Original Investigation

Determination of Nicotine Absorption from Multiple Tobacco Products and Nicotine Gum

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Abstract

Introduction: Snus is a smokeless tobacco product traditionally used in Scandinavia and available in pouched or loose forms. The objective of this study was to determine nicotine absorption for current pouched and loose snus products in comparison with a cigarette and an over-the-counter nicotine gum.

Methods: We conducted an open-label, randomized, 6-way, crossover study involving 20 healthy snus and cigarette users. One of 6 products (2 pouched snus, 2 weights of loose snus, a cigarette, and a nicotine gum) was administered at each of 6 visits. Blood samples were taken at intervals over 120 min and sensory perception assessed by questionnaire.

Results: For the 4 smokeless tobacco products and the nicotine gum, blood plasma levels of nicotine were ranked according to total nicotine content as follows: loose snus (27.1 mg nicotine) > pouched snus (14.7 mg nicotine) > loose snus (10.8 mg nicotine) = pouched snus (10.7 mg nicotine) > nicotine gum (4.2 mg nicotine). The area under the plasma concentration–time curve (AUC) and maximum plasma concentration (C_{max}) of nicotine ranged from 26.9 to 13.1 ng.h/ml and 17.9 to 9.1 ng.h/ml, respectively across all the products. Nicotine was absorbed more rapidly from the cigarette but systemic exposure was within the range of the smokeless tobacco products (AUC = 14.8 ng.h/ml; C_{max} = 12.8 ng.h/ml).

Conclusions: This study has generated new information on comparative nicotine absorption from a cigarette, loose snus, and pouched snus typical of products sold in Scandinavia. The similar nicotine absorption for 1 g portions of loose and pouched snus with approximately 11 mg of nicotine indicate that absorption kinetics were dependent on quantity of tobacco by weight and total nicotine content rather than product form.

Introduction

Snus is an oral moist snuff used in Scandinavia and commercially available in several countries. This noncombustible, smokeless form of tobacco has a history of use in Sweden that dates back several hundred years, although its composition and manufacturing processes have evolved over time. The main ingredients are finely ground tobacco, water, salt, humectants, and flavors. It is currently available in two distinct forms: a loose compacted tobacco or portions of tobacco sealed in small sachets termed "pouches." The pouch weight of tobacco ranges from approximately 0.3 to 1.5 g depending on the product.

Based on the epidemiology of tobacco-related disease in Sweden, snus has been reported to be significantly less risky than cigarettes (Foulds, Ramstrom, Burke, & Fagerström, 2003). Some health professionals consider snus to be a safer alternative to smoking for individuals who are unwilling or unable to give up tobacco entirely (Britton, 2008). While the determinants of tobacco use are complex and include environmental and social factors (Tobacco Advisory Group of the Royal College of Physicians, 2007), the rapid absorption of a sufficient dose of nicotine has been proposed to be an important factor for consumer acceptability of tobacco and nicotine products (Foulds et al., 2003). Nicotine replacement therapy (NRT) products, on average, provide the user much slower absorption and lower maximum plasma concentration (C_{max}) of nicotine compared with cigarettes (Benowitz, Porchet, Sheiner, & Jacob, 1988; Russell, Jarvis, Feyerabend, & Ferno, 1983; Sobue, Sekiguchi, Kikkawa, Akasaki, & Irie, 2006). Some authorities suggest this differing pharmacokinetic profile is a contributing factor to NRT products' limited success as aids for quitting smoking (Britton, 2008). There is little published information on nicotine absorption from snus compared with cigarettes, or for different forms of snus. In a review of smokeless tobacco and related health effects in Sweden, the rate of uptake and C_{max} of nicotine obtained from snus was reported to be intermediate between an NRT (such as nicotine gum or dermal patches) and cigarettes (Foulds et al., 2003). However, the composition of nicotine and tobacco products on the market today has changed somewhat since such earlier studies were carried out.

In 2007/2008, we conducted a consumption survey involving 2,914 Swedish snus users and found that the majority (96%) of snus consumers used either pouched or loose snus exclusively

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(Digard, Errington, Richter, & McAdam, 2009). The studied snus users typically kept pouches or portions in the mouth for 60–70 min, considerably longer than the 30 min indicated by prior anecdotal evidence. The average daily consumption of loose snus was higher than for pouched snus (10–12 g for pouched vs. 29–32 g for loose snus); the median number of portions per day was similar for pouched and loose snus users, but consumers of loose snus tended to use more tobacco per portion.

