Effect of smoking, smoking cessation, and nicotine patch on wound dimension, vitamin C, and systemic markers of collagen metabolism

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Background. *Postoperative wound disruption and tissue-destructive disorders are more frequent in smokers than in nonsmokers. Impaired wound healing and altered connective tissue turnover are suggested mechanisms, but exact details remain unknown.*
Methods. *Full-thickness, 5-mm punch biopsy wounds were made lateral to the sacrum in 48 smokers and were randomized double-blinded to continuous smoking, abstinence with transdermal nicotine patch (TNP), or abstinence with placebo patch and 30 never smokers. At 1, 4, 8, and 12 weeks, the wounds were excised and fixed for wound measurement, and blood was collected for measurement of vitamin C, procollagen I N-propeptide (PINP), matrix metalloproteinase 8 (MMP), MMP-9, neutrophils, and eosinophils.*
Results. *One week after wounding, smokers' wounds were 3.1 ± 0.1 mm (mean, standard error of the mean) wide and were 1.3 ± 0.1 mm deep compared with the never smokers' wounds, measuring 3.7 ± 0.1 mm wide and 1.5 ± 0.1 mm deep (*$P < .01$*, respectively). Abstinent smokers' wounds were 3.3 ± 0.1 mm wide (NS) and were 1.4 ± 0.1 mm deep (*$P = .02$ *compared with smokers). In smokers, vitamin C and PINP were 50.5 ± 9.0 μmol/L and were 52.7 ± 6.6 ng/mL, respectively, compared with 68.8 ± 14.5 μmolL and 64.7 ± 4.7 ng/mL in never smokers (*$P < .001$ *and* $P = .07$*). Both increased significantly after smoking cessation. Plasma MMP-8 and MMP-9 were correlated with neutrophil blood count, which significantly was affected by smoking status. No effect of TNP was found.*
Conclusion. *Smokers have smaller, more superficial wounds and lesser blood levels of vitamin C and PINP. Smoking cessation resulted in increased wound depth, vitamin C, and PINP as well as a decreased neutrophil blood count. These findings suggest that wound contraction and collagen metabolism are affected by a smoking-induced alteration in vitamin C turnover and by a change in inflammatory cell response. (Surgery 2010;■:■-■.)*

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In clinical practice, postsurgical healing complications and tissue destructive disorders are observed frequently in smokers compared with nonsmokers. For example, smokers have a higher incidence of wound and tissue dehiscence, anastomotic leakage, failure of dental implants, and loss of tissue flaps.[1-4] In view of that, delayed wound healing induced by smoking has been suggested. The effects may be seen months to years after operation with the development of incisional hernias and a recurrence of inguinal hernia occurring more frequently in smokers.[5,6]

The mechanisms by which smoking affects wound healing and connective tissue turnover are unclear. Biochemical studies have shown a decrease in collagen synthesis and deposition in skin and tissue and an increase in tissue protease levels,[7-9] whereas physiologic studies have shown a

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temporary tissue acidosis secondary to decreases in tissue blood flow and tissue oxygen after smoking.[10-12] Recently, delayed regeneration of epidermis in smokers has been demonstrated, suggesting impairment on cellular reparative mechanisms.[7]

Oxidative stress induced by smoking has a wide range of detrimental effects, but to date, studies on the specific contribution on wound healing have been limited. The blood levels of the antioxidant vitamin C are less in smokers compared with nonsmokers because of both a greater turnover as it becomes reduced by smoke-derived oxidant species and because of a lesser dietary intake of fruit and vegetables by smokers.[13-17] Accordingly, reports from population-based studies have shown that one third of male and one quarter of female smokers have a severe vitamin C deficiency.[18]

Vitamin C plays a critical role in wound healing by acting as an essential cofactor for collagen synthesis.[19] A vitamin C deficiency results in instability of procollagen and in subsequent defective collagen formation in the small blood vessels and connective tissue.[20] Experimental studies have found that prolonged dietary restrictions may lead to impaired healing and to disruption of test wound scars, which is suggestive of a scurvy-like condition.[21]

