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TOPICAL REVIEW

Breath acetone as a potential marker in clinical practice*

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Abstract

In recent decades, two facts have changed the opinion of researchers about the function of acetone in humans. Firstly, it has turned out that acetone cannot be regarded as simply a waste product of metabolism, because there are several pathways in which acetone is produced or broken down. Secondly, methods have emerged making possible its detection in exhaled breath, thereby offering an attractive alternative to investigation of blood and urine samples. From a clinical point of view the measurement of breath acetone levels is important, but there are limitations to its wide application. These limitations can be divided into two classes, technical and biological limits. The technical limits include the storage of samples, detection threshold, standardization of clinical settings, and the price of instruments. When considering the biological ranges of acetone, personal factors such as race, age, gender, weight, food consumption, medication, illicit drugs, and even profession/class have to be taken into account to use concentration information for disorders. In some diseases such as diabetes mellitus and lung cancer, as well as in nutrition-related behavior such as starvation and ketogenic diet, breath acetone has been extensively examined. At the same time, there is a lack of investigations in other cases in which ketosis is also evident, such as in alcoholism or an inborn error of metabolism. In summary, the detection of acetone in exhaled breath is a useful and promising tool for diagnosis and it can be used as a marker to follow the effectiveness of treatments in some disorders. However, further endeavors are needed for clarification of the exact distribution of acetone in different body compartments and evaluation of its complex role in humans, especially in those cases in which a ketotic state also occurs.

1. Introduction

New developments in measurement methods and detection techniques always provide an opportunity for the reinvestigation of old problems and open the way for the examination of new fields of interest. Such a development has occurred in the reliable and reproducible detection of volatile organic compounds (VOCs) in exhaled air that have been related to different kinds of disorders [1–3]. The key advantages of VOC detection in clinical practice are the non-invasive nature of technology and easy handling [1, 2]. In contrast, these techniques require skilled people and there are problems with sample storage [3].

Further limitations associated with breath testing are the high water content of breath samples, the high cost of analytical instruments, and the lack of clear-cut links between VOCs detected in breath and diseases [1, 3, 4]. This last limitation is in part due to the fact that even the samples of healthy persons show a significant variation with regard to both type and quantity of compounds. This hampers the comparison of data gained from different populations. A large number of VOCs have been detected in exhaled air and, although acetone is only one of them, it is always present [5–7].

The aims of this review are to give a historical overview of breath research with acetone, to delineate the metabolism of acetone, and to propose some linkage to the possible uses of exhaled acetone to diagnose and monitor various disease states. The objective is to
show that acetone in exhaled breath is a useful and promising probe for diagnosis and, in particular, that it can be used as a biomarker to follow the effectiveness of treatments.

2. From the odor of decaying apples to metabolic routes—the medical history of acetone research

The clinical value of the aroma of exhaled breath from humans was known even by early Greek physicians. It is enough to remind the reader here of the description of fetor oris or fetor hepaticus by Hippocrates, the great Greek doctor. However, the real emergence of VOCs in scientific thinking may be traced back to the end of the 18th century.

In 1798, John Rollo, an English physician, described an odor of decaying apple in the breath of a diabetic patient [8]. Although he did not realize it, this smell was a result of acetone in the breath. It took nearly sixty years later for acetone to be identified to be present in the human body, when Petters identified acetone in the urine of a diabetic patient [9]. He also found an apple- and violet-like odor in the breath of diabetic patients [9] that was attributed to acetone. Measurements on the amount of exhaled acetone were performed forty years later, when breath samples from chronically starved and diabetic patients were investigated. The average amounts measured were 3.6 grams/day for the starved and 0.343 grams/day for the patient suffering from diabetes [10, 11]. On the basis of Nebelthau’s work with starved humans, it was stressed that the ratio between the amounts of acetone excreted via urine and exhaled in breath was 1:10 [10]. All in all, according to the knowledge of the time, acetone was mainly regarded as a characteristic feature of diabetic coma and a waste product of metabolism [12–14]. This belief did not change until the Second World War [15].

In the 1940s and 1950s, the picture of the waste product nature of acetone started changing, because compounds labeled with radioactive isotopes were recognized as useful tools in biochemical research. Nevertheless, the changing of minds of scientists and physicians in this regard was very slow. Hence the notion of acetone as a waste product of metabolism persevered for many decades. Even in 1980, Robinson and Williamson reviewed ketone bodies in humans and wrote that ‘we make no mention of acetone, which is formed by non-enzymatic breakdown of acetoacetate and is unlikely to be important in metabolism of the intact animal’ [16]. However, the facts at that time did not support this note because experimental data were already available upon the incorporation of 14C-carbons of labeled acetone into cholesterol, fatty acids, urea, and glycogen [17–21]. Thus, one central dogma of biochemistry, which claimed the inability of mammals to metabolize acetone to intermediates of metabolism to a substantial degree, was strongly opposed. The oxidation of acetone to carbon dioxide exhaled in respiratory air was also reported [18, 21, 22]. Later, the possibility of in vivo formation of glucose from acetone in experimental animals was also published by several groups [21–28]. Similarly, 2-13C-acetone was reported to contribute to glucose production in fasting and diabetic humans [29, 30]. Last but not least, net glucose formation from acetone was measured in isolated rodent hepatocytes but not in perfused rat liver [31–33].

It was in 1980 that Coleman recognized the role of cytochrome P450 (CYP) type enzymes in acetone breakdown [34]. After a short time, the induction by acetone of several CYP isozymes was reported [35]. In 1984, Casazza et al published an article in which the pathways of acetone metabolism in rats were described [36]. This period was the second golden age of acetone research, and several communications were published on different aspects of acetone metabolism [36]. However, since the 1990s, the interest in acetone has gradually declined and the only still-developing area of research has been the investigation of its detection in exhaled breath. However, this new direction of investigation may allow for better understanding of background processes, thereby giving support to clinical practice.

3. The metabolic pathways for acetone

There are several pathways for acetone production and breakdown, with the routes for acetone degradation being particularly complex (figure 1). By keeping limitations of space in mind, the focus is strictly kept upon the aim to review acetone metabolism so as to establish clinical application. Those who are interested in details are directed to pertinent reviews.

3.1. Production of acetone

In the human body, there are two major physiological sources of acetone. The first source is the decarboxylation of acetoacetate, while the second is the oxidation of isopropanol. However, other sources cannot be excluded; these can be external, such as acetone inhaled through occupational hazard, or internal such as acetone as a byproduct of intestinal flora.

3.1.1. Conversion of acetoacetate into acetone

Acetoacetate is the major source of acetone production in mammals. It may arise from both lipolysis and breakdown of ketogenic amino acids, and is decarboxylated either in an enzyme-catalyzed way or non-enzymatically to acetone.

