



Original article

The effects of oral smokeless tobacco administration on endurance performance

Thomas Zandonai ^{a,b,*}, Enrico Tam ^{c,†}, Paolo Bruseghini ^c, Fabio Pizzolato ^c, Loretta Franceschi ^d, Massimo Baraldo ^d, Carlo Capelli ^{c,e}, Paola Cesari ^c, Cristiano Chiamulera ^a

^a Neuropsychopharmacology Laboratory, Department of Diagnostic and Public Health, University of Verona, Verona 37134, Italy

^b Mind, Brain and Behavior Research Center CIMCYC, Department of Experimental Psychology, University of Granada, Granada 18071, Spain

^c Department of Neurosciences, Biomedicine and Movement Sciences, School of Exercise and Sport Science, University of Verona, Verona 37131, Italy

^d Department of Experimental and Clinical Medical Sciences, University of Udine, Udine 33100, Italy

^e Department of Physical Performances, Norwegian School of Sport Sciences, Oslo N-0806, Norway

Received 24 June 2016; revised 14 September 2016; accepted 8 November 2016

Available online

Abstract

Background: Smokeless tobacco is widely used by athletes to enhance performance. Nicotine is a central nervous system stimulant and acts on cardiocirculatory and metabolic systems, involving tissue blood flow and circulatory vasoreactivity. The aim of this study was to investigate the effects of the oral smokeless tobacco (Swedish snus (SS)) on the perception of fatigue and time to exhaustion (TTE) during moderate-intensity aerobic exercise.

Methods: Fourteen healthy non-tobacco male users were recruited for a double-blind, controlled crossover design (SS vs. snus placebo (SP)). Subjects were tested for 3 sessions: experimental session 1 (Exp1) consisted of an incremental test to determine the maximal aerobic power output (W_{max}), whereas Exp2 and Exp3 consisted of exercising at 65% W_{max} until exhaustion in SS or SP conditions. During Exp2 and Exp3, muscle and cerebral oxygenation was assessed by means of near-infrared spectroscopy, and the rating of perceived exertion (RPE) was recorded.

Results: Comparing SS with SP tests, significant differences ($p < 0.05$) were found in the values of cerebral (~3%) and muscular tissues oxygenation (~4%) in the first 30 min of exercise. The RPE values were not significantly different between the 2 conditions (SS vs. SP). No significant difference was found in TTE (SS: 54.25 ± 21.84 min; SP: 50.01 ± 17.03 min).

Conclusion: This study showed that muscular and cerebral oxygenation increased significantly with snus administration during an endurance exercise until exhaustion, but this did not affect fatigue perception and TTE. The results showed that snus could not be considered an ergogenic substance in non-tobacco users.

© 2017 Production and hosting by Elsevier B.V. on behalf of Shanghai University of Sport. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Keywords: Exercise endurance; Maximal aerobic power; Nicotine; RPE; Smokeless tobacco; Snus; Time to exhaustion; Tissue oxygenation

1. Introduction

Snus is a smokeless, orally consumed tobacco traditionally produced and used in Scandinavian countries.¹ It is placed between the upper lip and the gums for approximately 30 min, and then discarded. Sweden is the only country in the European Union granted special exemption to manufacture and sell snus.^{2–4} In the USA, snus was introduced in 2006, and since then an upward trend on its use has been seen, as reported by Alpert et al.⁵ An analytical

chemistry study showed that ice hockey players take relevant amounts of nicotine.⁶ More recently, Marclay and colleagues⁷ assessed the amount of nicotine intake in athletes from various sports by measuring nicotine and metabolite levels following the analytical chemistry detection. An increased diffusion of this habit has also been documented in countries where the use of snus has never been popular. In this regard, some anecdotal reports have been recently published on the use of snus among alpine skiers in Italy: 74% of the athletes who practice winter sports have tried snus at least once and 50% of them continue to use it.⁸ However, up to now, no clear evidence is available for sustaining the positive effect that snus may have on sport performance.

To detect potential patterns of abuse, nicotine was listed in the World Anti-Doping Agency's Monitoring Program from

Peer review under responsibility of Shanghai University of Sport.

* Corresponding author.

E-mail address: thomas.zandonai@univr.it (T. Zandonai)

† Contributed equally to this manuscript.

<http://dx.doi.org/10.1016/j.jshs.2016.12.006>

2095-2546/© 2017 Production and hosting by Elsevier B.V. on behalf of Shanghai University of Sport. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

2012 to 2016.⁹ It therefore appears important to address the still unanswered questions on the sought and subjective reinforcing effects experienced by some athletes. From a psychobiological standpoint, snus is a delivery system of a psychoactive and addictive substance, that is, nicotine.^{10,11} Snus users have positive expectancies about the potential effects,¹² report subjective pleasure,¹³ and exhibit dependent behavior and withdrawal symptoms.^{14,15} Considering all the issues mentioned above, athletes with the desire to overcome their psychophysical limits are induced to believe that snus has positive effects on sport performance.¹⁶

Nicotine reduces heart rate variability, increases perception of mental fatigue and workload, and affects the perceived readiness level among amateur football players who are non-smokers and non-snus users.¹⁷ It also impairs myocardial perfusion during heart stimulation in healthy non-smokers¹⁸ and reduces stroke volume during submaximal intensity exercise.¹⁹ Therefore, the use of nicotine may negatively affect exercise capacity.²⁰