Smoking-induced oxidative stress also may lead to increased connective tissue degradation. Tobacco smoke contains a wide range of gaseous and particular compounds, some of which act as neutrophil chemotactic stimulants, which may induce chronic airway inflammation and tissue destruction.[22,23] The enhanced inflammatory response related to "primed" neutrophils leads to the release of both reactive oxygen species and potentially tissue-destructive enzymes like matrix metalloproteinases (MMP).[24-27] These mechanisms may be involved in the development of cardiovascular and pulmonary diseases and tissue-destructive disorders such as abdominal aortic aneurism, parodontosis, pulmonary emphysema, and skin wrinkling, which are more prevalent in smokers.[24,28-30]

In this study, we aimed to study the effect of smoking and smoking cessation on wound healing by measuring the dimensions of a full-thickness skin biopsy wound 1 week after wounding and the related changes in blood levels of vitamin C and procollagen I N-propeptide (PINP) and the matrix metalloproteases MMP-8 and MMP-9 as indicators of systemic collagen synthesis and degradation.

MATERIALS AND METHODS

Seventy-eight healthy volunteers were included in the study after obtaining written informed consent according to the Helsinki II declaration. Forty-eight subjects were daily smokers (24 women and 24 men aged 33 years [20–40] [median, range]). The subjects were smoking 20 (10–50) cigarettes per day and had a smoking history of 16 (3–50) pack years.* Thirty never smokers (15 women and 15 men), aged 26 (20–40) years, comprised the control group. Prior to inclusion, all subjects were assessed using the CO breath test to confirm smoking status.[31] Exclusion criteria were chronic medical disease, pregnancy, menopause, and current or recent medication with corticosteroids or nonsterroidal antiinflammatory drugs (also known as NSAIDs). The Copenhagen Ethical Committee on Biomedical Research approved the study (KF 02-037/00).

The study covered a period of 13 weeks. During the first week of the study, all smokers smoked 20 standardized filter cigarettes per day (Red Prince; Scandinavian Tobacco Company A/S, Copenhagen, Denmark). Subsequently, smokers were randomized double-blinded into 3 groups according to computer-generated randomization numbers, which had been drawn from sealed, opaque, and consecutively arranged envelopes at the day of inclusion. In the first group, the subjects continued to smoke 20 filter cigarettes per day. In the second group, the subjects refrained from smoking and used a transdermal nicotine patch (TNP) (15 mg/16 h plus 10 mg/16 h, Nicorette; Pfizer, Copenhagen, Denmark) 24 hours a day. In the third group, smokers refrained from smoking and used placebo patches. Each subgroup comprised 8 women and 8 men. The sample size and estimated dropout rate was calculated from previous wound healing studies performed by our group.[32,33]

A 5-mm, full-thickness punch biopsy wound was made on the skin over the medial gluteal muscle 4–6 cm lateral to the sacrum after the subjects had refrained from smoking the night before. The wound was dressed with a semipermeable, transparent dressing (Stabilon; Coloplast A/S, Humlebaek, Denmark). One week after wounding, the biopsy wound was excised and sutured as described previously.[34] The excised wound was fixed in a 4% buffered neutral formalin solution and stored at 4°C. The wounding sequence and subsequent excision was repeated at 4, 8, and 12 weeks after group allocation. The first and third wounds were made to the right of the sacrum, and the second and fourth wounds were made to the left of the sacrum. In 6 never smokers (3 men and 3

*Cigarettes per day/20 × years of smoking.