The enzyme designated acetoacetate decarboxylase (acetoacetate carboxylase: EC4.1.1.4.) was first identified in Clostridium acetobutylicum [38]. While the existence of acetoacetate decarboxylase activity was also found in rat tissues, liver, and plasma [39–42]
It has not been found, to date, in human tissue or fluid specimens. The activity of rat enzyme was attributed to a protein with a low substrate affinity, an optimal activity at pH 4.5, and the loss of activity in the presence of iodoacetate, urea, and HgCl$_2$. Acetone itself proved to be a competitive inhibitor for the activity. However, the enhancement of decarboxylation by an enzyme has been queried.

The most important concern was that the protein responsible for the enzymatic activity had never been purified to homogeneity and neither sequencing of the enzyme nor the identification of its coding gene have been done. Furthermore, details dealing with mammalian acetoacetate decarboxylase have not been reported in the last three decades. Hence, there remains a question as to what kind of protein this enzymatic activity may be attributed to in mammals.

The non-enzymatic decarboxylation of acetate and other $\beta$-keto-carboxylic acids was noted as early as 1929. As reported, carbon dioxide cleavage was enhanced by amines.

3.1.2. Conversion of isopropanol to acetone
The oxido-reduction between acetone and isopropanol is dominantly catalyzed by alcohol dehydrogenase (ADH)-type enzymes belonging to the class I isozymes of the ADH family (EC.1.2.1.3.), and catalase plays only a subordinate role.

The mechanism for the reaction yielding acetone is the following. First, the hydride from isopropanol goes to NAD$^+$ followed by deprotonation in two consecutive steps governed by histidine and serine residues, resulting in acetone formation. The opposite reaction, the reduction of acetone to isopropanol, is to be considered as degradation of ketone. As to the reaction mechanism, through the resonance forms of acetone a nucleophilic attack at the carbonyl by a hydride ion was suggested, followed by a proton donation by water, thus yielding an alcoholic group on the second carbon of the chain.

3.1.3. Acetone of bacterial origin
Bacteria can grow on different sources of carbon and produce various kinds of small organic molecules, including acetone. A wide variety of anaerobic and aerobic bacteria is capable of either using or producing acetone.

In the alimentary tract of ruminants, the levels of both acetone and isopropanol are easily detectable. Recent research has led to a recommendation that VOCs, such as, among others, acetone, can be used as markers in bacterial infections. This recommendation is mainly based on the analysis of headspace VOCs of cultures, a fact that raises doubts about clinical applicability at the present state of knowledge.

3.2. Degradation of acetone
Reviews summarizing acetone degradation, particularly the glyoxalase pathway, are abundant. Therefore, detailed discussion of the topic is avoided here and the reader is directed to the pertinent literature.

3.2.1. The role of CYPs
Two main routes of reactions are known for the degradation of acetone (figure 1), but the first step in both pathways is the same: conversion of acetone into acetal by a CYP isozyme, designated CYP2E1.

This isozyme is induced by the treatment of animals with acetone and a diverse range of exogenous compounds, as well as by fasting and chemically induced diabetes mellitus. In addition, acetone is considered not only as an endogenous substrate for CYP2E1 gene products, but also as the physiological inducer for them. The way of induction by acetone of CYP2E1 isozymes is very probably a
substrate-induced protein stabilization [56]. These isozymes are expressed in a wide variety of tissues and presumably show sex differences [57, 58].

3.2.2. C3 and C2 pathways
During acetone degradation, both three-carbon (C3) and two-carbon (C2) fragments are produced. In isolated rat hepatocytes, two C3 pathways with pyruvate as a common end-product have been identified; one of them mainly operating via the glyoxalase pathway was found to be entirely intrahepatic while the other pathway, the propanediol route, was suggested to involve additional extrahepatic step(s) [31]. Besides these pathways, only one C2 pathway exists that diverts intermediates of the propanediol route at the level of L-1,2-propanediol [53, 54]. The first step in the entirely intrahepatic C3 route is the conversion of acetal into methylglyoxal, a reaction that also needs the participation of CYPs. Methylglyoxal is further metabolized to pyruvate by two different metabolic routes. Alternatively, acetal can be phosphorylated via the propanediol pathway by a specific kinase, and acetal phosphate is subsequently converted into L-1,2-propanediol, which is either metabolized to L-lactate or split into formate and acetate [27, 53, 59, 60] (figure 1). It is assumed that the L-1,2-propanediol-1-P dehydrogenase catalyzed step probably cannot be managed in the liver [53, 60].

4. Chemical nature of acetone
The chemical history of acetone is longer than its medical history [61]. The chemical was probably produced 'by the dry distillation of metallic acetates during the Middle Ages' [61]. The correct elemental composition of acetone was, however, independently described by Dumas and Liebig only in the early 19th century [61].

Acetone, also known as dimethyl ketone, has a sweetish taste, a characteristic odor of decaying apple, and is miscible with water, alcohol, chloroform, ether, and most oils. It is very volatile (vapor pressure at 25 °C is 229.5 mm Hg [62]) and hence rapidly evaporates, even from water and soil. Once it enters the atmosphere it is degraded by photolysis, a reaction in which free radicals are involved or removed by wet deposits [63].

From biochemical and toxicological points of view, two notes are appropriate here. First, acetone freely crosses biological membranes, even the blood–brain barrier, owing to its miscibility with lipids [64, 65]. Second, it is to be noted that acetone covalently binds to macromolecules, e.g. to aminophospholipids and peptides [66, 67]. In the case of acetone–oxytotic formation, Schiff-base seems to be an intermediate [66]. Yet, due to the pH applied (approximately pH 5) and the concentration of acetone (60% aqueous acetone) used, this interaction is unlikely to be of physiological importance.

5. Detection of acetone in research and clinical practice
5.1. Measurement of plasma acetone levels
Historically, colorimetric methods were first developed for acetone determination. However, their lack of specificity and detection limits were common disadvantages [36]. In recent decades, gas chromatographs (GCs) equipped with flame ionization detectors (FIDs) or coupled with a mass spectrometer (MS) and high-performance liquid chromatography (HPLC) using 2,4-dinitrophenyl hydrazine (DNPH) as a derivatizing reagent became available [36, 68]. Using GCs, plasma acetone concentrations in healthy humans ranged between 8–15 μM and <30 μM with MS and FID, respectively, measured from gas headspace above plasma [69–73]. With the HPLC technique, plasma acetone concentration in healthy volunteers was detected between 34–120 μM [74].

All in all, GC-MS is recommended for detecting plasma acetone levels with appropriate accuracy.