Furthermore, data describing the possible effects of smokeless tobacco on endurance performance in healthy subjects are scant. One study has suggested that nicotine, administered by patch, significantly increases time to exhaustion (TTE) by ~17% during endurance performance.²¹ However, even if no effect on fatigue perception was detected when compared with placebo, the authors suggested that nicotine prolongs TTE via a central mechanism. Indeed, stimulation of nicotinic cholinergic receptors (nAChR) releases a variety of neurotransmitters in the brain.²² One of them, dopamine, has direct effects on brain microcirculation and underlies blood flow changes of brain functions.²³

In recent years, near-infrared spectroscopy (NIRS) has been introduced to investigate muscular and prefrontal cortex oxygenation during exercise.²⁴ Acute changes of oxyhemoglobin concentration [O₂Hb] connected to the increased cerebral blood flow are attributed to enhanced neural activation during exercise²⁴ and mental tasks.²⁵ Hence, NIRS could be used as a noninvasive tool to monitor the modulatory effect on central fatigue of a hexogen psychoactive substance like nicotine. Nevertheless, only 2 papers have reported a significant nicotine effect on neural metabolic activation in animals²⁶ and humans,²⁷ whereas no study has simultaneously investigated the muscular and cortical oxygenation under smokeless tobacco.

The purpose of this study was to investigate the effects of snus use during moderate-intensity endurance exercise and on perception of fatigue. Furthermore, the prefrontal cortex and muscular perfusion-oxygenation, as well as the cardiovascular and metabolic responses to exercise, were investigated. We supposed that nicotine administration through snus might increase TTE, decrease the perception of effort, and modulate muscular-cerebral blood perfusion.

2. Methods

2.1. Participants

We recruited 14 healthy male (18–45 years) non-smokers and non-snus users. Two subjects withdrew from the study

because of a leg injury during the washout week. The following were the participants' characteristics (mean ± SD): age = 23.1 ± 4.7 years; height = 178.0 ± 6.1 cm; weight = 74.5 ± 7.1 kg; the maximal oxygen uptake, $\dot{V}O_{2\max}$ = 48.9 ± 7.6 mL/kg/min; the maximal mechanical aerobic power output, W_{\max} = 316.3 ± 71.1 W; 65% W_{\max} = 205.6 ± 46.2 W; and HR_{\max} = 181 ± 9 beats/min. Three participants practiced soccer, 3 practiced running, 2 practiced tennis, and the remaining participants practiced gymnastics, gym, basketball, and swimming.

2.2. Experimental design

A double-blind, randomized crossover study was designed to compare the effect of Swedish snus (SS) and snus placebo (SP) on exercise endurance. The protocol consisted of 3 trials of cycling exercise. Experiment 1 (Exp1) consisted of an incremental exercise test to determine the W_{\max} , the $\dot{V}O_{2\max}$, and the oxygen uptake and workload ($\dot{V}O_2/W$) relationship at steady state. Experiment 2 (Exp2) and Experiment 3 (Exp3) involved exercising at 65% W_{\max} until exhaustion. A medical visit was conducted on the same day of Exp1, and after 3 days all participants carried out Exp2. Exp3 took place 1 week after Exp2. The participants were randomized to blindly receive either SS or SP on Exp2 or Exp3. All participants were informed about the procedures and risks of the study and signed an informed consent. The local ethical committee of the Department of Neuroscience, Biomedicine and Movement, University of Verona (Italy), approved the study protocol. The study was conducted in accordance with the current guidelines of the Declaration of Helsinki.

2.3. Exercise protocol

The subjects abstained from vigorous physical activity and alcohol or caffeine consumption during the 24 h before the tests. All tests were performed in the Exercise Physiology Laboratory of the University of Verona.

2.3.1. Exp1

First, the participants performed a submaximal test on a cycle-ergometer (Sport Excalibur; Lode, Groningen, The Netherlands) to obtain the $\dot{V}O_2/W$ relationship. Three minutes of rest were followed by 6 min of warm-up at 0 W, and then the workload was set at 30 W and increased by 30 W up to 150 W every 6 min. Pedaling frequency was kept constant at 75 rpm by means of a visual pacer. After 30 min of rest, a maximal incremental exercise test was performed. The initial workload was set at 50 W and increased by 30 W every 1 min until voluntary exhaustion. Voluntary exhaustion was defined as the inability to maintain the pedaling frequency (60–80 rpm) despite vigorous encouragement by the experimenters. Heart rate (HR), expiratory minute ventilation (\dot{V}_E), and $\dot{V}O_2$ and CO_2 production ($\dot{V}\text{CO}_2$) were continuously recorded (Quark b²; COSMED, Rome, Italy). The test was considered maximal if one of the following criteria was met: (1) final HR was within 10% of predicted maximum; (2) a clear plateau in oxygen uptake was noticed; and (3) respiratory exchange ratio ($\text{RER} = \dot{V}\text{CO}_2/\dot{V}O_2$) was equal to, or above, 1.10.²⁸ The global rating of perceived exertion (RPE) was recorded in the last 15 s of each step using

the 15-point Borg scale.²⁹ W_{\max} was identified as the workload corresponding to the intersection point between the individual linear VO_2/W relationship and $VO_{2\max}$.

2.3.2. Exp2 and Exp3

To minimize differences and ensure adequate muscle glycogen concentration, the participants were asked to follow a high carbohydrates diet 24 h before the tests.

