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Original Investigation

Pharmacokinetic Comparison of a Novel Non-tobacco-Based Nicotine Pouch (ZYN) With Conventional, Tobacco-Based Swedish Snus and American Moist Snuff

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Abstract

Introduction: The single-dose pharmacokinetics (PK) of a novel, non-tobacco-based nicotine pouch, ZYN, 3 and 6 mg, were compared with 8 mg General snus (part 1) and ZYN 8 mg was compared with 18 mg Longhorn moist snuff (part 2). The present study demonstrates the characteristics of three strengths of a novel tobacco-free oral snus, ZYN, viz. the extraction of nicotine from the oral cavity and its uptake into the systemic blood circulation. Comparison is made to Swedish General snus and American Longhorn moist snuff and from literature 4 mg Nicorette gum and mean of 13 brands of e-cigarettes.
Aims and Methods: A single-dose randomized crossover design was used. In vivo extraction and PK parameters were determined.
Results: *Part 1*. The AUC_{inf} of ZYN 3 mg was 27% smaller than that of 8 mg General and the AUC_{inf} of ZYN 6 mg was 34% larger than that of 8 mg General. Less nicotine was extracted from ZYN 3 mg (1.5 mg) and more from ZYN 6 mg (3.5 mg) than from 8 mg General (2.4 mg). The extracted fractions of nicotine for both ZYN products (56% and 59%) were significantly larger than for 8 mg General (32%).
Results: *Part 2*. Close to identical plasma nicotine curves, AUC_{inf} and C_{max} were found for ZYN 8 mg and Longhorn Natural 18 mg moist snuff. The extracted amount of nicotine from ZYN 8 mg (3.8 mg) was larger than the amount extracted from Longhorn Natural 18 mg (3.0 mg), but smaller than the extracted amount of nicotine from General 2 × 8 mg snus pouches (5.0 mg). The extracted fraction of nicotine for ZYN 8 mg (50%) was larger than for Longhorn Natural (19%) and General 2 × 8 mg snus pouches (33%).
Conclusions: The two higher doses of ZYN (6 and 8 mg) deliver nicotine as quickly and to a similar extent as existing smokeless products, with no significant adverse effects.

Introduction

Nicotine is the main driving force that motivates use of tobacco products. Several types of nicotine products have been marketed to deliver nicotine either for recreational use, smoking cessation, or relief of withdrawal. Cigarettes are the most efficient nicotine delivery system due to their very fast uptake and high levels of nicotine.[1] Smokeless tobacco products also contain and deliver nicotine enough to be self-administered. Pharmaceutical nicotine replacement therapy products, such as gum, lozenges, and patches, are effective but they are less acceptable to smokers in part because they deliver

nicotine more slowly and to a lower level than cigarettes and Swedish snus.[2,3] Recently, however, a higher strength of Nicorette gum, 6 mg, has been introduced that produced higher C_{max} and AUC_{inf} but only marginally improved craving control[4] since a common problem with nicotine replacement therapy has been underdosing.[5,6]

Recently, a novel non-tobacco oral nicotine product category seems to be established. The first product in this category was ZYN marketed by Swedish Match North America. ZYN comes in three strengths, 3, 6, and 8 mg, and is packed in a white pouch that is put under the upper lip. In the United States ZYN appears very popular among smokeless tobacco users.[7] One possible reason for its appeal may be that it is a non-tobacco product.

The purpose of the present paper is to study the pharmacokinetics (PK) of ZYN. Because the product has similar features to a smokeless tobacco product, we compared the PK of ZYN to the Swedish General snus, the first product to obtain a Modified Risk Tobacco Product order from Food and Drug Administration (FDA), and the American Longhorn moist snuff. From literature references comparison was made with 4 mg Nicorette and 13 brands of first-generation e-cigarettes.

Background and Rationale

When comparing the nicotine content of different nicotine-containing products it is important to consider that the nicotine extraction and uptake varies considerably depending on product type and formulation.[1]

Previous studies have indicated that on average about 15%–20% of the total nicotine content is extracted and absorbed, with large interindividual variation.[1–3] Extraction is generally not linear with pouch size: it is larger with small compared with larger pouches, which suggests that surface area, saliva penetration, and diffusion factors may be equally important determinants of nicotine uptake as pouch weight. ZYN is a novel non-tobacco-based nicotine pouch. ZYN does not contain nitrosamines or polycyclic aromatic hydrocarbons, the two most controversial classes of substances (which potentially may be carcinogenic) that can be found in conventional, tobacco-based snus, albeit at extremely low concentrations.