5.2. Techniques for detecting breath acetone
The first quantitative analysis of acetone in exhaled air was carried out using GC in combination with FID in 1971 [75]. Trotter used only 1 ml of breath, which was
directly injected to the GC column. The mean breath acetone concentration was 0.02 μmol l⁻¹ with a standard deviation of 0.01 μmol l⁻¹ in the 20 examined non-fasting subjects [75]. In 1995, Pleil and Lindstrom used evacuated electropolished canisters for breath sampling, which enabled the quantification of acetone and isoprene among other VOCs at 500–1000 parts per billion by volume (ppbV) concentrations using GC-MS [76].

GC-MS is recognized as a standard analytical method for breath VOC analysis allowing higher specificity. However, for detection of acetone as a single compound, this technique is not only bulky but also has a long response time. Furthermore, it requires preliminary concentration of the volatiles in order to reach sufficient sensitivity in the ppbV range [77–79]. Miniaturization resulting in shorter analysis time and lower power consumption, such as in MEMS-based micro GC systems, can highly enhance the attractiveness of this technique [80].

An attractive approach is the real-time detection of volatiles in exhaled breath. Chemical ionization mass spectrometers, such as proton transfer reaction mass spectrometry (PTR-MS) and selected ion flow mass spectrometry (SIFT–MS) enable the online tracking of compounds and determination of concentration changes in single ppb levels, even with breath-to-breath resolution. King et al examined the changes of acetone and isoprene levels in different physiological processes (exercise, sleep) and developed models under consideration of the affecting ventilator, cardiovascular, and endocrine factors [81–83].

Dummer et al applied SIFT–MS to determine a single-exhalation breathing maneuver for accurate analysis of breath acetone. In parallel, breath flow and volumes were recorded to further study the relationship between blood and breath acetone levels [84]. Prabhakar et al investigated 48 random subjects visiting an exhibition and demonstrated the screening value of breath analysis in an otherwise healthy population [85]. The reported level of breath acetone ranged between 300–1000 ppbV for the general population but in two cases—a child later diagnosed with type 1 diabetes mellitus and a fasting adult—the levels of breath acetone were 1500 and 2500 ppbV, respectively [85].

By now, ion mobility spectrometry in combination with GC separation is an often-applied technique for the detection of breath acetone [86]. Because of its proton affinity (812 kJ mol⁻¹) compared to water (697 kJ mol⁻¹), acetone can be detected with great sensitivity using the atmospheric pressure chemical ionization process used in IMS [87].

Generally, optical techniques enable a sensitive gas analysis due to the selective detectability of compounds in specific wavelength regions [88, 89]. An external cavity quantum cascade laser has been successfully applied for acetone detection [90]. Additionally, a stand-alone acetone analyzer based on the technique of cavity ring-down laser absorption spectroscopy was used in a clinical study for breath acetone analysis of 334 diabetic patients and 52 persons without diabetes [91]. Sensors are likely to become an analytical tool for detection of breath acetone due to their small size and low price. However, to be in competition with other laboratory or diagnostic systems, they should be sensitive and at the same time selective for the VOC to be tracked without cross-sensitivities for other volatiles. The following examples show that different sensors have a high potential to become the tool of choice for breath acetone detection. An ultrathin (10 nm) indium nitride (InN) gas sensor was applied for acetone detection in air at the sub-ppm level by Kao et al [92]. Also, Cr- or Si-doped WO₃ nanoparticles seem to be promising for detecting acetone, showing high sensitivity and selectivity at up to 90% relative humidity [93]. A portable acetone sensor consisting of Si-doped epsilon-WO₃ nanostructured films was developed with a detection limit of ~20 ppb and short response (10–15 s) and recovery times (35–70 s) [94]. Regarding spectroscopy-based systems, a sensor system on the basis of narrow-band absorption spectroscopy containing a laser-diode-based cavity-enhanced adsorption cell was successfully utilized for breath acetone measurements. The sensitivity of the handheld device was enhanced by an integrated miniaturized preconcentrator reaching a detection limit in a high ppbV range [95]. Finally, a sensitive detection (down to 20 ppbV) of acetone could be achieved by a fiber-optic biochemical gas-sensing system based on the reversed catalytic reaction of NADH-dependent secondary alcohol dehydrogenase employing a UV-LED with specific wavelength at 335 nm [96].

Despite these developments the collection of breath VOCs, including acetone, still lacks a standardized process. The reasons for this mainly lie in the different requirements of the various analytical systems applied and in the absence of a clinically based protocol. Breath can be sampled online when subjects exhale directly into a device or offline using different kinds of bags [97], canisters [76], or syringes to collect breath samples. Many factors have to be considered when sampling breath, including the chemical characteristics of the compounds, the constitution of the matrix, the physical parameters of the subjects during sampling (position, ventilation, etc.), temperature and material of the container, etc.

6. A brief summary of clinical implications

Implicit in this section is the idea to review diseases in which the disturbance of acetone metabolism may occur. It is intended to be done in such a way that, on the one hand, the biochemical background of disturbed acetone formation is presented and, on the other hand, the detection of breath acetone in different clinical...
states is discussed by proceeding disease by disease. Disturbed acetone metabolism is usually accompanied by an unequivocal change of the levels of other ketone bodies, acetoacate and β-hydroxy-butyrate. The physiological concentration of these ranges between 100–250 μM. The term ketosis is used when the actual ketone body concentration exceeds the upper limit. Both acetoacetate and β-hydroxy-butyrate are acidic, and therefore when the acid burden exceeds buffering capacity, blood pH falls below 7.35. This state is called ketoacidosis.

6.1. Starvation
6.1.1. Biochemical background and some clinical peculiarities
Limitations in food resources may lead to starvation-provoked mortality, and therefore the metabolic consequences of food deprivation appear in a strict order of events to enhance survival of an individual. The sequence of their appearance are the depletion of hepatic glycogen stores, the initiation of lipolysis, and finally the commencement of intensive proteolysis with a concomitant keto- and gluconeogenesis in the liver, which takes place in parallel with a decrease of glucose demand and an increase of ketone body utilization in peripheral tissues preferring vital organs [43, 98]. The adaptation to starvation happens at different levels and is regulated by hormonal changes [36, 43]. Notably, a modulation of the phosphorylation/dephosphorylation state of particular proteins (e.g. pyruvate dehydrogenase complex or glycogen synthase/phosphorylase) is seen at the molecular level, while on the other hand gene expression is also altered (e.g. enhanced expression of the enzymes of gluconeogenesis in the liver), resulting in a shift of focus in metabolic processes [56].

As a result of intensive lipolysis, the level of acetyl-coA exceeds the capacity of acetyl-coA oxidation and ketone bodies are produced, dominantly in the perivenous zone of the liver lobule [99]. They are secreted into the blood, thus elevating circulating ketone body levels by at least one order of magnitude. At maximal acetonemia, as much as 37% of the total acetate can be converted into acetone [29]. Increased acetone metabolism is also reflected by the rise of plasma concentrations of minor metabolites such as acetal and L-1,2-propanediol, and may provoke the appearance of not yet investigated compounds such as methylglyoxal [100]. In contrast, these metabolites were investigated and detected in plasma and tissues of rodents [101].

