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A comparison of cutaneous vascular responses to transient pressure loading in smokers and nonsmokers

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Abstract—Smoking has been recognized as a risk factor for pressure ulcer development. This study investigated the hypothesis that smoking causes alterations in cutaneous vascular perfusion, which may contribute to this increased risk. With the use of the laser Doppler fluximetry (LDF), the adaptive vasodilatory response to a transient pressure load at the sacrum was measured in nine healthy female smokers and their age, sex, body mass index (BMI), and menstrual cycle matched nonsmoker controls. In all subjects, removal of the pressure load resulted in a reactive hyperaemic response. The total hyperaemic response was approximately 45% smaller in smokers compared to nonsmoker controls. The reduction was due to a shortening of the duration of the response predominantly through an increase in the rate of recovery from peak, which was twice as fast in the smokers (2.4 ± 1.7 AU × seconds) compared with the nonsmoking controls (1.1 ± 0.9 AU × seconds) ($p < 0.005$). We conclude that changes in the vascular responsiveness can be measured objectively at skin sites at risk of pressure ulcers. We have also shown that vascular responsiveness is altered in light smokers compared to control subjects. These preliminary data open the way for further investigation into the risk factors associated with pressure ulcer development.

Key words: laser Doppler fluximetry, pressure loading, pressure ulcers, reactive hyperaemia, sacrum, skin microcirculation, smoking.

INTRODUCTION

Pressure ulcers are a frequent and costly problem in both primary and acute health care settings. The management of chronic wounds has been estimated to cost the National Health Service (NHS) in the United Kingdom (UK) approximately £1 billion a year [1]. The aetiology of pressure ulcers is multifactorial [2]. However, alterations in cutaneous vascular perfusion generally are recognized to play a major role in tissue breakdown [3], and thus factors that attenuate cutaneous vascular reactivity may contribute to the risk of developing pressure sores. An externally applied pressure load can result in a local reduction in blood flow and ischaemic damage. The subsequent removal of the load results in a hyperaemic response, which is usually beneficial. The magnitude of

Abbreviations: AU = arbitrary unit, AUC = area under the curve, BMI = body mass index, LDF = laser Doppler fluximetry, NHS = National Health Service, NO = nitric oxide, SD = standard deviation.

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the postischaemic hyperaemic response has been shown to be influenced by many factors, including age, sex, smoking history, menstrual status in women, and preexisting cardiovascular disease [4–10]. Several of these factors also are associated with an increased risk of pressure-sore development [11]. Furthermore, it has been suggested that impairment of the hyperaemic response following ischaemia contributes to tissue breakdown.

Various risk assessment tools have been developed to identify patients who are most at risk of developing pressure sores. These include Waterlow, Norton, and Braden scales [12–14]. However, there is little evidence that the use of such risk assessment tools is more effective than clinical judgment or that they improve outcome [14]. A need remains for an objective functional measure of risk that is relevant to disease progression and that can be used easily and effectively in a clinical setting.

Our hypothesis is that the adaptive vasodilatory response to a transient pressure load is altered in smokers as compared to nonsmoker controls. To investigate this hypothesis, we have sought to characterize the cutaneous reactive hyperaemic response following transient pressure loading to identify differences in the response in an "at-risk" group, smokers, and their age- and sex-matched nonsmoker controls.

METHODS

Study Population

The study was performed on a group of nine healthy female smokers and nine controls matched for age, sex, body mass index (BMI), and timing in the menstrual cycle. The smokers were light to moderate smokers with a smoking history of 1 to 4 pack years; the mean (± standard deviation [SD]) was 1.8 ± 1.2 pack years (**Table 1**). The number of pack years is calculated from the number of packs smoked a day multiplied by the number of years. The control subjects had no smoking history. The sample size of nine was calculated to give an 80 percent power to detect a difference in mean blood flow with a 5 percent two-tailed significance level. All volunteers were aged 19 to 27 years, with a mean age (± SD) in the smokers and control subjects of 22.3 ± 2.4 years and 22.2 ± 3.3, respectively. The BMI of the smokers and control subjects was 24.1 ± 4.2 and 24.2 ± 3.3 kg/m^2, respectively (see **Table 1**). Individuals with a history of cardiovascular or respiratory disease and those with recent pressure sores were excluded from the study. The study was performed according to the declaration of Helsinki and approved by the local research ethics committee (LREC) (LREC No. 211/01). It was conducted in the temperature-controlled environment of the clinical research areas of Southampton General Hospital, Southampton, UK.

