

# The effect of oral uptake of nicotine in snus on peripheral skin blood circulation evaluated by thermography

Ina Isabella Høiland<sup>1</sup>, Louis de Weerd<sup>2,3</sup>, and James B Mercer<sup>1,4,\*</sup>

<sup>1</sup>Cardiovascular Research Group; Department of Medical Biology; Faculty of Health Sciences; University of Tromsø, The Arctic University of Norway; Tromsø, Norway;

<sup>2</sup>Department of Clinical Medicine; Faculty of Health Sciences; University of Tromsø, The Arctic University of Norway; Tromsø, Norway; <sup>3</sup>Department of Plastic Surgery and Hand Surgery; University Hospital of North Norway; Tromsø, Norway; <sup>4</sup>Department of Radiology; University Hospital of North Norway; Tromsø, Norway

**Keywords:** nicotine, peripheral circulation, smokeless tobacco, skin blood perfusion, snus, thermography

**Abbreviations:** DIRT, dynamic infrared thermography; EP1, Experimental Protocol 1; EP2, Experimental Protocol 2; ISO, International Standards Organization; IR, infrared; ROI, regions of interest; ST, smokeless tobacco;  $T_{mh}$ , mean skin temperature for the dorsal side of the hand;  $T_{mf}$ , mean skin temperature for the dorsal side of the fingers.

While health risks from smoking cigarettes are well known, little is known about the health risks of using smokeless tobacco (ST). The aim of this study was to evaluate the effect that ST in the form of oral use of snus with nicotine and snus without nicotine has on peripheral skin blood circulation. 21 young habitual users of snus with nicotine participated in this study. Under controlled conditions the subjects were exposed to a 30 minute period of oral use of snus with nicotine (SN+) and snus without nicotine (SN-). The peripheral skin blood circulation was indirectly monitored on the hands by measuring skin temperature using infrared thermography. The skin blood circulation in the hands showed a statistical significant decrease in the SN+ experiments, while skin blood circulation was hardly effected in the SN- experiments. It is concluded that the use of smokeless tobacco in the form of oral use of snus containing nicotine causes a decrease in peripheral skin blood circulation while such an effect is not seen in snus without nicotine. This knowledge may be of use when treating patients that require adequate peripheral skin circulation or in the military when soldiers are exposed cold conditions.

## Introduction

In recent years cigarette sales have declined in the Western World, probably due to more awareness concerning the health risks associated with smoking. One of the well-known toxic substances in cigarette smoke is nicotine, a toxic alkaloid that is highly addictive. Interestingly, in a number of countries, especially in Scandinavia, the decline in cigarettes sales has been associated with an increase in sales of so-called smokeless tobacco (ST) products.<sup>1–3</sup> These products employ other routes for the uptake of nicotine in the body, for example through the mucosal membrane in the mouth by chewing gum, snuff or snus.<sup>4</sup> Since orally consumed ST products are not burned, this makes their use legal in places with anti-smoking regulations.

Snus is a moist form of orally consumed ST and can be used as loose grounded tobacco or contained in sachets or pouches similar to small teabags.<sup>5</sup> The placement is normally under the

upper lip.<sup>5</sup> The World Health Organization (WHO/Europe) has classified snus as carcinogenic<sup>6</sup> and smokeless tobacco users show symptoms of nicotine dependence at least as frequently as cigarette smokers.<sup>7</sup>

Snus users obtain a higher mean plasma blood concentration of nicotine compared to a cigarette smoker due to the higher concentration of nicotine in snus and the duration of use.<sup>8–10</sup> 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.<sup>11,12</sup> The aim of this experimental study was to evaluate the effects snus may have on peripheral skin blood circulation.

Peripheral circulation can be indirectly monitored by measuring skin temperature using thermography where the temperature of large skin areas of interest can be measured with a high degree of accuracy using an infrared camera.<sup>13–16</sup> Using dynamic infrared thermography (DIRT) skin perfusion dynamics can be

© Ina Isabella Høiland, Louis de Weerd, and James B Mercer

\*Correspondence to: James B Mercer; Email: james.mercer@uit.no

Submitted: 10/06/2014; Revised: 10/31/2014; Accepted: 10/31/2014

<http://dx.doi.org/10.4161/23328940.2014.984553>

This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

monitored by capturing multiple infrared images over time.<sup>17</sup> DIRT has been used to study the effect of a short period of nicotine abstinence in cigarette smokers on skin surface temperatures.<sup>18</sup> However, to our knowledge there are no studies that have used thermography as a technique to evaluate the effect snus may have on peripheral skin blood circulation. It is hypothesized that an effect on skin circulation may be expected from nicotine through its absorption via the mucosal membrane of the oral cavity. In this study we restricted our evaluation to the dorsal aspect of the hands and fingers.

