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Acute effect of Snus on physical performance and perceived cognitive load on amateur footballers

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Smokeless tobacco (Snus) is a substance that contains nicotine, which has been placed on World Anti-Doping Agency's 2014 Monitoring Program. A proliferation of nicotine use in sport has been observed in recent years, but little is known regarding its effects, especially on football players' performance. Therefore, the aim of this study was to assess the effect of Snus on physical performance, heart rate variability, subjective activation, mental fatigue, and perceived readiness before a physical test in non-smoker, non-Snus user, amateur football players. Participants were administered either Snus or placebo 40 min prior to a fitness test battery (handgrip test, countermovement jump, agility test, and Yo-Yo intermittent recovery test). Results showed that Snus intake (compared with placebo) increased perceived mental fatigue level and mental load, and reduced perceived readiness level and heart rate variability. No significant differences between the two experimental conditions were found in either performance in the physical tests or perceived physical fatigue levels. In light of these results, Snus could not be considered an ergogenic substance. On the contrary, based on the extant evidence linking mental load and fatigue with physical performance, we argue that the observed negative effects on mental fatigue, perceived readiness, and heart rate variability should be considered.

A recent review by Heishman et al. (2010) points to significant effects of nicotine on fine motor abilities and high-order cognitive functions such as attention and memory. Together with changes in cognitive functioning, nicotine administration has been related to significant physiological effects. For instance, in a study by Turner and McNicol (1993) participants were subject to treadmill exercise at an intensity corresponding to 60% and 85% of their maximal oxygen uptake (VO_{2max}) under the effects of oral smokeless tobacco (OST). Tobacco (in comparison with placebo condition) resulted in significantly increasing heart rate and blood lactate at rest, and decreasing stroke volume. Furthermore, due to OST-induced increase in plasma nicotine concentrations (boosting anaerobic energy production), a nicotine-induced cardiac sympathetic stimulation was suggested by Van Duser and Raven (1992). Several studies have analyzed smoked tobacco effects on cardiac functions showing that nicotine was able to produce heart rate modulations and decreased heart rate variability (HRV; e.g., Karakaya et al., 2007). However, a study by Mundel and Jones (2006) has shown that nicotine (administered via skin patch) improved exercise endurance in the absence of peripheral changes (ventilation, heart rate, and blood metabolites) concluding that physical enhancement was attained through a central nervous system mechanism.

In 2012, nicotine was included on World Anti-Doping Agency's Monitoring Program (WADA, 2012) after some analytic chemical studies showing a proliferation of nicotine use in the sport environment (e.g., Marclay et al., 2011), presumably due to its beneficial influence on physical performance. An easy and inexpensive way of consuming nicotine is through the use of smokeless tobacco products, like Snus. For example, in Sweden there is an increasing trend of Snus use (Norberg et al., 2011). Although Sweden is the only country in the European Union that grants special exemption to manufacture and sell this product (Ahlbom et al., 2007), there is recent evidence of Snus consumption in Switzerland (Fischer et al., 2014) and in Northern Italy (Zandonai et al., 2013). Snus is of growing popularity in the sport environment due to the absence of adverse effects on the respiratory system. Snus use has been associated to high-intensity sports in which athletes report lower cigarette consumption than people not engaged in sport practice

(Mattila et al., 2012). Sociological studies have shown that the use of Snus was more common among team sport athletes than athletes engaged in individual sports (Martinsen & Sundgot-Borgen, 2014), and in males than in females (Rolandsson et al., 2014).

Here, we focused in probably the most popular team sport in the world: football. The acyclical and unpredictable nature of high-intensity anaerobic efforts in football (Reilly, 2006) may induce footballers (at all levels) to look for ways to improve their performance via the administration of substances with low levels of adverse side effects. Snus could be one of these substances, given the reported positive effects of nicotine on anaerobic performance (e.g., Mundel & Machal, 2012) and the absence of respiratory side effects of Snus. However, to the best of our knowledge, there are no previous studies that have assessed the effects of this product in non-smoker, football players' physical performances.

