interpretations have evolved over time as we have made advances in the understanding of disease, epidemiology and statistics. Although commonly viewed as a checklist, this was never the intention; they should rather be considered as means of assessing causal inference in observational studies. The guidelines are presented below.

1. Strength of association: a stronger association indicates higher likelihood of causality. Today, statistical significance rather than magnitude of association determines the strength of association.
2. Consistency: if similar findings can be observed in other settings, it strengthens causality.
3. Specificity: there must be a one-to-one relationship between cause and outcome.

4. Temporality: exposure must precede outcome. This is often regarded as the most important criterion.
5. Biological gradient: if the exposure changes, so should the disease rate or other outcome (i.e., a dose–response relationship).
6. Biological plausibility: a potential underlying biological mechanism strengthens causality.
7. Coherence: the association agrees with current knowledge of the biology of the disease or other outcome.
8. Experiment: if the exposure is removed, the frequency of the outcome should decrease.
9. Analogy: there is less need for evidence if an exposure is similar to that of another agent similar to the first. Similarity can be in for example biological mechanism or disease progression pattern.

Applying the Bradford Hill criteria

Let's consider the above criteria with regard to this thesis.

Strength of association and consistency

Only results with a significance level of 5% has been regarded as statistically significant. The mortality among snus users has been evaluated in previous research; we repeated these analyses and found the results to be consistent despite different settings and adjustments for a large number of confounders. While established and emerging risk factors such as the metabolic syndrome and CRP have been investigated previously, the results are inconsistent or evaluated too few times, requiring further evaluation to meet the consistency criterion.

Temporality

Studies I, II and III are longitudinal cohort studies, where we measure a number of background factors at baseline and then follow the populations with regard to certain outcomes. In this way, longitudinal studies can establish a sequence of events, thus making sure that exposure precedes outcome, fulfilling the temporality criterion. They therefore give the most authentic measure of incidence among the observational studies.

Cross-sectional studies measure prevalence; in our case concentrations of biomarkers in snus users and non-users in Study IV. Although this type of study can expose associations, temporality cannot be established, as exposure and outcome are measured simultaneously. However, tobacco use is commonly begun during youth, with few new users recruited after the age of 25[171]. Our Study IV participants were between 25 and 79 years old with a mean of 49, indicating that the bulk of snus users would have used snus for quite some time, which strengthens the associations and makes causality more plausible.

Biological gradient

We performed dose–response analyses based on duration of snus use and weekly consumption, to evaluate a potential biological gradient. We found that while increasing duration of use often correlated with higher risk estimates, there was seldom such tendencies regarding weekly consumption. This is possibly explained in part by the variation in weight and nicotine content in different snus boxes, and that self-reports were limited to pre-formed categories (for example <2 boxes/week, 2–4 boxes/week, 5 or more boxes/week). Other possible contributing factors are individual differences in nicotine uptake and metabolism[180]. To further elucidate this, validation studies on amount of snus used and biomarkers of nicotine exposure are needed.

Another way of assessing biological gradient is by estimating risks among former snus users. A group of previously exposed individuals would, in accordance with the biology of other nicotine exposure (e.g. smoking), suffer from increased risk that would subside with time since cessation[181]. Our results generally showed no risk increases among former snus users, which is in accordance with the biological gradient guideline. It should be pointed out that the group of former users is vastly heterogenous. As former users were self-defined, participants quitting snus both the day before examination and 50 years ago would be included, and the previous use would range from very high to low. There would also be a higher-than-normal rate of people more inclined to change their lifestyle among former users, due to for example newly diagnosed disease or negative effects by snus use. With this in mind, the results of former users have been regarded as an indicator of biological gradient rather than an evaluation of risk by previous use in itself.

Plausibility, coherence, and analogy

Our hypotheses are based on confirmed or plausible biological mechanisms and current knowledge. We have studied known effects of snus use, smoking, use of nicotine replacement therapy, experimental nicotine exposure, and American smokeless tobacco (e.g., dipping tobacco), to use both as a basis of generating hypotheses and to identify possible mechanisms, but also to compare against our own results to evaluate any analogy.

Discussion of statistical methods

Alternative statistical models

Alternative statistical approaches may be considered. In study III we could have used techniques developed for correlated data, such as mixed models[182] and generalised estimating equations[183], instead of performing separate logistic regression analyses for each follow-up. In study IV, we considered analysing accelerated failure time[184,185], known to fit adult survival data very well, but decided on Cox regression[164] to increase comparability with previous studies.