However, a small study of loose and pouched snus users had previously indicated that the average systemic uptake of nicotine for these groups (based on nicotine equivalents in 24 hr urine), were comparable (Andersson, Bjornberg, & Curvall, 1994) despite a higher average level of daily consumption of tobacco by loose snus users compared with pouched. The loose and pouched product types in the study had comparable nicotine content, suggesting that differences in levels of nicotine extraction and/or bioavailability between the two forms of snus might account for the relatively higher proportion of nicotine uptake measured for pouched snus users.

Lunell and Lunell (2005) evaluated the extraction of nicotine from snus and measured steady-state plasma nicotine concentrations following repeated use of four Swedish pouched snus products with nicotine content ranging from 4.53 to 8.84 mg or a 2 mg nicotine gum over the course of 1 day. The amount of nicotine extracted as a percentage of the total nicotine content varied across snus products from 22% to 44%, compared with 44% extraction from the nicotine gum. Overall, the three moist snus samples gave rise to higher steady-state plasma nicotine levels than the dry snus and nicotine gum.

In a more recent study, Lunell and Curvall (2011) investigated single-dose pharmacokinetics and subjective effects for two pouched snus products, each containing approximately 8–10 mg of nicotine per pouch, and a high-dose (4 mg) nicotine chewing gum. They reported faster absorption of nicotine from the snus products, although the C_{max} was significantly higher for one snus product compared with the gum. All products were used for 30 min and there were no significant differences in t_{max} across products. The study concluded that the relatively rapid rise in plasma nicotine, faster onset of "head rush", and reduced urge to smoke in those using snus compared with the nicotine gum could partly explain the common use of snus in Sweden as an aid to quitting smoking.

However, significant gaps in knowledge remain. No published nicotine pharmacokinetics study to date has directly compared cigarettes with snus, tested types of snus with varying nicotine content, or compared loose with pouched snus. Therefore, in this study we investigated the effects of snus product form (pouched vs. loose) and nicotine content on nicotine absorption. We also compared these results with a cigarette and a high-dose (4 mg) nicotine chewing gum (an NRT product available over-the-counter [OTC] in Sweden).

The study was designed to evaluate nicotine absorption from use of a single product. The protocol of use for the pouched snus and the portion weights of loose snus was based on data from our snus consumption survey (Digard et al., 2009). Subjects used snus samples for 60 min, which was the median reported time period for an individual session of use observed in the survey. The weights of loose snus were selected to match the weight of the most frequently used pouched snus (1 g, also included in this study) and the median portion weight reported in the survey (2.5 g). A sensory assessment was performed by questionnaire for the snus products to investigate whether differences in nicotine absorption resulted in variation in reported subjective effects. CYP2A6 genotyping was included because the rate of nicotine metabolism is a potential variable affecting the measured pharmacokinetic endpoints.

Methods

Study Design

This was an open-label, randomized, 6-way, crossover study conducted at the Clinical Research and Trial Centre, Lund University Hospital, Sweden (trial registration number: ISRCTN11703777). The study was conducted in accordance with the "Declaration of Helsinki," ICH GCP and EU Directive 2005/28/EC. The study was approved by the Central Ethics Review Board, Stockholm, Sweden. Written informed consent was obtained from all subjects before entering the study and undergoing any study-related procedures. The Investigator site ensured that subjects were offered health advice and information on tobacco cessation helplines.

The study enrolled 20 healthy subjects. Each subject visited the clinic seven times: once for screening and physical examination and six times for product administration. All 20 subjects tested all the six products; one product per visit was assigned in random order using a validated system. Each product administration visit lasted for 4 hr and there was a gap of at least one whole day between product administration visits. Subjects were asked not to consume tobacco or nicotine products or foods and drinks that could act as cytochrome P450 enzyme inhibitors (specifically caffeine or herbal teas, cruciferous vegetables) for 12 hr prior to product administration. In addition, no food intake was allowed 1 hr prior to product administration. Subjects were excluded if they had used medications with a known mechanism of action on the cyclooxygenase pathway 14 days prior to first product administration.

Screening of urine for drugs of abuse was performed using a standard test kit SYVA® Rapidtest d.a.u® 10. Safety monitoring and reporting, including concomitant medications, was performed throughout the study period and 1 week after completion of last product use. The subjects were also monitored for cardiovascular changes prior to and at intervals after test product administration (pulse rate, blood pressure at 15, 30, and 60 min; heart rate via ECG records at 30, 60, and 90 min).

