MSc Thesis in Biomedical Engineering

The development and evaluation of a novel, non-invasive measuring system for the prevention of diabetic ketoacidosis

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THE DEVELOPMENT AND EVALUATION OF A NOVEL, NON-INVASIVE MEASURING SYSTEM FOR THE PREVENTION OF DIABETIC KETOACIDOSIS

MSc Thesis in Biomedical Engineering
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by

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ABSTRACT

INTRODUCTION A potentially life-threatening complication of diabetes is diabetic ketoacidosis (DKA). The measurement of ketones can be an important tool in preventing DKA. Current technologies do not meet the requirements to be used by diabetic patients in the daily practice. A new sensor, called the PuppyNose, has been developed for the continuous non-invasive measurement of ketones. This device is worn on the arm and measures skin acetone with a metal oxide sensor. It is the goal of this thesis to help the PuppyNose progress through different stages of the research and development process and to reach technology readiness level 4.

METHODS During four sub-studies the technical feasibility of the device is evaluated and the results of these sub-studies are used at each stage to improve the design of the PuppyNose. The device is tested in-vitro and in-vivo in participants under normal physiological conditions and in a participant who is in a state of ketosis.

RESULTS Laboratory testing has shown that the PuppyNose gave an accurate and linear representation of acetone concentrations in a range of 1 to 100 ppm with a limit of detection at 0.5 ppm. In contrast to this, the sensor demonstrated a decreasing sensor response as a result of humidity and low inter-observer and test re-test reliabilities. During an in-vivo fasting experiment (n=1) no significant correlation was found between the VOC-output of the device and fasting βHB levels rising to 1.7 mmol/L. An in-vivo sub-study with multiple participants (n=6) demonstrated that moving or shaking the device on the skin had no significant disturbing effect on the average VOC output of the device. Comfort and usability research with the same participants resulted in a System Usability Score of 80 and a Comfort Rating Scale that indicated that participants had the most negative emotions on how well they felt the device on their arm and how the device changed their physical appearance.

CONCLUSION The PuppyNose in its current form is not able to reach a higher technology readiness level than level 4, due to unstable behavior demonstrated in-vitro and due to not being able to measure ketones in-vivo. The device should be fundamentally redesigned in order to have a successful impact in predicting the risk of DKA.

CONTENTS

1 INTRODUCTION

1.1 PROBLEM DEFINITION

In the Netherlands 1,2 million people are diagnosed with Diabetes Mellitus. Of this group, 1 in 10 is diagnosed with Diabetes Mellitus Type 1 (DM1) [1]. Diabetes Mellitus is a chronic auto-immune disease characterized by the failure of the body to produce insulin. A feared, potentially life-threatening complication of diabetes is diabetic ketoacidosis (DKA). DKA is caused by the production of ketones, which are produced as an alternative form of energy in the absence of insulin. The measurement of ketones could inform patients on the risk of DKA and could prevent severe complications and hospitalization [2].

Current measuring methods exist for the determination of ketones, but have shortcomings. They are either invasive, unreliable, not patient friendly or lack the ability to measure continuously [2–4]. Therefore, current technologies do not meet the requirements to be used by diabetic patients in the daily practice. A new ketone sensor, to prevent complication of DKA, should be able to measure ketones timely, accurately and with selectivity. In addition the device should be non-invasive, patient-friendly and be able to measure continuously. Albert van Gool RD and Linetec RD have developed a prototype ketone sensor for the non-invasive continuous monitoring of ketones, this sensor system is called the PuppyNose. It is a portable device based on a metal oxide semiconductor sensor design. By measuring acetone on the skin it determines the state of ketosis of a patient. At this point in time a prototype has been made. However, the PuppyNose device has not been properly tested in-vitro and in-vivo. Research needs to be conducted to evaluate the technical feasibility and usefullness of this device.

1.2 OBJECTIVES

This report describes the research effort to evaluate the PuppyNose device in its early stages of its development. Research of development projects often consist of many iterations, where new developmental progress leads to new questions that need to be researched. At each new iteration the technology will have matured and changed. This is also the case for the nature of the research that goes along with it. As a guideline to indicate the goals of this project Technology Readiness Levels (TRL) are used. TRL are an evaluation framework to describe the progress of technologies (TRL will be described in more detail in the next section). The first two levels have already been reached. A non-invasive acetone sensor with the intended use of detecting diabetic

ketoacidosis in patients has been designed and at the start of this project, a first prototype is in the end stages of development. It is the goal of this thesis project to reach TRL 4 and make the first efforts to reach TRL 5.

In order to reach this goal a group of specific objectives have been formulated. These objectives are based on progress of the PuppyNose device through the different stages of the research and development process, going from simple in-vitro experiments to more complex in-vivo experiments:

OBJECTIVE 1 Testing the technical feasibility of the device in a laboratory setting.

OBJECTIVE 2 Testing the technical feasibility of the device in a single healthy individual during daily life activities, fasting and heavy exercise.

OBJECTIVE 3 Testing the technical feasibility of the device in healthy individuals who are not in a state of ketosis.

OBJECTIVE 4 Testing the technical feasibility of the device in healthy individuals who are in an elevated state of ketosis *(this objective might not be reached due to time constraints).*

1.3 APPROACH AND ORGANIZATION OF THE THESIS

Each new objective in this report represents a new stage in the developmental process. Thus, at each new objective new knowledge will be gathered, which is used for changes in the design of the device and for assumptions on which research of the next objective is based. Therefore, it has been chosen to divide this report in to four different sub-studies based on each objective. Each sub-study is conducted in the same order, having its own research questions, methods, results and discussions (see chapter 3, 4, 5 and 6). At the beginning of this report, background information is given on all the relevant topics for this research and regarding this technology (chapter 2). In the same chapter, a detailed overview is given the working of the device and how its design changed after each sub-study (see section 4.2.3). Finally, at the end of this report, a conclusion is given regarding the goal of this project (chapter 7).

2 BACKGROUND INFORMATION

2.1 PATHOPHYSIOLOGY OF DIABETES AND DIABETIC KETOACIDOSIS

Insulin is a hormone produced by the beta cells in the pancreas and is essential for the metabolism and regulation of glucose concentrations. Insulin is released when glucose levels are high. The hormone promotes the uptake of glucose into cells and stimulates cells to convert glucose to glycogen for storage. This will lower the concentration of glucose in the blood, which in turn leads to a decrease in the secretion of insulin. This way, glucose and insulin act in a negative feedback loop effectively regulating glucose levels [5]. Patients with diabetes lack the ability to produce insulin when sugar levels are high in the bloodstream. In the absence of insulin, cells will not be able to extract glucose from the bloodstream leading to glucose depletion in the cells [6]. Due to the energy depletion, the body will start metabolizing stored fat, a process which is called lipolysis, to meet its energy requirements. Fat molecules are broken down to form free fatty acids (FFA), which can be turned into energy [6]. At the same time, another process called ketogenesis, a biochemical reaction resulting in the creation of ketone bodies, is induced in the liver. This process is initiated when liver cells produce more Acetyl-CoA than can be used in the citric acid cycle for their own energy consumption. The surplus of Acetyl-CoA is via an enzymatic pathway transformed into acetoacetate (AcAc), one of the ketone bodies [2, 7, 8]. Acetoacetate can enzymatically be converted to beta-hydroxybutyric acid (β-HB) or spontaneously break down into acetone and carbon dioxide [7, 8] (see figure 2.1). Acetone, AcAc and β-HB together are called ketone bodies or ketones (KB). Acetone has no further use to the human body and is disposed in the urine or in gaseous form through the breath or skin. Ketone bodies are highly soluble and travel through the blood stream from the liver to other tissues. In humans they are mainly leveraged to supply the energy demand of neuronal tissues in the brain in glucose deprived situations, because brain tissue cannot convert FFAs into energy [9]. Beta-hydroxybutyric acid is the most stable ketone body [10] and can be found in the highest concentrations in the blood. For these reasons, β-HB is usually the gold standard bio-marker in determining the state of ketosis in humans and animals. However, all ketone bodies can be used as an effective bio-marker for ketosis.

As β-HB and AcAc or both strong organic acids [7], too high concentrations of these KBs would lower the pH. As a natural protection mechanism against this, ketone bodies have a self-controlling effect preventing uncontrolled increase of KB concentrations [11]. In healthy humans, ketone bodies inhibit lipolysis and stimulate the release of

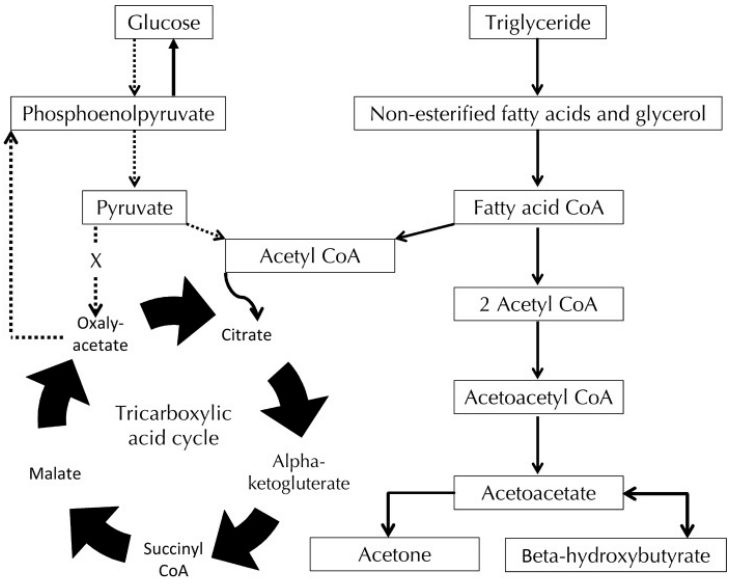


Figure 2.1: Figure shows metabolic pathway of ketogenesis. When glucose and insulin are low, high amounts of triglycerides are being metabolized into Acetyl-CoA. This is more than can be used in the citric acid cycle of liver cells. The excess amount is transformed into acetoacetate and subsequently acetone and β-hydroxybutyrate. In peripheral tissues β-HB can be converted back to Acetyl-CoA and transformed into ATP. Image taken from [2].

insulin, which in turn reduces lipolysis, FFA formation and ketogenesis in a negative feedback loop. However, in DM1 patients, the insulinotropic effects of ketone bodies are significantly reduced or absent, which makes these patients very susceptible for uncontrolled KB production [11]. Left untreated with insulin medication, this can result in an acute complication called diabetic ketoacidosis (DKA) [2, 3, 7, 8, 12–14]. DKA is characterized by a combination of high levels of ketones, metabolic acidosis and hyperglycemia [15]. Metabolic acidosis is a result of the large amount of acidic ketone bodies overloading the natural bicarbonate buffer in the blood and lowering the pH below safe physiological limits, where enzyme failure and organ failure can occur [16]. Hyperglycemia, due to the decreased cellular uptake of glucose, can lead to osmotic diuresis causing patients to lose large amount of water and sodium and potassium [6]. These events may develop in a life-threatening situation when left untreated.

2.2 INDUCING KETOSIS IN HEALTHY PERSONS

Ketone bodies are not only formed in patients with diabetes. Ketosis happens in all humans, and in many other singular or multi-cellular organisms, when there is a temporary or long term shortage of available glucose. Different concentrations of ketone bodies can also be induced in healthy persons under certain circumstances, albeit that this process happens more controlled than in diabetics. With the following

methods ketosis can be induced in healthy individuals: with fasting, a ketogenic diet, exercise or with exogenous ketone supplements. These will be discussed below.

FASTING During fasting glycogen reserves will be depleted within 12 hours. After that, glucose can still be formed from amino acids to meet the body's energy requirements. But after 24 hours ketone bodies will be the dominant energy supply for the brain and concentrations will begin to rise quickly [17]. Within the first 24 hours β-HB levels remain on average under 0.5 mmol/L [18–20]. β-HB levels then increase from around 1.5 in 2 days to 1.5-2.5 mmol/L in 3 days [18, 20]. Total major ketone body concentrations (AcAc + β-HB) show the same trend. A concentration is reached of 0.5 mmol/L after a 1 day period [17, 18]. This increases from 3-5 mmol/L in 3 days to 5-7.5 mmol/L at 7 days on average [14, 18]. Balasse et al. reports a model were steady state values of ketones are reached in 5 to 6 days with values ranging between 7-10 mmol/L [11]. Subjects that were followed-up up to 24 days showed remarkable steady concentrations still falling into that range. This seems to be the result of a ketone regulation mechanism against severe ketoacidosis, indicated by the insulinotropic and antilipolytic effects of high concentrations of ketones.

KETOGENIC DIET Ketogenic diets are diets which are very low in carbohydrates. These diets usually contain 5 to 10% of the total calories in carbohydrates, whereas this is around 50% for regular diets. Keto-diets induce a low level state of ketosis called nutritional ketosis. The low-carbohydrate intake ensures that ketone levels do not become to high and make blood pH too acidic. Therefore, the keto-diet is relatively safe and can be continued for periods of weeks or months at a time. Two different studies on healthy subjects have shown how β-HB increased significantly. Harvey et al. reported a rise to 1.8 mmol/L after 2 weeks on a 5% CH diet [21]. However, in this study the chain length of the fat molecules greatly determined the ketogenic response. Medium chain triglycerides are not incorporated into lipids and are therefore more easily metabolized into ketones resulting in higher states of ketosis [22]. Buga et al. showed an increase of 1.2 mmol/L after 6 weeks on a 10% CH diet, although there was almost no increase in the last 3 weeks [23]. Another study on epilepsy patients with an average age of 16 showed β-HB levels of 1.37 mmol/L at 3 months and 1.67 mmol/L at 6 months [24].

EXERCISE Ketogenesis can also be activated during long-duration, low to moderate intensity exercise. As the body is stressed during exercise, the total energy demand, determines from what available sources to the body the energy is derived. During low-intensity exercise, most of the energy is derived from free fatty acids in the blood and inter-muscular triglycerides. This results in an increase in lipolysis and fat oxidation, which in turn results in an increase in ketone bodies. During periods of exercise muscles rely more on ketone utilization, and can metabolize five times as much ketones as during rest [14]. Lipolysis and fat oxidation will increase until exercise intensities of 70% of the VO2max are reached, thereafter glycogen is predominantly used to meet the energy requirements [25] (see figure 2.2). VO2max is the peak volume of oxygen that the body can transport and metabolize per unit time. In order to stimulate maximal ketogenesis exercise intensities should not exceed the 70% VO2max. It is hard

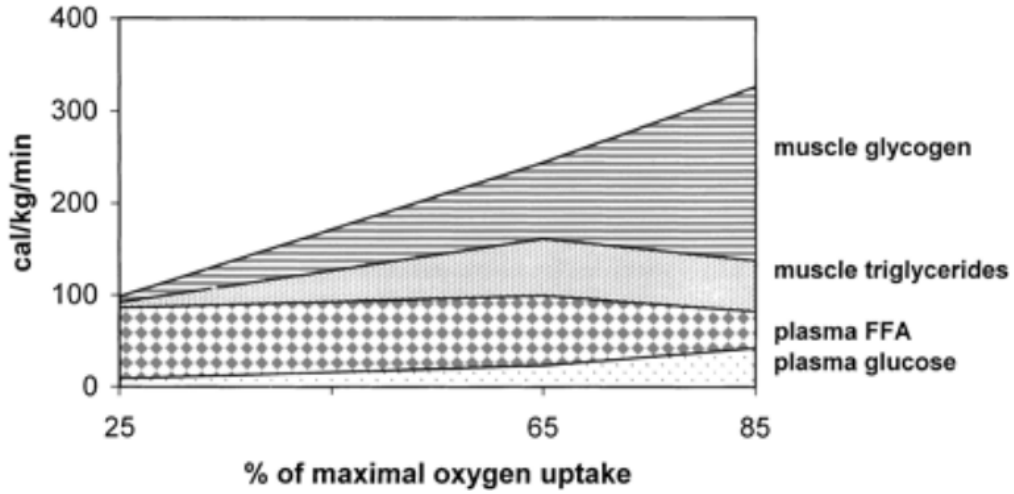


Figure 2.2: The amount and ratio of different energy sources is plotted against the percentage of the VO2max. At 65-70% are FFA, triglycerides and consequentially the amount of ketones the highest.

to give a clear representation of the effects of exercise on ketosis, because in the literature differences exist in fasting status, age, duration and intensity of the exercise and the training status of the subjects. As a result, findings from studies varied greatly. Some articles reported ketone levels between 0.5-0.6 mmol/L after longer duration low-intensity exercise [11, 23]. Three other experiments [11, 18, 26] showed that an one or two day fast or ketogenic diet in combination with higher intensity exercise (2h walking at 60% VO2 max, 1h running at 70% VO2 max and 2h running) led to much higher states of ketosis than fasting alone. In these studies maximal values of 2-5 mmol/L ketones were reached. In all cases, the highest ketone levels were reached $>$ 4 hours post-exercise, whereas FFA concentrations were at their peak at the end post-exercise. In an article by Koeslag et al. different groups of trained and untrained young adults performed exercise varying from low to high intensities and with durations from 30 to 90 minutes [27]. Remarkably, in none of the groups or sub-groups any levels of ketones could be detected that were significantly higher than non-exercising controls.