Handling missing data

In the cases of large number of missing results in certain variables, multiple imputation could have been performed to a larger extent, as was done in Study IV on risky drinking. However, the amount of missing data was low in most covariates and therefore not likely to have had any substantial effect on the results.

Type I error

To minimise the risk of committing a type I error; that is, of rejecting a true null hypothesis, we chose the well-established significance level of 0.05[186]. We chose not to adjust for multiple comparisons in our studies[187]. Hence, outcomes with *p*-values close to 0.05 may be due to spurious findings and should be interpreted with caution.

Type II error and power

Statistical power was adequate for our main research questions in most studies. In Study III, the sample size was reduced as we excluded current and previous smokers, resulting in only 37 current snus users at age 43. Power calculations show that with this sample size, small or even moderate risk increases will not be detected. This means that there is a risk of a potential association between snus use and metabolic syndrome or its components that we could not identify (a type II error). The same problem arose in sex-stratified analyse in Studies II and IV, due to a low number of female exclusive snus users. We therefore had too low power to, for example, perform analyses on cause-specific mortality in women.

Results discussion

All-cause mortality—Studies I and II

Our two studies on snus use and all-cause mortality showed increased risk among snus users compared to non-users. Study I found a risk increase of 1.28 (HR, 95% CI 1.20; 1.35), and in Study II the HR was 1.23 (95% CI 1.04; 1.46). The first study included only men, and adjustments for age, BMI, and education were made. Sensitivity analyses that also included adjustments for alcohol consumption and physical activity supported the main results, as did several other sensitivity analyses and dose–response analyses on duration of snus use.

Our second study gave us an opportunity to sharpen our adjustments for socioeconomic factors. It also included women. While adjusting for age, sex, calendar year, area of residence, co-habitation, education, income, and physical activity, there was still a significantly increased risk of all-cause mortality, both in the mixed-sex and men only groups.

Earlier research show increased risk of all-cause mortality among non-smoking snus users at roughly the same levels as our studies. Bolinder et al. found a RR of 1.4 (95% CI 1.3; 1.8), with a more pronounced risk among younger snus users (35–54 years at inclusion)[41]. Roosaar et al. found a HR of 1.23 (95% CI 1.09; 1.40) for death among ever snus users (current and former users combined) compared to non-users[40].

While point estimates differs somewhat between the studies on snus use and all-cause mortality, we consider the results concordant. This is further supported by the results from our meta-analysis displaying low heterogeneity between study results. However, differences in the studied populations such as inclusion of women, former snus users, and socioeconomic and health differences between a cohort of construction workers as compared to population-based samples may contribute to varying point estimates between studies. Interestingly, when adjusting for an identified interaction between snus use and area of residence in Study II, the overall mortality risk was somewhat inflated (HR 1.42, 95% CI 1.15; 1.76), underlining the importance of careful consideration of socioeconomic factors in analyses on snus use and health.

Thus, considering our consistent results on snus use and all-cause mortality that are in line with the previous studies on the topic, there is now further evidence for snus use as a risk factor for increased all-cause mortality. The size of the risk increase is estimated to be 1.32 (HR, 95 %

CI 1.21; 1.42) based on the meta-analysis of study I, II and the previous studies by Bolinder, Roosaar and colleagues.

Cardiovascular mortality

Looking deeper at cause-specific mortality, the main driving factor for increased all-cause mortality among snus users seems to be death from cardiovascular diseases. This was apparent in Study II, both in the mixed-sex and men only groups. Study I did show the same tendency, however, the supportive analyses were inconsistent in the case of restricting follow-up time to 10 years, making us interpret those results more carefully. Given the solid support for increased cardiovascular mortality in Study II and in previous literature[40,41,43,46,53,57,58] (Table 1a–1b), and at least partial support from study I, with plausible biological mechanisms and coherence to disease pathology, we find that there is now robust evidence for an association between snus use and increased risk of cardiovascular death.

The risk estimates in Study I, Study II and the previous study by Bolinder et al.[41] are somewhat different. This could be caused by heterogeneity between the eight pooled cohorts in Study I; for example with regard to measurements of exposure, time periods, and population characteristics such as socioeconomic background that may lower precision, and thus attenuate the association. Also, the results from Study II suggest that socioeconomic factors that were unaccounted for may have lowered the risk estimates in Study I.

Cancer mortality

Studies I and II both showed a tendency toward increased total cancer mortality among snus users. As the group of malignant diseases is vast and heterogeneous, it is reasonable to think that while some types of cancer may entail increased mortality among snus users, others will not, and thus this measure of all cancer-related mortality becomes an average, with the highest influence coming from the most common or deadliest types of cancer. To gain further insights into this interesting area, there is a need for more research on mortality from subtypes of malignant diseases among snus users.