The measurement of exhaled carbon monoxide level provided an immediate method of assessing smoking status, and it was recorded before each test using the EC50 Micro Smokerlyzer (Bedfont Scientific Ltd., Maidstone, UK). The participants rested on the cycle-ergometer for 5 min while baseline measurements were obtained. Blood samples were taken 3 min before SS or SP administration to determine blood lactate ($[La]_b$, mmol/L), blood glucose ($[Glu]_b$, mmol/L), and hemoglobin ($[Hb]_b$, g/dL) concentrations. SS or SP sachet was placed in the anterior part of the mouth between the upper gingiva at time zero (T_0). The participants kept the sachets in their mouth until the end of the exercise. Exercise started at T_0 (warm-up) and the subject pedaled at a constant frequency of 75 ± 5 rpm for 5 min. The workload was set at 100 ± 50 W to impose the same delta workload between warm-up and $65\%W_{\max}$. At the end of warm-up, the participants started pedaling at individual $65\%W_{\max}$ until exhaustion. The arteriolar pulse pressure profile at a fingertip was continuously measured using a noninvasive photoplethysmographic method (Portapres, FMS, Amsterdam, The Netherlands). Cardiac output (Q) and stroke volume (SV) were obtained by means of the Modelflow algorithm (BeatScope software Version 1.1a; FMS, Amsterdam, The Netherlands).³⁰ Muscle and cerebral oxygenation was continuously recorded (50 Hz sampling rate) by means of NIRS (OxiplexTS; ISS, Champaign, IL, USA).³¹ Muscular NIRS probe was positioned on the vastus lateralis muscle 15 cm above the patella. NIRS cerebral probe was positioned on the forehead and attached to the skin. The forehead side was the opposite of the dominant leg.²⁴ Tissue oxygenation index (TOI) was calculated as the ratio between absolute $[O_2Hb]$ and total hemoglobin $[THb]$. All the NIRS variables were normalized with respect to their related values at rest. RPE was recorded every 5 min until the end of the test using the 15-point Borg scale.²⁹ Blood samples to determine $[La]_b$, $[Glu]_b$, and $[Hb]_b$ were taken every 10 min from T_0 until the end of the test (Fig. 1).

At the end of the exercise, the participants spat out snus. Afterwards, we immediately interviewed them about the adverse

events of nicotine (if yes: mild, moderate, or serious). Finally, 5 mL of venous blood was sampled without stasis to assess the nicotine and cotinine levels.

2.4. Snus and blinding procedure

We administered 8 mg of nicotine in a portion of SS (1.0 g Catch White Eucalyptus from Swedish Match, Stockholm, Sweden). SS is a moist oral tobacco product with water content of 45%–55% and a pH of 8.5.¹ The placebo was completely free of tobacco and nicotine, and had the same pH, fragrance, form, and appearance as of the SS sachets (1.0 g portion of placebo, Onico Peppermint from Swedish Match). The SS and SP boxes were coded and covered so that neither the investigators nor the participants were aware of the contents.

2.5. Blood sample

Nicotine and cotinine were determined by means of high-performance liquid chromatography LC-200 pump technique. $[La]_b$, $[Glu]_b$, and $[Hb]_b$ (g/dL) were assessed in arterialized capillary blood (Biosen C-Line; EKF Diagnostic, Barleben, D, Germany; and HemoCue Hb 201; HemoCue AB, Ängelholm, Sweden).

2.6. Statistical analysis

All data were analyzed up to 30 min and at TTE to include the maximum number of subjects. Exercise data were analyzed to verify normal distribution using Shapiro-Wilk W test. TTE data were analyzed using a paired Student's *t* test (SS vs. SP).

Respiratory, cardiovascular, and NIRS-derived values were averaged over a 30 s interval for every 5 min of exercise. Cerebral and muscle NIRS data were normalized to baseline concentration values (Δ values). All data were reported as mean \pm SD.

A full within 2-way analysis of variance repeated measures (time \times SS/SP) was used to determine the differences in RPE, cardiorespiratory, blood, and NIRS average values. When significant effects were detected, a *post hoc* analysis was performed using Bonferroni's multiple comparison test. Statistical significance was always accepted at $p < 0.05$. Analysis was done using the Prism 6 statistical software (GraphPad, La Jolla, CA, USA).

3. Results

3.1. Smoking status assessment and nicotine and cotinine levels

The level of carbon monoxide was 0.9 ± 0.5 ppm before SS test and 1.0 ± 0.9 ppm before SP test, respectively. The values were distinctive of non-smokers (< 6.5 ppm).³² At the end of the trials at $65\%W_{\max}$, the concentrations of plasma nicotine were 4.49 ± 3.48 ng/mL in SS and 0.50 ± 0.90 ng/mL in SP ($Z = 0.0$, -45.0 ; $W = -45$; $p = 0.039$), whereas those of the plasma cotinine were 27.83 ± 17.65 ng/mL in SS and 5.00 ± 9.04 ng/mL in SP ($Z = 0.0$, -66.0 ; $W = -66.0$; $p = 0.010$).

3.2. Adverse events

Five participants reported adverse events at the end of the experiments at $65\%W_{\max}$. One subject reported a mild

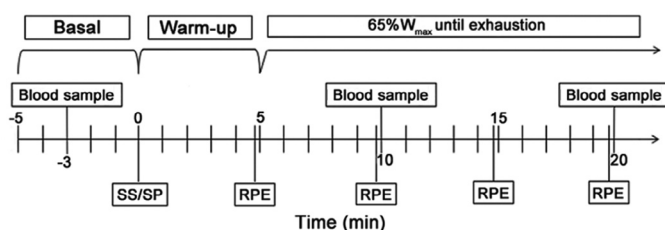


Fig. 1. Graphical representation of the experimental protocol in Exp2 and Exp3. RPE = rating perception of effort (Borg scale²⁹); SP = snus placebo; SS = Swedish snus.