Table 1.
Baseline data from two study groups of female smokers and nonsmokers. Data are mean (± standard deviation).

Variable	Nonsmokers (n = 9)	Smokers (n = 9)
Age (yr)	22.2 ± 3.3	22.3 ± 2.4
Skin Temperature (°C)	33.4 ± 1.2	31.4 ± 1.2
Body Mass Index (kg/m^2)	24.2 ± 3.3	24.1 ± 4.2
Smoking (pack years)	0	1.8 ± 1.2

Skin blood flux and skin temperature were measured by laser Doppler fluximetry (LDF) (DRT4, Moor Instruments Ltd., Axminster, UK) with the use of a single-point fluximeter probe (DPT1, Moor Instruments Ltd., Axminster, UK). The probe head was mounted in a rigid plastic indenter 50 mm in diameter and held in contact with the skin with a double-sided sticky O-ring (Moor Instruments Ltd., UK). The indenter was mounted vertically and supported by a cantilevered arm fixed to the side of the bed. Skin thickness was measured at the experimental sites with the use of ultrasonography (Dermascan C, Cortex Technology, Hadsund, Denmark).

Study Protocol

All volunteers were asked to refrain from exercising excessively and eating or consuming caffeine-containing drinks or alcohol for 2 hours before attending the laboratory. Smokers were also asked to refrain from smoking a cigarette for at least 2 hours before the study. The volunteers rested for 10 min to acclimatize to room temperature (19.5 °C to 22 °C) before the start of the experiment.

For the experiment, the subjects were in a prone position with the area of the sacrum exposed. The LDF probe, mounted in the rigid indenter, was placed in contact with the skin. Baseline skin blood flux and skin temperature were continuously recorded for 10 min. Following this action, loads of 500 g were applied at 2 min intervals over the indenter to give a final skin loading of 2,500 g, equivalent to a force of 25 N. The load was then removed and measurements continued for a further 10 min or until blood flux had returned to baseline levels. The loading pattern used was similar to that used by Schubert and Fagrell [15].

At the end of the experiment, we measured skin thickness at the sacrum using ultrasonography. Five measurements of the thickness of the epidermis were taken and an average calculated.

In six volunteers, skin blood flux was also measured in the skin of the forearm to obtain a measure of biological zero [16]. Biological zero is the residual laser Doppler signal in the absence of blood flow. We achieved this by arterial occlusion using a sphygmomanometer cuff placed around the upper forearm and inflated to 200 mmHg for 2 min.

Data Analysis

We characterized the cutaneous vascular response to pressure loading by the following parameters obtained from each experimental tracing using the manufacturer's software (see **Figure 1**). Blood flux is expressed in arbitrary units (AUs).

- Mean baseline flux measured over the 2 min period immediately before loading.
- Minimum blood flux recorded at the end of the loading period, with 2,500 g loaded.
- Peak hyperaemic response after removal of the load—the highest flux reached after load removal.
- Time to peak flux from load removal (seconds).
- Duration of hyperaemic response: Time from load removal to recovery to baseline flux (seconds).
- Rate of recovery (AU × s^{-1}).
- $T_{1/2}$: Time required for flux to fall to 50 percent peak (seconds).
- Total hyperaemic response (area under the hyperaemic response flux curve (AU × s).

