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SHORT REVIEW

Breath alkanes as an index of lipid peroxidation

A. Van Gossum*, J. Decuyper

Breath alkanes and lipid peroxidation. A. Van Gossum, J. Decuyper.
ABSTRACT: Formation of free radicals and lipid peroxidation are mechanisms that are involved in many conditions including cellular damage. In a human body, there are many antioxidant systems likely to limit the production of free radicals and to reduce their deleterious effect. The peroxidation of polyunsaturated fatty acids, such as linoleic acid and linolenic acid which are cell membrane components, induces the formation of volatile alkanes that are excreted in the breath. The determination of breath alkanes (pentane and ethane) is considered to be a valuable and elegant method to assess lipid peroxidation. The method for collecting a breath sample and the chromatographic analysis of the collected gas require a strict methodology. Moreover, we must take into consideration some factors, such as the hepatic function, which may influence the pentane metabolism itself. We critically describe the results of breath alkane determination obtained in humans in different clinical conditions.
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*Dept of Gastroenterology, Laboratory of Clinical Chemistry, Erasmus Hospital, Free University of Brussels, Brussels, Belgium.

Correspondence: A. Van Gossum, Dept of Gastroenterology, Hôpital Erasme, route de Lennik, 808–1070 Brussels, Belgium.

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Formation of free radicals and lipid peroxidation have been incriminated in many conditions which induce cell damage. Since it was discovered that ethane production was correlated with lipid peroxidation in the livers of mice treated with carbon tetrachloride, measurement of exhaled alkanes, especially ethane and pentane, has been used as a non-invasive technique for studying lipid peroxidation *in vivo* [1].

Before discussing the value of exhaled alkane measurements in humans, we briefly describe mechanisms of free radical generation and lipid peroxidation.

Oxygen activated species

The oxygen activated species are superoxide radical ($O_2^{-\cdot}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$). The latter is an intermediate which is formed during photosensitized reactions. The others are the intermediates of the four monoelectronic step reduction of molecular oxygen into water [2]:

$$O_2 + e^{-\cdot} \rightarrow O_2 + e + 2H^+ \rightarrow H_2O_2 + e + H^+$$
$$-H_2O- \rightarrow OH^{\cdot} + e^- + H^+ \rightarrow H_2O$$

In the respiratory chain, these intermediates remain tightly bound at the active site of the enzyme (cytochrome oxidase). However, "leakages" cause release of some $O_2^{-\cdot}$ [3]. The respiratory chain constitutes the first source of oxygen radicals. In other enzymatical systems where oxygen is the electron acceptor, oxygen reduction is limited to the first step. As an example, during the respiratory "burst" in activated polymorphonuclear leucocytes, one can observe a reduced nicotinamide-adenine-dinucleotide phosphate (NADPH) oxidase catalysed reduction of O_2 into $O_2^{-\cdot}$ by NADPH [4].

Another important source of oxygen radicals is during ischaemia-reperfusion [5]. During the ischaemia (the anaerobic phase), adenosine triphosphate (ATP) is consumed and converted into hypoxanthine, and xanthine dehydrogenase is converted into oxidase by a protease. During the reperfusion (the aerobic phase), xanthine oxidase catalyses the reduction of O_2 into $O_2^{-\cdot}$ by hypoxanthine which is oxidized into xanthine.

Oxygen radicals are also formed during the metabolism of some xenobiotics (for example, paraquat) [6]. The initial mechanism of oxygen radical generation is the reduction of O_2 into $O_2^{-\cdot}$.

The oxygen radicals are interrelated [6]. $O_2^{-\cdot}$ is transformed into H_2O_2 in a dismutation reaction which is catalysed by superoxide dismutase (SOD). In the presence of Fe^{++}, H_2O_2 can lead to the formation of $OH^{\cdot}$ *via* the Fenton reaction. *In vivo*, iron is at the ferric stage but in the presence of $O_2^{-\cdot}$ it can be reduced to the ferrous stage, and the Haber-Weiss reaction occurs:

$$H_2O_2 + O_2^{-\cdot} \rightarrow OH + OH^{\cdot} + O_2$$

$O_2^{-\cdot}$ and H_2O_2 are, in biological conditions, almost unreactive. However, in hydrophobic aprotic media, $O_2^{-\cdot}$ can promote some cellular component alterations. The deleterious species are $^1O_2$ and $OH^{\cdot}$. They can react with almost all of the cellular components: proteins, nucleic acids and lipids [2]. Their reaction with

polyunsaturated fatty acids (PUFA) leads to the lipid peroxidation which will be discussed in some detail below.