Swedish snus products with different nicotine content have previously been compared with 2 and 4 mg nicotine chewing gum.[2,3] We here extend previous observations on Swedish Snus by comparing the novel non-tobacco-based product (ZYN) with conventional, tobacco-based Swedish snus and American moist snuff.

Study Objectives

Primary Objective

To compare each subject's area under the plasma concentration time curve from time zero to infinity (AUC_{inf}) after administration of a single dose of a non-tobacco-based nicotine pouch (ZYN) containing 3 and 6 mg of nicotine, respectively, to that of a single dose of General Swedish snus containing 8 mg of nicotine (part 1) and a single dose of ZYN 8 mg to that of American Longhorn moist snuff (18 mg) and 2 pouches General snus (part 2).

Secondary Objectives

1. To compare $AUC_{60\ min}$, maximum concentration (C_{max}), time to maximum concentration (T_{max}), AUC_{0-t}, and terminal half-life.
2. To compare the estimated in vivo extracted amount of nicotine.
3. To compare pulse rate and subjective effects (head buzz) after study product administration.
4. Collection of adverse events (AEs).

Material and Methods

Study Design

An open, randomized, crossover, single-dose administration trial was performed in two parts that both were approved by the Independent Ethics Committee (IEC) in Uppsala, Sweden. The trials were performed by CTC Clinical Trial Consultants AB, Uppsala and adhered to the requirements of the EU Clinical Studies Directive 2001/20/EC and ICH Guideline for Good Clinical Practice.

Subjects

Healthy subjects aged greater than 19 years who had used tobacco-based snus for greater than 1 year with a weekly consumption of three or more snus cans (brands with nicotine content <1%) or two or more cans (brands with nicotine content >1%) were considered eligible to participate in the study. Before study entry, subjects signed an informed consent form and subsequently underwent screening evaluations including smoking and snus use, and medical history. Subjects who were pregnant or who had a history of hypertension or any cardiovascular disease were excluded.

Before study entry, subjects signed an informed consent form and subsequently underwent screening evaluations including smoking and snus use, medical history, and pulse measurements before/after application of their usual brand of snus.

Part 1

In total, 39 subjects were screened and 18 subjects were enrolled into the study. Each subject participated in the study for 22–41 days. One subject withdrew his consent after visit 2, ie, after 6 days in the study. In summary, the number of subjects in part 1 that compared ZYN to Swedish snus was 17.

Part 2

In total, 36 subjects were randomized, six subjects were discontinued due to protocol noncompliance and loss to follow-up. The number examined in part 2 that compared ZYN to American moist snuff and two pouches General snus was 30; one additional subject was excluded from the PK analysis due to nonacceptable Lambda *Z* criteria, leaving 29 subjects in the PK analysis set.

Methods

Subjects visited the clinic on separate days for each experimental session. The subjects were instructed to abstain from snus, cigarettes, or other nicotine delivery products from 8.00 PM the evening before. All sessions were performed during the morning hours to facilitate abstinence. Abstinence was determined by asking the subjects prior to each dosing and there was no exclusion of data based on baseline nicotine plasma concentrations. The highest value was 6.9 ng/mL. However, baseline nicotine plasma concentrations generally support the subjects' self-reported abstinence. Adjustment for baseline nicotine for the calculation of PK parameters gave values between 6% and 16% lower in study 1 and between 3% and 8% lower in study 2. Baseline adjustment did not result in any change in which of the differences that were significant (with the exception of T_{max} for ZYN 8 mg vs. General snus 2 × 8 mg, see discussion), nor did adjustment result in any meaningful change in the comparisons. Thus, data without adjustment for baseline concentrations are presented, mirroring the real-life situation.

The investigational products (IPs) were administered as single doses according to a computer-generated randomization list. A Latin square approach was used. All IPs were administered at the research clinic under supervision of the clinical staff to ensure compliance.

The subjects kept the pouch still between the upper lip and the gum for 60 min and were instructed not to manipulate the pouch with the tongue or lips. This is the way ZYN and other smokeless products are actually used by consumers.[8] The subjects were also instructed not to eat, drink, chew chewing gum, or brush teeth from 30 min before application of treatment, during application of the IP and 30 min after the IP had been taken out.[9] Each used pouch was collected and frozen pending analysis of nicotine.