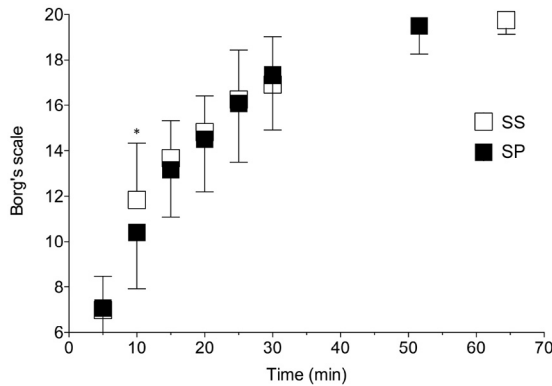


Fig. 2. Borg scale values (mean \pm SD) in the first 30 min and at TTE during SS and SP. * $p < 0.05$, compared with SP; $n = 12$. SP = snus placebo; SS = Swedish snus; TTE = time to exhaustion.

discomfort in the throat and another one reported mild nausea and dizziness at the end of the trial. A moderate nausea and dizziness was reported by other 3 participants. None reported adverse events under SP condition.

3.3. Exercise capacity and perceived exertion

The average TTE was 54.25 ± 21.84 min during the SS session, and 50.01 ± 17.03 min during the SP session. However, the difference was not statistically significant ($p = 0.4503$).

Borg scale values at the 10th minute of exercise were significantly larger than those assessed every 5 min of exercise in both Exp2 and Exp3. The mean difference between the 2 scores was 1.42 ± 0.42 ($F(6, 66) = 162.6$, $p = 0.0090$). Although RPE score kept increasing during exercise, score values were not significantly different (Fig. 2).

3.4. Lactate, glucose, and hemoglobin concentration

$[La]_b$ significantly increased during the first 10 min of exercise from ~ 1 mmol/L at rest in SS and in SP ($p < 0.001$) to remain afterwards stable until TTE. $[Glu]_b$ significantly decreased from rest in SS and SP ($p < 0.001$). $[Hb]_b$ remained stable from rest to TTE. There were no significant differences between SS and SP conditions (Table 1).

Table 1

Blood sample concentrations of lactate ($[La]_b$), glucose ($[Glu]_b$), and hemoglobin ($[Hb]_b$) in SS and SP conditions before prolonged exercise at baseline and at exhaustion (mean \pm SD).

	SS		
	Pre	Post	p
$[La]_b$ (mmol/L)	0.91 ± 0.21	5.01 ± 3.01	<0.001
$[Glu]_b$ (mmol/L)	4.66 ± 0.56	3.86 ± 0.39	<0.001
$[Hb]_b$ (g/dL)	17.21 ± 1.35	16.91 ± 0.95	0.328
	SP		
	Pre	Post	p
$[La]_b$ (mmol/L)	1.03 ± 0.42	4.51 ± 2.55	<0.001
$[Glu]_b$ (mmol/L)	4.74 ± 0.47	3.79 ± 0.47	<0.001
$[Hb]_b$ (g/dL)	16.97 ± 1.26	17.22 ± 1.37	0.264

Abbreviations: SP = snus placebo; SS = Swedish snus.

3.5. Metabolic and respiratory responses

VO_2 was, on average, 0.36 L/min at rest in both conditions and increased during exercise to attain a value of 2.64 ± 0.56 L/min and 2.63 ± 0.59 L/min in SS and SP, respectively. It slightly increased from the 10th minute of exercise to TTE by 0.16 L/min in SS and by 0.25 L/min in SP ($p = 0.9999$). No significant differences between SP and SS were observed throughout the trials as for V_E , VO_2 , and VCO_2 . The average RER during exercise was the same (1.03 ± 0.04) in both SS and SP.

3.6. Cardiovascular responses

Q at rest was 6.41 ± 0.75 L/min in SS and 6.48 ± 1.05 L/min in SP; it increased during exercise to 20.2 ± 2.42 L/min in SS and to 19.36 ± 2.06 L/min in SP. HR at rest was equal to 67.3 ± 7.9 bpm in SS and to 67.3 ± 11.8 bpm in SP; it increased during exercise to 167.0 ± 11.6 bpm in SS and to 163.1 ± 11.1 bpm in SP. However, Q and HR in SS and SP conditions were not significantly different during the time trial.

SBP and DBP increased at the exercise onset, reaching their zenith after the 15th–20th minute of exercise (Fig. 3). DBP at TTE was significantly smaller in SS (73.10 ± 8.53 mmHg) than in SP (80.70 ± 8.56 mmHg) ($p = 0.0068$).

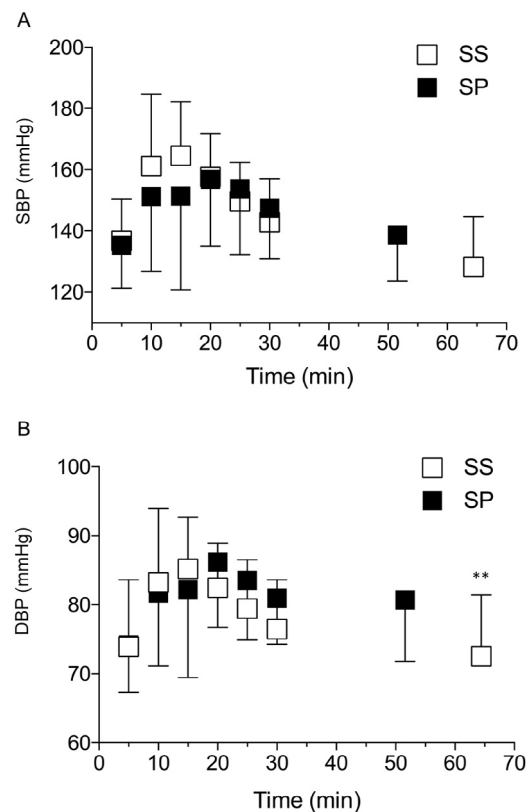


Fig. 3. SBP (A) and DBP (B) values (mean \pm SD) in the first 30 min and at TTE during SS and SP. ** $p < 0.01$, compared with SP; $n = 12$. DBP = diastolic blood pressure; SBP = systolic blood pressure; SP = snus placebo; SS = Swedish snus; TTE = time to exhaustion.