EXOGENOUS KETONE SUPPLEMENTS Ingestion of exogenous ketones are able to raise blood ketone levels, while not burdening subjects with fasting, dieting or exercise. Ketones can be ingested exogenously with ketone salts and ketone esters. These compounds are ketone bodies (β-HB and AcAc) bound to a different molecule for stability. The problems with ketone salts is that the blood β-HB reached are low and that large amounts of salts enter the body [28]. In research conducted by Stubbs et al. and O'Malley et al. β-HB levels in cyclists and healthy persons were never higher than 1 mmol/L [28, 29]. On the other hand, ketone esters are more stable and get hydrolyzed to actual, naturally occurring ketones upon first pass through the liver [30]. This results in much higher β-HB concentrations post-consumption. There is only one β-HB ester commercially available: (R)-3-hydroxybutyl-(R)-3-hydroxybutyrate. The effects

Table 2.1: The risk levels associated with certain β-HB levels. Table is based on guidelines by the National Health Service [39].

β-HB level	**Risk level**
$<$ 0.6 mmol/L	normal reading
0.6 - 1.5 mmol/L	slightly increased risk of DKA
1.6 - 2.9 mmol/L	increased risk of DKA
$>$ 3 mmol/L	very high risk of DKA

of this ester has been studied in different articles. Cox et al. reported that in healthy volunteers and athletes an ester of 573 mg/ kg bodyweight (BW) increased β-HB levels in 30 min to 6 mmol/L, a level which remained stable for 60 min. [31]. These results were confirmed by Shivva et al., where dosages of 291, 395 and 573 mg/kg BW raised β-HB concentrations to 3-5 mmol/L in 1.5 hours [32], and two articles by Stubbs et al., where 400 mg/kg BW ester induced a maximum of 3 mmol/L β-HB [28, 33]. Interestingly, Clarke et al. needed much higher dosages of 714 mg/kg BW to reach values of 3.3 mmol/L β-HB [34]. By ingesting ketones exogenously only β-HB is initially increased. When using different ketones as bio-markers of ketosis, it is relevant to see if the ratio β-HB:AcAc is the same under normal physiological conditions as it is after exogenous ingestion. During normal ketosis, this ratio is in well-fed state approximately 1:1 (β-HB:AcAc) [11, 14, 18, 35]. After prolonged fasting or a ketogenic diet this ratio rises, for unknown physiological reasons, to around 3:1 to 4:1 [11, 14, 18, 35–37] and can even in severe DKA go to 10:1 [37]. Investigation of these ratios after KE ingestion, show that for low β-HB levels ($<$ 0.3 mmol/L) ratios were 1:1 [34], but for β-HB levels (2-3 mmol/L) ratios rose to 2.5:1 to 4:1 [28, 34] . This demonstrates that after ketone ester ingestion, the ratios between β-HB and AcAc are the same as when ketone bodies are created under normal physiological conditions.

2.3 MEASURING KETONE BODIES

According to guidelines of the American Diabetes Association, to diagnose a patient with diabetic ketoacidosis, a set of criteria needs to be fullfilled based on the levels of glucose, the equilibrium of the bicarbonate buffer and the pH of the blood [16]. Although this gives a clear indication when a patient has this complication, it only can be used as cut-off point classifying patients in two groups: DKA or no DKA. In order to timely detect high states of ketosis leading to DKA, which is key in preventing its negative consequences, ketone bodies should be measured. For this, β-HB, which is the most dominant ketone in the blood, is primarily used in the literature and patient guidelines to indicate the risk of DKA [2, 3, 13, 38]. Different β-HB levels are an indicator for the severity of the risk of a DKA and when to seek medical treatment, even if symptoms or complaints are not present yet. Table 2.1 displays these risk levels.

Besides β-HB measurements, other methods measuring acetone and acetoacetate are also used for determining the DKA risk in diabetes patients. An overview of all the methods is given below.

β-HB BLOOD TESTS The main advantages of measuring β-HB in the blood is that it gives a real-time indication of the concentration and that the ketone β-HB can be accurately detected, mainly due to its high concentration in the blood [2]. Furthermore, plasma β-HB has a good sensitivity and specificity for determining DKA. When taken at a cut-off point of 5 mmol/L β-HB the sensitivity and specificity in detecting DKA are 100% and 94% respectively [3]. Traditionally, β-HB is determined with laboratory tests. However, in recent years point-of-care finger prick devices have entered the market, which can be used daily by patients themselves. However, these finger prick devices are invasive, painful and require active measurements by patients [2]. This makes them less suitable as a patient-friendly tool for day-to-day use.

URINE TESTS Another, frequently used method to measure ketones are via urine samples. Urine dipstick tests measure acetoacetate. They are cheap, non-invasive and easy to use, making them suitable for day-to-day use by patients. However, these tests have many shortcomings. Studies have shown that while urine dipstick tests are excellent for ruling out DKA (sensitivity: 98-99%), they also result in many false positives (specificity 35-77%) [3, 12]. This means that while almost no patients with DKA are missed, many have to be admitted to the hospital to undergo expensive laboratory testing to exclude DKA. Furthermore, the test readings are an average of plasma AcAc since the last void and testing can only be done when the patient is able to produce urine, which can take many hours in patients dehydrated due to their hyperglycaemia [2]. Additionally, urine dipstick can be used for diagnosing DKA in patients, but have often low accuracy in determining the ketotic state of the patient leading up to DKA.

BREATH ACETONE TESTS In recent years, technologies have been developed towards measuring ketones with acetone, either in the breath (BrAce) or on the skin (SkAce). Measuring acetone has the advantage that it is not invasive, can be monitored by small wearable or handheld devices and can support continuous measurements. The link between breath acetone and ketosis has been a well-studied topic, focusing on fasting and diabetes. Mostly, studies base the evaluation of this technology on the correlation between BrAce and β-HB measurements [26, 40–43]. In these studies BrAce values varied between 0.1 and 50 ppm. As a result of different measuring techniques in these studies, the same amounts of β-HB corresponded sometimes to very different BrAce concentrations. Multiple studies have shown that good correlations could be obtained between BrAce and β-HB (r= 0.82, 0.68, 0.92) [26, 41, 42]. Another review by Anderson combined the data of four different studies (avg. R^2 = 0.77 [Range: 0.54 to 0.94]) and fitted it with an exponential function [43]. This nonlinear relationship fitted the data well and indicates that a good model can be constructed, predicting β-HB values from breath acetone measurements. However, the problem with the determination of acetone concentrations in the breath, is that acetone exchange happens mainly in the airways and not in the alveoli. Unlike the alveoli physical parame-

ters such as air flow, temperature and pressure tend to fluctuate more. As a result, acetone exchange is influenced by exhaled air volume, breathing pattern and breath temperature [43]. Therefore, in almost all studies researching BrAce relatively strict measurement protocols have to be constructed relying on breathing in a sample bag, breathing through a straw or only collecting end-tidal breath. The collected gasses have to be analyzed in highly specialized laboratories using mass-spectrometry, gas chromatography or with complex chemical procedures [26, 40–42].

In recent years, portable devices have been designed for day-to-day use to measure breath acetone [4, 26]. For these devices, controlling the factors determining acetone exchange remains a challenge. Devices that have become more popular in recent years are sensors based on a Metal Oxide Semiconductor (MOx) design. While MOx sensors have gained attention in literature, there is only one study with available clinical data based on a commercially available sensor, the PBAM made by Readout, Inc [4]. In this study a moderate correlation between BrAce and β-HB was found of $r^2 = 0.57$.

SKIN ACETONE TESTS Measuring acetone on the skin has the advantage over breath measurements in that continuous measurements can be done and no complex compensation techniques are required to regulate acetone concentration in the breath. Although ketone measurement with BrAce has been studied intensively for over four decades, only a handful of studies have measured acetone concentrations induced by ketogenesis [44–49]. Two studies compared BrAce and SkAce values during light exercise and fasting (r =0.752 and r = 0.802, respectively), clearly showing that correlation between the two exist [44, 47]. In another study diabetic patients underwent a fast during which SkAce and β-HB was measured. A correlation was found between the two measures ($p<0.001$, r = 0.669)[46]. In all three studies a closed plastic bag over an arm, hand or finger was used. The air was then analyzed with mass-spectrometry or gaschromatograpy. Acetone skin concentrations ranged most of the time between 0.1 and 1 ppm, fluctuating strongly based on the type of measurement method used.
A problem with the measurement of acetone on the skin is that many different skin gasses emanate from the skin, mostly volatile organic compounds (VOCs) [50, 51]. Under normal physiological conditions these other compounds can be present in almost the same concentrations as acetone [51]. Sensors that are not selective to only acetone, such as MOx sensors, can suffer from the interference of these VOCs. However in the case of DKA or overnight fasting, these acetone concentrations can increase more than tenfold, from 0.08 to 0.8-0.98 ppm [48, 49]. In contrast to potentially increasing acetone, these other compounds, except for ethanol after alcoholic consumption and compounds used in cosmetics, remain stable at around 0.1 ppm [49].

2.4 METAL OXIDE GAS SENSORS

Metal oxide (MOx) gas sensors were developed in the 1950s and 1960s when it was shown that the characteristics of some semiconductor materials are modified in presence of certain gasses [52, 53]. The sensing principle of these sensors is that the

conductivity of the sensor changes as a result of the concentration of reducing agents reacting with the sensor surface [52, 54, 55]. This change in resistance can be measured to determine the concentration of the gas.
The sensor surface is made from a metal-oxide layer, usually tin-oxide (SnO_2) or zinc-oxide (ZnO), which has a crystalline structure comparable to silicon [55]. The material is doped with metal atoms, such as Fe or Al, determining the material's conductivity. The operating mechanism of MOx sensors is based on interaction with oxygen. When the sensor is heated, oxygen is adsorbed to the metal-oxide surface and acts as an oxidizing agent ($O_2 + e^- \rightarrow 2O^-$). As more oxygen becomes trapped on the surface of the material, the number of free electrons in the upper regions of the material decreases. This creates a so-called depletion zone [54, 55], a location with no free charge carriers. Consequently, when two electrodes would be attached at the end of the metal-oxide material parallel to the surface, a higher voltage is needed to let a minimal charge travel between the two electrodes. In other words the resistivity of the material becomes larger, when more oxygen atoms are adsorbed on the surface of the metal-oxide. When other reducing agents are in the air they compete with the oxygen on the surface of the sensor surface. In natural conditions the only electron donors existing in air are volatile organic compounds, small chain carbohydrates [56]. These compounds react with oxygen to form water and carbon dioxide. More VOCs in the air will result in less oxygen available and a lower the sensor resistance. As such, MOx sensors can be used as a sensor for acetone. The type of material and its structure determines how easily oxygen or reducing gasses can diffuse into the material and react with its surface to produce changes in its resistivity [57, 58].
Advantages of MOx sensors are that they react fast and that they are sensitive to small changes in the environment. Besides that, they are very low-cost to produce, especially in comparison to other sensors for acetone, which are normally based on mass spectrometry or gas chromatography [58]. An inherent disadvantage of MOx sensors is that they react to all reducing agents and thereby have low selectivity [58]. A MOx sensor cannot be used to distinguish between different gasses. Furthermore, the output is sensitive to the relative humidity as water water can also react as a reducing agent ($2H_2O \rightarrow O_2 + 4e^- + 4H^+$) [54, 55, 58].

2.5 TECHNOLOGY READINESS LEVELS

In a design project it can be hard to set clear goals, because it consists of many phases and iterations, which can comprise many years. An evaluation framework is an essential tool in demarcating goals and planning within such a project. To do this in this project, Technology Readiness Levels (TRL) have been chosen [59]. Technology Readiness Levels are a way to measure the maturity of a technology during its development. This evaluation framework was originally developed by NASA, but is now widely adopted in many areas of research and development and is even used by the European Commission as a tool to direct funding [60]. The TRL scale consist of nine stages, which go from early research and technology development in laboratory stage to proving the technologies effectiveness in a practical environment. For the

PuppyNose project the scale has been adapted to indicate the project's progress (see figure 2.3).

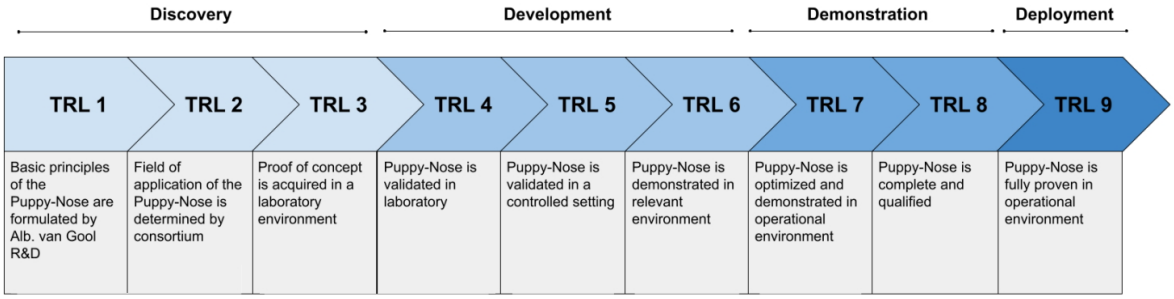


Figure 2.3: The Technology Readiness Levels graphical scale adapted specifically for this project.

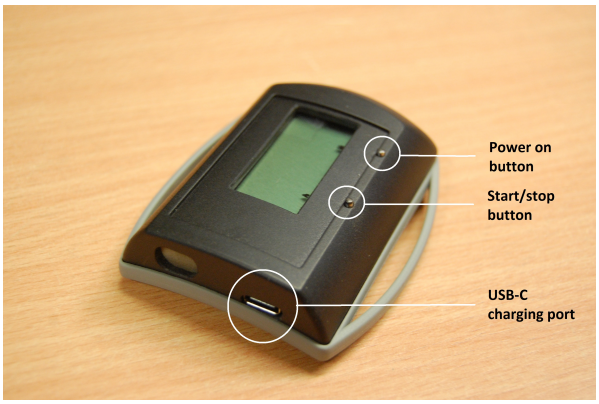
2.6 DESCRIPTION OF THE PUPPYNOSE DEVICE

The PuppyNose is a wearable device which is used as a sensor for acetone. Because acetone dissipates through the skin, it can be measured on the skin's surface. The PuppyNose utilizes this principle to measure ketosis non-invasively. To measure acetone the PuppyNose has a metal oxide semiconductor sensor.

2.6.1 Overview

The outside of the PuppyNose device consist of a case that can be kept in place with a tightening strap (figure 2.4a). The front side of the case of the device integrates a screen, two buttons and on the side a charging port (figure 2.4b). The PuppyNose has been designed to be worn on the upper arm with the buttons pointing towards the torso of the user. This location is chosen, because the device can remain in a fixed position during daily life activities, while this position still allows the user to continuously read the values on the display. On the backside of the case there is an opening for skin gas. This opening is covered by a hydrophobic mesh through which acetone can diffuse (figure 2.4c). An elliptical cushion surrounds the gas opening. Behind the mesh the acetone sensor is placed. When the device is placed on the arm the cushion makes a tight connection with the skin and seals off the gas opening from the surrounding air. This way, a semi-closed chamber is formed between the skin and the gas opening, ensuring stable concentrations of VOC that only depend on the gas emanating from the skin. The sensor then measures the concentration within this semi-closed chamber.
The electronics inside the device manage the measurement of VOCs and environmental variables. The information from the sensors is processed and displayed on the LCD screen or sent to an external computer via a Bluetooth connection. The components that are responsible for the functioning of the device and their interaction are

(a) The PuppyNose with the tightening strap.



(b) The buttons with their functionality.

(c) The backside of the PuppyNose. A mesh covers the opening and an elliptical cushion seals off the skin and forming a semi-closed chamber.

Figure 2.4: The PuppyNose viewed from different angles.

shown in figure 2.5.
In the next sections the working of the electronics and software is described in more detail.

2.6.2 The electronics

The electronics inside the device consists of a controller chip with an ESP32 design which is connected to a digital sensor chip, the BME680 made by Bosch [61] and a LCD screen. All elements of the device are powered by a 3.7V 500 mAh lithium ion battery, which has an integrated voltage and current regulator and charging circuit.
The ESP32 is a module which integrates a processor, memory and Bluetooth feature on the same chip. It runs all software tasks and instructs the BME680 to perform measurements.