Table I. Wound diameter and wound depth after 7 days of healing in never smokers, smokers, and abstinent smokers

	Wounds (n = 47) in never smokers (n = 30)		*Wounds (n = 93) in smokers (n = 48)*		*Wounds (n = 87) in abstinent smokers (n = 32)*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Diameter (mm)	3.7	0.1	3.1*	0.1	3.3†	0.2
Depth (mm)	1.5	0.1	1.3*	0.1	1.4‡	0.1

The groups represent actual smoking status at the time of wounding and excision. The abstinent smokers represent participants randomized to TNP and placebo. Values are mean.
*Different from never smokers' value ($P < .01$).
†Different from never smokers' value ($P < .05$).
‡Different from smokers' value ($P < .05$).

women), similar wounds were made simultaneously to control for a potential period effect, whereas wounding and excision were made once at a later time in the remaining 12 male and 12 female never smokers. All participants received a financial reimbursement, and cigarettes or patches were provided free of charge depending on group allocation.

After fixation, the tissue was embedded in paraffin and 4-μm-thick sections were made perpendicular to the skin surface. All sections were stained with hematoxylin-eosin. After mounting, representative sections were measured under a light microscope for wound diameter and depth. The microscope was equipped with a video camera, which projected the stained sections onto a slide micrometer-calibrated video monitor (Zeiss, Oberkochen, Germany). A 4 × front lens was used throughout for measuring. One senior and one junior histopathologist performed 2 independent assessments of wound dimensions in random order blinded to the subjects' group assignment.

At the day of inclusion, dietary supplementation was recorded as well as physical activity, body mass index, and dietary preferences. The subjects were instructed to maintain their ordinary diet and physical activity but to refrain from any more dietary supplementation intake until they had terminated the study. Two days after group allocation and wounding, venous blood was sampled between 8 a.m. and 10 a.m. All subjects had been fasting from midnight, except for pure water intake, and had refrained from smoking. The blood sampling was repeated after 4, 8, and 12 weeks between the same hours.

Whole blood for vitamin C analysis was drawn into tubes containing ethylenediamineteraacetic acid (EDTA) and metaphosphoric acid for sample stabilization and was centrifuged and stored at –80°C. Plasma vitamin C analysis used high-performance liquid chromatography (HPLC) analysis.[35] Samples were prepared in the following 3 steps: oxidation of ascorbate to dehydroascorbate (DHAA) with iodine/potassium iodide, removal of excess oxidant using thiosulphate, and derivatisation with dimethyl-phenylene-diamine (DMPD). The DHAA–DMPD derivative was separated using a Waters 717 HPLC system (Waters, Milford, MA) and was detected with a Waters 474 fluorimetric detector set to an excitation wavelength of 360 nm and an emission wavelength of 440 nm.[35]

The plasma PINP was determined from blood drawn in citrate tubes and measured by an enzyme-linked immunosorbent assay (ELISA) technique as described previously.[36] The concentration of total MMP-8 and MMP-9 in plasma (pro and active form) was determined from blood drawn in potassium-EDTA test tubes and in citrate test tubes, respectively. Commercially available ELISA test kits (Amersham; Little Chalfont, Buckinghamshire, UK) were used for the analysis as described previously and according to the manufacturers' instructions.[37] Whole blood drawn in potassium-EDTA tubes was used to determine neutrophil and eosinophil count (Technicon, New York, NY).

The compliance with the smoking or nonsmoking regime was assessed using 3 methods. First, blood analysis of carboxyhemoglobin using heparinized blood (ABL 700; Radiometer A/S, Copenhagen, Denmark) and of cotinine (HP 5890A Gas Chromatograph; Hewlett-Packard, Los Angeles, CA) was undertaken. Second, twice-weekly CO breath tests (MicroSmokerlyzer; Bedfont Instruments, Rochester, UK) were performed. Third, structured interviews on compliance were conducted, which included the number of cigarettes smoked by those randomized to continuous smoking and the use of nicotine or placebo patches by those randomized to abstinence.