The present study aimed at investigating the effects of Snus on physical performance of a group of non-smoker, non-Snus user, amateur footballers. Physical performance was evaluated by means of a test battery developed to assess various capacities related to physical demands in football (Reilly, 2006). Crucially, together with testing footballers, the novelty of our study relied on the assessment of the effects of Snus on HRV, as an objective physiological index (of autonomic balance), and on the level of various subjective (cognitive) indexes, such as perceived effort, perceived mental fatigue, perceived activation, perceived mental workload, and perceived readiness (PR) prior to a physical test. This is not trivial as previous studies have highlighted the close relationship between physical and cognitive effort (e.g., Zijdewind et al., 2006).

Methods

Participants

A total of 18 male amateur football players [age: 22.5 ± 1.5 years old; height 180 ± 6 cm; weight: 75.60 ± 7.4 kg; body mass index: 23.30 ± 1.4; mean ± standard deviation (SD)], non-smokers, and non-Snus users took part in this study. All participants were recruited from the Faculty of Sport Sciences of University of Granada (Spain). The study was approved by the Ethics Committee of the University of Granada and conformed to the 1964 Declaration of Helsinki.

General design

The study was a double-blind randomly assigned crossover design, comparing the effect of Snus vs placebo on physical performance (measured by a fitness test battery), and perceived effort [measured by the rating of perceived exertion (RPE)], perceived activation, perceived mental fatigue, and perceived mental workload (measured using visual analogue scale). The fitness test battery consisted of four tests: handgrip dynamometry test, countermovement jump test, 5 × 10 m agility test, and Yo-Yo intermittent recovery test. The protocol consisted of two sessions (EXP1 and EXP2) with 5 days of recovery and substance washout between each session. A training session was carried out a week before EXP1 to ensure familiarization with the tests. Half of the participants blindly received Snus on EXP1 day and placebo on EXP2 day, and the remaining half of the participants received placebo on EXP1 day and Snus on EXP2 day. Participants were randomly assigned to one of the two counterbalanced conditions. Each experimental session lasted for about 90 min. Before and after the fitness test battery, participant's subjective activation, physical fatigue, and mental fatigue was assessed (Fig. 1).

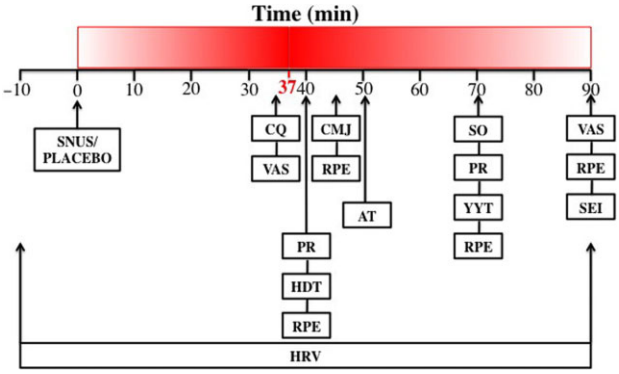

Fig. 1. Schematic description of the experimental session. Red bar is the estimated nicotine plasma concentration (the mean of T_{max} is 37.1 ± 10.2 min according to Lunell & Curvall, 2011). AT, agility test; CMJ, countermovement jump; CQ, control questions; HDT, hand dynamometric test; HRV, heart rate variability; PR, perceived readiness; RPE, rating of perceived exertion (Borg's scales); SEI, side effect interview; SO, substance out (Snus/placebo); VAS, visual analog scale (subjective activation and mental workload); YYT, Yo-Yo recovery intermittent test.

Protocol

Familiarization session: participants arrived at the laboratory by 08:00 h. They were informed about the study, its procedure and risks, and then signed an informed consent before starting the experiment. Then, participants performed the fitness test battery. The experimenters supervised the correct performance of the tests.