3.7. Muscular and cerebral NIRS responses

Cerebral (C) and muscular (M) Δ TOI, $[\Delta$ THb], and $[O_2Hb]$ values in SS were systematically larger than the ones observed in SP (Fig. 4A–F).

In particular, TOI (C) was significantly larger in SS than in SP at the 10th ($p = 0.0184$), 15th ($p = 0.0077$), 20th

($p = 0.0096$), and 30th ($p = 0.0152$) minute of exercise (Fig. 4A); Δ THb (C) and ΔO_2Hb (C) in SS were significantly larger than in SP at the 25th minute ($p = 0.0256$) and at the 10th minute ($p = 0.0166$) of exercise, respectively (Fig. 4C and E).

On average, Δ TOI (M) was significantly greater in SS than in SP at the 15th minute ($p = 0.0213$) and 30th minute ($p = 0.0219$) of exercise (Fig. 4B). Δ THb (M) in SS and SP

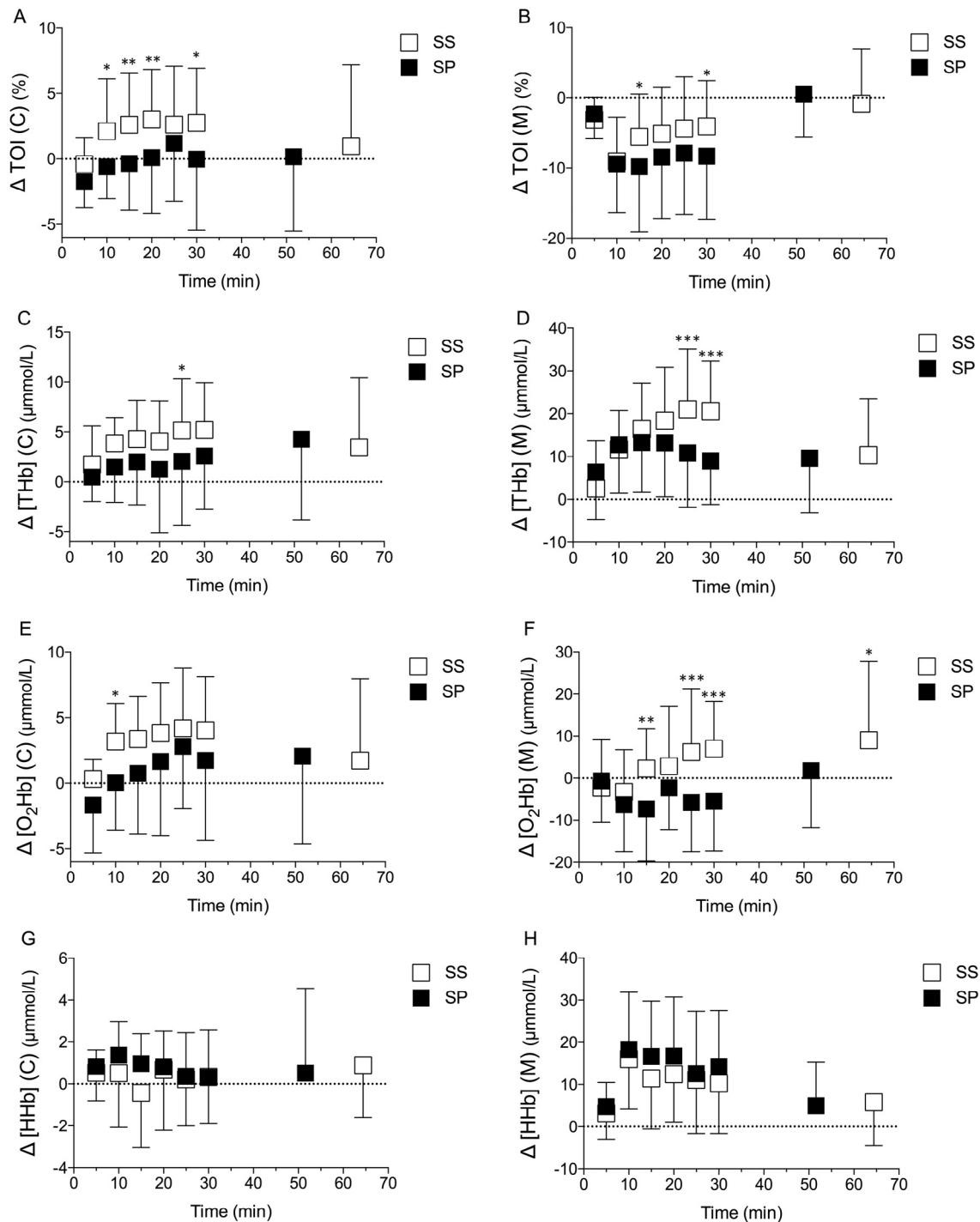


Fig. 4. Values of Δ TOI (A, B), Δ THb (C, D), ΔO_2Hb (E, F), and Δ HHb (G, H) in the first 30 min of exercise and at TTE during SS and SP measured in the prefrontal cortex (C) and on vastus lateralis muscle (M) (mean \pm SD). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $n = 12$. HHb = deoxyhemoglobin; O_2Hb = oxyhemoglobin; SP = snus placebo; SS = Swedish snus; TOI = tissue oxygenation index; THb = total hemoglobin; TTE = time to exhaustion.

