

## CLINICAL REPORT

# Smoking and Skin: A Study of the Physical Qualities and Histology of Skin in Smokers and Non-smokers

ANINA KNUUTINEN<sup>1</sup>, MATTI KALLIOINEN<sup>2</sup>, KIRSI VÄHÄKANGAS<sup>3</sup> and AARNE OIKARINEN<sup>1</sup>*Departments of <sup>1</sup>Dermatology, <sup>2</sup>Pathology, and <sup>3</sup>Pharmacology and Toxicology, University of Oulu, Oulu, Finland*

**Tobacco smoke is toxic to cells and could be a factor contributing to accelerated skin ageing. The aim of this study was to provide new information on the possible effects of smoking on the physical qualities of skin and the morphology of elastic fibres. The study population consisted of 98 men, including 47 current smokers and 51 never-smokers. Skin thickness and elasticity were measured from cheek, temple, abdomen, dorsal forearm and non-sun-exposed upper inner arm. Verhoeff-stained punch biopsies from the non-sun-exposed upper inner arm were assessed with a computerized image analyser in a blinded fashion to assess the amount and width of elastic fibres. The thickness of cheek skin was increased in the smokers, but skin thickness in other measured sites did not differ between the groups. The amount and width of elastic fibres in the sun-protected skin of the smokers and non-smokers did not differ significantly, nor did skin elasticity in this or any other region under evaluation, suggesting that smoking alone affects neither the amount and width of dermal elastic fibres nor the elasticity of skin in male smokers. Key words: ageing; elasticity; elastic fibres; skin thickness; tobacco.**

(Accepted January 2, 2002.)

Acta Derm Venereol 2002; 82: 36–40.

Knuutinen Anina, Department of Dermatology, University of Oulu, P.O. 5000, FIN-90014 Oulu, Finland. E-mail: anina.knuutinen@oulu.fi

Ultraviolet radiation is known to accelerate skin ageing, and photo-ageing of skin presents with distinct clinical and histological features (1, 2). Tobacco smoke could also be considered capable of affecting skin ageing as an extrinsic factor, but surprisingly little is known about the possible effects of smoking on the physical and morphological qualities of skin. When investigating the appearance and wrinkling of skin, Daniell (3) observed significantly higher wrinkle scores in smokers than in non-smokers. These findings have later been supported in larger and more carefully standardized studies in which cigarette smoking has been estimated to contribute a 2.3 to 4.7-fold risk of increased facial wrinkling, independently of sun exposure (4, 5). There are also contradictory findings questioning the significance of smoking as a causative agent for facial wrinkling (6–8).

Smoking is associated with skin diseases such as pustulosis palmoplantaris, hidradenitis suppurativa, psoriasis and infectious eczematoid dermatitis (9–12), and smokers have an increased risk of contracting squamous cell carcinoma of the skin (13). Because of the effects of tobacco on microcirculation and tissue oxygenation, smoking affects wound healing. Adverse effects of smoking on the surgical outcome of numerous procedures have been reported, including facelifts, breast reconstructions and transfers of cutaneous flaps (14). However, the mechanisms by which tobacco smoke affects the skin are poorly known. Alterations in the function of the immune system of smokers could play a role in the association between smoking and certain skin diseases (15, 16). Tobacco smoke extract alters the function of human skin fibroblasts and affects the extracellular matrix turnover *in vitro* (17, 18). Recently, significantly higher levels of matrix metalloproteinase-1 mRNA were observed in the buttock skin of smokers compared to non-smokers, using quantitative real-time reverse transcription PCR (19). Smoking is not known to affect skin thickness (20), but elastic fibre abnormalities have been observed in both the sun-protected and sun-exposed skin of smokers (21, 22). Surprisingly few data are available on the possible effects of smoking on the structure and function of the skin.

The present study was performed to achieve a larger perspective of the effects of smoking on skin, including assessments of the effects of smoking on the physical qualities of skin, such as skin thickness and elasticity in various body regions and evaluation of skin histology in terms of the proportional areas and width of dermal elastic fibres in a well-defined cohort of smokers and non-smokers living in Northern Finland.

## MATERIAL AND METHODS

### Patients

The study population consisted of 98 male volunteers, 47 of whom were current smokers and 51 never-smokers. They were recruited through advertisements of the study in a local newspaper and in weekly reports of some working offices. All participants were of Finnish origin from Northern Finland and all gave written informed consent. The study protocol was approved by the ethics committee of the Medical Faculty, University of Oulu. The age range was from 34 to 71 years, the mean age of the smokers being 50 (SD 8.5, range 34–71), and that of the non-smokers 53 years (SD 8.3, range 39–69).

