J Oral Pathol Med 1995; 24: 244–250
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JOURNAL OF
Oral Pathology&Medicine
ISSN 0904-2512

Reduction in nicotine intake and oral mucosal changes among users of Swedish oral moist snuff after switching to a low-nicotine product

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Andersson G, Axéll T, Curvall M: Reduction of nicotine intake and oral mucosal changes among users of Swedish oral moist snuff after switching to a low-nicotine product. J Oral Pathol Med 1995; 24: 244–250. © Munksgaard, 1995.

The purpose of this investigation was to assess nicotine regulation among users of portion-bag Swedish oral moist snuff (*snus*) when switching from an ordinary *snus* product (Brand A) to a low-nicotine product having only half the concentration of nicotine (Brand B). Two studies were performed to compare the short-term effects on consumption and nicotine intake of switching to low-nicotine *snus* with those of long-term effects. In Study 1, consumption data, soft tissue changes and nicotine intake were measured in a group of 24 habitual users of Swedish portion-bag *snus*, both during use of their ordinary *snus* (Brand A) for 2 weeks and during consumption of the low-nicotine product (Brand B) for 10 weeks. In study 2, the same data were measured during 2 weeks in a reference group of 18 *snus* users who had been habitual users of the low-nicotine *snus* (Brand B) for at least one year. Although there was no increase in number of hours of daily consumption, the amount of *snus* consumed increased on average by 2 grams a day (+15%) when switching from Brand A to the low-nicotine Brand B (Study 1). The Brand B reference group (Study 2) consumed about 3 grams less *snus* a day during the same number of hours as the subjects in Study 1 who had switched to Brand B. These results indicate that *snus* users compensate to a small extent for the lower nicotine delivery by increasing their consumption on short-term switching but the same does not apply to long-term users. There was a significant reduction in nicotine intake when switching to the low-nicotine brand. The individual average saliva cotinine levels decreased from 336 ng/ml to 153 ng/ml and total amounts of nicotine equivalents excreted during 24 h decreased from 25.2 mg to 14.4 mg (Study 1), reaching about the same levels found in the reference group in Study 2 (159 ng/ml and 14.3 mg, respectively). After switching from Brand A to Brand B in Study 1, there was a decrease in the frequency of Degree 3 clinical lesions and an increase in Degree 2 lesions. Also, Degree 3 lesions were less frequent, and Degree 1 lesions more frequent, in Study 2 than in Study 1. These observations point to the development of less pronounced changes after both long-term and short-term switching to a low-nicotine *snus*.

Key words: nicotine; oral moist snuff; oral mucosal changes; smokeless tobacco

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Accepted for publication November 30, 1994.

In Sweden, the use of oral moist snuff, *snus*, is a widespread habit. In 1993, about 10% of the population over 15 years of age were users of *snus* and 23% were cigarette smokers. Two types of *snus*, loose and portion-bag packed *snus*, are produced in Sweden. Of the Swedish *snus* users, mainly males, 73% consume only loose *snus*, 13% only portion-bag packed *snus* and 14% are mixed users. The most common way to use *snus* in Sweden is to deposit 1–2 g loose *snus* or a pouch of portion-bag *snus* (1 g) in the vestibular area inside the upper lip.

Previous studies have shown that soft tissue changes of the oral mucosa and the gingival margin are less pronounced

among those who use portion-bag packed *snus* than among those who use loose *snus* (1). No correlations were recorded between clinical severity of oral mucosal changes and either the amounts of nicotine or tobacco-specific nitrosamines extracted from the tobacco or the biological markers for uptake of tobacco constituents, i.e. the total amount of nicotine and metabolites excreted during 24 h and steady-state saliva cotinine concentrations (2). In these studies it was assumed that the difference in tissue response between portion-bag *snus* users and loose *snus* users is partly due to the pH differences of the two types of products, i.e. the higher the pH of *snus*, the more severe are the changes in the mucosa.

However, besides pH, there are several properties of *snus* that can affect the oral mucosa, such as the concentration of nicotine and other tobacco constituents, particle size, humidity, molality etc. The effects of some of these constituents have been evaluated in experimental model studies (3), but not in clinical trials on human beings. The tendency for smokers to compensate for the reduction in tar and nicotine yields by smoking more cigarettes or by increasing the intensity of puffing and inhalation have been studied both in experimental and natural switching studies (4–8). At present the results are somewhat conflicting. Whether *snus* users compensate or not when switching from normal to low-nicotine *snus* has not been investigated.

