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ABSTRACT

Background: Smokeless tobacco is an efficient delivery vehicle for nicotine and can contain significant amounts of carcinogens. However, few studies have examined factors that might moderate levels of nicotine or carcinogen exposure.

Aims: To determine the effect of duration of smokeless tobacco use on the uptake of nicotine and a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

Methods: Questionnaires on use of smokeless tobacco were administered, and urine samples from 212 smokeless tobacco users were analysed for biomarkers of uptake of nicotine and NNK. The biomarkers were cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Male smokeless tobacco users were recruited for studies designed to investigate methods of reducing smokeless tobacco use. The questionnaire and biomarker data were obtained at baseline, prior to reduction.

Results: Levels of cotinine ($p<0.001$) and total NNAL ($p<0.001$) were significantly correlated with duration (in years) of use of smokeless tobacco products. Median cotinine and total NNAL were 2.4 and 2.1 times higher, respectively, in the $\geqslant$21 years of use than in the 0–5 years of use category.

Conclusions: Smokeless tobacco users adjust their intensity of use with experience in order to increase their nicotine dose, resulting in a corresponding increase in exposure to NNK, a powerful carcinogen. These results indicate the importance of educating smokeless tobacco users about the effects of prolonged use of these products.

Smokeless tobacco, the predominant form of tobacco use at the beginning of the twentieth century before the dramatic ascendance of cigarettes, re-emerged as a formidable product in the US in the 1970s with the strong promotion of oral snuff products, and has been used for decades in other locations of the world such as Scandinavia and southeast Asia.[1] Smokeless tobacco products continue to increase in popularity in Western countries, and some tobacco control experts have proposed that "low nitrosamine" products be substituted for cigarettes by people who cannot quit tobacco, foreshadowing a potential second re-emergence of these products.[2 3] While smokeless tobacco lacks the combustion products of cigarette smoke, and is certainly less harmful, it is not without harm. Smokeless tobacco is an efficient delivery vehicle for the addictive agent nicotine, contains multiple carcinogens—most notably tobacco-specific nitrosamines—and is considered by the International Agency for Research on Cancer as "carcinogenic to humans", causing cancers of the oral cavity and pancreas.[1]

There have been no previous large studies examining the long-term effects of smokeless tobacco use on the uptake of nicotine and carcinogens. In this study, we demonstrate that biomarkers of exposure to nicotine and a powerful tobacco-specific nitrosamine carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK),[4] increase significantly with duration of smokeless tobacco use. The biomarkers are urinary cotinine, an accepted measure of nicotine uptake, and urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (total NNAL), an established metric of NNK uptake.[5] Only one small preliminary study from our group (n = 54), which included some of the subjects in the larger study reported here, previously examined the relationship of these biomarkers to duration of smokeless tobacco use, and reported a relationship to cotinine levels but not to total NNAL.[6]

METHODS

Subjects

This research was approved by the University of Minnesota Research Subjects' Protection Programs Institutional Review Board Human Subjects Committee, and involved smokeless tobacco users who were seeking treatment for smokeless tobacco reduction. Baseline data from three studies were used. They examined the effects of tobacco free snuff use (a herbal snuff-like product (study 1),[7] brand switching (study 2),[8] or use of a nicotine lozenge (study 3) compared to control groups on reduction of smokeless tobacco use. Subjects were recruited from the Minneapolis/St. Paul (Minnesota) metropolitan area through newspaper and television advertisements, and were screened over the telephone to determine interest and eligibility. During this screening, they were informed that the study compared different interventions for smokeless tobacco use reduction. Interested participants were asked to attend a meeting for orientation and screening and to obtain informed consent. Potential subjects were eligible for enrolment if they were: (a) between the ages of 18–70 years, (b) interested in reducing smokeless tobacco use but not quitting (ie, having an established quit date within the next 90 days), (c) using smokeless tobacco daily ($\geqslant$6 dips/day) for the past 6 months but not smoking more than 10 cigarettes per month, (d) in good physical health (ie, absence of an unstable medical condition or use of a medication that might affect tobacco use or be

Table 1 Levels of cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in the urine of 212 smokeless tobacco users stratified by categories of years of daily use