CYP2A6 has been reported to play a major role in the metabolism of nicotine and a number of genetic polymorphisms have been described which affect the rate of nicotine metabolism and inactivation (Haberl et al., 2005; Schoedel, Hoffmann, Rao, Sellers, & Tyndale, 2004). Therefore, CYP2A6 genotyping was performed to identify subjects' metabolic status and any resulting impact on plasma nicotine concentrations. Subjects were characterized as either extensive (wild-type) or intermediate metabolizers by genotyping for CYP2A6 alleles *1 (wild-type), *2, *4, *9B, and *12B.

Study Population

Adult male and female healthy volunteers were recruited provided that they reported that they consumed snus daily (pouch weight

Table 1. Product Information

Product (brand)	Target weight (g)	Measured nicotine content per product (mg)	Product parameters		
			pH	Moisture (%)	Weight (g)
Cigarette (Lucky Strike Red)	NA	14.6[a]	NA	NA	NA
Loose snus (Granit)	1	10.8	8.0–8.3	50.5–53.5	0.90–1.10
Loose snus (Granit)	2.5	27.1	8.0–8.3	50.5–53.5	2.40–2.60
Pouched snus (Lucky Strike Original, Brown)	1[b]	10.7	8.0–8.2	48.0–52.0	0.90–1.10[b]
Pouched snus (Lucky Strike Bold)	1[b]	14.7	7.9–8.1	47.0–51.0	0.90–1.10[b]
Nicotine gum (Nicorette®)	NA	4.2	NA	NA	NA

Note. NA = not applicable.

[a]Total nicotine present in the tobacco blend.

[b]Total weight including that of the pouch.

≥ 0.8 g) in the preceding 6 months and smoked 9–10 mg ISO Tar factory-made cigarettes occasionally (≤40 cigarettes/week).

Subjects were between the ages of 19–55 years, had a body mass index (BMI) of 18–30 kg/m^2 and agreed to comply with the study restrictions and procedures. Subjects were screened for good health by the Investigator, based on medical history, physical examinations, vital signs, 12-lead ECG, urine drug screen, and reports of routine clinical laboratory tests (analyzed at Lund University Hospital Laboratory, Sweden). Premenopausal females were required to have a negative pregnancy test at screening and be willing to use contraception during the study period.

Study Test Products

The study evaluated five different tobacco products (two pouched snus products with different nicotine contents, one loose snus product that was used at two different portion sizes, and a cigarette) and an OTC nicotine chewing gum (Table 1). All the products were commercially available in Sweden at the time of the study but were unbranded at the point of administration. The subjects were given instructions on how to use each product. Snus was placed under the upper lip for 60 min and the subjects were asked not to move it within the mouth during this time period. The subjects were instructed to smoke the cigarette naturally according to their usual smoking behavior for 5 min or until the cigarette had been smoked to 30 mm from the mouth end (if this occurred in less than 5 min), at which point the cigarette was extinguished. The nicotine gum was used for 30 min according to the manufacturer's guidelines specified on the pack. For the purpose of a standardized instruction, subjects were asked to chew the gum every 2 s (in time with a metronome) and swallow the formed saliva, once every minute for 30 min.

To minimize the effects of product variability, a new container of each snus product was used at each visit and a sample of each snus product (from the container being used for that visit) was checked for pH, moisture content and, in the case of pouched snus, target portion weight prior to administration. Samples from a container were only used if the sample parameters fell within set ranges for each of the snus products (Table 1). For the loose snus products, portions were formed by the clinic staff, using an adapted syringe device and weighed to ensure they were within the target weight range (Table 1).

The snus and nicotine gum portions were collected after use. A corresponding unused portion was also collected from the same container as each test portion. All were placed in individual sealed labeled vials and stored frozen prior to analysis. Used and unused snus and gum samples were analyzed for nicotine, which was expressed on a whole portion, wet-weight basis. The analysis of the snus samples was performed at British American Tobacco (Investments) Ltd., Group R&D, Southampton, United Kingdom by GC-MS. The nicotine gum samples were analyzed at Eurofins Food & Agro Sweden AB, Lidkoping, Sweden by LC-MS.

Sensory Evaluation

While using the administered snus products, subjects were requested to complete a short sensory questionnaire, consisting of four questions, to assess their sensory experiences relating to irritation of lips and throat, level of salivation, or other perceived sensations such as any "buzz" feeling during use of the snus. Subjects scored their experiences on a scale of 1–5 (low to high, respectively). The purpose of this was to evaluate any association between the reported strength of snus' sensory effects and measured nicotine uptake. The questionnaire responses were obtained after 5, 10, 20, and 30 min of placing snus in the mouth.

