

Impact of consumption factors on soft tissue changes in Swedish moist snuff users: a histologic study

Gunilla Andersson¹, Tony Axéll¹ and Åke Larsson²

Department of ¹Oral Surgery and Oral Medicine, ²Oral Pathology, Faculty of Odontology, Lund University, Malmö, Sweden

Andersson G, Axéll T, Larsson Å: Impact of consumption factors on soft tissue changes in Swedish moist snuff users: a histologic study. *J Oral Pathol Med* 1990; 19: 453–8.

The purpose of this study was to analyze the relative importance for histologic changes of the oral mucosa of such consumption factors as years with regular snuff use, hours and grams of daily snuff use. The material consisted of biopsies of selected cases (two groups) from 252 regular snuff users of whom 184 used exclusively loose and 68 exclusively portion-bag packed snuff. Group 1 comprised 8 pairs of loose snuff users with large differences (14–45 yr) in terms of years with regular habit. Many years of snuff use did not per se seem to result in tissue changes which significantly differed from changes seen in subjects with only a few years of loose snuff use. Group 2 included 5–10 subjects showing the lowest and highest daily consumption of loose or portion-bag packed snuff. Among those with a low daily consumption, portion-bags seem to be related to less pronounced changes than loose snuff. High daily snuff use was associated with relatively somewhat more pronounced epithelial surface changes but histologic differences between the two habit groups were difficult to identify.

Key words: mouth, diseases; oral cavity; snuff; tobacco, smokeless.

Gunilla Andersson, Department of Oral Surgery and Oral Medicine, Faculty of Odontology, Lund University, S-214 21 Malmö, Sweden.

Accepted for publication July 16, 1990.

It is well known that snuff causes changes of the oral mucosa, and that the clinical appearance may vary with differences in consumption factors (1–3). Recently it was found by means of step-wise logistic regression calculation that both the form of the snuff (loose versus portion packed) and the placement of the quid (one site versus multiple sites) had a relatively significant impact upon the clinical appearance of the mucosal changes. Number of years with regular snuff habit seemed to have less impact than daily exposure to snuff in terms of hours using snuff daily and grams of snuff used daily (4). In other previous studies (5, 6) these factors have been applied to a simple cumulative snuff exposure index based on the assumption that each of the individual consumption factors has an equally important influence on the development of the oral lesions. This has, however, not been clearly verified.

The purpose of the present study was to analyze the relative importance of consumption factors, such as years with regular snuff use, hours of daily snuff use and grams of snuff used daily on the histologic changes found at the site

Table 1. Age and snuff exposure data of study population

	Loose can snuff <i>n</i> = 184	Portion-bag snuff <i>n</i> = 68	Total <i>n</i> = 252
Mean age, yr	36.0 ± 11.6	36.9 ± 9.9	36.3 ± 11.2
Range, yr	19–80	17–66	17–80
Hours of daily snuff use	10.8 ± 3.8	10.3 ± 3.2	10.6 ± 3.6
Grams of snuff used daily	23.6 ± 12.2	11.3 ± 4.9	20.3 ± 12.0
Years with regular snuff habit	13.1 ± 8.2	3.1 ± 2.5	10.4 ± 8.4

Table 2. Eight matched pairs of loose snuff users according to daily consumption and with large duration differences

Pair No.	Age, yr	Accession No.	Consumption			Clinical degree	Predominant histologic type
			Year	g/day	h/day		
1	51	321	20	12.5	2	3	1
	46	310	3	12.5	4	1	normal
2	38	354	20	14.3	6.5	2	2
	21	399	1	14.3	5	3	2
3	50	246	35	12.5	7	2	1
	25	278	4	12.5	6	3	1
4	41	272	17	16.7	10	3	1
	39	292	3	16.7	9	3	1
5	66	239	50	25	10	3	2
	27	224	5	25	10	3	2
6	47	234	25	20	15	3	1
	20	349	5	20	13	3	1
7	60	248	40	25	15	3	1
	26	364	5	25	13	3	1
8	63	306	40	33.3	14	3	2
	45	266	6	33.3	14	3	1



Fig. 1. Matched pair No. 3 (acc. No. 246, Table 2), 50-yr-old man with clinical degree 2 lesion (loose snuff, 7 h daily, 12.5 g a day, 35 yr with regular habit). Type 1 surface changes with vacuolated surface cells, a thin necrotic, eosinophilic surface zone (arrow) and a slight inflammation in connective tissue. This biopsy showed increased mitotic rate but no dysplasia. Cf. Fig. 2 for comparison with short duration. $\times 200$.

