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Letter to the Editor

Response: scientific basis of the breath methylated alkane contour

We thank Drs. Mitsui and Kondo for this opportunity to clarify the scientific basis of the breath methylated alkane contour (BMAC). We agree with them that oxidative stress results in excretion of other alkanes in breath besides ethane and pentane [1]. However, we do not agree that their concerns detract from the scientific validity of the BMAC.

First, we agree that the concentrations of these VOCs are very low—our study of C4–C20 alkanes in breath and air detected these compounds in picomolar (10^{-12} mol/l) concentrations [2]. However, low concentrations do not present a problem provided one employs a sufficiently sensitive technique that minimizes potential sources of error and contamination. Over a period of years, we have identified the major sources of error in breath collection and analysis [3] and developed a dependable technique for routine collection and assay of breath and air VOCs in picomolar concentrations [4,5].

Second, Mitsui and Kondo assert "...atmospheric alkanes come mainly from industrial or automotive emissions...". This may not be correct. Atmospheric alkanes appear to come mainly from biogenic sources such as soil microorganisms [6] and cattle. Ruminant livestock can produce 250–500 l/day of methane, raising concerns that cattle may be a serious cause of global warming [7]. Human breath may also be an important source of VOCs in indoor air [8].

Third, they raise a concern that pentane dissolved in body fat may be a "potent source" of pentane in breath, and "...Thus, a positive alveolar gradient does not always mean that alkanes derive from lipid peroxidation...". In response, we note that they base this assertion on a study of rats that had been exposed to high concentrations of pentane (2000–4000 pm) for 20 h prior to breath testing [9]. This was a toxicology study in animals, and the findings are not relevant to unexposed humans. All of our subjects sat quietly for at least 15 min prior to the breath collection so that VOCs in their circulating blood should have been at or near equilibrium with VOCs in room air. Had any of our subjects been previously exposed to exogenous pentane and were releasing it from fat stores, we would have observed dramatically higher concentrations of pentane in their breath.

Fourth, we agree with Mitsui and Kondo that "...The high concentration of breath pentane reported by some researchers is thought to be isoprene interference...". This problem was first reported by Kohlmuller and Kochen in 1993 [10] who found that gas chromatography (GC) columns frequently failed to separate pentane from coeluting isoprene. However, this is not a problem with appropriate modern GC columns which separate VOCs with greater resolution. We employed mass spectroscopy (MS) to confirm that our GC column clearly separated isoprene from pentane [11]; similarly, MS objectively confirmed that the alkanes and methylated alkanes comprising the BMAC were pure compounds that were not contaminated by other VOCs [12].

Fifth, Mitsui and Kondo state "...No information on why C4–C12 alkanes apart from isopentane have negative alveolar gradients, while C12–C20 are all positive is given...". In fact, we have devoted considerable effort to explaining this observation [2,12]. We have shown by kinetic analysis that the alveolar gradient of a breath VOC varies with the rate of synthesis minus the rate of clearance [5]. Synthesis of C4–C20 alkanes and methylated alkanes increases with the intensity of oxidative stress, and these VOCs are cleared mainly by oxidation in the liver via cytochrome p450 enzymes [13,14]. Consequently, the alveolar gradient of an alkane or methylated alkane in breath is positive or negative depending upon which of the two processes is dominant: either synthesis by oxidative stress causing lipid peroxidation, or clearance, which varies with the degree of

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induction of hepatic cytochrome p450. We found that the intensity of cytochrome p450 induction in patients with lung cancer affected the BMAC sufficiently to provide an accurate marker of disease [15].

Sixth, they state "...there is no available data to support the contention that methylated alkanes derive not from environmental contamination but from endogenous lipid peroxidation". This is not correct. We showed that the abundance of these VOCs in human breath increased significantly with age [12], and that oxidative stress caused by breathing oxygen resulted in a significant increase in the alveolar gradients of alkanes and methylated alkanes in the BMAC [16].

Seventh, they assert that our data are not consistent because we reported that the mean alveolar gradient of heptane was positive in one study [17] but negative in another [5]. We applaud their close reading of our earlier publications, but an equally close reading of the paper that provoked this response would have provided them with the obvious explanation: the intensity of oxidative stress varies with age [18]. The volunteers in the 1994 study were older, on average, than the group we studied in 1999. Consequently, the mean age-related oxidative stress (and hence the alveolar gradient of heptane, a component of the BMAC) was higher in the first group than in the second. Not till some years later did we learn that age is a major determinant of the alveolar gradient of heptane and the other components of the BMAC. In science as in life, experience is the greatest of all teachers.

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