EXP1: Participants arrived at 08:00 h to the laboratory. Before the start of the experimental session they were asked the following control questions: "How many hours did you sleep last night?" and "Have you had any stimulant such as tea or coffee this morning before coming to the laboratory?" The use of the visual analogue scale, the PR, and RPE scales was subsequently explained to the participants. Then, measurement of exhaled carbon monoxide (CO) level was performed using the EC_{50} Micro Smokerlyzer (Bedfont Scientific Ltd., Kent, UK; see Meredith et al., 2014). After this test, participants laid down for 10 min in a supine position to record their basal HRV that was recorded during the entire session with a Polar RS800 (Polar Electro, Kempele, Finland). Subsequently, participants took the sachet of either Snus or placebo from the box (for further details, see Snus and Placebo in Materials section). They were instructed to place the Snus or the placebo substance in the anterior part of the mouth within the upper gingiva (time zero = T0) and to keep it in their mouth until performance of the Yo-Yo test. After taking the Snus or placebo, participants were invited to remain lying on a mat during 35 min in a supine position to record their HRV. Thirty-five minutes after intake (T0), psychological parameters (subjective activation, mental fatigue, and physical fatigue) were recorded by means of a visual analogue scale. After 40 min from

T0, participants stood up and started the hand dynamometry test. Next, after a warm-up, participants performed a vertical jump test as indicator of instantaneous power production. The vertical jump test involved two countermovement jumps (Ergojump, Rome, Italy) interspersed by 1-min rest. Only the best jump from each subject was used for data analysis. Next, agility was assessed by means of a 10×5 m agility test. After 70 min from T0 they spat out the corresponding substance (Snus or placebo) and started the Yo-Yo test (Fig. 1). At the end of the Yo-Yo test, subjective activation, perceived mental, and physical fatigue were again recorded by means of the visual analogue scale. Then participants were interviewed about any nicotine adverse effects (in case of "yes" answer, if it was mild, moderate, or serious). RPE and readiness perception were recorded several times during the fitness test battery. EXP2 was performed exactly following the same procedure and timetable of EXP1 for each participant, and took place 5 days later.

Materials

Snus and placebo

Swedish Snus is a low-nitrosamine, moist oral tobacco product with water content of approximately 45–55% and a pH of approximately 8.5. For Snus and placebo delivery and uptake, we followed the methodology of Lunell and Lunell (2005). According to Lunell and Curvall (2011), the mean time to maximum nicotine plasma concentration (T_{max}) is 37.1 ± 10.2 (SD) min (range: 24–60 min) for 1 g of Swedish portion Snus. The placebo was almost identical to the Snus in physical appearance, mouth feel, pH, flavoring, and other sensory characteristics, but it did not contain tobacco or nicotine. In this study, we administered a 1.0-g (8 mg of nicotine) portion of Snus (*Catch White Eucalyptus* from Swedish Match, Stockholm, Sweden), and a 1.0-g portion of placebo (*Onico peppermint* from Swedish Match).

Smoking status

The exhaled CO concentration was measured using the EC_{50} Smokerlyser (Bedfont Instruments). The Smokerlyser measures breath CO levels in parts per million (ppm) based on the conversion of CO to carbon dioxide (CO_2) over a catalytically active electrode. Exhaled CO concentrations are reported to closely correlate with blood carboxyhemoglobin concentration in smokers and in non-smokers (Deveci et al., 2004).

Activation, mental fatigue, and physical fatigue

Before Snus or placebo ingestion and immediately after finishing the fitness test battery, participants were asked to rate their perceived activation and perceived mental and physical fatigue using a visual analogue scale (Chalder et al., 1993), ranging from 0 (nothing) to 10 (top) values in response to the following questions:

(a) "What is your activation level now?" (b) "What is your physical fatigue level now?" and (c) "What is your mental fatigue level now?"

At the end of the session, participants were asked to use the visual analogue scale again following these questions:

(a) "Finally, how would you rate the overall mental load for this experimental session?" and (b) "How do you feel now, once the session has finished?"