The aim of the present investigation was to evaluate both short- and long-term effects of switching to a *snus* with reduced nicotine concentration by observing changes in consumption, nicotine intake and the oral mucosa.

Subjects and methods

In Study 1, consumption data, soft tissue changes and nicotine intake were measured in a group of habitual users of Swedish portion-bag *snus*, both during use of their ordinary *snus* (Brand A) and after switching to a low-nicotine portion-bag *snus* (Brand B).

In Study 2, the same data were measured in a group of habitual *snus* users who had consumed the low-nicotine portion-bag *snus* (Brand B) for at least one year.

Moist snuff

Both types of portion-bag *snus* used in this investigation were brands on the Swedish market and manufactured from ground tobacco using a heat-treatment process. Brand A, which is a regular type of Swedish *snus*, contains 0.8–0.9% nicotine, while Brand B is a low-nicotine product which has about half the concentration of nicotine, 0.4–0.5%. The portion bags of the two brands are of the same size and weight, have the same taste and contain the same amount of water (about 50%). The pH is initially in the range 8.2–8.5 in Brand A and in the range 7.8–8.2 for Brand B. On storage, the pH of Brand A tends to decrease, while the pH of Brand B tends to increase. Accordingly, the pH difference between the two products at use is small, if any.

Subjects

Study 1 – The subjects selected for this study were 24 habitual users of Brand A. Their mean age was 37 (s=10.7) years. They were selected from a population of 68 *snus* users (mean age 36.9 (s=9.9) yrs recruited for a previous study (1).

Study 2 – Eighteen users of Brand B made up a reference group. These subjects were of mean age 36.6 (s=10.1) yrs.

Subjects had no other tobacco habit and reported daily consumption of Brand A in Study 1 and Brand B in Study 2 for at least one year.

All participants were healthy volunteers. They were given written information on the purpose of the studies before giving their written consent to participate. The studies were reviewed and approved by the institutional ethics committee at Lund University.

Study procedures

Before the start of either study the participants visited a dental clinic where age, number of years with regular *snus* habit and daily consumption (hrs/day and g/day) were recorded. A thorough clinical examination of the oral mucosa was carried out. Sites where the portion-bag was placed were recorded.

Study 1 – The study period covered 12 weeks. During weeks 1 and 2 the participants continued to use their ordinary *snus* (Brand A), *ad libitum*. At the start of week 3, they switched to the low-nicotine *snus* (Brand B) and continued to use it for another 10 weeks, *ad libitum*. Each participant recorded the daily consumption (h/day and g/day) for each day during weeks 1, 2 ,4, 8 and 12. At the end of the same weeks, they visited the dental clinic for clinical examination of the oral mucosa, collection of saliva and urine samples and collection of consumption protocols.

Study 2 – The study period included 2 weeks during which the participants continued to consume their ordinary low-nicotine *snus* (Brand B), *ad libitum*. Each participant recorded the daily consumption (h/day and g/day) during the whole study period. At the end of the weeks 1 and 2, the subjects visited the dental clinic for clinical examination of the oral mucosa, collection of saliva and urine samples and collection of consumption protocols.

Clinical examination

At each visit to the dental clinic, a thorough examination was carried out and lesions in the oral mucosa were recorded. Changes at the site(s) where the *snus* was regularly placed, "snuff dipper's lesion", were registered according to the degree of clinical severity on a four-point scale suggested by AXELL *et al.* (9). This scale is based on clinical criteria including wrinkling, thickening and colour changes of the oral mucosa.

Sample collection

On Day 6 of Weeks 1, 2, 4, 8 and 12 in Study 1 and of Weeks 1 and 2 in Study 2, urine samples were collected for 24 h. The volume and pH of the urine voids were measured and then 10 ml aliquots were transferred into glass tubes.

On Day 7 of the same weeks, a saliva sample was gathered in the following way: 30 min after the intake of one portion-bag of *snus*, the snus was removed. After another 30 min the mouth was rinsed with water and a 2 ml sample of resting whole saliva was collected directly into a glass tube. The saliva and urine samples were kept frozen (−18°C) until analyzed.

