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Chapter

Organic Volatile Compounds Used in Type 2 Diabetes

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Abstract

Analysis of volatile organic compounds (VOCs) in exhaled breath is non-invasive method and appears as a promising tool for metabolic monitoring. Diabetes is a complex syndrome, metabolic diseases that is characterized by hyperglycemia associated with major changes in lipids and proteins. The pathophysiology of the link between diabetes, hypertension, inflammatory syndrome and oxidative stress is complex. We conducted a study and applied quantitative analysis of exhaled ethylene and ammonia in patients with type 2 diabetes mellitus (T2DM) and a healthy control group. For breath gas analysis, a very sensitive CO_2 laser photoacoustic spectroscopy (CO_2LPAS) was applied. The concentration of exhaled VOCs differed between T2DM patients and healthy group, in particular, T2DM patients exhaled significantly higher amounts of ethylene and ammonia compared to healthy control group. The data obtained by the CO_2LPAS system revealing that the increased breath VOCs has a close relationship with high glucose levels and with healthy complications.

Keywords: type 2 diabetes, exhaled breath, volatile organic compounds, ethylene, ammonia, CO_2 laser photoacoustic spectroscopy

1. Introduction

The analysis of volatile organic compounds (VOCs) represents a rapid and non-invasive method of early diagnosis. Some of the detected VOCs can be used as biomarkers for certain diseases or can reflect the metabolic profile of an organism and are presented in the exhaled breath, skin secretions, saliva, blood, urine and feces [1]. Breath analysis is considered to be a promising tool for noninvasive analysis of biochemical processes in the human body [2–5]. Exhaled breath contains both exogenous VOCs that come from environmental exposures as the ingestion of food, smoking cigarettes or/and air pollution, or endogenous VOCs that are produced by biological processes in the human body like oxidative stress (OS), inflammation, infectious disease. Breath analysis is currently used in the diagnosis of different pathologies, including gastrointestinal and liver disease, renal failure, lung disorders, cancer, and diabetes [6–8].

Diabetes Mellitus (DM) affects millions of people worldwide, and the incidence is increasing every year. Diabetes is a chronic condition characterized by hyperglycemia caused by a defect in insulin secretion or insulin action. Diabetes is a heterogeneous syndrome, characterized by a complex disorder in the regulation of the body's energy metabolism, which affects the use of carbohydrates, lipids and proteins, as well as other metabolisms [1–3]. The most prevalent type of DM is:

type 2 diabetes mellitus (T2DM), type 1 diabetes mellitus (T1DM) and gestational diabetes mellitus (GDM). According to International Diabetes Federation (IDF) 1 of 11 adults (20–79 years) have diabetes (463 million people), 1 in 2 adults with diabetes are undiagnosed (232 million people) and 2 in 3 people with diabetes lives in urban areas (310.3 million) [9, 10].

Individuals with T2DM presents increased risk for microvascular and macrovascular complications due to hyperglycemia. The complications related to diabetes are: eye disease (diabetic retinopathy), cardiovascular disease (one-third do half of all are diabetes related deaths), kidney disease, oral, nerve and/or vascular damage and diabetic foot complications (diabetic foot and lower limb complications affect between 40 and 60 million people with diabetes globally), diabetes-related complications of pregnancy (approximately 20.4 million of live births were affected by hyperglycemia in pregnancy in 2019) [9–14].

Studies over time have shown that oxidative stress contributes to the development and progression of diabetes, in T2DM particularly [15]. In diabetics, through hyperglycemia, hyperlipidemia, and hypertension is induced oxidative stress that affects multiple organs, leading to various complications including coronary artery disease, stroke, neuropathy, nephropathy, retinopathy [16, 17].

The aim of this chapter is showing the role of breath analysis in the evolution of type 2 diabetes mellitus by measuring ethylene and ammonia as oxidative stress breath biomarkers at T2DM and healthy subjects, using a CO_2 laser photoacoustic spectroscopy (CO_2LPAS) system. At the same time, it was determined the glycated hemoglobin HbA1c and blood glucose levels. Breath tests were compared between the two groups (healthy and with T2DM) to see if the breath analysis can discriminate between diabetic and healthy subjects, and if the breath tests are in accordance with blood tests.

2. Oxidative stress in diabetes mellitus

When the human's cells are perturbed by nutritional imbalance, infections, bacteria, toxins or disease reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed. ROS molecules are highly reactive and, the pathological consequence is damage to proteins, lipids and DNA [18, 19]. This oxidative damage may lead cell death and disease such as cancer or diabetes. Thus, oxidative stress may fi define as an imbalance between production of such reactive species and the body's ability to detoxify.