		Cotinine (nmol/ml)		Total NNAL (pmol/ml)	
Duration (years of daily use)	n	Geometric mean (95% CI)	Median (range)	Geometric mean (95% CI)	Median (range)
0–5	14	23.6 (13.5 to 41.2)	29.0 (3.03 to 71.9)	2.47 (1.42 to 4.31)	2.12 (0.39 to 16.7)
6–10	54	37.3 (30.9 to 45.1)	36.9 (4.91 to 112)	3.21 (2.62 to 3.92)	3.11 (0.57 to 22.4)
11–15	57	43.9 (37.4 to 51.6)	46.3 (5.88 to 143)	4.26 (3.60 to 5.03)	4.06 (0.66 to 23.0)
16–20	52	44.3 (35.2 to 55.7)	56.9 (5.93 to 155)	4.07 (3.34 to 4.94)	4.00 (0.84 to 14.7)
21+	35	55.9 (40.4 to 77.3)	62.0 (4.16 to 232)	5.21 (4.06 to 6.70)	4.53 (1.09 to 25.8)

affected by tobacco use reduction), and (e) in good mental health (ie, not taking psychotropic medications or manifesting a psychiatric co-morbidity within the past 6 months). If subjects met these criteria, they were scheduled for two baseline clinic visits during which tobacco use histories and first morning urine samples were obtained. Only the urine samples obtained at the second baseline visit were analysed. Tobacco use histories included inquiries on brand of smokeless tobacco used, date of first daily use, and amount of use (dips/day, min/dip, tins/week).

Biomarker analyses

Total NNAL and total cotinine were determined essentially as described previously.[9–11]

Analysis of tobacco

Copenhagen long cuts (US Smokeless Tobacco, Stanford, Connecticut), Skoal straight long cuts (US Smokeless Tobacco), and Kodiak premium wintergreen (Conwood, Memphis, Tennessee) were purchased at retail stores in three locations chosen randomly in the Minneapolis/St. Paul area. Tobacco from three tins of each product purchased at each location was thoroughly mixed. Thus, for each product, three representative samples were obtained. NNK, nicotine, and pH were analysed essentially as described previously and mean values calculated.[12–14]

Statistical analyses

In exploratory data analyses, cotinine and total NNAL were summarised using geometric means, medians, and ranges because of the skewedness of their distributions. The non-parametric Spearman correlation coefficient was used for describing the relationship of duration of daily use to biomarker levels and amount of use. Scatter plots with Loess smooth curves were used to illustrate the relationship between biomarker levels and duration. Multiple regressions were applied to assess the contribution of duration of daily use, amount of use, and brand to the inter-individual variability of cotinine and total NNAL. Biomarker levels were transformed to the natural log scale in both scatter plots and regression analyses.

RESULTS

The 212 smokeless tobacco users in this study were all male, 98% caucasian, and had a mean age of 33.8 (95% CI 32.8–34.8) years. They consumed an average of 4.2 (95% CI 3.9–4.4) tins of smokeless tobacco per week and took 9.9 (95% CI 9.1–10.6) dips/day. Their mean duration of daily use of smokeless tobacco was 14.1 (95% CI 13.3–15.0) years. They currently used Copenhagen fine and long cuts (31%), Skoal long cuts, mint and straight (14%), Kodiak premium wintergreen (42%), and other brands (14%). The highest brand prevalence amounts in each category of duration of smokeless use were Kodiak (63%) for 0–10 years of use, and Copenhagen (39% and 49%) for 11–20 and 21 plus years of use, respectively.

The relationship of cotinine and total NNAL to duration of daily smokeless tobacco use is illustrated in fig 1A,B. Cotinine (Spearman correlation 0.27, $p<0.001$) and total NNAL (Spearman correlation, 0.23, $p<0.001$) were both correlated with duration of use. Geometric means and median amounts of cotinine and total NNAL for five categories of years of daily use are summarised in table 1. Median cotinine and total NNAL were 2.4 and 2.1 times higher, respectively, in the ≥21 years of use category than in the 0–5 years of use category.

Dips of smokeless tobacco/day, min/day, and dips/tin of smokeless tobacco were correlated with duration of daily smokeless tobacco use (table 2). Values for min/dip and tins/week were not correlated with duration. Values for min/dip of smokeless tobacco, dips/day, min/day, and dips/tin, but not tins/week, were significantly correlated with cotinine and total NNAL (table 2).

