Effect of cigarette smoking on pentane excretion in alveolar breath

DAVID E. EULER,* SHASHANK J. DAVE, and HONGSHENG GUO

The concentrations of acetone, isoprene, and pentane in alveolar breath were examined in 50 smokers and 50 nonsmokers by gas chromatography. The baseline pentane in smokers was 0.17 ± 0.03 nmol/L (mean ± SE), which was not different from pentane in nonsmokers (0.23 ± 0.03 nmol/L). There were also no differences between smokers and nonsmokers in the concentrations of acetone and isoprene. Serial breath samples were obtained from 15 smokers before smoking and at 5, 15, and 60 min after smoking. Although acetone was not altered by smoking, isoprene increased by 86% ± 26% 5 min after smoking (P <0.001) and returned to baseline 10 min later. Pentane increased by 456% ± 156% 5 min after smoking (P <0.001) and remained increased 10 min later (204% ± 73% of baseline, P <0.05). Isoprene concentrations in mainstream cigarette smoke were >5000 times greater than breath concentrations, whereas pentane could not be detected in mainstream smoke. Because pentane is produced from the peroxidation of n-6 polyunsaturated fatty acids, the results provide evidence that cigarette smoking causes an immediate increase in lipid peroxidation.

INDEXING TERMS: free radicals • lipid peroxidation • gas chromatography • hydrocarbons • alkanes • acetone

Cigarette smoking has been implicated in the etiology of respiratory disease, cancer, and atherosclerosis [1–3]. Tobacco smoke contains large numbers of free radicals that are capable of initiating or promoting oxidative injury [4]. Reactive oxygen species may also be generated in smokers by phagocytes that are activated in response to pulmonary inflammation [5, 6]. Several studies have suggested that oxidative injury may play a seminal role in mediating the health risks associated with cigarette smoking [4]. Cigarette smokers have higher lipid peroxidation products in their blood than do nonsmokers [7–10], and smoking increases the serum concentrations of malondialdehyde [7–9] and F2-isoprostanes [10]. The increased serum concentrations of malondialdehyde were reduced by dietary supplements of vitamin E [9], and the high concentrations of F2-isoprostanes fell significantly (P = 0.03) after 2 weeks of abstinence from smoking [10]. Chronic smoking has also been shown to increase the rate of oxidation of isolated low-density lipoprotein cholesterol and its uptake by macrophages [11].

Although numerous methods are available for measuring oxidative stress in human subjects, the measurement of alkanes in expired breath is the only noninvasive method [12]. Pentane is generated from peroxidation of n-6 polyunsaturated fatty acids and the subsequent beta-scission of the intermediate hydroperoxides [13]. Pentane is only a minor reaction product, and its formation depends on the presence of transition-metal ions [14]. Despite these limitations, the use of pentane as an in vivo marker of lipid peroxidation has been validated extensively in experimental animals [12].

Pentane excretion has been shown to be higher in smokers than nonsmokers [15, 16] and to be decreased in smokers by dietary supplements of either vitamin E [15] or beta-carotene [16]. A major difficulty with the measurement of pentane in human breath is the coelution of isoprene on most chromatographic columns [17–20]. Isoprene is also the predominant hydrocarbon in tobacco smoke [21, 22], and the passive increase of this effluent in the breath of smokers might be misinterpreted as an increase in pentane. In addition to alkane formation, lipid peroxidation has been shown to produce acetone [23, 24] in experimental animals. Because acetone is a major constituent of human breath [18, 20, 25–28], conceivably the amount of this effluent might change if tobacco smoke initiates or promotes lipid peroxidation.

The purpose of the present study was to measure acetone, isoprene, and pentane in the alveolar breath of smokers and nonsmokers by injecting single-breath samples directly into a gas chromatograph. Serial sampling of alveolar air was performed to determine the effects of smoking one cigarette on the excretion of these three effluents. Ambient air was analyzed to determine the contribution of exogenous contaminants to breath effluents. Mainstream cigarette smoke was also analyzed to determine the passive contribution of tobacco combustion products to breath effluents.