Diabetic subjects tend to have an increase in ROS generation and a decrease in antioxidant defenses [18–24], in this case oxidative stress is a response to glucose and/or lipid overload. Thus, oxidative stress is involved in the occurrence of complications in subjects with T2DM [20–24], and also affect the two major mechanisms failing during diabetes: insulin resistance and insulin secretion [25–28].

According to Giugliano et al. patients with diabetes present a high level of oxidative stress leads to the appearance of atherosclerosis [29]. Also, according to Ceriello, hyperglycemia generates oxidative stress, which leads to endothelial dysfunction in blood vessels [30]. Atherosclerotic disease is the most important and frequent macrovascular complication in diabetics and it means the chronic inflammation and injury to the arterial. Oxidized lipids accumulate in the endothelial wall of the arteries, and this accumulation can lead to acute vascular infarction [20].

The link between diabetes and oxidative stress was conducted by measuring certain biomarkers such as lipid peroxidation products, biomarker for protein oxidation or DNA damage biomarker. These biomarkers are the result of free radical's damage on lipids, proteins and DNA [18–20].

Responsible for oxidative stress is the attack on proteins induce by the reaction between ROS and some amino acids [24]. Proteins are the principal target of ROS causing protein glycation and oxidative degeneration. Therefore, glycated hemoglobin HbA1c measure the protein alteration and is considered as biomarker of oxidative stress [31]. Ammonia is a biomarker of protein metabolism, and at subjects with T2DM, insulin deprivation is associated with an increase in amino acids and by an accelerated protein catabolism.

In diabetes the lipid profile is modified thus leading to the occurrence of lipid peroxidation. Another target of free radicals are the polyunsaturated fatty acids in cell membranes [32]. The end-products of LP that can be used to measure the effects of free radicals on lipids are malondialdehyde (MDA) measured in blood and ethylene as breath biomarker [33].

3. VOCs biomarkers in exhaled breath

3.1 Overview of breath analysis

Breath analysis can be considered as a potentially tool for the diagnosis and study of medical diseases. Respiratory analysis has been used in medicine since ancient times, from the time of Hippocrates (460–370 BC), when ancient Greek doctors assessing human health using human breath aroma [34–39]. The first breath analysis was conducted by Lavoisier in 1782, this showing that carbon dioxide from breathing is a product of combustion produced in the body [2, 38–40]. Respiratory studies continued later, when it was shown that human respiration contains VOCs, important in assessing human health [3–5]. The first invention that use gaseous compounds exhaled breath to assess human health was made in 1931 by Dr. Rolla N. Harger. He invented the 'drunkometer a breath-alcohol test, used to determine the alcohol concentration and has been widely used since 1938. An important step in breath analysis was conducted by Pauling et al. in 1971 when using a gas chromatograph were able to detect more than 200 VOCs in human breath [40]. Many studies on exhaled breath have been carried aiming to characterize these VOCs, and the studies carried out of Phillips estimated 1259 compounds in normal subjects in 1997 [41], and over 3000 compounds in 1999 [42]. The analysis of exhaled air is under investigation as a promising tool for express and noninvasive analysis of biochemical processes in the human body. The techniques currently used are promising to translate into clinics not for a specific disease diagnostic but more for providing information about biochemical processes that arise from underlying diseases. Nowadays, there are several commercially available devices for monitoring of breath biomarkers, such as ^{13}C used to diagnose Helicobacter pylori [43], CH_4 in breath accompanied by electrochemical sensors to detect H_2 and O_2 for diagnosis of gastrointestinal disorders [44], asthma detection by exhaled nitric oxide, breath alcohol testing [45], lung cancer detection [46], fructose malabsorption with hydrogen breath test, monitoring uptake of disinfection by-products following swimming [47] chronic kidney disease (CKD) and diabetes mellitus [48].

3.2 Exhaled breath

Human respiration is a gaseous mixture, and the main compounds are nitrogen, oxygen, carbon dioxide, inert gases, water vapor. In addition to these compounds that are found in high concentration in respiration, VOCs and inorganic molecules can also be found [49]. The presence of the latter in the composition of respiration depends on several external or internal factors. Thus, the composition of human

respiration is influenced both by exogenous compounds from exposure of the human body to various external environmental factors, and from endogenous compounds resulting from biological processes produced in the human body (in the lung tract, blood or peripheral tissues) [50].

Humans breath is a mixture of nitrogen, oxygen, carbon dioxide, inert gases, water vapor and thousands of VOCs traces and inorganic molecules [49]. The composition of breath contains exogenous compounds that originate from environmental exposures, and endogenous compounds that are produced by biological processes in the human body (in the pulmonary tract, blood, or peripheral tissues) [50]. The complex matrix of breath varies from each person both quantitatively and qualitatively. The development of different methods for gas samples analysis has allowed the detection of gaseous compounds traces from respiration, and research has shown that the most common VOCs in human respiration are nitric oxide (10–50 ppb), 1–20 ppb), ammonia (0, 5–2 ppm), carbon monoxide (0–6 ppm), hydrogen sulfide (0–1.3 ppm), acetone (0.3–1 ppm), methane (2–10 ppm), pentane (0–10 ppb) etc. [51–56].