Blood Sampling

Venous blood was collected by cannulation of the antecubital vein for both nicotine analysis (5 ml at each sampling time) and CYP2A6 genotyping (4 ml at visit 1, prior to any other blood sampling). Each sample was split into two aliquots. The sampling time points for nicotine analysis were preadministration (0) and at 5, 7, 10, 20, 30, 45, 60, 90, and 120 min after the start of each test product use. An additional sampling time point at 2 min was included while the cigarette was smoked.

Bioanalytical Methods

Nicotine

The bioanalysis of nicotine from plasma samples was conducted by Advanced Bioanalytical Service Laboratories Ltd., United Kingdom. Samples were analyzed by HPLC interfaced with an AB/MDS Sciex 4000 mass spectrometer. Quantification of nicotine was by peak area ratio. The determined lower limit of quantification for nicotine in plasma using this method was 0.5 ng/ml.

Genotyping

Genotyping of CYP2A6 alleles *1, *2, *4, *9B, and *12B was performed using whole blood samples collected into K2 EDTA VacutainerTM tubes, stored at −20 °C and analyzed by QIAGEN Manchester Ltd., Manchester, United Kingdom (formerly DxS Ltd.). DNA preparation was performed using a QIAGEN spin column kit. Prepared DNA samples were tested with a standard real time fluorescence assay to ensure quality of the test material. Specific DNA sequences were amplified by quantitative polymerase chain reaction (qPCR). All samples met the qPCR-QC acceptance criterion of Cycle Threshold <30.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic analysis was conducted by Covance Clinical Research Unit (Leeds, United Kingdom) using WinNonlin Enterprise Version 5.2 (Pharsight Corporation, Mountain View, CA). The pharmacokinetic population consisted of all subjects who received at least one test product and had evaluable pharmacokinetic data area under the plasma concentration–time curve from time 0 to t_{last} ($AUCt_{0-tlast}$) (nominal t_{last} for this study was 120 minutes post start of product administration), C_{max} and t_{max} were determined from the plasma concentrations of nicotine using noncompartmental procedures (Committee for Medicinal Products for Human Use, 2010).

Summary statistics for pharmacokinetics were calculated with and without the criterion for exclusion of a preadministration plasma concentration of greater than 4 ng/ml. This criterion was chosen based upon a previously reported cutoff level for noncompliance with the preadministration nicotine and tobacco product abstinence period (Lunell & Lunell, 2005). Comparison of products was performed using a mixed model, including sequence, period, and product as fixed effects and subject (sequence) as a random effect. Statistical comparisons of the plasma nicotine parameters $AUC_{0-tlast}$ and C_{max} following use of the snus products were conducted by ANOVA and adjusted using the Tukey multiple comparison test, at $p < .05$, to show statistically significant differences between products. All statistical analyses were performed using SAS v. 8.2 (SAS Institute Inc., Cary, NC). All figures were produced using SAS v. 9.2.

Results

Subjects

The 20 eligible healthy subjects enrolled onto the study consisted of 19 males (18 caucasians and 1 mixed race) and 1 female (caucasian). The average age of subjects at the time of enrollment was 29 ± 8.8 years (mean ± *SD*). Height, weight, and BMI (mean ± *SD*) were 180 ± 6.8 cm, 77.5 ± 11.1 kg, and 23.9 ± 3.52 kg/m^2, respectively. Based on the genotyping analysis for CYP2A6 alleles, 12 subjects were identified as homozygous wild-type (CYP2A6*1) and hence extensive metabolizers. Eight subjects were heterozygous, possessing one wild-type, and one of the variant alleles or, in the case of one subject, two different variant alleles. All these eight subjects would be expected to metabolize nicotine but at a slower rate compared with wild-type (Benowitz, Swan, Jacob, Lessov-Schlaggar, & Tyndale, 2006). In the absence of more detailed or quantitative metabolic profiling they were classified as intermediate metabolizers for the purpose of this study.

While four subjects were found to have preadministration nicotine plasma concentrations greater than 4 ng/ml and, therefore, were candidates for exclusion due to evident noncompliance with the abstinence guidelines, there was no apparent difference in the summary statistics and statistical analyses when calculated for all subjects or excluding these subjects. Therefore, the reported pharmacokinetic data and safety data include all 20 subjects.