Analysis of nicotine and metabolites

Urine samples from the *snus* users were divided into five portions. Portion one was analyzed for nicotine and cotinine, portion two for glucuronic acid conjugates of nicotine and cotinine, portion three for *trans*-3′-hydroxycotinine, portion four for glucuronic acid conjugate of *trans*-3′-hydroxycotinine and portion five for nicotine-N′-(1)-oxide and cotinine-N-(1)-oxide. Saliva samples were analyzed for concentrations of cotinine.

Nicotine and cotinine – The concentrations of cotinine in saliva and of nicotine and cotinine in urine were determined by modified methods according to CURVALL *et al.* (10) and FEYERABEND *et al.* (11). Appropriate amounts of the internal standards, N′-methylanabasine for nicotine and N′-ethylnorcotinine for cotinine were added to samples of urine (0.5 ml) and the internal standard for cotinine was added to the samples of saliva (0.5 ml). The samples were extracted with dichloromethane (1 ml) under basic conditions (0.5 ml 5 M NaOH) and the organic layer was separated and evaporated. Quantitative analysis was performed on a gas chromatograph (Varian Model 3700, Walnut Creek, California) equipped with an all glass capillary split/splitless injector, a fused silica capillary column coated with free fatty acid phase (FFAP, Hewlett-Packard Ltd, Avondale, Pensylvania), a thermionic specific detector (TSD, Varian) a Varian Model 8200 autosampler and a Shimatzu Model C-R3A (Tokyo, Japan) reporting integrator. The average coefficient of variation for nicotine and cotinine over the range 10 to 500 ng/ml was 3.4% and 3.6%, respectively.

Nicotine and cotinine glucuronides – The total amounts of nicotine and cotinine, i.e. both free and conjugated alkaloids, were determined after enzymatic hydrolysis of urine samples. To 0.5 ml of urine, which was diluted with an equal amount of sodium acetate buffer (0.05 M, pH 4.7), was added β-glucuronidase from *Helix Pomatia* (6000 U, EC 3.2.1.31, Type HP-2, Sigma Chemical Co, USA) dissolved in sodium acetate buffer (0.2 ml). After incubation at 37°C for 4 h, the reaction mixture was analyzed for nicotine and cotinine using the same analytical procedure as described above. The concentrations of glucuronic acid conjugates of nicotine and cotinine were calculated from these data (12).

Trans-3′-hydroxycotinine – Appropriate amounts of the internal standard N′-ethylnorcotinine were added to samples of urine (0.5 ml). The samples were extracted with dichloromethane (2 ml) under alkaline conditions (3 ml K_2CO_3) for 30 min. The organic layer was separated and evaporated. The quantitative analysis was performed by high resolution gas chromatography as described under the section "Nicotine and cotinine" above. The average coefficient of variation over the *trans*-3′-hydroxycotinine range 0.5 to 16 μg/ml was 4.7%.

Trans-3′-hydroxycotinine glucuronide – The total concentration of *trans*-3′-hydroxycotinine was determined after enzymatic hydrolysis of urine samples. To 0.5 ml of urine, which was diluted with an equal amount of sodium acetate buffer (0.05 M, pH 4.7), was added β-glucuronidase from *Helix Pomatia* (6000 U) dissolved in sodium acetate buffer (0.2 ml). After incubation at 37°C for 24 h, the reaction mixture was analyzed for the total concentration of *trans*-3′-hydroxycotinine and the concentration of glucuronic acid conjugate was calculated from these data (12).

Nicotine-N′-oxide and cotinine-N-oxide – The reduction reagent, titanium chloride (200 mg/ml of conc. HCl, 0.2 ml), was added to samples of urine (0.5 ml). After reaction at room temperature for 2 h, the mixture was alkalized with sodium hydroxide (2 ml 5 M) and then analyzed using the procedure described for analysis of nicotine and cotinine.

Statistical calculations

Means and population standard deviations (s) were calculated for all parameters. This investigation was planned as a repeated measures design. Repeated measures analysis of variance was used estimating effects on consumption, exposure and uptake data. For assessing effects on lesions, repeated measures analysis of a general logits model and logistic regression were used (13).