Oxygen radicals are too short-lived to be detected in clinical conditions. It is the products of their reaction with biological substrates which are used to monitor their implication in the processes under study.

Defence systems against oxygen activated species

During evolution, aerobic living organisms have developed defence systems against these oxygen radicals. They act at different levels. On the first line, we find systems acting in order to reduce the possibility of the Haber-Weiss reaction: superoxide dismutase (SOD) acts on $O_2^{\cdot-}$, catalase and other peroxidases act on H_2O_2, and ceruloplasmin acts by converting Fe^{++} into Fe^{+++}.

On a second line, we find oxygen radical scavengers. These compounds react with oxygen radicals and, thus, decrease their concentration. The most well known oxygen radical scavengers are: vitamin E (alphatocopherol), vitamin C, B-carotene, bilirubin, uric acid and glutathione (GSH). On a third line, we find the repair systems (especially the deoxyribonucleic acid (DNA) repair systems) [6–8]. However, in some clinical conditions these defence systems can be overwhelmed and oxygen radicals can, thus, cause deleterious effects. For example: during ischaemia-reperfusion in organ transplantation, in myocardial infarction, open heart surgery, rheumatoid arthritis, cigarette-smoke effect, and emphysema, during hyperoxia, in the adult respiratory distress syndrome, and idiopathic haemochromatosis [6].

Lipid peroxidation

Lipid peroxidation is a radicular mechanism which can be initiated by $OH^{\cdot}$. The initiation step is a hydrogen atom abstraction from a polyunsaturated fatty acid (LH) leading to a carbon centred radical ($L^{\cdot}$) which after reaction with oxygen forms a peroxide radical ($LOO^{\cdot}$):

$$OH^{\cdot} + LH \rightarrow H_2O + L^{\cdot}$$
$$L^{\cdot} + O_2 \rightarrow LOO^{\cdot}$$

This peroxide radical is able to abstract a hydrogen atom from another LH molecule (reinitiation) leading to the formation of a hydroperoxide (LOOH). In the presence of iron, LOOH is converted into alkoxyl radical. In the case of linoleic acid, this alkoxyl radical is degraded into an aldehyde and the pentanyl radical, which abstracts a hydrogen atom, leading to pentane $CH_3 - (CH_2)_3 - CH_3$.

Pentane formation is observed with the w-6 PUFA series. In the same reaction schema, ethane formation is observed with the 3 PUFA series. Lipid hydroperoxides can also lead to the formation of malonaldehyde which can react with proteins to form fluorescent compounds [9].

The most deleterious effects of lipid peroxidation are perturbations of membrane fluidity, and of calcium ion transport capacity. *In vivo*, alpha tocopherol and the glutathione-glutathione peroxidase system are able to limit the extent of lipid peroxidation. Alpha tocopherol can react with lipid peroxides, the resulting alpha tocopheryl radical can restore alpha tocopherol by a reaction with vitamin C [7]. Glutathione peroxidase is able to use the lipid hydroperoxides as substrates for the oxidation of glutathione reduced form (GSH) into glutathione oxidised form (GSSG) [8].

Methods for monitoring lipid peroxidation

Direct detection of lipid hydroperoxides, measurements of malonaldehyde, conjugated dienes or fluorescent compounds are all subject to artifacts [2, 6, 9]. However, detection of unsaturated aldehyde and detection-characterization of lipid hydroperoxides after high performance liquid chromatography (HPLC) separation are promising methods. The currently accepted technique for measurements of lipid peroxidation *in vivo* is the measurement of alkanes, especially pentane, in the exhaled air.

Alkane measurement methodology in exhaled air

The methodology for measuring alkanes in the breath must avoid contamination with alkanes present as contaminants in room air. For this reason, before collecting a breath sample, it is necessary to perform a wash-out of the lungs with hydrocarbon free air. The wash-out time proposed varies between authors from 4–40 min [10–14].

In practice, subjects breathe through a mask or mouthpiece connected to a Rudolph valve from a bag containing hydrocarbon free air. During the wash-out, the exhaled air is discarded. The initial atmospheric air with its hydrocarbon content is flushed from the lungs. Afterwards, whilst hydrocarbon free air is still inspired, breath is collected for pentane analysis.