significantly differed at the 25th minute ($p < 0.001$) and 30th minute ($p < 0.001$) of exercise (Fig. 4D). $\Delta\text{O}_2\text{Hb}$ (M) in SS was larger than in SP at the 15th ($p = 0.0013$), 25th ($p < 0.001$), and 30th ($p < 0.001$) minute of exercise and at TTE ($p = 0.0260$) (Fig. 4F). Finally, deoxyhemoglobin $\Delta\text{HHb(c)}$ and HHb (M) seemed not to be affected by SS and SP or by the duration of the exercise (Fig. 4G and H).

4. Discussion

This study investigated the effects of snus administration on TTE and on the perception of fatigue during endurance exercise. The data showed that tissue (muscular and cerebral) oxygenation was significantly altered by nicotine administered through snus, but TTE and the capacity to sustain aerobic exercise (exercise tolerance)³³ were unaffected.

Cerebral ΔTOI , $\Delta[\text{O}_2\text{Hb}]$, and $\Delta[\text{THb}]$ in the prefrontal cortical areas suggested that cerebral oxygenation was higher in SS than in SP. This fact supported the hypothesis that nicotine, acting as a central stimulator, induced a larger cerebral activity.²¹ In agreement with this finding, Gehricke et al.²⁷ showed that nicotine administration produced a rapid initial increase in cerebral $\Delta[\text{O}_2\text{Hb}]$, followed by subsequent decrease. This effect could be a consequence of the nicotine-induced dopamine release in the prefrontal cortex via stimulation of nicotinic acetylcholine receptors (nAChR).³⁴ Our data on cerebral NIRS responses confirmed these findings, as we found significant differences in the cerebral tissue values from the 10th to the 30th minute of exercise in SS vs. SP. However, this probable higher activation did not impact on the exercise tolerance and TTE.

A parallel effect on muscular tissue was also evident. Muscular ΔTOI , $\Delta[\text{THb}]$, and $\Delta[\text{O}_2\text{Hb}]$ were significantly higher in SS than in SP in the later stages of exercise, and they showed a delayed response (25th–30th minute). In particular, TOI (M) was significantly different at the 15th and 30th minute of exercise under the effect of SS (plus 4% on the average). This might be directly related to the increase of muscular blood flow induced by nicotine during SS, as found in other studies.³⁵ The higher values of ΔTOI (M), $\Delta[\text{THb}]$, and $\Delta[\text{O}_2\text{Hb}]$ induced by snus seemed to be a consequence of a potentiated blood redistribution mechanism mediated by nicotine. Exercise stimulated splanchnic and renal vasoconstriction causing blood redistribution,³⁶ which was potentiated by nicotine.¹⁹ Despite a better muscle oxygenation obtained with snus administration, however, there were no improvements in performance at this exercise intensity.

Individual factors such as tolerance to nicotine effects or changes in nicotinic acetylcholine receptors' availability could influence the response to nicotine absorbed through snus and may explain these inconclusive results.³⁷ In addition, one can speculate that the larger corollary discharge that originated from a larger cortical activation³⁸ might be somehow opposed by a weaker afferent feedback due to a smaller perturbation of the muscular milieu.³⁹ The combination of these 2 contrasting mechanisms could lead to an unchanged TTE.

In our non-smokers and non-snus users, nicotine induced diastolic hypotension at exhaustion. In 4 studies, SBP and DBP

blood pressure increased in smokeless tobacco users.^{19,20,37,40} Conversely, others studies reported no effects on blood pressure.⁴¹

Anecdotally, we noticed a relationship between the drop in DBP and reported adverse events. The diastolic average difference between subjects with adverse events and subjects with no adverse events was $\sim 10.5 \pm 2.6$ mmHg (Fig. 3). Nicotine effect by smokeless tobacco administration has been reported to lead to sympathoadrenal activation effect,^{40,42} which could affect cardiovascular responses, inducing and increasing blood flow and resulting in higher HR and blood pressure with muscular tremor.⁴³ In our subjects, nicotine intake level was pharmacologically equal to a high dose, and it might be responsible for the significant difference in DBP at TTE.⁴¹ Ruiz and Strain⁴⁴ reported that concentrations of nicotine comparable with the ones prevailing in our study produced bradycardia, hypotension, and depressed mental state. Dempsey et al.⁴⁵ observed that pharmacokinetic and genetic factors could influence sensitivity in never-smokers, and this may explain the variable cardiovascular response of our subjects to nicotine.

Statistical analysis indicated a non-significant difference in RER values at steady state until exhaustion. Therefore, we might infer that there were no differences on the use of energy substrates. However, our data did not confirm that the anaerobic energy turnover might be higher after the administration of snus, as suggested by Van Duser and Raven's,¹⁹ as no differences in $[\text{La}]_b$ were detected in the 2 conditions.