The relationship of duration of use to dependence, as indicated by reported time to first dip of the day, was examined. These data were available for 188 of the 212 subjects. Duration was longer in subjects (n = 135) who reported using smokeless tobacco within 30 min of waking than in subjects (n = 53) who did not; mean (SD) 14.9 (6.09) vs 12.4 (5.79) years ($p = 0.01$).

Duration of use was significantly greater for Copenhagen than Skoal or Kodiak ($p<0.001$). Brand was not related to the

Table 2 Relationship of duration of smokeless tobacco use and levels of cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine to parameters of smokeless tobacco use in 212 users

	Spearman correlation (p Value)		
Parameter	Duration of use	Cotinine	Total NNAL
Min/dip	0.11 (0.12)	0.20 (0.004)	0.16 (0.02)
Dips/day	0.26 (<0.001)	0.15 (0.03)	0.19 (0.005)
Min/day	0.28 (<0.001)	0.29 (<0.001)	0.28 (<0.001)
Tins/week	0.04 (0.54)	0.08 (0.23)	0.13 (0.055)
Dips/tin	0.21 (0.003)	0.27 (<0.001)	0.23 (<0.001)

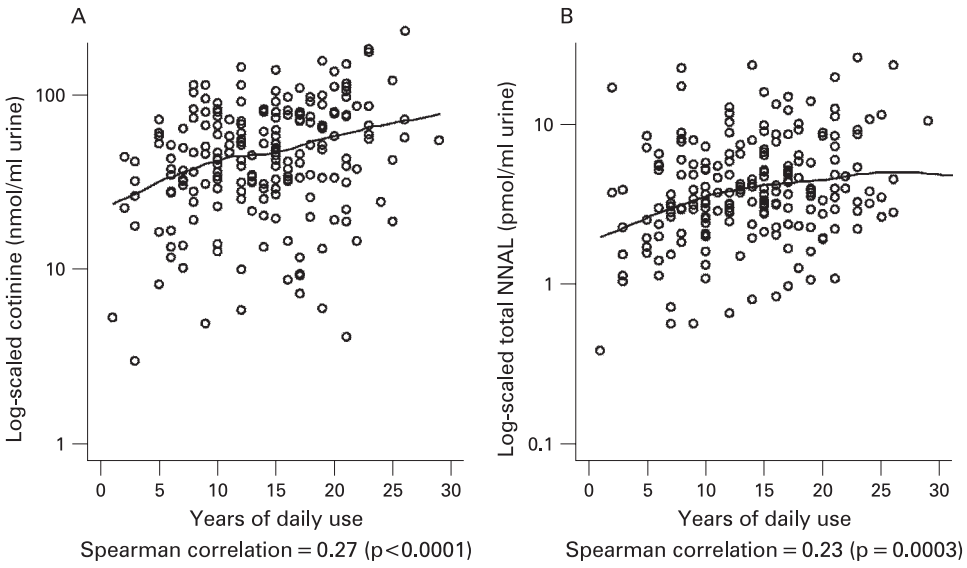

Figure 1 Log-scaled levels of urinary cotinine (A) and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (B), plotted by years of daily use of smokeless tobacco in 212 men. Spearman correlations: cotinine, 0.27 ($p<0.001$); total NNAL, 0.23 ($p<0.001$).

biomarker levels after adjustment for duration and amount of use in multiple regression models. Levels of NNK in recent analyses of the most common smokeless tobacco products used here were similar (μg/g wet weight): Copenhagen long cuts (0.47), Skoal straight long cuts (0.63), and Kodiak premium wintergreen (0.55). Levels of nicotine and unprotonated nicotine (mg/g wet weight) were: Copenhagen (11.6, 3.1), Skoal (11.4, 2.7), and Kodiak (8.9, 5.5).

DISCUSSION

The results of this study demonstrate a significant increase in levels of urinary cotinine and total NNAL with duration of daily smokeless tobacco use. This increase can be explained mainly by corresponding increases in min/day, dips/day, and dips/tin of smokeless tobacco use with duration of use, and was not related to brand, or to levels of NNK or nicotine in different brands. The results strongly suggest that smokeless tobacco users adjust their intensity of use with experience in order to increase their nicotine dose, resulting in a corresponding increase in exposure to NNK, and presumably other constituents as well.