A telephone follow-up was conducted 1 week after last dose to assess any adverse effects.

IPs and Labeled Dosage

In addition to pharmaceutical grade nicotine, microcrystalline cellulose, maltitol, and gum arabic are used as fillers, sodium carbonate and sodium bicarbonate regulate pH (8.3), and all flavors are food grade.

Part 1

Test products:

1. ZYN 3 Smooth containing 3 mg nicotine per dose (pouch).
2. ZYN 6 Smooth containing 6 mg nicotine per dose (pouch).

Reference product:

General portion snus 1.0 g (8 mg nicotine/g).

Part 2

Test products:

1. ZYN 8 Smooth containing 8 mg nicotine per dose (pouch).
2. General portion snus 1.0 g (8 mg nicotine per pouch); one dose = two pouches (one on each side of the upper lip).

Reference product:

Longhorn Natural portion snus 18 mg nicotine per dose (pouch).

Efficacy Assessments

Nicotine plasma concentrations were determined at preset time points, before (0) and at 5, 10, 15, 30, and 60 min, 1.5, 2, 4, and 6 h after administration of each product.

PK parameters were calculated by noncompartmental analysis according to the linear up–log down method using Phoenix WinNonlin ver. 8.1 (Pharsight Corporation). AUC_{inf} = area under the plasma concentration versus time curve extrapolated to infinity, AUC_{0-t} = area under the plasma concentration versus time curve to last measuring point, $AUC_{60\ min}$, C_{max}, T_{max}, and terminal half-life were calculated.

The time for the predose sample was set to 0 and plasma concentrations below the quantification limit was set to 0 before T_{max} and to missing thereafter. Baseline measurement was defined as the latest measurement prior to each dosing.

The elimination constant (Kel) was calculated and the threshold for acceptance of regression was defined by: ≥0.85 for R^2_{adj}30% for % residual AUC, and ≥1.0 for the half-life span. The elimination constant for subjects not fulfilling all three acceptance criteria was calculated based on the mean calculated eliminations constants for the same subject at other dosing occasions. Subjects not fulfilling acceptable criteria for eliminations constant determination at any dosing occasion were excluded from the PK population.

Vital signs—Heart rate was measured at the following time points: before (0) and at 5, 10, 15, 30, and 60 min after administration of each product. Heart rate was measured using an automatic device in sitting position after 10 min of rest.

Nicotine Plasma Assays

Frozen plasma samples collected for nicotine determinations were shipped to a certified contract laboratory, ABS Laboratories Ltd, United Kingdom. The analysis of the plasma samples was performed by a validated liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay. To quantify nicotine, a multilevel calibration at eight concentrations was performed over a range of 0–50 ng/mL. The calibration line was fitted by means of linear regression weighted by 1/concentration^2. The samples were assayed once.

Incurred sample reproducibility was performed according to the European Medicines Agency (EMA) and FDA guidelines so that 10% of the analyzed study samples up to 1000 were reanalyzed and then 5% of the number above 1000. The calibration standards must have a back-calculated accuracy within 100 ± 15%, and within 100 ± 20% at the lower limit of quantification. The standard curve was constructed from at least three quarters (ie, 12) of the calibration standards, excluding the zero concentration calibration standards. Duplicate quality control samples at low, medium, and high concentrations were included in each analysis batch. The accuracy of at least two-thirds of the quality control samples was within 100 ± 15%. Half of the quality control samples at each concentration were within 100 ± 15%. At least half of the blank samples with internal standard and half of the blank samples without internal standard, placed immediately before the calibration standards, were free of interference. Internal method on file with ABS Laboratories. The lower limit of quantification is 0.5 ng/mL.

Collection and Analysis of Pouches

Pouches for the determination of nicotine after administration of the IP were collected after 60 ± 1 min. All the pouches were collected and frozen immediately at –20°C. All the pouches were analyzed in Swedish Match laboratories. Used pouches from all evaluable subjects excluding withdrawn or dropout subjects were analyzed. Nicotine was extracted from the snus using sodium hydroxide and methyl-tert-butyl ether containing quinoline as an internal standard. The nicotine present in the extract was determined by using a gas chromatograph equipped with a flame ionization detector.

Subjective Effects

Each subject's rating of "head buzz" using a Visual Analogue Scale (VAS), anchored with "not at all" to "extremely" was obtained before (0) and at 5, 10, 15, 30, and 60 min after administration of each product.

