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# The effect of oral uptake of nicotine on skin blood perfusion of the face and hands in snus users as determined by thermography.

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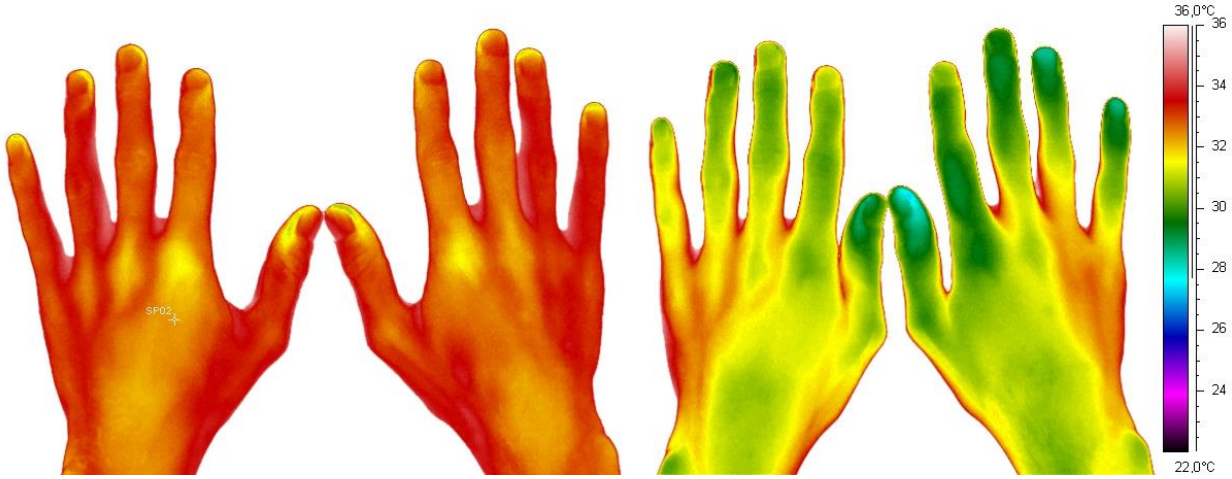
Master thesis in Biomedical sciences May 2014




UNIVERSITY OF TROMSØ

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**Ina Isabella Høiland** **Tromsø, May 2014**

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## Abbreviations

**ANS**: Autonomic nervous system

**CNS**: Central nervous system

**DA**: Mesolimbic dopamine

**DIRT**: Dynamic infrared thermography

**EP1**: Experimental Protocol 1

**EP2**: Experimental Protocol 2

**ES1**: Experimental Setup 1

**ES2**: Experimental Setup 2

**ISO**: International Standards Organization

**IR**: Infrared

**Ach**: Acetylcholine

**NA**: nucleus accumbens

**nAchR's**: Nicotinic acetylcholine receptors

**NNAL**: Cotinine 4-(methylnitrosamino)-1-(3-pyridyl)-butanol, tobacco specific nitrosamine

**ROI**: regions of interest

**ST**: Smokeless tobacco

$\mathbf{T_{me}}$: maximum skin temperature in the inner canthus of the eyes

$\mathbf{T_{mf}}$: mean facial skin temperature

$\mathbf{T_{mh}}$**:** mean skin temperature for the back of hand

$\mathbf{T_{mm}}$: mean skin temperature for the ROI covering the mouth area

$\mathbf{T_{ms}}$: mean finger skin temperature

## Summary

In recent years the number of people using cigarettes in Norway has significantly declined, with intake of nicotine being replaced by smokeless tobacco (ST) products such as snus placed in the oral cavity. While health risks from smoking cigarettes are well known little is known about health risks of using ST. The main aim of this thesis was to compare the effects of oral use of snus with nicotine (SN+) and snus without nicotine (SN-) on skin perfusion in the hands and face in young habitual users of snus. Skin perfusion was indirectly monitored by measuring changes in by skin temperature using infrared thermography. The main findings of this study were a strong decrease in skin perfusion in the hands in the SN+ subjects, with little or no effect in the SN- controls. SN+ had no effect on the skin temperature of the inner canthus of the eye, which will be of interest to those using thermography for fever detection in pandemic screening situations. The clear physiological effects of using snus containing nicotine demonstrated in this study justify advising patients to avoid using snus containing nicotine prior to surgery and in the immediate period after surgery. To my knowledge this study is the first to show the negative effects of SN+ on peripheral circulation.

# Introduction

It is a well-known fact that cigarette smoking has several different negative effects on health [1]. There are approximately 4000 different chemical substances in cigarette smoke and a significant amount of these are toxic [2, 3]. Some of these toxic substances are known to influence the cardiovascular system, sexual function and lung function [3]. One of the well-known toxic substances in cigarette smoke is nicotine, a toxic alkaloid that can be isolated from the tobacco plant. The inhalation of cigarette smoke is probably the most known route of administration of nicotine. However, there are other routes for the uptake of nicotine in the body, for example through the mucosal membrane in the mouth by chewing gum, snuff and snus, or through the skin by transdermal plasters.

Over the last decade the use of snus has become increasingly popular in Norway and Sweden, especially among the younger generation. This is presumably related to changes in the law that made cigarette smoking in public buildings illegal from the year 2004. Because of the increased use of snus it is important to be aware of the potential negative health effects that snus may have on bodily functions. Snus contains, among other things, nicotine which is known for its addictive effects but also for its physiological effect on peripheral circulation [4]. Peripheral circulation can be indirectly measured by measuring skin temperature using thermography where the temperature of large skin areas of interest can be simultaneously measured with a high degree of accuracy using an infrared camera [5, 6].

The aim of this thesis was to evaluate the possible effects snus may have on skin perfusion by measuring skin temperature changes following administration of snus after a short period of abstinence. It is hypothesized that an effect on skin perfusion may be expected from nicotine through its absorption via the mucosal membrane of the oral cavity. The dorsal aspect of the hands was selected to represent the main area of investigation. In addition, skin temperature on the face was also measured in order to see whether there were local effects on skin perfusion around the area where the snus was normally placed within the oral cavity, that is, under the

upper lip as well as the inner canthus of the eyes since this area is of interest in relationship to fever detection using mass screening thermographic systems [7].

## Nicotine

Nicotine is a toxic alkaloid that is found in a family of plants named Nightshades or Solanacae. The plant which is used for isolating nicotine for commercial use is the *Nicotiana tobacum* plant, which is named after the French ambassador Jean Nicot in Portugal [8]. It is believed that tobacco was sniffed, chewed, eaten and drunk by the Native Americans for medical and ceremonial purposes before 1 B.C. [8]. However, tobacco use can be traced back to as early as 1000 B.C., with traces of cultivation sites of the tobacco plant in Mexico dating back to 1000-1400 B.C. [9]. In 1828 Posselt and Reimann isolated and characterised nicotine as a poison and identified nicotine as the main pharmacoactive ingredient in tobacco [9]. The chemical formula was discovered in 1843 by Melsens and in 1893 and the first synthetic nicotine was made in 1904 by Pictet and Crepieux [9].

In the human body, nicotine resembles the endogenous signal molecule acetylcholine (Ach) (Fig. 1). Nicotine binds to nicotinic acetylcholine receptors (nAchR) [10]. These receptors are ligand gated ion channels and binding of a ligand, that be nicotine or acetylcholine, can lead to depolarization of the cell, release of transmitters or gene transcription [11]. Nicotinic acetylcholine receptors are found in the central nervous system, throughout the brain on both excitatory and inhibitory neurons. Nicotine has the ability to stimulate antagonistic pathways in the central nervous system (CNS) through several nAchR subtypes that possess different sensitivities to activation and desensitization by nicotine. Stimulation can increase inhibition of circuits when excitation is high and increase excitation when circuits are less active [12]. The result of this circuit-level integration is that nicotine can modulate behavioral function in a bidirectional way [10]. The main target of nicotine is located in the brain where a subfamily of nAchR’s are responsible for depolarization and transmitter release when stimulated [12]. The released transmitters can be both glutamate (excitatory) and GABA (inhibitory), which makes the effect of nicotine complex [12].

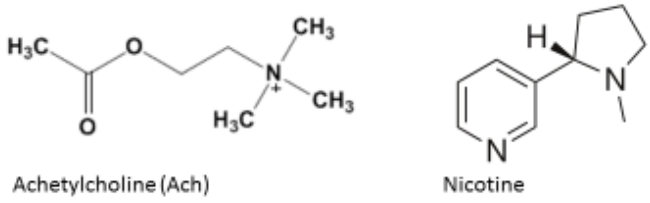


Fig. 1. The chemical structures of Ach and nicotine respectively.

Nicotine stimulates nAchRs in the mesolimbic dopamine (DA) system which is a central mediator of drug reward and reinforcement located in the ventral tegmental area [13, 14]. One of the brain areas where the DA system projects to is the nucleus accumbens (NAcc). NAcc is the brain's pleasure centre. Stimulation of the DA system leads to an increase in dopamine release in the NAcc which gives a feeling of wellbeing. This is also called the reward circuit. Stimulation of the DA system will also greatly attenuate nicotine self-administration [10] as sstimulation leads to a positive feedback resulting in a desire to continue the stimulating behaviour (that is using nicotine). This mechanism, together with the fact that nicotine stimulation of nAchRs leads to synthesis of more nAchRs makes nicotine a highly addictive substance [14, 15]. Nicotine can have various behavioural effects in humans and animals since nicotine influences distinct neuronal pathways that express different subtypes of nAchRs. Small differences in the activation state or sensitivity of neuronal pathways can therefore result in large differences in the behavioural responses produced by nicotine among individuals [10].

Nicotine has a half-life of approximately 1-2 hours. Around 1% of the absorbed nicotine is excreted by the kidneys and can be detected in the urine, depending on the pH of the urine. 70-80% is metabolized in the liver to cotinine by Cytochrome P450 2A6 enzymes [16]. Cotinine has a longer half-life than nicotine and is further metabolized to trans-3′-hydroxycotinine (3-OH-Cot) that has a half-life of 7hours [17]. In order to obtain an accurate level of serum nicotine it is necessary to measure the concentration of nicotine in combination with the concentration of these two metabolites [18] .

## *Smokeless tobacco -ST*

In recent years cigarette sales have declined in the Western World probably due to more awareness concerning the health risks associated with smoking. In addition, the decline may be explained by new and more restricted regulations in use and sales of cigarettes. In Norway smoking is forbidden in public buildings and tobacco advertising is prohibited. The tobacco industry is trying to promote a "new face" for tobacco by developing products that are a less health risk choice compared to cigarettes. Smokeless tobacco (ST) is one such product. In 2011, 25% of the young people between 18 and 30 years in Norway were smoking, and 31% reported using ST on a daily basis [19]. ST is orally consumed and not burned and this makes its use legal in places with anti-smoking regulations. There are a variety of different types of ST products and the use of the different types is, to a great extent, linked to geography [8, 20].

In India smokeless tobacco is used by over 25% of the people, with Gutkha being the most common [20, 21]. This is an oral ST product and is placed inside the cheek and chewed and the salvia is either spat out or swallowed. In America, snuff is widely used. This is a fine grounded dry tobacco that is inhaled through the nose. Dipping tobacco is a moist form of snuff which is placed between the lower lip and gum, not chewed and the saliva is spat out. Snus is another oral smokeless tobacco product which is placed between the lip and gum, without any spitting. The traditional Swedish snus is the preferred snus type in the Scandinavian countries including Norway. This snus type was developed during the early 1800s. It was made of ground tobacco leaves, water, salt and potash, and its composition has hardly changed today. Up to about the early 1940s snus was the predominant form of tobacco used in Sweden, until cigarette smoking took over in the late 20$^{th}$ century [22]. Snus has never been marketed as a product designed to help people to cease smoking although many Scandinavian smokers have replaced cigarettes with snus after the health hazards of smoking became clear [22, 23]. There are several differences between Swedish- and American snus. American snus has generally lower moisture content, American snus having a water content of 15-30% compared to a water content of around 50% in the Swedish snus. The placement is normally under the upper lip for the Swedish type and under the lower lip for the American type, neither is chewed [24, 25]. The manufacturing processes is also different between the two snus types resulting whit the Swedish snus has a lower pH than the

American type and thus, a higher bioavailability of nicotine [24, 26]. There is on average a higher nicotine content in Swedish snus compared to both American snus and cigarettes.

ST produces sympathomimetic effects similar to those produced by cigarette smoking. Smoking and ST increase heart rate and epinephrine levels immediately after administration [27] and both has been associated with death from cardiovascular disease, cerebrovascular disease and cancer [28]. ST-users and cigarette smokers have an increased risk of developing diabetes type 2 after long-term use [29]. Nicotine's action as a sympathomimetic drug is responsible for the increase in heart rate and blood pressure seen in ST-users and cigarette smokers [30, 31]. Most current studies have focused on the effect of cigarette smoking or ST products in general, with few studies designed specifically to look at the effect of snus. In a study by Overland, *et al.* they found higher levels of high density lipoproteins and higher systolic blood pressure in snus-users compared to non-snus users [32].

Snus contains about 2500 different chemical substances compared to 4000 in cigarette smoke [33]. The Norwegian health authorities (Helsedirektoratet) states that at least 30 of these substances are carcinogenic and 40-50 substances from tobacco have a possible negative significance on health [22, 34]. Snus contains a high level of tobacco specific nitrosamines although these levels are not as high as in cigarettes [8]. One of these nitrosamines is Cotinine 4-(methylnitrosamino)-1-(3-pyridyl)-butanol (NNAL) [34], a carcinogenic substance that can be used as an exposure biomarker. Interestingly, when switching from cigarettes to snus there is a 50 % decrease in urinary NNAL. It is still unclear if a 50% reduction in NNAL in urinary output leads to a reduction in cancer [35]. Since 1992 snus has been illegal in the European Union, however Sweden is an exception to this rule, and Denmark has a partial exception [33]. The use of snus in Norway is legal [4]. The World Health Organization (WHO/Europe) has classified snus as carcinogenic and the Norwegian health institute (Folkehelseinstituttet) states that snus is as addictive as cigarettes, if not more so [36]. Recently, Swedish snus was launched commercially in the USA [19, 32]. In Norway there is a clear trend showing that the use of cigarettes is declining and that more people are using snus. In 1983, 247 tons of snus were sold in

Norway. That amount had increased to 1125 tons in 2008 [37]. In 2013, 14% of Norwegian men (16-74 years) used snus compared to 4% of Norwegian women [37].

Snus is chemically basic and the target pH in the production of traditional snus brands is approximately 8.5 [22]. This makes the nicotine un-protonated, and thus more easily absorbed by the body (higher bioavailability) [15]. Snus is a moist form of tobacco, used as loose grounded tobacco or contained in sachets or pouches similar to small teabags [25]. Snus users can obtain high plasma concentrations of nicotine [38, 39] and snus users need nicotine plasters with a higher dose of nicotine compared to cigarette smokers for successful ST cessation [39]. There are large individual differences in the time of use but generally it ranges from 20 minutes to 4 hours. The concentration of nicotine may differ from sachet to sachet. The amount of nicotine in each portion of snus also depends on the serving size [25]. In contrast to cigarettes, where nicotine is taken up by the lungs, nicotine from snus is taken up though the mucous membrane in the mouth before entering the blood stream. The absorption of nicotine is slower when absorbed over the mucous membrane [33]. An average cigarette contains approximately 9 mg of nicotine while one portion of snus (1g) contains 4-18 mg of nicotine, depending on the type and brand. The fact that the snus users obtain a higher mean plasma blood concentration of nicotine compared to a cigarette smoker [13, 39] is most likely due to the higher concentration of nicotine in snus and the duration of use [40].

