

METHODS FOR MEASURING ETHANE AND PENTANE IN EXPIRED AIR FROM RATS AND HUMANS

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Abstract—Numerous studies in animals and humans provide evidence that ethane and pentane in expired air are useful markers of *in vivo* lipid peroxidation. The measurement of breath hydrocarbons, being noninvasive, is well suited for routine use in research and clinical settings. However, the lack of standardized methods for collecting, processing, and analyzing expired air has resulted in the use of a wide variety of different methods that have yielded highly disparate results among investigators. This review outlines the methods that we have developed and validated for measuring ethane and pentane in expired air from rats and humans. We describe the advantages of these methods, their performance, as well as potential errors that can be introduced during sample collection, concentration, and analysis. A main source of error involves contamination with ambient-air ethane and pentane, the concentrations of which are usually much greater and more variable than those in expired air. Thus, it appears that the effective removal of ambient-air hydrocarbons from the subject's lungs before collection is an important step in standardizing the collection procedure. Also discussed is whether ethane or pentane is a better marker of *in vivo* lipid peroxidation. © 2000 Elsevier Science Inc.

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INTRODUCTION

Ethane and pentane are the main volatile hydrocarbons formed during the breakdown of peroxidized n-3 and n-6 fatty acids, respectively [1]. The formation of ethane and pentane in the process of lipid peroxidation represents

only a secondary pathway [2], but is of special importance because these hydrocarbons are exhaled through the breath and can thus be studied noninvasively.

The first documentation of breath ethane as a marker of *in vivo* lipid peroxidation dates from 1974, when Reily et al. [3] reported that mice exhaled large amounts of ethane after exposure to carbon tetrachloride, a potent catalyst of *in vitro* lipid peroxidation. In 1977, Tappel's group extended these findings by demonstrating the presence of markedly increased amounts of not only ethane, but also pentane in the breath of vitamin E-deficient rats [4]. Since these initial reports, a number of compelling studies have shown increased exhalation of ethane and/or pentane in response to various oxidant stresses. In animals, for example, substantial increases have been observed after exposure to hyperbaric oxygen [5], iron excess [6], and phenylhydrazine [7]. In humans, increases in ethane and/or pentane have been observed subsequent to hyperbaric oxygen [8], cigarette smoking [9], total body irradiation [10], and acute aerobic exercise [11]. Consistent with the relationship between ex-

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haled ethane and pentane and in vivo lipid peroxidation is the observation that various antioxidants have decreased the exhalation of these hydrocarbons in studies of animals [12,13] and humans [14–16]. Taken together, these studies provide substantial evidence that expired-air ethane and pentane are representative and valid markers of in vivo lipid peroxidation.

Yet despite the growing number of reports on breath ethane and pentane there exist no widely accepted methods for collecting and analyzing expired air. As a result, most investigators have had to develop their own techniques. However, descriptions of these techniques often provide limited methodological detail and limited documentation of method validation and performance; various investigators have noted this fact [17–20]. The lack of extensive validation and the manifold differences between different techniques are very likely responsible for much of the striking variability in expired-air ethane and pentane values reported by different investigators. The large variability in normal adult values has been reviewed elsewhere [20–22].

Our interests in methodological detail and method performance motivated us to develop and validate techniques for the collection and analysis of expired air from laboratory rats [23] and humans [20]. The purpose of this review is to outline these methods, and to focus on their advantages as well as the potential errors and difficulties that can be introduced during sample collection, storage, concentration, and analysis. Most of these potential errors and difficulties are relevant to other methods as well. Also discussed are comparisons of ethane and pentane as sensitive and reliable markers of in vivo lipid peroxidation. For a comprehensive treatment of technical and physiological aspects of measuring breath ethane and pentane, the reader is referred to the excellent review by Kneepkens et al. [21].