Results

Snus habit data

Study 1 – The 24 subjects who took part in this study had used *snus* of Brand A regularly for 6.7 (s=4.2) yrs. Of 21 participants who reported previous tobacco use, 15 had used *snus* of brands with similar pH and nicotine content as Brand A for 9.2 (s=7.5) yrs and 6 had smoked cigarettes for 13.8 (s=8.3) yrs. During consumption of Brand A in Weeks 1 and 2, the subjects kept the *snus* in the mouth for 14.1 (s=4.0) h/day. Twenty-two subjects reported the same daily exposure time during Weeks 3–12 with use of Brand B as they reported during use of Brand A in Weeks 1 and 2; two subjects increased their daily exposure by 2 and 4 h, respectively, during use of Brand B.

Study 2 – The 18 subjects who took part in this study had used *snus* of Brand B for 2.8 (s=1.2) yrs. All subjects reported previous tobacco use. Fourteen had used *snus* of other brands with similar pH and nicotine concentration as Brand A for 8.6 (s=5.1) yrs, while 4 had smoked cigarettes for 21.8 (s=12.6) yrs. During Weeks 1 and 2 of this study, the daily exposure time was 13.1 (s=3.0) h/day.

Consumption

As is illustrated in Fig. 1, there were great inter-individual variations in daily

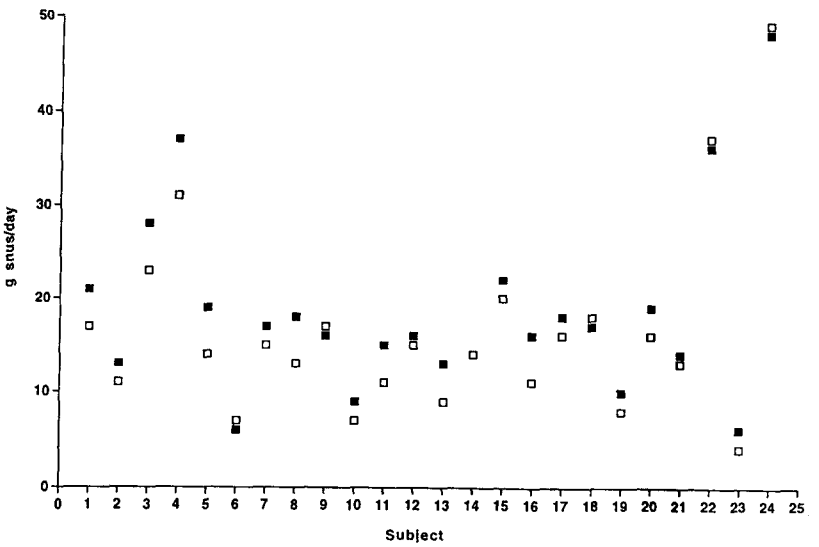

Fig. 1. Study 1. Individual average daily intake of *snus* during use of Brand A in weeks 1 and 2 (open squares) and during use of Brand B in weeks 4, 8 and 12 (closed squares).

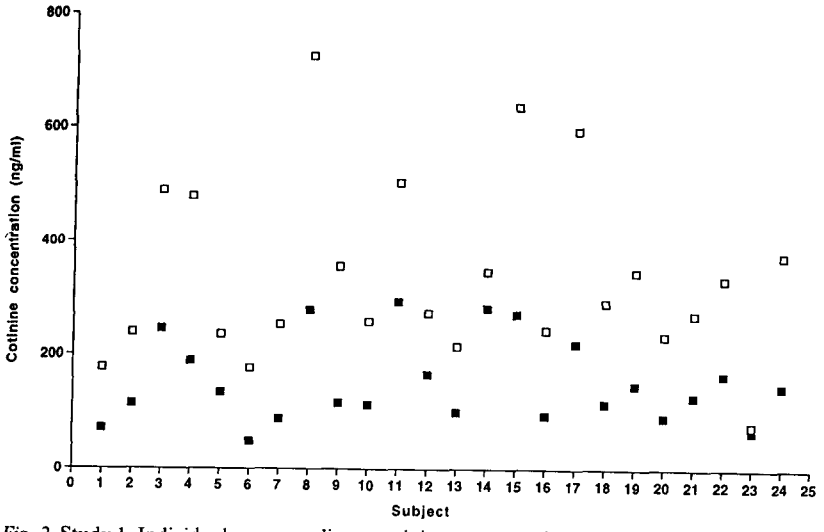

Fig. 2. Study 1. Individual average salivary cotinine concentration (ng/ml) during use of Brand A in weeks 1 and 2 (open squares) and during use of Brand B in weeks 4, 8 and 12 (closed squares).