The BME680 sensor chip measures the change in resistance of a metal oxide semiconductor (MOx) sensor when volatile organic compounds are present. The MOx sensor is integrated with a heating element, which is placed underneath the sensor surface. The heating element warms the sensor surface to a temperature of around

350 °C before measurement. Bosch does not provide information what exact material it uses for its sensors although the ideal operating temperature indicates a metal oxide based on tungsten, zinc or tin (e.g. based on WO_3, ZnO or SnO_2) [62, 63]. Besides a VOC sensor, a temperature, pressure and relative humidity sensor are embedded on the BME680. For this sensor extensive processing support is available e.g. to compensate for temperature and humidity influences. All four sensors are outfitted on a 3x3x0.93 mm package on the chip. The sensor heating and the read-out of the sensors is an energy demanding process and the BME680 is therefore not designed to operate continuously. The sensor chip runs normally in low-power inactive mode and only performs a sequence of heating up the MOx sensor and a measurement of all sensors, when it gets a command from the ESP32 controller.

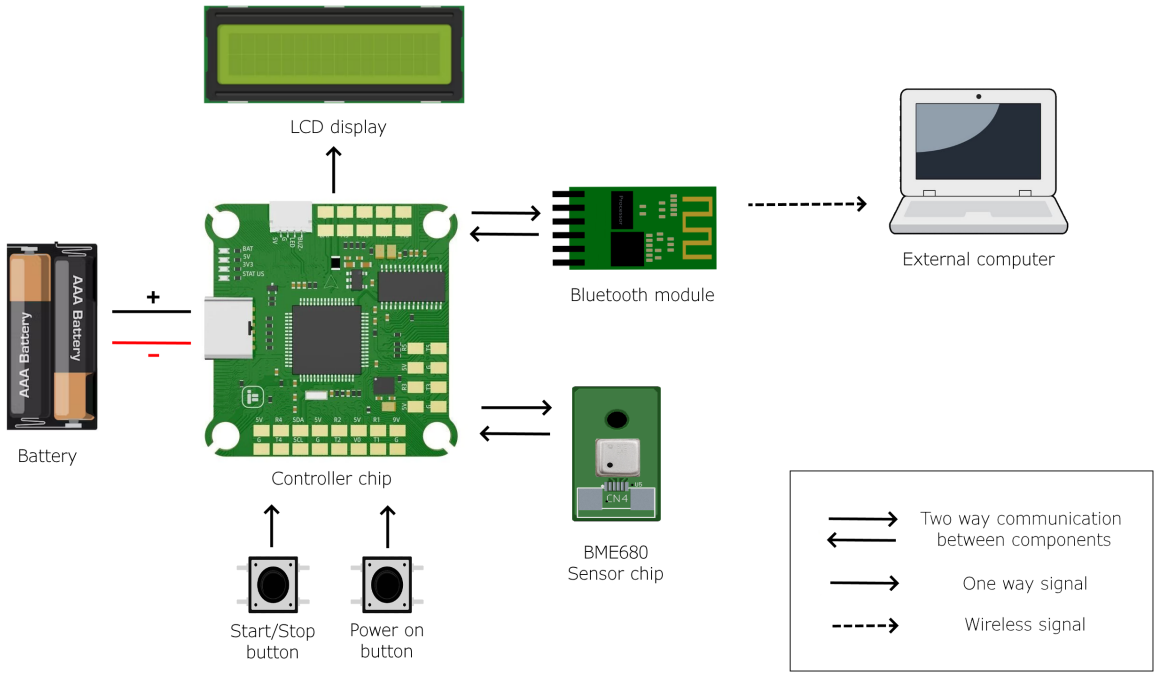


Figure 2.5: A schematic representation of how the different components of the PuppyNose operate. The controller chip receives power from the battery and acts as the central commanding unit of the device. After the user has powered the device on and starts a measurement with the buttons, the controller chip will command the sensor chip to start measuring at fixed intervals. The sensor chip will send the data of all its measurements back to it. The BME680 has four sensors on board. One sensor measures volatile organic compounds, while the three other sensor measure temperature, relative humidity barometric pressure. The controller uses the temperature, humidity and pressure data to compensate the VOC measurements. After compensating and transforming the data, the controller sends the data to the screen for display and the Bluetooth module. The Bluetooth module will send the data to an external computer where it can be stored.

2.6.3 The software

The software of the PuppyNose runs on the controller chip and is designed to read in the data from the sensor chip, apply the necessary conversion and calculations and to send the data over Bluetooth as time efficiently as possible. It has to do this while also keeping the power consumption to a minimum. To keep power low, the device is normally kept into deep sleep mode. When the power button is pressed the controller chip and the LCD become active and the device will be detectable for a Bluetooth connection. A start/stop button activates the sensor chip and start a measurement until the button is pressed again. When the start-button is triggered the controller chip will run two parallel tasks at the same time at fixed intervals: an acquisition task and a communication task (see figure 2.6).
During the acquisition task power and an activation signal is sent to the sensor chip and raw data is sent back from it at a rate of 1 Hz. This data is then converted on the controller chip with a library provided by Bosch called the BSEC library [64]. This library offers an algorithm that corrects for relative humidity and temperature influences. It is not known, which compensation and filtering techniques the algorithms of the BSEC library uses, as Bosch has not made this publicly available. This library can produce a set of output measures from the raw input data. These include the indoor air quality (IAQ) and an estimation of the VOC concentration in the air. The processed data and the raw data together is then appended to a Bluetooth send buffer. At the same time the communication task runs. The communication task checks which buffers are filled and not in use by the acquisition task. If a buffer is filled, this task sends it to an external computer via Bluetooth.
The usage of the compensation algorithms in the BSEC library is essential, because raw sensor values of the BME680 can change strongly due to drift and other external factors in matter of hours. For the compensation algorithm to reduce these effects, the device has to run a calibration cycle in clean air after it is activated from deep sleep mode. The clean air value acts a reference point for the software to base its concentrations on. When the device is running, long-term drift is continuously corrected by the software [61].

2.6.4 The data

The PuppyNose measures and computes a range of data variables. A part of these variables consists of the raw measurements of the sensor chip, another part are the compensated values calculated by the BSEC library and the last part are values indicating the accuracy of the measurements and the calibration process. All this data is sent via Bluetooth to an external device. However, the most important variables are displayed on the PuppyNose's screen (see figure 2.7). A description of these variables:

- Gas – the uncompensated value for the raw sensor resistance in Ω. This is the resistance that is measured by the sensor chip's internal electronics.
- VOC – the amount of volatile organic compounds in the air in parts per million (ppm). This value is considered the most stable and realistic measure of the acetone concentration on the skin. For this value the raw sensor resistance is com-

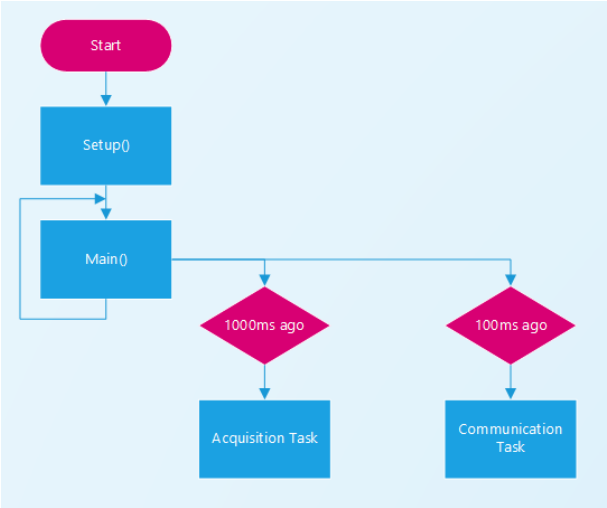


Figure 2.6: A block diagram depicting how the communication and acquisition tasks run independently and continuously.

pensated for relative humidity and temperature influences by an algorithm from the BSEC library in the PuppyNose's controller unit. The value produced by the library represents the total concentration of compounds in an exhaled breath of healthy humans (ethane, butadiene, ethanol, acetone and carbon monoxide). In the case of the PuppyNose only acetone will be measured and hence, the concentration displayed on the screen is not an exact 1:1 representation of the concentration of acetone present.

- Comp IAQ – compensated Indoor Air Quality. An index ranging from 0 to 500 for reporting the cleanness of the air. This variable is corrected for influences of temperature and humidity on the sensor output. Values below 50 represent good air quality, while values above 100 represent unhealthy air quality. When measuring on the skin the IAQ can be interpreted as a different scale for the amount of acetone present.
- ... % – Relative Humidity (RH) on the skin.
- ... C – Temperature (T) on the skin.

For this study, only the variables VOC, relative humidity and temperature are used.

Figure 2.7: The screen of the PuppyNose displaying the five different variables.

2.6.5 Usage of the PuppyNose

All functionalities of the PuppyNose device can be operated with the power-on and start/stop buttons. In order to improve usability, it does not matter how long a button is pressed and each button only has a single function at a certain time. To start a measurement the start/stop button must be pressed. This will start the calibration period that lasts for about five minutes, in which no valid measurement can be processed. After the calibration is complete, the device will internally process and show values for the six different variables (figure 2.7). After starting a measurement the data is automatically transmitted to an external computer via Bluetooth if a connection has been set up. When the measurement is to be stopped, the user presses the start/stop button again. The device stops measuring and goes to sleep after five minutes.

The device has a battery life of about 4-6 hours when measuring continuously. If battery power gets to a minimum, the device will display 'battery low' when powered on. At this point the user should charge the device via the USB-C port. The charging takes about 3-4 hours, although the device can sustain longer charging times due to a built-in overcharging protection circuit.

2.7 DEVELOPMENTAL CHANGES OF THE PUPPYNOSE DEVICE

The design and functionalities of the PuppyNose were not the same over the course of this project. Changes to the design were made based on the results of research of the sub-studies in this report. Sometimes these findings were made as a result of an experiment, but in some cases the finding of weaknesses in the design was serendipitous. During this process there were four notable different designs that were used. These versions and their moment of introduction during the project are described below.

Laboratory experiments

During the first laboratory experiments the PuppyNose device only consisted of the the sensor chips without the casing (figure 2.8a). The sensor chip was connected to the main controller chip via a long connector cable. This simple design was convenient for the laboratory experiments, because the sensor chip could be inserted in a closed glass container where well controlled acetone concentrations were created, when the device in its entirety would not have fitted.

First design for humans

The first design for usage by humans was with the casing of figure 2.8b. The sensor chip was placed directly behind the opening on the backside of the device. A circular cushion on the outside of the device and a silicone washer on the inside prevented any leakage of skin gas. A metallic mesh was placed on the sensor opening against dirt and against small amounts of sweat. This design proved to be unsuccessful due to

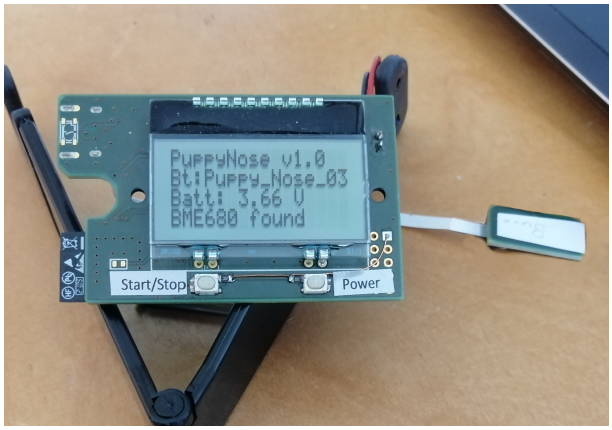
quick entrapment and accumulation of skin gasses, which increased the VOC output to 1000 ppm in several minutes. Therefore, for the first single person experiments the outdoor cushion was removed from the device to guarantee more exchange of air surrounding the sensor. With this improvement concentrations stabilized to lower values in the range of 10 to 100 ppm. Concentrations that were measured with the first PuppyNose design for humans were still considered high and the removal of the cushion made the sensor very sensitive to external changes, including changes in the surrounding air.

Hydrophobic mesh

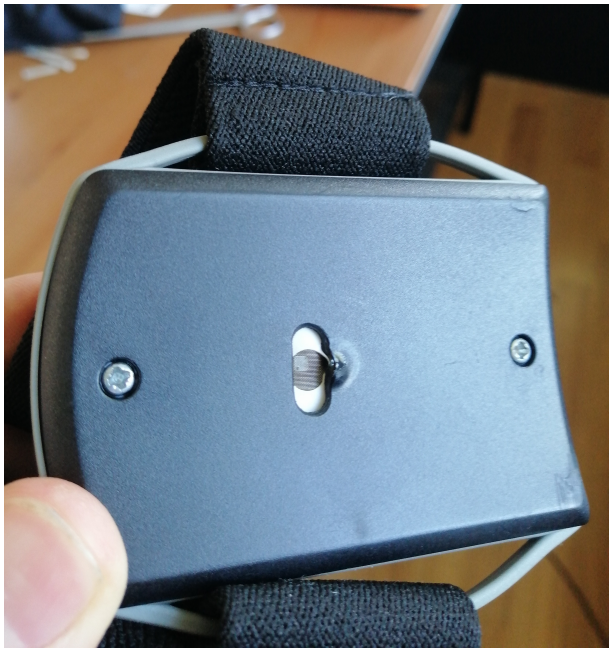
What triggered the need for a new design, was when during an exercise experiment involving heavy sweating, sweat permeated through the mesh and made a short circuit between the metal of the mesh and the power connector of the sensor chip. This resulted in total malfunctioning of the device (figure 2.8c).
In order to improve this design, the mesh was replaced by a hydrophobic mesh and the sensor chip and washer were placed in a way that only the sensing element was directly under the opening and the rest of the sensor chip was sealed of to avoid a short circuit. To test the strength of this new approach the same exercise experiment was conducted again. Unfortunately, during this experiment a high enough amount of sweat was produced that the newly installed hydrophobic mesh was overloaded, resulting again in a short circuit of the sensor chip.

Latest design

To avoid sweat and accumulation of skin gasses influencing the device from working properly, the design was fundamentally changed. A different, theoretically better hydrophobic mesh (HD10BHY material) was installed. This mesh was tested by placing a column of salinated water placed above it to test its hydrophobic ability. It was able to withstand a water column of 5 cm, while the mesh also proved to be almost completely permeable to acetone. Other changes included, the removal of the washer and the displacement of the sensor chip not directly under the opening but in a corner of the casing of the device. To ensure that enough skin gasses would be captured the circular cushion was again placed on the exterior of the device. This design was used in the remainder of all the in-vivo experiments.

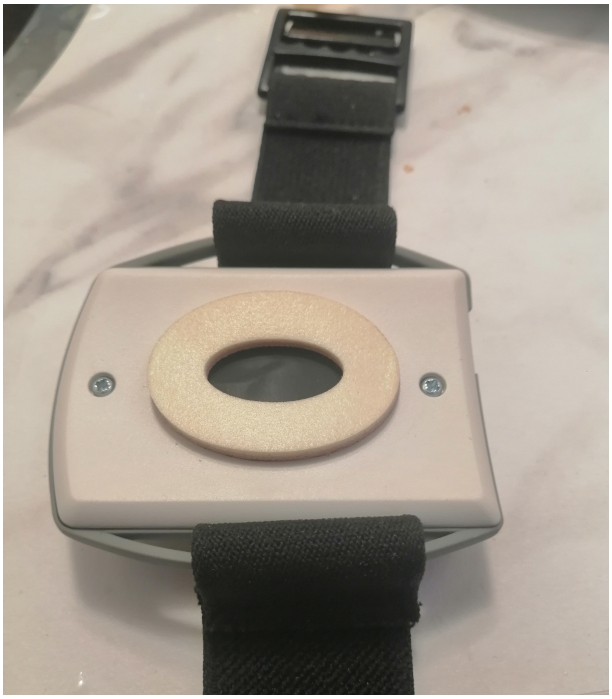


(a) First version of the PuppyNose with only the sensor chip connected to the controller chip via a cable.



(b) The second design with a simple metallic mesh that resulted in a short-circuit during the exercise experiment.

(c) The PuppyNose with a hydrophobic mesh and without a washer under it. This design resulted in a sensor malfunction.



(d) The final design with the HD10BHY hydrophobic mesh and the elliptical cushion to seal of the skin.

Figure 2.8: The different designs of the PuppyNose.

3 SUB-STUDY 1: LABORATORY SETTING

3.1 RESEARCH QUESTIONS

OBJECTIVE 1 Testing the technical feasibility of the device in a laboratory setting.

1. Can the device accurately measure a relevant range of acetone values?
2. What is the inter-observer reliability of different devices measuring under the same circumstances? Or in other words, are the outputs of different sensors consistent?
3. What is the test-retest reliability (or intra-observer reliability) of a single device measuring under the same circumstances?
4. What is the external influence of relative humidity on the output of the device?
5. How does the device react to other volatile organic compounds emanating from the skin other than acetone?

3.2 METHODS

3.2.1 Study design

During the first sub-study the device is tested in varying circumstances in the laboratory. In each laboratory test all variables except one is kept constant. This reveals what the technical specifications of the sensor are and which factors have an effect on the sensor output besides the acetone concentration.