3.3 Breath biomarkers

Biomarkers are chemicals, usually VOCs, which indicate the normal or abnormal process that take place in the human body and can suggest the presence of a disease or a recent exposure to a drug or an environmental pollutant. The most important breath biomarkers are ammonia, acetone, isoprene, nitic oxide, hydrogen sulphide, methane, ethane and pentane [30–42, 51–58].

3.3.1 Ammonia

Ammonia (NH_3) is very important for the human body and is involved in many physiological processes. The ammonia originates from the catabolism of the amino acids (which are produced mainly by degradation of proteins), can penetrate the blood-lungs and appears in the exhaled breath. Ammonia is absorbed into the portal circulation, taken up by the liver and converted to urea by the urea cycle. Urea cycle is the metabolic pathway that converts urea nitrogen excretion from the body.

Ammonia (NH_3) is highly toxic to humans, it is converted into urea which is non-toxic, highly soluble and easily excreted by the kidney. Urea is formed in the urea cycle from NH_4^+, CO_2, and the nitrogen of aspartate [59, 60]. The cycle occurs mainly in the liver. Ammonia travels to the liver from other tissues, mainly in the form of alanine and glutamine and is released from amino acids in the liver by a series of transamination and deamination reactions. Urea cycle enzyme deficiency will result in insufficient elimination of NH_4^+ or hyperammonemia which leads to central nervous system deterioration in the form of mental retardation, seizure, coma, and death [60]. The deamination of amino acids, transamination of most amino acids with α-ketoglutaric acid to form glutamic acids, and operation of glutaminase enzyme in the kidney represent the main source of ammonia in the human body. In addition to these sources of ammonia, this can be produced during purine and pyrimidine catabolism, by the action of intestinal bacteria on the non-absorbed dietary amino acids, or by the action of monoamine oxidase enzyme. Therefore, ammonia can be considered to be an important biomarker monitored in the blood, urine, saliva or breath [57–65]. The ammonia concentration is dependent on a range of factors including the health status of the patient, the route of sampling (nasal or oral), contribution from oral bacteria, diet, pharmaceutical use, physical activity and levels of metabolic activity. High levels of ammonia are associated with a variety of pathological conditions, such as hepatic and renal dysfunction, Reye's syndrome, errors

in the metabolism of urea cycle (urea cycle disorders, UCD) and is also a potential biomarker in exercise physiology and studies of drug metabolism. Normal concentration of ammonia in exhaled breath is 50–2000 ppb (parts per billion) [61–66].

3.3.2 Ethylene

Ethylene (C_2H_4) is produced by the oxidation of cellular lipids [67, 68]. The relation among ethylene, free radicals, and diabetes can be explained by the oxidative stress. The free radicals attack cellular biomembranes causing cell damage and even cell death [69, 70]. A free radical is an unstable and highly reactive molecular species with an unpaired electron that can donate or accept an electron from other molecules [71, 72].

There are many types of radicals, but the species highly unstable and concern in biological systems are derived from oxygen and known as reactive oxygen species (ROS), such as superoxide radicals, $O_2^{\cdot}$ -, hydrogen peroxide, H_2O_2, and hydroxyl radicals, $HO^{\cdot}$ [70–72]. Under stress, ROS levels increase and this can lead to significant cell damage, damage known as oxidative stress. The term "oxidative stress" (OS) is defined as the imbalance between the production of free radicals and the body's ability to defend itself [18, 19]. This process is kept in balance by the antioxidant defense system. Certain chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular disease, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in humans can be caused or their process it can be accelerated by the appearance of oxidative lesions at the biomolecular level in (lipids, proteins or DNA) when there is an imbalance in the production of free radicals [18, 19, 69–72].

The human body contains a high percentage of lipids (including polyunsaturated fatty acids - PUFA) vulnerable to free radical attack. Lipid peroxidation (LP) occurs as a result of oxidative degradation of polyunsaturated fatty acids induced by free radicals. Therefore, in LP process a peroxidative sequence is initiated by the attack of any free radical species which can extract a hydrogen atom from the group of methylene (CH_2), together with an electron on the carbon atom (•CH). By molecular rearrangement the resulting carbon radical is stabilized and is produce a conjugated diene that formed a lipid peroxyl radical (LOO•). But, the propagation of LP continues because these radicals can still extract hydrogen atoms from other lipid molecules to form the lipid hydroperoxides (LOOH). The LP process is finished with the end products that includes malondialdehyde, 4-hidroxinonenal and hydrocarbons, such as pentane, ethane and ethylene [18, 19, 68–74]. The stable end-products of LP, such as ethane, ethylene and pentane are suitable for the estimation of cell damage, because these species are excreted in the breath within a few minutes of their formation in tissues. Excess ethylene in the exhaled breath is associated with biochemical events around LP and can be considered a direct measurement of oxidative stress [67–69, 74]. Ethylene was one of the first breath compounds studied, being reported to range between 3 and 100 ppb [75, 76].