Measurement of ethane and pentane in expired air from laboratory rats

We use an open-flow respiratory chamber system to collect expired air from rats. This involves placing a rat in a hermetically sealed glass jar through which hydrocarbon-free air (HCFA) is passed for an initial 20-min washout period. The washout period serves to flush ambient-air ethane and pentane from the chamber and the rat's lungs. During the next 40 min, two consecutive 4 l air-breath samples are collected into gas sampling bags and stored until analysis for ethane and pentane. An advantage of the collection system is that it is constructed from simple and inexpensive equipment; this enables multiple collection systems to be set up and used concurrently.

Because ethane and pentane concentrations in expired

air are usually below the detection limit of most chromatographic methods, samples are concentrated before analysis. To do this, we have developed a trap-and-purge technique that concentrates samples at least 80-fold. Briefly, a 4 l air-breath sample is passed through a precolumn of Indicating Drierite (W. A. Hammond Drierite, Co., Ltd., Xenia, OH, USA) and soda lime (to remove water vapor and carbon dioxide, respectively) and then through a cooled loop containing adsorbents that trap alkanes, but very little nitrogen and oxygen. Next, the alkanes are desorbed with heat and analyzed by gas chromatography. We find that adult rats typically exhale ethane and pentane at rates of about 1 pmol/100 g body wt/min.

We feel that the primary advantage of this method is that it traps highly volatile ethane (b.p. -88.6°C) very efficiently. Indeed, our motivation for developing our trap-and-purge technique was because we were unable to trap ethane effectively with techniques that use adsorbents cooled to -130°C in an ethanol-liquid nitrogen slurry [4]. Other investigators have reported similar difficulties [24]. Thus to trap ethane, we cool the adsorbents in liquid nitrogen (b.p. -196°C); at this temperature, trapping efficiency is essentially 100%, even over a range that far exceeds ethane concentrations found in expired air from rats (and humans). Moreover, the use of liquid nitrogen is far less cumbersome and subject to less temperature variations than is an ethanol-liquid nitrogen slurry.

Measurement of ethane and pentane in expired air from humans

We collect expired-air samples from humans using an apparatus we have designed. Briefly, a seated subject breathes hydrocarbon-free air from a 100–1 bag. The subject wears a noseclip and breathes through a mouthpiece to prevent inhaling ambient air. After 6 min, the subject's lungs are flushed of ambient-air ethane and pentane, and two consecutive 3–1 expired-air samples are collected into gas sampling bags. Concentrations of ethane and pentane are then determined using the same trap-and-purge and analysis procedures as described above. The total volume of the collected expired air and the time required for the collection of each sample are used for calculations of concentration (pmol alkane/l) and exhalation rate (pmol alkane/kg body weight [b.wt./min]). We find that normal adults typically exhale ethane and pentane at rates of about 2 pmol/kg b.wt./min.

One advantage of the method we have developed is that we offer a detailed description of the components of the breath collection apparatus, along with explicit instructions for the collection and analysis of samples [20]. Methodological details are of paramount importance to

the reliability of the measurements, mainly because contamination from ambient air may render the result unreliable, as discussed below.

Various features of the method make it readily adaptable for routine use in the clinic. First, the collection apparatus can be made portable. Second, the 10-min collection procedure, which is well tolerated by subjects, makes the test ideal for repeated measurements of the same subject. Third, the expired-air samples can be collected and stored (or transported) for later analysis. Finally, but perhaps most important, is that the method is both reliable and practicable.

Evaluation of method performance

The performance of analytical methods should be judged on two factors: (i) reliability and (ii) practicability [25]. The reliability is assessed on the basis of the magnitude of analytical variation, the specificity, and the limit of detection. The practicability of a method depends on numerous factors such as speed, dependability, required technical skill, cost, and safety.

As for the reliability of our methods, we have determined that our trap-and-purge technique has an acceptably low analytical variation of 6% (expressed as % coefficient of variation, % CV). The 6% CV represents an average of three values that span the usual range of ethane and pentane concentrations in human expired air. We have evaluated the specificity of our method by demonstrating adequate gas chromatographic separation between significant components in breath that could possibly interfere with ethane and pentane, i.e., ethylene, propane, butane, isopentane, hexane, and isoprene [20,23]. However, the use of mass spectrometry is still needed for unequivocal peak identification. The limit of detection for ethane and pentane are 0.22 and 0.77 pmol, respectively. These limits are about 30 \times and 10 \times less, respectively, than the ethane and pentane signals obtained from the analysis of a typical sample of human expired air.