Extraction of Nicotine from Snus and Gum

The nicotine content of snus and gum portions was measured in separate samples taken both before and after use. The amount of extracted nicotine, as mg/portion and as a percentage of total nicotine, was calculated as follows:

Amount extracted = Quantity in an unused portion – Quantity in the used portion

Transfer (%) = 100 * (Amount extracted / Quantity in unused portion)

As shown in Table 2, the percentage of nicotine extracted from the products was highest for the nicotine gum, with 63% extracted. For snus products the mean extraction during use was between 24% and 32%.

Nicotine Pharmacokinetics

In this study, the primary pharmacokinetic parameters for measuring uptake of nicotine from the test products were C_{max}, t_{max}, AUC_{0-120}, and the results are given in Table 3. The plasma nicotine concentration/time profiles are shown in Figure 1. Nicotine uptake as measured by nicotine concentration in plasma from the venous circulation is characterized herein as systemic exposure to nicotine.

Table 2. Nicotine Content and Extraction for Snus and Nicotine Gum

Product	Nominal portion weight (g)	Measured nicotine content (g), mean ± *SD*, *N* = 20		Extracted nicotine, mean ± *SD*, *N* = 20	
		Unused	Used	mg/portion	% of total
Pouched snus	1.0	10.72 ± 0.91	7.34 ± 1.96	3.38 ± 1.92	31.41 ± 18.30
Pouched snus	1.0	14.67 ± 0.76	10.14 ± 2.32	4.53 ± 2.09	31.06 ± 15.08
Loose snus	1.0	10.79 ± 0.31	7.34 ± 1.48	3.45 ± 1.42	32.05 ± 13.34
Loose snus	2.5	27.09 ± 0.71	20.67 ± 2.58	6.42 ± 2.35	23.75 ± 8.86
Nicotine gum	1.0	4.15 ± 0.10	1.55 ± 0.36	2.62 ± 0.36	63.13 ± 8.75

Table 3. Pharmacokinetic Parameters of Plasma Nicotine Following Single Use of Different Tobacco Products and a Nicotine Gum

	Product (nicotine content) $N = 20$					
	Cigarette (14.6 mg)	Loose snus (10.8 mg)	Loose snus (27.1 mg)	Pouched snus (10.7 mg)	Pouched snus (14.7 mg)	Nicotine gum (4.2 mg)
AUC_{0-120} ng.h/ml						
Geometric mean*	14.8	16.0[a]	26.9[b]	16.8[a]	20.4[c]	13.1
Geometric coefficient of variation %	30.4	31.2	23.8	39.6	37.6	28.3
C_{max} ng/ml						
Geometric mean*	12.8	10.8[a]	17.9[b]	10.8[a]	13.4[a]	9.1
Geometric coefficient of variation %	41.3	34.4	22.8	41.4	39	28.6
t_{max} h						
Median	0.117	1.00	1.00	1.00	1.00	0.75
Range: minimum–maximum	0.083–0.517	0.75–1.50	0.75–1.50	0.33–1.50	0.75–1.50	0.33–1.50

Note. *Means with different letters are significantly different according to Tukey's multiple comparison test ($p < .05$).

The study showed that systemic exposure (C_{max}, AUC_{0-120}) to nicotine from snus was dependent on the total nicotine content of the portion (Figure 1, Table 3). The AUC_{0-120} for all six test products were ranked as: loose snus (27.1 mg) > pouched snus (14.7 mg) > loose/pouched snus (10.8 mg and 10.7 mg, respectively) > cigarette (14.6 mg) > 4.2 mg nicotine gum. C_{max} followed a similar ranking: loose snus (27.1 mg) > pouched snus (14.7 mg) > cigarette (14.6 mg) > loose/pouched snus (10.8 mg and 10.7mg, respectively) > 4.2 mg nicotine gum. Unlike the oral products, the total amount of nicotine available from the cigarette would have been limited to that inhaled by the smoker. The transfer of nicotine into smoke can be determined by machine smoking. However, no single smoking regime can fully account for the individual and temporal variation in smoking behavior and predict exposure in all smokers. To give an indication of transfer, we measured nicotine yield with the two most commonly used smoking regimes: the ISO standard test method 10315:2000 and Health Canada Method T-115, which yielded 0.9 and 2.1 mg nicotine per cigarette, respectively.