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Materials and Methods

Breath samples were collected from 100 healthy volunteer subjects, 50 smokers and 50 nonsmokers. Subjects were excluded if they reported a history of arthritis, cirrhosis, colitis, diabetes, or ischemic heart disease. Subjects were also excluded if they were taking dietary supplements of vitamin E, vitamin C, or beta-carotene. The nonsmokers were excluded if they reported a history of chronic exposure to secondhand smoke. The smokers group included only those who smoked at least 20 cigarettes per day for at least 2 years. All subjects were breathing smoke-free ambient air for 1 h or more before breath sampling. Serial breath samples were obtained from 15 of the smokers, alveolar air being sampled before and at 5, 15, and 60 min after these subjects smoked a single cigarette. All 15 subjects inhaled the smoke and smoked the cigarette to completion. The protocol was approved by the Institutional Review Board of Loyola University Medical Center.

Alveolar air samples (750 mL) were collected with a commercially available breath collection system (Quinton, Menomonee Falls, WI). Preliminary studies showed that the concentrations of organic effluents in breath were stable in these multilaminate collection bags for 24 h. Breath samples were aspirated from the collection bags into air-tight glass syringes (60 mL) within 5 h of breath sampling. Samples of ambient air (500 mL) were also obtained at the time of breath sampling. To examine the organic components in cigarette smoke, we collected mainstream smoke in a 150-mm length of polyvinylchloride tubing (6.4 mm diameter), drawing ambient air through a lit cigarette into the tubing at a flow rate of ~800 mL/min. When the cigarette had burned to a butt length of 10 mm (~2 min), the air flow was terminated and a smoke sample (10 mL) was aspirated through a side port in the tubing into an air-tight glass syringe and diluted with 1500 mL of hydrocarbon-free air. A total of four different filter cigarette brands were tested (three cigarettes per brand). The brands were chosen on the basis of their frequency of use by the 50 smokers.

Alveolar air, ambient air, and mainstream cigarette smoke were analyzed by gas chromatography according to previously published methods [20]. We modified our original method to include a 25 m × 0.53 mm Poraplot Q capillary column (Chrompack, Raritan, NJ), which had a capacity ratio of 6.0 (33 000 theoretical plates). Helium was used as the carrier gas at a flow rate of 10 mL/min. The temperature of the sampling loop was ambient, the injector temperature was 90 °C, and the detector temperature was 200 °C. The column temperature was held at 30 °C for 1 min, then increased by 40 °C/min to 95 °C, by 2.0 °C/min to 119 °C, and by 50 °C/min to 200 °C and was held there for 2 min. A single run required ~30 min.

Calibration was performed with a commercially available reference gas mixture of hydrocarbons (C2-C9) at a concentration of 100 μL/L (Alltech, Deerfield, IL) and appropriate dilutions. A calibration curve was constructed from four pentane concentrations: 0 nL/L, 12.5 nL/L (0.51 nmol/L), 50 nL/L (2.04 nmol/L), and 250 nL/L (10.2 nmol/L). The slope of the pentane calibration curve was 16.6 (SE 0.04), with S_p = 0.42.

The concentration of isoprene was estimated from the pentane calibration curve [20, 27, 28]. A calibration curve was also prepared for acetone (Sigma, St. Louis, MO) by diluting 100–400 μL of liquid acetone with 2000 mL of organic-free water. An aliquot of each dilution (10 μL) was vaporized into 1500 mL of hydrocarbon-free air. The slope of the acetone calibration curve was 12.5 (SE 0.05), with S_p = 1.3.

The precision of the breath assay was determined by measuring the intra- and interassay CVs for repeated measurements in one nonsmoker. The intraassay CV was determined by drawing 10 air samples (60 mL) from one breath collection bag and was 1.8% for acetone, 2.3% for isoprene, and 13% for pentane. The interassay CV, determined by collecting 10 sequential alveolar air samples (750 mL) into different bags over a 10-min interval, was 3.5% for acetone, 5.9% for isoprene, and 24% for pentane.