Data are expressed as mean ± SD. Statistical analysis was performed using the nonparametric Wilcoxon signed rank test to compare the two study groups. A nonparametric statistical test was the most appropriate test, because we cannot be certain that the population has a normal distribution. A p value of <0.05 was taken as significant.

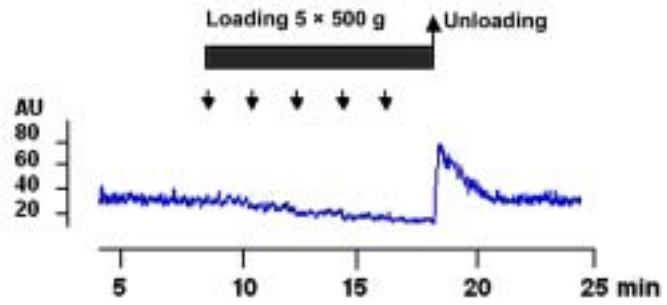

Figure 1.
LDF trace obtained during loading and unloading of skin of sacrum.

RESULTS

Baseline Values

No significant difference was found in the cutaneous baseline flux measured at the sacrum before loading in the smokers and the control group (22.3 ± 19.6 AU and 22.0 ± 8.1 AU, respectively) (**Table 2**). The skin temperature of the smokers (31.4 °C ± 1.2 °C), however, was significantly lower than that of the nonsmoking control group (33.4 °C ± 1.2 °C) ($p < 0.01$) (**Table 1**). No significant difference was measured in skin thickness at the sacrum in smokers (0.600 mm ± 0.006 mm) and nonsmokers (0.610 mm ± 0.021 mm).

Effects of Loading

Loading of the sacrum resulted in a significant reduction in blood flux in all subjects, which did not differ significantly between the two groups (**Table 2**). At the end of the loading period, the mean loaded flux was 7.3 ± 5.9 AU in smokers and 9.1 ± 5.3 AU in nonsmokers. The mean blood flux recorded in the skin of the forearm following arterial occlusion (biological zero) was 4.3 ± 1.8 AU, thus confirming that the pressure load used was sufficient to cause local ischaemia.

Reactive Hyperaemic Response

Removal of the pressure load from the sacrum resulted in a rapid increase in skin blood flux in all volunteers, which reached a peak within ~20 s. The increase in blood flux then decayed and returned to the preload baseline flux within 120 s (**Figure 1**). The total hyperaemic response, calculated as the area under the curve (AUC) of the flux trace, was reduced by more than 45 percent in the smokers to a value of 2565.0 ± 2177.6 AU × s compared with that of 4697.7 ± 3543.3 AU × s in nonsmokers ($p < 0.05$) (**Figure 2(a)**). Further analysis of the flux curve showed no significant difference in the peak flux or in the time to peak between the two groups (**Table 2**). However, a significant reduction was shown in the duration of the response in the smokers (61.2 s ± 34.7 s) compared with nonsmokers (111.4 s ± 53.0 s, $p < 0.05$) and in the rate of recovery from peak, which was twice as fast in the smokers (**Figure (2b)**). The $T_{½}$ of the response

Table 2.
Characterization of hyperaemic response at sacrum in smokers and nonsmokers before, during, and following application of a 25 N pressure load. Data are mean (± SD).

Parameters	Control Group (n = 9)	Smokers (n = 9)	Differences (n = 9)
Basal Flux (AU)	22.0 ± 8.1	22.3 ± 19.6	0.3 ± 23.4
Loaded Flux (AU)	9.1 ± 5.3	7.3 ± 5.9	1.8 ± 8.1
Recovery Flux (AU)	24.0 ± 6.4	24.9 ± 23.2	0.84 ± 27.0
AUC (AU × s)	4697.7 ± 3543.3*	2565.0 ± 2177.6*	2132.6 ± 1990.5
Time To Peak (s)	19.3 ± 22.4	13.8 ± 5.7	5.4 ± 24.6
Peak Hyperaemia (AU)	79.5 ± 17.3	83.6 ± 31.1	4.2 ± 41.6
Recovery Rate (AU × s^{-1})	1.1 ± 0.9*	2.4 ± 1.7*	1.3 ± 1.4
Total Duration (s)	111.4 ± 53.0*	61.2 ± 34.7*	50.2 ± 57.8
$T_{1/2}$ (s)	19.8 ± 12.9	12.2 ± 7.7	7.6 ± 13.8

*$p < 0.05$, Wilcoxon signed rank test.

in smokers was 12.2 s ± 7.7 s compared with 19.8 s ± 12.9 s in nonsmoking controls.