Statistics. The data were analyzed initially for compliance to the study plan, and data from

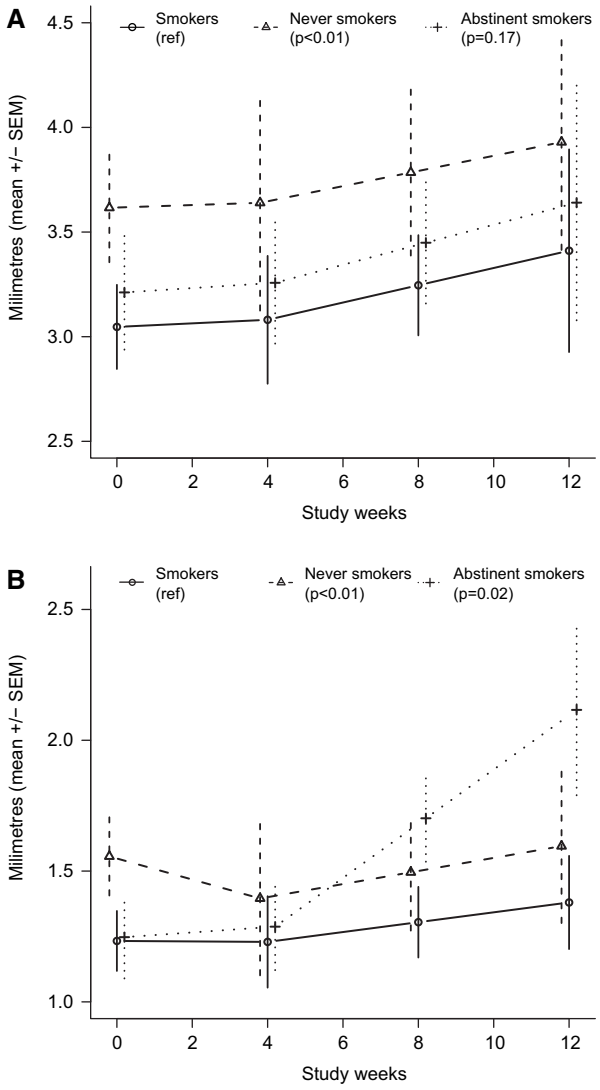

Fig 1. (*A*) Wound diameter after 7 days of healing in smokers, never smokers, and abstinent smokers. The y-axis is in millimeters (mean ± SEM). The x-axis is study weeks. The bold line is smokers (ref). The interrupted line is never smokers ($P < .01$). The dotted line is abstinent smokers ($P = 0.17$). The footnote shows that values are estimated from the model. (*B*) Wound depth after 7 days of healing in smokers, never smokers, and abstinent smokers. The y-axis is in millimeters (mean ± SEM) The x-axis is study weeks. The bold line is smokers (ref). The interrupted line is never smokers ($P < .01$). The dotted line is abstinent smokers ($P = .02$). The footnote shows that values are estimated from the model adjusted for change over time.

noncomplying subjects were discarded. Validated compliance to abstention was defined as a CO breath test less than 6 ppm and a carboxyhemoglobin fraction less than 0.03.[31]

Linear regression and random effects models for repeated measurements were applied to analyze for differences in development over time and change in values between groups.[38] All models were adjusted for time of wounding or blood sampling. In addition, the models applied for analysis of wound diameter and wound depth were adjusted for inter-rater variability, and the model for analysis of vitamin C was adjusted for dietary supplementation taken until commencement of the study. The correlation between vitamin C in the blood and wound diameter and the depth and plasma PINP, respectively, and among the neutrophil blood count and the plasma MMP-8 and MMP-9, respectively, were calculated in similar models and presented as regression coefficients (β value). Finally, delta values of change in carboxyhemoglobin and cotinine levels were compared between groups by the Mann–Whitney *U* test.

All statistical analyses were performed by use of the Statistical Analysis Software 9.1 or "R" 2.6.2 statistical packages (SAS Institute Inc., Cary, NC). According to the subjects' actual smoking status at the time of wounding and blood sampling, the results were presented as mean and standard error of the mean (SEM). Because none of the models disclosed a significant difference in values between the TNP and the placebo groups, data from these groups were pooled for analysis and presentation. A threshold of $P \leq .05$ was considered statistically significant.