4. Laser photoacoustic spectroscopy as a method for breath VOCs detection

The analysis of trace gases from human breath for medical monitoring and diagnostics and require gas sensors characterized by high sensitivity and selectivity (to avoid interference from other potential interfering species), multi-component capability, real time measurements, large dynamic range, in situ measurements, ease to use. Laser photoacoustic spectroscopy (LPAS) is sufficiently sensitive and

rapid to allow the simultaneous analyses of several trace gas metabolites in single breath exhalations. Over the years, the LPAS technique has demonstrated its ability to detect traces of gas in fields such as biology and medicine due to several factors, such as: real-time detection of one or more volatile compounds, detection limits ranging from ppm (parts-per-million) to ppb (parts-per-billion), high sensitivity and selectivity, use of a single breath collection from a small sampling volume (few 100 ml) without the need for further preparation [66–68, 75–82].

4.1 Laser photoacoustic spectroscopy: basic principles

Laser photoacoustic spectroscopy is based on the photoacoustic effect that occurs at the interaction between light and matter with the generation of a sound wave. In 1880, Alexander Graham Bell discovered these phenomena [83] while trying to find wireless communication. Thus, he discovered that certain optically absorbing solids emit a sound when illuminated by a modulated light. In 1881, Bell [84] Tyndall [85], Röntgen [86] and Preece [87] have demonstrated that the photoacoustic (PA) effect occurs not only in solid but also in liquid and gas. They found also, that the sound was stronger when the sample was placed in a cavity called photophone or spectrophone. With the appearance of sensitive microphones, increased interest in this technique. Afterwards, techniques based on this phenomenon have been known a continuous development, and today can be applied in almost all disciplines of Science and Technology.

An instrument based on the PA effect and which uses a laser as a radiation source, has important advantages for the analysis of gas traces such as high sensitivity ppb or even ppt (parts per trillion) concentrations and selectivity, high dynamic range, high accuracy and precision, good time resolution, versatility, reliability, robustness and is easy to use.

Over the years, photoacoustic spectroscopy (PAS) has proven its ability to detect traces of gas and has been used successfully as a gas sensor in biological and medical applications [88–93].

In gases, the PA effect is produced as a result of the following sequences (see **Figure 1**) [76]: absorption of incident laser radiation modulated in frequency or amplitude by the target gas molecules; local heating due to non-radiative relaxation; the extension and contraction of the gas sample that determines the pressure variation, which is an acoustic wave; detection of acoustic waves using microphones.

4.2 Experimental section for VOCs breath analysis

Exhaled breath was analyzed using a LPAS system and we have measured ammonia and ethylene concentration from the exhaled breath in subjects with T2DM and healthy subjects [66, 75, 76]. The block diagram of the laser PA spectrometer is presented in **Figure 2**.

LPAS main components of the system are: a CO_2 laser (home-built) emitting in the 9.2–10.8 μm range (area where ammonia and ethylene shows a high

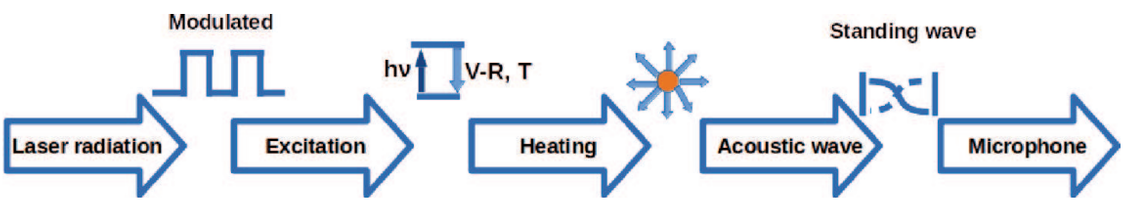

Figure 1.
Schematic of the physical processes occurring in PAS [76].