DISCUSSION

The aim of this study was to investigate the hypothesis that the adaptive vasodilatory response to a transient pressure load at the sacrum is altered in smokers compared to nonsmoker control subjects. All tests were completed on female volunteers to eliminate potential gender-related differences in vascular reactivity [17]. The smoking volunteers involved had a short smoking history. Although the deleterious effects of smoking are considered to be time-dependent, studies have concluded that chronic effects of smoking occur relatively early in a person's smoking history [8]. Subjects were also matched for age, BMI, and timing in the menstrual cycle, so any

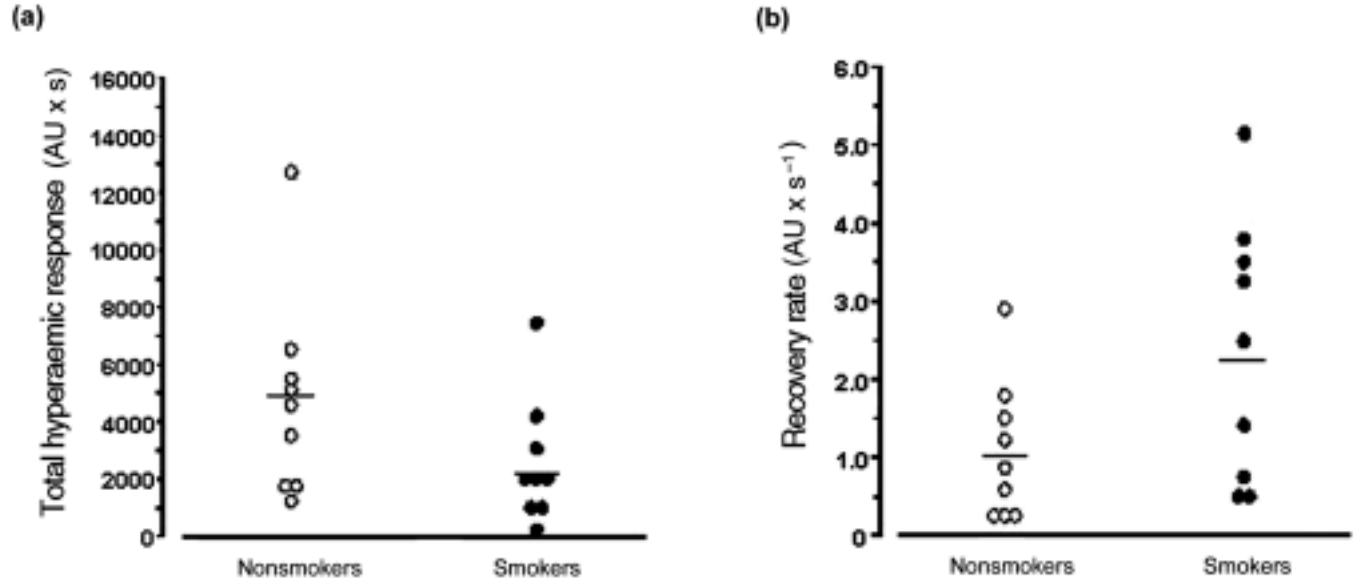

Figure 2.
(a) Total hyperaemic response calculated as area under LDF flux curve (AUC) and **(b)** rate of recovery from peak hyperaemic response, following removal of a 25 N pressure load at sacrum. Data are mean ± SD from nine smokers and nine matched nonsmoker controls. *$p < 0.05$, Wilcoxon signed rank test.

variation in the response because of these parameters was minimized.