RESULTS

Seventy-four of the 78 subjects (95%) completed the study. Four smokers, 1 of whom was randomized to continuous smoking and 3 were randomized to abstinence, withdrew from the study because they did not wish to continue participation. In addition, data from 2 subjects were discarded because they failed to meet the compliance criteria.

Wound diameter and depth. A total of 227 biopsy wounds were measured. The smokers' wounds were smaller and more superficial than the wounds of the never smokers (Table I). After smoking cessation, the abstinent smokers' wound diameter remained unchanged compared with the smoker's wounds (Fig 1, *A*). The wound depth was not affected after the first 4 weeks of abstinence, but thereafter, the abstinent smoker's wound depth increased ($\beta = 0.19 \pm 0.08$; $P < .01$) (Fig 1, *B*). No significant difference in wound depth was found between the abstinent smokers and the never smokers. Also no significant difference was found between TNP and the placebo group (data not shown). When vitamin C was

Table II. Vitamin C, systemic markers of collagen synthesis and degradation, and inflammatory cells in never smokers and abstinent smokers

	Blood samples in never smokers (n = 47)		*Blood samples in smokers (n = 93)*		*Blood samples in abstinent smokers (n = 87)*	
	Mean	*SEM*	*Mean*	*SEM*	*Mean*	*SEM*
Vitamin C (μM/mL)	110.6	5.92	54.13*	5.71	35.15*‡	7.25
PINP (ng/mL)	64.72	4.71	52.73	4.85	56.87§	1.83
P-MMP-8 (ng/mL)	3.74	1.05	6.00	0.81	4.74	1.33
P-MMP-9 (ng/mL)	17.19	1.9	18.00	1.50	16.36	2.42
Neutrophils (billions/L)	3.17	0.19	4.60*	0.19	4.00*‡	0.2
Eosinphils (billions/L)	0.25	0.05	0.32†	0.02	0.28§	0.02

The groups represent actual smoking status at the time of blood sampling. The abstinent smokers represent participants randomized to TNP and placebo. Values are mean.
*Different from never smokers' value ($P < .01$).
†Different from never smokers' value ($P < .05$).
‡Different from smokers' value ($P < .01$).
§Different from smokers' value ($P < .05$).
P, Plasma.

included in the wound depth model, a linear correlation was found ($\beta = 0.002 \pm 0.001$; $P = .01$). The wound diameter was not correlated with vitamin C.

Vitamin C. Plasma vitamin C in smokers was significantly less than the level of never smokers (Table II). After 4 weeks, vitamin C levels decreased equally below baseline level in all subjects independent of smoking status and group allocation (Fig 2, *A*). In the abstinent smokers' group, an interaction between vitamin C levels and a period of abstinence was found ($\beta = 2.23 \pm 0.86$; $P = .01$), indicating a significant increase in vitamin C after smoking cessation. No effect of TNP in the abstinent smokers' group was found (data not shown). Dietary supplementation taken until commencement of the study did not affect vitamin C in either of the groups.

PINP. Plasma PINP tended to be less in smokers than never smokers ($P = .07$; Table II and Fig 2, *B*). In the abstinent smokers' group, PINP was at a greater level than in the smokers. A trend was found suggestive of an interaction between PINP and time from smoking cessation ($\beta = 0.7 \pm 0.37$; $P = .07$). When vitamin C was included in the PINP model, a linear correlation between PINP and vitamin C was found ($\beta = 0.08 \pm 0.03$; $P = .03$). No significant effect of TNP in the abstinent smokers' group was found.