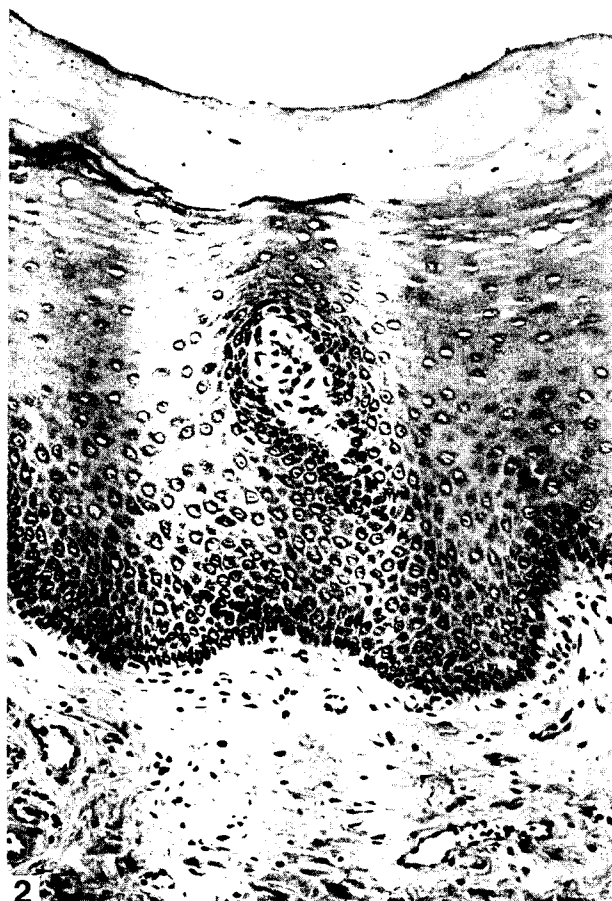


Fig. 2. Matched pair No. 3 (acc. No. 278, Table 2), 25-yr-old man with a clinical degree 3 lesion (loose snuff, 6 h daily, 12.5 g a day, 4 yr with regular habit). Type 1 surface changes with vacuolated cells combined with insignificant connective tissue inflammation. No increased mitotic rate, no dysplasia. Cf. Fig. 1 for comparison with long duration. $\times 200$.

where the snuff quid was regularly placed.

Material and methods

Included in the study were 252 healthy men with a regular snuff habit for at least the last 3 months and with no other current tobacco use. For a detailed description of the recruitment procedure, see ANDERSSON & AXÉLL (4). Age and snuff exposure data are shown in Table 1.

All subjects were called to a dental clinic and asked about type and brand of snuff used, years with snuff usage, daily use in terms of hours and grams and whether they placed the snuff quid at one or more sites. Further questions were asked about general health, medi-

cation, other tobacco habits and alcohol consumption. A careful clinical examination was carried out including registration of snuff dipper's lesions classi-

fied according to a four grade clinical scale (7).

From the central part of each changed mucosal area a biopsy was

Table 3. Selected cases with low daily consumption of portion-bag packed and loose snuff

Type	Age, yr	Accession No.	Consumption			Clinical degree
			g/day	h/day	yr	
P-bag	43	009	8	5	1	2
	66	020	4.4	5	7.5	1
	33	025	6.9	5.5	2	1
	40	030	5.3	5	4	2
	48	048	6.9	7	9.0	1
Loose	37	217	7.1	2.5	10	2
	32	274	3.6	1	16	1
	33	339	7.1	3.5	13.5	1
	44	281	6.7	5.0	10.0	3
	33	288	7.1	6.0	18	1