One feature of the practicability of our method is demonstrated by its speed (and thus high throughput): it takes 10 min to collect two 3 l expired-air samples, 5 min for the trap-and-purge procedure, and less than 10 min for gas chromatographic analysis. Another feature is its dependability. Method failures, from collection to analysis, are rare. Indeed we have used our hydrocarbon adsorbent trap for the analysis of well over a thousand samples with no detectable changes in quantitation.

Potential errors introduced during the collection and storage of samples

A potential error introduced during the collection of expired air involves the failure to distinguish between

Table 1. Range of Ambient-Air Ethane and Pentane Concentrations Reported by Various Investigators

Ref.	<i>n</i>	Ethane (pmol/l)	Pentane (pmol/l)
26	7 ^a	70–220	110–340
20	23	68–224	2–76
27	●●● ^b	73–726	51–209
28	5 ^c	800 \pm 390	30 \pm 60

^a Values represent the range of values from seven urban centers. Chromatographic peak assignments were verified using mass spectrometry.

^b Not available.

^c Values are mean \pm SD.

endogenous and exogenous hydrocarbons. Exogenous ethane and pentane, i.e., those in ambient air, are usually found in much greater concentrations (Table 1) than those in washed-out human breath. In their comprehensive review of breath ethane and pentane, Kneepkens *et al.* [21] conclude that according to the majority of studies that use a washout period, ethane and pentane are exhaled at rates not exceeding about 5 pmol/kg b.wt./min, or about 50 pmol/l (assuming a b.wt. of 70 kg and a minute ventilation of 7 l/min). We usually obtain mean values of about 25 pmol/l for ethane and about 18 pmol/l for pentane. Therefore, considering that ambient-air ethane and pentane concentrations can sometimes be 15–30 \times greater than those in expired air, it seems that the accurate measurement of endogenous ethane and pentane production would benefit by eliminating the atmospheric component.

A relatively easy way to do this is to have subjects breathe HCFA for a period of time in order to flush ambient hydrocarbons from the lungs before collecting expired air. Perhaps it is feasible that the ambient hydrocarbons could be measured simultaneously and then subtracted out; but, this introduces more error because ambient levels are not only much higher than those in expired air, they are also much more variable. Indeed, ambient-air ethane can fluctuate “very quickly from moment to moment” [29], particularly if the air intake vent is near a source of pollution, such as a parking lot. Thus, the inclusion of a washout period to remove ambient-air ethane and pentane seems desirable as a means of establishing methodological standardization. In healthy subjects, we demonstrated that 4 min of breathing HCFA was adequate to flush high levels of ethane and pentane from the lungs, and that washout times up to 30 min resulted in no further reductions in breath hydrocarbons [20]. However, longer washout times are probably necessary in patients with compromised lung function. For example, Habib *et al.* found that an 8-min washout including multiple vital capacity breaths was needed to flush high levels of ambient ethane from the lungs of patients with chronic obstructive pulmonary disease [29].

Errors may also be introduced during the storage of expired-air samples. Drury et al. [30] found that pentane concentrations in 1 l Tedlar (Norton Performance Plastics, Akron, OH, USA) gas sampling bags did not change significantly after 24 or 48 h of storage, but did increase by 30% (range 22–108%, $n = 5$) after 72 h. Using 3 l Tedlar bags, we found that ethane and pentane concentrations can increase appreciably after only 24 h of storage. The increases are likely due to a slow diffusion of ambient hydrocarbons into the gas sampling bags. In the study by Drury et al. [30], the large variability in pentane accumulation after storage might reflect differences between individual bags. We have noted that Tedlar bags invariably develop leaks after extensive use. We therefore test the bags periodically by filling them with nitrogen and leaving them overnight; bags with small leaks will be partially deflated the next morning. The finding of large differences in ethane and pentane values between consecutive breath samples can sometimes also help identify a bag with leaks.