MMP-8, MMP-9, and neutrophil blood count. The total plasma MMP-8 level in smokers tended to be greater than in never smokers ($P = .09$) (Table II). After smoking cessation, MMP-8 was not significantly different from the never smokers' level. The total plasma MMP-9 was not different from smokers and never smokers and did not change after smoking cessation (Table II). In both analyses of MMP-8 and MMP-9, no effect of TNP was found.

The neutrophil blood count was greater in smokers compared with never smokers (Table II). After abstinence, the neutrophil blood count decreased significantly toward the level of never smokers. This change occurred within the first 4 weeks after smoking cessation (data not shown). Eosinophil count showed similar time-related changes to the neutrophil count being significantly greater in smokers and decreasing after smoking cessation (Table II). No TNP-associated changes were observed for either neutrophil or eosinophil count.

The total plasma MMP-8 was correlated with neutrophil blood count ($\beta = 1.94 \pm 0.42$; $P < .001$). A similar correlation was found between the neutrophil blood count and total plasma MMP-9 ($\beta = 0.04 \pm 0.01$; $P < .001$).

Control measurements. The carboxyhemoglobin and cotinine values confirmed that the subjects complied with the study plan (Table III). After 4 weeks of abstinence, the carboxyhemoglobin fraction and cotinine decreased to a minimum and remained less than in the continuous smokers throughout the study. Subgroup analysis of values in the TNP and placebo group confirmed compliance to TNP or placebo patch, respectively.

DISCUSSION

This study shows that smokers have smaller and more superficial wounds 1 week after wounding and tend to have less procollagen I levels in blood

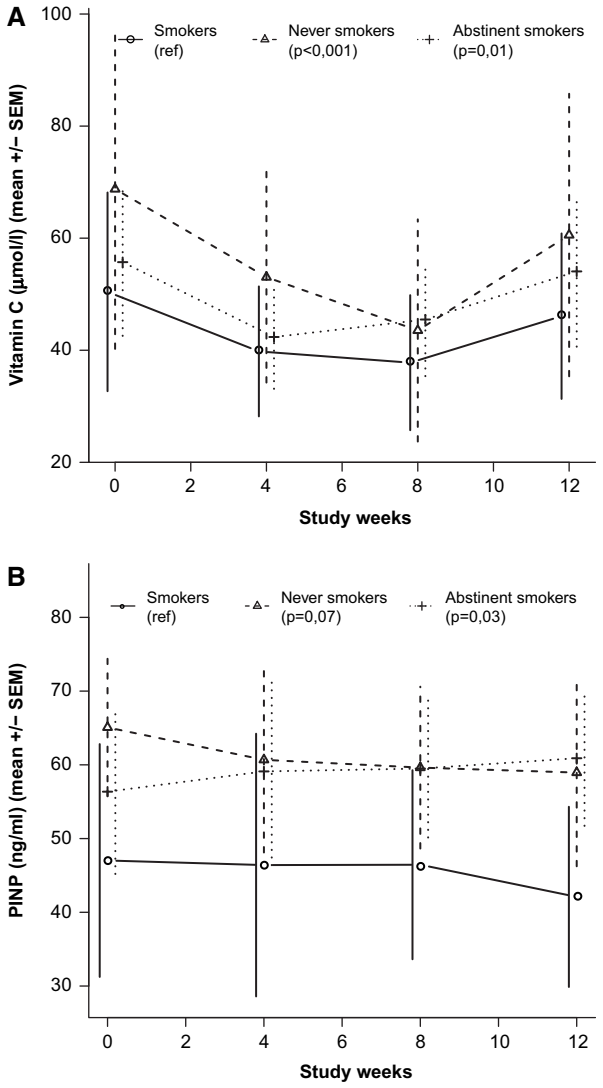

Fig 2. (*A*) Blood levels of vitamin C in smokers, never smokers, and abstinent smokers The y-axis is vitamin C (μmol/L) (mean ± SEM). The x-axis is study weeks. The bold line is smokers (ref). The interrupted line is never smokers ($P < .01$). The dotted line is abstinent smokers ($P = .01$). The footnote shows that values are estimated from the model adjusted for change over time. (*B*) Plasma PINP in smokers, never smokers, and abstinent smokers. The y-axis is P-PINP (ng/mL) (mean ± SEM). The x-axis is study weeks. The bold line is smokers (ref). The interrupted line is never smokers ($P = .07$). The dotted line is abstinent smokers ($P = .03$). Values are estimated from the model adjusted for change over time.