amounts of *snus* intake. In Study 1, the individual average daily consumption ranged from 4 g/day to 48 g/day both during consumption of the usual brand (Brand A) and after switching to the low-nicotine brand (Brand B). In Study 2, the individual average daily consumption ranged from 6 to 48 g/day. Although there was a considerable difference in consumption between subjects, there was almost no variation over time within subjects when consuming the same brand.

There was a slight but significant increase in daily amount of *snus* intake in Study 1 when switching from Brand A to Brand B (+2.5 g/day; $p<0.001$). The average *snus* intake of 16.2 (s=10.2) and 16.4 (s=9.9) g/day during Weeks 1 and 2 in Study 1 increased to 18.8 (s=10.0), 18.7 (s=10.6) and 18.6 (s=9.4) g/day during Weeks 4, 8 and 12. The reference group in Study 2 had an average daily intake of 15.0 (s=9.5) and 15.2 (s=9.8) g/day during Weeks 1 and 2, respectively.

Exposure and uptake data

At steady-state, salivary cotinine concentrations correlate well with the nicotine intake during tobacco consumption (14–16). In Study 1, the individual average salivary cotinine concentration ranged from 70.4 to 731 ng/ml during consumption of the usual brand (Brand A) and from 31 to 335 ng/ml after switching to Brand B (Fig. 2). In Study 2, the individual average salivary cotinine concentrations ranged from 57 to 442 ng/ml during Weeks 1 and 2.

The average salivary cotinine concentrations during the two weeks of using the usual brand (Brand A) were similar, 321 (s=162) ng/ml and 352 (s=155) ng/ml, respectively. After switching to Brand B, the average salivary cotinine concentrations fell to about half the concentration, i.e. 157 (s=117), 152 (s=64) and 150 (s=68) ng/ml for Weeks 4, 8 and 12, respectively. The average salivary cotinine concentrations in the reference group (Study 2) during Weeks 1 and 2 were 162 (s=92) and 155 (s=84) ng/ml, respectively. The reduction on switching to the low-nicotine brand (Brand B) in Study 1 was statistically significant ($p<0.001$).

Nicotine intake was estimated as the total amount of nicotine and metabolites excreted during 24 h expressed as nicotine equivalents. The individual values ranged during the two weeks of using the usual brand (Brand A) from 4 to 65 mg, while the individual values during Weeks 4, 8 and 12 ranged from 2 to 41 mg (Fig. 3), which were similar to the values obtained in Study 2 (3 to 37 mg). The individual average nicotine dose during usage of the normal nicotine brand (Brand A) in Weeks 1 and 2 in Study 1 were 26.4 (s=13.3) and 23.9 (s=11.3) mg, respectively; this decreased after switching to the low-nicotine brand (Brand B) to 12.5 (s=6.5), 15.8 (s=8.9) and 14.9 (s=9.1) mg, respectively, during Weeks 4, 8 and 12. The corresponding values for the reference group in Study 2 were 15.2 (s=8.9) and 13.5 (s=7.1) mg during Weeks 1 and 2. The decrease in daily nicotine intake after switching to the low-nicotine *snus* was statistically significant ($p<0.001$).

Clinical observations

The pattern of *snus*-induced oral mucosal lesions related to study periods is shown by clinical severity in Table 1.

