Pentane and Isoprene in Expired Air from Humans: Gas-Chromatographic Analysis of Single Breath
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Both pentane and isoprene are excreted in human breath. Although pentane is considered an index of lipid peroxidation, the significance of isoprene is unknown. Having a similar boiling point, these two hydrocarbons are difficult to separate by gas chromatography. We separated pentane from isoprene on both a Poraplot Q and a Poraplot U column, injecting single-breath samples directly into a gas chromatograph. The breath samples were pressurized to 800 mmHg to increase the amount of sample volume delivered to the column. In a group of 43 healthy volunteers, the concentrations of end-expiratory pentane and isoprene were 0.57 ± 0.3 and 7.05 ± 3.53 nmol/L, respectively. There was a significant linear correlation (r = 0.57, P <0.0001) between age and pentane concentration in expired air; isoprene showed no correlation with age or pentane concentrations. The age-related increase in pentane production suggests that oxidative stress may play a role in the aging process in humans. The method described should allow for rapid, inexpensive, serial measurement of expired pentane and isoprene.

Indexing Terms: lipid peroxidation/hydrocarbons/free radicals/respiratory gases

The formation of free radicals and lipid peroxidation has been implicated in many conditions that lead to cell injury. The measurement of breath alkanes, especially ethane and pentane, has been used as a noninvasive index of lipid peroxidation. Although both gases have been measured by gas chromatography (GC) in the breath of experimental animals, studies in humans are usually limited to the measurement of pentane (1, 2). For measurement of breath pentane to be widely accepted as a diagnostic indicator of cell injury, one must recognize some of the limitations of the methodology used to perform the assay. Several studies have identified isoprene as the major hydrocarbon of human breath (1, 3–6), and two reports describe the coelution of pentane and isoprene on most GC columns (1, 6). However, isoprene was separated from pentane on a porous-layer open-tubular column (Poraplot U) (1, 6).

Because the amount of pentane in expired air is less than the detection limit of most flame-ionization detectors (FIDs), it is often necessary to concentrate pentane before analysis. This has been accomplished by passing large volumes of expired air through cold- or ambient-temperature traps containing hydrocarbon adsorbents (6–10). Some previously reported methods also included a rebreathing circuit as part of the apparatus so as to concentrate the pentane in the lungs and reduce the volume of expired air required for analysis (2, 7).

Recently, a technique for the analysis of pentane from a single-breath sample injected directly into a gas chromatograph has been described (11). Here, we report the use of porous-layer open-tubular columns to separate isoprene from pentane in combination with a single-breath sampling technique. Using this method, we investigated the pentane and isoprene concentrations of expired air in normal volunteers and the variation of the concentration of these hydrocarbons in relation to subject age.

Materials and Methods
We collected end-expiratory breath samples (125 mL) from 43 healthy volunteers (20 men, 23 women) by use of a modified Haldane–Priestly tube (12). The volunteers were nonsmokers between ages 22 and 75 years. Subjects breathed into a polyvinylchloride tube (1.2 cm (i.d.) with a disposable mouthpiece at one end and a one-way valve at the other. Breath samples were aspirated into 60-mL polyethylene syringes (Fortuna Syringe; Aldrich Chemical Co., Milwaukee, WI) through a stopcock inserted just proximal to the one-way valve. Samples were collected in the morning, 3 h after the last meal, and then analyzed within 3 h. All subjects were breathing room air for at least 1 h before the breath sampling. Procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Gas Chromatography
To analyze the hydrocarbons in expired air, we used a gas chromatograph (5890 series II; Hewlett-Packard, Naperville, IL) equipped with a gas sampling valve, a 10-mL sampling loop, and a FID operating in at 1 nA/V. The sample loop was flushed with 40 mL of the breath sample and manually pressurized to 800 mmHg with the last 20 mL of the sample by use of a digital manometer (UM 200/200; Netech, Hicksville, NY). The hydrocarbons were eluted by passing the sample through a 10
m × 0.53 mm Poraplot Q capillary column (Chrompack, Raritan, NJ) with helium as the carrier gas at a flow rate of 4 mL/min. The temperature of the sampling loop was 70°C, of the injector 90°C, and of the detector 225°C. To minimize peak broadening from the large sample volume, we held the column temperature at 30°C for 2 min to concentrate the hydrocarbons at the head of the column. The column temperature was then increased by 20°C/min to 95°C and held for 1 min. Thereafter, the column temperature was increased by 0.5°C/min to 103°C, held for 1 min, and then increased to 200°C at 20°C/min and held for an additional 2 min.