3.2.2 Study procedures

System set-up

In order to test the PuppyNose in a controlled environment, the setup of figure 3.1 is used. In this setup acetone concentrations are created in a glass container with metal lid with a volume of 2,23 L. With this installation relative humidities from 20 up to 90% can be formed. This is done by sending air from a compressor through a water chamber, with saturated water vapor, to the glass measurement container. Desired humidity levels can be reached by measuring the relative humidity (RH) with an external sensor and controlling the airflow through the water chamber (see figure

3.1a and 3.1b). Accurate dilutions of acetone are made by extracting saturated acetone vapor from a bottle with liquid acetone and injecting it in the measurement container. For this, a syringe accurate to 0,01 mL and customized vapor pressure tables are used. To ensure a stable concentration during an experiment the container can be completely closed off with no airflow passing through it.
Besides the method using syringes, an acetone concentration can also be established in the measurement container via a continuous flow method. Figure 3.1b shows that the humid air can be mixed with saturated acetone vapor supplied by a peristaltic pump. This produces a continuous supply of acetone. The concentration cannot be calculated exactly with this method. However, using another pre-calibrated VOC sensor developed by Van Gool RD called the DogNose the acetone concentration in the glass container can be determined. The DogNose device is an analog, grid powered sensor system based on the TGS822 metal oxide sensor developed by Figaro [65]. The DogNose can only determine acetone accurately at a RH of 50%, because the system is only calibrated for this humidity.

Measurement of acetone

A set of experiments is conducted to test the response of the sensor to different acetone concentrations. First, a calibration experiment is performed to investigate the linearity of the output response. Acetone is injected in the measurement container in concentrations of 0, 5, 10, 20, 30, 50, 70 and 100 ppm at a fixed relative humidity of 50%. Furthermore, to study the correlation of the outputs of different devices under the same circumstances, the experiment is done with two PuppyNoses.
Secondly, the hysteresis of the sensor is studied. Acetone is added to the measurement container via the continuous flow method. With this method a perpetual flow of humid air mixed with increasing amounts of acetone is sent to the measurement container in which the PuppyNose is present. Acetone values between 1 to 330 ppm are reached first in increasing, then in decreasing order. The acetone concentration cannot be calculated with the continuous flow method and has to be measured directly with the DogNose device to determine its concentration. An external humidity sensor is used to validate the RH levels. This experiment is designed to see whether hysteresis affects the sensor output.

Limit of detection

The limit of detection (LoD) is determined to indicate what the lowest concentration of acetone is that can be measured by the PuppyNose device. The device measures ten times a blank sample (air without acetone present) and ten times a sample containing 1 ppm acetone. The concentration of 1 ppm is chosen, because it is the lowest concentration that can be reached with this experimental set-up. Working at 95% certainty, the LoD is given by the following equations [66]:

$$LoB = mean_{blank} + 1.645 \cdot \sigma_{blank} \tag{3.1}$$

$$LoD = LoB + 1.645 \cdot \sigma_{1\ ppm\ sample} \tag{3.2}$$

where:

LoB = Limit of Blank, the highest apparent concentration when only blanks are tested.

Influence of humidity

To test how the humidity affects the sensor output, two experiments are conducted. In both experiments various relative humidity levels are created with the set-up of figure 3.1b and an external RH sensor to validate the correct RH level. The first experiment, investigates whether the sensor's sensitivity changes at different humidity levels. Four RH levels are created of: 20, 40, 60 and 80%. At each RH level, acetone concentrations are formed by the injection method. Concentrations of acetone are created ranging from 5, 10, 20, 30, 50, 70 to 100 ppm. The calibration curve with the injected acetone concentrations for each RH level are compared to reveal if the sensitivity and linearity of the VOC output is disturbed by the humidity. In a second experiment, the effects of humidity alone are studied. The PuppyNose is exposed to a range of RH levels between 10 and 90 %. The output of the on-board humidity sensor of the PuppyNose is assessed by comparison with the output of that of the external sensor. With this experiment, the effects of the relative humidity on the VOC output can be studied when no acetone is present.

Selectivity of the sensor

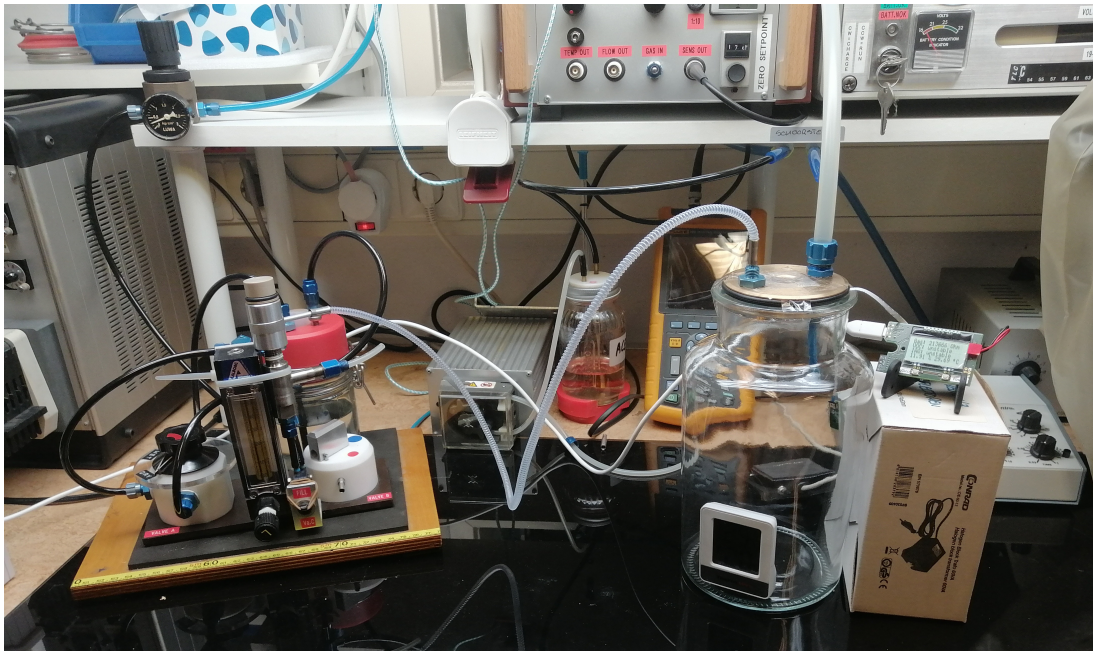
The selectivity of the device is tested by doing the injection experiment with ethanol for the concentrations of 0, 5, 10, 20, 30, 50, 70 and 100 ppm. Besides acetone, ethanol is the only VOC that is tested for this experiment. The reason for this, is that only ethanol rises to the level of potential skin acetone concentrations after alcoholic consumption, while all other VOCs exist in relative constant levels on the skin [49].

Test-retest reliability

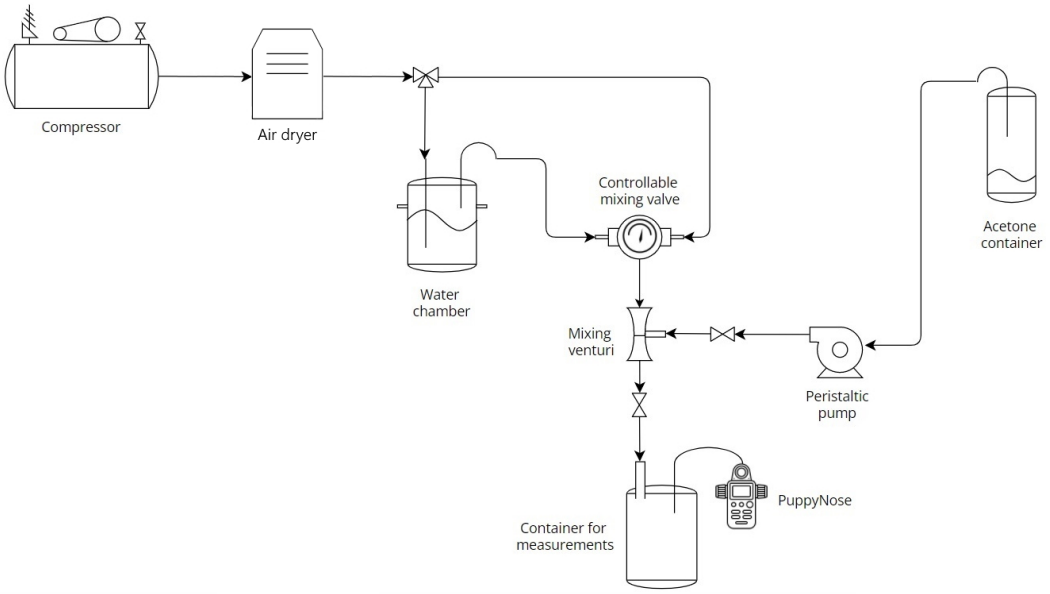
To test the intra-observer or test-retest reliability of a single PuppyNose device two measurement sessions are conducted two hours apart. At the beginning of each session the PuppyNose is calibrated in clean air and then acetone is injected in concentrations of 0, 5, 10, 20, 30, 50, 70 and 100 ppm at a relative humidity of 50%.

3.2.3 Outcome measures and analysis

The main outcome measure that is used is the VOC output from the PuppyNose device. In some cases the sensor response is also reported. The sensor response is often used for MOx sensors and is defined as the ratio between the raw resistance value at clean air and at a given concentration of gaseous acetone (R_a/R_g). It can be interpreted as an intrinsic measure for the sensitivity of the sensor material for a certain compound. To study sensor dynamics, response times (τ_{rs}) and recovery times (τ_{rc}) are used. Response time is the time needed for a signal to get to 90% of a new equilibrium value. The recovery time is the time to reach 10% of the original baseline value.



(a) A photo of the set-up as used for the experiments.



(b) The schematic of the architecture of all the components.

Figure 3.1: This setup was used to create controlled humidity levels and a continuous flow of acetone. Dried air from the compressor flows partly through the water chamber with saturated water vapor. By setting the mixing valve to a certain level, the ratio humid air v. dry air is controlled and with that the relative humidity level. When acetone should be added continuously the peristaltic pump lets acetone vapor flow through the mixing venturi. In the venturi a negative pressure is created due to a flow increase in middle of this component. This ensures good mixing of the gasses. The humid, acetone rich air flows to the measurement container. Measurements can be taken during continuous inflow of air or, when a certain relative humidity is reached, the flow can be stopped and measurements can be done at a constant level. Instead of a continuous flow, acetone can also be added via a syringe at the top of the container via a special opening.

During all experiments, the output of the device is tested against a known benchmark value created by a known amount of acetone or ethanol. The strength of the correlation between the independent and dependent values is studied with Pearson's correlation coefficient. If a significant result is obtained, the relationship is further studied by constructing a regression model.
Furthermore, to study the humidity and hysteresis effects, results are analyzed differently. Humidity effects are displayed with a sensor response diagram. In this diagram the relative effects of varying the humidity on the output of the sensor can be studied. The effects of hysteresis are studied by comparing the output values of the series of measurements in increasing order with those in decreasing order. The difference between the values in a certain order indicates if sensor exposure to high concentrations of acetone has an effect on the measurement.

3.3 RESULTS

In this section, the results of each experiment will be discussed separately.

Measurement of acetone

CALIBRATION Figure 3.2 display the results of the experiment where the acetone concentration was increased from 1 to 100 ppm at a RH of 50%. This was done simultaneously for two devices: PuppyNose 3 (PN3) and PuppyNose 5 (PN5). In both cases, a linear relationship was observed between the acetone concentrations made in the glass concentrations and the VOC output of the PuppyNose sensor. This linearity was indicated by a strong Pearson correlation for both sensors of r = 0.99 for PN3 and r = 0.99 for PN5 with both having a significance of $p < 0.001$ (see figure 3.2). In figure 3.2 a linear regression model describes the exact relationship between the VOC output of the sensor and the original concentration.
Similar regression model were fitted to the raw sensor resistance values and the real acetone concentrations. This was done to study if the raw sensor values could also be used to measure acetone. For both linear and logarithmic transformed regression models a significant relationship could be observed ($p < 0.05$). However, the predictive value of these models was worse than by using the VOC output. For the models regarding the raw sensor values, goodness-of-fit R^2 varied between 0.587 (logarithmic model) and 0.815 (linear model). This illustrates the effect of the compensation algorithm in transforming the data and increasing the predictive value of the output of the PuppyNose.

LIMIT OF DETECTION After testing the sensor (PN2) ten times on a blank sample and ten times on a sample of 1 ppm, the limit of detection was determined to be 0.5 ppm.

CORRELATION BETWEEN TWO SENSORS Pearson's correlation between the two outputs showed a strong, significant correlation of r = 1.00 ($p < 0.001$). However, figure 3.2 shows that the output sensitivity of the two different sensors do not correspond

in a 1:1 relationship. A new linear regression model between PN3 and PN5 revealed that for the same acetone concentrations the responses reported by the two sensors differed by a factor of 4.85 ($p < 0.001$). The sensor gas response (R_a/R_g) was also determined at 50 ppm for both sensors. For PN5 this was 1.83 and for PN3 1.40. Baseline resistance values of the sensors differed by 35%, being $2.33 \cdot 10^5$ Ω for PN5 and $1.72 \cdot 10^5$ Ω for PN3.

HYSTERESIS OF THE SENSOR During this experiment, the acetone concentration was gradually increased to a point of 330 ppm and then gradually decreased again, using the DogNose measurements as a reference for the actual concentration. The humidity should be kept stable at 50% to ensure that the DogNose measurements were accurate, but fluctuated between 50% and 42% during the experiment. The temperature remained stable at 27°C. Figure 3.3 displays the measured VOC concentration of the PuppyNose (PN3). Notably, in almost all cases the VOC output was lower for the measurement during the decreasing part of the experiment. To model these effect a linear model was created (figure 3.3). This showed that the values in descending order were 12% lower than those in ascending order. The same effect was observed in the raw sensor resistance output, where all values were higher in decreasing order. This indicates that a hysteresis effect is both present in the raw and compensated output of the device. This effect could be seen in the sensor response R_a/R_g. At first, it was 1.35 at 66 ppm, rising to 1.94 at 330 ppm. The second time the concentration reached 66 ppm the response had decreased to 1.28.

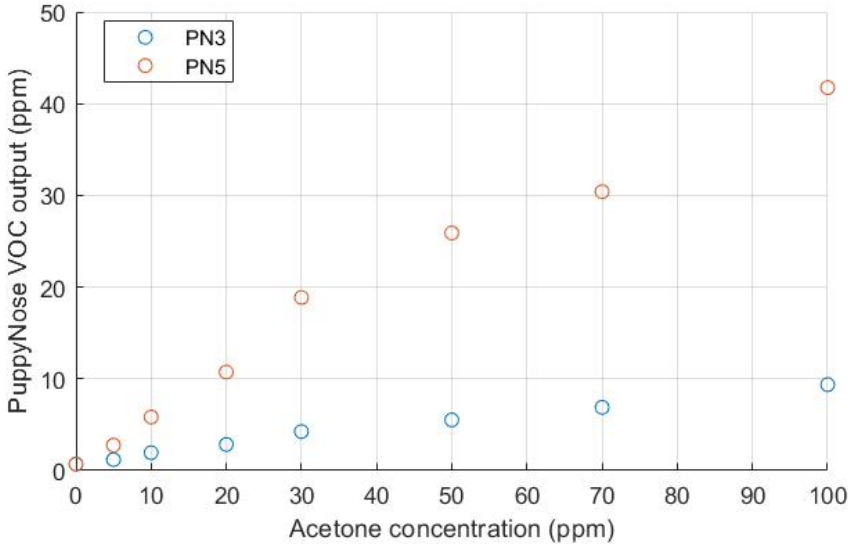


Figure 3.2: Scatter plot of the acetone concentration measured by the PN3 and PN5 devices plotted against the real injected concentrations. To model the measurements to the real concentrations, linear regression was performed.
PN3: $y_{real} = -11.66 + 11.57 \cdot x_{meas}$ ($p_{const} = 0.004$, $p_{slope} < 0.001$, $R^2 = 0.99$)
PN5: $y_{real} = -4.97 + 2.37 \cdot x_{meas}$ ($p_{const} = 0.183$, $p_{slope} < 0.001$, $R^2 = 0.97$)

Humidity influences on acetone measurements

For four humidity levels (20, 40, 60, 80%) acetone concentrations between 1 and 100 ppm were created and measurements were taken with the PuppyNose (PN3). In the case of the 20% and 80% experiments, the relative humidity was not entirely stable over the complete duration of the experiment. The RH level of 20% increased by about 4-5 percentage points during the 30 minutes experiment. A decrease in the same amount of percentage points was seen for the RH level of 80%. For each humidity level, the correlation was tested between real and measured values with Pearson's r. If a significant result was achieved, a regression model was fitted to the data of each humidity level. In table 3.1 the results of these models are displayed. For all humidity levels a strong correlation could be identified. However, as the regression models show, how sensitive the sensor reacted to acetone was strongly influenced by the humidity. Especially, at a low humidity level of 20% the sensitivity, indicated by the slope of the regression line, was more than two times as high as at the other humidity levels. This is also shown visually in figure 3.4b, illustrating the sensor response as a function of the humidity levels. This difference in output remained the same after the raw resistance data has been transformed by the compensation algorithm (see figure 3.4a). Note that if the compensation algorithm would have effectively reduced the humidity effects on the sensor response, the graph in the sensor response diagram (figure 3.4b) would have been a horizontal line.