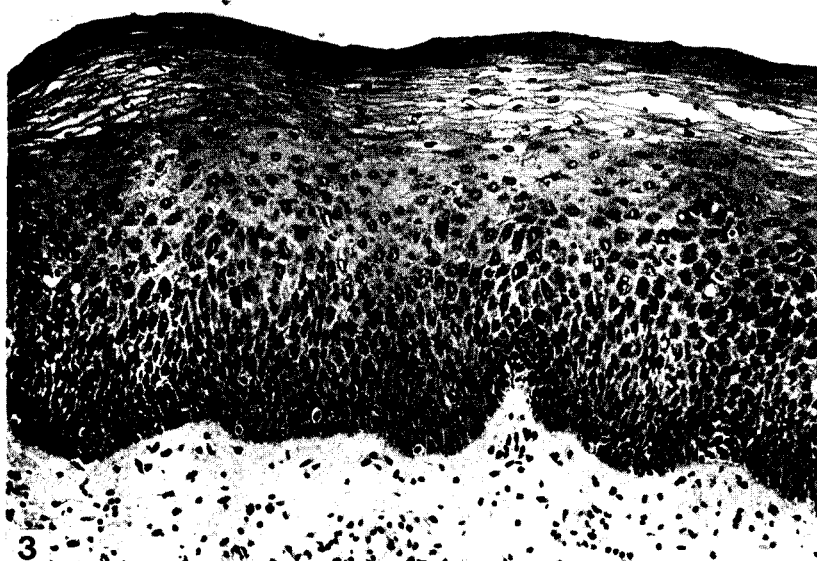


Fig. 3. Low consumer of portion-bag packed snuff (acc. No. 030, Table 3). 40-yr-old man with a clinical degree 2 lesion (5 h daily, 5.3 g a day, 4 yr with regular habit). Close-to-normal surface epithelium. The surface layer is eosinophilic with pyknotic nuclei but with no evidence of keratinization (rhodamine B neg.). A slight degree of inflammation in connective tissue. No increased mitotic rate. Cf. Fig. 4 for comparison with low consumption of loose snuff. $\times 190$.

taken with a 6 mm punch instrument. The tissue was fixed in 10% neutral buffered formalin and embedded in paraffin. Five micron thick sections were stained with hematoxylin-eosin and PAS. Sections were also stained with rhodamine B and examined by fluorescent light (8) to evaluate the degree of keratinization.

Sections of all 252 biopsies were examined light microscopically. An array of structural changes appearing in varying combinations were identified among which, "type 1" or "type 2" surface changes were characteristic findings (9). In type 1, the surface layer is variably thickened, combined with vacuolated cells and often showing "chevron-type patterns". This feature is commonly encountered in snuff dipper's lesions (10) but occasionally type 2 changes appear. They are characterised by a variable degree of keratinization, as evidenced by an eosinophilic and a more or less continuous rhodamine B stain (8).

Two main groups both including only subjects placing the quid at one site, were selected for the present study.

This selection was made by one of the authors (GA) and the microscopic examinations by another (AL). The histologic analysis was carried out without any information available about the subject's tobacco consumption patterns.

Group 1 – These patients were selected from the 184 users of loose snuff in

order to evaluate the relative importance of duration of snuff habit (number of years). Based on close to equal daily consumption (hours/day, grams/day) and large differences in terms of years with regular habit (range differences 14–45 yr) we were able to identify 8 pairs according to Table 2. No portion-bag users were included because of lack of cases fulfilling our criteria of long duration.

Group 2 – These patients were selected from the total material of 252 subjects with the purpose of evaluating the relative impact of daily consumption factors (hours/day, grams/day). Based on the findings obtained in Group 1,

these patients were selected irrespective of years with snuff habit, according to the following. From the loose and portion-bag users, respectively, the 5–10 subjects showing the lowest and highest daily consumption were selected. The consumption data of these four categories are summarized in Tables 3 and 4.

Results

Many vs. few years of loose snuff use (Group 1)

Data of the eight pairs are summarized in Table 2. In three of these pairs (Nos. 1–3), all the six patients had a daily snuff consumption of no more than 7 h, the corresponding figure being 9–15 h for the remaining five pairs (Nos. 4–8). Clear-cut histologic differences between the individual cases of each separate pair were difficult to identify in the biopsies. The different types of surface changes (type 1, 2) were evenly and seemingly randomly distributed among the subjects, with the type 1 predominating. Four of five cases of epithelial hyperplasia appeared within two of the matched pairs (Nos. 3 and 7) and they all showed the typical type 1 surface change (Figs. 1–2). There was a slight difference in mitotic rate between subjects in some of the matched pairs, with five (acc. Nos. 321, 354, 246, 234, 248) of seven such cases (also acc. Nos. 292 and 364) being long-time users. Notably case No. 292, with only a 3 yr history of snuff use showed an increased mitotic rate whereas it matched case No. 272, with a 17 yr history, showed no such change. Neither did cases Nos. 306 and 239, with a 40 and 50 yr history, respectively, show any increased mitotic rate. In