## Methods

This study is composed of 2 experimental protocols; Experimental Protocol 1 (EP1) and Experimental Protocol (EP2). EP1 refers to the main study and EP2 to a follow up study. Both protocols employed the same experimental set up.

### Subject recruitment

In EP1 15 young healthy volunteers aged between 19 and 32 y (4 males and 11 females) participated. They were recruited via Facebook or from the University of Tromsø community. In EP2 6 young healthy volunteers aged between 19 and 25 y (3 males and 3 females) were recruited from the University of Tromsø community. Only daily snus users were included, with no considerations as to how often they used snus, how much snus they used, or to the nicotine strength of the snus.

The study was approved by the regional ethical committee. The participants were informed verbally and in writing and signed a consent form before participation.

### Snus

The SN+ was of the Swedish brand Skruf® (Skruf sterk #3), a pouched snus with a nicotine content of 8 mg per portion of 1 g. The SN- was the brand Onico®, which is supposed to taste, look- and smell like regular SN+. For both types a single pouch weighed 1 g.

### Infrared cameras (IR cameras)

All thermal images were taken with a high resolution (0.1°C) IR camera (FLIR ThermalCAM™ SC645). The emissivity was set to 0.98. The rainbow palette was used in this study as recommended by the ISO.<sup>19</sup> The images were stored on a pc and later processed using image analyzing software ThermaCAM Researcher pro 2.8 SR-1 (FLIR Systems AB, Boston, MA, USA).

### Experimental setup

In EP1 and EP2, each subject was subjected to 2 experiments – one in which they received SN+ and a second experiment in which they received (SN-) as a control experiment. The order of SN+/SN- was randomized and the subjects were not informed whether they were receiving SN+ or SN-. In each subject the experiments were performed on different days between the hours of 08.00 and 17.00. Room temperature could not be independently controlled and was usually between 22°C and 23°C, but

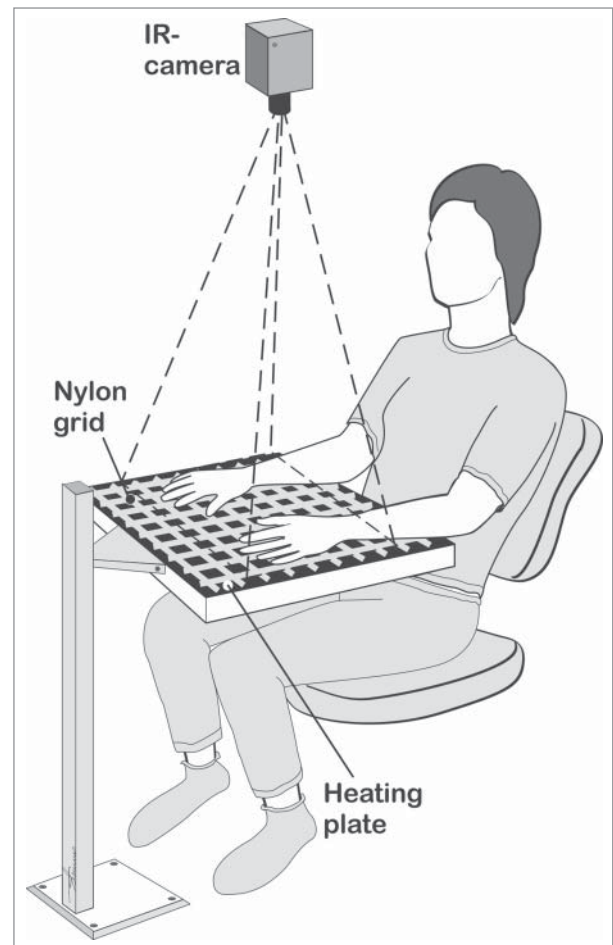


Figure 1. Schematic illustration of experimental setup.

on 3 occasions in EP1 room temperature was as low as 19°C for a short period at the start of the experiment. Air movement within the windowless room was measured at head height of the sitting subjects using a high precision air movement sensor (AirFlow TA-5 Thermal Anemometer, AirFlow developments limited, United Kingdom) and was less than 0.1 m/sec.