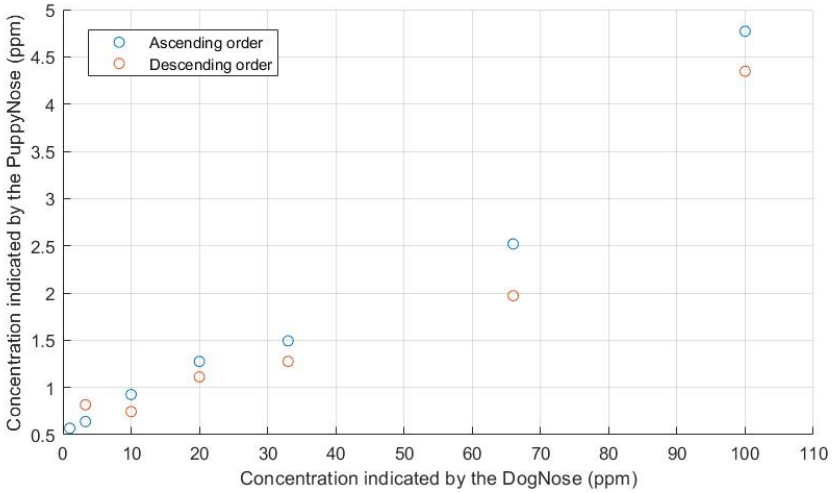
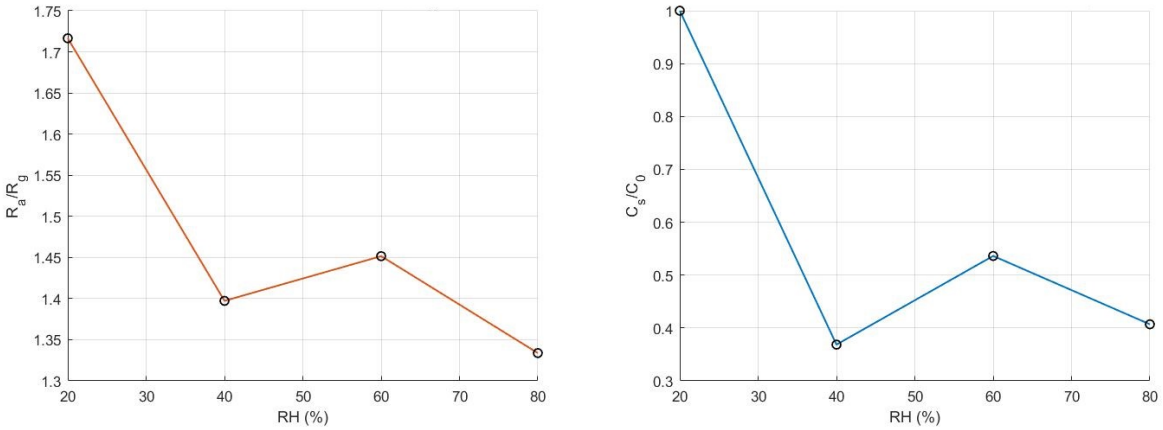


Figure 3.3: Scatter plot of the measurements of the PuppyNose (PN3) against the acetone concentrations created by the continuous flow method. The concentrations were controlled by DogNose measurements. In this figure 330 ppm is not shown for scale. A linear regression model was created of all the data points:
$y_{real} = -6.22 + 24.63 \cdot x_{meas}$ ($p_{const} = 0.25$, $p_{slope} < 0.001$, $R^2 = 0.96$)
The mean errors (ME) were then calculated between the predictions of this model and the points in ascending and descending order separately:
$ME_{ascend} = -2.78$ and $ME_{descend} = 2.78$

Table 3.1: The table shows for each humidity level the Pearson's correlation and the regression model that were performed on the data. In each case a significant result was obtained. Note that for a significant regression model only $p_{slope} < 0.05$ is needed.

Humidity level	Pearson's correlation	Regression model	Significance of model
20%	$r = 0.99$ $(p < 0.001)$	$y_{real} = 3.61 + 4.54 \cdot x_{meas}$	$p_{slope} < 0.001$ $p_{const} = 0.24$
40%	$r = 0.99$ $(p < 0.001)$	$y_{real} = -1.74 + 14.23 \cdot x_{meas}$	$p_{slope} < 0.001$ $p_{const} = 0.48$
60%	$r = 1.00$ $(p < 0.001)$	$y_{real} = -3.55 + 11.00 \cdot x_{meas}$	$p_{slope} < 0.001$ $p_{const} = 0.03$
80%	$r = 1.00$ $(p < 0.001)$	$y_{real} = -7.77 + 15.53 \cdot x_{meas}$	$p_{slope} < 0.001$ $p_{const} < 0.001$



(a) Sensor response of the resistance (R_a/R_g) for increasing humidity level. For each RH, the response was determined at an acetone concentration of 50 ppm.

(b) Response diagram of the normalized VOC output and the humidity level. All measurements were taken at 50 ppm acetone concentration and normalized based on the 20% RH value.

Figure 3.4: The response diagrams of the raw sensor resistance and the VOC output at increasing RH of PN3.

Independent humidity effects

In this experiment the influences of humidity on the PuppyNose (PN3) was monitored, while no acetone was added to the system. The device's initialization and calibration process was done in clean air at a humidity level of 40%. The total time series of these measurements when the humidity level was continuously varied, is shown in figure 3.5. When comparing the performance of the RH-sensor of the PuppyNose with that of the external humidity sensor, the measurements of the PuppyNose do show highly linear correlation with the real RH (Pearson's $r = 1.00$, $p < 0.001$). In spite of that, the relative humidities measured by the PuppyNose do not accurately represent the real ones in a 1:1 fashion, as linear regression showed (see figure 3.6). Furthermore, the VOC output (figure 3.5) was also affected by the change in RH, despite the presence of the compensation algorithm and no acetone being present

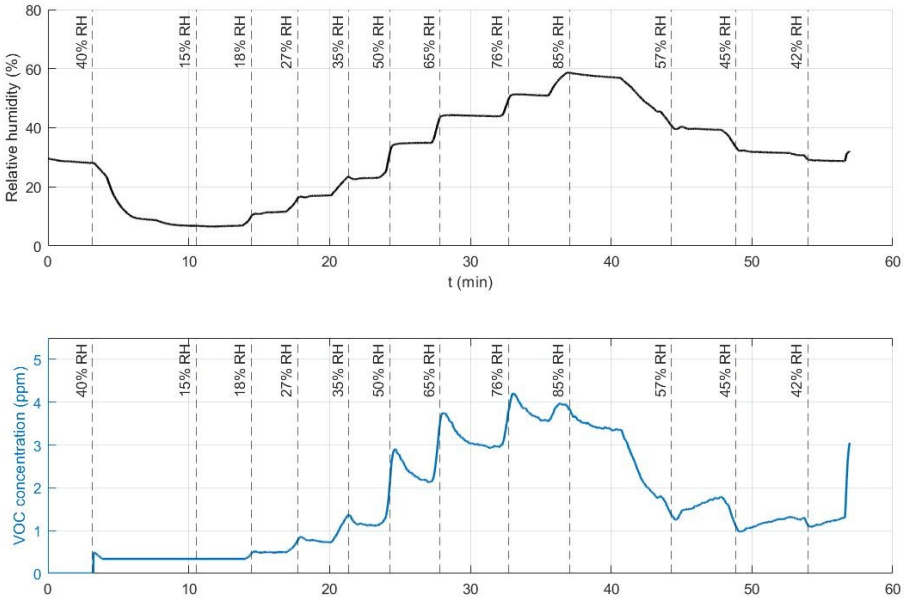


Figure 3.5: The time series graph of the RH and VOC output of the PuppyNose. The acetone concentration was kept at 0 ppm and only the humidity was increased during this experiment. The markers in the figure show the RH that was measured by an external sensor.

during the measurements. Interestingly, hysteresis is present as the humidity affected the VOC output of the sensor differently at different time points, e.g. at 25 min a 57% RH resulted in a lower VOC output than the 50% RH at 45 min.

Selectivity

Figure 3.7 shows the results of the PuppyNose's (PN3) exposure to the injection of alcohol at a RH of 50%. Strong linearity is present indicated by Pearson's r = 0.98 ($p < 0.001$). What became apparent, is that the same concentrations of alcohol results in higher output reactions than acetone. This was illustrated by figure 3.7 and a R_a/R_g of 1.85 at 50 ppm. Another notable difference was that more time was needed for the sensor to reach a stable output. At the 100 ppm concentration, it took more than 40 minutes to reach the reported value. However, even after the 40 minutes a stable value was not reached, but due to time constraints it was not possible to wait for a more stable output and the experiment was ended. For comparison sake, two linear regression models were calculated, one with and one without the 100 ppm data point (figure 3.7).

Test-retest reliability

Figure 3.8 shows that the output sensitivity of the device (PN2) changed when the same experiment was performed two times two hours apart. Linear regression between the two outputs indicated a significant linearity that had no 1:1 relationship ($p < 0.001$, $R^2 = 1.00$). The outputs from the two sessions differed by a factor of 1.94,

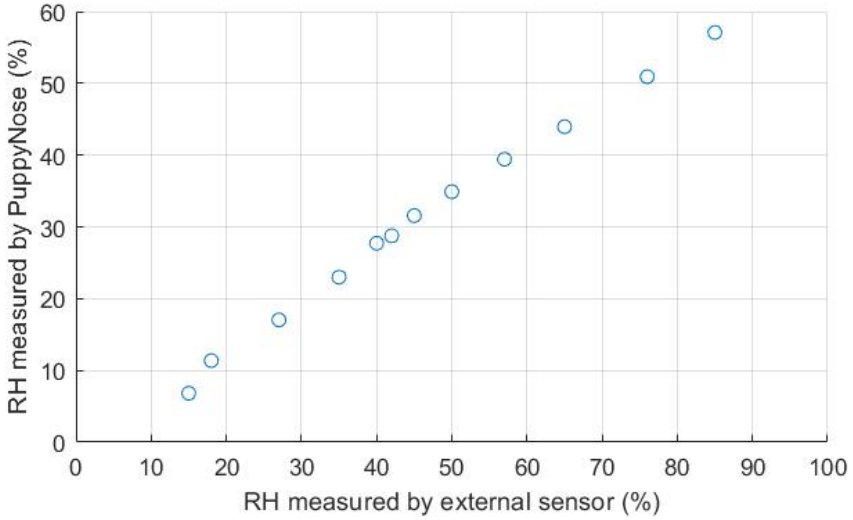


Figure 3.6: Scatter plot of the relative humidity measured by the PuppyNose (PN3) and an external sensor. The relationship between the two outputs is summarized in a linear regression model:
$RH_{external} = -1.43 + 0.70 \cdot RH_{PN}$ ($p_{const} = 0.114$, $p_{slope} < 0.001$, $R^2 = 0.99$)

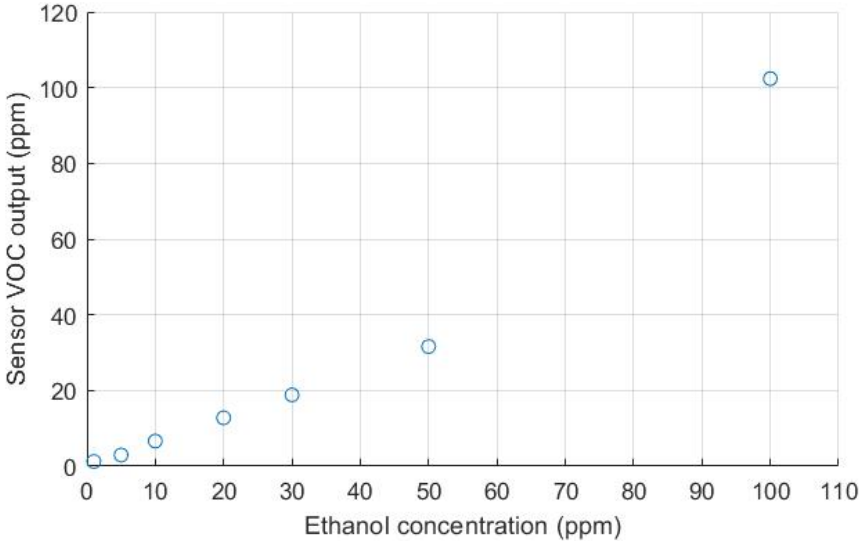


Figure 3.7: Scatter plot of the association between ethanol measured by PN3 and the real ethanol concentration. Two linear regression models were performed. In one model the 100 ppm alcohol measurement was regarded as an outlier and it was excluded and in one model all measurements were kept in:
With 100 ppm: $y_{real} = 6.74 + 1.00 \cdot x_{meas}$ ($p_{const} = 0.12$, $p_{slope} < 0.001$, $R^2 = 0.96$)
Without 100 ppm: $y_{meas} = -0.40 + 1.60 \cdot x_{real}$ ($p_{const} = 0.25$, $p_{slope} < 0.001$, $R^2 = 1.00$)

during the measurement of the same acetone concentrations. The sensor response at 50 ppm was 1.18 and 1.28 (increase of 7.98%) for session 1 and 2 respectively. In contrast, the baseline sensor resistance remained relatively constant at 5.48 and 5.55 $\cdot 10^5$ Ω (increase of 1.22%). However, over longer periods of time the baseline sensor resistance of the MOx sensor began significantly deviating from its original position. While this device was kept in clean air for 24 hours, its resistance value changed almost 50%. This behavior was also seen during the other experiments, where the sensor resistance and starting sensor response of the same device could be fundamentally different when the same experiment was done on different days.

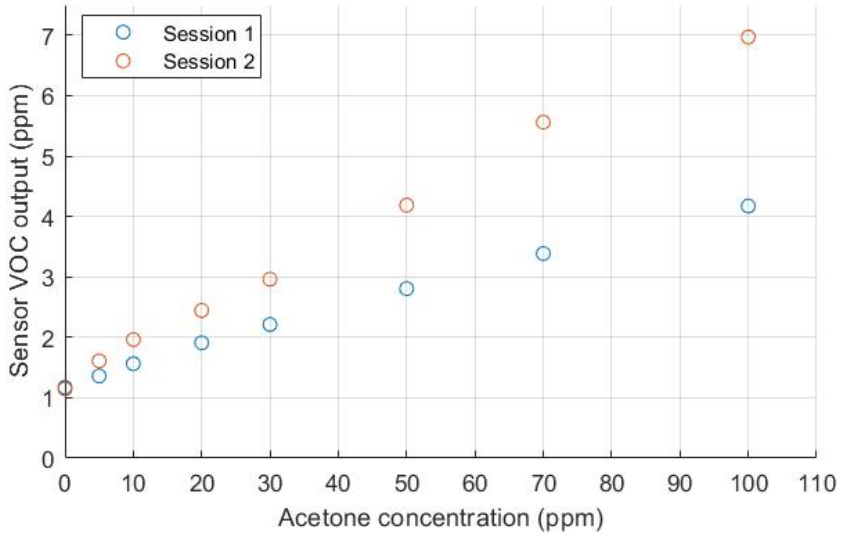


Figure 3.8: Scatter plot testing the test-retest reliability of PN2. Acetone was measured on two different occasions two hours apart. Linear regression was performed on the data of the two sessions.
Session 1: $y_{real} = -41.70 + 33.29 \cdot x_{meas}$ ($p_{const} < 0.001$, $p_{slope} < 0.001$, $R^2 = 1.00$)
Session 2: $y_{real} = -21.84 + 17.13 \cdot x_{meas}$ ($p_{const} < 0.001$, $p_{slope} < 0.001$, $R^2 = 1.00$)

Sensor dynamics

The dynamics of the sensor (PN3) was studied over time after the injection of acetone and ethanol. The time series graphs are shown in Appendix A.1. Response (τ_{rs}) and recovery times (τ_{rc}) were studied for the raw sensor resistance output and VOC output of the device. Regarding the raw sensor resistance, τ_{rs} was 15-20 s and τ_{rc} was 40-60 s for all concentrations between 0 and 100 ppm. In the VOC output small spikes of < 5 s were filtered out and the τ_{rs} had increased by a factor of three, while τ_{rc} remains the same. This demonstrated that the compensation algorithm smooths out sharp increases in the original signal. The sensor responded different to ethanol. After injection, the signal produced an overshoot and then decreased to the equilibrium value. The response to ethanol seemed underdamped, whereas the sensor response could be considered critically damped for acetone. As a result, sensor response times for ethanol were larger than for acetone and changed with concentration. Because the sensor was not removed from the measurement container during the injection of

ethanol, the response times to each increased concentration could not be determined exactly. However, it was estimated that τ_{rs} was between 15-30 minutes for most concentrations. In contrast to this, the τ_{rc} at the end of the experiment (100 ppm) was only 20 seconds.