The t_{max} for all snus products in this study was 1 hr, which was also the time of use specified in the study. In comparison, the time of use for the nicotine gum was 30 min and the t_{max} was 0.75 hr (45 min). The t_{max} for the cigarette was considerably less at 0.117 hr

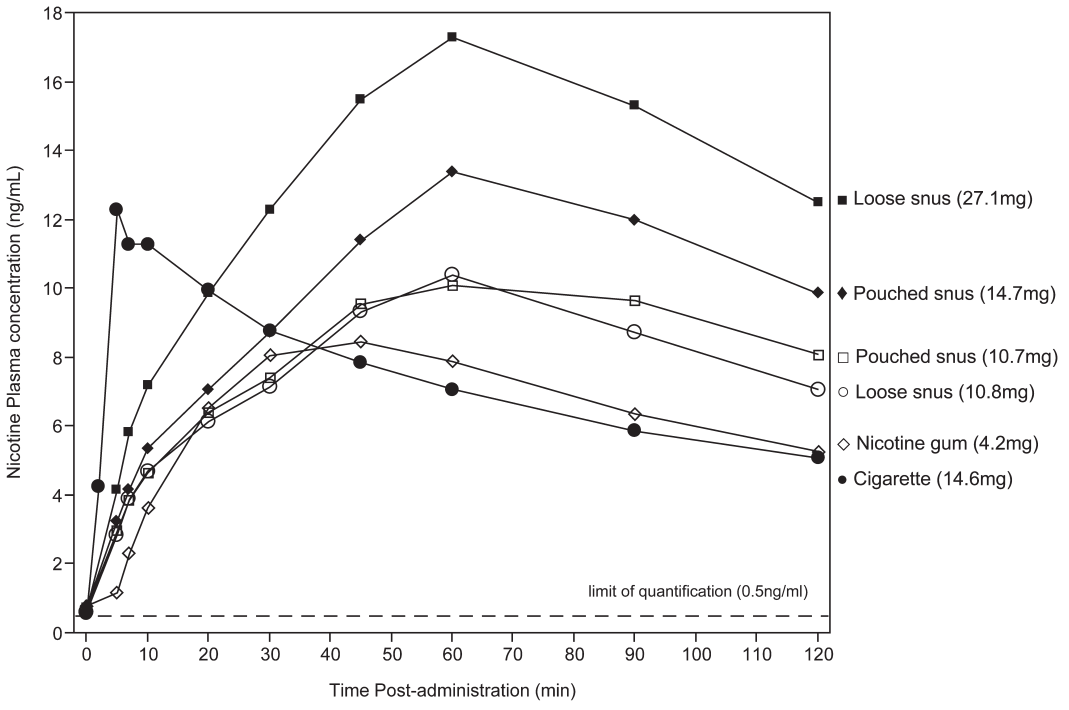

Figure 1. Mean plasma nicotine concentrations at each time point following single use of the different tobacco products and nicotine gum. Products (nicotine content): • Cigarette (14.6 mg); □ Pouched snus (10.7 mg); ○ Loose snus (10.8 mg); ♦ Pouched snus (14.7 mg); ■ Loose snus (27.1 mg); ◊ Nicotine gum (4.2 mg). The dashed line represents the limit of quantification (0.5 ng/ml).

(7 min) consistent with the short time of use (5 min). The range of t_{max} for the cigarette reported in Table 3 (0.083–0.517 hr) was influenced by the accidental loss of one blood sample at 5 min. When this subject was removed from the analysis the range was reduced to 0.083–0.333 hr but the t_{max} remained the same. While nicotine was absorbed more rapidly from the cigarette, systemic exposure was within the range of the smokeless tobacco products ($AUC_{0–120}$ = 14.8 ng.h/ml; C_{max} = 12.8 ng.h/ml).

Comparison of the ratios of geometric means for $AUC_{0–120}$ and C_{max} for increasing nicotine content in snus showed that the increase in plasma nicotine was subproportional to the nicotine content of snus; for loose snus a 2.5 times increase in nicotine content from 10.8 to 27.1 mg was associated with a 1.7 times increase in $AUC_{0–120}$ and C_{max}. Similarly, for pouched snus a 1.4 times increase in nicotine content from 10.7 to 14.7 mg was associated with a 1.2 times increase in $AUC_{0–120}$ and C_{max}.