As discussed previously, snus contains known carcinogenic substances, and has been shown to increase the incidence of some forms of cancer[188]. Adding the two current studies on snus use and overall cancer mortality, there are now four studies showing, at least in parts, increased risk of cancer mortality among snus users[39,40], while one study showed no risk increase[41]. Although causality between snus use and increased cancer mortality cannot be assured in Study I and II, our results together with the previous findings on cancer-related mortality suggest that there may be certain malignant diseases in which snus use leads to impaired survival, or there may be an increased incidence in particularly lethal cancers among snus users.

Other causes of mortality

“Other cause mortality” in Study I was defined as all cause-specific mortality that was not generated by cardiovascular or cancer diseases. This risk was clearly increased, and the result was supported by additional analyses and dose–response analyses. In Study II, we chose to subdivide this group further into “external causes of death”, and the remaining “other causes”. The results showed that risk of death from external causes (e.g., accidents and suicide) may be increased, at least in some socioeconomic strata. This suggests that snus users may have increased risk of death from mental health disorders and risk-taking behaviour. This in turn may be caused by pre-existing conditions or personality traits that lead to tobacco addiction[32,33]. It is also possible that snus use in and of itself may lead to other behaviours with negative health consequences, as indicated in a study by Norberg et al. showing increased risk of alcohol dependence for snus users[155]. One may speculate that snus use might cause biochemical or structural changes in the brain, e.g., in the reward centre, that renders the user more prone to other types of dependence (demonstrated in animal models and genetic studies[8]), to mental health symptoms, or increased risk-taking.

Interestingly, in Study II, the remaining other causes of death were not increased among snus users, suggesting that higher risk of other cause mortality may be limited to external causes of death.

Socioeconomic aspects

A main concern when investigating health consequences of snus use is that SES is associated with snus use as well as with negative health outcomes, including mortality[144]. In Study II we demonstrate that despite taking several important socioeconomic factors into consideration, the risk of overall and cause-specific mortality remained increased at a level relevant from both an individual and public health perspective. Thus, socioeconomic differences between users and non-users of snus cannot explain the associations between snus use and increased mortality.

The mortality risks for snus users were increased in both more and less favourable socioeconomic groups. Using an overall SES classification, increased risks were found in the medium SES group. That we found the associations between snus use and increased all-cause and cardio-vascular mortality in groups of varying social background indicate that health hazards by snus use are not restricted to certain socioeconomic strata. Our analyses further identified significant interaction between area of residence and snus use, demonstrating the importance of controlling for socioeconomic factors in analyses of health consequences of snus use.

Pathological pathways

Moving forward from the two first studies, we asked ourselves what the mechanisms behind this increased mortality among snus users might be. After finding that previous research shows conflicting results with regard to established cardiometabolic risk factors and is apparently lacking with regard to more recently discovered ones, we proceeded with studies within these two fields.

Established risk factors

Despite the well-demonstrated effects of increased heart rate and blood pressure, increased lipolysis and vascular constriction, we could not find any increase in the commonly-established risk factors of hypertension, obesity, diabetes mellitus, abnormal lipid profile (high triglycerides, low HDL-C), nor in metabolic syndrome prevalence. As mentioned, previous literature shows conflicting evidence, or no increased prevalence among snus users, for many of these risk factors. The association between snus use and diabetes mellitus[81,90,94,97], however, has support in earlier work, but not in our results, perhaps because our participants were still too young to have developed T2D, or due to low power. Obesity has been associated with snus use in several studies [49,84,85,45]. However, many of these studies had not excluded former smokers. A study that did[88] found that the increased risk of obesity in snus users was in fact restricted to former smokers. There is a need for more research on this topic, to evaluate whether the previously reported association perhaps is due to residual confounding.

In Study II, we added a number of potentially-mediating factors to our fully adjusted regression model, to evaluate their importance on the causal path. In line with the results of Study III, there was no major impact on estimates after this addition. Taken together, the increased mortality risks for snus users that we have demonstrated do not seem to be mediated primarily via traditional cardiometabolic risk factors. However, even if blood pressure among snus users is not raised during abstinence (non-use), it will be while snus is used[25,189]. This could influence cardiovascular health, because users generally have a dip in place during a substantial part of the day[1].