In EP1 the subjects were dressed in normal indoor clothing and were seated on a chair throughout the experiment. During the periods when thermal images were not being recorded the subjects were allowed to let their hands hang freely but without contacting any surface. During the short period (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. 1). The nylon grid minimized skin contact with the surface supporting the hands. To provide a constant background temperature an electric heating plate with a surface temperature of ca. 40°C was placed 3 cm below the grid. In the image analysis software it was possible to select a cut-off temperature value which was 0.1C lower than the highest skin temperature measured such that all skin temperatures above this value were colored white, leaving a clear thermal image of the hands on a white background. The short period during which the hands

were placed on the grid avoided heating of the hands by the heating plate. The IR camera was directed toward the dorsal side of the hands (Fig. 1). During the experiments the subjects were able to see the real time IR images on a monitor.

In EP2 the subjects sat with the hands resting on the nylon grid during the entire experimental period (30 minutes). 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 (15 sec) when IR images were being recorded. In contrast to EP1 the subjects were unable to observe the real time thermal images.

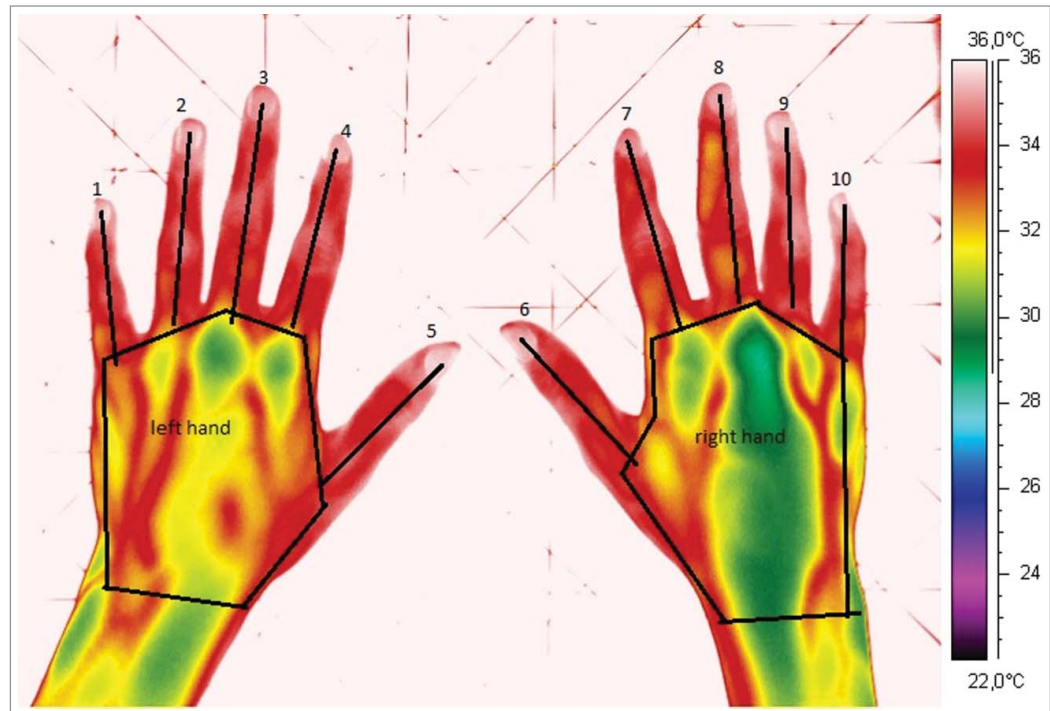
As mentioned above in EP1 there were a few occasions when there was some instability in the hospital room heating system and as a result air temperature fell by a few degrees from the normally controlled level of 22–23°C. To avoid this problem in EP2 a small heating fan was used to ensure a room temperature of 22–23°C at the start of the experiment. The fan heater was turned off during the experiments and the room temperature remained stable thereafter.

#### 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. They were not allowed to use lotions on the hands and to have no nail varnish and were instructed to avoid any cold exposures on the day of the experiment and to refrain from washing their hands before the experiment. They were required to remain indoors for at least 20 minutes before entering the laboratory (stabilization period). After the stabilization period the subject received 2 bags of either SN+ or SN- which were placed by the researcher in the mouth. The subject used their tongues to place the snus under the upper lip.