Although both normal and overweight humans produced elevated levels of ketone bodies under starvation, overweight people did so much slower than their normal counterparts [29, 102]. Furthermore, the length of fasting had an influence on the rate of acetone oxidation. In 3-day and 21-day fasting by obese humans, the estimated rates of acetone oxidation accounted for 60% and 25% of the rates of acetone production, respectively [29]. Noteworthy is the fact that pregnant females were more susceptible to developing ketosis than their nonpregnant counterparts [103]. In studies with rats, maternal and fetal plasma acetone levels equilibrated in non-fasted case and did not differ from plasma acetone concentration of non-pregnant controls [42, 104]. Fetal plasma acetone levels, however, exceeded maternal values when animals were subject to food deprivation [42, 104]. According to estimations, about 2%–30% of endogenous acetone is eliminated in breath and urine, and hepatic production of glucose starting from acetone may account for as much as 11% [29].

In refeeding, which is an opposite process to starvation, experimental data of acetone levels are not yet available. One preliminary report can be found [105]. Nevertheless, one can speculate as to whether breath acetone may be a valuable marker to follow up changes, particularly in monitoring patients with anorexia nervosa.

6.1.2. Breath acetone measurements
In the literature there is an agreement that an elevated level of acetone is present in the breath of starved humans, but the data are inconsistent regarding the range where the linear relationship between plasma and breath acetone concentrations exists [29, 68, 102]. In one study, the ratios of plasma concentrations to breath concentrations were in the range of 500:1–600:1 when plasma acetone concentrations were below 500 μM whereas, at higher plasma concentrations, the ratios were 350:1–500:1 [29]. In other reports, a linear relationship was reported at up to 4 mM plasma acetone levels [68, 102]. Blood β-hydroxy-butyrate levels of fasting obese patients also correlated to breath acetone in a linear manner in a range up to 3 mM plasma β-hydroxy-butyrate levels [106]. Overnight fasted obese but otherwise healthy people had lower breath acetone than their normal controls [68]. An almost linear relationship between blood ketone and exhaled acetone was documented in a two-day study undertaken with 11 healthy volunteers, in which breath acetone was detected by the SIFT-MS technique [85]. On the first day, the subjects received an isocaloric meal with different fat content and fasted on the second day of the study [85].

No gender-related effect on breath acetone production could be observed in overnight fasted volunteers without diabetes [107]. This is also the case observed with fasting [85].

The influence of different meals after overnight fasting was examined by different groups. The effect of a protein-calorie meal on exhaled acetone after 12 h fasting was examined by Smith et al using SIFT-MS [105]. A reduction in acetone concentrations from maximum was observed after consumption of the protein meal, reaching its nadir between 4–5 h [105]. A ketogenic breakfast induced an increase in acetone, β-hydroxy-butyrate, and insulin levels, as well as a
reduction of blood glucose levels over 6 h after the consumption of the meal [108].

Righettoni and coworkers found a correlation between blood glucose and breath acetone levels after overnight fasting measured with PTR-TOF MS and a complementary relationship using nanostructured Si-doped WO3 sensors [109].

6.2. Diabetes mellitus

6.2.1. Biochemical background and some clinical peculiarities

Diabetes mellitus affects approximately 8% of the world population [110]. In this disease, an absolute or relative inadequacy of the effect of insulin gives rise to a complex disturbance of metabolism. Whatever the reasons diabetes mellitus develops—autoimmune disorder, obesity, taking medicines, post-transplantational state, alcoholism, pancreatic injury—it is dominated by two metabolic changes: rise of blood sugar level and intensive lipolysis [37]. In the absence of insulin, fatty acids are promptly mobilized in and released from the adipose tissue. Parallel to this phenomenon, fatty acid synthesis is repressed in the liver. An adaptation at the molecular level in which, e.g., the enzymes of gluconeogenesis/glycolysis and CYP2E1 isozymes are involved, is also seen [55]. All in all, malonyl-coA concentration drops and results in an increase of carnitine–palmitoyl transferase activity which, through the rise of β-oxidation, leads to an elevated level of acetyl-coA. Since the tricarboxylic acid cycle (TCA) is insufficient to use up all acetyl-coA produced, β-ketothiolase catalyzes the reverse reaction and ketone body generation increases. Importantly, the increased rate of β-oxidation results in an elevation of hepatic NADH+/H+ concentration. NADH+H+ is certain to donate hydrogen directly to acetoacetate, and in this way the disturbance of NADH+H+∕NAD+ ratio prompts the reduction of acetoacetate to β-hydroxy-butyrate with high probability. As an indicator of hepatic intramitochondrial redox-state, β-hydroxy-butyrate∕acetocetate ratio is augmented. In peripheral tissues, the reverse reaction is promoted, and in this way intra- and extra-hepatique mitochondrial redox status can be poised. Another indicator for the change of redox status is the appearance of isopropanol in the plasma of diabetic patients [49, 111]. However, the scientific merit of this finding is still a subject of debate. Finally, it should be noted that about 2.1% of 2-C14-acetone may be converted to glucose in diabetic humans, suggesting that acetone also contributes to glucose formation in this state [100].

Two points ought to be stressed from the clinical point of view and considered as states in which the detection of breath acetone may be of high impact. The first is that plasma acetone concentrations in diabetic humans, in general, elevate by at least two orders of magnitude in ketotic states and its concentration may exceed the level of 12 mM in extreme cases [70]. Even in treated diabetic patients, the plasma acetone concentration is higher than in the normal population [112]. Approximately 50% of acetoacetate produced in diabetic ketotic humans is converted into acetone and there is a linear relationship between their plasma concentrations [30]. It is, however, to be added that there may be a different propensity to present severe diabetic ketoacidosis (DKA), at least among diabetic children belonging to different races under age of 5 [113]. The second point is the ability of sodium-glucose cotransporter 2 inhibitors to provoke DKA without hyperglycemia [114–116]. These drugs are indicated for the treatment of type 2 diabetes mellitus and result in renal glycosuria, and in this way a shift from carbohydrate to fat utilization is seen as posing an opportunity for ketoacidosis [114].

6.2.2. Breath acetone measurements

In 1966, Rooth and Östenson measured an acetone level in the breath of juvenile diabetic patients (type 1) that was four times higher than found in healthy controls using GC with a flame ionization detector [117]. A smaller difference was found between exhaled acetone values of elderly diabetic patients (type 2) and controls (around 1.5 times) [117]. Eleven years later, Crofford et al suggested that breath acetone measurements, in combination with blood glucose levels, were useful in following the management of diabetic outpatients [68]. According to their classification, a greatly elevated glucose level and breath acetone concentration higher than 50 nM indicated insulin insufficiency [68].