3.4 DISCUSSION

This first sub-study aimed to get an insight in the performance and limitations of the PuppyNose device under controlled circumstances. The device was tested under different circumstances to test its relevant measurement range, accuracy, response to humidity, test-retest reliability, inter-observer reliability and its selectivity to another volatile organic compound.

The detection limit of the device was determined at 0.5 ppm. The lowest concentration of acetone that was measured in this sub-study with the device was 1 ppm, because it was not possible to reach lower concentrations with the used experimental method. The reported LoD of the PuppyNose is on par with a review on 21 different types of MOx acetone sensors with most detection limits ranging from 0.1 to 1 ppm [62]. However, in another review on 10 different types of MOx sensors, a LoD as low as 10 ppb could be reached [62, 63].
Most of the reported MOx sensors have much higher sensor responses (R_a/R_g) of > 10 at acetone concentrations around 50 to 100 ppm [62, 63]. In comparison, the R_a/R_g of the PuppyNose's BME680 was between 1.5 and 2 also for 50 ppm of acetone, demonstrating a lower sensitivity and measurement accuracy. A low LoD and high sensor response over a short range seem preferable compared to the opposite in the detection of health related bio-markers. Differences between diabetic and healthy states can be in a low range where a high resolution is needed to reach good diagnoses [67]. In that regard the MOx BME680 sensors seems to be outperformed by more recently developed materials. However, it should be noted that most of these sensors are in a very early developmental stage and often not commercially available.

During almost all experiments it was demonstrated that the PuppyNose was accurate enough to measure acetone concentrations ranging from 1 to 100 ppm. Furthermore, the VOC output always showed strong linearity. However the injection experiment, experiments with humidity and test-retest experiment showed the sensor sensitivity (R_a/R_g) and sometimes the baseline sensor resistance was significantly affected by humidity, hysteresis and drift. The compensation algorithm was not able to correct for these changes as indicated by figure 3.3, 3.4b and 3.8. The humidity experiments showed that the sensor response of the PuppyNose was decreased by the RH. This decrease in sensitivity was seen in a decrease in the slope of the raw resistance and in the compensated VOC output. In spite of the fact that these changes were picked up by the PuppyNose's RH sensor, these changes were left uncorrected by the internal algorithm (figure 3.6). The effect of humidity on the raw sensor resistance and sensor response corresponds with the that of MOx sensors described in litera-

ture. In most cases, the sensor response is maximum in dry air and decreases when the RH increases [62, 68–70], although there exist specific sensor materials where the maximum sensor response is positioned around RH levels $>$ 60% [71]. A different experiment in this report demonstrated that not only the sensitivity to acetone was changed as a result of the RH, but that humidity alone resulted in a change in the VOC output. This phenomenon was also investigated by a study by Yadav et al. [70]. This study reported how an increase in humidity, with the sharpest effect between 20% and 40%, changed the response of the sensor by a factor of five.
Hysteresis was also studied in this report. Measurements with the continuous flow set-up demonstrated hysteresis affecting the sensor after high values of acetone were reached (figure 3.3). Unfortunately, there was a serious flaw in the design of the experiment that was initially overlooked. The DogNose sensor was taken as reference measurement system to validate the actual amount of acetone present. However, it was not recognized that this MOx sensor would be potentially susceptible to hysteresis as well. Alas, it cannot be distinguished whether the measured hysteresis was caused by the PuppyNose or the reference DogNose sensor. In the literature, it is described that MOx sensors can be significantly affected by hysteresis. Nguyen et al. demonstrated that multiple cycles of a SnO_2 metal oxide gas sensor to the same exposure to acetone decreased the baseline resistance value of the sensor and the sensor response at each cycle [72]. Although it could not be confirmed in this study for acetone, it was observed in this study that humidity induced hysteresis in the VOC output of the PuppyNose sensor (figure 3.5). This effect is also reported in the literature. Yadav et al. showed hysteresis in the sensor resistance due to humidity changes, albeit that the effect was only $\pm$5% of the total output when the RH was varied from 10 to 90% [70].

Measuring on two different session two hours apart, had a large impact on the test-retest reliability of the PuppyNose. This change was caused by a sensitivity change in the sensor material with the sensor response changing by 7.98% over the two sessions, although the baseline resistance value only changed by 1.22%. Over larger amounts of time, in the order of days, baseline resistance values did also fluctuate. These changes seem too large for long-term sensor drift in resistance and sensor response values that is observed normally in MOx sensors, where significant changes take weeks to months to be in the same order of magnitude [73, 74]. Alternatively, it appears that these changes in sensitivity and baseline resistance have to do with the stability of the BME680 sensor, which can fluctuate strongly causing aggressive short-term drift. As the sensor response usually has values within a small range, this can have a large impact on the eventual VOC output of the device.
The inter-observer reliability of the PuppyNose devices was low, with VOC concentrations that differed by a factor of five. This is the result of the large difference in sensor responses, 1.40 and 1.83 at 50 ppm and baseline output resistances. This large difference is likely caused by the instability of each individual sensor that also resulted in low test-retest reliability and fluctuating baseline resistances that were observed. In the literature no studies could be found that compared the inter-observer reliability of MOx sensor materials. Hence, no information is available whether this is a common issue with MOx sensors.

Acetone induced VOC outputs that were more than two times as low for the same concentrations as ethanol. Furthermore, the sensor response acetone : ethanol was 0.76 measured at 50 ppm. In other studies, the sensor response of acetone versus ethanol varied from 0.85, 1.2, 2 and 3 times [75–78], although in one case a specially designed material of $Si : WO_3$ made for acetone sensing in the breath, resulted in sensor responses that was 7 times as high for acetone as they was for ethanol [74]. When evaluating the dynamical sensor response it was found that ethanol induced a many times stronger reaction than acetone. This behavior was not observed in the literature where concentrations of acetone or alcohol usually have comparable response times (30s to several minutes) and where ethanol response times are mostly twice as high as those of acetone [79, 80]. In contrast to this, it appears that the BME680 is very reactive to high concentrations of certain compounds, such as ethanol, whereas it is not to others.
As this sub-study has demonstrated, problems with selectivity can arise when ethanol is present on the skin. Strategies to counter this have been described in the literature. Two studies have investigated the possibility to distinguish acetone from ethanol using specific heating methods and a classification algorithm [81, 82]. Because ethanol and acetone produce a different output at each temperature, unique classification profiles can be created. Palacín et al. uses sixteen BME680 sensors with different heating temperatures. With this strategy successful classification of butane, ethanol and acetone was possible to an accuracy of 100% [81]. Shaposhnik et al. presented a method to distinguish acetone and ethanol in concentrations between 0.1 to 100 ppm [82]. They did this by performing a heating cycle between 100 and 450°C and selecting 25 different time points during this cycle. This strategy could be used for enhancing the selectivity performance of the PuppyNose in situations where users have consumed alcohol before measurement.

3.5 CONCLUSION

The aim of this first sub-study was to test the technical feasibility of the PuppyNose device in a laboratory setting. The conclusion to this objective is addressed by answering the individual sub-questions:

1. The acetone injection experiment showed that the PuppyNose can detect acetone values in a range between 1 to 100 ppm accurately and linearly, with a detection limit of 0.5 ppm. At this point in time, this range seems appropriate for the detection of skin acetone in ketotic humans. However, the BME680 sensor had a small sensor response range in comparison to different MOx sensors.

2. The inter-observer reliability of the PuppyNose was poor. Two sensors showed large differences in response to the same amount of acetone. No environmental factors could be found that would predict this difference in sensitivity. It is most likely that these effects are caused by inherent instabilities in the sensor itself.

3. The test-retest reliability of the PuppyNose was poor. The BME680 sensor is unstable, causing short term drift that affects the sensor output. This effect was

present in both raw and compensated outputs, illustrating that the algorithm by Bosch was not able to filter these errors out.

4. The relative humidity had a strong impact on the sensor response, especially between 20 and 40% RH. The humidity compensation of the Bosch algorithm had little effect in improving these results. Additionally, the effect of sensor hysteresis on the output was studied. However, due to flaws in the design of the experiment, no reliable conclusions could be drawn from the results.

5. The PuppyNose device reacted more strongly to ethanol than to acetone. Furthermore, the response time to reach a stable value for ethanol was many times larger than for acetone.

Overall, the PuppyNose is able to detect and accurately represent amounts of acetone higher than 1 ppm. However, the sensitivity and baseline resistance are often unstable when measurements are taken at different time points. It appears that the BME680 sensor was designed to measure large changes in the VOC concentrations in a relatively stable environment, while the device operates in a continuous session. Its performance drops when stable, reproducible measurements have to be performed at different moments under varying conditions.

4 SUB-STUDY 2: SINGLE PERSON EXPERIMENTS

4.1 RESEARCH QUESTIONS

OBJECTIVE 2 Testing the technical feasibility of the device in a single healthy individual during daily life activities, fasting and heavy exercise.

1. Does the output of the device remain stable when it is worn under circumstances of heavy exercise and sweating?
2. Does the output of the device remain stable when it is worn during daily life activities, while the individual is not in a state of ketosis?
3. Is there a correlation between ketone levels measured by the PuppyNose and β-HB levels measured by finger prick tests during multiple days of fasting?

4.2 METHODS

4.2.1 Study design

The second sub-study is the first research exploring the behavior of the sensor under circumstances which cannot be modeled in-vitro. For this study, three type of experiments are designed. For the first experiment measurements are taken during a high intensity cycling exercise. This shows how the sensor operates under more extreme circumstances. Under these circumstances the sweat production of the arm is expected to increase rapidly, while ketone levels should still remain low. The effect of rising relative humidity can then be studied in-vivo. During the second experiment, the individual wears the sensor for longer duration daily life activities. During this experiment, short perturbations, consisting of moving the device on and from the arm and shaking it on the arm, can indicate how the sensor operates under circumstances that are likely to happen with daily use. The final experiment investigates if the device is able to measure ketones in-vivo on a clinically relevant level. This is done by measuring two times per day during a 72h fast. For accurate comparison, measurement is also done when the individual is on a normal diet. At each measurement point, blood β-HB measurements are taken, so the PuppyNose data can be compared with the gold standard method.

4.2.2 Study population

The individual that is enrolled in this study is the researcher himself. As such, no informed consent was needed and the study was not subjugated to the WMO.

4.2.3 Study procedures

Exercise experiment

During the exercise experiment the PuppyNose measures ketone values while indoor physical exercise or training is performed by the participant. The exercise consists of two 20 minute sessions of indoor cycling at varying intensities. In between the sessions there is a 15 minute break in which the sensor is taken from the arm, but not re-calibrated. Each session consists of 5 minute cycling at 175 Watt, 12.5 minutes at 300 Watt and finally cooling down for 2.5 minutes at 175 Watt again. This program is designed with the intend to quickly increase sweat production and cardiovascular output, while being too short in duration to directly increase ketone body production. There are two sessions planned to see if there are longer lasting effects due to the high humidity that influences the output of the device.

Daily life experiment

This experiment requires the participant to wear the PuppyNose device during a period of three hours and it consists of three different parts. These parts are conducted in continuous order during a single session:

i *Wearing the sensor doing normal activities.*
To assess how the stability of the output behaves during a simple in-vivo experiment.

ii *Wearing the sensor for a period of time, not wearing the device and wearing it for the same period again.*
To assess the in-vivo test-retest reliability of the device.

iii *Wearing the sensor while applying perturbations.*
To asses the sensitivity of the output to perturbations and user interactions likely to occur in real life.

All these parts are combined as follows in a 3-hour long session. Part i and ii are conducted in the first two hours and part iii in the last hour. The participant wears the device from the start for 45 minutes. Then from 45 minutes to 1:15 the device is taken off. From 1:15 to 2:00 hours the participant wears the device again. During the first two hours the participants should not interact with the device in another way than wearing it. Besides that, the participant can perform his daily activities normally, such as working behind a laptop and eating. From 2:00 to 3:00 hours perturbations are applied to the device at every 20 minutes (at 2:00, 2:20 and 2:40). The first perturbation is moving the device on the skin. The second perturbations is removing the device from the skin for a short period of time. The final perturbations is shaking the arm

with the device heavily without further touching the device. At the 3:00 hour time mark the experiment ends.

Fasting experiment

During this experiment, measurements are done during a 72 hours fasting period. Only water is consumed for this period of time. The fast starts on Sunday night at 21:00 hours and lasts until Wednesday night at 21:00 hours. In the course of this period, measurement are taken with the PuppyNose at everyday around 10:00 hours in the morning and around 21:00 hours in the evening. When a measurement is taken, the PuppyNose is first calibrated in clean air for 5 to 10 minutes and then the device is worn on the arm for 30 minutes. At the beginning of each measurement time point the blood β-HB is also determined with a finger prick test. These finger prick test consist of a meter called CareSens Dual and Ketosens test strips, both made by i-sens. During the entire duration of the fasting experiment and 12 hours before the start, no alcohol can be consumed by the participant in order to avoid false positive measurements. After the fasting period, two control measurements are planned at 21:00 hours each on a different day. These new two moments should be at least three days apart from the fasting period, in which the participant has started his regular diet again. These measurements act as a comparison to the PuppyNose measurements during the fasting period.

4.2.4 Outcome measures and analysis

Results from the first two experiments are analyzed qualitatively based on the time series graphs of the VOC, relative humidity and temperature. This approach is chosen as the behavior of the device in-vivo remain unclear and can be best studied this way. For the last experiment the PuppyNose VOC values and the β-HB values are tested for correlation with a regression model. To obtain a single VOC value for a given time point, per 30 minute measurement period, the last 25 minutes of data was averaged. In the literature, the relationship between breath acetone and β-HB is not unambiguously understood and is sometimes evaluated with linear models, but in other research with logarithmic ones [26, 40–43]. Therefore, both model types are used in the statistical analysis of this study to see if a significant relationship can be detected.

4.3 RESULTS

Exercise experiment

During this experiment the performance of the PuppyNose (PN5) was studied under exercise conditions including heavy sweating and increased body temperature. The experiment was conducted two times in total. During the first moment, the device broke down before the end of the experiment due to moisture entering the internal circuit via the metal mesh. After that, changes were made to the mesh to make it

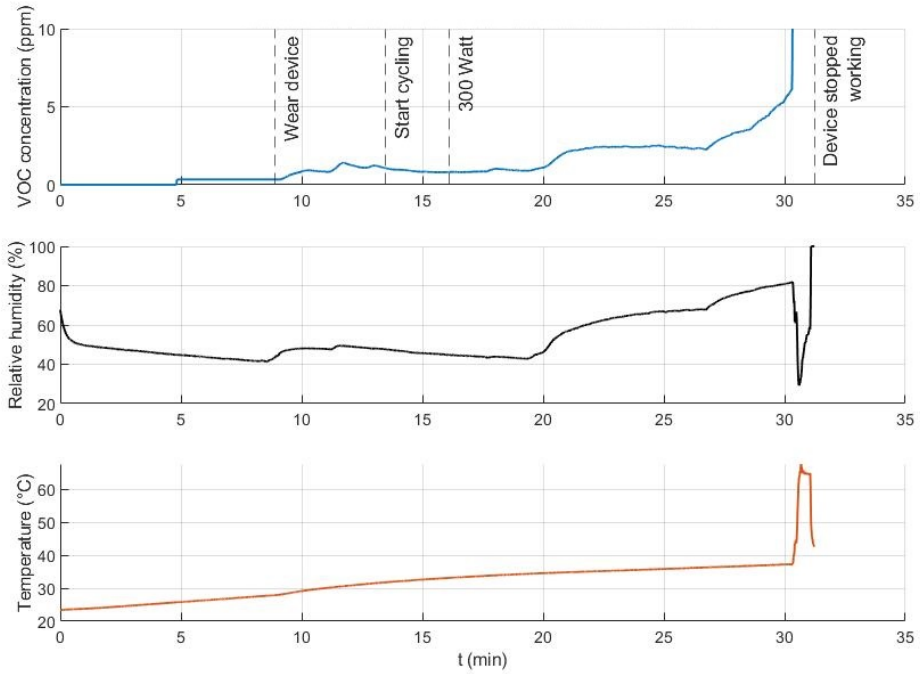


Figure 4.1: The time series graph of the VOC, RH and temperature data as measured by PN3 during exercise. At the t = 31 min. the VOC output reached 1000 ppm for a short period of time (not shown for scale) before the sensor stopped working.

more hydrophobic. Because PN5 broke down as a result of this short circuit, a new device had to be selected (PN3). This new hydrophobic mesh proved to be unsuccessful, as the sensor broke down a second time for the same reason. After the second breakdown, the device was designed with a new location of the sensor chip, not directly under the opening on the backside (see section 2.7 for a full description of the changes). However, the experiment was not carried out again with the latest design of the device due to time constraints.