Recently, ZARLING and CLAPPER [15] proposed a method for measurement of volatile alkanes from single-breath samples from which they analysed pentane concentration in room air, total breath and alveolar breath. The alkane quantifications in the breath are performed by gas chromatography using direct injection of a gaseous sample, or after trapping alkanes on different trapping media.

PINCEMAIL *et al.* [11] proposed the use of charcoal; the alkanes are extracted with carbon disulphide and an aliquot of the extract is injected. LEMOYNE *et al.* [10] proposed use of cold alumina which can trap alkanes which are liberated after heating the alumina.

Human studies

Measurement of alkane production has been recognized as a reliable index of lipid peroxidation. Studies on expired alkanes performed in animals are numerous and have shown the protective effect of vitamin E in peroxidation situations such as ozone exposure, [16] or carbon tetrachloride (CCl_4) [17], paraquat [18, 19] or heavy metal

ingestion [20]. On the contrary, despite an increasing interest in lipid peroxidation, studies performed in humans using alkane breath tests are few. This is probably, in part, related to the technical problems of methodology.

Nevertheless, alkane (mostly pentane) breath tests have been used in humans in various conditions: oxygen therapy, hepatic cirrhosis, physical exercise, long-term parenteral nutrition, intravenous administration of lipid emulsion, and smoking. We will present and briefly discuss the most striking results of pentane studies carried out in human beings.

It is well recognized that lipid peroxidation is a central feature of oxygen-associated tissue damage. Indeed, hyperoxic exposure, especially in neonates, may induce severe damage including bronchopulmonary dysplasia and retrolental fibroplasia [21]. Results of alkane measurements in animals exposed to hyperoxia are conflicting [22–24]. Using a specially assembled circuit, MORITA *et al.* [12] measured excretion of pentane in healthy adult volunteers exposed to oxygen for 2 h. After breathing 100% oxygen, pentane excretion was augmented by 42–62% within 30–120 min. This phenomenon was rapidly reversible. Measurement of exhaled pentane confirmed a rapid increase in lipid peroxidation in hyperoxic conditions. Further studies are needed to determine the point at which lipid peroxidation overwhelms repair mechanisms and produces irreversible oxygen toxicity.

Increases in pentane output have also been observed in cirrhotic patients with liver dysfunction [25, 26]. However, in this condition, one must be careful in interpreting pentane levels. Increases in pentane output may correspond more to a decreased metabolism of pentane than to a real increase in the lipid peroxidation process. Indeed, pentane and, to a lesser extent, ethane appear to be excellent substrates for cytochrome P-450 and, in particular, for the ethanol-inducible form of this haemoprotein [27]. ALLERHEILIGEN *et al.* [28] recently confirmed the main role of cytochrome P-450 in pentane metabolism using an elegant model. They studied the clearance of pentane from arterial blood of the rat following an injection of the hydrocarbons into a closed chamber containing the animal. Clearance was estimated from the analysis of arterial blood and chamber air concentration-time curves using a three compartment model which included the chamber. The 85% decrease in the clearance of pentane in animals pretreated with either a metabolic inhibitor or a toxin which destroys cytochrome P-450 suggests that the rate at which pentane is metabolized by the cytochrome P-450 enzyme system may contribute significantly to the overall rate of elimination of pentane. It follows that any influence on cytochrome P-450 activity (a disease or a drug) must be taken into account in using breath pentane excretion as an index of lipid peroxidation.

Increased production of exhaled pentane has been described in normal volunteers subjected to heavy exercise [11, 29]. A previous oral supplementation of vitamin E was shown to decrease this phenomenon, reenforcing the role of lipid peroxidation in this condition. However, there have been criticisms regarding these conclusions. Because physiological stress may mobilize tissue stores of the alkanes, the increased pulmonary excretion of pentane during muscular exercise may not represent lipid peroxidation but merely the passive washout of previously equilibrated exogenous environmental pentane from muscle secondary to increased blood flow [28].