Where possible, the results were expressed as the mean ± SE. An unpaired t-test was used to compare group means when group variances were equivalent. When an F-test showed unequal group variances, then group means were compared with a Mann–Whitney test. When the data were expressed as the frequency of an event or characteristic, a Fisher’s exact test was used to make comparisons. Repeated-measures analysis of variance (ANOVA) was used to compare serial breath samples in smokers. If the ANOVA yielded a significant F-value, then individual group means were compared with a Student–Newman–Keuls test.

Results

Of the 50 subjects in each group, 60% of the smokers and 64% of the nonsmokers were men (P = 0.20). The mean age of the smokers was 44.7 ± 1.6 years (range, 23–72) and not significantly different from the mean age of the nonsmokers (48.0 ± 2.3; range, 22–75). The smokers smoked an average of 22.2 ± 0.7 cigarettes per day. The cumulative total of cigarettes smoked ranged from 5 to 120 pack years (mean 27.7 ± 2.7). Of the 15 smokers in the subgroup that had serial breath sampling, 73% were men, ranging in age from 23 to 70 (mean 45.2 ± 4.1). This group smoked a cumulative total of from 5 to 69 pack years (mean, 29.1 ± 5.0).

Table 1 shows the concentrations of organic effluents that were measured in the breath of smokers and nonsmokers. Acetone concentrations were identical in the two groups of subjects. Although isoprene and pentane tended to be lower in smokers, the differences did not reach statistical significance. The concentrations of acetone, isoprene, and pentane were also measured in ambient air to determine the passive contribution of exogenous effluents to breath effluents. The number of ambient air samples (n = 50) was less than the total number of subjects in the study because breath samples were often obtained from multiple subjects breathing the same ambient air. The concentrations of acetone, isoprene, and pentane in ambient air were significantly less (P < 0.001) than the concentration of these effluents in the breath of both smokers and nonsmokers. For acetone and isoprene, a positive gradient from alveolar air to ambient air was present in 100% of smokers and 100% of nonsmokers. For pentane, 80% of smokers and 90% of nonsmokers had a positive alveolar-to-ambient gradient. The dif-
ference in the pentane gradient between the two groups was not significant (\( P = 0.26 \)).

To determine the passive contribution of smoke effluents to breath effluents in smokers, we also measured the concentrations of acetone, isoprene, and pentane in mainstream cigarette smoke (Table 1). The average concentration of acetone in mainstream smoke was ~800 times greater than the average concentration in alveolar air. Isoprene showed a 5500-fold gradient from smoke to alveolar air. In contrast, the concentration of pentane in mainstream smoke was below the detection limits of our method (0.02 nmol/L).

Figure 1 shows chromatograms of alveolar air samples obtained from a representative subject before and 5 min after smoking. This subject was a 33-year-old man who had smoked 1 pack of cigarettes per day for 7 years. The retention times of the peaks that were identified were 9.0 min (acetone), 11.6 min (isoprene), and 12.6 min (pentane). Comparison of the chromatograms recorded before and after smoking shows a dramatic increase in the amplitude of the pentane peak. The pentane area increased from 2.0 to 13.0 (mV·s). There was also an increase in the area of the isoprene peak (from 137 to 157 mV·s) and the acetone peak (from 81 to 100 mV·s). Fig. 1C shows a chromatogram of a sample of mainstream cigarette smoke from the same brand of cigarettes smoked by this subject. The smoke sample was diluted 150:1 with hydrocarbon-free air before it was injected into the gas chromatograph. The most striking feature of this chromatogram is the absence of any component with a retention time close to where pentane should have eluted (arrow). The absence of a pentane peak was not due to the scale on the ordinate, since no pentane peak was observed even at 50-fold magnification.