RPE

RPE was measured with the Borg 15-point scale (RPE 6–20; Borg, 1998) and with a 10-point category ratio (RPE CR10; Noble et al., 1983) immediately after the handgrip test, countermovement jump, and Yo-Yo test. Participants were given verbal encouragement during the fitness tests.

PR

The value for perceived readiness (Nurmekivi & Karu, 2001) was also taken prior to the handgrip and Yo-Yo tests. This scale determines the grade of recovery that subjects perceived from 1 point ("not recovered at all") to 5 points ("completely recovered").

HRV measurement

Recordings were performed using a Polar RS800 heart rate monitor set to the RR interval mode (Polar Electro, Kempele, Finland) together with an electrode transmitter belt (Polar WearLink Wind, Polar Electro), after application of conductive gel. Data were transferred to Polar Pro Trainer 5 software (Polar Electro) and analyzed by means of Kubios 2.1 HRV analysis software (The Biomedical Signal and Medical Imaging Analysis Group, Department of Applied Physics, University of Kuopio, Finland).

Handgrip dynamometry

An electronic dynamometer (Takei TKK-5401, Tokio, Japan) was used to determine maximal handgrip strength in both right and left hands. The dynamometer was adjusted for each subject's hand size, and the participants were kept in stand position with the arms parallel to the ground and with the elbow joint maintained at 90 degrees of flexion. The participants were instructed to perform a maximal isometric contraction. Each subject was allowed two non-consecutive trials per arm, and the highest value was recorded.

Countermovement jump

Muscle power was evaluated using a wireless inertial measurement unit (FreePower, Sensorize, Rome, Italy). Two standardized countermovement jumps separated by a 2-min rest interval were performed. The wireless inertial measurement unit was positioned approximately at

the body mass centre, placing the belt around the waist. Participants started from a standing position with the hands on their hips, and were instructed to perform a fast downward movement up to 90 degrees of knee flexion followed by an upward movement trying to jump as high as possible. Maximum jump height was used for further analysis.

Agility test (5 × 10 m)

Agility was evaluated using a 5 × 10-m maximal shuttle run on an indoor running track. After one practice trial, a maximum test was carried out. Performance time was recorded with using a chronometer.

Yo-Yo recovery intermittent test

The Yo-Yo recovery intermittent test was performed using the level 1 version of the test, following the guidance defined by the test's creator (Bangsbo, 1996). The level 1 Yo-Yo test is a progressive shuttle running test that allows 10 s of active recovery after every 20-m shuttle. Running speeds are dictated by an audible cue played from a CD. Participants must be at one end of a 20-m base every time a signal is played. Yo-Yo test performance was considered as the total distance covered by the subject when they drop out.

Data analysis

A repeated measures analysis of variance (ANOVA) with the within-participant factors substance (Snus, placebo) and measurement (first, second) was performed for variables subjective activation, perceived mental fatigue, perceived physical fatigue, and HRV. A repeated measure ANOVA with the within-participant factors substance (Snus, placebo) and test (handgrip dynamometry, countermovement jump, agility 5 × 10, Yo-Yo) was performed to analyze the data from the RPE 6–20 and RPE CR-10 scales. Data from the PR scale were analyzed with a repeated measures ANOVA with within-participant factors substance (Snus, placebo) and test (handgrip dynamometry, Yo-Yo). Data from the handgrip dynamometry test were submitted to a repeated measures ANOVA with the factors of substance and hand. Data from the remaining dependent variables were analyzed using a one-way ANOVA with the within-participants factor substance (Snus, placebo).

For HRV data, recordings were pre-processed to exclude artifacts by eliminating RR intervals that differed more than 25% from the previous and the subsequent RR intervals (Malik et al., 1989). Removed RR intervals were replaced by conventional spline interpolation so that the length of the data did not change (i.e., resulting in the same number of beats). We used the smoothness prior method with a Lambda value of 500 to remove disturbing low frequency baseline trend components (Tarvainen et al., 2002). The mean R-R interval (RRi), root mean square difference of successive normal R-R intervals (rMSSD), and geometric Poincaré Plot index (SD1) were calculated as indices of HRV.