There are some health benefits associated when switching from cigarettes to smokeless tobacco, e.g. decreased risk of lung cancer and less negative effects on the respiratory system [41]. In Sweden the use of cigarettes has declined and is the country with the lowest percentage of daily smokers in the Scandinavian countries. This is thought as being due to the fact that cigarette smokers switch to the less harmful tobacco products such as snus [25]. However, the use of smokeless tobacco, including snus is associated with health risk as it can lead to periodontal disease, cancer and cerebrovascular and cardiovascular diseases [4, 42]. Nicotine is highly addictive and it is hard to refrain from using nicotine once the brain and body has become addicted. The withdrawal symptoms of a person who tries to cease using snus are the same as seen in a person trying to cease smoking. Ebbert *et al*. found that nicotine replacement therapy

does not increase the long term tobacco abstinence period in ST users [42], supporting the finding that the overall nicotine exposure in ST users is greater.

## Peripheral blood circulation

The skin is the largest organ of the human body. Its blood supply can change many fold in response to thermal requirements. The core of the body is maintained at a more or less constant temperature [43]. From a thermoregulatory point of view it is the heat carrying capacity of blood and the physiological regulation of blood flow that are important for the heat exchange with the environment. In a situation where the body needs to conserve heat, skin blood perfusion is dramatically decreased due to vasoconstriction, especially in the acral body parts (hands and feet) [44, 45]. When the body needs to loose heat in warm situations or when exercising, heat and skin blood perfusion increases dramatically through vasodilatation of the blood vessels in the skin. In the cold, blood flow to the skin represents only a few percent of cardiac output whereas in the heat this can increase to as much as 30%. The skin surface temperature is determined by the rate of heat exchange between the surroundings and the body core [43].

The human skin consists of two layers, the outer epidermis that forms a waterproof barrier and the underlying dermis that supports the epidermis. The blood supply to the skin has three major functions: 1) Transporting nutrition and oxygen to and removing waste products and carbon dioxide from cells, 2) immune defence and 3) the regulation of body temperature (thermoregulation). The skin is supplied from two different vascular plexi: the subdermal plexus and the superficial plexus. The subdermal plexus is located just below the dermis and branches from this plexus supply the superficial plexus, which lies at the junction between the dermis and epidermis [46]. There are so called arteriovenous anastomoses (AVAs) in the fingers (in the finger tip, under the finger nail), face and other acral parts of the body. This allows thermoregulation within a wide range in response to temperature changes [47].

Most of the skin blood supply is directed to the dermis, with being located in the subcutaneous fat layer located below the dermis. The fat layer is a supporting- and isolation-layer for the arteries, as well as an energy depot for the body. In the dermis there are many dividing cells that require an adequate blood supply for their metabolic needs [46]. The arteries and vessels that are thought of as being involved in thermoregulation are located near the surface of the skin and blood perfusion in these vessels can be indirectly monitored with thermography [48, 49].

Blood transports heat from the core of the body to the surface of the skin where it is released to the surroundings, assuming that the temperature of the surroundings is less than the body temperature. In other words, when the blood vessels in the skin vasodilatate and skin blood perfusion is increased there is a rise in skin surface temperature. Vasoconstriction of the blood vessels will lead to a drop in skin surface temperature. This temperature change can be monitored by an infrared (IR) camera. Measuring the skin temperature with an IR camera provides us with an indirect measurement of blood flow. That thermography provides us with a reliable indirect method for measuring skin blood perfusion has been confirmed in studies comparing thermography with other methods for measuring skin blood flow, such as Doppler [50, 51].

### *Control of peripheral circulation*

The autonomic nervous system (ANS) is part of the peripheral nervous system and is involved in involuntary regulatory processes in the body, such as respiration, cardiac regulation, vasomotor activity and certain reflex actions [52]. The ANS is divided into the sympathetic and the parasympathetic nervous systems which, for the majority of the body's homeostatic regulatory systems, have opposing regulatory functions. In both systems there are normally two connecting neurons between their origin and the target organ, the so called pre-ganglionic and post-ganglionic neurons. The main preganglionic neurotransmitter for both divisions of the autonomic nervous system is Ach [53]. This is also the post ganglionic neurotransmitter for the parasympathetic nervous system, working at the target organs. For the sympathetic nervous system, the post ganglionic neurotransmitter is noradrenalin that acts on adrenergic receptors on the target. Ach is responsible for the release of excitatory neurotransmitters as noradrenalin and

adrenaline. Nicotine has an effect on this part of the nervous system, stimulating sympathetic nerves to release noradrenalin and adrenaline due to its spatial similarities to Ach. Other sedative relaxant neurotransmitters like serotonin and β-endorphin are also released by the nicotinic acetylcholine receptor mechanism and their effects become more apparent at higher dose of nicotine [54].

In response to heat and cold stresses thermoregulatory reflexes regulates blood flow to the skin. These reflexes exert their effects on the skin circulation through two branches of the sympathetic nervous system - a noradrenergic vasoconstrictor branch and a vasodilator branch. The active vasodilator system is responsible for most of the vasodilator response to heat stress and can increase skin blood flow > 10-fold [55, 56].

Noradrenaline is a neurotransmitter that leads to contraction of the peripheral blood vessels in response to cold exposure to the skin. This reaction can also be seen after nicotine administration. There is a nicotine-dependent stimulation of the sympathetic system and an inactivation of vagal cardiovascular control [57]. Acute smoking causes a marked increase in sympathetic nerve activity to the skin and thus cutaneous vasoconstriction [58]. Decrease in skin temperature in the hands was reported after smoking a cigarette, possibly due to the effects of nicotine [55]. The result was explained by a contraction of peripheral blood vessels leading to a decrease in skin perfusion as indicated by a fall in skin temperature seen during thermography. The same effect is expected to be seen in those using smokeless tobacco.

## Thermometry and physics of heat transduction

As early as the time of Hippocrates the importance of body temperature in medicine was recognised. Wet mud was smeared onto the skin to observe how fast it dried out, the warmer the skin, the faster it dried [50]. This was the approach for measuring temperature until the 16$^{th}$ century [59]. The first thermoscope was made by Galileo from a glass tube, but measuring temperature was

a subjective skill until Santorio Sanctorius developed the first thermometer in 1611 [50]. The first medical thermometer was developed by Dr. Carl Wunderlich in 1868. This thermometer had a scale that was limited to around 37 degrees Celsius [50]. Today measuring the body core temperature in different bodily cavities such as rectum, oral cavity, ear canal, bladder, oesophagus and in the axilla are commonly carried out as part of normal clinical routine. Measurements of skin temperature are rarely carried out even though there are many situations, as we know from studies employing thermography, were such measurements do provide valuable clinical information [60].

Heat transfer to and from the body can occur in four different ways: 1) Conduction, direct contact between an object and a heat source. 2) Convection, heat transfer between a solid form and a liquid or gas which moves relatively to the solid form. 3) Radiation, exchange of heat by electromagnetic energy between objects having different temperatures. 4) Evaporation, conversion of liquid in to vapour. The main avenues of heat exchange in a lightly clothed person placed in a room at normal room temperature (22-23ºC) are shown in Fig. 2, where approximately 60% of the heat loss from the body is by infrared radiation [61, 62]. Both convection and radiation are used in remote temperature detection methods [59]. Convection from the human body can be visualized by a technique called Schlieren photography. This makes use of changes in refractive index and which are made visual by special illumination. This technique is mostly used to monitor heat loss in experimental subjects, e.g. designing clothing for people working in extreme physical environments [59]. Heat loss from radiation can be measured by thermography [59].

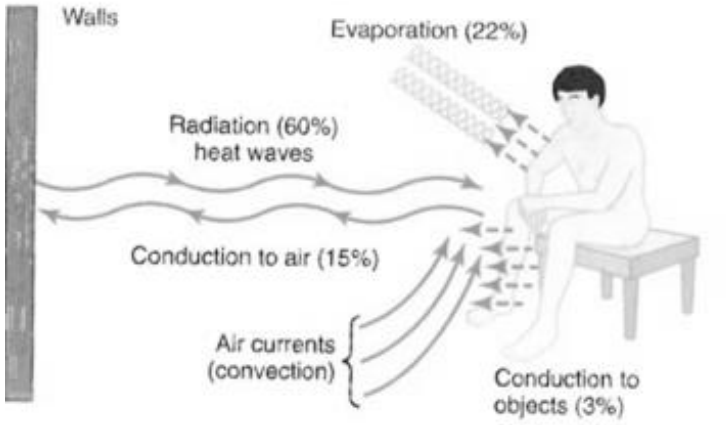


Fig. 2. Heat transfer from the human body [63].

## Thermography

Thermography (infrared thermal imaging) makes use of the fact that all objects above absolute zero (-273 $^{o}$C or 0$^{o}$K) emit energy in the form of electromagnetic radiation [43]. These waves are called infrared radiation and are not visible to the human eye. Since all objects on earth are above absolute zero, all objects emit this radiation called natural or thermal radiation [43]. The wavelengths of the infrared spectra are between 700nm-1mm and lie between that of visible light and microwaves (Fig. 3).

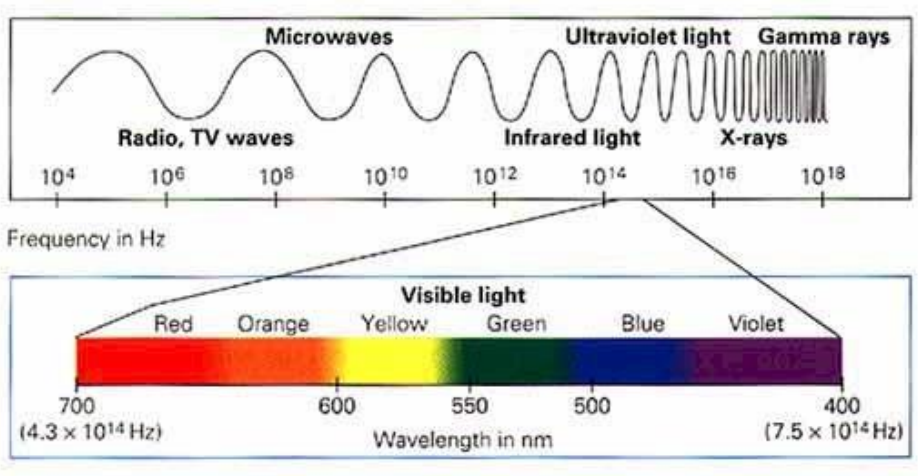


Fig. 3. The electromagnetic spectrum.

The intensity of the emitted infrared radiation from an object directly varies with the objects surface temperature. Infrared cameras provide us with visible images of the otherwise invisible emitted infrared light.

## *Emissivity*

Emissivity is the ability an object has to emit energy by radiation. The emissive power of an object is defined as the heat flux per unit time and reflects the energy it radiates [64]. The radiation is compared to the radiation of a so-called perfect black body. A perfect black body is an object that absorbs all incident radiation and radiates in a continuous spectrum as described by Planck's law [46]. The energy an object emits depends on its temperature and emissivity, the warmer the object the more energy it radiates. The human body has an emissivity coefficient of 0.98 which is close to the emissivity of a perfect black body (1.0) [54, 65]. Modern infrared (IR) cameras have settings which can be adjusted to the emissivity coefficient of the object examined. The distance to the object and air humidity are other factors that can influence the readings from an infrared camera. Corrections for these conditions can be independently adjusted in modern IR-cameras.

## *Radiometric images, sensitivity and colour coding*

An IR camera detects and records the electromagnetic radiation from an object. Through digital processing the camera provides a visual display of the amount of electromagnetic radiation emitted, transmitted and reflected by an object [65]. Recent advances in digital infrared camera technology, including state of the art image analysis software, have greatly improved the usefulness of this technology for examining skin blood perfusion. In a fraction of a second it is possible to measure skin surface temperatures over large areas to a high degree of accuracy. The IR cameras used in this thesis have a high thermal resolution of 0.1°C (methods).

The electromagnetic video signal is a direct measurement of temperature. Modern camera software provide highly detailed values of temperature in the recorded thermal images-

radiometric images. The final detail is dependent on the number of thermal sensors contained within the camera sensor system, called focal plane arrays. The processed visual images provided by IR-cameras are initially processed as grey scale images (from black to white). However, in order to make the temperature distribution easier to visualize further digital processing can produce coloured images, where the different colours represent different temperatures. For medical thermography the International Standards Organization (ISO) recommend that the so-called rainbow palette should be used (Fig.4), which was also the colour palette used in this study [66]. The temperature range can be set according to the object measured, for human skin a range of 22$^{o}$C- 37$^{o}$C is normally suitable.

Fig.4. The rainbow pallet used in the IR pictures. The scale goes from cold (purple/black) to red/white (warm).

## *Thermography today*

The use of Infrared thermography or infrared thermal imaging in medicine is called medical thermography. This method is based on analysis of skin surface temperatures as a reflection of normal or abnormal human physiology using specialized IR-cameras. Today, thermography has become one of the most efficient techniques for the study of skin temperature. Modern infrared digital cameras employing focal-plane array technology provide a sensitive diagnostic tool for a multitude of clinical and experimental situations [67]. Many studies have shown thermography to be a useful tool in research as well as being helpful in the diagnosis of various forms of cancer, nervous system disorders, metabolic disorders, neck and back problems, pain syndromes, arthritis, vascular disorders, and soft tissue injuries, among others [68-72]. Thirty years of clinical use and several thousand peer-reviewed studies in the medical literature have established thermography as a safe and effective means to examine the human body [73]. This technique is completely non-invasive and does not require the use of ionizing radiation or other potentially harmful elements. The use of medical thermography in reconstructive surgery is well established

and routinely used at the University Hospital in Northern Norway (UNN) where this study was carried out.

In recent years there has been a large interest in using thermography for detecting whether a person is febrile or not in a mass screening scenario, for example during a pandemic situation [74]. Implementation of pandemic infrared thermographic screening is based on the detection of febrile temperatures, usually using the inner canthus of the eyes as the target site. Using thermography for fever screening is not without debate and there is some concern about the exactness of the relationship between deep body temperature and infrared thermal images of selected skin areas on the head [7]. There are few scientific validation studies that have specifically carried in this area, most been made on children [7]. To my knowledge there are no studies that have specifically examined the effect that nicotine use may have on facial skin temperature, especially around the inner canthus of the eye.

### *Static and Dynamic Infrared Thermography*

There are 2 basic forms of infrared thermal imaging, static and dynamic. In static IR thermography a single image is taken [65]. From these images the spatial temperature distribution over the area of interest can be measured. The interpretation of such an image depends on the temperature distribution over the area. In clinical medicine the distribution of body surface temperature is basically symmetrical between the left and right side of the body [75]. The face hands, arms, feet and legs show a high degree of thermal symmetry [76, 77]. Clinical thermographers often make use of this fact where an asymmetrical thermal distribution between two identical skin areas on the left and right side of the body, may indicate a pathological condition on one of the sides.

Static thermography gives little information on the dynamics of skin blood perfusion. Dynamic infrared thermography (DIRT) is a technique that involves taking a sequence of images over time and provides the user with useful clinical information on the dynamics of skin perfusion. The technique involves increasing the recording frequency of individual images. In modern infrared

cameras the recording frequency is selected by the operator. Some modern IR cameras have scanning frequencies as high as 50 Hz. By using DIRT changes in skin temperature can be continually monitored and recorded, for example following some sort of intervention such as a thermal challenge using fan cooling, water immersion or by applying cold/warm object to the skin surface. Alternatively one can monitor dynamic changes in skin perfusion following a chemical intervention. This was the form used in this study when monitoring the effect of nicotine containing snus on skin blood perfusion.