Safety Assessments

AEs and serious adverse events were recorded from start of IP administration until the last follow-up visit. Medical events occurring between screening and first treatment with IP were reported separately as baseline events. AEs were coded using the Medical Dictionary of Regulatory Activities (MedDRA).

Table 1. Part 1: In Vivo Extracted Amount of Nicotine From Each Test Product (n = 17), Mean and (95% CI)

Product	Weight (g)	Nicotine (mg/dose)		Extracted nicotine	
		Unused	Used	mg/dose	% of total
ZYN 3 mg	0.398 [0.387 to 0.406][a]	2.86 [2.75 to 3.03][a]	1.26 (0.98 to 1.53)	1.59[b] (1.32 to 1.86)	55.9[b] (46.3 to 65.5)
ZYN 6 mg	0.404 [0.394 to 0.414][a]	5.89 [5.22 to 6.49][a]	2.42 (1.91 to 2.93)	3.51[b] (2.99 to 4.02)	59.1[b] (50.5 to 67.7)
General snus pouch 8 mg	0.993 [0.970 to 1.046][a]	7.57 [7.25 to 8.08][a]	5.11 (4.76 to 5.46)	2.41 (2.04 to 2.79)	32.0 (27.1 to 36.9)
Nicorette gum 4 mg[c]	0.990 [0.977 to 1.013][a]	3.80 [3.74 to 3.89][a]	1.24 (1.08 to 1.40)	2.56 (2.40 to 2.73)	67.4 (63.2 to 71.7)
E-cigarette[d]	0.169 (0.088 to 0.251)	1.33 (0.87 to 1.79)	0.10 (–0.04 to 0.25)	1.22 (0.80 to 1.66)	93.8 (84.6 to 103)

CI = confidence interval; N.A. = not applicable.

[a]Ranges in brackets are minimum and maximum.

[b]Wilcoxon signed rank sum test compared with General 8 mg, $p < .05$.

[c]Values for Nicorette 4 mg (n = 15) based on data from ref. [3] and were not included in significance testing.

[d]Values for e-cigarettes (n = 13) taken from ref. [10] and were not included in significance testing. Unused and used exposure correspond to the vaporized and exhaled dose, respectively, during 15 puffs.

Statistical Methods

A previous study[3] made the calculation of sample size possible. In vivo nicotine extraction from a 1 g Swedish portion snus containing 8 mg nicotine/pouch was estimated at 2.18 ± 0.92 mg per portion. Under the assumption of a complete dissolution and extraction of the 3 and 6 mg ZYN products, respectively, versus the 2.18 ± 0.92 mg nicotine, and a standard deviation of 5.0 the estimated sample size was 16 with a power of 80% and alpha = 0.05 for part 1, and 32 for part 2.

AUC_{inf} based on plasma concentrations of nicotine after administration of one single dose of the non-tobacco-based nicotine pouch containing 3 and 6 mg of nicotine, and that of single doses of a 1 g Swedish snus pouch 8 mg, was described using summary statistics and nonparametric Wilcoxon signed rank sum test for within-subject difference.

Summary statistics was used to present all continuous variables and frequency tables for categorical variables. A significance level of 5% with two-sided tests was used in all comparisons. The test products were compared with the reference product in all analyses. In addition, pairwise comparisons between the test products were performed.

The extracted dose of nicotine was analyzed using the Wilcoxon signed rank sum test within-subject difference. AUC_{inf}, $AUC_{60\ min}$, AUC_{0-t}, C_{max}, T_{max}, and terminal half-life of nicotine after use of the ZYN pouch versus the General snus pouch (part 1) were analyzed using Wilcoxon signed rank sum test for within-subject difference. The same statistical methods were applied for the comparisons with Longhorn moist snuff (part 2). Heart rate and VAS scales for "head buzz" were analyzed using the Wilcoxon signed rank sum test for within-subject difference.

All AE data were fully listed by Investigator terms and MedDRA Preferred Term (PT). AE data were summarized by System Organ Class (SOC) and PT.

Results

Part 1

Less nicotine was extracted from ZYN 3 mg (1.5 mg, 95% confidence interval [CI]: 1.3–1.8 mg, p = .002) and more from ZYN 6 mg (3.5 mg, 95% CI: 3.0–4.0 mg, p = .002) than from 8 mg General (2.4 mg, 95% CI: 2.0–2.8 mg). The extracted fractions of nicotine for both ZYN products (56% and 59%, 95% CI: 46%–65% and 50%–68%) were significantly larger than for 8 mg General (32%, 95% CI: 27%–37%). See Table 1 for a summary of nicotine exposure for the different products.