Partial eta squared was reported as an index of effect size. The Mauchly's sphericity test was performed when appropriate. This test did not reach significance in any of the analyses.

Results

The results from the ANOVAs revealed a significant main effect of measurement for subjective activation, perceived mental fatigue, and perceived physical fatigue, with greater values in the second measurement than in the first one (see Tables 1 and 2). The main effect of test was significant for both the RPE 6–20, and RPE CR-10, measures (see Tables 1 and 3). Fisher's least significance difference (LSD) post-hoc tests showed significant differences between the handgrip dynamometry and the Yo-Yo test (both Ps < 0.001), and between the countermovement jump and the Yo-Yo test (both Ps < 0.001). Crucially, perceived mental fatigue, perceived overall mental workload, and PR were affected by Snus intake. Snus also affected HRV (see Tables 1 and 4). Fisher LSD post-hoc tests revealed that RRi values were reduced from baseline to the second measurement in the Snus session, $P < 0.001$, with no effect in the placebo session ($P = 0.81$). rMSSD and SD1 indices followed a similar trend, with a reduced HRV after substance intake with respect to baseline in the Snus session ($P = 0.05$ and $P = 0.04$, for rMSSD and SD1, respectively) and no effect in the placebo session ($P = 0.10$ and $P = 0.11$, for rMSSD and SD1, respectively). On the contrary, performance in the physical tests was not modulated by substance intake. It is important to point out that the side effects of the Snus resulted in missing data from some participants during the experimental session.

Side effects

Twelve participants reported adverse symptoms at the end of the Snus condition session and only one reported adverse effects at the end of the placebo condition session – mild, moderate, or serious – (Table 5). Because of these adverse effects some participants could not complete all tests during the experimental session.

Discussion

Nicotine is the main psychoactive substance present in tobacco, targeting neuronal nicotinic acetylcholine receptors. This psychoactive substance has been shown to increase muscle blood flow (Weber et al., 1989), heart rate, blood pressure, level of circulating catecholamine, and vasoconstriction during light exercise (e.g., Walker

Table 1. Results of the data analysis

Control variables (between sessions)			
Sleep hours			$F < 1$
Smokerlyzer			$F < 1$
Subjective measures			
	Substance (Snus, placebo)	Measurement (first, second)	Substance × measurement
Subjective activation	$F(1,15) = 1.75$, $P = 0.2$	$F(1,15) = 20.7$, $P < 0.001$, $\eta_{partial}^2 = 0.58$	$F < 1$
Perceived physical fatigue	$F < 1$	$F(1,15) = 43.6$, $P < 0.001$, $\eta_{partial}^2 = 0.74$	$F < 1$
Perceived mental fatigue	$F(1,15) = 13.18$, $P = 0.002$, $\eta_{partial}^2 = 0.46$	$F(1,15) = 34.96$, $P = 0.001$, $\eta_{partial}^2 = 0.69$	$F < 1$
	Substance (Snus, placebo)	Test (handgrip dynamometry, Yo-Yo)	Substance × test
Perceived readiness	$F(1,9) = 36.24$, $P = 0.001$, $\eta_{partial}^2 = 0.80$	$F(1,9) = 1.76$, $P = 0.21$	$F(1,9) = 1.09$, $P = 0.32$
	Substance (Snus, placebo)	Test (handgrip dynamometry, agility, Yo-Yo)	Substance × test
RPE 6–20	$F(1,14) = 1.23$, $P = 0.28$	$F(2,28) = 51.70$, $P < 0.001$, $\eta_{partial}^2 = 0.78$	$F(2,28) = 1.07$, $P = 0.35$
RPE CR-10	$F < 1$	$F(2,28) = 44.18$, $P < 0.001$, $\eta_{partial}^2 = 0.76$	$F < 1$
Perceived mental load	$F(1,15) = 20.86$, $P < 0.001$, $\eta_{partial}^2 = 0.58$		
Physical performance			
	Substance (Snus, placebo)	Hand (right, left)	Substance × hand
Handgrip dynamometry	$F < 1$	$F(1,17) = 57.95$, $P < 0.001$, $\eta_{partial}^2 = 0.77$	$F(1,17) = 2.68$, $P = 0.12$
	Substance (Snus, placebo)		
Agility 5 × 10	$F(1,16) = 2.19$, $P = 0.15$		
Yo-Yo test (cm)	$F(1,14) < 1$		
Yo-Yo test (VO_{2max})	$F(1,14) < 1$		
Countermovement jump	$F(1,16) < 1$		
HRV			
	Substance (Snus, placebo)	Measurement (first, second)	Substance × measurement
RRi	$F(1,15) = 5.79$, $P = 0.02$, $\eta_{partial}^2 = 0.27$	$F(1,15) = 47.32$, $P < 0.001$, $\eta_{partial}^2 = 0.76$	$F(1,15) = 33.06$, $P < 0.001$, $\eta_{partial}^2 = 0.68$
rMSSD	$F(1,15) = 3.86$, $P = 0.06$	$F < 1$	$F(1,15) = 7.13$, $P = 0.01$, $\eta_{partial}^2 = 0.32$
SD1	$F(1,15) = 4.04$, $P = 0.06$	$F < 1$	$F(1,15) = 7.69$, $P = 0.01$, $\eta_{partial}^2 = 0.34$