Table 4. Selected cases with high daily consumption of portion-bag packed and loose snuff

Type	Age, yr	Accession No.	Consumption			Clinical degree
			g/day	h/day	yr	
P-bag	42	017	24	17	6	3
	44	023	16	14	1.5	2
	34	061	24	13	5	3
	37	015	16	13	5	2
	31	063	16	13	1	2
	44	066	24	13	1	3
	39	070	16	13	1	2
Loose	24	286	33.3	16	9	3
	41	340	66.7	16	21	3
	34	343	50	16	17.5	3
	57	264	25	17	30	4
	38	249	50	15	16	3
	60	248	25	15	40	3
	25	316	50	15	12	3
	39	384	66.7	15	24	3



Fig. 4. Low consumer of loose snuff (acc. No. 281, Table 3). 44-yr-old man with a clinical degree 3 lesion (5 h daily, 6.7 g a day, 10 yr with regular habit). Close-to-normal surface epithelium. A thin eosinophilic surface zone lacks evidence of keratinization (rhodamine B neg.). A few inflammatory cells in connective tissue. No increased mitotic rate. Cf. Fig. 3 for comparison with low consumption of portion-bag packed snuff. $\times 190$.

cases Nos. 234 and 354 a slightly increased cellular density was found. No case suggestive of dysplasia (cf. 8) could be demonstrated in any of the 16 cases.

From these findings, we conclude

that many years of loose snuff use does not per se result in tissue changes which significantly differ from changes seen in subjects with only a few years of loose snuff use.

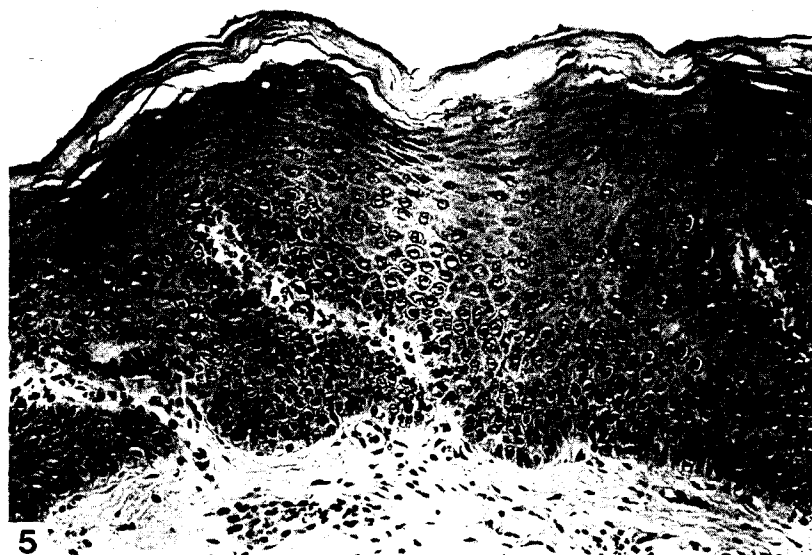


Fig. 5. High consumer of portion-bag packed snuff (acc. No. 066, Table 4). 44-yr-old man with a clinical degree 3 lesion (13 h daily, 24 g a day, 1 yr with regular habit). Type 1 surface change, with moderately thickened vacuolated surface layer accompanied by an increased mitotic rate in the underlying epithelium (not illustrated) but no dysplasia. Discrete inflammation in connective tissue. For comparison Cf. Fig. 3 = low consumption of portion-bag packed snuff and Fig. 6 = high consumption of loose snuff. $\times 190$.

High vs. low daily consumption of loose and portion-bag packed snuff (Group 2)

Low consumption of portion-bag packed snuff – Five cases were identified, using 8 g snuff or less for no more than 7 h a day (Table 3). Three of these five had a close-to-normal surface epithelium (Fig. 3). One had a well-developed type 1 and one had a type 2 surface change, the latter also showing slightly atrophic epithelium. No case with increased mitotic rate was recorded.