Each experiment lasted 60 minutes, a 30 min period with the snus in the oral cavity followed by a 30 min recovery period.

The skin surface temperatures were separately analyzed for the hands and the fingers. The regions of interest (ROI's) used are



**Figure 2.** Infrared thermogram with overlay of the ROI's measured on dorsal side of the fingers and the hands. The temperature scale is shown on the right.

shown in Figure 2. For the hand the average temperature within a polygonal ROI extending from the wrist to the base of the digits was used. For the finger the average temperature along a straight line 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 were recorded at the start of the experiment before SN+ or SN- was given (time-point 0) and at time-points 10, 15, 20, and 30 minutes with snus under the lip. Following removal of the snus images were taken at time-points 40, 45, 50 and 60 minutes.

#### Experimental protocol 2 -EP2

In EP2 the subjects were all asked to use snus up to 4 hours before the start of the experiments. In other words the period of abstinence in these subjects was exactly 4 hours. In EP2 the subjects were more heavily dressed than in EP1 with the purpose to obtain vasodilation of the blood vessels at the start of the experiment. Otherwise the participation requirements as used in the EP1 were followed.

After the end of the stabilization period (30 minutes) 2 bags of SN+ or SN- were given to the subjects as described above for EP1 without information to the subjects on the type of snus being administered.

In EP2 thermal images were taken 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. Temperature measurements were restricted to the dorsal aspect of the 10 digits and calculated from straight lines at the ROI's as described above for EP1.

### Skin temperature calculations

All statistical calculations were carried out using Excel® (Microsoft Corporation, USA). The skin temperatures of the hand and the fingers were analyzed separately. For the polygonal ROI 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 temperature along the single line ROI used for the fingers was calculated in a similar manner.

### Statistics

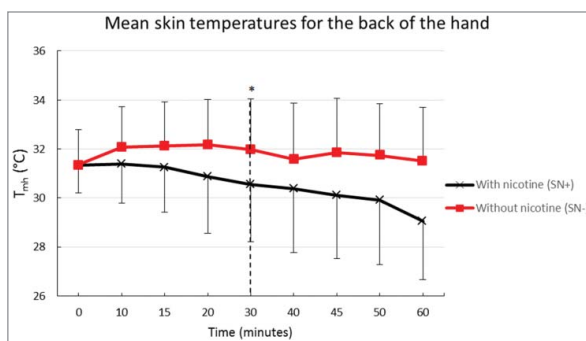
A t-test (hypothesis test) based on pooled measurements was used to calculate the statistical significance of the results. The difference in temperature between the experiment with SN+ and SN- for each person for each set time-point was used for the statistical analysis.

## Results

### Experimental Protocol 1

#### Mean skin temperatures for back of the hand

The mean skin temperature for the hand ( $T_{mh}$ ) throughout the time-course of the experiments in the SN+ and SN- subjects are presented in Figure 3.  $T_{mh}$  at the start of the experiment was very similar in both SN+ and SN- groups (31.3°C and 31.4°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°C). For all other time-points there were no differences in  $T_{mh}$  between SN+ and SN-. In the SN+ group there was a statistically significant drop in  $T_{mh}$  between the start of the experiment and time-point 30,



**Figure 3.** Time course of changes in mean skin temperature of the hands ( $T_{mh}$ )  $\pm$  SD's for the SN+ and SN- subjects in EP1. The dotted line represents the end of snus period (30 minutes). \* = statistical significant difference in  $T_{mh}$  between SN+ and SN- ( $t < t_a = -1.761$ ).

the time the snus was removed. The further drop in  $T_{mh}$  during the 30 minute recovery period was also statistically significant for SN+. No statistical significant change in skin temperature was found for the SN- subjects throughout the time-course of the experiment.

### Mean finger skin temperatures ( $T_{mf}$ )

$T_{mf}$  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 Figure 4.  $T_{mf}$  at the start of the experiment in the 2 groups were practically identical (32.8°C). In the SN+ subjects there was a statistically significant drop in  $T_{mf}$  already after 10 minutes. This trend continued and after 30 minutes  $T_{mf}$  had fallen by 2.8°C. In the SN- groups there were no statistically significant changes during the same period. In both groups  $T_{mf}$  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.