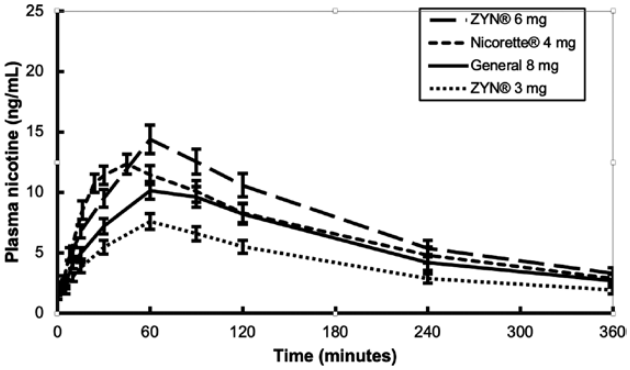

Figure 1. Part 1. Diagram showing mean plasma concentrations (ng/mL) versus time (min). The upper curve shows ZYN 6 mg nicotine per pouch. The middle curves show General snus 8 mg and Nicorette 4 mg gum from ref. [3]. The lower curve shows ZYN 3 mg nicotine per pouch. Error bars correspond to 95% CIs. CI = confidence interval.

The plasma concentrations from the ZYN product containing 3 mg nicotine were lower than those of 8 mg General snus and the plasma concentrations from ZYN containing 6 mg were higher than the 8 mg General snus at almost all time points, see Figure 1. See Table 2 for PK parameters for the different products. For comparison, literature data for 4 mg Nicorette gum[3] and mean of 13 brands of e-cigarettes[10] are included in Tables 1 and 2.

The C_{max} of ZYN 3 mg was 25% lower (95% CI: 14%–37%, p < .001) than that of 8 mg General snus, whereas the C_{max} of ZYN 6 mg was 42% higher (95% CI: 24%–60%, p < .001) than that of 8 mg General. The AUC_{inf} of ZYN 3 mg was 27% smaller than that of 8 mg General (95% CI: 19%–36%, p < .001) and the AUC_{inf} of ZYN 6 mg was 34% larger than that of 8 mg General (95% CI: 16%–52%, p = .0056).

For the secondary PK parameters, there were statistically significant differences in AUC_{0-t}, $AUC_{60\ min}$, and C_{max}, ZYN 3 mg being lower and ZYN 6 mg higher than 8 mg General snus. No statistically significant differences were seen in terms of terminal half-life and T_{max}.

The maximum increase in pulse rate was 2.7 beats per min larger (95% CI: 0.2–5.2 beats per min, p = .027) for the ZYN 6 mg product than for the ZYN 3 mg product, whereas neither of the ZYN products differed significantly from 8 mg General snus. The maximum increase in head buzz for 8 mg General snus was larger than for ZYN 3 mg; the median difference was 7 mm (interquartile range: 0–27.5 mm, p = .033), see Table 5.

Table 2. Part 1: Mean and 95% CI Pharmacokinetics Results of Each Test Product (n = 17)

	AUC_{inf} (ng/mL h)		C_{max} (ng/mL)		T_{max} (min)		$T_{½}$ (min)	
Product	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
ZYN 3 mg	32.0[a]	23.3–40.7	7.7[a]	6.3–9.0	61	56–66	152	126–178
ZYN 6 mg	57.7[a]	43.9–71.6	14.7[a]	12.3–17.1	66	59–72	140	120–160
General snus 8 mg	45.9	29.8–62.1	10.6	8.9–12.3	69	60–78	144	119–168
Nicorette 4 mg[b]	52.5	40.0–65.1	12.8	11.1–14.5	46	37–55	171	152–190
E-cigarettes[c]	14.1	8.8–19.3	8.4	5.4–11.5	5.1	0.5–9.7	106	84–129

CI = confidence interval.
[a]Wilcoxon signed rank sum test compared with General snus 8 mg, $p < .05$.
[b]Values for Nicorette 4 mg (n = 15) based on data from ref. [3] and were not included in significance testing.
[c]Values for e-cigarettes (n = 13) taken from ref. [10] and were not included in significance testing.