The major point of strength of this study consisted of a novel evaluation of the psychoactive effects of snus on submaximal intensity exercise tolerance and fatigue in parallel with the assessment of several physiological parameters. However, the study was not without limitations. In particular, it did not address the possible effects of nicotine in daily snus users under abstinence and satiety conditions. In addition, Q measured with Modelflow was not calibrated against values obtained using gold standard methods. However, it was shown that a non-calibrated Modelflow method could provide a reliable estimate of the relative change in Q during submaximal exercise in healthy young humans.⁴⁶

This study showed that snus did not modify fatigue perception during endurance exercise and TTE, suggesting no increase in endurance performance. However, nicotine intake might negatively affect cardiovascular responses in non-smoker athletes. For this reason, coaches should monitor athletes on the use of snus and on nicotine's addiction properties. Further investigations on the effects of snus on performance are required.

5. Conclusion

Despite an increase in cerebral and muscular tissue oxygenation, snus use in healthy, non-smoker participants did not modify perception of effort and did not increase TTE during continuous exercise performed at 65% W_{max} . Further research is necessary to test the perception of effort and TTE at higher exercise intensities (and maybe systematically modulating the levels of nicotine concentrations). In general, our results

showed that snus could not be considered an ergogenic substance at submaximal exercise level.

Acknowledgments

This work was supported by research funds allocated to the following departments at the University of Verona: Neurosciences, Biomedicine and Movement Sciences, and Diagnostic and Public Health. The authors heartily thank the volunteers without whom this study would not have been possible.

Authors' contributions

TZ, CCh, ET, PC, and CCA planned the experiment; TZ and ET submitted the project to ethical committee for approval; TZ, ET, PB, FP, LF, and MB carried out the experiments and analyzed the data; TZ and ET drafted the manuscript; CCh, PC, TZ, ET, and CCA revised the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

None of the authors declare competing financial interests.

References

- Rutqvist LE, Curvall M, Hassler T, Ringberger T, Wahlberg I. Swedish snus and the GothiaTek® standard. *Harm Reduct J* 2011;**8**:11. doi:10.1186/1477-7517-8-11
- Ahlbom A, Bridges J, Jong WD, Hartemann P, Jung T, Mattsson MO, et al. *Health effects of smokeless tobacco products: Scientific Committee on Emerging and Newly Identified Health Risks*. Brussels: European Commission. Health and Consumer Protection DG; 2007. Available at: http://ec.europa.eu/health/ph_risk/committees/04_scenihp/docs/scenihp_o_013.pdf; [accessed 20.06.2016].
- Anon. *Directive C. 92/41/EEC of 15 May 1992 amending Directive 89/622/EEC on the approximation of the laws, regulations and administrative provisions of the Member States concerning the labelling of tobacco products*. Available at: <http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31992L0041:EN:HTML>; [accessed 20.06.2016].
- Fagerström KO, Schildt EB. Should the European Union lift the ban on snus? Evidence from the Swedish experience. *Addiction* 2003;**98**:1191–5.
- Alpert HR, Koh H, Connolly GN. Free nicotine content and strategic marketing of moist snuff tobacco products in the United States: 2000–2006. *Tob Control* 2008;**17**:332–8.
- Marclay F, Saugy M. Determination of nicotine and nicotine metabolites in urine by hydrophilic interaction chromatography–tandem mass spectrometry: potential use of smokeless tobacco products by ice hockey players. *J Chromatogr A* 2010;**1217**:7528–38.
- Marclay F, Grata E, Perrenoud L, Saugy M. A one-year monitoring of nicotine use in sport: frontier between potential performance enhancement and addiction issues. *Forensic Sci Int* 2011;**213**:73–84.
- Zandonai T, Baraldo M, Franceschi L, Zappamiglio T, Chiamulera C. *Effects of smokeless tobacco (snus) administration on exercise endurance in men. SRNT Annual Meeting, Boston, MA, USA, POS3-3, p.165*. Available at: https://c.ymcdn.com/sites/srnt.siteym.com/resource/resmgr/Conferences/Past_Annual_Meetings/2013_Annual_Meeting_Abstract.pdf; [accessed 20.06.2016].
- World Anti-doping Agency (WADA). *Monitoring Program 2016*. Available at: <https://wada-main-prod.s3.amazonaws.com/resources/files/wada-2016-monitoring-program-en.pdf>; [accessed 20.06.2016].
- Henningfield JE, Fant RV, Tomar SL. Smokeless tobacco: an addicting drug. *Adv Dent Res* 1997;**11**:330–5.
- Fagerstrom KO, Rutqvist LE, Hughes JR. Snus as a smoking cessation aid: a randomized placebo-controlled trial. *Nicotine Tob Res* 2012;**14**:306–12.
- Wium N, Aarø LE. Outcome expectations and use of smokeless tobacco (snus): a cross-sectional study among young Norwegian snus users. *Scand J Psychol* 2011;**52**:64–70.
- Caldwell B, Burgess C, Crane J. Randomized crossover trial of the acceptability of snus, nicotine gum, and Zonnic therapy for smoking reduction in heavy smokers. *Nicotine Tob Res* 2010;**12**:179–83.
- Hatsukami DK, Gust SW, Keenan RM. Physiologic and subjective changes from smokeless tobacco withdrawal. *Clin Pharmacol Ther* 1987;**41**:103–7.
- Timberlake DS. A latent class analysis of nicotine-dependence criteria and use of alternate tobacco. *J Stud Alcohol Drugs* 2008;**69**:709–17.
- Bujon T. Positive to nicotine (Positifs à la nicotine). *Psychotropes* 2008;**14**:59–76. [in French].
- Morente-Sánchez J, Zandonai T, Mateo-March M, Sanabria D, Sánchez-Muñoz C, Chiamulera C, et al. Acute effect of snus on physical performance and perceived cognitive load on amateur footballers. *Scand J Med Sci Sports* 2015;**25**:e423–31. doi:10.1111/sms.12321.
- Kajiser L, Berglund B. Effect of nicotine on coronary blood-flow in man. *Clin Physiol* 1985;**5**:541–52.
- Van Duser BL, Raven PB. The effects of oral smokeless tobacco on the cardiorespiratory response to exercise. *Med Sci Sports Exerc* 1992;**24**:389–95.
- Chagué F, Guenancia C, Gudjoncik A, Moreau D, Cottin Y, Zeller M. Smokeless tobacco, sport and the heart. *Arch Cardiovasc Dis* 2015;**108**:75–83.
- Mündel T, Jones DA. Effect of transdermal nicotine administration on exercise endurance in men. *Exp Physiol* 2006;**91**:705–13.
- Carpenter CM, Wayne GF, Connolly GN. The role of sensory perception in the development and targeting of tobacco products. *Addiction* 2007;**102**:136–47.
- Benowitz NL. Nicotine addiction. *N Engl J Med* 2010;**362**:2295–303.
- Perrey S. Non-invasive NIR spectroscopy of human brain function during exercise. *Methods* 2008;**45**:289–99.
- Durant G, Gagnon JF, Tremblay S, Dehais F. Using near infrared spectroscopy and heart rate variability to detect mental overload. *Behav Brain Res* 2014;**259**:16–23.
- Calderan L, Chiamulera C, Marzola P, Fabene PF, Fumagalli GF, Sbarbati A. Sub-chronic nicotine-induced changes in regional cerebral blood volume and transversal relaxation time patterns in the rat: a magnetic resonance study. *Neurosci Lett* 2005;**377**:195–9.
- Gehricke JG, Polzonetti C, Caburian C, Gratton E. Prefrontal hemodynamic changes during cigarette smoking in young adult smokers with and without ADHD. *Pharmacol Biochem Behav* 2013;**112**:78–81.
- American College of Sports Medicine (ACSM). *ACSM's guidelines for exercise testing and prescription*. 9th ed. Philadelphia, PA: Wolters Kluwer/Lippincott Williams & Wilkins Health; 2014.
- Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982;**14**:377–81.
- Wesseling KH, Jansen JR, Settels JJ, Schreuder JJ. Computation of aortic flow from pressure in humans using a nonlinear, three-element model. *J Appl Physiol* 1993;**74**:2566–73.
- Mancini DM, Bolinger L, Li H, Kendrick K, Chance B, Wilson JR. Validation of near-infrared spectroscopy in humans. *J Appl Physiol* 1994;**77**:2740–7.
- Deveci SE, Deveci F, Açık Y, Ozan AT. The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. *Respir Med* 2004;**98**:551–6.
- Coyle EF, Coggan AR, Hopper MK, Walters TJ. Determinants of endurance in well-trained cyclists. *J Appl Physiol* 1988;**64**:2622–30.
- Wing VC, Payer DE, Houle S, George TP, Boileau I. Measuring cigarette smoking-induced cortical dopamine release: a [¹¹C] FLB-457 PET study. *Neuropsychopharmacology* 2015;**40**:1417–27.
- Andersson K, Arner P. Systemic nicotine stimulates human adipose tissue lipolysis through local cholinergic and catecholaminergic receptors. *Int J Obes Relat Metab Disord* 2001;**25**:1225–32.
- Rowell LB. *Human cardiovascular control*. New York, NY: Oxford University Press; 1993.
- Benowitz NL. Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. *Annu Rev Pharmacol Toxicol* 2009;**49**:57–71.