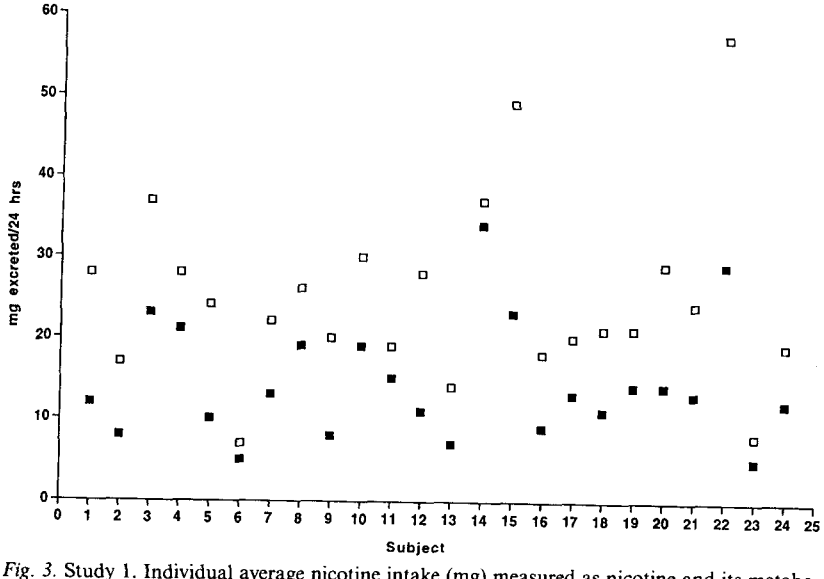

Fig. 3. Study 1. Individual average nicotine intake (mg) measured as nicotine and its metabolites excreted during 24 h expressed as nicotine equivalents. Average nicotine intake during weeks 1 and 2 (open squares) and during weeks 4, 8 and 12 (closed squares).

Table 1. Cross tabulation of frequencies of clinical grading versus study period in Study 1 and 2.

	Study period	Degree 0 n (%)	Degree 1 n (%)	Degree 2 n (%)	Degree 3 n (%)
Study 1	Week 1	0 (0)	4 (17)	11 (46)	9 (37)
	Week 2	0 (0)	4 (17)	11 (46)	9 (37)
	Week 4	0 (0)	5 (21)	17 (71)	2 (8)
	Week 8	0 (0)	5 (21)	14 (58)	5 (21)
	Week 12	1 (4)	4 (17)	18 (75)	1 (4)
Study 2	Week 1	0 (0)	6 (33)	8 (45)	4 (22)
	Week 2	0 (0)	5 (28)	10 (55)	3 (17)

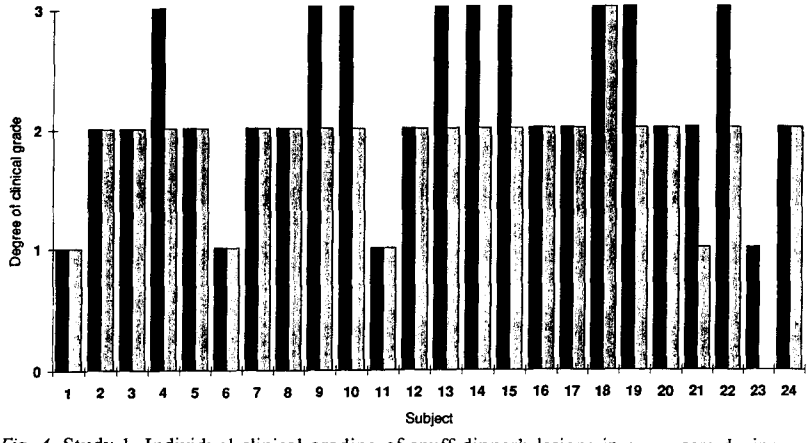

Fig. 4. Study 1. Individual clinical grading of snuff dipper's lesions in *snus* users during use of Brand A in week 2 (dark grey bars) and during use of Brand B in week 12 (light grey bars).