The theory that the increase of blood sugar level represses fatty acid oxidation was confirmed in the investigations of Turner et al, who detected reduced breath acetone levels following glucose ingestion in healthy volunteers using SIFT-MS [118]. The same authors also made the observation that the absolute levels of breath acetone in people with controlled type 1 diabetes were not invariably higher than those seen in healthy subjects [118]. They found a linear relationship between breath acetone levels and fixed circulating insulin levels during carefully controlled experimental hypoglycemia [119]. In a study in which exhaled acetone concentrations of uncontrolled type 2 diabetic patients were investigated in a Japanese population, the results showed positive correlations of acetone with plasma glucose when glucose values decreased during hospitalization [120]. In a small group of children, breath acetone was found to be elevated in diabetic patients regardless of whether measurements were undertaken in nonfasting, fasting, or postprandial states [121]. Interestingly, in healthy controls, a negative linear relationship was observed between breath acetone and age, the latter being the independent variable [121].

Breath acetone and plasma acetone in DKA patients were related to each other in a linear manner.
up to 10 mM plasma concentration \cite{30}. Similarly, there were also linear relationships between acetone and acetol as well as acetone and L-1,2-propanediol levels up to 8 mM plasma acetone concentrations, whereas neither acetol nor L-1,2-propanediol are present in the plasma of healthy humans at detectable levels \cite{100}. Because of the high variation of breath acetone concentration even within the healthy population, the collected evidence does not support the concept that breath acetone alone would be a reliable parameter of diabetic control \cite{122}. Careful patient recruitment, standardization of breath sampling, and analytical procedures of breath acetone could probably improve study outcomes.

Other compounds such as isoprene (because of the coherence with the mevalonic acid pathway of cholesterol synthesis) and methyl nitrate (due to its relation to oxidative stress and modulation of lipolysis) were implicated with diabetes \cite{121, 123}. Indeed, on the one hand, the isoprene level in breath is strongly influenced by physical activity, and apparently there is not a significant relationship between breath isoprene and serum cholesterol concentrations; on the other hand, the concentration of methyl nitrate was found to be very low (in the low parts per trillion range), which makes their use as a volatile marker practically difficult \cite{124}.

**6.3. Acetone as a toxic agent and occupational hazard**

**6.3.1. Biochemical and clinical background**

Acetone is toxic \cite{63}. It is metabolized in humans in the ways discussed above (figure 1) or eliminated unchanged via the lungs (figure 2) and kidneys, but probably only elimination plays a role in the cases of intoxication, and no biochemical reactions have to be taken into account.

Cases of acetone poisoning resulting from inhalation of vapor, absorption through skin, and ingestion due to accidental and occupational exposure or suicide attempts have been reported \cite{63, 125}. Hepatorenal damage, bone marrow hypoplasia, and neurotoxic effects including coma have been reviewed in relation to acetone toxicity \cite{63}.

From a methodological point of view, it is to be noted that chronic studies were usually undertaken in an occupational environment. In these cases, the coincidental exposure to other chemicals could not be fully excluded and the exposures were usually, but not exclusively, followed by urinary excretion of acetone, as is done in acute cases as well \cite{126–129}. When it is detected, breath acetone may be a useful tool in monitoring workers exposed to occupational hazards \cite{130, 131}.

Inhalant abuse is quite frequent, particularly among young people and down-and-outs \cite{132}. Among inhalants abused, acetone is preferred for its narcotic properties \cite{133}. If inhaled intentionally with the purpose of recreational intoxication (usually in combination with other solvents as an ingredient of commercial products) or suicidal intent, breathing is difficult \cite{134–136}. This raises the question as to whether breath acetone detection is of clinical value; it is an issue that needs to be evaluated.

It is, however, a fact that the administration of a single dose of acetone to pregnant rodents resulted in the appearance of acetol and L-1,2-propanediol in

\begin{figure}
\centering
\includegraphics[width=\textwidth]{acetone_metabolism.png}
\caption{Compartments involved in acetone metabolism. C3 designates the following three-carbon compounds: L-1,2-propanediol, acetol, and pyruvate. The elimination of acetone through the kidneys is not shown.}
\end{figure}
fetal tissues and plasma [101]. Although transplacental transport of either acetal or L-1,2-propanediol cannot be excluded, the above-mentioned observation strongly suggests that the inhalation of acetone by a pregnant female may lead not only to acetone intoxication of the fetus, but also to its fetal metabolism.

6.3.2. Breath acetone measurements
Measurement of acetone and other VOCs in the breathing zone of workers and the follow-up of plasma as well as urinary levels of these compounds to characterize their personal exposure to hazardous volatiles belongs to the field of inhalation toxicology. Another field of interest in this regard is the uptake and detection of acetone and other VOCs in exhaled breath. The latter approach was used by Wigaeus et al., who exposed eight volunteers to acetone for two hours and afterwards detected its concentration in alveolar air [130]. Sampling and analysis of the exhaled breath revealed that only 20% of inspired acetone was eliminated in air and 1% was found in urine, stressing two points that cannot be distinguished on the basis of their data [130]. The first opportunity is that acetone entered fatty tissue to be released, later causing a flashback-like effect; the second is the metabolism of acetone. However, the current available data are not sufficient to solve the problem. Further research would be needed to evaluate the situation. In another study, by monitoring workers in acetate fiber plants for acetone in blood, exhaled air, and urine by GC, the strongest correlation was found between urinary acetone and the degree of exposure, whereas a positive correlation was also seen between exposure to acetone and its concentration in alveolar air or blood [131].

6.4. Isopropyl-alcohol intoxication
6.4.1. Biochemical and clinical background
Isopropanol in the human body essentially originates from either metabolic processes (diabetes mellitus, amino acid breakdown) or external sources resulting from an exposure. Isopropanol is a clear volatile with a bitter taste and a characteristic odor resembling a mixture of ethanol and acetone [137, 138]. These features are probably the reasons why it was the fifth on the list of 12 most common poisons in a study undertaken in the USA, accounting for 8% of the total intoxications assigned to the listed drugs, and has been deliberately consumed by alcoholics [139, 140]. Its ingestion occurs either accidentally (mainly by children) or purposefully, but occupational exposure by inhalation has also been observed [139, 141].

The essential factor in a biochemical context is the conversion of isopropanol to acetone by ADH. This reaction is a simple oxido-reduction using NADH +H+/NAD⁺ as a cofactor, and acetone formation is preferred to the reverse reaction [142]. Hence, the elevation of plasma isopropanol concentration results in a rise of acetone level, and there is a positive correlation between these factors [141–146]. The lack of acetone in a case of severe isopropanolemia has, however, been reported for a Japanese male, stressing the importance of ADH polymorphism in events of isopropanol intoxication [147]. The occurrence of symptoms during an isopropanol intoxication episode usually depends on the ingested dose, and the symptoms range from slight clinical signs to serious, life-threatening symptoms [137, 144, 145, 148, 149].