## Aim of study

The main aim of this master thesis was to investigate the effects nicotine has on skin blood perfusion using the facial and hand skin as target sites in healthy young subjects when using snus. A secondary aim was see whether the temperature of face, in particularly the inner canthus of the eye, a facial temperature site normally used in mass screening systems for fever detection is affected by snus containing nicotine

Infrared thermography was used to indirectly monitor skin blood perfusion during a period of snus use. Infrared thermography has been used in similar studies in the past. For example in a study by Miland *et. al* (2006) the effect of a short period of nicotine abstinence on skin surface temperatures was studied. In their study skin blood perfusion changes on the dorsal side of the hands was studied in both young and old smokers and non-smokers using thermography and thermocouples before, during and after immersing the right hand for 2 min in water at 10°C [55]. To my knowledge there are no studies that have used thermography as a technique to evaluate the effect snus may have on skin blood perfusion on the hands and the facial skin.

The study was carried out in a special laboratory for thermography (LAB 3) at the Department of radiology, University Hospital of Northern Norway (UNN), supervised by Professor James Mercer, Medical Imaging Research Group, Department of Medical Biology, Faculty of Health Science, The Arctic University of Norway (UiT).

# Methods

In this study 2 experimental protocols were used, a main study, Experimental Protocol 1 (EP1) and a follow-up study, Experimental Protocol (EP2). The two protocols employed two slightly different experimental setups as described below (Experimental Setup 1 (ES1) for EP1 and Experimental Setup 2 (ES2) for EP2).

## *Subject recruitment*

In the main study (EP1) 15 young healthy volunteers aged between 19 and 32 year (4 males and 11 females) participated (hereafter referred to as the subjects). They were recruited via Facebook or from the University of Tromsø community. Only daily snus users were accepted, with no considerations as to how often they used snus, how much snus they used or the nicotine strength of the snus. Each subject was subjected to 2 experiments – one in which they received snus containing nicotine and a second experiment in which they received snus without nicotine as a control experiment. The order of nicotine/no nicotine was randomized and the experiments were performed on different days. The subjects were not informed whether they were receiving snus with or without nicotine.

The study was approved by the regional committee for medical and health research ethics in Northern Norway (Regionale Komiteer for Medisinsk og Helsefaglig Forskningsetikk Nord). The participants were informed verbally as well as receiving written information and had to sign a consent form before participating in the experiment.

## *Snus*

The snus used in the experiments was of the Swedish brand Skruf (Skruf sterk #3 – Fig. 5). This is a pouched snus with a nicotine content of 8 mg per portion of 1 g. The nicotine free snus was the brand Onico, which is supposed to taste, look- and smell like regular snus with nicotine. For

both types of snus a single pouch weighted 1 gram. Throughout the remainder of this thesis snus with nicotine and snus without nicotine will be referred to as SN+ and SN- respectively.


Fig. 5.The type of snus used in the experiment A) Skruf Stark #3 with nicotine B) Onico without tobacco or nicotine.

## *Infrared cameras (IR cameras)*

Two different infrared (IR) cameras were used in the main study (ES1). One was used for taking images of the facial area (FLIR ThermaCAM™ S65 HS, FLIR systems, Sweden) and one for taking images of the dorsal aspect of the hands (FLIR ThermalCAM™ SC645). Both cameras have a resolution of 0.1 $^{o}$C. The emissivity of both cameras was set to 0.98. In the follow-up study (ES2) only one of the cameras was used (FLIR ThermalCAM™ SC645).

The rainbow palette was used in this study as recommended by the ISO [66]. The images were stored on a pc for further analysis. The stored image were processed using image analysing software ThermaCAM Researcher pro 2.8 SR-1 (FLIR Systems AB, Boston, MA, USA).

### *Experimental Setup 1-ES1*

The experiments were carried out at different times of the day (08.00-17.00). Room temperature could not be independently controlled and was usually between 22°C and 23°C, but on a few occasions was as low as 19°C. Air movement within the windowless room was measured at head height of the sitting subjects using a sensitive air movement sensor (AirFlow TA-5 Thermal Anemometer, AirFlow developments limited, United Kingdom) and found to be less than 0.1 m/sec.

In ES1 the subjects were dressed in normal indoor clothing and were seated on a chair throughout the experiment. During periods when thermal images were not being recorded the subjects were allowed to let their hands hang as free as possible without contacting any surface. During the short period (ca. 15 sec) when thermal images were being recorded, the hands were positioned palms down on a grid made of thin nylon netting strung on a wooden frame (Fig. 6). The nylon grid also minimized skin contact with the surface supporting the hands. To provide a constant background temperature an electric heating plate which had an even surface temperature of ca 40ºC was placed 3 cm below the grid. The short period during which the hands were placed on the grid avoided unnecessary heating of the hands by the underlying heating plate. During the experiments the subjects were able to observe a monitor on which the live thermal images were displayed. In this experimental setup both IR cameras were used. One of the cameras was directed towards the facial area and the other towards the dorsal surface of the hands (Fig. 6).

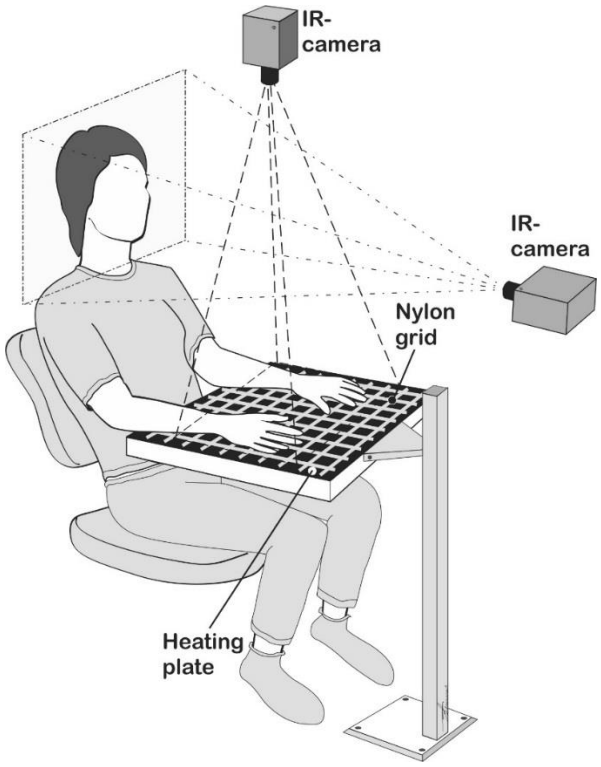


Fig. 6. Schematic illustration of Experimental Setup 1.

## *Experimental Setup 2-ES2*

The setup for ES2 was similar to that described above for ES1 (Fig 7) with the following differences. The facial area was not examined and only the temperatures of the dorsal aspect of the hands were monitored.

During the entire experimental period (30 minutes) the subjects sat with the hands resting on the nylon grid. In the intervals when IR images were not being recorded a thick piece of cloth was placed between the palmar surface of the hands and the nylon grid to avoid heating of the hands by the underlying warming plate. The cloth was removed during the short periods (ca. 15 sec) when IR images were being recorded. In contrast to ES1 the subjects were unable to observe the live thermal images on a monitor.

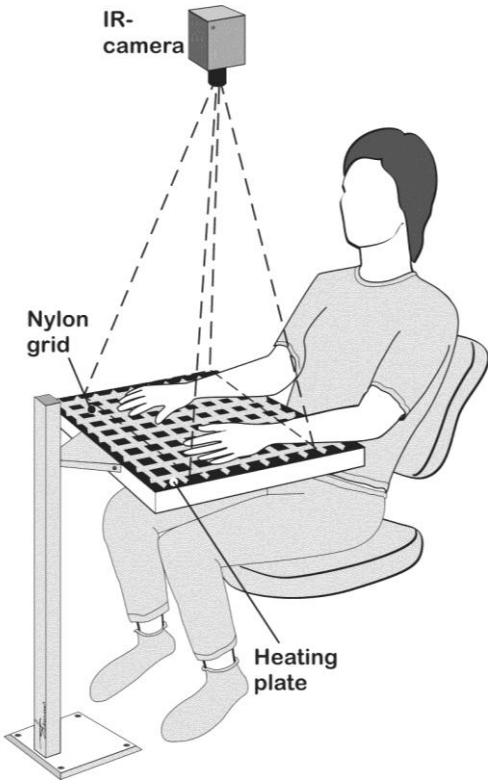


Fig. 7. Schematic illustration of Experimental Setup 2.

In ES1 there were a few occasions when there was some instability in the hospital room heating system, especially in the mornings and as a result air temperature fell by a few degrees from the normally controlled level of 22-23$^{o}$C. To avoid this problem in ES2 a small heating fan was used to raise room temperature back to 22-23°C before the experiment started. The fan was switched off before the subject entered the room. No extra heating was employed during the experiment and the room temperature remained stable.

## *Experimental protocol 1 -EP1*

The subjects were asked to abstain from nicotine (snus, cigarettes, nicotine plasters etc.), as well as to abstain from using caffeine, alcohol or other stimulants for a minimum period of 4 hours prior to the start of an experiment. To prevent incorrect readings with the IR camera they were

asked not to use lotions on the hands or face and to have nails free from nail varnish. The subjects were also asked to avoid any cold exposures on the day of the experiment and to refrain from washing their hands or face before the experiment. They were required to remain indoors for at least 20 minutes before entering the laboratory (stabilization period). The male subjects were required to be clean shaven and for both sexes the subjects had to be free from facial jewellery such as piercing. Long hair was secured so as not to obscure the facial skin. After the stabilisation period the subject received two bags of either Skruf stark # 3 (SN+) or Onico (SN-). The two bags were placed by the researcher on the surface of the subjects tongue. The subjects had then to use their tongues to place the bags into the position that they routinely used when using snus (always under the upper lip).

Each experiment lasted 60 minutes, a 30 min period during which the subjects kept the snus in their oral cavities followed by a 30 min recovery period. After the 30 minutes of using snus, the subject spat out the pouches into a bowl that was held up for them.

When recording the facial images the subjects were asked to look directly into the IR camera which was positioned at head height across the table in front of them (see Fig. 6). One of the bare walls of the room was used as a uniform background for the facial pictures. On the face, skin temperatures were analysed from seven differently shaped regions of interest (ROIs): the tip of the nose, left and right cheek, the chin, in between the eyes (bridge of nose), around the mouth and the inner canthus of the eyes (Fig. 8).

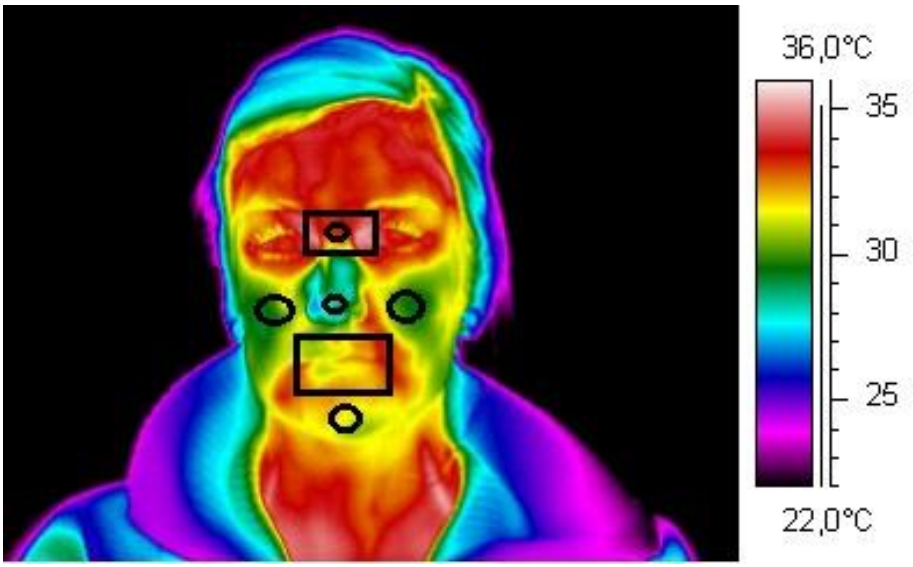


Fig. 8. The seven ROIs used to measure the temperature of the face during the experiment. The temperature scale is shown to the right.

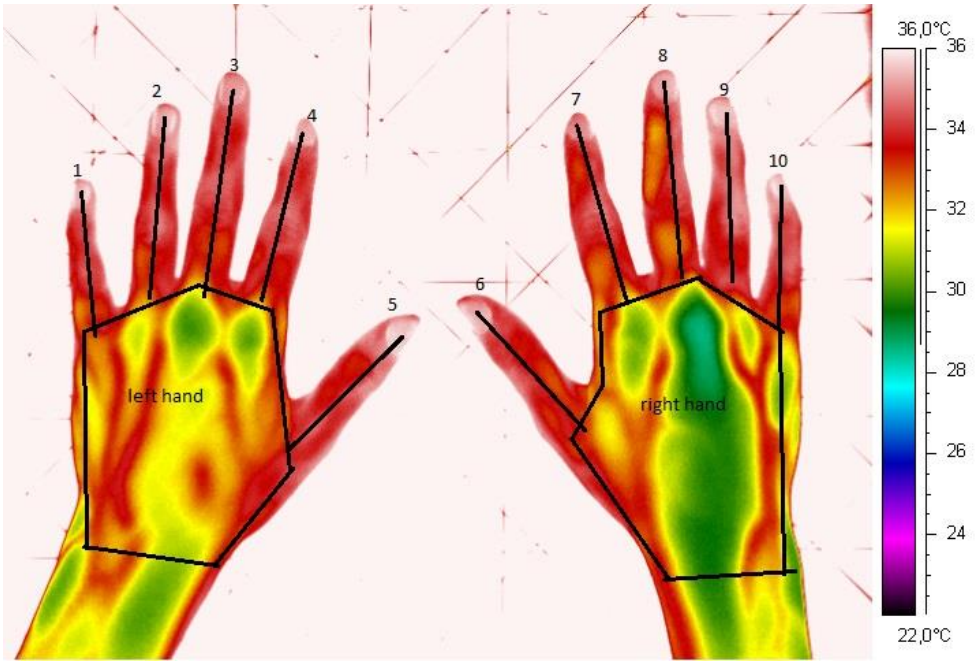


Fig. 9. The regions of interest (ROI's) measured on the fingers and the back of the hands. The temperature scale is shown on the right.

For the hands, dorsal skin surface temperatures were separately analysed for the back of the hand and the fingers. The ROI's used are shown in Fig. 9. For the dorsal surface of the back of the hand the average temperature within a polygonal ROI extending from the wrists to the base of the digits was used. For the fingers the average temperature along straight lines which extended from the tip of the finger (middle of the fingernail) to the base of the finger was used. With regard to the latter, test images prior to the study had revealed that the average temperature along these profile lines was very similar when compared to the average temperature within a traced ROI of the entire finger.

IR images of the face and hands were recorded at various set time-intervals during the 60 minute experiment: at the start of the experiment before SN+ or SN- were given (time-point 0) and at time-points 10, 15, 20, and 30 minutes while the person had snus under the lip. Following removal of the snus further images were taken at time-points 40, 45, 50 and 60 minutes (Fig. 10).