HRV, heart rate variability; rMSSD, root mean square of successive differences; RPE, rating of perceived effort; RRi, R-R interval; SD1, instantaneous beat-to-beat variability of the data.

Table 2. Parameters assessed by means of the visual analog scale (values from 0 to 10)

Parameter	Activation level				Physical fatigue				Mental fatigue			
Substance	Snus		Placebo		Snus		Placebo		Snus		Placebo	
Measurement	First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
Mean	4.81	7.33	5.07	7.42	4.13	7.71	3.65	7.76	4.19	7.13	3.06	6.06
SD	1.81	2.01	1.75	1.41	2.06	1.73	1.80	1.52	2.07	1.71	1.84	1.34

SD, standard deviation.

et al., 1999). Together with physiological effects, nicotine has also been related to changes in cognitive functioning (Heishman et al., 2010). For this reason, the World Anti-Doping Agency has decided to include nicotine in its Monitoring program since 2012. A simple way of consuming nicotine avoiding the negative respiratory side effects is via OST like Snus. Here, we investigated the effect of Snus on physical performance, HRV,

Table 3. Descriptive statistics (mean and standard deviation) for Snus and placebo conditions for the dependent variables with a single measure (control questions, countermovement jump, handgrip dynamometry, agility test, Yo-Yo recovery intermittent test, mental load after session, perceived readiness, perceived mental workload)

	Snus		Placebo	
	Mean	SD	Mean	SD
CQ1 Sleeping hours	6.88	1.08	6.81	0.87
CQ2 Smokerlyzer (ppm)	2.94	1.42	3.54	3.84
Maximum jump height (m)	0.43	0.05	0.43	0.05
Handgrip right (kg)	42.22	5.61	42.12	4.59
Handgrip left (kg)	37.26	6.20	38.38	4.90
Agility test (s)	18.78	0.77	18.62	0.58
Distance YYT (m)	1328.00	507.31	1368.33	453.72
Mental load after session (0–10)	6.41	2.06	5.53	1.73
Perceived readiness before handgrip test (1–5)	3.64	0.92	4.38	0.65
Perceived readiness before YYT (1–5)	3.57	0.79	3.89	0.78
RPE 6–20 after handgrip test	14.89	2.85	14.44	2.62
RPE 6–20 after CMJ	14.59	2.55	14.83	2.15
RPE 6–20 after YYT	18.67	0.98	18.50	1.42
RPE CR10 after handgrip test	6.06	2.11	5.67	2.14
RPE CR10 after CMJ	6.06	1.89	5.94	2.11
RPE CR10 after YYT	9.36	1.15	9.06	1.39

CMJ, countermovement jump; CQ, control questions; RPE, rate of perceived exertion; SD, standard deviation; VO_{2max}, maximal oxygen uptake; YYT, Yo-Yo recovery intermittent test.

and on various subjective (cognitive) indexes in a group of amateur football players. As noted in the Introduction, the nature of the physical efforts involved in football practice makes this sport vulnerable to Snus use.