than never smokers. Abstinence from smoking increased wound depth and procollagen I levels but did not affect wound diameter. Both wound depth and procollagen I was correlated linearly with blood levels of vitamin C. Total plasma MMP-8 was marginally affected in smokers but changed, like total plasma MMP-9, with the neutrophil and eosinophil blood counts, which were enhanced in smokers and restored after smoking cessation.

The smokers' smaller and more superficial wounds suggest that smoking enhance wound contraction during early wound healing. Abstinence from smoking seems, in part, to reverse this mechanism beginning 4 weeks after smoking cessation. These findings suggest that healing mechanisms associated with fibroblast function seem to be altered by smoking as reported previously,[39] but also they are reversible to some degree by abstinence from smoking. The following mechanisms may be involved: First, activated fibroblasts may have caused contracture of dermal and adipose tissues, thus changing the wound volume.[40] Second, more fibroblasts may have been differentiated into myofibroblasts possibly stimulated by fibronectin,[41] which in the wound clot, serves as an initial scaffold for wound healing.[42] Plasma fibronectin levels in smokers have been found to be 10-fold greater than in nonsmokers, presumably secreted by vascular endothelial cells injured by reactive oxygen species.[43,44] Also a greater fibronectin level released by activated platelets from the wound clot during hemostasis as a consequence of smoking-induced oxidative stress may have been involved.[45] Third, transforming growth factor-β (TGF-β), which promotes fibroblast differentiation into myofibroblasts,[41] may have been expressed in greater levels during early wound healing, considering previous findings of greater levels of TGF-β in smokers' lung tissue.[46] Finally, mechanical traction, which is a notable factor known to stimulate wound contraction, may have been involved as well, but given that all wounds were located in the same anatomic region, this mechanism is not likely to have contributed to the differences observed.

As found by this model, enhanced wound contraction in smokers during early healing was unexpected but may indicate a faster wound closure, which has been demonstrated in smokers' wounds after excision for pilonidal disease.[47] It is not clear whether contraction and wound closure occurs prematurely in smokers, but it is likely that smoking disturbs these mechanisms. This concept is supported by the fact that wound volume increased after 4 weeks of abstinence and by the substantial evidence from our group and others demonstrating impaired epidermal regeneration, bone fusion, and collagen deposition in smokers indicative of a systemic alteration in smokers' wound healing.[7,9,48]

Table III. Compliance to the study plan

	Blood samples in never smokers (n = *47*)		*Blood samples in smokers* (n = *93*)		*Blood samples in abstinent smokers* (n = *87*)	
Carboxyhemoglobin	0.01	(0.01–0.02)	0.05*	(0.02–0.08)	0.01†	(0.01–0.05)
P-cotinine	0	(0–125)	293*	(100–597)	99†	(0–480)

The groups represent actual smoking status at the time of blood sampling; the abstinent smokers represent participants randomized to TNP and placebo. Values are median and range.
*Different from never smokers' value ($P < .01$).
†Different from smokers' value ($P < .01$).

The lesser blood levels of vitamin C in the smokers and the increase in vitamin C levels as a function of time from smoking cessation suggest a decrease in oxidative stress over time in the abstinent smokers and confirms previous reports.[49,50] The origin of these changes in vitamin C during the course of the study is unclear. Seasonal variation or change in dietary intake of fruit and vegetables by the subjects during the study may explain these findings.[51] Also, our method of stabilizing vitamin C may have affected the results and overestimated the true vitamin C values.[52] Finally, supplementary dietary intake also could have affected vitamin C, but this confounder was recorded at inclusion and was adjusted for in the statistical analysis.