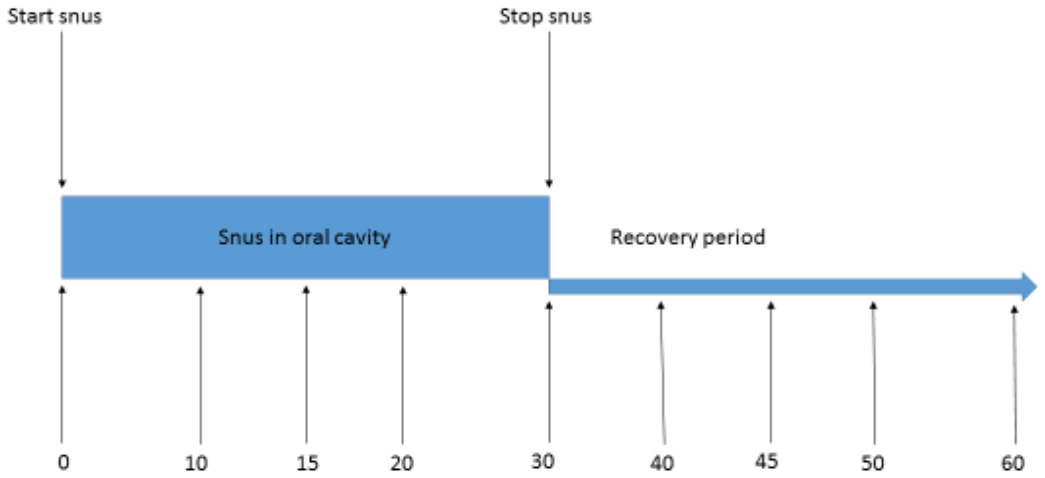


Fig. 10. Timeline for images capturing in Experimental Protocol 1

## *Experimental protocol 2 -EP2*

In the follow-up study 6 young healthy volunteers aged between 19 and 25 year (3 males and 3 females) were recruited from the University of Tromsø community. In EP2 the subjects were asked to use snus with nicotine 4 hours prior to the start of the experiment, with no future use before the experiment. During the experiment the subjects were more heavily dressed than in EP1 in order to ensure that all subjects had warm hands at the start of an experiment with the purpose to obtain vasodilatation of the blood vessels. Otherwise the participation requirements used in the EP1 were followed.

After the end of the stabilisation period (30 minutes) two bags of SN+ or SN- were given to the subjects as described above for EP1. The subjects were not informed beforehand whether the bags of snus contained nicotine or not.

In this follow up study thermal images were only taken of the dorsal aspect of the hands at time-point 0, before snus and at 5 minute intervals during the 30 minute period in which snus was placed under the upper lip (Fig. 11). Temperature measurements were restricted to the dorsal aspect of the 10 digits and calculated from straight lines ROI's as described above for ES1 (see Fig. 12).

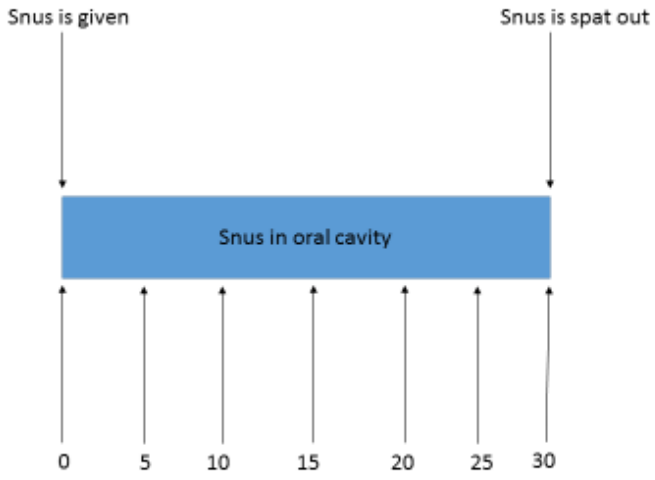


Fig. 11. Timeline for image capturing in Experimental Protocol 2

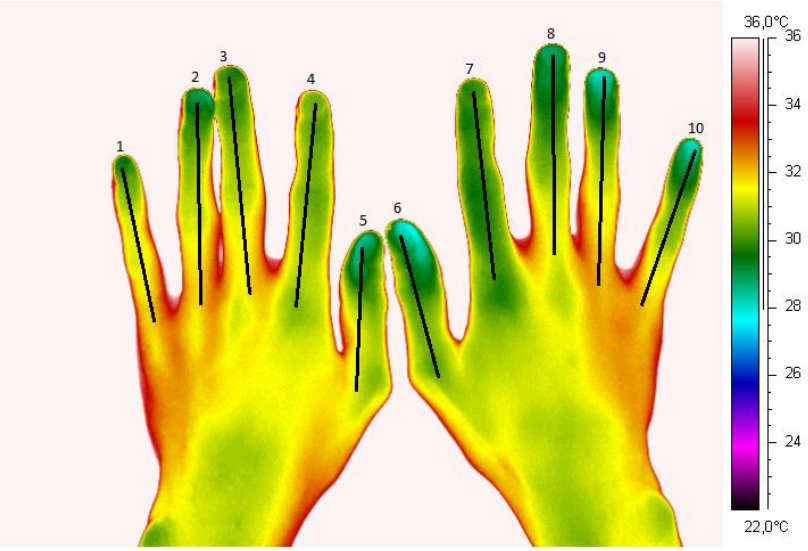


Fig. 12. The linear regions of interests (ROI's) measured on the hands in EP2. The temperature scale is shown to the right.

## *Skin temperature calculations*

All statistical calculations were carried out using Excel® (Microsoft Corporation, USA).

### *Facial temperatures*

In EP1 the average temperature within the seven facial ROI's for each subject was calculated at each time-point. This data was then used to calculate a total average for the 7 ROI's for each subject for each time-point (average of average) for SN+ and SN-. The mean facial skin temperature ($T_{mf}$) value for the 15 subjects for each time-point was calculated from this data. The mean temperature for the ROI in the area around the mouth at each time-point for each subject was also calculated. From these values the average for the group was calculated (average of average). To test whether facial temperature was affected by nicotine a statistical comparison (Student T-test) was made of the mean facial temperature difference between the nicotine and nicotine free experiments at all the set time-points in all subjects. The same was done for the temperatures measured in the ROI around the mouth. For the ROI covering the inner canthus of the eyes maximum skin temperature at this site was additionally calculated for both the SN+ and

SN- subjects at the start of the experiment and at the end of the 30 minute period of using snus. Student T-test was used for comparing differences and the temperature change after 30 minutes of snus (SN+ and SN-).

### *Hand temperatures*

For the hand skin temperatures the back of the hand and the fingers were analysed separately. For the polygonal ROI on the back of the hand the average temperature for each subject at each time-point (0,10,15,20,30,40,45,50 and 60 minutes after start) was calculated for SN+ and SN-. A mean of these values was calculated with SD's. The mean temperatures along the single line ROI's used for the fingers was calculated in a similar manner.

## *Statistics*

The statistical analysis was carried out using Excel®, and a hypothesis test based on pooled measurements was used to calculate the statistical significance of the results.

The difference in temperature between the experiment with SN+ and SN- for each person for each set time-point was used for the statistical analysis. The design of ES1 and ES2 allows each subject to be their own control (the control values were the experiment with SN-). The central limit theorem assumes that the results (the change in temperature) are (approximately) normally distributed [78].

The average temperature change at a given time-point for the group is described as $\mu$ and the standard deviation for the temperature change is described as $\sigma$. Both of these are unknown in this case and their value has to be estimated. Statistical t is the natural statistic to use in this case [78].

*Equations*

Estimators:

$$\bar{X} = estimated\ change \in temperature$$

$$\hat{\mu} = \bar{X} = \frac{1}{n}\sum_{i=1}^{n} Xi$$

Equation 1.1: estimation of the average temperature difference (μ)

$$\hat{\sigma}^2 = S^2 = \frac{1}{n-1}\sum_{i=1}^{n}(Xi - \bar{X})^2$$

Equation 1.2: estimation of the variance (σ)

$$S = \sqrt{\hat{\sigma}^2}$$

Equation 1.3: estimation of standard deviation (SD)

$$t = \frac{\bar{X} - \mu}{\sqrt{s^2}/\sqrt{n}} \sim T(n-1)$$

Equation 1.4: calculation of the statistical t.

From this set of equations the following hypothesis can be formulated

Hypothesis $H_0$: $\mu=0$ (no change in temperature following intake of snus with nicotine)

Hypothesis $H_1$:$\mu<0$ (a negative change in temperature following intake of snus with nicotine)

The hypothesis was that there is a significant drop in skin temperature on the face, back of hands and fingers in EP1 and in the fingers in EP2.

### *t -value calculations*

$t_\alpha$ is the *critical value* for the statistical t. In this case $\alpha$= 0, 05= 100(1-0.05) % = 95%

In EP1 15 subjects participated thus, $t < t_\alpha$= -1.761 (for the sample of 15, that is, 14 degrees of freedom). For a sample of 15 persons the t-value needs to be lower than the critical value of -1.76 for the results to have a certainty of 95%. The estimated temperature change is constant but the certainty tells us how likely it is that the temperature change is as a result of using snus.

In EP2 6 subjects participated thus $t < t_\alpha$= -1.48 (for the sample of 6, that is, 5 degrees of freedom). For a sample of 6 persons the t-value has to be lower than -2.01 for the results to have a certainty of 95%.

# Results

## *Experimental Protocol 1*

### *Mean facial skin temperatures*

Example images at different time-points for a single subject from SN+ and SN- experiments are shown in Fig.13.The mean facial skin temperature ($T_{mf}$) throughout the time-course of the experiments in the SN+ and SN- subjects as well as statistical evaluation of the difference between the SN+ and SN- subjects at each time-point are presented in Fig 14 and Table 1 respectively. $T_{mf}$ at the start of the experiment were practically identical (32.3ºC) in both groups. As can be seen in the figure there was a small, but statistically significant increase in $T_{mf}$ in both groups during the first 10 minutes after receiving snus. Thereafter $T_{mf}$ remained stable throughout the remainder of the experiment. At time-points 10, 15, 20 & 30 there was a statistically significant difference between the SN+ and SN- values, in all cases the SN+ values being lower (Table 1). For all other time-points there were no differences between SN+ and SN-. The time-points with statistical significant difference between SN+ and SN- subjects are marked with * in the graphs.

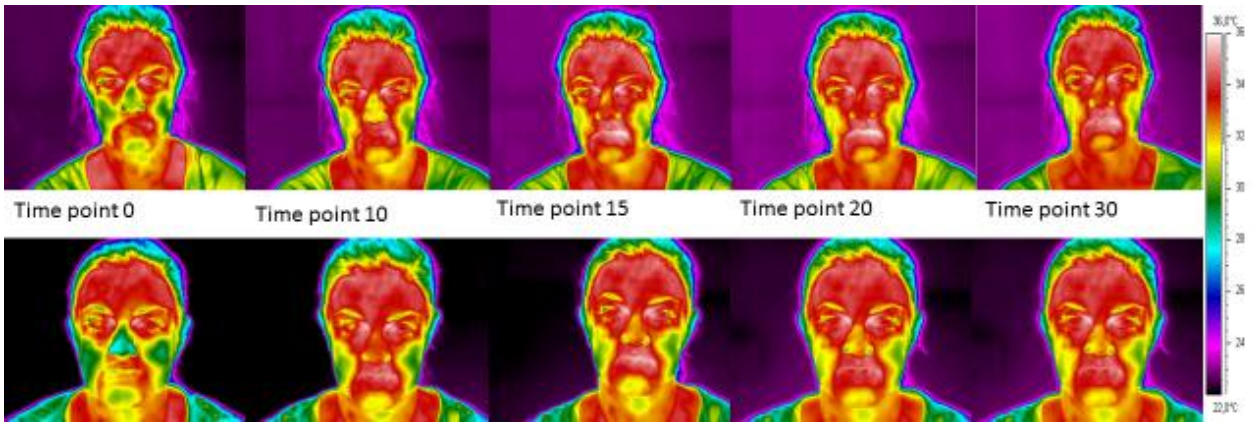


Fig. 13. IR images of the face during the snus period, for SN+ upper row and SN- lower row.

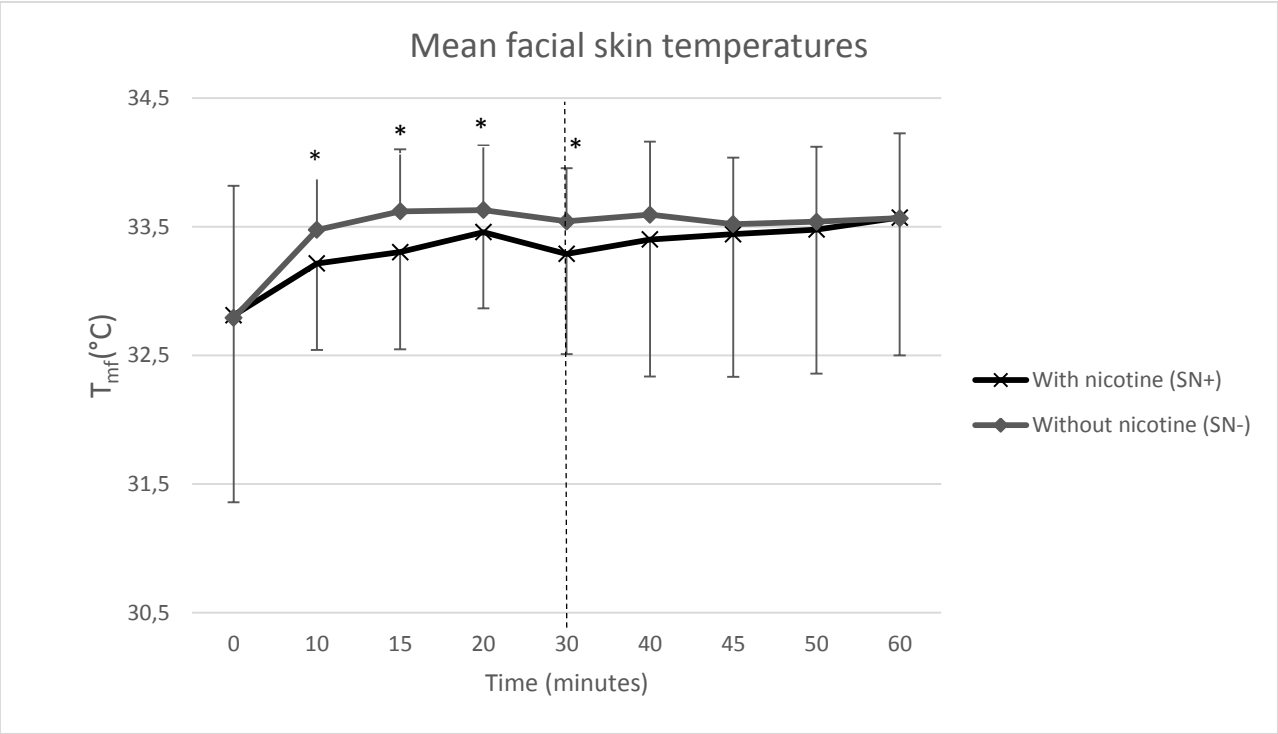


Fig.14. Mean facial skin temperatures ($T_{mf}$) ± SD's, during the time-course of the experiments in the SN+ and SN- subject. The dotted line represents the end of snus period (30 minutes). * = statistical significant difference in temperature between SN+ and SN-.