Table 3. Part 2: In Vivo Extracted Amount of Nicotine From Each Test Product (n = 30), Mean and (95% CI)

		Nicotine (mg/dose)		Extracted nicotine	
Product	Weight (g)	Unused	Used	mg/dose	% of total
ZYN 8 mg	0.530 [0.511–0.544][a]	7.52 [6.95–7.83][a]	3.74 (3.23–4.25)	3.79 (3.29–4.29)	50.4 (43.6–57.1)
General 2 × 8 mg (16 mg)	2.009 [1.909–2.081][a]	15.43 [15.03–16.04][a]	10.44 (10.05–10.83)	5.04[b] (4.66–5.42)	32.6[b] (30.1–35.0)
Longhorn Natural 18 mg	1.507 [1.449–1.549][a]	15.62 [14.37–16.87][a]	12.75 (11.84–13.66)	2.99[b] (2.06–3.93)	18.9[b] (13.1–24.8)

CI = confidence interval.
[a]Ranges in brackets are minimum–maximum.
[b]Wilcoxon signed rank sum test compared with ZYN 8 mg, $p < .05$.

Part 2

The extracted amount of nicotine from ZYN 8 mg (3.8 mg, 95% CI: 3.3–4.3 mg) was larger (p = .0042) than the amount extracted from Longhorn Natural 18 mg (3.0 mg, 95% CI: 2.1–3.9), but smaller (p < .001) than the extracted amount of nicotine from General 2 × 8 mg snus pouches (5.0 mg, 95% CI: 4.7–5.4 mg), see Table 3.

The extracted fraction of nicotine from ZYN 8 mg (50%, 95% CI: 44–57%) was significantly larger than the fraction extracted nicotine from General snus 2 × 8 mg (33%, 95% CI: 30%–35%, p < .001) and Longhorn Natural 18 mg (19%, 95% CI: 13–25, p < .001), see Table 3. In addition, the extracted fraction of nicotine of General snus 2 × 8 mg was significantly higher than the extracted fraction nicotine of Longhorn Natural 18 mg (p < .001).

The plasma concentration versus time curves were similar for all test products with the highest concentrations of nicotine observed at 1 h after start of product administration, in association with removal of the test product. Nicotine plasma concentrations are summarized in Figure 2.

There were no statistically significant differences in mean AUC_{inf}, AUC_{0-t}, mean C_{max}, or extracted amount of nicotine between ZYN 8 mg and Longhorn Natural 18 mg, see Table 4. ZYN 8 mg gave 17% lower AUC_{inf} (95% CI: 9%–25%, p < .001) and 12% lower C_{max} (95% CI: 3%–22%, p = .0084) than General snus 2 × 8 mg. For all products but General snus 2 × 8 mg, there was a strong correlation between the AUC_{inf} and the extracted amount of nicotine (not shown).

The minor differences in T_{max} or terminal half-life between the different products were not statistically significant.

Safety

Administration of single doses of nicotine-containing pouches was safe and well tolerated by the healthy subjects in this study. A total of 16 AEs were reported by eight subjects during the study and two AEs (dry mouth) were judged to have a possible or probable relationship to treatment. There were no serious adverse events or discontinuations due to AEs during the study.

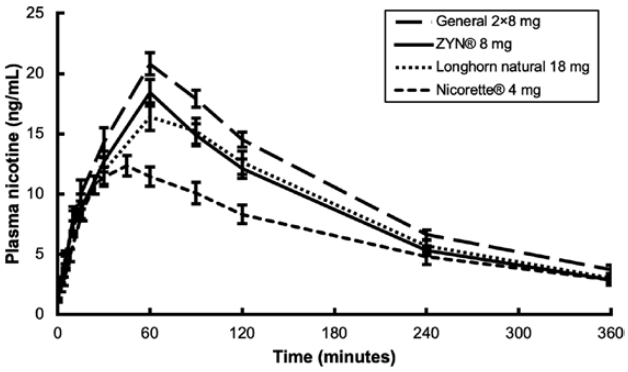

Figure 2. Part 2. Diagram showing mean plasma concentrations (ng/mL) versus time (min). The upper curve shows General snus 2 × 8 mg nicotine per pouch. The middle curves show ZYN 8 mg nicotine per pouch and Longhorn Natural 18 mg per pouch. The lower curve shows 4 mg Nicorette gum from ref. [3]. Error bars correspond to 95% CIs. CI = confidence interval.