Low consumption of loose snuff – Five cases were found using 7.1 g or less for no more than 6 h daily (Table 3). All of these five showed a thin, homogeneous eosinophilic surface zone (Fig. 4). Two of them were combined with a thin type 1 change. No case with increased mitotic rate was recorded.

High consumption of portion-bag packed snuff – Seven cases were identified, using 16 g snuff or more for at least 13 h a day (Table 4). Five of these had a typical type 1 surface change, being prominent in three and less extensive in two (Fig. 5), but none of the five showing hyperplasia (9). Two of the seven cases showed a type 2 change. An increased mitotic rate was found in six cases.

High consumption of loose snuff – Eight cases were identified using 25 g or more of loose snuff for at least 15 h a day (Table 4). One case showed type 2 and seven the typical type 1 surface change, being prominent in five and accompanied by hyperplasia in four (Fig. 6). One of the two cases, showing a less prominent type 1 change, also had areas of type 2. This case (acc. No. 264, Table 4) was the only clinical grade 4 case, and was interpreted as suggestive of dysplasia also showing an increased density and increased mitotic rate (Fig. 7). The latter was also being found in five more of the eight cases.

In summary, in comparison with low consumption, high daily consumption of portion-bag packed or loose snuff results in more pronounced surface epithelial changes. Predominant among these are the appearance of type 1 changes, with swollen cells variably accompanied by a thin necrotic surface zone, and with an increased mitotic rate. Among those with a high consumption histologic differences between the two snuff habit groups were difficult to identify with the exception of a single case using loose snuff. Among those with a low consumption, portion-bag packed snuff tends to cause less changes than

loose snuff. Only one case suggestive of dysplasia was recorded in the subgroups.

Discussion

Supporting previous findings in the clinical part of this study, it was difficult to clearly identify definite histomorphologic differences in biopsies taken from subjects with a long history (17–50 yr) compared with a short history (1–6 yr) of loose snuff use. Within this group, with the two subjects of each of the eight pairs matched with respect to daily exposure (h/day and g/day) of loose snuff, there was a slight tendency among those with a very long history to show an increased mitotic rate. However exceptions to this were also seen, indicating that long duration may not be a decisive factor for the development of this particular change.

In order to further analyze the impact of daily consumption upon tissue changes, cases were selected based on low (less than 6–7 h/day) and high (more than 13–17 h/day) consumption of each type of snuff. The differences in tissue reactions between users of portion-bag and loose snuff which we identified in the low consumption groups, were no longer observed between the high consumers, with the exception of one single case using loose snuff and also exhibiting the only clinical degree 4 lesion of this selected material. The present findings clearly showed that tissue changes (evidence of surface etching with development of type 1 change; mitotic rate) were more pronounced among subjects exposed to snuff for many hours daily in contrast to a few hours daily.

In a recent clinical study (4), it was suggested that years with regular snuff habit as compared to daily consumption had a rather low influence on the development of changes in the oral mucosa associated with snuff use. Thus, a simple cumulative index, as used in a few previous studies (5, 6), may not truly reflect these changes. This conclusion was further supported by the present histopathologic study, based on the same material as the clinical study referred to above. The daily but intermittent use of snuff causes a mixed tissue reaction of injury and repair. Hence, the tissue response observed at any given time must be a result of the combined effects of hours and grams of snuff used daily and of the time permitted for tissue repair. Also, the tissue response should some-