Results showed that amateur footballers' HRV and PR before the fitness tests (handgrip and Yo-Yo tests) were reduced by acute Snus intake. In addition, perceived mental fatigue and overall mental load after the experimental session were increased as a consequence of Snus administration. Regarding HRV, in line with Karakaya et al. (2007) findings, results showed a decrease after Snus administration even before the beginning of the fitness test battery. Results appear to confirm that nicotine leads to a reduced vagal tone.

Our study goes beyond previous research that has investigated the effect of nicotine on HRV (e.g., Barutcu et al., 2005). Indeed, these studies used smoked tobacco, and consequently, they did not control for possible respiratory disorders, which could have influenced the observed HRV patterns. On the contrary, in our study, we were able to avoid possible respiratory disruption focusing on vagal alteration produced by nicotine by administering the product orally.

In spite of scientific literature showing significant beneficial effects of nicotine on motor abilities and other high-order cognitive functions (see Heishman et al., 2010, for a review) suggesting performance enhancement (Pesta et al., 2013), the present study did not show any improvement in physical performance in a sample of non-smoker, non-Snus user, amateur footballers. Many published studies support that nicotine causes cognitive enhancement in smokers, but the influence of nicotine on human performance in non-smokers is less clear (Heishman et al., 2010). Although some studies have failed to detect nicotine-induced cognitive enhancement (e.g., McClernon et al., 2003), others have also found impairment effects (Heishman et al., 1993).

In our study, participants showed reduced PR before the handgrip dynamometry test in the Snus condition compared with the placebo condition. In the same way, 35 min after Snus intake, larger mental fatigue values were reported than after placebo intake. Furthermore, once the Yo-Yo test was finished (at the end of the experimental session), participants reported higher mental workload in the Snus condition than in the placebo condition. Therefore, Snus had a negative effect on perceived mental fatigue, even before starting the physical tests. Perceived activation was also greater in the Snus condition than in the placebo condition. Regarding this latter result, research suggests that the effects of nicotine on subjective activation are dose dependent (Perkins & Stitzer, 1998) with mild positive effects at low doses, moderate positive effects at intermediate doses, and negative effects at high doses. Therefore, it is possible that the nicotine intake level achieved in our study was pharmacologically equivalent to a high dose, considering the lack of tolerance in our non-smoker participants. This lack of tolerance would have produced the side effects reported here that could be responsible of the negative effects of Snus. Differences in nicotine dose–response and tolerance levels could also explain the apparent inconsistent results from studies with non-smokers mentioned above.

In contrast to the above-mentioned results, no differences between the Snus and placebo conditions were found for performance in the fitness tests used in this study: handgrip dynamometry test, countermovement jump, agility test, and Yo-Yo test. Interestingly enough, the null effect of Snus (compared with placebo) on physical performance was accompanied by a similar null effect on RPE (see Mundel & Jones, 2006, for a similar result). Therefore, taken together, the results of the physical tests and the RPE scales would suggest that Snus could not be considered an ergogenic substance. However, there is evidence of a direct negative relationship between mental load, mental fatigue, and physical performance (e.g., Marcora et al., 2009). One could then expect that in long exercise sessions (longer than in the present study), the adverse effects of Snus on perceived mental fatigue and mental workload would result in an

Table 4. Heart rate variability (HRV) measurements

HRV parameters	RR				rMSSD				SD1			
Substance	Snus		Placebo		Snus		Placebo		Snus		Placebo	
Moment	Basal	Effect	Basal	Effect	Basal	Effect	Basal	Effect	Basal	Effect	Basal	Effect
Mean	948.85	665.19	895.42	887.29	62.09	50.11	63.59	71.73	43.99	35.22	44.94	50.81
SD	119.72	97.70	131.75	137.55	28.30	28.29	22.88	29.69	20.06	19.45	16.26	21.04

rMSSD, root mean square of successive differences; RRi, R-R interval; SD, standard deviation; SD1, instantaneous beat-to-beat variability of the data.