Emerging risk factors

Low-grade inflammation

Our results on hs-CRP levels suggest that snus users have lower hs-CRP-concentrations than non-users. If true, snus might have certain anti-inflammatory properties that that may be protective against diseases such as CVD and cancer. The only previous study on the subject show non-increased CRP concentrations among users[73]. Our study is larger and has excluded current or previous smokers, and is therefore more reliable. We conclude from this that low-grade inflammation is unlikely to be an important mechanism linking snus use to adverse health outcomes. However, to more closely evaluate snus effects on CRP, longitudinal studies are needed.

Low vitamin D-concentrations

Study IV showed evidence of lower 25(OH)D levels, and higher odds ratios of having 25(OH)D in the lowest quartile among current snus users. This is to our knowledge the only study on snus use and 25(OH)D, but previous work on smoking and other forms of smokeless tobacco have shown lower concentrations among users. One shortcoming in Study IV is that we did not have possibility to adjust for dietary intake of vitamin D. This should be evaluated in future studies, as it may be an important confounder of the association between snus use and vitamin-D status.

The mechanisms for lower 25(OH)D levels among smokers are still unclear, and there is still uncertainty as to whether it is nicotine or other toxic substances in cigarette smoke that is the cause[133]. Our results on snus use indicate that it may be nicotine that is primarily driving this phenomenon.

Vitamin D has multiple functions in the body, including improved cardiac contractility[190] and immune system enhancement[191]. A number of diseases and conditions have been linked to low 25(OH)D, including cardiometabolic risk factors, mental health symptoms[192], CVDs, cancer[132] and increased overall mortality rates[130–132]. A greater risk of 25(OH)D deficiency among snus users could in itself contribute to, or be a part of another mechanism explaining the increased mortality rates among snus users with regard to both all-cause, cardiovascular, and cancer mortality.

Surprisingly, trials of D vitamin supplementation as a way of preventing morbidity and mortality have not succeed in their mission, possibly due to factors such as ethnicity, vitamin D-status, and genetic variability[193]. This makes preventing low vitamin D levels, for example by tobacco use prevention, even more important.

Increased testosterone concentrations

We found increased levels of testosterone among male snus users in Study IV. This is supported by a recent study showing increased testosterone and reduced sperm count in snus users[194], and by reports of raised testosterone among users of other types of smokeless tobacco[136] and cigarettes[135]. It has been suggested that these associations may be mediated by stimulation of gonadotropin-releasing hormone and luteinising hormone[195] and inhibition of testosterone break down by cotinine[135]. The association between raised testosterone concentrations and nicotine-containing products strengthen the plausibility of an association also with snus use, and the proposed underlying biochemical mechanisms provide a credible explanation of how it may arise.

The evidence base on health effects by testosterone show primarily that low testosterone levels among men is associated with various adverse health outcomes, including increased risk of cardiovascular events and mortality. However, supraphysiological levels of testosterone also have negative effects on the body, increasing the risk of for example hypertension, worsened metabolic profiles and, according to some reports, higher risk of CVD[196,197]. As it appears, testosterone may have both protective and non-beneficial effects[134], and further studies are needed to establish potential health risks by moderately increased testosterone concentrations. Thus, raised free testosterone levels may contribute to increased mortality among snus users, but are not likely to be a main underlying mechanism of this association.

The modern snus user

As previously described, the snus-using population has changed over the last few decades to include men from all socioeconomic strata, and more women (where the socioeconomic patterns are not the same as for men). These societal changes may mean that in older snus-using cohorts, we are studying a population that is different from the modern group of snus users.

While we want to investigate the effects of snus use on health, excluding current and previous smokers is essential. However, whether this approach produces results that are valid for the snus-using population, as a large proportion of snus users also smoke at some point in life, merits discussion. Dual use, and use of snus among previous smokers, may lead to unknown multiplicative interactions that we do not identify in our studies on exclusive snus users. Perhaps it would be closer to reality to study snus use without excluding smokers, although it must then be made clear that it is not the pure snus effect that is studied. We suggest, as a complement to studies on exclusive snus use, deepened research into the dual effects of smoking and snus use.

Aspects on sex and gender

It is known that cardiometabolic risk factors have different impact on the health of men and women. For example, in a meta-analysis using data from 75 cohorts, the relative risk for CVD from smoking was 25% higher in women compared to men[141]. It is unknown whether this is due to socially-mediated differences in smoking patterns or risk-taking behaviours, or related to biological mechanisms, possibly involving sex hormones. As nicotine is the main constituent in both cigarettes and snus, it is clearly important to evaluate the possible health effects of snus use for men and women separately. However, due to few female exclusive snus users in current cohorts, this has been difficult so far.