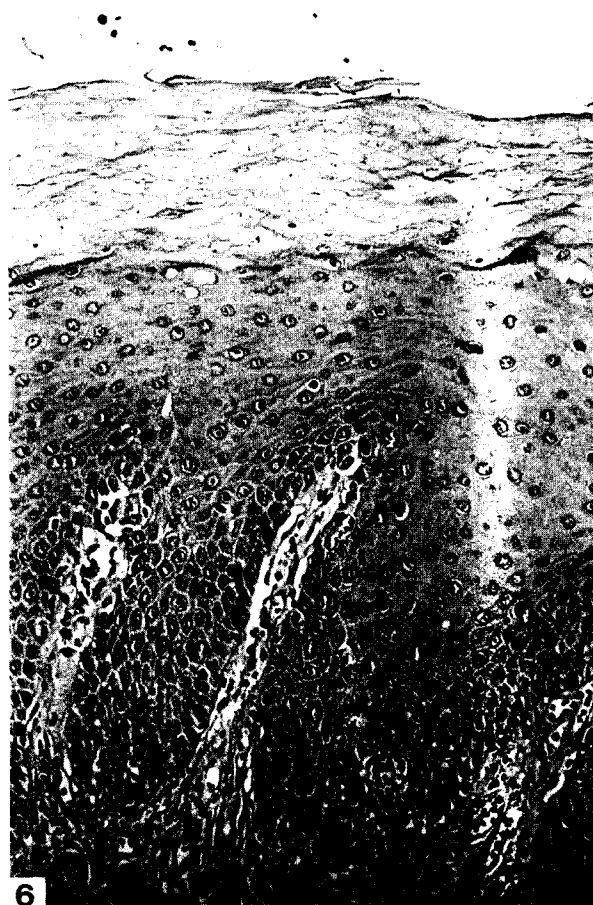


Fig. 6. High consumer of loose snuff (acc. No. 248, Table 4). 60-yr-old man with a clinical degree 3 lesion (15 h daily, 25 g a day, 40 yr of regular habit). Prominent type 1 surface change with swollen cells ("hyperplasia") and evidence of chevron-pattern, also accompanied by an increased mitotic rate (not illustrated) but no dysplasia. A moderate degree of inflammation in connective tissue. For comparison, Cf. Fig. 4 = low consumption of loose snuff and Fig. 5 = high consumption of portion-bag packed snuff. $\times 200$.

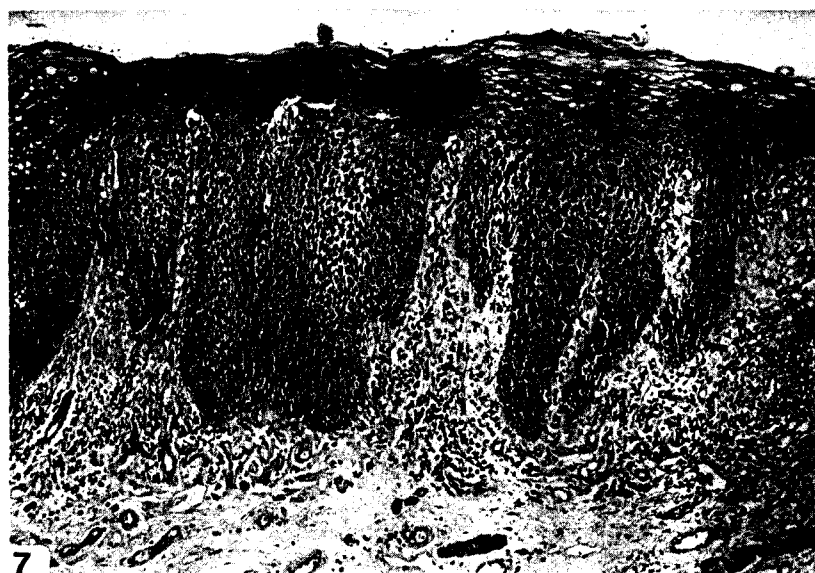


Fig. 7. High consumer of loose snuff (acc. No. 264, Table 4). 57-yr-old man with a clinical degree 4 lesion (17 h daily, 25 g a day, 30 yr with regular habit). Type 1 and 2 surface changes are accompanied by slightly irregular rete pegs. An increased cell density is present in lower part of epithelium, accompanied by evidence of loss of cohesion (arrow) as well as increased mitotic rate (not illustrated) and connective tissue inflammation. These changes were interpreted as suggestive of dysplasia. $\times 190$.

how be modified by the duration, i.e. the number of years with regular snuff habit.

We conclude that the tissue response following a certain snuff habit is rather consistent and predictable, being predominated by surface etching. Isolated cases will however always appear showing a slightly aberrant tissue response such as our acc. No. 264, a heavy consumer of loose snuff, showing the only clinical degree 4 lesion of the present study (Fig. 7). It is tempting to believe that such aberrations may be related to an individually variable capacity of the mucosa to repair and recover from the snuff-induced changes. Such a capacity seems to be little influenced by many years of regular snuff habit. Rather, our present findings indicate that a decisive factor may be the daily consumption. The histology of possible irreparable changes among snuff users has however not been very well characterized, due to lack of experimental data. Such studies

may require repeated biopsies and are in progress as part of the current project.

Acknowledgments – This study was supported by grants from Swedish Tobacco Research Council (Project No. 8716).

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