Statistical comparisons of $AUC_{0–tlast}$ and C_{max} were made for the snus products where the protocol of use and the time of exposure were the same in all cases. Notably, there was no statistically significant difference ($p < .05$) in systemic exposures between pouched and loose snus when the nicotine contents of the products were equivalent (10.7 mg and 10.8 mg, respectively; Table 3). All other pairwise comparisons of $AUC_{0–tlast}$ for snus products did show a significant difference ($p < .05$). Whereas for C_{max}, only loose snus 27.1 mg compared with all other snus products showed a statistically significant difference ($p < .05$; Table 3).

Sensory Evaluation

For all snus products, the degree of irritation of lips and throat, level of salivation, or other perceived sensations such as any "buzz" feeling that subjects reported when using snus were generally low on the scale provided by the questionnaire. Overall there were no trends associated with product form or nicotine content noted in snus sensory questionnaire responses, suggesting that these product parameters had little effect on the level of sensations that subjects reported when using the snus products.

Effect of Genotype

CYP2A6 genotyping classified 12 subjects as extensive metabolizers and eight subjects as intermediate metabolizers. The mean $AUC_{0–120}$ was approximately 10%–30% lower for extensive metabolizers across all products, apart from the 27.1 mg loose snus for which the $AUC_{0–120}$ values were similar for intermediate and extensive metabolizers. However, due to the variability of individual exposure levels across test products for all subjects, regardless of metabolic status, the results of the genotyping analysis were not considered to have any significant impact on the interpretation of the pharmacokinetic data.

Safety

Some changes were observed in blood pressure, pulse rate, and heart rate during the study which were consistent with the recognized effects of nicotine on the sympathetic nervous system (Omvik, 1996; Robertson, Tseng, & Appalsamy, 1988). Increases from baseline systolic and diastolic blood pressure and pulse rate (mean increases of 3–10 mmHg, 4–10 mmHg, and 8–13 bpm, respectively) and heart rate as determined by ECG (mean increases of 6–12 bpm) were noted 15–30 min after product administration. There were no apparent associations between changes in blood pressure, pulse, or heart rate and the nicotine content of the products. No serious or severe adverse events were reported during the study.

Discussion

In this study, we have generated new information on comparative nicotine absorption from a cigarette, loose snus, and pouched snus. The tobacco products were typical of those commercially available in Europe (cigarette) and Scandinavia (snus) at the time of the study. In addition, nicotine absorption from use of a high-dose OTC pharmaceutical nicotine gum was measured for all subjects.

Nicotine plasma levels from smoking the cigarette rose more rapidly than for the oral products, as expected from the literature (Foulds et al., 2003). The total plasma nicotine concentration over the sampling period of 120 min ($AUC_{0–120}$) was higher for all the snus products than for the nicotine gum and cigarette, likely due to the higher total nicotine content of the snus and longer duration of use. The latter is an important variable; most nicotine pharmacokinetic studies on snus are based on a usage time of 30 min. However, in this study we applied the median time of 60 min reported in a survey of Swedish snus users as this was potentially more consistent with actual product use (Digard et al., 2009). These data showed that nicotine was continually absorbed from the snus portions over the entire 60-min period, which may partly explain the usage time observed in Swedish consumers. Nevertheless, the percentage of nicotine extracted from snus measured in this study (24%–32%) was similar to that observed by Lunell and Lunell (2005; 22%–44%).

The mean quantity of nicotine extracted from the used portions of snus and the nicotine gum followed the same trend as the pharmacokinetic AUC results and was positively correlated with the total nicotine content. However, the $AUC_{0–120}$ for the 11 mg snus was only about 1.3 times higher than for the 4.2 mg gum, suggesting that the nicotine in the oral tobacco may be less bioavailable compared with that in the gum. Overall, the measured CYP2A6 metabolic status of the subjects did not have an impact on the pharmacokinetic end-points measured.

The similar nicotine pharmacokinetics for 1 g portions of loose and pouched snus, both containing similar levels of nicotine (10.8 mg and 10.7 mg, respectively), suggested that product form was not a major influencing factor in this study. Rather, it was dependent on the total amount of nicotine in the snus portion (quantity by weight of tobacco × the nicotine content of the blend). However, the data for the three different levels of total nicotine in snus indicated that the relationship was subproportional.

It is important to note that various behavioral factors not investigated in this study could also affect nicotine absorption such as moving the snus portion around the mouth, spitting saliva during use, or swallowing some amount of loose snus. Hence, while the protocol of use applied in this study was based on observed median usage patterns, in particular the portion sizes and 60-min duration of use (Digard et al., 2009), the data presented here are not representative of all snus users.