In clinical practice, the appearance of isopropanol in plasma was considered to be an unambiguous mark of alcoholism. However, this has been changed since the application of advanced detection techniques that have identified isopropanol as a minor but physiological intermediate of metabolism, as an elevation of blood isopropanol after physical exercise was reported [150]. Under pathological conditions, isopropanol was detectable in plasma not only in the case of alcohol intoxication, but also in diseases such as diabetes mellitus as well as liver and gastrointestinal diseases, and the plasma acetone concentration was at the same time increased [49, 143–145, 150, 151]. Moreover, higher isopropanol level resulted in a false-positive ethanol test outcome for a man, who was under a ketogenic diet, during traffic control [152]. Transplacental isopropanol exposure in pregnancy was also reported [153]. In ketotic states, particularly when the NADH/H⁺/NAD⁺ ratio is high—for example, in diabetes mellitus or chronic alcoholism—isopropanol production may function as a shunt regenerating NAD⁺, thus contributing to the maintenance of metabolic stability. In this way, its role is just like that of lactate and β-hydroxy-butyrate formation under special metabolic circumstances.

In livers investigated in the course of autopsies, the amount of isopropanol always exceeded that of acetone, but in other tissues (kidney, brain, blood) the picture was contradictory, showing an opposite relationship [49].

6.4.2. Breath acetone measurements
Breath isopropanol concentration is rather small compared to breath acetone levels. Rudnicka et al. reported an isopropanol concentration range of 3–20 parts per billion (ppb) in exhaled air, thus one hundredth of the breath acetone concentration [154]. It is difficult to estimate how much isopropanol is converted to acetone in one day. In an experiment by Ruzsanyi et al., 1 μL (0.8 mg) deuterated isopropanol was self-administered [155]. Considering that 100% of the labeled d3-isopropanol (1.12E-5 mol) was metabolized to d3-acetone by ADH enzymes, around 6.2% of the produced d3-acetone (7.90E-07 mol) was exhaled in the first 7.4 h of investigation [155]. To approximate the daily isopropanol amount converted from acetone, ADH could hypothetically be saturated, e.g. using ethanol, and thus the formation of acetone from isopropanol would be
blocked (which corresponds to a methanol intoxication where ADH is blocked through ethanol).

6.5. Ketotic state(s) in alcoholics and the effect of disulfiram on acetone level
6.5.1. Biochemical background and some clinical peculiarities

The fundamental biochemical consequence of etilalcohol (ethanol) consumption is the modulation of the NADH+H+/NAD⁺ ratio. The rise of this ratio results from the function of alcohol and aldehyde dehydrogenases and slows down TCA-cycle, inhibits gluconeogenesis, and results in a rise of acetyl-coA levels, thus establishing a favorable situation in the liver for the ignition of fatty acid synthesis, ketone body production, and acetate liberation [156, 157]. The elevated level of NADH+H⁺ pushes the balance of oxido-reductions towards reduction that is reflected by the rise of lactate and isopropanol levels in body fluids [156, 158]. Greater is the burden by organic acids and higher is the change of blood pH.

Even a single dose of ethanol results in an increase of isopropanol and acetone concentrations in human blood specimens [150]. In alcoholics, during their alcohol consumption period, isopropanol, acetate, aceton, and methanol increases in the plasma, raising the potential that these compounds could perhaps be used as markers of the alcoholic state [159]. Beside aceton, ethanol, and the aforementioned compounds, the role of abnormal metabolites (acetoin, 2,3-butanediol, 1,2-propanediol) was also strongly highlighted [160–163]. The formation of these compounds is a result of disturbed NADH+H⁺ formation that directs reactions toward the production of minor metabolites. Notably, these compounds can also be detected in exhaled breath as VOCs [150].

Beside alcohol consumption itself, there are two states in alcoholics which require further attention; one of these is alcoholic ketoacidosis (AKA), while the second one is disulfiram administration.

Although AKA is well known, paradoxically it is still an underdiagnosed state in clinical practice [164]. For a long time, it was suggested that it could easily be recovered from when treated, but recent research revealed that sudden death of unknown origin in alcoholics can frequently be attributed to AKA [164–166]. As a syndrome, it results in metabolic acidosis and occurs in chronic alcoholic patients after malnutrition and cessation of alcohol consumption with a recent history of binge drinking [164, 167]. It starts within 2–4 days after an alcoholic has stopped consuming alcohol, proven by the inability to detect ethanol in the plasma, usually occurring when alcoholics have become depleted of their carbohydrate fuel stores and water, and ketone body accumulation is seen as an accompanying event [159, 164, 168]. In post mortem analysis, β-hydroxy-butyrate is thought to be a more reliable marker for AKA than acetone [168]. Clinically, AKA is characterized by nausea, vomiting, abdominal tenderness/pain, dehydration, and an acetone-like odor on the person’s breath[164].

As a result of overlapping events, the metabolic background of AKA apparently shows a barely detectable difference from diabetic ketoacidosis (DKA). However, a marked and characteristic feature of AKA is an elevated ratio of β-hydroxy-butryate/acetoacetate in comparison to DKA, accompanied by normo/hypoglycemia that is not present in DKA as hyperglycemia is seen [168, 169]. Hormonal predisposition may be a factor, whereas AKA occurs in pregnancy as well [170].

The main point of the action of disulfiram is the inhibition of aldehyde dehydrogenase [170, 171]. Nevertheless, its inhibitory effect on microsomal mixed function oxygenases, CYP 450 s (e.g. CYP2E1 playing a role in ethanol metabolism), as well as on other enzymes has also been reported [172–175]. These inhibitory effects lead to the elevation of acetaldehyde concentrations during ethanol consumption and cause adverse symptoms and sometimes death. However, its action is metabolism-dependent [172–174]. A metabolite of disulfiram, diethyldithiocarbamate, is converted by CYP2E1 to a reactive intermediate that inactivates the enzyme, thus leading to mechanism-based inactivation [176]. Therefore, it is not the increased precursor supply but rather the diminished metabolism that is responsible for the rise of plasma acetone concentration in the presence of disulfiram. In addition, disulfiram depletes hepatic glutathione content, probably through the potentiation of the action of acetaldehyde, elevates plasma acetone levels, and has a protective effect against chloroform-induced liver injury [177–179]. Clinically noteworthy is that the elevation of blood acetone levels happens in the absence of a commensurate increase of its precursor molecule, acetoacetate [180, 181].