The time series data from the second time the experiment was conducted, is shown in figure 4.1. Only this experiment is shown here as the events from both experiments were comparable due to the occurrence of the same events. The other time series graph can be found in Appendix A.1. In figure 4.1, the relative humidity data shows an increasing trend as training intensity and consequentially sweat production increased during the course of the measurement. The measured RH finally stabilizes at a maximum of 80%. This seems to be the limit of the sensor, which would correspond with the results from the humidity calibration experiment. The temperature also shows a continuously increasing trend until a maximum of 40 °C. The VOC output of the sensor appears to be correlated with the increase in humidity, as no rise in ketones is expected in this short time frame. This effect is not filtered out by the compensation algorithm. At 31 minutes the device broke down, before the final breakdown the relative humidity rose to 100% and the VOC concentration to 1000 ppm (not shown in figure 4.1 for scale), which is the maximum value of the sensor output.

Daily life experiment

After the failure of the PuppyNose 5, all remaining experiments in this sub-study were carried out with the newly designed PuppyNose 3. What was noticed with the first testing of the latest design, was that the VOC output did not stabilize around several hundreds of parts per million after it was attached to the arm. This was the case in some of the old designs that had the sensor much closer to the skin.
Figure 4.2 shows the time series results of the daily life activities with all the activities marked on the graph. The figure shows how the VOC output remains fairly stable despite the decreasing relative humidity in the first 40 minutes of measurement. The VOC sensor remained stable during normal wearing and not-wearing. However, the sensor output appears very sensitive to movement of the device on the skin and by pulling it from the skin. These events go along with minute long spikes in the output. Remarkably, spikes in the output occur also at 112, 134 and 168 minutes when no noticeable external changes occur. In particular the outlier at 168 minutes stand out, lasting twelve minutes and reaching values of 90 ppm. Furthermore, unstable events appear to become more heavy as time increases. Nonetheless, after each outlier stability restored itself.

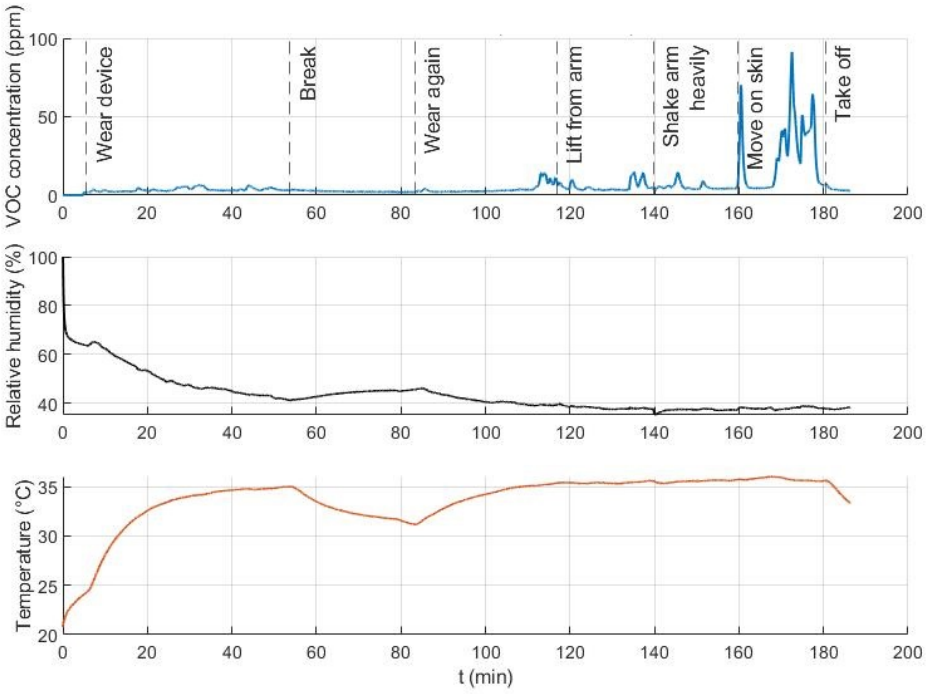


Figure 4.2: Time series graph of the VOC, RH and temperature data as measured by PN5 during daily life activities. Markers in the graph indicate which perturbations were applied at which moment.

Fasting experiment

The data from the 72 hours fasting period consisted of VOC data from the PuppyNose (PN5) and β-HB data taken at the same time from finger prick tests. Special attention

was given to reduce artifacts by moving the device as little as possible and the VOC outputs of 25 minutes were averaged to decrease noise and reduce the effects of outliers. However, this did not reduce the normal baseline outputs to values lower than 1 to 5 ppm. Multiple linear regression analyses were performed. The results of these models are summarized in table 4.1. All models failed to reach statistical significance. This indicates that measuring during a state of ketosis resulted in output signals that were not distinguishable over the normal spread of values.

Table 4.1: Table shows all the regression models that were evaluated for significance. Three models were evaluated, because in the literature these different types are all used to review the relationship between BrAce and β-HB. No model reached statistical significance.

Type of regression	Regression model	R^2	Significance
Linear	$\beta HB = 2.89 - 0.378 \cdot Voc_{pn}$	0.031	$p_{slope} = 0.627$ $p_{const} = 0.006$
Logarithmic	$\beta HB = 0.35 - 0.014 \cdot log(Voc_{pn})$	0.001	$p_{slope} = 0.918$ $p_{const} = 0.037$
Double logarithmic	$log(\beta HB) = 0.32 - 0.07 \cdot log(Voc_{pn})$	0.012	$p_{slope} = 0.764$ $p_{const} = 0.016$

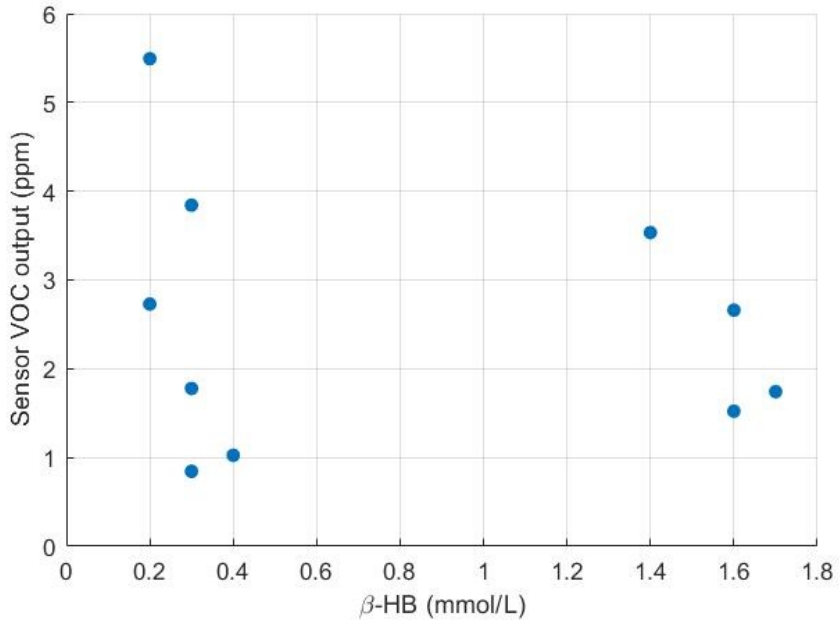


Figure 4.3: Scatter plot of the averaged PuppyNose's VOC output and the β-HB measurements that were taken the same time. The four points on the right part of the figure were all measured after 42 hours of fasting.

4.4 DISCUSSION

It was the aim of this sub-study to test the technical feasibility of the PuppyNose device in a controlled in-vivo environment by testing various real-life scenarios with single-person experiments.
The exercise experiment demonstrated that the sensor is sensitive to increasing humidity levels as a result of heavy sweating. Despite the fact that the increase is slower than during the experiments in laboratory setting, it is not corrected by the algorithm. Furthermore, this experiment shows the vulnerability of the device to moisture. The eventual breakdown of two sensors appears to be caused by the entry of moisture, as high RH levels did not damage the circuitry. The newest device design, with the sensor chip on a different location and better hydrophobic mesh, has been used from this point on, but has not yet been tested in a new exercise experiment. Even if this new design repels liquid water effectively, it would be interesting to know if it also reduces or slows down the relative humidity in the air entering the sensor. This could greatly improve the stability of the VOC output against humidity.

The daily life experiments indicated that expected real-life actions and perturbations could have a high impact on the output of the sensor, although their effects may be short lived. These perturbations could be avoided by giving the user specific instructions or measure for longer periods of time and average the results. However, the outliers that occur seemingly spontaneously, and especially the one at 168 min in figure 4.2, are harder to predict and filter out. These outliers could be the result of a measurement error at the level of the sensor or of a sudden change in the air quality. To differentiate between the two, new human-bound research should measure simultaneously with two sensors on a single person.

The final experiment investigated the correlation between SkAce and β-HB. No other studies, to the researcher's knowledge, have investigated the relationship between skin acetone and β-HB for metal oxide sensors. There was one study that investigated this relationship for a breath acetone MOx sensor by Suntrup et al. [4]. This study did find a significant correlation of R^2 = 0.57 with BrAce values ranging from 0 to 35 ppm. Taking 1.5 mmol/L β-HB as a cut-off point, the BrAce measurements could classify the participants in the correct group with a sensitivity of 82.0% and a specificity of 87.1%. The 1.5 mmol/L cut-off corresponds to an increased level for DKA given by table 2.1 and is a good indicator for self-monitoring of DKA. Although the PuppyNose used a type of sensor and measuring method to measure SkAce in a way that has not been described by literature yet, some studies suggest that the relevant measurement range of acetone samples in human samples may be of the scale of 0.1 to 1 ppm [46, 49, 62]. This is in contrast to the breath, where concentrations can increase to 100 ppm [43]. However, these values might be much higher for sensors with a similar design as the PuppyNose, as skin gasses can accumulate in the sensor chamber before measurement. Skin acetone measurement is further complicated by the fact that there always other VOCs present on the skin [49], which appear to cause of the baseline VOC output that was always present in the PuppyNose's measurements.

4.5 CONCLUSION

It was the aim of this sub-study to test the technical feasibility of the PuppyNose device in a controlled in-vivo environment by testing various real-life scenarios with single-person experiments. All the sub-questions will be answered individually:

1. Exercise experiments showed that the output of the PuppyNose was sensitive to heavy sweating. The slowly increasing humidity raised the VOC output of the sensor without these effects being filtered out. Additionally, this experiment revealed weaknesses in the design regarding the leakage of moisture through the mesh. A new hydrophobic mesh was installed, but it is not known of this new design provides better protection against moisture.

2. During normal wearing, the output remained relatively stable over time in daily life experiments. However, moving the device on the skin caused unstable behavior of the sensor that induced spikes raising the VOC output to more than ten times that of the stable values. To avoid the effects of these spikes the PN should be worn for longer periods of time ($>$ 20 minutes) and the output should be averaged to obtain a reliable result. However, the negate these effects completely, it is advisable to not move the sensor at all during measurements.

3. The fasting experiment shows no significant correlation between the PuppyNose's VOC output and the β-HB concentration elevated to 1.7 mmol/L after three days of fasting. Although 1.7 mmol/L is lower than could be expected from literature, this level indicates an increased DKA risk. The PuppyNose should detect risk levels of DKA to be a valuable tool in its prevention. This study indicates that the device is not able to do so.

In conclusion, the PuppyNose sensor is not able to detect ketone levels corresponding to an increased risk in DKA. It is not exactly known what the reason is for this. This might be caused by other VOCs emanating from the skin, which obscure the acetone measurement, or the PuppyNose not being stable or accurate enough to measure the changing acetone levels. Other problems that were found are that the device is sensitive to humidity changes and movement on the skin, although these problems could be minimized by averaging over longer periods of time and advising participants not to move the sensor. Despite these precautions some fluctuations may still occur spontaneously.

5 SUB-STUDY 3: INDIVIDUALS NOT IN KETOSIS

5.1 RESEARCH QUESTIONS

OBJECTIVE 3 Testing the technical feasibility of the device in healthy individuals who are not in a state of ketosis.

1. What is the test-retest reliability of the device when it is worn by the same individuals under normal daily life conditions?
2. Does the output of the device remain stable when it is worn by multiple individuals when the device is moved on the skin, the individual shakes their arm or when the device is pulled from the skin?
3. What is the inter-observer reliability of different devices when measuring under normal daily life conditions?
4. How is the usability and comfort of the device perceived by people who use it?

5.2 METHODS

5.2.1 Study design

Sub-study 3 focuses on how the device operates in an in-vivo environment for a larger group of healthy people. Participants not in ketosis will wear the device during normal daily activities while they are supervised by the researcher. This study is designed to see how the output changes when only possibly disturbing influences affect the output. The concept of this study is the same as the daily life experiments of sub-study 3 (see 4). However, this time the use of a larger group of participants with no prior knowledge is used. As a result, confirmation bias is reduced and different user interactions with the device might occur as compared to when the researcher, who was already familiar with the device, uses it. Hence, attention is also given to how participants interact with the device and how they perceive its comfort.

For this sub-study, healthy participants are enrolled. To ensure this study is not subjected to the Medical Research Involving Human Subjects (non-WMO) it is conducted in a way that it is observational and participants are not required to follow any rules of behavior. This study is approved by the ethical committee of the University Twente and all participants gave their informed consent (see Appendix A.3).

5.2.2 Study population

Healthy volunteers are selected from the employees of the University of Twente and family members of the researcher. In total four to six participants are enrolled. Participants were included based on the criteria that they were older than 18 and that they do not consume alcohol the day before and on the same day of the experiment.

5.2.3 Study procedures

During this sub-study the performance of the PuppyNose is assessed in-vivo, when no or only negligible amounts of elevated ketone levels are expected during the observational period. The measurement procedure is nearly the same as the single person experiments (see section 4.2.3) consisting of three parts, which are done in a single three hours session. The exception is that the perturbations are done in a different order. First participants have to shake their arm, then move the device on the skin and finally pull the device from the skin. Participants are supervised by the researcher, who resides in the same room during the experiment. Half to two thirds of the participants are asked to wear two sensors at the time on both arms. This is done to investigate the inter-reliability of two different sensors. Written instructions on how the device works and what the participants need to do during the experiment, are given beforehand (see Appendix A.4 and A.5). During the session, the researcher helps the participants with the procedures of the experiment and as little as possible with the operation of the device in order for the participants to get an unbiased view of the perceived usability of the device. After the experiment, all participants are asked to fill in an usability and comfort questionnaire to evaluate their experiences on these topics.

5.2.4 Outcome measures and analysis

PuppyNose data

The outcome measures are the VOC concentration measured by the PuppyNose and the scores retrieved from the usability and comfort questionnaire. Conclusions are based on a combination of statistical tests and on a qualitative analysis of the VOC data. The qualitative analysis gives a visual explanation on how the different events affect the sensor output. The temperature and relative humidity data will be used to explain the effect of external influences on the primary sensor output.
Statistic tests are used to evaluate the test-retest reliability and to see if using a different device (inter-observer reliability) or applying perturbations significantly affects the output. In order to do this, first all the data points within the time period of each event are averaged: the first and second 45 minutes the device is worn and each 20 minutes period when a new perturbation is applied. Then, two types of tests are run. A paired sample t-test is performed between the first 45 minutes of wearing (control event) and each of the other events (intervention events). The comparison between the control and intervention events, shows if measuring under the same circumstances (test-retest reliability) or if applying perturbations significantly affect the

output. For the second statistical test, an independent samples t-test is performed between each event of the two sensors. This shows if any of the two sensor performs statistically differently while measuring the same events.

Comfort and usability questionnaires

The comfort and usability questionnaire consists of sixteen closed and three open questions. The closed questions are formed by two established rating scales: the System Usability Scale (SUS) [83] and the Comfort Rating Scale (CRS) [84]. The SUS is a widely used questionnaire consisting of ten questions giving a good overview how participants perceive the ease-of-use and intuitive design of a device. Each question is based on a 5-point Likert scale. The SUS produces a score from 0 to 100 based on the following formula:

$$Score = 2,5 \cdot \sum (Q_{odd} - 1) + (5 - Q_{even}) \tag{5.1}$$

where:

Q_{odd} = value for an odd question
Q_{even} = value for an even question

The final score gives a single score in the overall perceived usability. This score can be coupled to an absolute judgment of usability with Adjective Rating Scale (table 5.1). Which SUS score corresponds to which judgement is displayed in table 5.1.

Table 5.1: Table linking the System Usability Scores to a corresponding Adjective Rating.