The short-term effects of smoking on the excretion of volatile organic compounds in alveolar air are summarized in Fig. 2. The alveolar concentration of acetone 5 min after smoking (13.2 ± 1.7 nmol/L) was not significantly different from baseline (12.6 ± 2.0 nmol/L). Similar acetone concentrations were also measured at 15 and 60 min after smoking. In contrast to acetone, the alveolar concentration of isoprene increased from 6.5 ± 0.84 to 10.3 ± 1.1 nmol/L 5 min after smoking. This represented a relative increase in isoprene concentration of 68% ± 26% (\( P < 0.001 \)). Isoprene returned to baseline values 15 min after smoking and showed no further changes at 60 min. Compared with the brief changes in isoprene excretion, the effect of smoking on pentane excretion was more prolonged. Five minutes after smoking, the concentration of pentane in alveolar air increased from 0.23 ± 0.06 to 0.96 ± 0.24 nmol/L. This represented a relative increase in pentane of 456% ± 198% (\( P < 0.001 \)). Despite a partial return towards baseline 15 min after smoking, the mean pentane concentration was still significantly (\( P < 0.05 \)) increased (0.57 ± 0.19 nmol/L). By 60 min the mean pentane concentration (0.38 ± 0.16 nmol/L) was not significantly different from baseline.

### Table 1. Organic effluents in breath, cigarette smoke, and ambient air.

<table>
<thead>
<tr>
<th></th>
<th>Conc. nmol/L</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Acetone</td>
<td>Isoprene</td>
<td>Pentane</td>
</tr>
<tr>
<td>Non-smokers</td>
<td>14.7 ± 2.2</td>
<td>7.2 ± 0.5</td>
<td>0.23 ± 0.3</td>
</tr>
<tr>
<td>Smokers</td>
<td>14.7 ± 1.9</td>
<td>6.2 ± 0.4</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Ambient air</td>
<td>1.4 ± 0.10*</td>
<td>0.18 ± 0.03*</td>
<td>0.05 ± 0.01*</td>
</tr>
<tr>
<td>Mainstream smoke</td>
<td>11 789 ± 920*</td>
<td>34 086 ± 3238*</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not detected.

*P<0.0001 compared with concentrations in smokers and non-smokers.

Fig. 1. Chromatograms of a breath sample obtained before smoking (A) and 5 min after smoking (B) in one subject and (C) mainstream cigarette smoke from the same brand of cigarettes smoked by this subject.

Peaks: (1), acetone; (2), isoprene; (3), pentane. The arrow in C marks the time that pentane should have eluted had it been present (see text).
Discussion

The oxidative properties of the tar and gas phases of cigarette smoke have been elucidated [4]. Carbon- and oxygen-centered radicals are thought to form in the gas phase from the interaction of nitrogen dioxide with other organic components such as isoprene. One puff of smoke is estimated to contain $10^{17}$ organic free radicals in the gas phase [4]. The tar phase of cigarette smoke contains a quinone/semiquinone complex, which can reduce oxygen to superoxide anion. The highly reactive radicals from the gas and tar phases of cigarette smoke would be exposed initially to the alveolar surface of epithelial cells. Free radicals or their reaction products might also diffuse through the epithelium, interstitium, and endothelium to peroxidize lipids in blood cells or serum lipoproteins. Lipid peroxidation may also result from the sequestration and activation of neutrophils in the pulmonary microvasculature.

In the present study, we used the excretion of pentane in alveolar breath as an indirect index of lipid peroxidation. A limitation of our method is that for peak identification we used the retention time of reference standards rather than mass spectrometry. However, we have also analyzed breath by using a more polar column (Poraplot U) to change the elution order of the peaks [20, 27, 28]. Because we observed the same breath concentrations of acetone, isoprene, and pentane with both columns, our peak identification is quite likely accurate. Furthermore, Kohlmueller and Kochen [18] have confirmed our peak identification in breath by using a Poraplot U column and mass spectrometry.