### Experimental Protocol 2

$T_{mf}$  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 Figure 5.  $T_{mf}$  was similar in both groups at the start of the experiment (33.8°C for SN+ and 34.0°C for SN-). Throughout the time-course of EP2 there was a statistically significant difference between SN+ and SN- subjects only at time-points 20 and 25.

The decrease in  $T_{mf}$  from time-point 0 to time-point 25 for the SN+, subjects was statistically significant, however, not at time-point 30. A small but statistically insignificant increase in  $T_{mf}$  can be seen in the SN- subjects throughout the first 25 minutes in which the subjects have snus under the upper lip.

## Discussion

In this study the effect of nicotine uptake over the oral mucosa on skin blood circulation of the hands of young healthy habitual users of snus containing nicotine has been studied. The results show that peripheral skin blood circulation decreased when using snus containing nicotine, but not when using snus without nicotine. The most likely explanation of this effect is that the substance nicotine in snus causes a vasoconstriction of the peripheral blood vessels and therewith causes a decrease in skin temperature. While this finding was not unexpected due to the well documented effects of nicotine uptake through smoking,<sup>20</sup> to our knowledge this is the first study in which such an effect has been demonstrated through the oral administration of nicotine by using snus. 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+. For ease of clarity the results of the 2 protocols used, EP1 and EP2, are separately discussed, followed by some general conclusions.

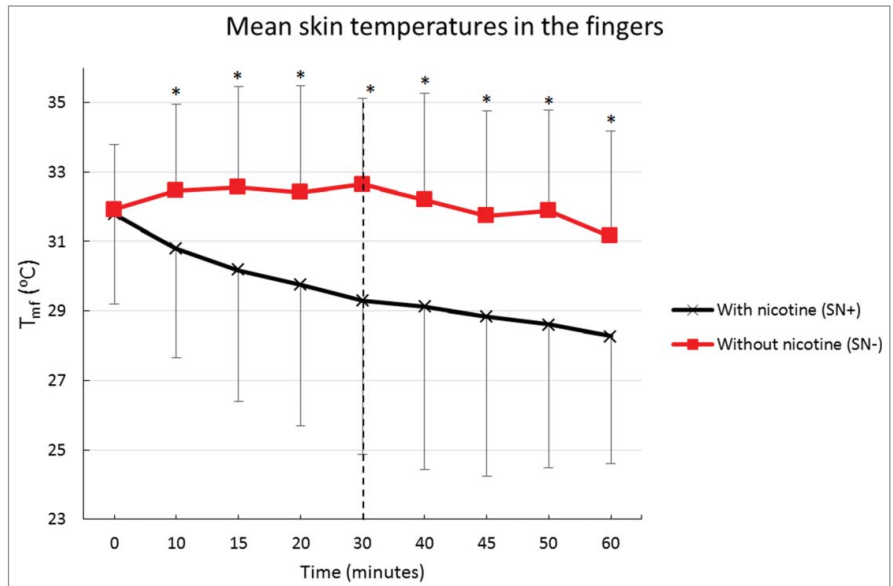
### Experimental Protocol 1

When considering the skin temperature measurements in both EP1 and EP2 an influence of the heating plate (40°C) that was positioned below the nylon grid in order to ensure a uniform background temperature in the IR images (Fig. 1) 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 this heating, which was limited to 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 would cause a common error for both the SN+ and SN- experiments.

The vasoconstrictive effect of nicotine intake during exposure to SN+ was more pronounced on the fingers (Fig. 4) compared to the hand (Fig. 3). Interestingly, the time-course of the changing pattern of hand skin temperature caused by 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.<sup>21</sup> This temperature fall is known to be associated with the shutting down of AVAs.<sup>22</sup>