For comparison, we also analyzed the breath samples of 17 subjects on a 10 m × 0.53 mm Poraplot U capillary column (Chrompack), using helium at a flow rate of 3 mL/min. The temperature was held at 30°C for 2 min and increased by 20°C/min to 90°C. Thereafter, the column temperature was increased by 5°C/min to 115°C and by 20°C/min to a final temperature of 190°C, which was held for 3 min.

Chromatogram data acquisition, storage, and peak area integration was done with a 386 PC and Peaksimple II software (SRI Instruments, Menlo Park, CA). Linear regression analysis was performed to examine the relationship between variables. Results are expressed as the mean ± SD. Statistical analyses were carried out with a PC version of Arcus Professional Statistical Analysis Software (Arcus, West Lancs, UK).

Calibrations

Calibrator gas mixtures were prepared by using a commercially available mixture of hydrocarbons (C₂–C₁₀) at a concentration of 100 μL/L (Alltech Associates, Deerfield, IL). An aliquot of the C₄–C₁₀ gas (1.0–10.0 mL) was drawn into a gas-tight syringe (Hamilton Co., Reno, NV) and injected into a Teflon bag that had been flushed with hydrocarbon-free air. Using either a 500- or 1500-mL gas-tight syringe (Hamilton), we injected various amounts of hydrocarbon-free air into the bag to achieve the desired dilution. To mix the commercial gas and air in the bag, we repetitively withdrew the entire contents of the bag into a 1500-mL syringe and then refilled the bag. The diluted calibrator gas mixtures were injected into the gas chromatograph in the same manner as the breath samples. Calibration curves were plotted for four pentane concentrations: 0 nL/L, 20 nL/L (0.82 nmol/L), 50 nL/L (2.04 nmol/L), and 100 nL/L (4.09 nmol/L). The CVs (n = 5) for the latter three calibrators were 6.5%, 4.1%, and 5.8%, respectively. The calibration slope for the Poraplot Q column was 0.62 ± 0.005 (n = 5); for the Poraplot U column, it was 0.59 ± 0.05 (n = 4). For all calibration curves, r > 0.95.

To determine the retention times of isoprene, acetone, and ethanol, we vaporized 50 μL of each reagent (Aldrich) separately and diluted each sample with hydrocarbon-free air. The concentration of isoprene was estimated from the pentane calibration curves; these two hydrocarbons have the same number of carbon atoms, and the FID response to hydrocarbons is thought to be predominantly determined by the carbon content (13). To verify that the FID responses to pentane and isoprene were equivalent, we vaporized 50 μL of liquid isoprene and pentane in a Tedlar bag and diluted as above. The response of the FID to isoprene was 9% greater than for pentane. Because we felt that a 9% difference was within the range of experimental error, we considered these responses to be equivalent.

Results

Isoprene and pentane could not be detected in room air. The CVs for measuring pentane and isoprene in repeated breath sampling of a single subject every 2 min over a 10-min period were 20.5% and 17.0%, respectively (n = 5). Pressurization of the sample loop to 800 mmHg doubled the area of individual peaks but did not alter the peak widths or retention times. Injection of hydrocarbon-free air samples between runs showed no carryover of any component from one run to the next.

The elution order of breath hydrocarbons on the Poraplot Q column was acetone (14.2 min), isoprene (19.6 min), and pentane (20.5 min) (Fig. 1A). On the Poraplot U column the elution order was ethanol (10.9 min), pentane (11.1 min), isoprene (11.8 min), and acetone (12.3 min) (Fig. 1B). When a breath sample from the same subject was run on both columns (Fig. 1), the concentration of pentane obtained with the Q column

![Fig. 1. Chromatogram of an expired breath sample from a single subject, eluted on a Poraplot Q capillary column (A) and on a Poraplot U capillary column (B).](image-url)
(0.38 nmol/L) was similar to the pentane concentration determined with the U column (0.42 nmol/L). Likewise, the isoprene concentration obtained with the Q column (7.75 nmol/L) was similar to that obtained with the U column (8.12 nmol/L).

The mean concentration of end-expiratory pentane for all 43 subjects included in the study was 0.57 ± 0.3 nmol/L; the mean isoprene concentration was 7.05 ± 3.53 nmol/L. To determine the influence of age on pentane concentration, we assessed these two variables by linear regression analysis. We found a direct relation between age and pentane concentration (r = 0.57, P < 0.0001), but isoprene concentration did not vary significantly with age (Fig. 2). Likewise, there was no significant relation between isoprene concentration and pentane concentration.