6.5.2. Breath acetone measurements

Using a sensitive GC assay, acetone levels were studied in three groups of subjects (healthy volunteers, acutely intoxicated alcohol abusers, abstinent alcohol abusers) [182]. Acetone levels were significantly higher in the acutely intoxicated subjects in comparison to the two other groups [182]. Bloor et al used SIFT-MS to measure breath acetone after ingestion of a single dose of disulfiram and found acetone levels elevated from 300 to over 4000 ppb during the course of the 20 h investigation [183]. The robustness of the finding is reduced by the fact that only one subject was exposed to disulfiram [183]. To the authors’ best knowledge, breath acetone levels have not been investigated in AKA patients. Nevertheless, the likelihood of interference between acetone and ethanol would not be of falsifying clinical value in the case of AKA, as ethanol is usually not present (see above). Furthermore, using infrared breath-alcohol analyzers, the interference can
be avoided even in the case of elevated breath acetone concentrations [184].

Alcohol consumption and smoking are two activities occurring together, and therefore the influence of a smoking habit is of particular interest. The effect of a smoking habit upon exhaled breath composition is an often-discussed topic owing to the fact that, among others, unsaturated hydrocarbons, aromatic compounds, and aldehydes and their metabolites are often related to cigarette smoking [185]. Regarding acetone, 1-propanol, and 2-propanol, no significant differences were found between smokers and non-smokers [185].

6.6. Special diets (ketogenic and Atkins diets)

6.6.1. Biochemical background

Anecdotal remarks regarding the beneficial effect on seizure control of starvation were already available in Biblical times (see Matthew 17). The appearance of a scientific approach, called the ketogenic diet, to use as an alimentary supplementation in the treatment of intractable epilepsies can be traced back to 1920s [186]. The ketogenic diet is a high-fat, low-carbohydrate diet mimicking starvation and leading to the elevation of plasma levels of all the three members of the ketone body family [187, 188]. Seizure-suppressing effects and plasma ketone body concentrations were interrelated [187]. Whatever materials or mechanisms are responsible for the antiepileptic action of the ketogenic diet, the location of the antiseizure activity of any chemical compound has to be the brain, and this rule necessarily applies to acetone as well [189]. Patients successfully treated with a ketogenic diet had elevated levels of acetone in their brains [190]. As acetone freely crosses the blood–brain barrier, its concentrations in blood and cerebrospinal fluid were found to be similar in rats, and it exhibited a rapid uptake into and relatively slow clearance from the brain [65, 191]. Though acetone is not the only metabolite whose concentration is changed during the diet, neither acetoacetate nor \( \beta \)-hydroxy-butyrate are able to pass through the blood–brain barrier as both need transport proteins [192]. As a result of a linear relationship between plasma and breath acetone, the latter is likely to be a valuable marker for a follow-up study of the ketogenic diet via exhaled breath analysis [193, 194].

The Atkins diet and its modified versions are popular diets promising weight loss, but scientific evidence concerning them is scant [195–197]. The idea of the original diet is to switch the body’s metabolism from the consumption of glucose to the conversion of stored body fat; this is the process that leads to ketosis. Both plasma acetone concentration and the levels of acetone metabolites (acetol, methylglyoxal) increased in humans on the Atkins diet [198].

6.6.2. Breath acetone measurements

The relationship between breath acetone and urinary as well as plasma concentrations of acetoacetate and \( \beta \)-hydroxy-butyrate in children and adults during a ketogenic diet was already examined [193]. A non-linear relationship between breath acetone and plasma or urinary acetoacetate was found [193, 199, 200]. This is the reason as to why breath acetone was dedicated as an adequate predictor for ketosis and monitoring seizures in treatment-refractory epileptic cases [193, 199–201].

6.7. Inherited diseases in which ketosis occurs

There are several inherited diseases in which ketotic state occurs, two of which are mentioned here.

The clinical features of congenital propionic and methylmalonic acidemia are marked with early onset of disease, retardation of growth and mental development, and death in childhood in a remarkable number of patients [202]. The most important metabolic event in these states is severe ketoacidosis. Elevated levels of plasma propionate and/or methylmalonate, glycine, and isoleucine as well as acetone, ketones (methyl-ethylketone, pentanone), and diols (L-1,2-propanediol, 2,3-butanediol) in the urine are seen [202]. The appearance of diols in the urine raises the possible role of CYP2E1 isozymes [202]. Complex enzymatic defects give a basis for the increase of plasma acetone levels. The biochemical principle of these diseases lies on the dysfunction of either the propionyl-coA carboxylase or the methylmalonyl-coA mutase, either of which blocks the entry of some amino acids (isoleucine, methionine, valin) into the TCA cycle at the level of succinyl-coA [202].

Deficiency of mitochondrial succinyl-coA:3-ketoacid coA transferase (SCOT) was first described in 1972 [203]. Since then, several reports have been published on its occurrence [204, 205]. SCOT insufficiency is a rare inborn error of metabolism characterized by either asymptotic or permanently ketogenic intermittent ketoacidotic crises between episodes [203–205]. Clinically, ketoacidotic episodes are precipitated by acute infection, starvation, and fever or by the administration of protein under 24 months of age [203, 205]. In accordance with this clinical observation, temperature sensitivity to the mutant protein has been recognized [206].

Biochemically, unusual metabolite(s) cannot be found in the plasma of probounds, which is otherwise the case with most of the organic acidemias. Variable increases in plasma values for glycine, proline, lysine, and uric acid were observed with concomitant elevation of ketone body levels [203]. However, ketonuria became minimal when glucose was applied as a sole substrate [203]. The biochemical core of metabolic events is the reversible CoA transfer from succinate to acetoacetate by SCOT that is a pivotal reaction in the interrelationship between carbohydrate metabolism
and ketone body oxidation [203, 207]. This reaction is the first and rate-limiting step of ketone body utilization in peripheral tissues. By transferring CoA moiety from succinyl-CoA to form acetoacetyl-CoA, the entry of acetoacetate into the TCA cycle becomes possible and it can be used for energy production [207].

To date, breath acetone measurements are not known.

6.8. Lung tumors

Epidemiological studies have established that the worldwide incidence, prevalence, and mortality attributed to lung malignancies have risen since the 1930s, making lung cancers the leading cause of cancer deaths in developed countries and reaching alarming rates in developing countries [208, 209]. This increase is predominantly due to the popularity of cigarette smoking but recently the role of inhaling smoke from recreational drugs, particularly marijuana and cocaine, have also been revealed as causal factors in these carcinomas [209, 210]. Owing to the large health burden and poor prognosis, efforts have been made in favor of early diagnosis. One of the tools for this is the analysis of VOCs in exhaled breath, in which acetone is one of the constituents showing a change [211, 212]. Nevertheless, the real biochemical reason for the increase of acetone formation in this disorder is as of yet unclear.