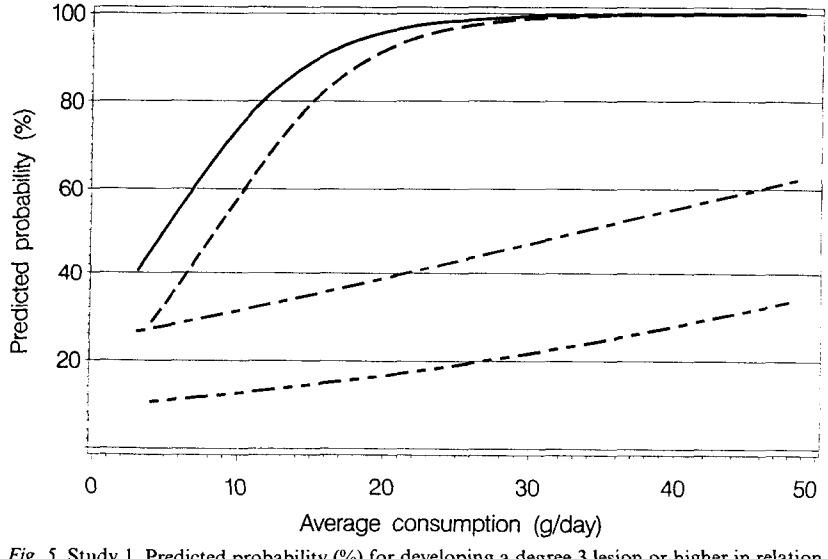

Fig. 5. Study 1. Predicted probability (%) for developing a degree 3 lesion or higher in relation to average daily consumption (g/day) during use of Brand A (- - -) and Brand B (- - - - -). Predicted probability (%) for developing a degree 2 lesion or higher in relation to consumption (g/day) during use of Brand A (—) and Brand B (– –).

It is obvious from these data that Degree 2 is the most frequently observed type of lesion at all occasions both in Studies 1 and 2. However, compared to the distribution of degrees of clinical severity during Weeks 1 and 2 in Study 1, there is a decrease in Degree 3 lesions and accordingly an increased number of Degree 2 lesions during Weeks 4 to 12. On comparison of the distribution of degrees of clinical severity during the first two weeks in Study 1 with the distribution during the first two weeks of Study 2, it is obvious that the Degree 3 lesions are less frequent, while Degree 1 lesions are more frequent in Study 2 than in Study 1. These observations indicate the development of less pronounced changes after switching to Brand B, which is further illustrated in Fig. 4. Ten subjects in Study 1 exhibited a reduction of one index point in the oral mucosal index between Weeks 2 and 12. Fig. 5 illustrates the differences in predicting the probability of developing a Degree 2 (or higher) lesion when consuming Brand A or Brand B. This difference is obvious when the daily consumption is 20 g or less, but is not found at higher consumption levels. It is also demonstrated in Fig. 5 that the probability of inducing a Degree 3 lesion is about three times as large when consuming Brand A as when consuming Brand B; this occurred at all consumption levels in Study 1.

To some extent the degree of oral mucosal changes seems to be covariate with the concentration of salivary cotinine levels ($r=0.2605$; $p<0.01$) and the nicotine dose ($r=0.3848$; $p<0.001$).

Discussion

Subjects and consumption

The subjects in Study 1 were about the same age as the participants in a previous study (1) from which they were recruited. Further, the mean age was almost the same for the participants in Study 2. The number of years with regular use of Brand A in Study 1 was significantly higher than for Brand B in Study 2. However, the number of years with a regular tobacco habit in the two groups of *snus* users was almost the same. This is also valid for the daily exposure time.

Average consumption data were increased concerning h/day and g/day compared to previous studies (1, 2). In the present investigation, the subjects kept 4–5 g more *snus* in the mouth 3–4 h longer, and 1–2 g more *snus* for 1–2 h longer, than the 68 and 23 portion-bag users, respectively, participating in the two studies reported by ANDERSSON *et al.* (1, 2).

The differences in *snus* exposure data may be explained by the different outlines of the studies. It might be assumed that the longer subjects are under observation, the more reliable are the values on consumption obtained.

Both in Study 1 and 2 there were considerably variations in daily consumption between subjects, but there were almost no variations over time within subjects when consuming the same brand. When the subjects in Study 1 switched from their usual *snus* (Brand A) to the low-nicotine brand (Brand B), consumption increased on average by about 2 g a day. The available amount of nicotine was decreased by about 50% when switching from Brand A to Brand B, but the increase in average daily consumption was only 15%. Except for two subjects, the participants in Study 1 kept the *snus* in the mouth for about the same number of hours daily when using Brand A as when using Brand B. Accordingly, the *snus* users in Study 1 compensated only to a small extent for the lower nicotine delivery by increasing consumption. The reference group in Study 2, who had used the low nicotine *snus* (Brand B) for at least 1 year (range 1–6 years), consumed about 3 g less *snus* a day during the same number of hours than the subjects in Study 1 who had switched to Brand B. This indicates that *snus* users compensated to a small extent for the lower nicotine delivery by increasing consumption on short-term switching but not on long-term use. It should be borne in mind, however, that the two studies involved different subjects and it is conceivable that other factors may have been responsible for the difference observed between them in relation to their reactions to *snus* use.