We have demonstrated that it is possible to detect differences in the hyperaemic response, measured using LDF, in the skin of smokers and that of nonsmokers. We have further shown that the pressure-induced reactive hyperaemia response in smokers is significantly reduced and that this reduction is a result of an attenuation of the duration of the vasodilatory response.

The response to a short-term occlusion of the circulation is the result of both a myogenic or flow-mediated vasodilatation and the postischaemic release of metabolic factors, such as nitric oxide (NO), adenosine, or cyclooxygenase products. These will determine the peak hyperaemic response and the longer-lasting vasodilatory capacity of the vascular bed, respectively [5,18,19]. Thus, the magnitude of the response is thought to reflect the integrity of the vascular bed and is widely used to assess vascular function.

Various methods have been used to produce a transient ischaemia, including arterial occlusion of a limb and the application of a local pressure load [20,21]. In our study, pressure loading at the sacrum caused a significant and maintained reduction in laser Doppler blood flux in all volunteers, smokers and nonsmokers. The reduction in blood flux at the sacrum while the load was applied was similar to that measured as biological zero in the skin of the forearm, during arterial occlusion. This confirmed that loading to 25 N for 10 min resulted in a local ischaemia. The postischaemic increase in blood flux on removal of the pressure load at the sacrum was similar to that observed at other skin sites, including the forearm and heel [9,22], with both the time to peak and duration of the hyperaemic response measured in the skin of the healthy nonsmoking volunteers lying within the ranges reported previously [23,24].

Smoking is recognized as a potential risk factor in tissue breakdown [8,25]. However, few studies have been performed to characterize the effects of smoking on the reactive hyperaemic response or to determine whether quantification of this may be used in risk assessment in a clinical setting [24]. The 45 percent reduction in total hyperaemic response that we observed indicates a considerable modulation of vascular reactivity even in the young, healthy population of light smokers studied.

While we saw no change in the magnitude of the peak response after a 10 min loading, we did see attenuation in the duration of the response and in the rate of recovery from peak and $T_{½}$. It has been suggested that the later part of the hyperaemic response is NO-dependent, and it has been shown that inhibition of NO synthesis by N^G-monomethyl-L-arginine (L-NMMA) can attenuate a postischaemic vasodilatation in the human forearm [18]. Components of cigarette smoke can inhibit endothelial nitric oxide synthase (eNOS), reduce the bioactivity of NO, and impair endothelium-dependent vasodilatation [25–28]. Thus, it seems likely that the failure to maintain a hyperaemia in our group of smoking volunteers is a result of an attenuation of the direct effect of NO on the microvasculature. However, the mechanisms underlying this in human skin have yet to be explored fully.

We also found that the skin temperature of the smokers was more than 1 °C lower than that of nonsmokers, acclimatized to the same environmental conditions. This finding may be directly related to a reduction in local thermoregulatory blood flow in the smokers consequent to a reduction in the bioactivity of NO. However, as in previous studies, we were unable to detect a difference in the basal blood flow [21,29]. While unlikely, one possible explanation for this is that the fluximetry method of measuring superficial blood flux is not sensitive enough to detect these small changes against such a variable baseline. Alternatively, other vasocontrol mechanisms may be in place in the smokers to maintain basal blood flow.

CONCLUSION

In conclusion, we have demonstrated that it is possible to measure significant changes in the response to a transient pressure load in the skin of an “at-risk” group of smokers, compared with their age-, sex-, hormonal status-, and BMI-matched nonsmoker controls. Furthermore, we have shown that such changes can be measured objectively using an easy, safe, and minimally invasive technique at a skin site common for the development of pressure ulcers. Our findings provide the basis for further studies into the mechanisms underlying the altered responses in smokers and other at-risk groups. The implications of the altered vascular responsiveness in terms of tissue breakdown require further investigation.

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