Only males were included in the study to avoid confounding factors that might arise in a female population, such as usage of hormonal replacement therapy. All smokers were current smokers and had been smoking for at least 15 years. Non-smokers were defined as men who had never been habitual smokers. The exclusion criteria consisted of diagnosed diabetes, psoriasis, rheumatoid arthritis and other diseases requiring long-term corticosteroid treatment. Of the smokers, 42 were cigarette smokers, three were pipe smokers and two smoked cigars. All pipe and cigar smokers were previous cigarette smokers and were therefore likely to have inhaled tobacco smoke (23). The mean number of years smoked was 33 (SD 8.4, range 15–56) and the mean number of cigarettes smoked per day was 19 (SD 6.6, range 5–40). Pack years of smoking averaged 30, and were calculated by multiplying the number of years smoked by the number of packs smoked per day. To confirm the smoking status of the participants, urinary cotinine and other nicotine metabolites of all participants were analysed using a commercial double antibody nicotine metabolite kit (Diagnostic Corporation, Los Angeles, CA, USA).

#### Assessments of skin thickness and elasticity

Skin thickness and elasticity were measured from five different skin regions: sun-protected upper inner arm, dorsal forearm, temple, cheek and abdomen. Three measurements were made at each site, after which means were calculated and compared. Skin thickness was measured with a 20 MHz Dermascan-A<sup>®</sup> ultrasound device (Cortex Technology, Hadsund, Denmark) and elasticity was measured with a Dermalab<sup>®</sup> suction device (Cortex Technology, Hadsund, Denmark), which consists of a main unit, a built-in printer and a probe. The probe, which provides a vacuum chamber, is attached to the skin surface with the help of adhesive tape. Elasticity is calculated on the basis of the differential force needed to elevate the skin surface 1.5 mm between two infrared detection levels inside the probe chamber. The elasticity modulus is reported as MilliPascals, MPas. The probe was placed perpendicular to the skin, in order to avoid the impact of gravitation on the measurements. Three measurements were made in each skin region, slightly changing the placement of the probe each time, and means of the three measurements were calculated. Five suction cycles were used with all measurements to control the reliability of the method. During the measurements, the study subjects were immobile in a supine position.

#### Skin biopsies and assessments of elastic fibres

Skin biopsy specimens were 6 mm punch biopsies obtained from the sun-protected upper inner arm of 81 study subjects (42 smokers, 39 non-smokers) under local anaesthesia. The biopsy site was chosen in order to minimize the confounding effect of solar irradiation on histological findings. Facial biopsies would have enabled the combined effects of solar irradiation and smoking on skin ageing to be assessed, but the aim, which was established at the beginning of the study, was to evaluate the effects of smoking solely, and the biopsy site was chosen accordingly. Histological evaluations were made from Verhoeff-stained 3- $\mu$ m thick sections in a blinded fashion and included assessments of the mean proportional areas of elastic fibres in the papillary and reticular dermis and of the mean width of elastic fibres in the reticular dermis. For an assessment of the proportional areas occupied by elastic fibres, four fields of both papillary and reticular dermis were analysed. The width of elastic fibres was assessed from three fields of reticular dermis. All visible elastic fibres in each field were included in the assessments of both the proportional areas and the widths of elastic fibres. All the assessments were made with a Nikon TMD Optiphot light microscope and a 40  $\times$  Plan

Nikon objective connected to an MCID/M4 3.0 Rev 1.1 (Imaging Research Inc) image analyser, after which means were calculated and compared.

#### Statistics

Statistical analyses were performed with the SPSS 8.0 for Windows software. The statistical methods included Student's *t*-test for independent samples when analysing parameters with Gaussian distributions and non-parametric Mann-Whitney tests when dealing with skewed distributions.  $P < 0.05$  was considered statistically significant. Correlations were analysed with the Pearson correlation coefficient or Spearman's rank correlation coefficient, depending on the distributions of variables.

## RESULTS

#### Background characteristics of the study subjects

The mean age of the study subjects was 52 years, and 77% of the subjects were between 40 and 59 years of age, 5% were below 40 and 18% were over 60. The mean urinary concentration of nicotine metabolites, normalized by urinary creatinine concentration, was 465 ng/nmol creatinine (range 49–1100 ng/nmol) in the smokers and 9 ng/nmol creatinine (range 3–29 ng/nmol) in the non-smokers. The frequencies of alcohol consumption reported by the participants did not differ significantly between the smokers and non-smokers, but the smokers consumed more alcohol per occasion ( $p < 0.001$ ). The differences in the amount of sun exposure during the Finnish summer months (from the 1st of June until the end of August) were not statistically significant ( $p > 0.1$ ). The frequencies of holidays in southern countries and outdoor occupations were also similar in the groups of smokers and non-smokers ( $p > 0.1$ ). In general, the frequencies of occasional topical steroid use were similar in smokers and non-smokers ( $p = 0.24$ ). None of the study subjects had used topical steroids on the measurement sites.