A decrease in systemic oxidative stress by smoking cessation suggested by the increase in blood levels of vitamin C, by a probable concurrent change in substances stimulating fibroblast function, and possibly by myofibroblast differentiation may explain why the abstinent smokers' wounds depth changed as a function of time from abstinence. The change in wound depth was correlated linearly with the vitamin C blood levels, which has not been reported before in vivo. Previous in vitro studies have found that wound contraction mediated by fibroblasts and keratinocytes is attenuated when vitamin C enriched substrate is added to the cell culture.[53] In view of this finding, it is not clear why only wound depth and not wound diameter was affected by abstinence from smoking. Nevertheless, our findings add to other reports suggesting that the clinical importance of vitamin C deficiency in smokers extends beyond that of manifest scurvy.[54]

In the smokers, the plasma level of PINP tended to be less than in never smokers and increased after smoking cessation. Previous studies have demonstrated that the level of amino-terminal collagen I propeptides that reach the circulation are a direct marker of ongoing type I collagen synthesis.[36] In tissue samples, PINP has been associated with fibroblast activity, and immunohistochemical studies have demonstrated an increased synthesis and deposition of PINP during wound healing.[55] In view of that, our findings suggest that smoking affects collagen synthesis systemically, which adds to previous reports of decreased collagen synthesis in the skin and in wound healing models in smokers,[8,9] Similarly, the increase in plasma PINP level after smoking cessation is confirmed by a recent study from our group showing enhanced PINP deposition after 3 weeks of validated smoking cessation in a subcutaneously implanted expanded polytetrafluoroethylene wound healing model.[32] In that study, we found that TNP-enhanced PINP deposition, which is contrary to this study where TNP failed to affect PINP systematically.

The blood levels of vitamin C and PINP were correlated significantly, suggesting that vitamin C predicts collagen synthesis in healthy subjects irrespective of smoking status, confirming experimental evidence from a rodent wound healing model.[56] The current findings also suggest that smoking-induced changes in blood levels of vitamin C may explain the observed alterations in procollagen I levels, thus offering a possible pathophysiologic mechanism for defective collagen synthesis in smokers.

The neutrophil and eosinophil blood counts were greater in smokers than never smokers, whereas total plasma MMP-8 and MMP-9 levels only marginally were affected by smoking status. Four weeks of abstinence from smoking decreased neutrophil and eosinophil blood count toward the level of never smokers, which is consistent with a decrease in oxidative stress.[57,58] The matrix metalloproteases MMP-8 and MMP-9 in plasma changed similarly to neutrophil count, which is not surprising given that proteases are products of this cell type. The lack of a significant association between the plasma levels of these matrix metalloproteases and smoking in the present study compared with previous studies using serum may be related to a lesser level in plasma compared with serum.[25,26,59,60] Yet, the correlation among neutrophil blood count and both MMP-8 and MMP-9 suggest an association between smoking status and

oxidative stress and the release of proteolytic enzymes. This observation supports the view that smoking is a predisposing factor for tissue-destructive disorders and is potentially reversible by smoking cessation.[61]

In conclusion, after 7 days postwounding, smokers have smaller and more superficial wounds, which after smoking cessation, maintain diameter but increase in depth; this outcome is correlated with vitamin C in blood. This correlation may suggest a premature wound closure in smokers and a probable stimulatory impact on wound contraction and fibroblast function by mechanisms modulated by changes in oxidative stress. In addition, oxidative stress in smokers has a detrimental impact on blood levels of vitamin C and inflammatory cells, which affect procollagen I, MMP-8, and MMP-9 in plasma. These effects may be restored in part by abstinence from smoking. Overall, these findings suggest that smoking-induced oxidative stress is a probable pathophysiologic mechanism for the detrimental effect of smoking on wound healing, collagen synthesis, and connective tissue degradation.

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