The data on breath acetone in lung tumor cases are contradictory. These not unequivocal findings are probably due to the application of different breath sampling and analytical procedures, and clinical protocols. The possible role of the tumor type cannot be excluded either. Ulanowska et al measured acetone among other VOCs, and a higher acetone concentration in the breath of patients suffering from lung cancer was observed in comparison to controls [213]. In contrast, Bajarevic et al detected a significantly lower level of acetone in the exhaled air of patients with lung carcinoma [214].

To examine metabolic alterations at the cellular level, the headspace VOCs of cultured cancerous cells were analyzed and compared with those from with non-cancerous cell lines. Filipiak et al, as well as Schallsschmidt et al, did not observe significant changes in acetone, but a strong ethanol production in the headspace of cancerous A549 IC and Lu7466 cell lines was evident [215, 216]. However, this was not the case for normal lung cells [215]. Moreover, a significantly higher level of ethanol was detected in tumor tissues than in healthy lung tissues [217]. Supporting these results, ethanol was detected in higher concentrations in the breath gas of patients with lung carcinoma than in healthy controls [217]. However ethanol is formed, the mechanism of its formation and its relation to acetone production needs to be evaluated. Particularly, its interference with the CYP2E1 isozyme ought to be uncovered.

Exhaled nitric oxide (NO) measurements are also offered in predicting diagnosis and disease progression in lung tumors [218]. Despite the uncertainties just discussed, acetone has a significant potential to detect disease and, in contrast to NO, it is easier to measure [212]. Nonetheless, further research is warranted.

6.9. Infections

There are several pathogens producing acetone detectable in exhaled breath; among others Pseudomonas aeruginosa, Salmonella typhimurium, and Staphylococcus aureus [31]. Although the application of VOCs of bacterial origin is promising as a non-invasive bacterial detection method, the sensitivity and specificity of the tests have to be improved, particularly in the case of acetone. One reason for this is that investigations of cultured Streptococcus pneumoniae and Haemophilus influenzae did not reveal any rise of acetone in the headspace of cultures, whereas elevated concentrations of other VOCs (acetic acid, acetaldehyde, methyl methacrylate, 2,3-butandione, and methanethiol) were obvious [219]. Therefore, acetone cannot be used as the only marker, but rather specific patterns of VOCs will be representative in infectious diseases. Furthermore, the question arises as to whether and how, in the presence of more bacterial strains, these strains cooperate in the in vivo situation. It can be considered that not all the strains release acetone but rather use it as carbon source (see above). In other words, the specific VOC profile of each strain ought to be established. The clinical value of breath acetone in bacterial infections is unclear and the widespread use of this method is still awaited.

However, there are some clinical and animal data. For example, during the analysis of breath samples of Plasmodium falciparum-infected volunteers, acetone was one of the nine VOCs whose level varied significantly over the course of malaria [220]. Unfortunately, the correlation between parasitemia and breath acetone was not investigated [220]. Fink et al examined VOC profile during sepsis, endotoxemic shock, and hemorrhagic shock in rats using ion mobility spectrometry, and measured decreasing acetone and 3-pentanone concentrations during endotoxemic shock and sepsis [221]. Nevertheless, it is unknown whether expired breath markers of inflammation and sepsis can be detected before clinical conditions become apparent [221].

7. Conclusions and clinical perspectives

In this paper, the basic concepts and methodologies of breath analysis of acetone are briefly presented along with an overview of the literature. The excretion of acetone via breath is discussed with regard to its
clinical implications. Breath analysis offers an attractive alternative to invasive blood analysis and is valuable for screening populations. Moreover, it is a good motivational tool for those enrolled in weight-loss programs because the changes of breath acetone sensitively follow fat loss [222, 223]. Finally, although historically acetone has been measured in exhaled breath to monitor ketosis in diabetic patients, owing to the development of gas-detection techniques, follow-up studies of patients suffering from various diseases can be performed in clinical practice.

As shown (figure 1), acetone metabolism is a complex network and breath acetone is a function of its formation, degradation, as well as elimination via urine (figure 2). Hence, there are several factors that can affect breath acetone even among patients with the same disease. Therefore, its level varies from disease to disease and from patient to patient. Influencing factors—among others race, age, food consumed, medicines, addiction behavior, lifestyle, and profession—have to be mentioned [105, 107, 109, 120, 224, 225]. This highlights the difficulty of investigations since recruiting a homologous group of patients is troublesome, and clinical setting is important and has to be standardized. At the same time, it is important to mention that a patient may have several disorders affecting breath acetone, which raises the question as to how one can identify the real reason for the change of breath acetone. In the authors’ opinion, a deeper insight into acetone metabolism is still needed, particularly in evident ketotic states other than DKA. This will enable an understanding of the clinical impact of metabolic changes and open a way for a more rational therapy.

Most importantly, even the difference among the members of general population poses a problem for control group selection. There is a huge variation among personnel with regard to both the quantity of exhaled compounds and their quality [5, 85, 120, 226]. The former applies to the levels of breath acetone, too, as a one to ten difference may be detected [85, 120]. Increasing the number of controls may in part solve the problem, but the higher the number of patients, the more expensive the research. However, further research cannot be avoided in order to ascertain the racial, sex, and age-dependent differences in fat and acetone metabolism. Furthermore, the relation of these factors to breath acetone concentrations of either healthy or sick individuals has to be further explored, particularly focusing on the fact that there is an overlap between the ranges of exhaled acetone of healthy controls and disordered patients [227]. Perhaps the HLA typing as well as the characterization of drug-metabolizing capacity may be of help in answering how genetic polymorphism affects acetone-degrading capacity and, in this way, breath acetone levels in humans. Unless reliable control groups can be formed, the measurements will present bad fluctuations.

Since sensitive gas-detection methods are currently available for an accurate acetone analysis even with breath-to-breath resolution (e.g. PTR-MS, SIFT-MS) the planning of the clinical protocol and breath sampling are crucial points in getting reproducible results in clinical trial. On the other hand, the accelerated evolution of sensor technology will allow miniaturization of gas-sensing systems in the near future and enable the use of sensor arrays specific for selected single VOCs, such as acetone, which will be applicable in the clinical routine. The other subject of future research has to be how to replace blood analysis with a wherever-applicable VOC detection. A new frontier for innovation would be the development of such a mobile instrument (such as mobile phones) that makes home blood glucose or fatty acid control possible via measuring exhaled acetone among other VOCs. Despite these concerns and still-existing limits, there are diseases in which breath acetone detection is of clinical value. Therefore, we conclude that the detection of acetone in exhaled breath is a useful and promising tool for diagnosis that can be used as a state marker to follow the effectiveness of treatments in some disorders.

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