#### Skin thickness

Overall, the mean skin thickness of various body regions was similar in the smokers and non-smokers (Table I).

Table I. Mean skin thickness (mm) in smokers and non-smokers

Skin region	Smokers (n = 47)			Non-smokers (n = 51)		
	Mean	Range	SD	Mean	Range	SD
Temple	1.7	(1.3–2.1)	0.2	1.6	(1.2–2.0)	0.2
Cheek	1.9*	(1.6–2.3)	0.2	1.7*	(1.4–2.0)	0.2
Upper inner arm	1.1	(0.8–1.5)	0.1	1.1	(0.8–1.4)	0.1
Dorsal forearm	1.4	(1.0–1.9)	0.2	1.4	(1.1–1.9)	0.2
Abdomen	1.8	(1.4–2.2)	0.2	1.8	(1.3–2.7)	0.2

\*Significant difference,  $p < 0.001$ .

The thickness of cheek skin was significantly increased in the smokers both when the groups of smokers and non-smokers were compared as such ( $p < 0.001$ ) and when they were divided into sub-groups aged below and over 50 years ( $p < 0.005$ ; Fig. 1).

*Elasticity*

Elasticity of the skin was similar between the smokers and non-smokers (Table II). The elasticity modulus measured from the upper inner arm of the smokers was slightly less than that of the non-smokers, indicating looseness of skin in this region, but the difference between the groups was not statistically significant ( $p = 0.07$ ).

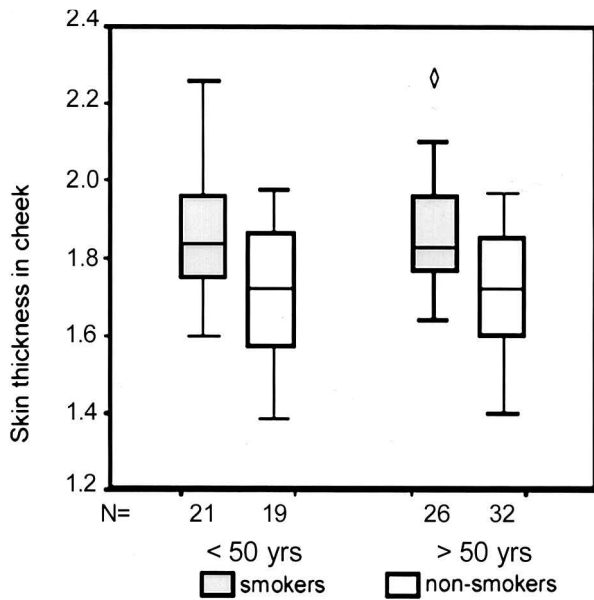


Fig. 1. Effect of smoking on skin thickness in the cheek. The line across each box represents the median value. The edges of the boxes represent lower and upper quartiles and the whiskers extending from the edges show lowest and highest values within a defined region. Outliers are plotted outside the box plot.

Table II. Mean elasticity (MPa) of skin in smokers and non-smokers

Skin region	Smokers (n = 47)			Non-smokers (n = 51)		
	Mean	Range	SD	Mean	Range	SD
Temple	4.6	(0.5–13.8)	3.3	4.7	(0.9–12.5)	3.3
Cheek	7.7	(2.5–16.3)	3.6	7.8	(3.2–15.5)	3.1
Upper inner arm	9.8	(3.0–18.1)	3.4	11.2	(4.2–20.4)	4.0
Dorsal forearm	18.9	(13.0–22.6)	2.3	19.0	(13.6–22.2)	1.9
Abdomen	5.7	(1.6–15.3)	3.0	6.1	(1.7–15.2)	3.3

MPa = MilliPascal.

*Elastic fibres*

The number of elastic fibres and their width were not affected by smoking in skin samples obtained from the sun-protected upper inner arm. The mean proportional areas of elastic fibres in the papillary dermis were 6.6% in the smokers and 6.5% in the non-smokers ( $p = 0.9$ ). The mean proportional areas of elastic fibres in the reticular dermis were 8.7% in the smokers and 9.1% in the non-smokers ( $p = 0.5$ ; Fig. 2). The mean width of elastic fibres was 1.8  $\mu\text{m}$  in both smokers and non-smokers.