Potential errors introduced during sample concentration and analysis

A potential problem introduced during sample concentration involves the trapping of oxygen while an expired-air sample is passed through the cold loop. Although our adsorbent-containing loop does not trap oxygen while immersed in liquid nitrogen, subsequent loops we have built trap oxygen to various degrees. The packing characteristics seem critical. Incidentally, we have found that the trapped oxygen can be completely vented without affecting the recovery of ethane and pentane. This is done by taking the loop out of the liquid nitrogen after an expired-air sample has been passed through; as the loop starts to warm, the trapped oxygen expands rapidly and can be vented into a syringe. The loop is then heated, and the trapped hydrocarbons are purged with a small amount of nitrogen into another syringe. Venting the trapped oxygen has the advantage of allowing the samples to be concentrated into volumes as small as 10–15 ml (an overall 200- to 300-fold concentration).

Contamination with ethane and pentane from system components can be another source of error. For example, we have found that Drierite and soda lime (Fisher Scientific, Santa Clara, CA, USA) are consistently contaminated with high levels of hydrocarbons, especially pentane. These materials can only be used after being adequately purged with a stream of nitrogen gas [20].

During sample analysis, the presence of methane in expired air can sometimes pose a problem. We have identified some subjects who consistently exhale unusually large amounts of methane. When these breath sam-

ples are concentrated, the methane yields a large chromatographic peak that can run into that of ethane. We have since determined that better separation between methane and ethane can be achieved using a PoraPak Q, 100/120 mesh (Waters Corp., Milford, MA, USA) column (2 m \times 3.2 mm outer diameter) at 40°C. This column however is not optimized for pentane.

General technical aspects that promote optimal results

Despite the lack of a widely accepted standardized method for measuring ethane and/or pentane in expired air, we feel that optimal results can be obtained by any method provided it includes several technical aspects. First, the method should include a means of effectively dealing with the high and very variable concentrations of ambient-air ethane and pentane. We feel that this is best achieved by using a washout period, and by scrupulously avoiding ambient-air contamination during sample collection, storage, concentration, and analysis. Contamination with even small amounts of ambient air may render the results unreliable. Second, the concentration procedure must be reliable; the documentation of recovery, linearity, within- and between-day variability, and long-term stability of measurements is desirable. Assessment of these aspects will particularly enhance the reliability of detecting changes by repeated measurements of the same subject. Third, the chromatography should adequately resolve significant breath constituents that may coelute with ethane or pentane—namely, methane, ethylene, isopentane, and isoprene. If these aspects are incorporated, then it seems that the researcher or clinician should obtain accurate and reproducible results, even without between-laboratory standardization of methods.

Which is the better marker of in vivo lipid peroxidation: ethane or pentane?

In our studies of iron-related lipid peroxidation in rats [31] and humans [32,33], we found that exhaled ethane was a more sensitive indicator than pentane. Others have also reported the greater response of ethane than pentane to iron excess [6,34].

From a methodological point of view, we have determined that a single ethane measurement is more reliable than one of pentane. This is based on the finding that the between-day biological variation of ethane in expired air was less than that of pentane (24% CV vs. 38% CV) [20]. The greater biological variation in pentane exhalation rates may reflect changes in rates of pentane metabolism. Hydrocarbons are metabolized by hepatic mono-oxygenases at rates

that correlate with molecular weight [35]. Accordingly, Wade and van Rij, using human subjects who breathed through a rebreathing circuit, measured pentane metabolism to be considerably faster ($T_{1/2} = 52$ min) than that of ethane ($T_{1/2} = 4.1$ h) [36]. They subsequently showed that pretreatment with a blocker of hepatic mono-oxygenases increased ethane exhalation by 70% and pentane by 1250% [37]. These studies demonstrate that pentane exhalation is influenced more by hepatic metabolism, and that under some circumstances, increases in breath pentane may represent altered hepatic metabolism rather than increased *in vivo* lipid peroxidation.