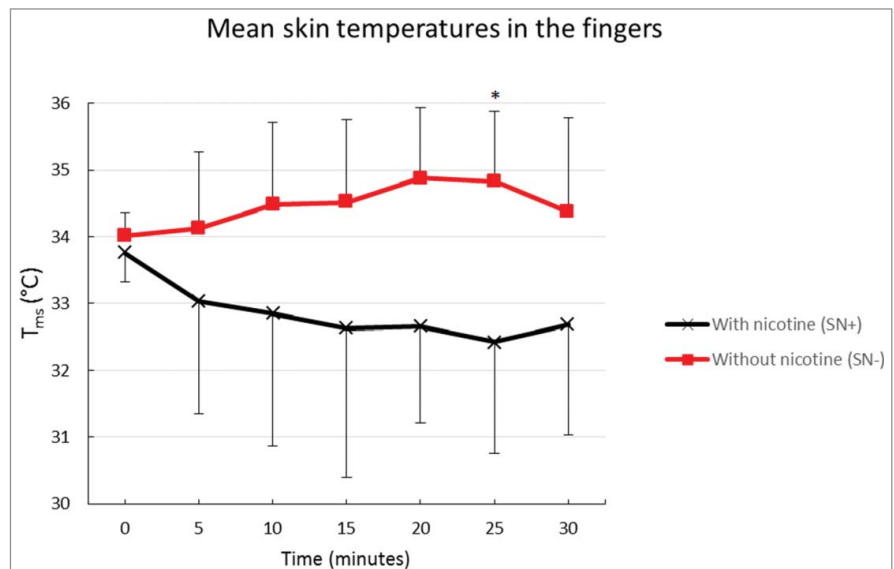
For the hand the fall in skin temperature during the period in which SN+ was in the oral cavity only became statistically significant at time-point 30, the end of the snus period. The  $T_{mh}$  in the SN- group changed very little throughout the entire experiment while for the SN+ group  $T_{mh}$  not only fell during the snus period but continued to fall during the recovery period. The temperature fall in the SN+ experiment, 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.<sup>23,24</sup> 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.<sup>25</sup>

The vasoconstrictive effect of nicotine was most evident on  $T_{mf}$  and it is



**Figure 4.** Time course of changes in mean skin temperature of the fingers ( $T_{mf}$ )  $\pm$  SD's for the SN+ and SN- subjects in EP1. The dotted line represents the end of snus period (30 minutes). \* = statistically significant difference in temperature  $T_{mf}$  between SN+ and SN- ( $t < t_a = -1.761$ ).

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<sup>26</sup> 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



**Figure 5.** Time course of changes in mean skin temperature of the fingers ( $T_{mf}$ )  $\pm$  SD's for the SN+ and SN- subjects in EP2 during the 30 minute period snus was placed in the oral cavity. \* = statistically significant difference in temperature between SN+ and SN- ( $t < t_\alpha = -2.00$ ).

connections between the terminal arteries and veins.<sup>27</sup> 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 vasodilated subjects the opening of the AVAs is easily visible in the IR-images due to its effect on peripheral skin blood 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 the SN+ subjects in this study a clear temperature fall at the finger tips could be seen.

Ideally it was hoped that at the start of each experiment all subjects would be in a similar vasomotor state (vasodilated) and have similar mean skin temperature values. However, this was not the case in EP1 and, although the  $T_{mh}$  and  $T_{mf}$  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,  $T_{mf}$  fell by nearly 3°C for the SN+ group during the 30 minutes of snus compared to  $T_{mf}$  for the SN- group which had almost an 0.5°C increase in the same period.

In this study 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.<sup>28</sup> 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  $T_{mf}$  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 2 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 hand. A similar finding was also seen for  $T_{mh}$  of the hands in the SN+ subjects. In support of the former suggestion it was noticed that room temperature was as low as 19°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  $T_{mf}$  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  $T_{mf}$  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 vasoconstrictive effect nicotine has on skin blood vessels.

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. This 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 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 EP2.

### Experimental Protocol 2

The protocol used in EP2 clearly reduced the standard deviation in both the SN+ and SN- subjects at the start of the experiment. However, the overall results of EP2 were similar to EP1. The implications of the results from this study may be found in health care and the military. For example a recent study has shown that surgical site infections occur more frequently in smokers compared to non-smokers.<sup>29</sup> The infections may be explained by a compromised skin circulation in the operated area by nicotine. A negative effect on skin blood circulation could lead to a higher risk for postoperative wound healing complications. Patients with peripheral vascular diseases or wound healing problems may be advised not to use snus containing nicotine. In the military non-freezing cold injuries and frost bites are still a major problem. The interference of snus containing nicotine with peripheral skin blood circulation may predispose those soldiers using snus to these forms of cold injuries.