*Correlations of the parameters studied with age and with the amount of urinary nicotine metabolites*

None of the parameters correlated significantly with age or with the amount of urinary nicotine metabolites. The thickness of cheek skin had a weak positive correlation with the amount of urinary nicotine metabolites ( $r = 0.3$ ), in contrast to the thickness of upper inner arm skin, which had no correlation with the amount of urinary nicotine metabolites ( $r = -0.1$ ).

DISCUSSION

In the present study, skin thickness in various body regions was similar between the smokers and non-smokers apart from the cheek, where the mean skin

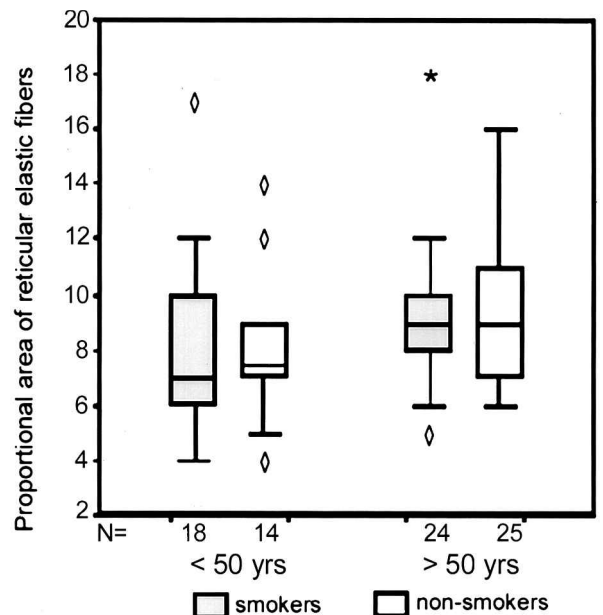


Fig. 2. Proportional areas occupied by elastic fibres in reticular dermis of the upper inner arm. The line across each box represents the median value. The edges of the boxes represent lower and upper quartiles and the whiskers extending from the edges show lowest and highest values within a defined region. Outliers are plotted outside the box plot.

thickness was significantly greater in smokers. This observation could be explained, at least in part, by the combined effects of smoking and cumulative sun exposure. Sun alone is an unlikely explanation because both the amount of sun exposure during the Finnish summer months and the frequency of holidays to southern countries were similar between smokers and non-smokers. Overall, skin thickness in various body regions did not vary significantly between the groups, which is in line with the observations by Whitmore & Sago (20), who compared skin thickness of the volar forearm in black and white women, taking smoking into account as a possible confounding factor. To our knowledge, only one previous study has compared elastic fibres of the non-sun-exposed skin of smokers and non-smokers (21). Elastic fibres were observed to be wider and more numerous in the skin of smokers than in the non-smokers (21). However, the study sample consisted of 10 smokers and 10 non-smokers only, and the authors do not mention having made the assessments in a blinded fashion (21). Altered amounts and morphologies of elastic fibres have been observed in the sun-exposed skin of smokers also (22). In the present study, skin samples for histological evaluation were obtained from non-sun-exposed skin only. Histological evaluations were made in a blinded fashion and the sample size of 42 smokers and 39 non-smokers was larger than that in the previous studies. Furthermore, elasticity of skin was assessed dynamically, using a suction device with which elasticity of skin was measured from five different skin regions, including the upper inner arm, which was the site of skin biopsies also. Our study suggests that smoking alone affects neither the amount and width of dermal elastic fibres nor the elasticity of skin in male smokers.

The slight difference in mean ages of the smokers and non-smokers was not statistically significant and is unlikely to explain the lack of significant differences in skin thickness, elasticity and proportional areas and width of elastic fibres between smokers and non-smokers.

This study was conducted in Northern Finland, where the cumulative yearly sun exposure is considerably lower than that in countries of the previous studies (3–5), in which positive associations between skin ageing and smoking were found. Since smoking and sun-exposure have been observed to mutually potentiate each other's effects on skin ageing (4), smokers living in regions with intensive sun exposure may be more prone to the possible adverse effects of smoking on skin than those living in countries with less sun-exposure. On the other hand, most people in Nordic countries have fair skin, which is sensitive to deleterious effects of UV radiation.

#### ACKNOWLEDGEMENTS

We acknowledge Oulu University Hospital, the Finnish Medical Foundation and Research Foundation of Orion

Corporation for grants supporting the study. Mrs. Riitta Vuento and Mrs. Tuula Lujala are acknowledged for performing the immunohistochemical stainings, Mrs. Kirsi Salo for the urinary nicotine metabolite measurements and Mrs. Soili Manninen for expert secretarial assistance.