Discussion

These open, randomized, crossover studies compared the PK and subjective effects of nicotine following a single dose of a non-tobacco-based nicotine pouch (ZYN) containing either 3, 6, or 8 mg nicotine and single or double doses of conventional, tobacco-based Swedish General 8 mg snus and Longhorn Natural 18 mg, respectively. We also looked at the in vivo extraction of nicotine to explain differences in AUC_{inf} and C_{max}.

Table 4. Part 2: Mean and 95% CI Pharmacokinetics Results of Each Test Product ($n = 29$)

	AUC_{inf} (ng/mL h)		C_{max} (ng/mL)		T_{max} (min)		$T_{½}$ (min)	
Product	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
ZYN 8 mg	58.4	50.7–66.1	18.5	16.1–20.8	59	55–62	109	103–115
General snus 8 × 2 mg	70.3[a]	63.9–76.6	21.2[a]	19.0–23.4	63	58–68	114	107–121
Longhorn Natural 18 mg	60.6	50.9–70.2	16.9	14.4–19.5	65	58–72	115	108–122

CI = confidence interval.
[a]Wilcoxon signed rank sum test compared with ZYN 8 mg, $p < .05$.

Table 5. Part 1: Maximum "Head Buzz" and Maximum Change in Heart Rate

	Maximum "head buzz" VAS (mm)		Maximum change in heart rate (bpm)	
Product	Median (Q1; Q3)	Range	Median (Q1; Q3)	Range
ZYN 3 mg	9 (4; 19)	0–59	8.5 (5.5; 14.5)	4.0–18.0
ZYN 6 mg	11 (5; 26)	0–63	10.5[a] (9.5; 16.5)	4.5–22.5
General snus 8 mg	24[a] (12; 47)	0–62	11.0 (4.0; 15.0)	0.0–22.0

VAS = Visual Analogue Scale.
[a]Wilcoxon signed rank sum test compared with ZYN 3 mg, $p < .05$. All other comparisons had $p > .05$.

Part 1

The in vivo extracted amount of nicotine from ZYN 3 mg was significantly lower than from 8 mg General snus and from ZYN 6 mg significantly higher. This was an unexpected finding. Rather, it was expected that the extraction and absorption of nicotine would correlate with the amount of nicotine in each product. However, these findings may be explained by differences in the extracted fraction of nicotine. Thus, the extracted fraction nicotine for both ZYN products was significantly larger (56%–59%) compared with the 32% of the reference General snus 8 mg. As expected, there was a statistically significant difference in the extracted amount of nicotine between the ZYN 6 mg and ZYN 3 mg. Retrospectively comparing a 6-mg gum formulation to ZYN 6 mg, the gum was seen to release more nicotine, 4.9 versus 3.5 mg, which corresponds to extracted fractions of 82% and 59%, respectively.[6] Despite the higher nicotine delivery of the 6 mg gum compared with the 4 mg version the effect on craving for cigarettes was small.[11]

In line with the extraction data, ZYN 6 mg resulted in significantly higher nicotine AUC_{inf}, compared with the General portion snus 1.0 g (8 mg nicotine). As for the extraction of nicotine, systemic absorption, AUC_{inf}, did not correlate with the amount of nicotine in each product. Hence, the AUC_{inf} was lower for the ZYN 3 mg pouches and higher for the ZYN 6 mg pouches compared with that from the General 8 mg pouch. In general, corresponding results to those obtained for AUC_{inf} and extracted amount of nicotine were obtained also for AUC_{0-t}, $AUC_{60\ min}$, and C_{max}. There were, however, no statistically significant differences between the ZYN products and the General portion snus 1.0 g (8 mg nicotine/g), in terms of terminal half-life and T_{max}.

Despite significant differences between each of the ZYN products and the reference product General snus for all primary and secondary PK endpoints.

The extraction and plasma data were supplemented with assessments of subjective effects of "head buzz" and heart rate measurements, shown in Table 5, both of which constitute proxies for systemic nicotine uptake. Interestingly, the maximum VAS score for "head buzz" was higher following intake of General 8 mg than after intake of any of the ZYN products (3 and 6 mg) despite a higher AUC_{inf} following ZYN 6 mg, ie, there was no obvious correlation between nicotine levels and head buzz (although for ZYN 6 mg versus General 8 mg the difference was not statistically significant). Potentially, another component of conventional snus, eg, tobacco, could have contributed to the feeling of head buzz. The subjective effects could also have been due to classical conditioning to the long-term use of a specific product, in this case General snus, or in tobacco-based snus in general.