*Table 1. The average difference in $T_{mf}$ between SN+ and SN- and the standard deviation at the different time-points is presented. The t value as calculated from equation 1.4 are shown for each time-point. The statistical significant and the statistical insignificant difference in temperature are marked with + and - respectively.*

| Minutes | Average difference in facial temperature $\mu$ | Standard deviation $\sigma$ | t-value | t < -1,7 (critical value 95%) |
|---|---|---|---|---|
| 0 | 0.0 | 0.9 | -0.1 | - |
| 10 | -0.3 | 0.6 | -2.3 | + |
| 15 | -0.3 | 0.6 | -2.2 | + |
| 20 | -0.2 | 0.4 | -1.8 | + |
| 30 | -0.3 | 0.6 | -1.8 | + |
| 40 | -0.3 | 0.8 | -1.2 | - |
| 45 | -0.2 | 0.9 | -0.8 | - |
| 50 | -0.1 | 0.8 | -0.5 | - |
| 60 | 0.1 | 0.7 | 0.4 | - |

***Mean skin temperatures of the mouth area***

While there were some differences in $T_{mf}$ between SN+ and SN- there were no clear differences when each of the 7 ROI's on the face were examined separately. Since the snus was placed under the upper lip it was suggested that in the SN+ subjects one might see a local effect on skin surface temperature in the ROI covering this area. However as can be seen in Fig 15 and Table 2 this was not the case. For this facial area there were no statistically significant differences in the mean skin temperatures values at any time-point throughout the time-course of the experiment.

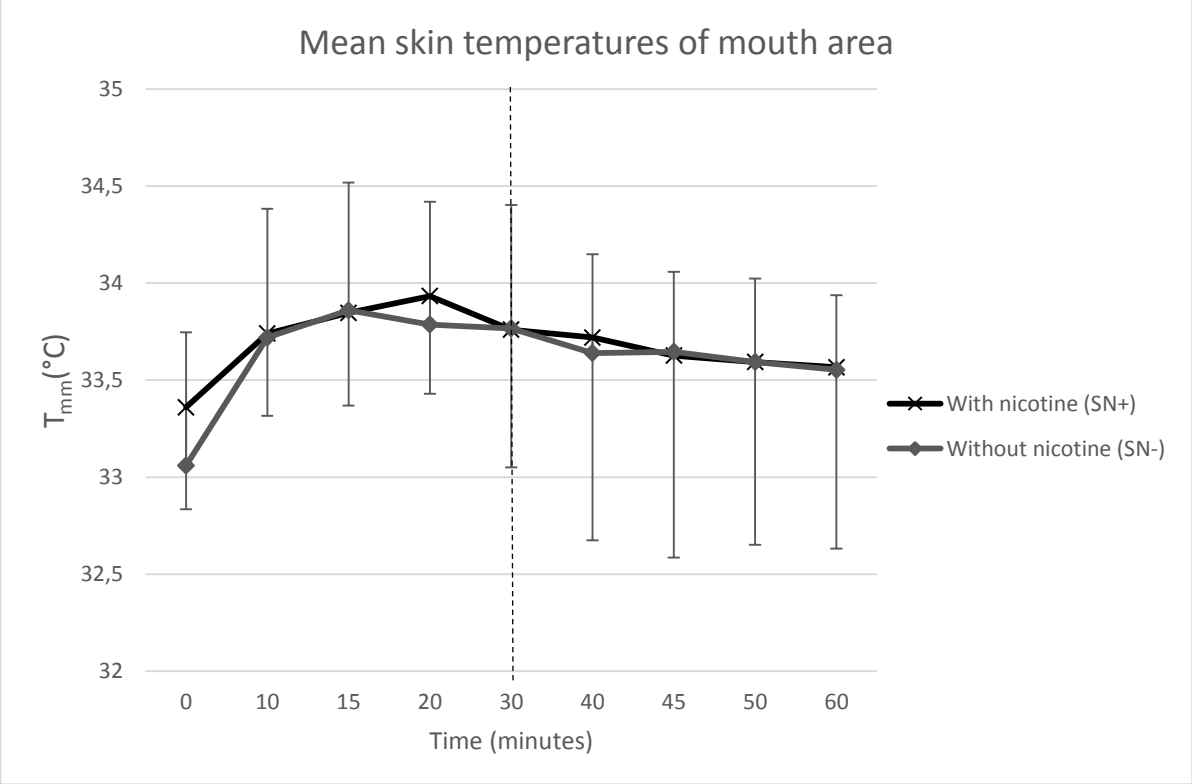


Fig. 15. Mean skin temperatures of the mouth area ($T_{mm}$) ± SD's. The dotted line represents the end of snus period (30 minutes). The dotted line represent the end of snus period (30 minutes).

*Table 2. The average difference in $T_{mm}$ between SN+ and SN- and the standard deviation at the different time-points are presented. The t-value as calculated from equation 1.4 are shown for each time-point. The statistical significant and the statistical insignificant difference in temperature are marked with + and - respectively*

| Minutes | Average difference in temperature in mouth ROI $\mu$ | Standard deviation $\sigma$ | t-value | t < -1,7 (critical value 95%) |
|---|---|---|---|---|
| 0 | 0.3 | 0.6 | 2.0 | - |
| 10 | 0.0 | 0.5 | 0.2 | - |
| 15 | 0.0 | 0.5 | -0.1 | - |
| 20 | 0.0 | 0.7 | 0.2 | - |
| 30 | 0.1 | 0.7 | 0.5 | - |
| 40 | 0.1 | 1.2 | 0.3 | - |
| 45 | 0.0 | 1.1 | -0.1 | - |
| 50 | 0.0 | 1.0 | 0.0 | - |
| 60 | 0.0 | 1.0 | 0.1 | - |

### *Maximum mean skin temperature of the inner canthus of the eye*

The maximum mean skin temperatures for the ROI' covering the inner canthus of the eyes ($T_{me}$) in the SN+ and SN- subjects at time-points 0 and 30 are presented in Fig 16 and the statistical comparisons are shown in Tables 3 and 4. There was no statistically significant difference between the SN+ and SN- subjects at these two time-points. However for both groups there was a statistically significant increase in maximum inner canthus temperature from time-point 0 to time-point 30.

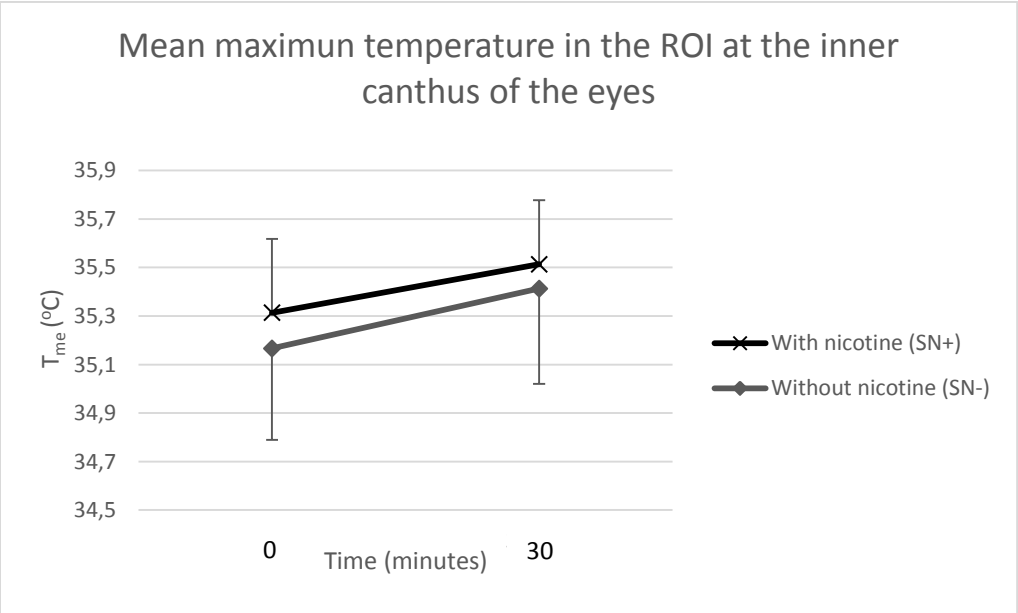


Fig.16. Mean maximum temperature in the ROI at the inner canthus of the eyes ($T_{me}$) at timepoint 0 and after 30 minutes of snus for SN+ and SN- subjects with SD's.

*Table 3. Statistical evaluation of differences in* $T_{me}$ *at the start and end of the snus period for SN+ and SN- subjects with SD's are presented, with the t-value for each time-point. The statistical insignificant difference in temperature is marked with -.*

| Minutes | Average difference in temperature μ | Standard deviation σ | t-value | t < -1,7 (critical value 95%) |
|---|---|---|---|---|
| 0 | -0.1 | 0.4 | -0.3 | - |
| 30 | -0.1 | 0.4 | -0.9 | - |

*Table 4. The average temperature change with SD's for* $T_{me}$ *from the start point and to the end of the snus period (30 minutes) for SN+ and SN- with the t-value for SN+ and SN- for the time series (0-30), are presented. The statistical insignificant decrease is marked with -.*

| 0-30 minutes | Average difference in temperature μ | Standard deviation σ | t-value | t < -1,7 (critical value 95%) |
|---|---|---|---|---|
| SN+ | 0.2 | 0.2 | 3.5 | - |
| SN- | 0.2 | 0.3 | 2.7 | - |

.

*Hand skin temperatures*

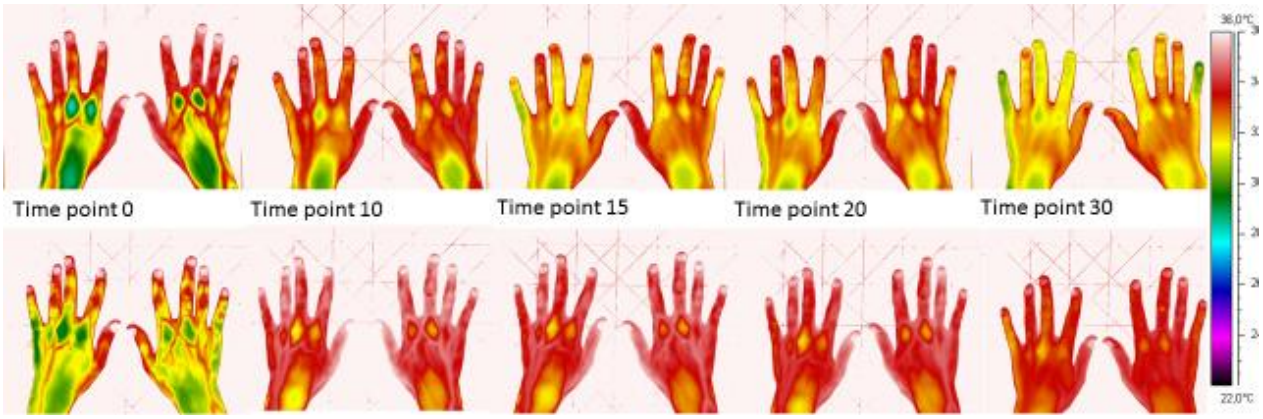


Fig.17. IR images of the dorsal side of the hands at different time/points during the snus period (0-30 minutes). Upper row: SN+, lower row: SN-.

In Fig. 17 sample IR images of the hands for an individual subject throughout the time-course of a single SN+ and a SN- experiment are shown, which give a visual presentation of the pattern of temperature changes seen. The decreasing skin temperatures with time in the SN+ subject are clearly evident.

*Mean skin temperatures for back of the hand*

The mean skin temperature for the back of the hand throughout the time-course of the experiments in the SN+ and SN- subjects are presented in Fig 18. The mean skin temperatures at the start of the experiment were very similar in both SN+ and SN- groups (31.3$^{o}$C and 31.4$^{o}$C respectively). At time-point 30 there was a statistically significant difference between the SN+ and SN- values, with the SN+ skin temperatures being lower (0.8$^{o}$C) as presented in Table 5 and with an * in Fig. 18. For all other time-points there were no differences between SN+ and SN-. In the SN+ group there was a statistically significant drop in skin temperature between the start of the experiment and time-point 30, when the snus was removed. Furthermore the further drop in mean skin temperature after 30 minutes of recovery was also statistically significant. No statistical significant change in skin temperature was found for the SN- subjects throughout the time-course of the experiment.

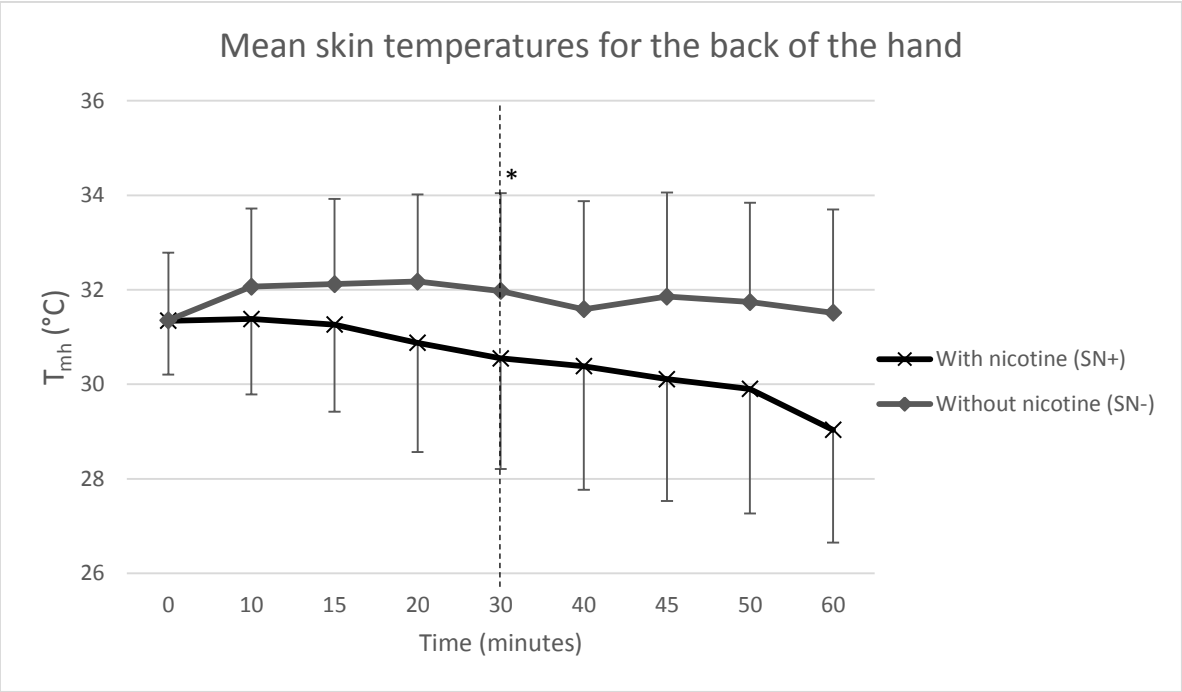


Fig.18. Mean skin temperatures of the back of the hand ($T_{mh}$) ± SD's for the SN+ and SN- subjects. The dotted line represents the end of snus period (30 minutes). *= statistical significant difference in temperature between SN+ and SN-.

*Table 5. Table 5 the average difference $T_{mh}$ between SN+ and SN- and the standard deviation at the different time-points are presented. The t-value as calculated from equation 1.4 are shown for each time-point. The statistical significant and the statistical insignificant difference in temperature are marked with + and - respectively.*

| Minutes | Average difference in temperature μ | Standard deviation σ | t-value | t < -1,7 (critical value 95%) |
|---|---|---|---|---|
| 0 | 0.4 | 1.0 | 1.4 | - |
| 10 | 1.5 | 2.0 | 0.8 | - |
| 15 | -0.5 | 1.6 | -1.1 | - |
| 20 | -0.4 | 1.6 | -1.0 | - |
| 30 | -0.8 | 1.6 | -1.9 | + |
| 40 | -0.9 | 2.1 | -1.7 | - |
| 45 | -0.9 | 2.2 | -1.5 | - |
| 50 | -0.9 | 2.2 | -1.6 | - |
| 60 | -1.0 | 2.3 | -1.7 | - |

*Mean finger skin temperatures*

Mean finger skin temperature ($T_{ms}$) for the back of the fingers throughout the time-course of the experiments as well as the statistical evaluation of the difference between the SN+ and SN- subjects at each time-point are presented in Fig 19 with the time-points with statistical significant difference between SN+ and SN- subjects marked with an * and in Table 6 respectively. Mean finger skin temperature at the start of the experiment in the two groups were practically identical (32.8ºC). In the SN+ subjects there was a statistically significant drop in temperature already after 10 minutes. This trend continued and after 30 minutes $T_{ms}$ had fallen by 2.8ºC. In the SN- groups there were no statistically significant changes during the same period. In both groups finger skin temperature fell during the recovery period (1.7°C and 0.9°C for the SN- and SN+ groups respectively), this fall only being statistically significant for the SN- group (Table 7).