It has been proposed that the high solubility of pentane in adipose may further complicate interpretations of the measurement of breath pentane [22]. Springfield and Levitt tested the hypothesis that adipose tissue would provide an enormous pentane sink and found that obese rats exposed to a high pentane environment excreted pentane more slowly than did normal rats [22]. Based on these findings, they predicted that many hours of breathing HCFA would be required to wash environmental pentane that is solubilized in the adipose of even a nonobese human. Although this prediction may be true, data from two studies of various washout times lead to the conclusion that if pentane measured in the breath represents a slow washout from the adipose, then its contribution to total breath pentane output must be very small. We studied washout times of 4, 10, 20, and 30 min in nine healthy subjects and found no significant differences between pentane exhalation rates after washouts of 4 and 30 min [20]. Indeed, the mean exhalation rate of pentane in these subjects was 1.1 pmol/kg b.wt./min—a value 2.5 times less than the mean exhalation rate of ethane. Morita *et al.* studied even longer washout times in 15 healthy volunteers [8]. They found that after subjects breathed HCFA for 0, 30, 60, and 90 min, mean pentane exhalation rates, in pmol/kg b.wt./min (\pm SEM), were 10.2 (\pm 1.5), 1.6 (\pm 0.2), 1.2 (\pm 0.9), 1.3 (\pm 0.4), and 1.3 (\pm 0.3), respectively. Thus, although it remains uncertain as to whether pentane measured in expired air represents endogenous production or a slow release of environmental pentane solubilized in body fat, the above studies demonstrate that relatively stable, low rates of pentane exhalation can be achieved with relatively short washout periods. This indicates that, at least in healthy volunteers, measured increases in exhaled pentane may represent true *in vivo* lipid peroxidation and not simply a release of pentane from the adipose. However, studies are still needed comparing pentane washout times from obese and normal human subjects after prior exposure (and equilibration) to a high pentane environment.

Overall, ethane seems to be a more reliable marker of *in vivo* lipid peroxidation: it is metabolized to a

much lesser extent than pentane, it is poorly soluble in tissues, and it displays less day-to-day biological variation than does pentane. Nevertheless, several studies have shown the exhalation of pentane, but not ethane, to increase under some circumstances [38–40]. This may be because pentane is derived from n-6 fatty acids, the predominant lipid class in the body. It seems, therefore, that the measurement of both ethane and pentane is still desirable.