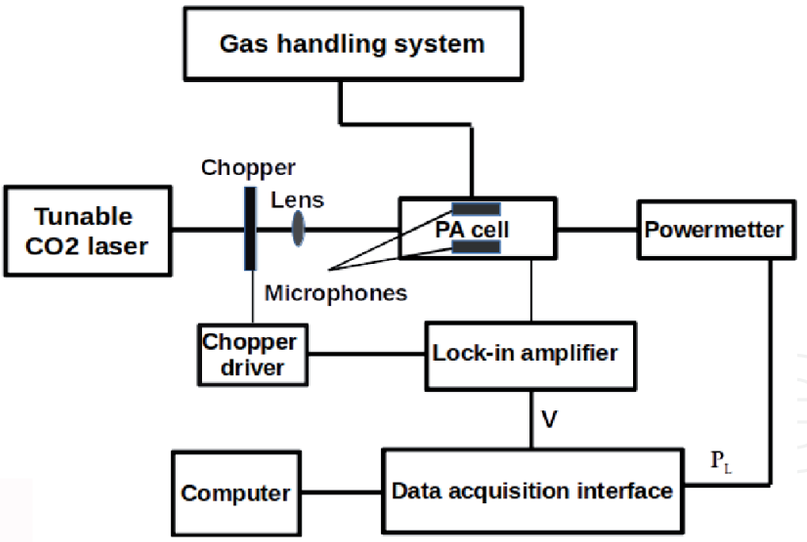

Figure 2.
CO_2LPAS system.

absorption), frequency-stabilized and an output power between 2 and 5 W, and an external PA cell (the external resonator home-build), inside it being mounted four microphones (sensitivity of 20 mV/Pa each), connected in series are mounted flush with the internal surface of the resonator tube. Before entering into the PA cell, the cw laser beam is modulated by a mechanical chopper that operate at the resonant frequency of the PA cell (564 Hz) and focused by a ZnSe lens. The laser beam power after passing through the PA cell is measured by a radiometer connected to a data acquisition interface module together with a lock-in amplifier. The acquisition interface is connected to a computer where all experimental data are processed in real-time and stored. The software allows the display of several parameters, such the values for the PA voltage, average laser power after chopper, and the trace gas concentration, and the response of the PA system is based on the formula:

$$V = \alpha C P_L S_M c.$$

Where:
V (V)—photoacoustic signal (peak-to-peak value);
α (cm^{-1} atm^{-1})—gas absorption coefficient at a given wavelength;
C (Pa cm W^{-1})—cell constant;
P_L (W)—cw laser power before chopper;
S_M (V Pa^{-1})— microphone responsivity;
c (atm)—concentration or partial pressure of the trace gas.

Another important part of the CO_2LPAS system is represented by the gas handling system. It has the role to ensure the purity of the PA cell, to introduce the sample gas into the PA cell at a controlled flow rate, to pump out the sample gas from the PA cell, and to monitor the total and partial pressures of mixture gas sample. This several functions can be performed by the gas handling system without being necessary any disconnections.

4.3 Breath collection

In breath analysis, depending on the desired result, is required a knowledge and understanding of respiratory physics. In the individual, normal, resting breathing is about 0.5 L, breathing known as tidal volume. The total volume of the lung is about

6 L, of which 4.8 L is called the vital capacity and the remaining 1.2 liters is called the residual volume and remains in the lungs [77–79].

Exhaled breath is an inhomogeneous gas mixture composed of "dead space" (roughly 150 milliliters) and the gas part represented by the "alveolar" respiration coming from the lungs (about 350 milliliters). Human exhalation contains both gas molecules resulting from the exchange of blood, but also compounds from the atmosphere. In the process of breath gases collection, there are three basic approaches [79, 80]:

1. Dead-space gas collection (or upper airway collection), which is the volume of air in the conducting airways where gas exchange cannot take place, where no exchange of oxygen or carbon dioxide occurs. There is also an alveolar dead space, which is the air in those alveoli that are ventilated but not perfused, where gas cannot be exchanged.

2. Alveolar collection (pure alveolar gas is collected), refers at the portion of exhaled breath that contain gases that have exchanged with blood. In the lungs there is a great flow of blood and the exhaled breath that contains both gas molecules resulting from the exchange of blood, but also compounds from the atmosphere. More specifically, excluding the dead-space from the analysis are excluded the chemicals that originate from the atmosphere. Most respiratory tests use this type of test because it contains VOCs resulting from endogenous activity and the influence of exogenous VOCs is eliminated.

3. Mixed breath collection means that total breath, dead-space air and alveolar gas, is collected.

For the desired results must be taken into account different factors such as the type of breath collected, single or multiple exhalation, and the technique used for sample analysis.

4.4 Protocols and procedures for breath analysis

Our measurement procedure to determine the concentrations of gases involves the following basic steps: cleaning the cell, calibration of the cell or measurement of the cell responsivity, and acquiring spectra of ethylene and ammonia [45, 56, 57].