## Conclusions

The main finding in this thermography based study is the demonstration of a strong decrease in peripheral skin blood circulation through the uptake of nicotine in snus, presumably due to the vasoconstrictive effect of nicotine. While similar responses are known from cigarette smoking, to our knowledge this study is the first to show this for habitual users of snus containing nicotine. 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.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest are disclosed.

### Acknowledgment

The authors wish to thank senior laboratory technician Knut Steinnes, Cardiovascular Research Group, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, Norway, for preparing **Figure 1**.

## References

- Røykevaner, 2013. Statistics Norway.
- Norwegian Institute of Public Health. Smoking and smokeless tobacco in Norway - fact sheet. [http://www.fhi.no/eway/default.aspx?pid=240&ctr=MainContent\\_6894&Main\\_6664=6894:0:25,7629:1:0:0:::0:0&MainContent\\_6894=6706:0:25,7640:1:0:0:::0:0](http://www.fhi.no/eway/default.aspx?pid=240&ctr=MainContent_6894&Main_6664=6894:0:25,7629:1:0:0:::0:0&MainContent_6894=6706:0:25,7640:1:0:0:::0:0)
- Foulds J, Ramström L, Burke M, Fagerström K. Effects of smokeless tobacco (snus) on smoking and public health in Sweden. *Tob Control* 2003; 12:349-59; <http://dx.doi.org/10.1136/tc.12.4.349>
- WHO International Agency for Research on Cancer (IARC) Monograph on the Evaluation of Carcinogenic Risks to Humans. Smokeless Tobacco and Some Tobacco-specific N-Nitrosamines 2007; 89:PP626; Available from: <http://monographs.iarc.fr/ENG/recent-pub/mono89.pdf>
- Krisberg K. New types of smokeless tobacco present growing risk for youth: Survey: Products mistaken for candy. *Nations Health* 2010; 40:1-14.
- Directive 2004/40/EU of the European Parliament and of the Council on the approximation of the laws, regulations and administrative provisions of the Member States concerning the manufacture, presentation and sale of tobacco and related products and repealing Directive 2001/37/EC [http://ec.europa.eu/health/tobacco/docs/dir\\_201440\\_en.pdf](http://ec.europa.eu/health/tobacco/docs/dir_201440_en.pdf)
- Post A, Gilljam H, Rosendahl I, Bremberg S, Galanti MR. Symptoms of nicotine dependence in a cohort of Swedish youths: a comparison between smokers, smokeless tobacco users and dual tobacco users. *Addiction* 2010; 105:740-746; PMID:20148785; <http://dx.doi.org/10.1111/j.1360-0443.2009.02852.x>
- Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci* 1992; 13:177-84; PMID:1604710; [http://dx.doi.org/10.1016/0165-6147\(92\)90060-J](http://dx.doi.org/10.1016/0165-6147(92)90060-J)
- Ebbert JO, Croghan IT, Schroeder DR, Hurt RD. A randomized phase II clinical trial of high-dose nicotine patch therapy for smokeless tobacco users. *Nicotine Tob Res* 2013; 15:2037-44; PMID:23873976; <http://dx.doi.org/10.1093/ntr/nts097>
- Digard H, Proctor C, Kulasekaran A, Malmqvist U, Richter A. Determination of nicotine absorption from multiple tobacco products and nicotine gum. *Nicotine Tob Res* 2013; 15:255-61; PMID:22585541; <http://dx.doi.org/10.1093/ntr/nts123>
- Benowitz NL. Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. *Prog Cardiovasc Dis* 2003; 46:91-111; PMID:12920702; [http://dx.doi.org/10.1016/S0033-0620\(03\)00087-2](http://dx.doi.org/10.1016/S0033-0620(03)00087-2)
- Benowitz NL, Jacob P 3rd, Yu L. Daily use of smokeless tobacco: systemic effects. *Ann Intern Med* 1989; 111:112-16; PMID:2742246; <http://dx.doi.org/10.7326/0003-4819-111-2-112>