The 456% increase in pentane we observed 5 min after smoking suggests a profound, sudden increase in lipid peroxidation. We waited 5 min to sample alveolar air in order to flush the gas-phase components of cigarette smoke from the lungs. The pentane concentrations possibly would have been even higher if sampled while smoke was still in the alveoli. The enhanced lipid peroxidation appeared to last for at least 15 min. The absence of pentane in mainstream cigarette smoke indicates that the pentane was of human origin. Although pentane was present in low amounts in ambient air, it was not detectable in mainstream smoke because the smoke samples were diluted with hydrocarbon-free air. The failure of tobacco combustion to generate pentane supports the results of Barrefors and Petersson [21], who used gas chromatography and mass spectrometry to measure the hydrocarbon content in sidestream and environmental tobacco smoke.

Because of the extreme reactivity of free radicals, it is not surprising that the increased lipid peroxidation was temporary. A similar transient increase in arterial concentrations of conjugated dienes was observed in anesthetized rabbits exposed to mainstream cigarette smoke for 4 s [29]. However, in contrast to our results, Morrow et al. [10] reported that smoking (3 cigarettes/30 min) had no immediate effects on plasma concentrations of F$_2$-isoprostanes. Perhaps the formation of F$_2$-isoprostanes is delayed relative to the formation of pentane or conjugated dienes.

Although smoking had rather dramatic short-term effects on pentane excretion, no long-term effects were observed. This finding conflicts with previous studies by Hoshino et al. [15] and Hallard et al. [16], who reported that pentane excretion was increased by ~50% in smokers. However, these previous investigations did not provide any chromatographic evidence of the separation of pentane from other volatile organic components in breath. An important limitation of the use of basal pentane concentrations as an index of lipid peroxidation is that pentane undergoes significant hepatic metabolism [30, 31]. Furthermore, the high lipid solubility of pentane should result in large stores of this compound in body fat, which exchanges very slowly with blood [19]. The sustained increase in plasma concentrations of malondialdehyde and F$_2$-isoprostanes reported previously in chronic smokers [7–10] may reflect differences in the formation, distribution, or metabolism of these products in comparison with pentane.

Previous studies that analyzed cigarette smoke by gas chromatography have shown isoprene to be the predominant hydrocarbon produced during tobacco combustion [21, 22]. The analysis of mainstream cigarette smoke in our study confirmed these results. Despite an isoprene concentration in mainstream smoke that was 5500 times greater than the concentration in alveolar air, smoking caused only an 86% increase in breath
isoprene. As a water-insoluble hydrocarbon, isoprene may not diffuse through interstitial fluid at a rate sufficient to equilibrate with pulmonary capillary blood. Alternatively, studies in experimental animals have shown that isoprene may be partially metabolized before it reaches the circulation [32].

Studies on the role of acetone as an index of lipid peroxidation have given controversial results. Exposure of isolated perfused hearts to a free-radical-generating system caused increased acetone release [24]. Likewise, the treatment of intact rats with several free-radical-generating xenobiotics caused increased urinary excretion of acetone [23]. However, in vitro studies of the iron-catalyzed decomposition of linoleic hydroperoxide showed no acetone formation, despite formation of hexanal and pentane [33]. Because acetone is a normal human metabolite produced by spontaneous decarboxylation of acetocacetate, it is difficult to determine the portion in breath that might be due to lipid peroxidation. In the present study, smoking had neither short- nor long-term effects on breath acetone. Despite an 800-fold gradient from mainstream smoke to alveolar air, acetone was not increased 5 min after smoking.

In summary, we have shown that basal pentane excretion in smokers is similar to pentane excretion in nonsmokers. However, the inhalation of smoke from a single cigarette is followed by a sudden increase in the concentration of pentane in alveolar air, which lasts <60 min. Because pentane was not present in mainstream cigarette smoke, it was most probably produced endogenously by free radical attack on polyunsaturated fatty acids. These findings provide the first evidence for an immediate, smoking-induced increase in lipid peroxidation in humans. Conceivably, the enhanced lipid peroxidation may be modifiable by antioxidant interventions. Additional investigation is needed to determine if the magnitude of the smoking-induced increase in pentane has any prognostic significance.

References