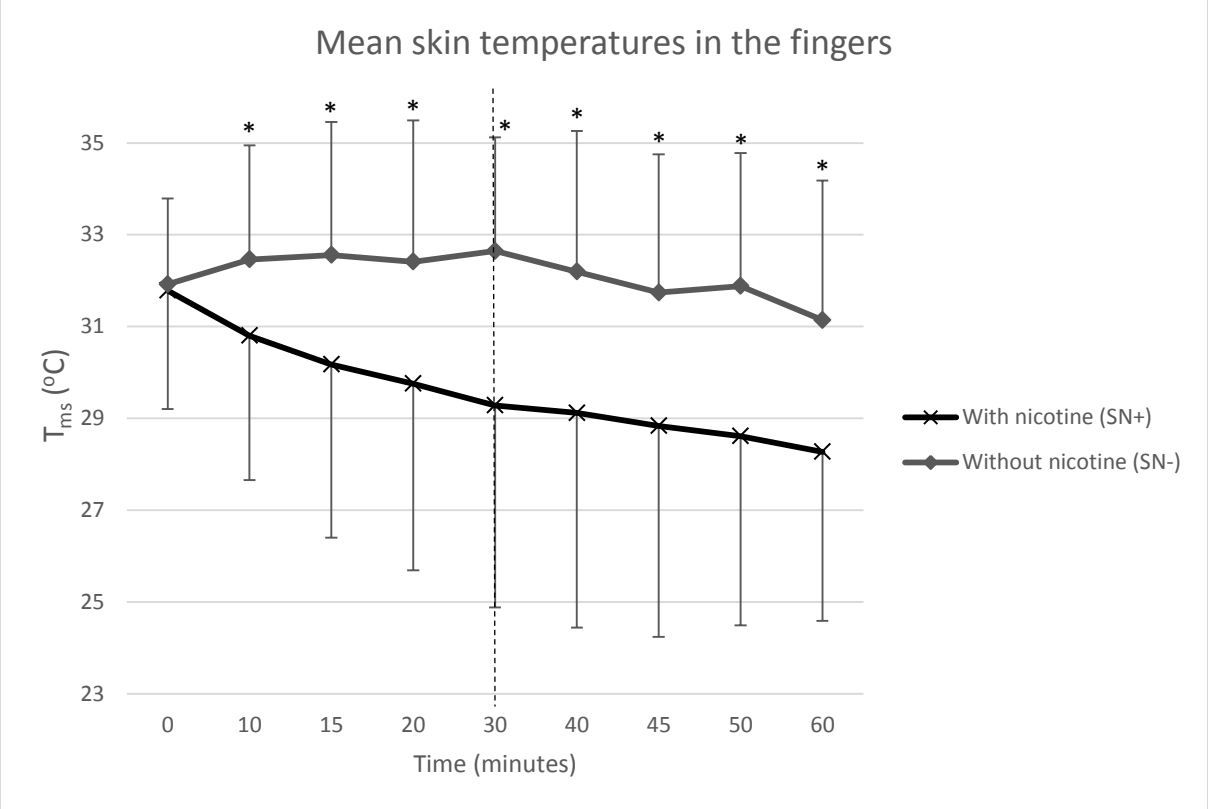


Fig.19. Mean skin temperatures of the fingers ($T_{ms}$) in SN+ and SN- subjects. The dotted line represents the end of snus period (30 minutes). *= statistical significant difference in temperature between SN+ and SN-.

*Table 6. the average difference in $T_{ms}$ between SN+ and SN- and the standard deviation at the different time-points is presented. The t-value as calculated from equation 1.4 are shown for each time-point. The statistical significant and the statistical insignificant difference in temperature are marked with + and - respectively.*

| Minutes | Average difference in temperature $\mu$ | Standard deviation $\sigma$ | t-value | t < -1,7 (critical value 95%) |
|---|---|---|---|---|
| 0 | -0.1 | 2.1 | -0.2 | - |
| 10 | -2.0 | 3.4 | -2.3 | + |
| 15 | -2.2 | 3.2 | -2.7 | + |
| 20 | -2.4 | 3.2 | -2.8 | + |
| 30 | -2.8 | 3.6 | -2.9 | + |
| 40 | -2.5 | 4.5 | -2.2 | + |
| 45 | -2.5 | 4.6 | -2.1 | + |
| 50 | -2.5 | 4.2 | -2.2 | + |
| 60 | -2.0 | 4.4 | -1.8 | + |

*Table 7. The statistical significant decrease in $T_{ms}$ with SD's for snus period (0-30 minutes) and the recovery period (30-60 minutes) are represented for SN+ and SN-. Statistically significant and insignificant decrease in $T_{ms}$ are marked with + and - respectively.*

| | Average change in temperature $\mu$ | Standard deviation $\sigma$ | t-value | t < -1,7 (critical value 95%) |
|---|---|---|---|---|
| **With nicotine** | | | | |
| 0-30 (snus period) | -2.1 | 2.7 | -3.0 | + |
| 30-60 (recovery) | -0.9 | 2.1 | -1.6 | - |
| **Without nicotine** | | | | |
| 0-30 (snus period) | 0.5 | 2.6 | 0.8 | - |
| 30-60 (recovery) | -1.6 | 1.9 | -3.2 | + |

## *Experimental Protocol 2*

The mean skin temperature for the back of the fingers ($T_{ms}$) throughout the time-course of the experiments as well as the statistical evaluation of the difference between the SN+ and SN- subjects at each time-point is presented in Fig 20 and Table 8 respectively. The mean skin temperature for the back of the fingers was similar in both groups at the start of the experiment

(33.8$^{o}$C for SN+ and 34.0$^{o}$C for SN-). Throughout the time-course of this experiment there was a statistically significant difference between SN+ and SN- subjects only at time-points 20 and 25.

The decrease in $T_{ms}$ from time-point 0 to time-point 25 for the SN+, subjects was statistical significant, although not at time-point 30. A small but statistically insignificant increase in skin temperature of the fingers can be seen in the SN- subjects throughout the first 25 minutes of having snus under the upper lip.

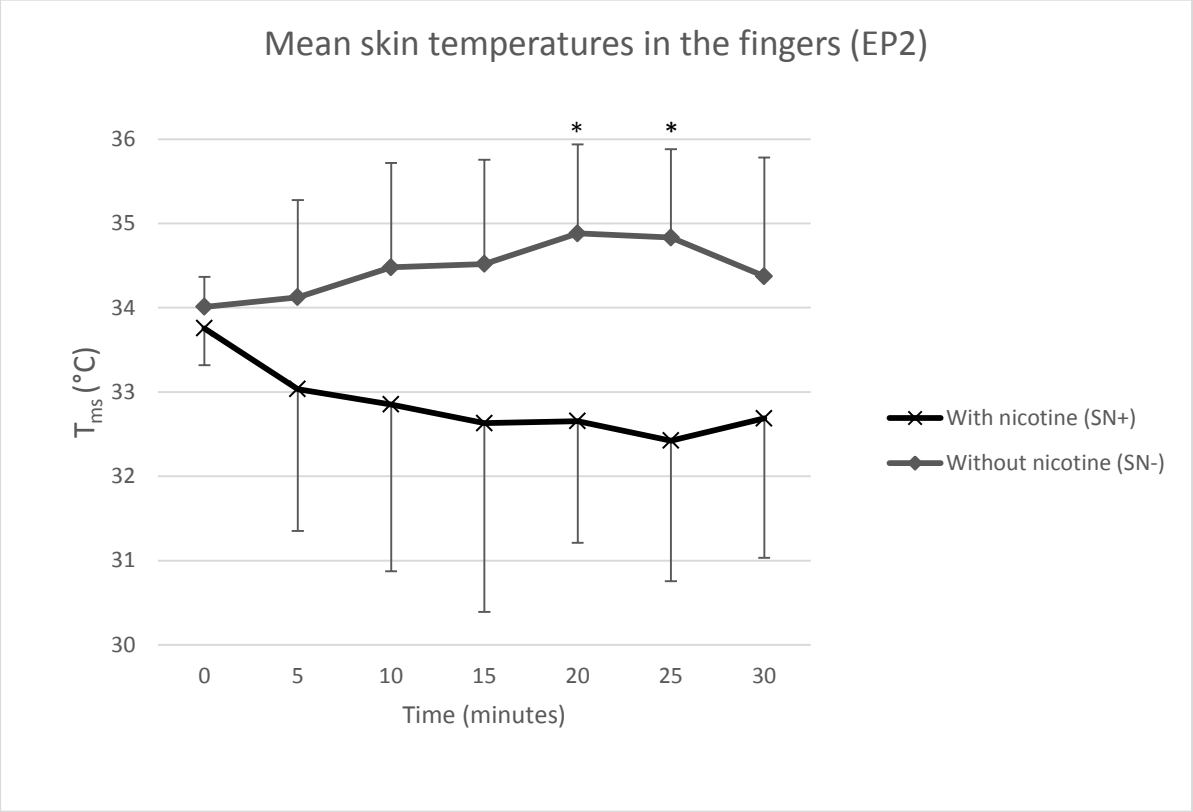


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Fig.20. The mean skin temperature ($T_{ms}$) of the fingers during the time-course of the experiments with SN+ and SN- . *= statisticaly significant difference in temperature between SN+ and SN-.

*Table 8 the average difference in $T_{ms}$ between SN+ and SN- and the standard deviation at the different time-points is presented. The t-value as calculated from equation 1.4 are shown for each time-point. The statistical significant and the statistical insignificant difference in temperature are marked with + and - respectively.*

| Minutes | Average difference in temperature $\mu$ | Standard deviation $\sigma$ | t-value | t < -2,0 (critical value 95%) |
|---|---|---|---|---|
| 0 | -0.1 | 0.3 | -0.7 | - |
| 5 | -1.1 | 2.0 | -1.3 | - |
| 10 | -1.6 | 2.1 | -1.9 | - |
| 15 | -1.9 | 2.7 | -1.7 | - |
| 20 | -2.2 | 2.0 | -2.7 | + |
| 25 | -2.4 | 2.3 | -2.5 | + |
| 30 | -1.7 | 2.5 | -1.7 | - |

*Table 9. The statistical significant average difference in $T_{ms}$ with SD's during the time series; 0-25 minutes and 0-30 minutes for SN+ and SN-. Statistically significant and insignificant decrease are marked with + and - respectively.*

| | Average difference in temperature $\mu$ | Standard deviation $\sigma$ | t-value | t < -2,0 (critical value 95%) |
|---|---|---|---|---|
| **With nicotine** | | | | |
| 0-25 | -1.3 | 1.5 | -2.1 | + |
| 0-30 | -1.1 | 1.5 | -1.7 | - |
| **Without nicotine** | | | | |
| 0-25 | 0.8 | 1.1 | 1.9 | - |
| 0-30 | 0.4 | 1.6 | 0.6 | - |

# Discussion

In this study the effect of nicotine uptake over the oral mucosa on skin perfusion of the face and hands of young healthy habitual users of snus containing nicotine has been studied. The results show that skin perfusion decreased when using snus, presumably through the effect of nicotine. While this finding was not unexpected due to the well documented effects of nicotine uptake through smoking [1, 79], to my knowledge this is the first study in which such an effect has been demonstrated through the oral administration of nicotine by using snus. In the following sections the results of the two protocols used, EP1 and EP2, will be individually discussed. This is followed by a more general discussion of the study

## *Experimental Protocol 1*

### *Facial skin temperatures*

In EP1 oral uptake of nicotine caused a small but statistically significant difference in $T_{mf}$ between the SN+ and SN- subjects, with $T_{mf}$ being lower in the SN+ group during the period in which snus was in the oral cavity. However, it is difficult to draw a firm conclusion concerning the effect that snus containing nicotine has on $T_{mf}$ since the results are confounded by the statistically significant 0.6ºC increase in $T_{mf}$ in both the SN+ and SN- subjects during the first 10 minutes of the experiments. The subjects $T_{mf}$ was actually higher at all other time-points compared to the start temperature in both SN+ and SN- experiments.

Mean facial skin temperature was determined by averaging skin temperature values from 7 different ROI's and it is important to keep in mind that there may be local differences in facial skin temperatures [80]. Local variations in facial skin temperatures were also found in this study. For example the largest increases during the initial 10 minutes of the experiments were found on the cheeks and nose. These local variations in skin temperature values in the individual ROIs provide information that allows one to suggest some tentative explanations for the increase in $T_{mf}$ during the first 10 minutes of the experiments.

First, snus can be regarded as being an irritant, causing severe reactions in the oral mucosa resulting in oral lesions and activation of the immune system at the mucosal area in contact with the snus [81, 82]. Since it is known that other irritants such as capsaicin and menthol applied to the mucosal tissue in the oral cavity cause an increase in facial skin blood flow [83] it is speculated that this may be, at least, partially responsible for the observed increases in $T_{mf}$ during the first 10 minutes. A second tentative explanation relates to local reflexes affecting mucosal circulation, caused by having an object placed in the oral cavity. Increases in mucosal circulation are known to be initiated, for example, when drinking or eating [84] which may also occur when using snus. A third, and perhaps, more plausible explanation may be that the equilibration period prior to the start of the experiments was too short. In this study the experiments were carried out in the winter months in Northern Norway and the subjects would be subjected to cold outside air temperatures prior to coming to the experimental laboratory. Even though the minimum equilibrium period in EP1 was 20 minutes, this period may have been too short. Evidence of this can be seen from the skin temperatures on the tip of the nose and the cheeks. The skin temperatures of the tip of the nose were on average 1-2$^{o}$C lower than the 6 other ROI's at the starting point (time-point 0). The low nose temperature strongly affects the $T_{mf}$ values for the 7 combined ROIs at the start of the experiment. The nasal vasculature has many arteriovenous anastomoses (AVAs) [85] which are normally closed at room temperature in normothermic subjects, causing the nose to appear as a relatively cold area compared to other facial areas [85]. After entering a warm environment the AVAs will slowly open, although to have fully open AVAs most subjects need to be slightly hyperthermic, for example during strenuous exercise [86]. It is suggested that in EP1 the subjects simply had too little time for the AVAs to become adapted to the room temperature in the laboratory. The cheek skin temperatures were also approximately 1$^{o}$C lower at the start of the experiment compared to other facial sites and perhaps, for similar reasons, had not fully equilibrated to the ambient temperature of the laboratory at the time-point when snus was administered.