For the measurement and detection of the gases from breath, the laser is kept tuned where ethylene and ammonia exhibit the strongest and most characteristic peaks. The cleaning of the cell must be carried out by successive washing with nitrogen of purity 6,0 (99,9999%) at atmospheric pressure, cleaning performed each time the contents of the cell are changed. An adequate degree of cell clearance is considered to be obtained if the PA signal measured in the nitrogen atmosphere has a rather low level, usually 30 μV. Absorption measurements are performed at room temperature, in the range 20° C - 22°C.

Exhaled breath samples are collected in aluminized bags (750 ml aluminum-coated bags) consisting of: a disposable mouthpiece, a tee-mouthpiece assembly (including a plastic tee and a removable one-way flutter valve), a bag with the role of collecting "dead air" (the first part of expired breath), while the alveolar air in the collection bag and a discard multi-patient collection bags are designed to collect multiple. The breath sample can be kept in multi-patient collection bags for up to 6 hours.

Ammonia is a highly adsorbed compound and the ammonia molecules adhere very well to the walls of the PA cell, so that to ensure the quality of each

measurement, an intense N_2 washing cycle of the PA cell is performed. In this way the PA signal measured is due exclusively to the absorption of ammonia or ethylene molecules. Before measurements the PA cell is filled with pure nitrogen and the background signal detected, and we started from a background signal ~25 μV.

After collecting the breath sample, the gas sample is transferred from the bag to the PA cell at a controlled flow rate 36 l h^{-1} (600 standard cubic centimeters per minute (sccm)). Before the PA cell is found a trap filled (with a volume > 100 cm^3) with KOH (potassium hydroxide) pellets replaced after each measurement, pellets used to retain interfering gases such as carbon dioxide (~4% in exhaled breath sample) and water vapor [94].

All of the collected samples were analyzed over a period of three months. To remove any residual contaminants, all of these bags were thoroughly cleaned by flushing with nitrogen gas (purity 99.9999%) and subsequently evacuated for breath sample collection. Following the procedure, the breath samples was introduced in the PA cell, the PA cell closed and used for measurements. The measurements were performed on the 10P (14) laser line (where ethylene exhibits a strong absorption with an absorption coefficient of 30.4 cm^{-1} atm^{-1}) and 9R (30) (where ammonia exhibits a strong absorption with an absorption coefficient of 57 cm^{-1} atm^{-1}). In this way the signal measured by microphones in PA cell is quantified and is proportional to the ethylene and to the ammonia concentrations.

5. VOCs measurement from the exhaled breath in type 2 diabetes

Oxidative damage was quantified by measuring breath ethylene and ammonia concentrations using CO_2LPAS system. PA detection provides necessary selectivity for analyzing multicomponent mixtures by the use of line-tunable CO_2 lasers. Breath samples were collected in special sample bags, aluminized multi-patient collection bags from QuinTron (750 mL aluminum-coated bags). All samples were given between 09:00 and 11:00 a.m. and analyzed using the CO_2LPAS system. Measurements of HbA1c and glucose were done using standard procedure. The subjects involved in this study are persons diagnosed with T2DM (n = 16), recruited from the family doctor, age between 42 and 71 years, body mass index BMI = 31.4–35.3, known stable cases of T2DM whose medical therapy had been unaltered over the last 12 months, and a healthy control group (n = 9), age between 29 and 42 years, non-smokers, non-diabetics and body mass index BMI = 19.8–23.4. From T2DM subjects, 7 present hypertension and inflammatory syndrome. No patients were on supplements with antioxidants. Informed consent was obtained from all individuals. The participants with hormonal disorders, benign or malignant disorders, renal failure, central nervous system disorders, and also smokers were excluded from the study.

As an observation of the results obtained, it can be seen that the average ethylene of T2DM subjects is higher than the average ethylene healthy subjects (see **Figure 3**). The ethylene values in healthy subjects are normal and in the range 10.73 ppb and 57.13 ppb, but at the subjects with T2DM the ethylene concentrations range was between 78 ppb and 444 ppb. The differences in exhaled breath ammonia concentration are presented in **Figure 4**, where the mean values of breath ethylene concentrations in healthy control group and subjects with T2DM are presented. The ammonia values in healthy subjects are normal and in the range 0.832 ppm and 1.76 ppm, but at the subjects with T2DM the ammonia concentration range was between 2.74 ppm and 10.16 ppm. Our measurements showed a significantly increase of ammonia concentrations in the exhaled breath at diabetic subjects compared to healthy subjects.

For the subjects involved in this study, among the exhaled breath analyses, were determined blood analyses such as blood glucose levels and glycated hemoglobin HbA1C. **Table 1** shows the values of HbA1c and glucose blood tests, as well as the values of the respiration results obtained with the CO_2LPAS system.