Table 5. Side effects reported by some of the participants at the end of the Snus session

Subject code	Mild	Moderate	Serious
01AAJ		Tachycardia	
02CCJ			Confuse, dizziness
03CGJ			Nausea, dizziness
04CBI	Dizziness	Stomach ache	Empty sensation, tiredness, confuse
05EGA	Tiredness		
07LMJ	Dizziness		
09RLF	Sore throat		
11SSS		Nausea, dizziness	
12BNV	Tiredness	Dizziness	
13HJE	Dizziness		Nausea
16PGM	Dizziness	Sweating, increase body temperature	Tremor
17MA		Dizziness, sweating	Empty sensation, nausea

impairment of physical performance (Wright et al., 2008).

Nicotine absorption varies greatly among individuals. However, according to Lunell and Lunell (2005) the variation in nicotine extraction rates among different Snus users could range from 50% to 300% in the same Snus user as the amount of extracted nicotine could be partially an effect of the intensity with which that portion is manipulated in the mouth. In addition, nicotine side effects may differ between non-smokers and smokers due to tolerance and neuroadaptive processes during prolonged nicotine use (Kobiella et al., 2011). Our study with non-smokers and non-Snus users showed that 12 participants reported adverse events after Snus administration, such as increased heart rate, dizziness, and nausea. Possible explanations could be found in the amount of nicotine absorbed and excessive sensitivity to nicotine. It is likely that, as non-smokers, participants were not tolerant to nicotine unpleasant effects. In fact, four participants had to suspend their participation in the fitness test battery due to serious side effects before starting the Yo-Yo test. It is important to point out here that, compared with rest, exercise can lead to increase in plasma nicotine levels and toxicity due to increased drug absorption during physical exercise (Lenz & Gillespie, 2011).

Apart from the side effects reported above, the effect of nicotine varies as a function of nicotine habituation. It would therefore be interesting for future research to investigate the effects of nicotine on smoker athletes and/or Snus users, comparing objective and subjective physical and mental performance under conditions of satiety and abstinence. Indeed, Snus effects could have been different on non-smoker participants after a familiarization process with this substance, although an unethical risk of addiction could appear, which is not in line with the "fair play" and the education of the ideal "athlete 2.0" that rejects the improvement of performance despite the possible health damage (Zabala & Atkinson, 2012).

Perspectives

In spite of being a substance with growing popularity in sport, particularly in Sweden, there is an important lack of knowledge about the potential impact of Snus on physical performance. In addition, World Anti-Doping Agency is interested on the effects of nicotine, the main component of Snus, on athletes' performance and health, and so nicotine has been included on its monitoring program since 2012. To date, there are no studies assessing the effects of Snus on physical performance, perceived physical effort, and perceived mental workload in the same experiment. Moreover, no previous study has investigated the effect of Snus on football players and their specific physical demands. The results of the present study are novel and relevant, and could lead to research in other sport contexts (e.g., different countries, type of sport, corresponding physical demands).

Snus intake, with respect to a placebo control condition, increased subjective perceptions of mental fatigue, mental workload and activation, together with a reduction of HRV, and PR in a group of non-smokers non-Snus users amateur football players. In contrast, it neither influenced physical performance in a fitness test battery nor the level of perceived physical fatigue and RPE. However, we suggest that this negative effect on mental fatigue could reduce athlete's physical

performance in long and exhausting exercise. This remains an open interesting question for future research. In light of the present results, we suggest that Snus should not be recommended as ergogenic aid for non-smokers, non-Snus user, football players.

Key words: Smokeless tobacco, Snus, nicotine, ergogenic aids, fitness, performance.

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