One could speculate that the placement of nicotine under the upper lip may affect the temperature of the overlying skin area. However, the changes in mean skin temperatures for the ROI covering the mouth (Fig.15) were very similar to the general pattern seen for $T_{mf}$ , with even smaller

differences between SN+ and SN- (Fig.14), indicating that the placement of snus under the upper lip had no clear local effect on skin temperature

As mentioned in the introduction the temperature of the inner canthus of the eyes is regarded as being one of the warmest skin temperature sites on the face. It is for this reason that this facial area has attracted the attention of those interested in using thermography for fever detection in mass screening situations, for example at airports. This study showed that there was a statistically significant increase in the temperature of the inner canthus at the end of the 30 minute period during which snus was in the oral cavity however, the increase occurred in both the SN+ and SN- subjects. Furthermore, there were no statistically significant differences between the SN+ and SN- subjects in the maximum skin temperature measured at either the start or the end of the experiment. It is therefore concluded that snus containing nicotine has no clear effect on the temperature of the inner canthus of the eyes, at least under the conditions used in this study.

*Hand skin temperatures*

When considering the mean skin temperatures of the hands in EP1 (also for EP2) an influence of the heating plate (40ºC) that was positioned below the nylon grid in order to ensure a uniform background temperature in the IR images (Fig 6 and 7) needs to be considered. Although the heating plate was situated 3 cm below the hands resting on the grid it could potentially cause a slight heating of the palmar sides of the hands, thus influencing the results. However, it is felt that this effect was negligible due to the short period of time that the palmar skin was actually exposed to the heating plate, which was limited to ca. 15 sec periods when an IR image was being taken. In addition, it is assumed that if the hands were slightly heated by the warm plate this this would cause a common error for both the SN+ and SN- experiments.

In comparison to $T_{mf}$ the vasoconstrictive effect of nicotine intake using SN+ was very evident for the hands. This effect was more pronounced on the fingers (Fig. 19) compared to the back of the hand (Fig. 18). Interestingly, the time-course of the changing pattern of hand skin temperature caused by the nicotine is very similar to that seen when a warm hand is exposed to cold. In such a situation the fingers also show the greatest fall in skin temperature [87]. This temperature fall is known to be associated with the shutting down of AVAs [88].

For the back of the hand the fall in skin temperature during the period in which the snus was in the oral cavity only became statistically significant at time-point 30, the end of the snus period. The skin temperature on the back of the hand in the SN- group changed very little throughout the entire experiment while for the SN+ group $T_{mh}$ not only fell during the snus period but continued to fall during the recovery period. The temperature fall in the snus period, up to time-point 30, and in the recovery period can only be described as a trend since at none of the time-points in this period was there a statistically significant difference between the SN+ and SN- subjects. Since it is known that the half-life of nicotine is 1-2 hours it is speculated that the trend of falling temperatures in the recovery period may have been due to the continuing effect of nicotine on the blood vessels [89, 90]. Such a trend may be more pronounced in users of snus compared to cigarette smoking since they are reported to have a higher plasma blood concentration of nicotine and cotinine [38].

The vasoconstrictive effect of nicotine was most evident on $T_{ms}$ and it is postulated that this vasoconstrictive effect was exerted on the AVAs as well as on other blood vessels in the fingers. As is known from sympathetically mediated responses to cold exposure [47] a vasoconstriction of the AVAs will have a strong effect on finger skin temperature, considering their role in thermoregulation. The AVAs in the fingers, which are primarily located at the tip of the finger, below the finger nails, consist of short direct connections between the terminal arteries and veins [91]. The AVAs have a rich supply of sympathetically controlled smooth muscle. In a hot environment a withdrawal of the sympathetic activity will cause the opening of the AVAs which allows rapid arterial blood supply to the veins. As a result the arterial blood supply to the finger tips increases as well as the venous return from the fingertips. In fully vasodilatated subjects the opening of the AVAs is easily visible in the IR-images due to its effect on skin perfusion, where this area shows up as the warmest areas on the fingers. The opposite occurs when subjects need to conserve heat, as in the cold, when sympathetic input is at its maximum and these connections are fully closed. In many of the SN+ subjects in this study a clear temperature fall at the finger tips could be seen, an example of which can be seen in Fig. 17.

Ideally it was hoped at the start of each experiment that all subjects would be in a similar vasomotor state (vasodilatated) and have similar mean skin temperature values. However, this

was not the case in EP1 and, although the mean skin starting values in the SN+ and SN- subjects were similar, the standard deviation was quite large at the starting point. Despite the large SD's, not only at the start of the experiment but at all other time-points, $T_{ms}$ fell by nearly 3°C for the SN+ group during the 30 minutes of snus compared to the SN- which had almost an 0,5$^{o}$C increase in the same period.

In this study the average temperatures along lines drawn on the fingers were used to calculate $T_{ms}$. In pilot experiments it was shown that this method of calculating $T_{ms}$ gives a very similar result to the average temperature determined by drawing a polygonal ROI that covers the entire finger. There are several advantages of using the average temperature along a straight line compared to a polygonal ROI. Firstly it is easier and less time consuming to draw a straight line than constructing a polygonal ROI. Secondly, it reduces the chance of including part of the background temperature in the calculations when drawing the line close to the edge of the finger. Thirdly, it also reduced the chance of including inaccurate temperature data from the edge of a curved object, such as the fingers. This is important since the temperature measuring accuracy of an IR- camera become uncertain along the border lines of rounded objects. It should be also noted that the straight line ROIs on the finger also covered the finger nail, an area that strictly speaking can not be included in true skin temperature. There is little information on emissivity values for human nails although there are studies that show human skin and nails have similar thermal diffusivity [92]. Since the temperature under the nailbed is greatly influenced by AVA' activity it was decided to include the nail in the measurements.

The continuing fall in $T_{ms}$ following removal of the snus in both the SN+ and SN- subjects during the recovery period is not fully understood. It is proposed that there are two possible factors which can be involved, a) air temperature/general thermal state of the lightly clothed subjects and b) a slow washout of the nicotine from the blood stream after removal of snus as mentioned for the skin temperatures for the back of the hand. A similar finding was also seen for $T_{mh}$ of the back of the hands in the SN+ subjects. In support of the former suggestion it was noticed that room temperature was as low as 19$^{o}$C on some of the days when the experiments were performed while on other days room temperature was between 22 -23°C. It is postulated that in the lightly clothed subjects who are sitting still, that the lower air temperature exerted a mild cold stress

causing the continuing fall in $T_{ms}$ during the recovery period. Indeed, some subjects reported feeling slightly chilly at the end of the experiment compared to the start of the experiment. The fact that SN- subjects and not SN+ subjects showed a statistically significant decrease in mean skin temperatures in the fingers during the recovery period, support the notion that extraneous factors, such as cold air temperature were responsible for the temperature decrease in the recovery period. Despite a possible effect due to slightly low room temperature it is interesting to note that in the recovery period the skin temperature values in the SN+ subjects were, at all time-points lower than in the SN- subjects, clearly indicating the vascoconstrictive effect nicotine has on skin blood vessels.

In summarising the findings from EP1it is clear that there were some shortcomings in regard to the protocol and experimental setup. While the overall effects of nicotine uptake using snus were as expected, the fact that there was no rise in temperature in the recovery period was unexpected. The results of EP1 indicate that the central vasomotor regulation from the hypothalamus through negative feedback regulation may have been influenced by peripheral cold sensors. It should also be noted that the SD's in EP1 were large for each time-point. These results gave rise to the question as to whether the results would have been different if the subjects had not been slightly cold stressed and if they had a more similar skin temperatures at the start of the experiment. It was for these reasons that it was decided to carry out the follow up study with a slight different experimental protocol (EP2).

## *Experimental Protocol 2*

The design of EP2 using more warmly dressed subjects at air temperatures between 22-23ºC was to ensure that all subjects were vasodilatated at the start of each experiment, in the hope that this might eliminate the putative cold input effect described above and thereby reduce the large SD's found in EP1. The fingers, rather than the back of the hand, were selected for investigation due to the strong effect SN+ had on skin perfusion in this area in EP1. The notion was that if there was to be an effect of nicotine in the more vasodilatated subjects, this would be clearly seen in the fingers. It was expected that the nicotine induced vasoconstrictor effect in the SN+ subjects

would be even more pronounced than in EP1. A further difference in EP2 was that the subjects were not allowed to observe the computer monitor on which the live thermal images were displayed. While this approach clearly reduced the standard deviation in both the SN+ and SN- subjects at the start of the experiment, the overall results of EP2 were similar to EP1 although the drop in mean skin temperature in the SN+ subjects was slightly less than that observed in the SN+ subjects in EP1. The number of subjects who participated in EP2 was small (6) and it is speculated that this is, at least part, of the reason that the vasoconstrictor effects of SN+ were not as pronounced as was hoped.

Despite these small differences the overall findings of EP1 and EP2 are similar and show that snus containing nicotine has a powerful effect on skin perfusion, especially in the hands. This response is very similar to the effect of nicotine from cigarette smoking.

### *General*

In this study there were 15 and 6 participants in EP1 and EP2 respectively and, due to these small numbers, caution must be applied in drawing conclusions on the effects of SN+. However, despite the low number of subject used in this study there was a marked negative effect on peripheral circulation. It is therefore felt justifiable to recommend further in-depth investigations comparing different age groups and subjects with different habits regarding the amount and frequency of snus usage to better characterize these findings.

Recent studies have shown that surgical site infections occur more frequently in smokers compared to non-smokers [93]. The infections may be explained by a compromised skin perfusion in the operated area by nicotine and carbon monoxide. In plastic surgery patients are often advised to stop smoking three months in advance of the scheduled operation to reduce the risk for postoperative wound infections. A negative effect on blood perfusion of the skin could lead to a higher risk for postoperative wound healing complications. Plastic surgical operations where skin perfusion becomes compromised due to wide skin undermining, such as occurs in abdominoplasties or in free flap surgery are at special risk [94]. As one of the conclusions of this study it is felt, in line with the recommendations regarding intake of nicotine through smoking,

that the negative effect that snus containing nicotine has on peripheral skin perfusion as demonstrated in this study justify advising patients to avoid using snus containing nicotine prior to surgery and in the immediate period after surgery.

The absorption of chemical substances over of mucosal tissue is well known. While, the mucosal layer of the lungs are only used for administration of substances in patients with pulmonary diseases like asthma and emphysema, the oral cavity, rectum and nose are more commonly used sites for administration of a wide variety of drugs. When a drug is absorbed directly from the oral cavity the absorption is fast. The drug avoids rapid metabolism in the liver and the low pH in the stomach [11]. For example, sublingual administration of nitroglycerine for angina treatment is very common. This ease of uptake via the oral mucosa provides an ideal site for nicotine uptake, and is one of the appealing aspects for achieving a desired nicotine effect using snus.

Snus contains many other chemical substances besides nicotine[33] and many of these are carcinogenic[34]. These chemicals may also be absorbed through the mucosal membrane in the oral cavity together with nicotine, and benefit from the fast absorption with high bioavailability of the substance in the body. One of the reasons for the rapid absorption may be the high vascularity on the oral mucosa. This study showed that the area around the mouth has a high skin temperature. Such may reflect the high vascularity of the oral area.

While it is suggested that the main peripheral effects of nicotine are due to stimulation of the sympathetic nervous system, such effects may also be due to nicotine's interactions with nicotinic receptors on visceral sensory nerves. It is thought that this action of nicotine is critical for the cardio-vascular effects of nicotine use [95]. Previous studies have shown that nicotine causes a decrease in skin temperature when injected intravenously at low doses, comparable to those obtained by self-administration [96].

Nicotine interacts with nAchR's in the CNS which is responsible for the addictive nature of nicotine. As is known for many substances nicotine use can lead to a desensitization of its receptors, nAchR. After prolonged use of nicotine the molecular and physiological reaction from nicotine may decrease [97]. This may partly explain some of the different responses observed in

this study where some of the subjects had a large decrease in temperature when given SN+ while in other subjects the decrease was small. The subjects with the lowest decrease were those reporting a high SN+ consumption. One can speculate if a person that had never used nicotine previously were to use the same amount of snus as the subjects in this study that one would expect a stronger physiological response compared to the response seen in habitual SN+ users. Nicotine poisoning or "nicotine shock" can be seen in persons unfamiliar to nicotine. Some of the symptoms are nausea, dizziness increased heart rate and a feeling of being cold while sweating, and can occur in habitual nicotine users as well as first time users [33, 98] .

There is a decreased risk in developing cardiovascular diseases, cancers (upper aerodigestive, pancreatic, bladder, renal and lung) and developing diseases in the respiratory system when switching from cigarettes to snus [41]. On the other hand snus contains many other chemical substances besides nicotine [33] and, as already mentioned, many of these are carcinogenic [34]. For example, there is evidence of snus having negative effect on cardiovascular health [32] and snus users have about twice the risk of pancreatic cancer compared with never-tobacco users [99]. A health hazard from SN+ is significant in those that would otherwise not use tobacco in any form [100]. The impact of snus containing nicotine on cancer in the oral cavity is still a matter of discussion [34, 101, 102]. The direct contact of carcinogenic substances with the oral mucosa makes it very plausible that the use of snus may contribute to the development of oral cancer.

In summary, the main finding in this thermography based study is the demonstration of a strong decrease in skin perfusion through the uptake of nicotine in snus, presumably due to the vasoconstrictive effect of nicotine. While similar responses are known from cigarette smoking, to my knowledge this study is the first to show this for habitual users of snus containing nicotine. A secondary finding is that nicotine uptake in users of snus has no effect on the skin temperature of the inner canthus of the eye, which will be of interest to those using thermography for fever detection in pandemic screening situations. The clear negative physiological effects of using snus containing nicotine demonstrated in this study justify advising patients to avoid using snus

containing nicotine prior to surgery and in the immediate period after surgery. In this study the number of participants was small and further studies comparing different age groups and subjects with different habits regarding the amount and frequency of snus usage are needed to shed more light on the physiological effects of using snus and similar smokeless products containing nicotine.

# References


1. Zhu, B.Q. and W.W. Parmley, *Hemodynamic and vascular effects of active and passive smoking.* Am Heart J, 1995. **130**(6): p. 1270-5.
2. Nordqvist, C. *What Chemicals Are In Tobacco Smoke? What Is Cigarette Smoke?* 2011 [cited 2014 01.05].
3. *National Cancer Institute* 2011 [cited 2014 01.05].
4. (SCENIHR), S.C.o.E.a.N.I.H.R. *Health effects of smokeless tobacco products*. 2008 [cited 2014 01.05].
5. Schlager, O., et al., *Correlation of infrared thermography and skin perfusion in Raynaud patients and in healthy controls.* Microvasc Res, 2010. **80**(1): p. 54-7.
6. Awwad, A.M., et al., *The effect of temperature on blood flow in island and free skin flaps: an experimental study.* Br J Plast Surg, 1983. **36**(3): p. 373-82.
7. Mercer, J.B.a.R., E.F.J, *Fever screening and infrared thermal imaging: concerns and guidelines.* Thermology International 2009. **19(3)**: p. 3.
8. Melikian, A.A. and D. Hoffmann, *Smokeless tobacco: a gateway to smoking or a way away from smoking.* Biomarkers, 2009. **14 Suppl 1**: p. 85-9.
9. Nordqvist, C. *What is nicotine*. 2014 [cited 2014 24.02].
10. Picciotto, M.R. and P.J. Kenny, *Molecular mechanisms underlying behaviors related to nicotine addiction.* Cold Spring Harb Perspect Med, 2013. **3**(1): p. a012112.
11. Dale, H.P.R.a.M.M., *Pharmacology*. Seventh ed. 2012, London: Elsevier
12. Picciotto, M.R., *Nicotine as a modulator of behavior: beyond the inverted U.* Trends Pharmacol Sci, 2003. **24**(9): p. 493-9.
13. Koob, G.F., *Drugs of abuse: anatomy, pharmacology and function of reward pathways.* Trends Pharmacol Sci, 1992. **13**(5): p. 177-84.
14. Mansvelder, H.D. and D.S. McGehee, *Cellular and synaptic mechanisms of nicotine addiction.* J Neurobiol, 2002. **53**(4): p. 606-17.
15. Tomar, S.L. and J.E. Henningfield, *Review of the evidence that pH is a determinant of nicotine dosage from oral use of smokeless tobacco.* Tob Control, 1997. **6**(3): p. 219-25.
16. H. Z. Ring, X.J.L., C.F. Thorn, N. L. Benowitz. *Nicotine Pathway, Pharmacokinetics*. 2012 30.04.2014].
17. Zevin, S., P. Jacob, 3rd, and N. Benowitz, *Cotinine effects on nicotine metabolism.* Clin Pharmacol Ther, 1997. **61**(6): p. 649-54.
18. Hukkanen, J., P. Jacob, 3rd, and N.L. Benowitz, *Metabolism and disposition kinetics of nicotine.* Pharmacol Rev, 2005. **57**(1): p. 79-115.
19. Larsen, E., J. Rise, and K.E. Lund, *Risk and protective factors of adolescent exclusive snus users compared to non-users of tobacco, exclusive smokers and dual users of snus and cigarettes.* Addict Behav, 2013. **38**(7): p. 2288-94.
20. *3rd International Conference on Smokeless Tobacco Advancing Science & Protecting Public Health*. 2002.
21. *The Tobacco Atlas*. [cited 2014 24.02].
22. Rutqvist, L.E., et al., *Swedish snus and the GothiaTek(R) standard.* Harm Reduct J, 2011. **8**: p. 11.
23. Lund, K.E., A. McNeill, and J. Scheffels, *The use of snus for quitting smoking compared with medicinal products.* Nicotine Tob Res, 2010. **12**(8): p. 817-22.