38. Marcora S. Perception of effort during exercise is independent of afferent feedback from skeletal muscles, heart, and lungs. *J Appl Physiol* 2009;**106**:2060–2.
39. Amann M. Exercise-induced metabolic perturbation: all roads lead to Rome. *Exp Physiol* 2010;**95**:765–6.
40. Benowitz NL, Gourlay SG. Cardiovascular toxicity of nicotine: implications for nicotine replacement therapy. *J Am Coll Cardiol* 1997;**29**:1422–31.
41. Asplund K. Smokeless tobacco and cardiovascular disease. *Prog Cardiovasc Dis* 2003;**45**:383–94.
42. Lunell E, Lunell M. Steady-state nicotine plasma levels following use of four different types of Swedish snus compared with 2-mg Nicorette chewing gum: a crossover study. *Nicotine Tob Res* 2005;**7**:397–403.
43. Yoshida T, Sakane N, Umekawa T, Kondo M. Effect of nicotine on sympathetic nervous system activity of mice subjected to immobilization stress. *Physiol Behav* 1994;**55**:53–7.
44. Ruiz P, Strain E. Lowinson and Ruiz's substance abuse: a comprehensive textbook. *JAMA* 2012;**307**:1869.
45. Dempsey DA, St Helen G, Jacob 3rd P, Tyndale RF, Benowitz NL. Genetic and pharmacokinetic determinants of response to transdermal nicotine in white, black, and Asian nonsmokers. *Clin Pharmacol Ther* 2013;**94**:687–94.
46. Sugawara J, Tanabe T, Miyachi M, Yamamoto K, Takahashi K, Iemitsu M, et al. Non-invasive assessment of cardiac output during exercise in healthy young humans: comparison between Modelflow method and Doppler echocardiography method. *Acta Physiol Scand* 2003;**179**:361–6.