Our results suggest growing dependence on smokeless tobacco use with duration of use. Our users used more dips/tin, and presumably smaller dips, with longer duration. It is possible that nicotine may be extracted more readily in the mouth from these smaller dips. Increased nicotine dependence with years of use followed by a plateau has been observed in smokers and probably occurs in smokeless tobacco users as well.[15 16] This pattern seems consistent with our observations. Our finding that time to first dip in the morning was related to duration is also consistent with increased nicotine dependence among those who used smokeless tobacco for longer periods of time. In smokers, higher nicotine dependence is associated with decreased likelihood of quitting and the same phenomenon may have influenced our results.[17]

We observed a greater increase in cotinine levels than in total NNAL levels with duration of use. While the reason for this is not clear, a related phenomenon has been observed in smokers, in whom levels of NNAL plateau at higher levels of cotinine.[18] This could be due to induction of alternate pathways of NNK metabolism at higher doses of nicotine and other cigarette smoke constituents.

What this paper adds

This study shows for the first time that exposure to nicotine and the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), as measured by their biomarkers cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in urine, significantly increases with duration of smokeless tobacco use.

While the driving force for the results observed here is undoubtedly nicotine, exposure to NNK also increases with duration. NNK is a powerful carcinogen, inducing tumours of the lung, pancreas, and nasal cavity in rodents at relatively low doses.[4 19] Oral cavity tumours were observed when NNK and the related tobacco-specific nitrosamine, *N′*-nitrosonornicotine (NNN), also present in relatively substantial quantities in smokeless tobacco, were co-administered to rats.[20] NNK and NNN are considered carcinogenic to humans by the International Agency for Research on Cancer.[1]

A limitation of this study was that our smokeless tobacco users were seeking to reduce their use. These individuals are dependent and may have a different pattern of use than smokeless tobacco users who are not seeking treatment. A cross-sectional population-based investigation might be a more appropriate design.

In summary, the results of this study clearly demonstrate that levels of urinary cotinine and total NNAL in smokeless tobacco users increase with duration of use. Smokeless tobacco users need to be educated about the effects of prolonged use. These findings should be considered when recommending smokeless tobacco use as a harm reduction strategy as increased duration of use may have unintended consequences due to higher than expected toxicant exposure.

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Competing interests: None.

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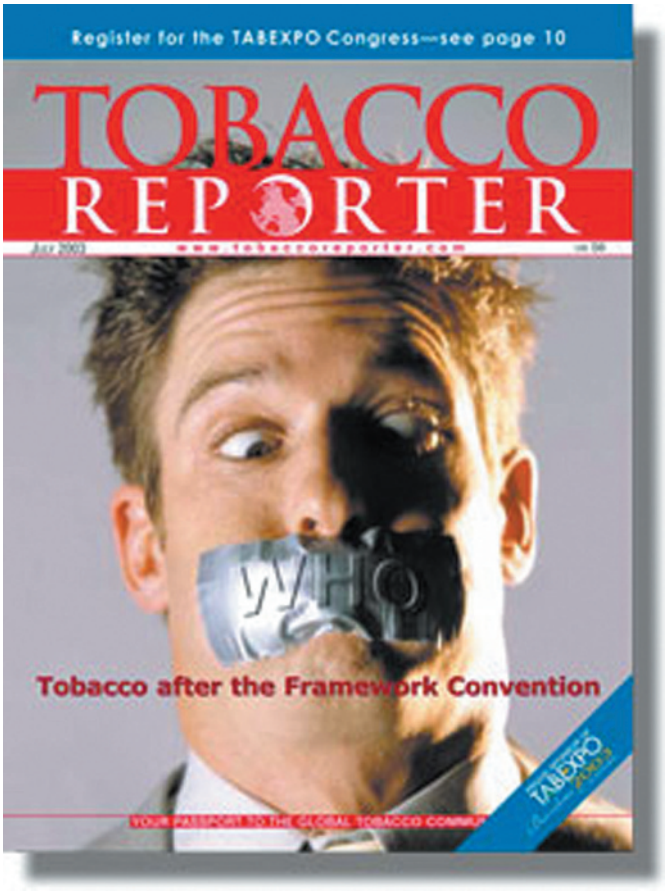
The Lighter Side



One of the leading trade journals, *Tobacco Reporter*, has been monitoring the FCTC for years. This year, the publisher and editor of *TR*, Noel Morris and Taco Tuinstra, have been blogging "The Counterfeit Trail", an account of their investigation "exposing the illegal tobacco trade": http://the counterfeittrail.blogspot.com/.