REFERENCES

- [1] Dumelin, E. E.; Tappel, A. L. Hydrocarbon gases produced during *in vitro* peroxidation of polyunsaturated fatty acids and decomposition of preformed hydroperoxides. *Lipids* **12**:894–900; 1977.
- [2] Frankel, E. N. Volatile lipid oxidation products. *Prog. Lipid Res.* **22**:1–33; 1983.
- [3] Riely, C. A.; Cohen, G.; Lieberman, M. Ethane evolution: a new index of lipid peroxidation. *Science* **183**:208–210; 1974.
- [4] Dillard, C. J.; Dumelin, E. E.; Tappel, A. L. Effect of dietary vitamin E on expiration of pentane and ethane by the rat. *Lipids* **12**:109–114; 1977.
- [5] Habib, M. P.; Eskelson, C.; Katz, M. A. Ethane production rate in rats exposed to high oxygen concentration. *Am. Rev. Respir. Dis.* **137**:341–344; 1988.
- [6] Dillard, C. J.; Tappel, A. L. Volatile hydrocarbon and carbonyl products of lipid peroxidation: a comparison of pentane, ethane, hexanal, and acetone as *in vivo* indices. *Lipids* **14**:989–995; 1979.
- [7] Clemens, M. R.; Remmer, H.; Waller, H. D. Phenylhydrazine-induced lipid peroxidation of red blood cells *in vitro* and *in vivo*: monitoring by the production of volatile hydrocarbons. *Biochem. Pharmacol.* **33**:1715–1718; 1984.
- [8] Morita, S.; Snider, M. T.; Inada, Y. Increased N-pentane excretion in humans: a consequence of pulmonary oxygen exposure. *Anesthesiology* **64**:730–733; 1986.
- [9] Habib, M. P.; Clements, N. C.; Garewal, H. S. Cigarette smoking and ethane exhalation in humans. *Am. J. Respir. Crit. Care Med.* **151**:1368–1372; 1995.
- [10] Arterbery, V. E.; Pryor, W. A.; Jiang, L.; Sehnert, S. S.; Foster, W. M.; Abrams, R. A.; Williams, J. R.; Wharam, M. D., Jr.; Risby, T. H. Breath ethane generation during clinical total body irradiation as a marker of oxygen-free-radical-mediated lipid peroxidation: a case study. *Free Radic. Biol. Med.* **17**:569–576; 1994.
- [11] Leaf, D. A.; Kleinman, M. T.; Hamilton, M.; Barstow, T. J. The effect of exercise intensity on lipid peroxidation. *Med. Sci. Sports Exerc.* **29**:1036–1039; 1997.
- [12] Dillard, C. J.; Gavino, V. C.; Tappel, A. L. Relative antioxidant effectiveness of alpha-tocopherol and gamma-tocopherol in iron-loaded rats. *J. Nutr.* **113**:2266–2273; 1983.
- [13] Dillard, C. J.; Downey, J. E.; Tappel, A. L. Effect of antioxidants on lipid peroxidation in iron-loaded rats. *Lipids* **19**:127–133; 1984.
- [14] Do, B. K.; Garewal, H. S.; Clements, N. C., Jr.; Peng, Y. M.; Habib, M. P. Exhaled ethane and antioxidant vitamin supplements in active smokers. *Chest* **110**:159–164; 1996.
- [15] Kanter, M. M.; Nolte, L. A.; Holloszy, J. O. Effects of an antioxidant vitamin mixture on lipid peroxidation at rest and postexercise. *J. Appl. Physiol.* **74**:965–969; 1993.
- [16] Steinberg, F. M.; Chait, A. Antioxidant vitamin supplementation and lipid peroxidation in smokers. *Am. J. Clin. Nutr.* **68**:319–327; 1998.
- [17] Kohlmuller, D.; Kochen, W. Is n-pentane really an index of lipid peroxidation in humans and animals? A methodological reevaluation. *Anal. Biochem.* **210**:268–276; 1993.
- [18] Mendis, S.; Sobotka, P. A.; Euler, D. E. Pentane and isoprene in