24. Piano, M.R., et al., *Impact of smokeless tobacco products on cardiovascular disease: implications for policy, prevention, and treatment: a policy statement from the American Heart Association.* Circulation, 2010. **122**(15): p. 1520-44.
25. Foulds, J., et al., *Effect of smokeless tobacco (snus) on smoking and public health in Sweden.* Tob Control, 2003. **12**(4): p. 349-59.
26. Foulds, J. and H. Furberg, *Is low-nicotine Marlboro snus really snus?* Harm Reduct J, 2008. **5**: p. 9.
27. Wolk, R., et al., *Hemodynamic and autonomic effects of smokeless tobacco in healthy young men.* J Am Coll Cardiol, 2005. **45**(6): p. 910-4.
28. Henley, S.J., et al., *Two large prospective studies of mortality among men who use snuff or chewing tobacco (United States).* Cancer Causes & Control, 2005. **16**(4): p. 347-358.
29. Ostenson, C.G., et al., *High consumption of smokeless tobacco ("snus") predicts increased risk of type 2 diabetes in a 10-year prospective study of middle-aged Swedish men.* Scand J Public Health, 2012. **40**(8): p. 730-7.
30. Benowitz, N.L., *Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment.* Prog Cardiovasc Dis, 2003. **46**(1): p. 91-111.
31. Benowitz, N.L., P. Jacob, 3rd, and L. Yu, *Daily use of smokeless tobacco: systemic effects.* Ann Intern Med, 1989. **111**(2): p. 112-6.
32. Overland, S., et al., *Snus use and cardiovascular risk factors in the general population. The HUNT3 study.* Addiction, 2013.
33. *Helsedirektoratet* [cited 2014 24.02].
34. Luo, J., et al., *Oral use of Swedish moist snuff (snus) and risk for cancer of the mouth, lung, and pancreas in male construction workers: a retrospective cohort study.* Lancet, 2007. **369**(9578): p. 2015-20.
35. Hatsukami, D.K., et al., *Developing the science base for reducing tobacco harm.* Nicotine Tob Res, 2007. **9 Suppl 4**: p. S537-53.
36. *Folkehelseinstituttet* [cited 2014 24.02].
37. *Statistisk sentralbyrå* [cited 2014 20.02].
38. Holm, H., et al., *Nicotine intake and dependence in Swedish snuff takers.* Psychopharmacology (Berl), 1992. **108**(4): p. 507-11.
39. Ebbert, J.O., et al., *A Randomized Phase II Clinical Trial of High-Dose Nicotine Patch Therapy for Smokeless Tobacco Users.* Nicotine Tob Res, 2013.
40. Digard, H., et al., *Determination of nicotine absorption from multiple tobacco products and nicotine gum.* Nicotine Tob Res, 2013. **15**(1): p. 255-61.
41. Gartner, C.E., et al., *Assessment of Swedish snus for tobacco harm reduction: an epidemiological modelling study.* Lancet, 2007. **369**(9578): p. 2010-4.
42. Ebbert, J., et al., *Interventions for smokeless tobacco use cessation.* Cochrane Database Syst Rev, 2011(2): p. CD004306.
43. Jones, B.F., *A reappraisal of the use of infrared thermal image analysis in medicine.* IEEE Trans Med Imaging, 1998. **17**(6): p. 1019-27.
44. Johnson, J.M., C.T. Minson, and D.L. Kellogg, Jr., *Cutaneous vasodilator and vasoconstrictor mechanisms in temperature regulation.* Compr Physiol, 2014. **4**(1): p. 33-89.
45. Charkoudian, N., *Skin blood flow in adult human thermoregulation: how it works, when it does not, and why.* Mayo Clin Proc, 2003. **78**(5): p. 603-12.

46. Miland, Å.O., *Dynamic Infrared Thermography in the assessment of skin perfusion- a clinical and experimental study* 2008.
47. Bornmyr, S., et al., *Finger skin blood flow in response to indirect cooling in normal subjects and in patients before and after sympathectomy.* Clin Physiol, 1998. **18**(2): p. 103-7.
48. Choi, J.K., et al., *Evaluation of mean skin temperature formulas by infrared thermography.* Int J Biometeorol, 1997. **41**(2): p. 68-75.
49. Love, T.J., *Thermography as an indicator of blood perfusion.* Ann N Y Acad Sci, 1980. **335**: p. 429-37.
50. Weum, S., *Imaging in plastic surgery- Aclinical and experimental study with notes on the history of medical imaging* 2013.
51. Stikbakke, E.a.M.J.B., *An infrared thermographic and laser Doppler flowmetric investigation of skin perfusion in the forearm and finger tip following a short period of vascular stasis.* Thermology International, 2008. **18**: p. 4.
52. Soler, N.G., *Laboratory Evaluation of the Autonomic System*, in *Clinical Methods: The History, Physical, and Laboratory Examinations*, H.K. Walker, W.D. Hall, and J.W. Hurst, Editors. 1990: Boston.
53. Jeannette Naish, P.R., and Denise Syndercombe Court, *Medical Sciences*. 1 ed. 2009: Elsevier.
54. FG, V.A.N.H., L. Kadic, and J.J. Driessen, *Skin temperature measured by infrared thermography after ultrasound-guided blockade of the sciatic nerve.* Acta Anaesthesiol Scand, 2013. **57**(9): p. 1111-7.
55. Miland, A.O. and J.B. Mercer, *Effect of a short period of abstinence from smoking on rewarming patterns of the hands following local cooling.* Eur J Appl Physiol, 2006. **98**(2): p. 161-8.
56. Taylor, W.F., et al., *Effect of high local temperature on reflex cutaneous vasodilation.* J Appl Physiol Respir Environ Exerc Physiol, 1984. **57**(1): p. 191-6.
57. Grassi, G., et al., *Mechanisms responsible for sympathetic activation by cigarette smoking in humans.* Circulation, 1994. **90**(1): p. 248-53.
58. Narkiewicz, K., et al., *Cigarette smoking increases sympathetic outflow in humans.* Circulation, 1998. **98**(6): p. 528-34.
59. Ring, E.F.J., *The development of thermometry and thermal imaging in medicine.* Journal of Medical Engineering & Technology, 2006.
60. Ring, E.F. and K. Ammer, *Infrared thermal imaging in medicine.* Physiol Meas, 2012. **33**(3): p. R33-46.
61. de Weerd, L., J.B. Mercer, and S. Weum, *Dynamic infrared thermography.* Clin Plast Surg, 2011. **38**(2): p. 277-92.
62. John, E.H.a.A.C.G., *Guyton and Hall Textbook of Medical Physiology* 12 ed. 2011: Saunders.
63. Hall, J., *Guyton and Hall Physiology Review (Guyton Physiology)*. 11th ed. 2011: Elsevier.
64. Engineering, D.O. 2012 [cited 2014 02.05].
65. de Weerd, L., *Free Perforator Flap surgery and Dymanic infrared Thermography- a clinical nd experimental study* 2010.

66. (ISO), I.S.O., *Medical electrical equipment -- Part 2-59: Particular requirements for basic safety and essential performance of screening thermographs for human febrile temperature screening*, 2014.
67. Smith, K.J.H.a.R.E., *Guidelines for specifying and testing a thermal camera for medical applications* Thermology International, 2009. **19**.
68. Hsieh, J.C., et al., *Clinical application of infrared thermography in diagnosis and therapeutic assessment of vascular ischemic pain.* Ma Zui Xue Za Zhi, 1990. **28**(4): p. 493-501.
69. Bruehl, S., et al., *Validation of thermography in the diagnosis of reflex sympathetic dystrophy.* Clin J Pain, 1996. **12**(4): p. 316-25.
70. Bergqvist, D., H.O. Efsing, and T. Hallbook, *Thermography. A noninvasive method for diagnosis of deep venous thrombosis.* Arch Surg, 1977. **112**(5): p. 600-4.
71. Hooshmand, H., *The role of infrared thermography in diagnosis and management of pain*, in *Engineering in Medicine and Biology Society. Proceedings of the 19th Annual International Conference of the IEEE (Volume:2 )*1997: Chicago, IL.
72. Howell, K.J., *Infrared thermography (IRT) for the assessement of microvascular skin blood flow in a specialist connective tissue diseasi unit*, 2010, University of Glamorgan/Prifysgol Morgannwg.
73. Ammer, E.F.R.R.a.K., *Infrared thermal imaging in medicine* Physiol Meas, 2012. **33**: p. 13.
74. Pascoe, D.D., Ring, E.F., Mercer, J.B., Snell, J., Osborn, D., Hedley-Whyte, J.,, *Clinical Thermometers, Project Team 1, Thermal Imagers for Human Febrile Temperature Screening (2010).* . International Standards for Pandemic Screening Using Infrared Thermography. In: Proceedings of SPIE Medical Imaging meeting., 2010. **7626 76261Z-6**.
75. Herry, C.L. and M. Frize, *Quantitative assessment of pain-related thermal dysfunction through clinical digital infrared thermal imaging.* Biomed Eng Online, 2004. **3**(1): p. 19.
76. Rustemeyer, J., J. Radtke, and A. Bremerich, *Thermography and thermoregulation of the face.* Head Face Med, 2007. **3**: p. 17.
77. Uematsu, S., et al., *Quantification of thermal asymmetry. Part 2: Application in low-back pain and sciatica.* J Neurosurg, 1988. **69**(4): p. 556-61.
78. Walpole R.E, M.R.H., Myers S.L, Ye K *Probability & Statistics, For engineers and Scientists* 2007: Person Education International
79. Trap-Jensen, J., *Effects of smoking on the heart and peripheral circulation.* Am Heart J, 1988. **115**(1 Pt 2): p. 263-7.
80. Marrakchi, S. and H.I. Maibach, *Biophysical parameters of skin: map of human face, regional, and age-related differences.* Contact Dermatitis, 2007. **57**(1): p. 28-34.
81. Poore, T.K., et al., *The effects of smokeless tobacco on clinical parameters of inflammation and gingival crevicular fluid prostaglandin E2, interleukin-1 alpha, and interleukin-1 beta.* J Periodontol, 1995. **66**(3): p. 177-83.
82. Johnson, G.K., et al., *Development of smokeless tobacco-induced oral mucosal lesions.* J Oral Pathol Med, 1998. **27**(8): p. 388-94.
83. Kashima, H. and N. Hayashi, *Facial skin blood flow responses to irritant stimuli in the oral cavity.* Auton Neurosci, 2013. **174**(1-2): p. 61-5.
84. Kashima, H., Y. Hamada, and N. Hayashi, *Palatability of tastes is associated with facial circulatory responses.* Chem Senses, 2014. **39**(3): p. 243-8.

85. Lang, J., *Clinical Anatomy of the Nose, Nasal Cavity and Paranasal Sinuses*. 1989, New York Thieme.
86. Mercer, P.J., 2014.
87. John W. Castellani, P.D.C.O.B., M.S. , *Peripheral Vasodilation Responses to Prevent Local Cold Injuries* Meeting Proceedings RTO-MP-HFM-126, Keynote 2. Neuilly-sur-Seine, France: RTO, 2005.
88. Krogstad, A.L., et al., *Arteriovenous anastomoses and the thermoregulatory shift between cutaneous vasoconstrictor and vasodilator reflexes.* J Auton Nerv Syst, 1995. **53**(2-3): p. 215-22.
89. Benowitz, N.L., et al., *Interindividual variability in the metabolism and cardiovascular effects of nicotine in man.* J Pharmacol Exp Ther, 1982. **221**(2): p. 368-72.
90. Kyerematen, G.A., et al., *Smoking-induced changes in nicotine disposition: application of a new HPLC assay for nicotine and its metabolites.* Clin Pharmacol Ther, 1982. **32**(6): p. 769-80.
91. Daanen, D.H.A.M., *Arterio-venous anastomoses and thermoregulation*, in *TNO Institute for Perception,Group: Thermophysiology*1991.
92. Dias, D.T., et al., *Thermal characterization in vitro of human nail: photoacoustic study of the aging process.* Photochem Photobiol, 2007. **83**(5): p. 1144-8.
93. Sorensen, L.T., et al., *Smoking as a risk factor for wound healing and infection in breast cancer surgery.* Eur J Surg Oncol, 2002. **28**(8): p. 815-20.
94. James B. Mercer, L.d.W., Åshild Odden Miland and Sven Weum *Pre-, intra-, and postoperative use of dynamic infraredthermography (DIRT) provides valuable information on skin perfusion in perforator flaps used in reconstructive surgery.* FLIR Technical Series Application Note for Research & Science Pre-, intra-, and postoperati, 2010.
95. Alimohammadi, H. and W.L. Silver, *Evidence for nicotinic acetylcholine receptors on nasal trigeminal nerve endings of the rat.* Chem Senses, 2000. **25**(1): p. 61-6.
96. Tang, J.S. and E.A. Kiyatkin, *Fluctuations in central and peripheral temperatures induced by intravenous nicotine: central and peripheral contributions.* Brain Res, 2011. **1383**: p. 141-53.
97. Quick, M.W. and R.A. Lester, *Desensitization of neuronal nicotinic receptors.* J Neurobiol, 2002. **53**(4): p. 457-78.
98. Nyberg, G., et al., *Cardiovascular effect of nicotine chewing gum in healthy non-smokers.* Eur J Clin Pharmacol, 1982. **23**(4): p. 303-7.
99. Foulds, J. and L. Kozlowski, *Snus--what should the public-health response be?* Lancet, 2007. **369**(9578): p. 1976-8.
100. Bates, C., et al., *European Union policy on smokeless tobacco: a statement in favour of evidence based regulation for public health.* Tob Control, 2003. **12**(4): p. 360-7.
101. Rodu, B. and P. Cole, *Smokeless tobacco use and cancer of the upper respiratory tract.* Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2002. **93**(5): p. 511-5.
102. Rodu, B. and C. Jansson, *Smokeless tobacco and oral cancer: a review of the risks and determinants.* Crit Rev Oral Biol Med, 2004. **15**(5): p. 252-63.