Among subjects with T2DM, 7 have hypertension and inflammatory syndrome. In those subjects averages of all analyzes, both blood tests and the breathing are

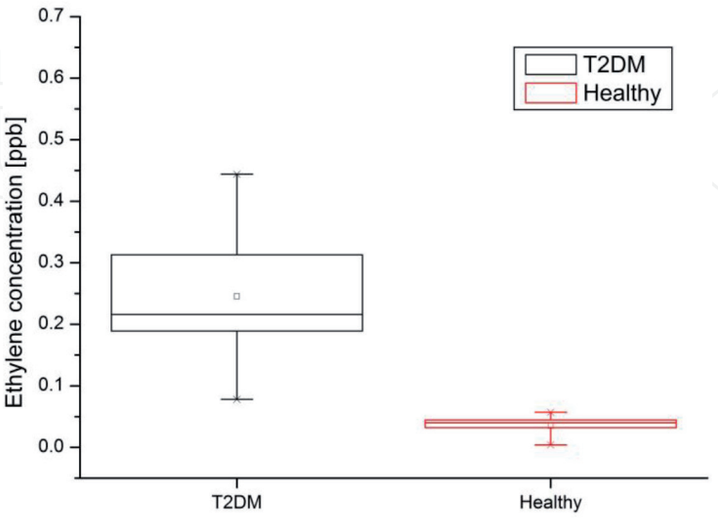

Figure 3.
Mean breath ethylene concentrations in healthy subjects and T2DM.

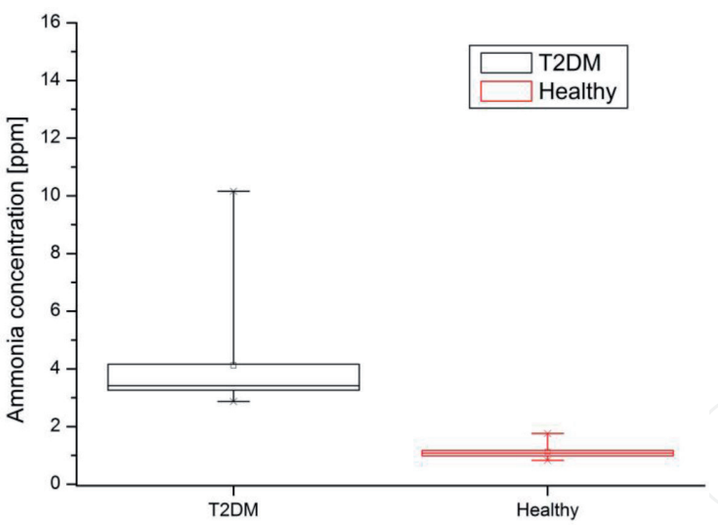

Figure 4.
Mean breath ammonia concentrations in healthy subjects and T2DM.

Parameters	Healthy subjects	T2DM	T2DM with hypertension and inflammatory syndrome
Blood glucose (mg dL^{-1})	83.94 ± 9.2	200.17 ± 44.01	200.17 ± 44.01
HbA1C [%]	4.79 ± 0.52	7.88 ± 0.66	8.21 ± 1.1
C_{C2H4} [ppb]	24 ± 3.35	238 ± 92	246 ± 59
C_{NH3} [ppm]	1.296 ± 0.18	3.96 ± 0.85	5.16 ± 1.12

Table 1.
Mean values for subjects involved with standard deviations (SD).

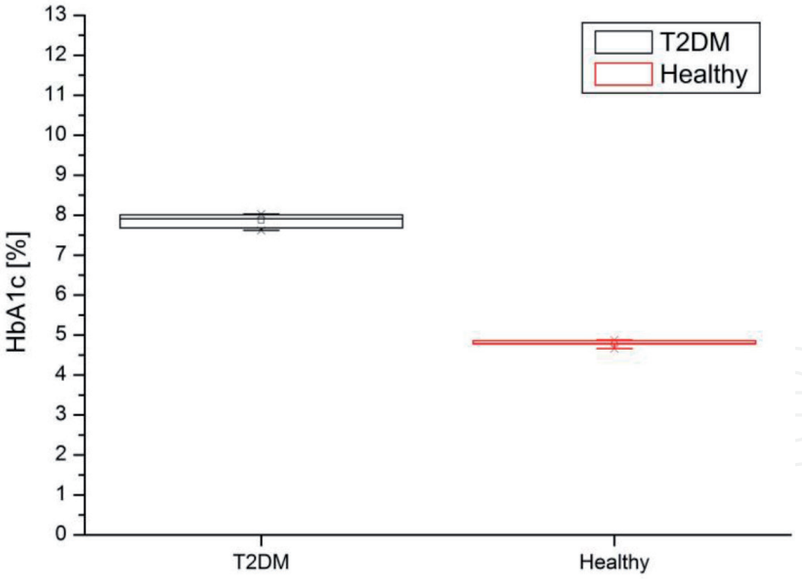

Figure 5.
Mean values of HbA1C in n healthy subjects and T2DM.

higher in subjects with T2DM who have hypertension and inflammatory syndrome compared to those who have no health complications.