- expired air from humans: gas-chromatographic analysis of single breath. *Clin. Chem.* **40**:1485–1488; 1994.
- [19] Seabra, L.; Braganza, J. M.; Jones, M. F. A system for the quantitative determination of hydrocarbons in human breath. *J. Pharm. Biomed. Anal.* **9**:693–697; 1991.
- [20] Knutson, M. D.; Lim, A. K.; Viteri, F. E. A practical and reliable method for measuring ethane and pentane in expired air from humans. *Free Radic. Biol. Med.* **27**:560–571; 1999.
- [21] Kneepkens, C. M.; Lepage, G.; Roy, C. C. The potential of the hydrocarbon breath test as a measure of lipid peroxidation (published erratum appears in *Free Radic. Biol. Med.* 1994 Dec; **17**(6):609). *Free Radic. Biol. Med.* **17**:127–160; 1994.
- [22] Springfield, J. R.; Levitt, M. D. Pitfalls in the use of breath pentane measurements to assess lipid peroxidation. *J. Lipid Res.* **35**:1497–1504; 1994.
- [23] Knutson, M. D.; Viteri, F. E. Concentrating breath samples using liquid nitrogen: a reliable method for the simultaneous determination of ethane and pentane (published erratum appears in *Anal. Biochem.* 1999; **270**:186). *Anal. Biochem.* **242**:129–135; 1996.
- [24] Lawrence, G. D.; Cohen, G. Concentrating ethane from breath to monitor lipid peroxidation in vivo. *Methods Enzymol.* **105**:305–311; 1984.
- [25] Statland, B. E.; Per Winkel, M. D. Sources of variation in laboratory measurements. In: Henry, J. B., ed. *Clinical diagnosis and management by laboratory methods* (16th edn.). Philadelphia: W. B. Saunders Co.; 1979:17–28.
- [26] Sexton, K.; Westberg, H. Nonmethane hydrocarbon composition of urban and rural atmospheres. *Atmos. Environ.* **18**:1125–1132; 1984.
- [27] Dumelin, E. E.; Dillard, C. J.; Tappel, A. L. Breath ethane and pentane as measures of vitamin E protection of *Macaca radiata* against 90 days of exposure to ozone. *Environ. Res.* **15**:38–41; 1978.
- [28] Zarling, E. J.; Clapper, M. Technique for gas-chromatographic measurement of volatile alkanes from single-breath samples. *Clin. Chem.* **33**:140–141; 1987.
- [29] Habib, M. P.; Tank, L. J.; Lane, L. C.; Garewal, H. S. Effect of vitamin E on exhaled ethane in cigarette smokers. *Chest* **115**:684–690; 1999.
- [30] Drury, J. A.; Nycyk, J. A.; Cooke, R. W. I. Pentane measurement in ventilated infants using a commercially available system. *Free Radic. Biol. Med.* **22**:895–900; 1997.
- [31] Knutson, M. D.; Walter, P. B.; Ames, B. N.; Viteri, F. E. Both iron deficiency and daily iron supplements increase lipid peroxidation in rats. *J. Nutr.* in press; 2000.
- [32] Knutson, M. D.; Walter, P. B.; Mendoza, C.; Ames, B. N.; Viteri, F. E. Effects of daily and weekly oral iron supplements on iron status and lipid peroxidation in women. *FASEB J.* **13**:A698 abstr.; 1999.
- [33] Mertz, S.; Donanagelo, C.; Knutson, M.; King, J. C.; Walter, P.; Ames, B.; Viteri, F. E. Breath ethane (Eth) excretion rate in women is increased by daily iron (Fe) but not by daily zinc (Zn) supplementation. *FASEB J.* **13**:A241 abstr.; 1999.
- [34] Filser, J. G.; Bolt, H. M.; Muliawan, H.; Kappus, H. Quantitative evaluation of ethane and n-pentane as indicators of lipid peroxidation in vivo. *Arch. Toxicol.* **52**:135–147; 1983.
- [35] Frank, H.; Hintze, T.; Bimboes, D.; Remmer, H. Monitoring lipid peroxidation by breath analysis: endogenous hydrocarbons and their metabolic elimination. *Toxicol. Appl. Pharmacol.* **56**:337–344; 1980.
- [356] Wade, C. R.; van Rij, A. M. In vivo lipid peroxidation in man as measured by the respiratory excretion of ethane, pentane, and other low-molecular-weight hydrocarbons. *Anal. Biochem.* **150**:1–7; 1985.
- [37] Wade, C. R.; van Rij, A. M. The metabolism of low molecular weight hydrocarbon gases in man. *Free Radic. Res. Commun.* **4**:99–103; 1987.
- [38] Allard, J. P.; Royall, D.; Kurian, R.; Muggli, R.; Jeejeebhoy, K. N. Effects of beta-carotene supplementation on lipid peroxidation in humans. *Am. J. Clin. Nutr.* **59**:884–890; 1994.
- [39] Toshniwal, P. K.; Zarling, E. J. Evidence for increased lipid peroxidation in multiple sclerosis. *Neurochem. Res.* **17**:205–207; 1992.
- [40] Bilton, D.; Maddison, J.; Webb, A. K.; Seabra, L.; Jones, M.; Braganza, J. M. Cystic fibrosis, breath pentane, and lipid peroxidation (letter; comment). *Lancet* **337**:1420; 1991.

ABBREVIATIONS

HCFA—hydrocarbon-free air
CV—coefficient of variation