Exposure and uptake data

During habitual consumption of tobacco products, the nicotine intake is well reflected by the steady-state concentrations of cotinine in saliva (14–16). Both in Study 1 and Study 2 the salivary cotinine concentrations were fairly consistent within subjects when consuming the same brand, but due to the large inter-individual variation in daily *snus* consumption and nicotine exposure, there were large variations in salivary cotinine concentrations between subjects. On switching from the normal nicotine brand (Brand A) to the low-nicotine brand (Brand B) in Study 1, the salivary cotinine levels were significantly reduced ($p<0.001$). On average the reduction was 55%, i.e. from 336 ng/ml down to 153 ng/ml, to about the same level that was found in the reference group (159 ng/ml) in Study 2.

At steady-state, the rate of excretion of metabolites reflects the generation rate. Thus, the daily nicotine intake by habitual tobacco users can be estimated by quantifying the 24 h-urine amounts of nicotine and its seven main metabolites, i.e. cotinine, *trans*-3′-hydroxycotinine, glucuronic acid derivatives of nicotine, cotinine and 3′-hydroxycotinine and nicotine-1′-N-oxide and cotinine-1-N-oxide. Mass balance studies of nicotine in tobacco users after intravenous infusion have shown that nicotine together with its seven main metabolites account for about 98% of the ingested dose (unpublished observation).

In accordance with the large variation in *snus* consumption and nicotine exposure, there was a large variation in nicotine intake between subjects. However, the nicotine intake was fairly consistent over time within subjects when they were consuming the same brand. There was a significant decrease in daily nicotine intake after switching from the normal to the low-nicotine *snus* ($p<0.001$). On average the reduction was 43%, i.e. a decrease from 25.2 to 14.4 mg, which was about the same level as in the reference group (14.3 mg).

The average reduction in salivary cotinine and total amount of nicotine equivalents excreted during 24 h were similar (about 50%) and proportional to the nicotine concentration of the *snus* products. These results clearly show that *snus* users do not compensate for the reduced delivery of nicotine when switching from their ordinary brand to a low-nicotine brand. Although there was a slight increase (about 15%) in consumption after short-term switching, both short- and long-term switching to a brand containing half the concentration of nicotine resulted in a corresponding reduction in nicotine intake.

This study on *snus* users has shown that the nicotine intake decreased by about 50% when switching from normal nicotine *snus* to a *snus* having half the concentration of nicotine. The average nicotine intake measured as nicotine equivalents excreted during 24 h and steady-state salivary cotinine concentrations decreased from 25.2 to 14.4 mg and from 336 to 153 ng/ml, respectively.

Clinical observations

The pattern of oral mucosal changes in subjects using Brand A is very similar to that found in a group of 68 subjects using the same or a similar product with regard to nicotine concentration and pH, but with a slightly lower consumption as expressed by hours of daily use and grams used a day (17). This indicates that the subjects selected for Study 1 show a fairly representative pattern and mode of clinical reaction to the application of this type and brand of *snus*.

The similarity of registered lesions according to clinical degree of severity between the present Study 1 and the study by ANDERSSON & AXÉLL (17) supports the reliability of the clinical criteria and classification used.

After switching to the low-nicotine product (Brand B) there was an obvious change in the oral mucosal reactions by way of a reduced degree of whiteness and clinical impression of thickening. Previously such mucosal changes have been suggested to relate to or be caused by the high alkaline pH value of *snus* (2, 9, 18). However, in the present study no decisive pH differences were encountered between Brand A and Brand B, thus making the theory relating to the importance of pH value questionable. The only recorded difference between the brands was the nicotine content. It remains to be investigated whether the severity of oral mucosal changes related to use of moist snuff is associated with nicotine content or if there is another still unknown factor in the product, as previously mentioned by PINDBORG *et al.* (19). Therefore, future studies will include investigations of the significance of decreasing either pH or nicotine on clinical and histological changes in the oral mucosa.

Acknowledgements – The authors thank BARBRO JURLAND for assistance in conducting the study, DJULI RUZESKOVIC for chemical analysis and BERTIL RUNSTRÖM for data analysis.

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