Part 2

The extracted amount of nicotine from General snus 2 × 8 mg was significantly larger than from the other products. Corresponding results were observed for mean AUC_{0-t} and mean C_{max}. The mean AUC_{inf} of General snus 2 × 8 mg was significantly higher than the mean AUC_{inf} of the other products. The extracted amount of nicotine from ZYN Smooth 8 mg was significantly larger than from Longhorn Natural.

As is seen in Figure 2, all plasma nicotine curves except General snus 2 × 8 mg are very similar despite very different nicotine contents in the unused products. The reason is differences in the fraction of nicotine that is extracted upon use. Thus, the extracted fraction of nicotine for ZYN 8 mg (50%) was significantly larger than Longhorn Natural 18 mg (19%; $p < .001$) as well as General suns 2 × 8 mg (33%; $p < .001$), see Table 3.

For all products but General snus 2 × 8 mg, there was a strong correlation between the AUC_{inf} and the extracted amount of nicotine. Both AUC_{inf} and C_{max} for General snus 2 × 8 mg were significantly higher than for ZYN 8 mg and Longhorn Natural 18 mg, respectively. See Table 4. For baseline-adjusted data, the T_{max} for ZYN 8 mg (59 min) was significantly shorter than for General snus 2 × 8 mg (64 min, $p = .042$).

In general, corresponding results to those obtained for AUC_{inf} and extracted amount of nicotine were obtained also for AUC_{0-t}, $AUC_{60\ min}$, and C_{max}. There were, however, no statistically significant differences between the products in terms of T_{max}. The nicotine gum and e-cigarette data given here for comparative reasons only are from other studies and thus need to be interpreted with caution. It seems though that the extracted amount of nicotine from 4 mg gum is higher than from ZYN 3 mg but lower than from the ZYN 6 and

8 mg doses. The same seems to be true for C_{max}, and the 4 mg gum peaks faster, ie, has a shorter T_{max}, although this could be explained by the shorter administration time (30 min). E-cigarettes clearly have a shorter T_{max}, but not necessarily a higher C_{max}.

Limitations

First the results can only be applied to snus users. The nicotine absorption may be different in smokers not used to nicotine pouches. Secondly, this study has two parts with different subjects and making comparisons across studies should be made with caution. Thirdly, in part 1 the number of subjects was relatively small.

Conclusions

Despite a lower nicotine content, the non-tobacco-based ZYN 6 mg product gave rise to a significantly larger uptake of nicotine to the systemic blood circulation, measured as AUC_{inf} than did the conventional, tobacco-based 8 mg General snus.

Conventional, tobacco-based General snus (8 mg) gave rise to significantly larger nicotine extraction and subsequent uptake in the systemic blood circulation than did the non-tobacco-based ZYN 3 mg product. ZYN 8 mg delivers similar amounts of nicotine as the Longhorn Natural moist snuff (18 mg), but significantly less than two pouches of General snus (2 × 8 mg).

In summary, the two higher doses of ZYN (6 and 8 mg) deliver nicotine as quickly and to a similar concentration compared with existing smokeless products, with no significant adverse effects. This suggests their efficacy in reducing withdrawal symptoms and helping smokers reduce or stop combustible tobacco use should be similar to that for existing smokeless products.

Supplementary Material

A Contributorship Form detailing each author's specific involvement with this content, as well as any supplementary data, are available online at https://academic.oup.com/ntr.

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Declaration of Interests

Dr Lunell has received grants from Swedish Match for a number of clinical trials on nicotine and tobacco products. He has not received any consulting fees from Swedish Match or any other tobacco company. Dr Pendrill is a Research Scientist at Swedish Match. Dr Fagerström has received consulting fees from many companies that develop or market pharmaceutical and behavioral treatments for smoking cessation. He currently receives consulting fees from Swedish Match and has received fees in the past from BAT and PMI to assist their development of less-risky tobacco products. Dr Hughes has received consulting and speaking fees from several companies that develop or market pharmacological and behavioral treatments for smoking cessation or harm reduction and from several nonprofit organizations that promote tobacco control. He currently receives consulting fees from the pharmaceutical company Achieve. He also receives fees from the tobacco companies of Swedish Match, Altria, and Philip Morris International to assist their efforts to develop less-risky tobacco products.

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