#### REFERENCES

1. Kligman LH, Kligman AM. The nature of photoaging: its prevention and repair. *Photodermatol* 1986; 3: 215–227.
2. Gilchrist BA, Yaar M. Ageing and photoageing of the skin: observations at the cellular and molecular level. *Br J Dermatol* 1992; 127 (Suppl 41): 25–30.
3. Daniell HW. Smoker's wrinkles: a study in the epidemiology of "crow's feet". *Ann Intern Med* 1971; 75: 873–880.
4. Kadunce DP, Burr R, Gress R, Kanner R, Lyon JL, Zone JJ. Cigarette smoking: risk factor for premature facial wrinkling. *Ann Intern Med* 1991; 114: 840–844.
5. Ernster VL, Grady D, Miike R, Black D, Selby J, Kerlikowske K. Facial wrinkling in men and women, by smoking status. *Am J Public Health* 1995; 85: 78–82.
6. Allen HB, Johnson BL, Diamond SM. Smoker's wrinkles? *J Am Med Assoc* 1973; 225: 1067–1069.
7. O'Hare PM, Fleischer AB Jr, D'Agostino RB Jr, Feldman SR, Hinds MA, Rasette SA, et al. Tobacco smoking contributes little to facial wrinkling. *J Eur Acad Dermatol Venereol* 1999; 12: 133–139.
8. Malvy DJ-M, Guinot C, Preziosi P, Vaillant L, Tenenhaus M, Galan P, et al. Epidemiologic determinants of skin photoaging: baseline data of the SU.VI.MAX cohort. *J Am Acad Dermatol* 2000; 42: 47–55.
9. Eriksson M-O, Hagforsen E, Lundin IP, Michaëlsson G. Palmoplantar pustulosis: a clinical and immunohistological study. *Br J Dermatol* 1998; 138: 390–398.
10. König A, Lehmann C, Rompel R, Happle R. Cigarette smoking as a triggering factor of hidradenitis suppurativa. *Dermatology* 1999; 198: 261–264.
11. Naldi L, Parazzini F, Brevi A, Peserico A, Veller Fornasa C, Grosso G, et al. Family history, smoking habits, alcohol consumption and risk of psoriasis. *Br J Dermatol* 1992; 127: 212–217.
12. Karvonen J, Poikolainen K, Reunala T, Juvakoski T. Alcohol and smoking: risk factors for infectious eczematoid dermatitis? *Acta Derm Venereol* 1992; 72: 208–210.
13. De Hertog SA, Wensveen CA, Bastiaens MT, Kielich CJ, Berkhout MJ, Westendorp RG, et al. Relation between smoking and skin cancer. *J Clin Oncol* 2001; 19: 231–238.
14. Chang LD, Buncke G, Slezak S, Buncke HJ. Cigarette smoking, plastic surgery and microsurgery. *J Reconstr Microsurg* 1996; 12: 467–474.
15. Holt PG. Immune and inflammatory function in cigarette smokers. *Thorax* 1987; 42: 241–249.
16. Meliska CJ, Stunkard ME, Gilbert DG, Jensen RA, Martinko JM. Immune function in cigarette smokers who quit smoking for 31 days. *J Allergy Clin Immunol* 1995; 95: 901–910.
17. Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst* 1999; 91: 1194–1210.
18. Yin L, Morita A, Tsuji T. Alterations of extracellular matrix induced by tobacco smoke extract. *Arch Dermatol Res* 2000; 292: 188–194.
19. Lahmann C, Bergemann J, Harrison G, Young AR. Matrix metalloproteinase-1 and skin ageing in smokers. *Lancet* 2001; 357: 935–936.

20. Whitmore SE, Sago NJG. Caliper-measured skin thickness is similar in white and black women. *J Am Acad Dermatol* 2000; 42: 76–79.
21. Francès C, Boisnic S, Hartmann DJ, Dautzenberg B, Branchet MC, Le Charpentier Y, et al. Changes in the elastic tissue of the non-sun-exposed skin of cigarette smokers. *Br J Dermatol* 1991; 125: 43–47.
22. Boyd AS, Stasko T, King LE Jr, Cameron GS, Pearse AD, Gaskell SA. Cigarette smoking-associated elastotic changes in the skin. *J Am Acad Dermatol* 1999; 41: 23–26.
23. Turner JAMcM, Sillett RW, McNicol MV. Effect of cigar smoking on carboxyhaemoglobin and plasma nicotine concentrations in primary pipe and cigar smokers and ex-cigarette smokers. *BMJ* 1977; 2: 1387–1389.