Public health relevance

Snus and tobacco harm reduction

There has been an interest in finding alternative ways of reducing the vast global burden of disease and death caused by tobacco smoking, known as tobacco harm reduction. Within this movement, there have been public health workers, researchers and representatives of the tobacco industry working to promote snus as a possible means of encouraging smoking cessation among smokers unable or unwilling to quit. Sweden has been viewed as a successful example of how the wide-spread use of snus has contributed to the low rates of cigarette smoking, and therefore also to lower death tolls in, for example, lung cancer.

In theory and at a first glance, this may seem an appealing strategy, but there are weak points. First, it is uncertain whether snus would actually be effective as a cessation aid or substitute for inveterate smokers[198,199]. There have been studies showing increased quit rates among Scandinavian snus users, but these studies have a low degree of evidence[200]. Other studies could not find any benefit, some even showing that snus use may serve as a gateway to smoking, thus increasing smoking rates when the intention was the opposite[201–204].

Secondly, snus is not a harmless alternative to smoking. As previously discussed, there are a number of known negative health consequences to snus use, and also several fields where there is too little knowledge. If snus is to be used as a recommended cessation aid, the possible public health gain must be clearly proven, which is currently not the case.

A third reason for the unsuitability of recommending snus use to smokers is that studies have shown that this may frequently result in dual use of snus and cigarettes[33,202]. Although by some seen as positive because of reduced number of cigarettes per day, dual use is not well understood[205]. It is possible that the use of cigarettes and snus may interact to pose serious health hazards to the user[206]. Experience from the introduction of light cigarettes—which failed due to in part individual changes in smoking behaviour, but also in part because the conversion of toxins was actually greater at lower exposures—bids us to thoroughly examine the effects of dual use before recommending snus for the purpose of smoking cessation[205]. Also, previous work show that dual users experience stronger dependence and increased withdrawal symptoms during quitting attempts[207].

Taken together, the evidence for snus use as a successful as a strategy to reduce smoking in society is low, and there are indications that such efforts would put individuals at greater risk and result in lower quit rates. At the same time, nicotine replacement therapy (NRT) offers safe and at least moderately-effective means of providing nicotine in controlled doses, without known carcinogens, to users who wish to quit or reduce their smoking[200]. While snus is not shown to be superior to NRTs, the latter should be chosen in accordance to evidence.

Clinical implications

The results from Studies I–IV are highly relevant from clinical and public health perspectives, especially in populations where snus use is widespread. They are also important in discussions on legal and harm reduction aspects involving snus. Further, they are easily incorporated into clinical practice. Apart from a general appeal to any snus user to quit this habit, it should be especially emphasised among individuals with high cardiovascular risk or manifest CVD. For public health benefits, efforts to prevent snus introduction should be intensified, and more resources put into snus cessation aid.

Future perspectives

There is still much to be learned about the potential health effects of snus use. Long-term health consequences of snus use among women should be addressed. Evaluating mortality rates of different cancers may reveal that mortality in some forms of cancer is associated with snus use while others are not. The associations seen between snus use and hs-CRP, 25(OH)D and testosterone should also be explored in more depth, for example regarding vitamin D-status and dietary factors in snus users, and a potential impact of snus use on fertility among men and women. Other interesting research fields include the microbiotic content of snus and its influence on health, and the dual effects of smoking and snus use.

Conclusions

From this thesis, in mixed-sex and men only-groups, we conclude that:

- Snus use is associated with increased all-cause and cardiovascular mortality, even after comprehensive adjustments for health measures, lifestyle, and socioeconomic factors. Snus use may also be associated with increased cancer mortality.

- Snus use is associated with increased mortality of causes other than cardiovascular and cancer diseases. This association may be restricted to death by external causes.

- Snus users of both more and less favourable socioeconomic background have increased risk of death. The associations between snus use and mortality cannot be explained by socioeconomic differences between snus users and non-users.

- Snus use over the life-course does not seem to be associated with metabolic syndrome or its individual components (obesity, hypertension, altered blood lipids, T2D or impaired glucose metabolism). It is unlikely that these risk factors would constitute the main pathways linking snus use to increased mortality.

- Snus use is associated with lower concentrations of biomarkers representing low-grade inflammation and vitamin D-status in both sexes, and higher concentrations of free testosterone in men. Lower vitamin D-concentrations and increased testosterone levels may contribute to, or be part of, mechanisms explaining higher mortality among snus users.

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