Our results show a high difference in the HbA1c mean value of healthy group and the and the mean values obtained in subjects with T2DM. These differences are presented in **Figure 5**. In diabetes, impaired detoxification of free radicals and this degree of damage can be seen in HbA1c, which is a biomarker of oxidative stress.

6. Discussions

Damage to proteins and lipids due to oxidation has been implicated in the pathogenesis of type diabetes. Previous studies on oxidative stress are based on invasive blood samples analysis, and these studies found significant high level of LP product such as malondialdehyde [25, 26, 95, 96]. Through this research, Nour Eldin et al. 2014 shows that malondialdehyde concentration in blood as LP biomarker was elevated in T2DM compared to healthy control group, and that there is an association between hyperglycemia and oxidative stress [27].

Diabetes mellitus and hypertension are interrelated diseases, and hypertension is about twice as frequent in individuals with diabetes as in those without diabetes. Breath ammonia concentrations in subjects with diabetes can be a consequence of oxidative stress after ROS attack on proteins, accelerated catabolism of proteins due to insulin deprivation and hepatic glucose production [28]. According to Erejuwa, 2012, ROS are involved in insulin signaling, and goes to development of insulin resistance [97]. Therefore, oxidative stress increased insulin resistance and increase the free radical's formation in diabetes, which leads to damage in proteins and lipids. Hyperglycemia generates an increased level of free radicals which can lead to dysfunctions [98, 99] and increases oxidative damage which cause in development of health complications in diabetes, complications associated with inflammation [100] or vascular disorders [100, 101].

Some of the subjects with T2DM involved in this study, present complications such as hypertension and/or inflammatory syndrome. T2DM is an inflammatory disease, and inflammation is caused by insulin resistance correlated with obesity, or by hyperglycemia and hyperlipidemia.

Breath ethylene concentrations in subjects with T2DM was found in higher level compared with healthy subjects. Breath ethylene is considered a marker of oxidative stress, being an end-product of LP, caused by the attack of free radicals on polyunsaturated fatty acids. The damage related to free radical's action increase and a direct measurement of the damage can be achieved by quantitative determination of ethylene concentrations.

Ammonia is a biomarker of protein metabolism, and at subjects with T2DM, insulin deprivation is associated with an increase in amino acids and by an accelerated protein catabolism. Moreover, we found that breath ethylene and ammonia concentrations are higher in T2DM subjects that present hypertension and/or inflammatory syndrome than in those without complications. It is known that T2DM lead to complications like kidney failure, heart disease, cerebrovascular disease, but there is a lack of information of ammonia level in subjects with T2DM and the relationship between ammonia level and diabetes complications.

In subjects with T2DM by measuring the percentage of HbA1c, clinicians are able to get an overall picture of the average blood sugar levels have been for the past 2–3 months. Through our measurements, the diabetics present a high level of glycated hemoglobin HbA1c. An increased level of HbA1c reflect a poor metabolic control of the patients with diabetes in uncontrolled T2DM [102–106].

The relation between level of ammonia and ethylene in the exhaled breath and T2DM could be explained by the inadequate insulin control with disease progression by development of complications such as oxidative stress, inflammatory syndrome, and hypertension. The studies show a relation between hyperglycemia, oxidative stress and inflammation coexist in pathological processes but also that hyperglycemia and free radicals increase the oxidative stress which will then activate the inflammatory processes [97, 107].

Future studies are needed to understand the relationship between them and the importance of breath ammonia and ethylene biomarkers.

7. Conclusions

Real-time breath ethylene and ammonia monitoring in subjects with type-2 diabetes using a CO_2LPAS system was realized.

This paper has presented accurate measurement of breath ethylene and ammonia concentrations and the results obtained in comparison with the blood samples analysis have demonstrated the suitability of the experimental PA system for trace gas detection.

This study shows a high level of oxidative stress in people with diabetes through a high level of glycated hemoglobin HbA1c, and high concentrations of ethylene and ammonia in respiration.

Our study suggests that sensitive, noninvasive, real-time analysis of oxidative stress, using ethylene and ammonia as breath biomarkers, distinguishes healthy subjects from those with type 2 diabetes and controlled by uncontrolled diabetes.

The breath analysis may also bring opportunities in molecular monitoring for other research fields by using an LPAS system.

Despite these advances, there is a continuing need for miniaturized devices, in addition to a precise and easy to use instrument, which should provide a quick response, preferably in real time.

Further research is therefore required to expand the applicability of breath analysis in clinical diagnosis.

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