Discussion

The mean pentane concentration in breath for 43 healthy subjects was 0.57 ± 0.3 nmol/L. It is difficult to compare this value with previously published values—which range from 0.004 nmol/L (9) to 5.4 nmol/L (14) in adults—given the absence of a commonly accepted method for performing the analysis. The large variability in pentane concentrations reported in the literature may be partly attributed to the techniques used to determine the concentration of hydrocarbons in expired air. The reproducibility and recovery rates of commonly used trapping techniques are often not documented. Furthermore, the use of a rebreathing circuit in some studies may have influenced the results: Pentane undergoes significant hepatic metabolism (2). Coelution of isoprene and pentane also represents a significant source of variability. Because commonly used columns fail to resolve isoprene from pentane (1, 6), many authors may have unknowingly reported the combination of isoprene and pentane as pentane.

In the present study, we obtained a clear separation of pentane and isoprene on both Poraplot Q and U columns (Fig. 1). Because we did not use mass spectroscopy, one might argue that comparison of retention times with those of the calibrators does not guarantee reliable peak identification. However, the peak elution order and the relative peak amplitudes we observed on the Poraplot U column were identical to those reported by Kohlmuller and Kochen (1), using mass spectroscopy. Although these investigators could not resolve pentane from isoprene on a Poraplot Q column, the temperature ramp they used may have been too steep to achieve adequate resolution. We were able to resolve pentane from isoprene on the Q column only when we used a temperature ramp of 0.5°C/min above 95°C. Given that the concentrations of pentane and isoprene were nearly identical for analysis of breath samples from one subject on the two different columns (Fig. 1), it is quite likely that our peak identification was accurate.

Kohlmuller and Kochen (1), using total breath sam-

ples, reported lower concentrations of pentane (0.13 ± 0.11 nmol/L) and isoprene (1.62 nmol/L) than we observed with the single-breath analysis. However, the subjects in their study were all ages 20–30 years. The nine subjects in that age range in our study had mean ± SD pentane of 0.28 ± 0.13 nmol/L and an isoprene of 5.54 ± 2.95 nmol/L. The higher concentrations of hydrocarbons we observed may be due to the sampling of end-expiratory air; Zarling and Clapper (11) found higher hydrocarbon concentrations in end-expiratory breath than in total breath. Alternatively, the isoprene concentrations that we determined from our pentane calibration curves may have been systematically biased if the concentrations of pentane calibrators were less than the measured isoprene concentrations.

The direct linear relation we observed between age and pentane concentration supports the hypothesis that aging is associated with increased lipid peroxidation. The aging process may be accompanied by increased production of free radicals and (or) a decrease in antioxidant defense mechanisms. Our results are in agreement with a preliminary study in humans that suggested that pentane excretion was altered in older subjects (15). Plasma malondialdehyde, another index of lipid peroxidation, also reportedly increases with age (16). Additionally, pentane production is directly related to age in rats (17), houseflies (18), and honeybees (19). In a study on the biochemical correlates of longevity in the housefly, longer-lived strains

Fig. 2. Simple linear regression analysis of age vs (A) pentane and (B) isoprene in expired air.
had higher superoxide dismutase/metabolic rates and higher activities of catalase, glutathione reductase, and thioredoxinase, supporting the view that longer-lived flies manifest lower levels of oxidative stress than do shorter-lived flies (20).

Kohlmuller and Kochen suggested that oxygen-derived free radicals might interact with polyisoprenes as well as with membrane lipids, so that both isoprene and pentane are produced (1). However, we found no correlation between isoprene and pentane excretion, which suggests that polyisoprenes may not be substrates for free-radical attack.

Increased concentrations of pentane in expired air have been reported in various conditions, e.g., exercise (9, 21), rheumatoid arthritis (22), oxygen exposure (23), smoking (24), vitamin E deficiency (25), lipid infusions (26), alcoholic cirrhosis (27), endogenic psychoses (16), acute myocardial infarction (28), Alzheimer disease (29), necrotizing enterocolitis (30), and multiple sclerosis (31). In view of the difficulties in separating pentane and isoprene, we recommend reevaluating the exhalation of pentane by humans as an index of lipid peroxidation in health and disease.

References