There was no significant difference in self-reported sensory perceptions between any of the snus samples. However, these data were derived from a questionnaire that had been adapted for this study and not fully validated. Therefore, while this was an interesting observation further and larger studies would be required to confirm the generality of the finding.

In summary, this study has provided new information on nicotine absorption for typical snus products, including loose snus, which demonstrates relationships between weight of tobacco and total nicotine content and systemic nicotine exposure. This study also provides data on nicotine absorption for a cigarette and an OTC nicotine gum.

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Declaration of Interests

Dr Malmqvist does not act as a consultant for British American Tobacco and has no financial interests in the Company.

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References

Andersson, G., Bjornberg, G., & Curvall, M. (1994). Oral mucosal changes and nicotine disposition in users of Swedish smokeless tobacco products: A comparative study. *Journal of Oral Pathology & Medicine*, *23*, 161–167. doi:org/10.1111/j.1600-0714.1994.tb01106.x

Benowitz, N. L., Swan, G. E., Jacob, P., III, Lessov-Schlaggar, C. N., & Tyndale, R. F. (2006). CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. *Clinical Pharmacology & Therapeutics*, *80*, 457–467. doi:10.1016/j.clpt.2006.08.011

Benowitz, N. L., Porchet, H., Sheiner, L., & Jacob, P. (1988). Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparison with cigarettes and nicotine gum. *Clinical Pharmacology & Therapeutics*, *44*, 23–28. doi:org/10.1038/clpt.1988.107

Britton, J. (2008). Should doctors advocate snus and other nicotine replacements? Yes. *British Medical Journal*, *336*, 358. doi:org/10.1136/bmj.39479.427477.AD

Committee for Medicinal Products for Human Use. (2010). *Guideline on the investigation of bioequivalence. European medicines agency*. Retrieved from http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf

Digard, H., Errington, G., Richter, A., & McAdam, K. (2009). Patterns and behaviors of snus consumption in Sweden. *Nicotine & Tobacco Research*, *11*, 1175–1181. doi:org/10.1093/ntr/ntp118

Foulds, J., Ramstrom, L., Burke, M., & Fagerström, K. (2003). Effect of smokeless tobacco (snus) on smoking and public health in Sweden. *Tobacco Control*, *12*, 349–359. doi:org/10.1136/tc.12.4.349

Haberl, M., Anwald, B., Klein, K., Weil, R., Fuss, C., Gepdiremen, A., et al. (2005). Three haplotypes associated with CYP2A6 phenotypes in Caucasians. *Pharmacogenetics and Genomics*, *15*, 609–624. doi:org/10.1097/01.fpc.0000171517.22258.f1

Lunell, E., & Curvall, M. (2011). Nicotine delivery and subjective effects of Swedish portion snus compared with 4 mg nicotine polacrilex chewing gum. *Nicotine & Tobacco Research*, *13*, 573–578. doi:org/10.1093/ntr/ntr044

Lunell, E., & Lunell, M. (2005). 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 & Tobacco Research*, *7*, 397–403. doi:org/10.1080/14622200500125468

Omvik, P. (1996). How smoking affects blood pressure. *Blood Pressure*, *5*, 71–77. doi:10.3109/08037059609062111

Robertson, D., Tseng, C. J., & Appalsamy, M. (1998). Smoking and mechanisms of cardiovascular control. *American Heart Journal*, *115*, 258–263. doi:10.1016/0002-8703(88)90646-1

Russell, M. A., Jarvis, M. J., Feyerabend, C., & Ferno, O. (1983). Nasal nicotine solution: A potential aid to giving up smoking? *British Medical Journal*, *286*, 683–684. doi:org/10.1136/bmj.286.6366.683

Schoedel, K. A., Hoffmann, E. B., Rao, Y., Sellers, E. M., & Tyndale, R. F. (2004). Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenetics*, *14*, 615–626. doi:org/10.1097/00008571-200409000-00006

Sobue, S., Sekiguchi, K., Kikkawa, H., Akasaki, M., & Irie, S. (2006). Comparison of nicotine pharmacokinetics in healthy Japanese male smokers following application of the transdermal nicotine patch and cigarette smoking. *Biological and Pharmaceutical Bulletin*, *29*, 1068–1073. doi:org/10.1248/bpb.29.1068

Tobacco Advisory Group of the Royal College of Physicians. (2007). *Harm reduction in nicotine addiction: Helping people who can't quit*. Royal College of Physicians. Retrieved from http://www.tobaccoprogram.org/pdf/4fc74817-64c5-4105-951e-38239b09c5db.pdf