- Schlager O, Gschwandtner ME, Herberg K, Frohner T, Schillinger M, Koppensteiner R, Mlekusch W. Correlation of infrared thermography and skin perfusion in Raynaud patients and in healthy controls. *Microvasc Res* 2010; 80:54-7; PMID:20144625; <http://dx.doi.org/10.1016/j.mvr.2010.01.010>
- Awwad AM, White RJ, Webster MH, Vance JP. The effect of temperature on blood flow in island and free skin flaps: an experimental study. *Br J Plast Surg* 1983; 36:373-82; PMID:6860870; [http://dx.doi.org/10.1016/S0007-1226\(83\)90064-4](http://dx.doi.org/10.1016/S0007-1226(83)90064-4)
- Ring EFR, Ammer K. Infrared thermal imaging in medicine. *Physiol. Meas* 2013; 33 R33-R46; <http://dx.doi.org/10.1088/0967-3334/33/3/R33>
- Stikbakke E, Mercer JB. An infrared thermographic and laser Doppler flowmetric investigation of skin perfusion in the forearm and finger tip following a short period of vascular stasis. *Thermol Int* 2008; 18: 107-11.
- De Weerd L, Mercer JB, Bøe Setså L. Intraoperative Dynamic infrared thermography and free-flap surgery. *Ann Plastic Surg* 2006; 57:279-84; PMID:16929195; <http://dx.doi.org/10.1097/01.sap.0000218579.17185.c9>
- Miland AO, Mercer JB. Effect of a short period of abstinence from smoking on rewarming patterns of the hands following local cooling. *Eur J Appl Physiol* 2006; 98:161-8; PMID:16874507; <http://dx.doi.org/10.1007/s00421-006-0261-2>
- ISO/TR 2009 Medical electrical equipment—deployment, implementation and operational guidelines for identifying febrile humans using a screening thermograph ISO/TR 13154:2009.
- Trap-Jensen J. Effects of smoking on the heart and peripheral circulation. *Am Heart J* 1988; 115:263-67; PMID:3276115; [http://dx.doi.org/10.1016/0002-8703\(88\)90647-3](http://dx.doi.org/10.1016/0002-8703(88)90647-3)
- Rasmussen L, Mercer JB. A comparison of the thermal responses in hands and feet of young and elderly subjects in response to local cooling as determined by infrared imaging. *Thermol Int* 2004; 14:71-6.
- Krogstad AL, Elam M, Karlsson Tm Wallin BG. Arteriovenous anastomoses and the thermoregulatory shift between cutaneous vasoconstrictor and vasodilator reflexes. *J Auton Nerv Syst* 1995; 53:215-22; PMID:7560758; [http://dx.doi.org/10.1016/0165-1838\(94\)00178-M](http://dx.doi.org/10.1016/0165-1838(94)00178-M)
- Benowitz NL, Jacob P 3rd, Jones RT, Rosenberg J. Interindividual variability in the metabolism and cardiovascular effects of nicotine in man. *J Pharmacol Exp Ther* 1982; 22: 368-72.
- Kyerematen GA, Damiano MD, Dvorchik BH, Vesell ES. Smoking-induced changes in nicotine disposition: application of a new HPLC assay for nicotine and its metabolites. *Clin Pharmacol Ther* 1982; 32:769-80; PMID:7140141; <http://dx.doi.org/10.1038/clpt.1982.235>
- Holm H, Jarvis MJ, Russell MA, Feyerabend C. Nicotine intake and dependence in Swedish snuff takers. *Psychopharmacology (Berl)* 1992; 108:507-11; PMID:1410167; <http://dx.doi.org/10.1007/BF02247429>
- Bornmyr S, Svensson H, Söderström T, Sundkvist G, Wollmer P. Finger skin blood flow in response to indirect cooling in normal subjects and in patients before and after sympathectomy. *Clin Physiol* 1998; 18:103-7; PMID:9568348; <http://dx.doi.org/10.1046/j.1365-2281.1998.00082.x>
- Daanen HAM. Arterio-venous anastomoses and thermoregulation. TNO Defence Research, Institute for Perception. Report IZF 1991 B-12, 1991: pp 1-20.
- Dias DT, Steimacher A, Bento AC, Neto AM, Baesso ML. Thermal characterization in vitro of human nail: photoacoustic study of the aging process. *Photochem Photobiol* 2007; 83:1144-8; <http://dx.doi.org/10.1111/j.1751-1097.2007.00074.x>
- Sørensen LT, Hørby J, Friis E, Pilsgaard B, Jørgensen T. Smoking as a risk factor for wound healing and infection in breast cancer surgery. *Eur J Surg Oncol* 2002; 28: 815-20; PMID:12477471; <http://dx.doi.org/10.1053/ejso.2002.1308>