A significant increase in breath pentane output has been observed in patients with long-term parenteral nutrition who are likely to develop nutrient deficiency [30]. There was a significant inverse correlation between breath pentane excretion and serum alphatocopherol levels. Also, serum alphatocopherol levels were noted to decrease with continued parenteral nutrition. Therefore, it appears that in some conditions, pentane output may be considered as a functional assessment of vitamin E content. On the other hand, a fast increase in pentane output has been described in adult humans after intravenous administration of lipid emulsions [31]. The same phenomenon was described in neonates and in newborn rabbits but only a few days after starting infusion of fat [13]. Interestingly, supplementation of vitamin E attenuated the rise of pentane release that was observed in this condition [23]. The explanation of this phenomenon is still uncertain. Indeed, administration of lipid emulsion may either be similar to an oxidative stress enhancing endogenous lipid peroxidation, or may cause an increased delivery of lipid substrate for lipid peroxidation in commercial lipid emulsions which contain large amounts of w-6 polyunsaturated fatty acids. Research is currently in progress in our laboratory to clarify this point. It has also been demonstrated that 10 days of oral supplementation in vitamin E (1,000 IU) lowers breath pentane output in normal volunteers [32]. Preliminary data have shown the same effect in heavy smokers for whom basal pentane output is higher than for nonsmoking subjects (Van Gossum *et al.*, unpublished data).

Another situation in which free radical release is involved is the ischaemia-reperfusion mechanism that occurs in various conditions such as stroke, myocardial infarction, pancreatitis, organ transplantation. With regard to this, BULKLEY [33] recently described a sharp increase of exhaled pentane output immediately after reperfusion of a transplanted liver in humans.

The origin of expired alkanes, which we consider to be a reliable reflection of lipid peroxidation remains a problem. Basically, alkanes are end-products of lipid peroxidation resulting from the destruction of polyunsaturated fatty acids which are contained within membrane cells. Because lipid peroxidation occurs in many organs, pentane and ethane measured in the breath may be considered as a global assessment of the lipid peroxidation processes in the entire body. However, we know that the liver, red cells and lungs are major sites of lipid peroxidation. In some conditions, it is possible to predict the organs in which lipid peroxidation will be increased. For instance, in cases of intoxication with paraquat, the liver is the organ where an enormous amount of lipid peroxidation will occur [34].

Another question is whether the gastrointestinal tract *per se* might be a site of pentane production.

GELMONT *et al.* [35] claimed that the major source of pentane was not membrane lipid peroxidation but was the action of intestinal bacteria on linoleate hydroperoxide. However, they also observed an increase in pentane production associated with vitamin E deficiency and a rapid reversal in the increase with the administration of vitamin E. Because bacteria in the human bowel are mainly in the colon and vitamin E is absorbed in the small bowel, it is unlikely that the reduced pentane observed after vitamin E administration was due to its action on colonic bacterial products.

Other studies performed on animal or human newborns did not support an intestinal production of pentane. ROBERTS *et al.* [24] observed that starved newborn rats, delivered by Caesarian section and consequently without gastrointestinal flora, produced high amounts of hydrocarbon gas. WISPE *et al.* [23], studying newborn infants, failed to observe an increase in hydrocarbon gas in the breath over the first few days after delivery. Moreover, it was noted with interest that two home parenteral nutrition patients with complete bowel resections (and no intestinal flora) had high breath pentane outputs [30].

Conclusions

Free radical formation and lipid peroxidation are mechanisms involved in many clinical situations. Measurement of breath alkanes appears to be an elegant and valuable method to assess lipid peroxidation and the protective role of some compounds (such as vitamin E) on this phenomenon. Breath alkane measurements require adequate methodology and consideration of factors that are likely to influence expired alkane output. If these measurements prove to be accurate and reliable, they have potential to become routine clinical tests.

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Les hydrocarbures volatiles comme un index de la peroxydation lipidique. A. Van Gossum, J. Decuyper.
RÉSUMÉ: La formation de radicaux libres et la peroxydation lipidique sont des réactions intervenant dans de nombreuses conditions entraînant un dommage cellulaire. L'organisme humain possède plusieurs systèmes antioxydants (ex.: l'enzyme superoxide dismutase, la vitamine E, etc) capables de limiter la production de radicaux libres et leurs effets délétaires sur la cellule. La peroxydation d'acides polyinsaturés, comme l'acide linoléique et l'acide linolenique qui sont des constituants de la membrane cellulaire, entraîne la formation d'hydrocarbures volatiles qui sont excrétés dans l'expirat. La mesure dans l' expirat de ces hydrocarbures (pentane ou ethane) est considérée comme une méthode élégante et fiable pour évaluer la peroxydation lipidique. La technique de récolte d' un échantillon d'expirat ainsi que l'analyse chromatographquie des gaz recueillis exigent une methodologie rigoureuse. Par ailleurs, il faut tenir compte de facteurs, comme la fonction hépatique, pouvant influencer le taux de pentane expiré. Nous décrivons de manière critique les résultats des tests respiratoires mesurant le pentane ou l'éthane obtenus chez l'homme dans différentes circonstances cliniques.
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