Adjective Rating	SUS score
Best imaginable	90.9
Excellent	85.5
Good	71.4
OK	50.9
Poor	35.7
Awful	20.3
Worst imaginable	12.5

The CRS is a rating scale developed for the assessment of comfort of wearable computers. It bases its definition of comfort on six dimensions, which are summarized in table 5.2. Each dimension is scored on a 21-point Likert scale (0 to 20). Higher scores indicate more negative interpretation of that particular dimension of the CRS. The CRS is not intended to provide an absolute judgment on the comfort of the device, but should be used as a comparative tool. This way, the CRS can be used to highlight differences in comfort between different versions of the PuppyNose for different aspects of comfort.

Lastly, the questionnaire incorporates three open questions. These allow the participants to voice the problems they had with the device and what they wanted to see changed. The questionnaire is added to Appendix A.2.

Table 5.2: Table describing the six categories of the Comfort Rating Scale. The description in the table was also used on the survey handed to the participants.

Comfort dimension	Description
Emotion	I am worried about how I look when I wear this device. I feel tense or on edge because I am wearing the device.
Movement	The device affects the way I move. The device inhibits or restricts my movement.
Harm	The device is causing me some harm. The device is painful to wear.
Attachment	I can feel the device on my body. I can feel the device moving.
Perceived change	Wearing the device makes me feel physically different. I feel strange wearing the device
Anxiety	I do not feel secure wearing the device.

5.3 RESULTS

In total six participants partook in this study, three males and three females. Two participants, one male and one female, were employees of the University of Twente. The four other participants were recruited from the family of the researcher. The participants that were university employees wore one PuppyNose device and the other four wore two devices at the same time. In all cases PuppyNose devices 6 and 8 were used for this study.

5.3.1 PuppyNose data

Due to a problem with the Bluetooth, large pieces of data (1-2 hours) were missing for the two persons who wore a single device. The data from the four other participants, who all wore two devices, were, except for less then ten minutes data loss, entirely complete. The time series data from two participants is displayed in figure 5.1 and 5.2. All the data from all the participants can be found in Appendix A.1.

QUALITATIVE ANALYSIS When looking at these time series graphs, a few things stand out. First of all, it looks that the disturbances caused by each event (wearing the sensor again, shaking the arms, moving the device on the skin and pull the device from the skin), have almost no or only short-lived effects on the output of the device. Moreover, over the long term the time series of the two devices show good correspondence. Long lasting trends are visible in both cases and for the humidity and temperature sensors alike. These trends in the VOC output seem to correlate with same trends in temperature and humidity. In the time series of most participants, there was a long-term shift visible in the raw baseline resistance data. However, this was often not present in the VOC ouput. This seems to have been filtered out by the compensation algorithm. In spite of these long term trends, in the short term sudden, high spikes can be visible in the VOC data of figure 5.2 at 24, 95 and 188 minutes of PN6. These fluctuations seems sensor-specific as they are not measured by PN8. These fluctuations also affect the output after averaging. For instance the 'Pull from

skin' event had an average of 23.85 ppm for PN6 against 2.14 ppm for the same event for PN8. These fluctuations in one sensor were also seen in the time series of one other participant.

Both figure 5.1 and 5.2 showed that temperature levels increased slowly from 25°C, before the device was worn, to temperatures between 35 and 40°C. An external sensor indicated that the room temperature for both participants was just below 20°C. It appears that the PuppyNose measurements overvalue the actual temperature.

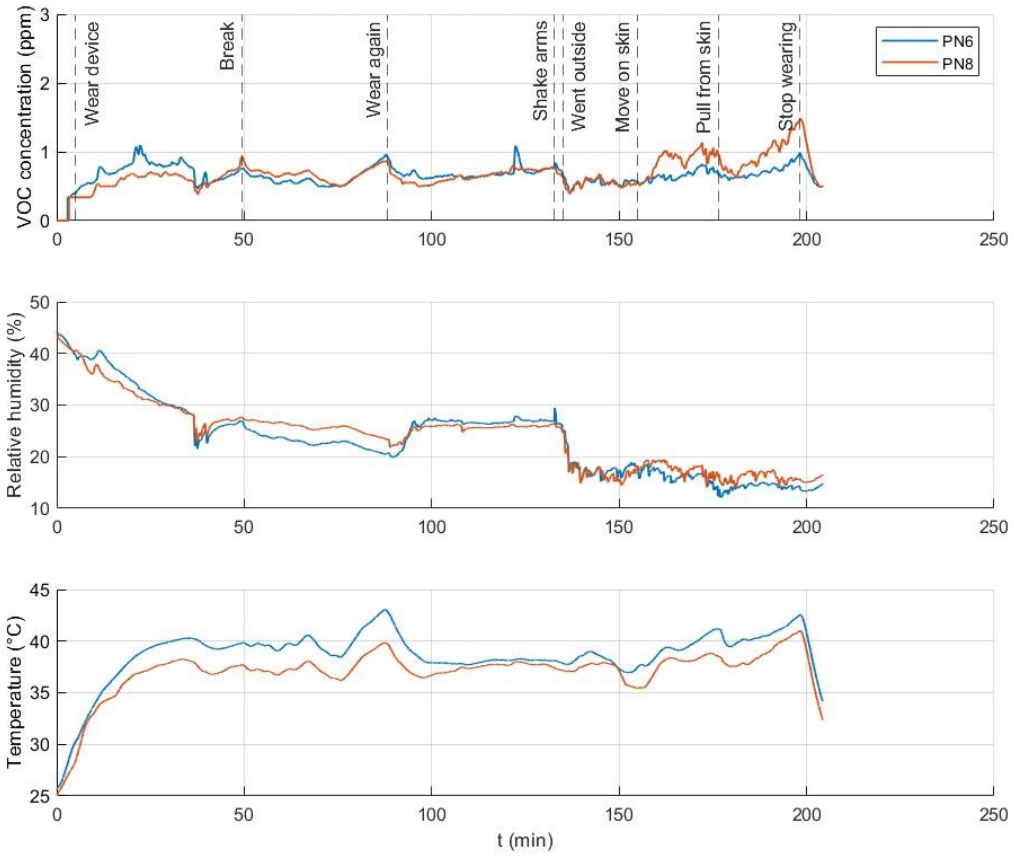


Figure 5.1: Time series graph of the VOC, RH and temperature data from participant 5. Markers indicate the beginning of each event. After 132 min. the participant went outside, where the temperature was around 10°C lower.

STATISTICAL TESTS For the calculation of the paired t-test, it was not possible to use the time series of the employees of the university. The time series missed such large pieces of data that in both cases either the control or all of intervention events were missing and performing a paired t-test was not possible. However, for the independent sample t-test some events of these two participants had complete data and could be used for analysis.

Figure 5.3a and 5.3b show the results of the paired samples t-test. For each of the two sensors, there were no significant differences between the average VOC output

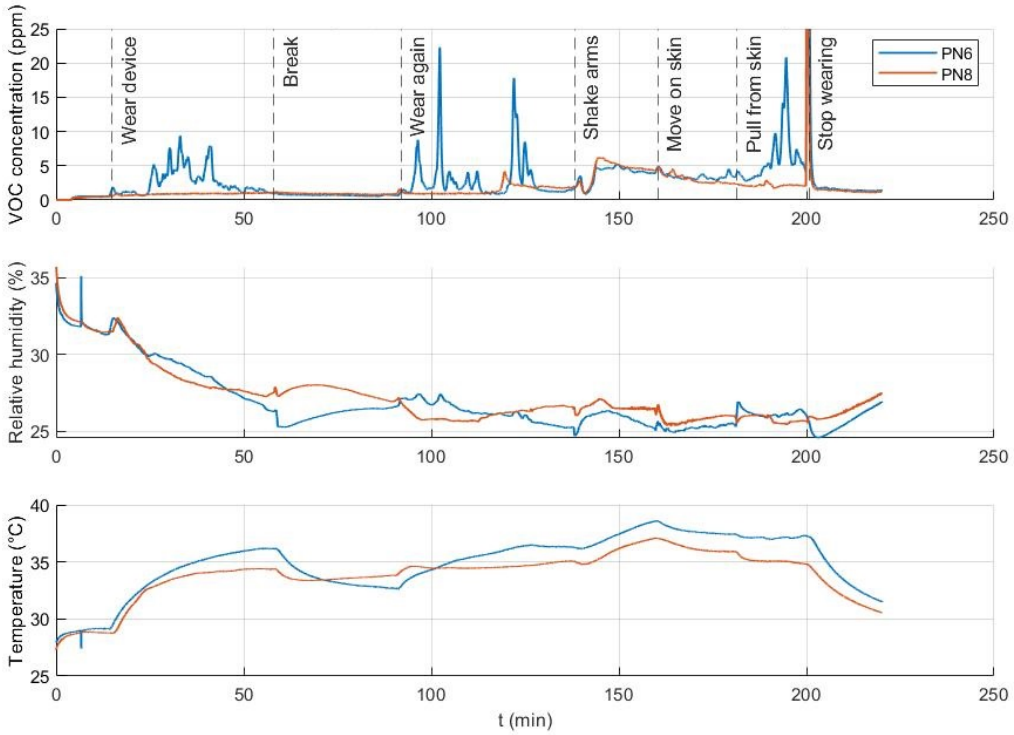
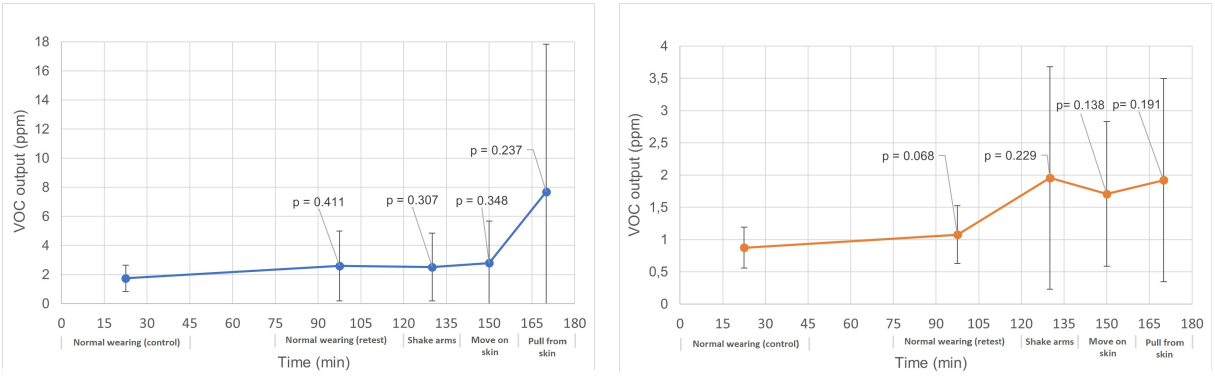


Figure 5.2: The time series graph of the data from participant 3. Markers indicate the beginning of each event. The spike at 198 min reached the maximum value of 1000 ppm, but this is not shown for scale.

of the control event and the intervention events. In other words, wearing the same PuppyNose twice on different times points or applying perturbations to the device had no statistical effect on the averaged output.
Likewise for the comparison of the two sensors at each event, no statistical significance could be found from the independent t-test (see table 5.3). This indicates that the individual devices did not significantly deviate in their averaged output.

Table 5.3: Table shows the mean of the participants' VOC data at each event for the two sensors. Outputs of different sensors were compared with an independent samples t-test. The statistical significance was calculated between the two sensors for each event. For no event $p < 0.05$ was reached.

Event	Mean (std) PN6	Mean (std) PN8	Significance
Wear normally 1st time	1.74 (0.91)	0.87 (0.32)	p = 0.152
Wear normally 2nd time	2.59 (2.40)	1.08 (0.45)	p = 0.149
Shake arm	2.51 (2.32)	1.96 (1.73)	p = 0.692
Move device on skin	2.79 (2.89)	1.71 (1.12)	p = 0.473
Pull device from skin	7.69 (10.14)	1.92 (1.57)	p = 0.274



(a) PuppyNose 6 **(b)** PuppyNose 8

Figure 5.3: Figures showing the mean of the participants' VOC data at each event. The results of the paired t-test between the control (first normal wearing) and each of the other events is displayed as a p-value above each measurement. None of the events showed significant difference compared to the controls.

5.3.2 Usability and comfort

All six participants completed the SUS and CRS questionnaire. The mean score of the SUS among all participants was 80. According to the adjective rating scale (table 5.1), this score corresponds to having 'good' usability. The distribution of all the individual questions of the SUS can be found in figure 5.4. Most of the questions received a score in the desirable range. A few participants expressed that they found the third question confusing: "I found the different functionalities of the PuppyNose well-integrated", because the sensor system only consisted of one device with a simple measuring functionality.

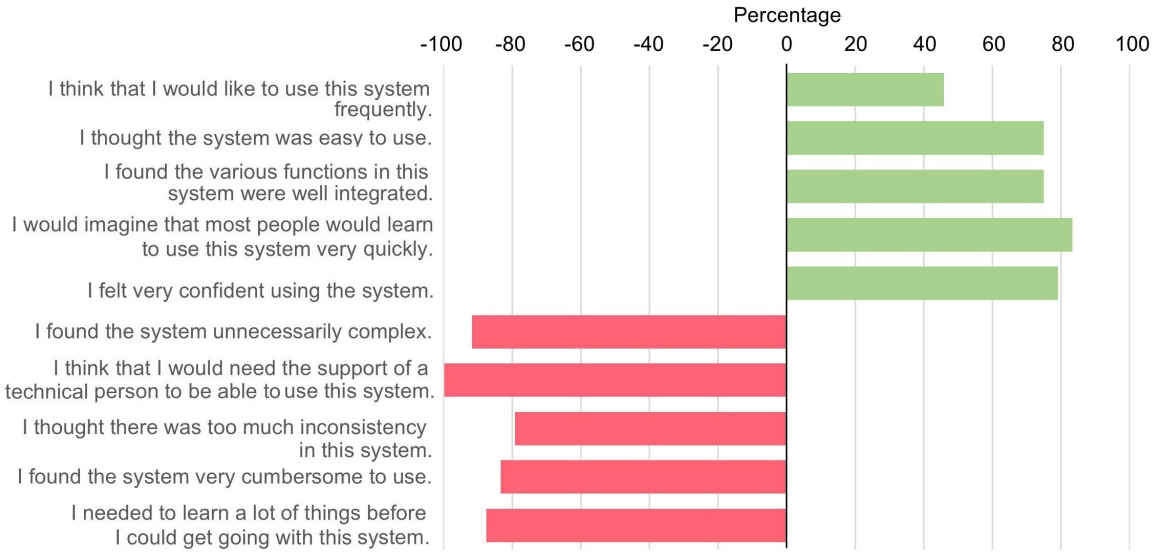


Figure 5.4: The distribution of the SUS for each question. For the first five questions (green) a positive response to the questions resulted in a higher score, whereas for the last five questions (red) this was reversed.

The results of CRS are shown in figure 5.5 The distribution of the scores are displayed per dimension of comfort. The figure shows that the dimensions on 'Attachment' and 'Perceived change' scored high. This indicates that participants were aware of how the device changed their appearance and that the device was present on their arms. Participants were positive about the freedom of movement ('Movement') that the design offered and the perceived safety of the device ('Anxiety'), which received low scores as compared to the other comfort dimensions.

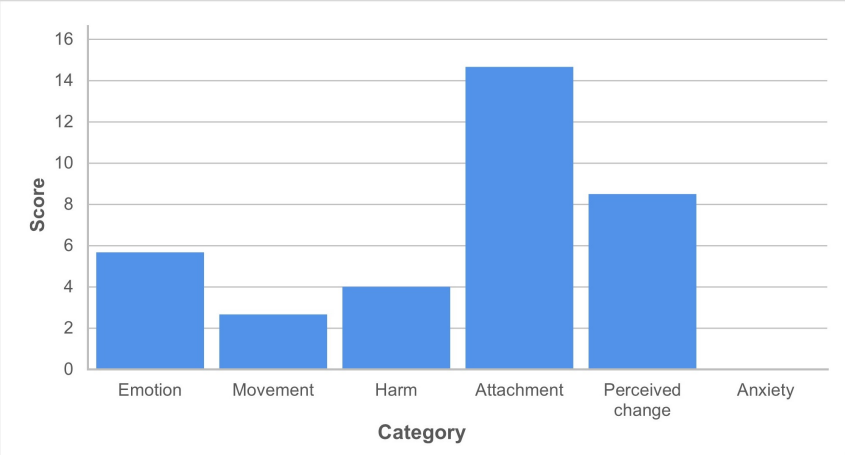


Figure 5.5: The average score of all the participants for each question of the CRS.

Lastly, participants were asked to voice their concerns and possible improvement regarding the design of the device. There were similar themes that recurred on most responses. Five participants stated that they found the size of the device too large. Two of them indicated that it would hinder their willingness to use the device in daily life situations, because it was not convenient to wear the device in combination with clothes. One participant said: "It should be smaller. In comparison with a glucose meter it is a large device." Four of the respondents made remarks on the difficulty to close the gasp of the band. One suggested that a velcro band would be more convenient to use. Finally, three respondents made remarks on the size and amount of values on the screen of the device. They stated that the values were too small and in dark surroundings hard to read. Also, a suggestion was made to improve the readability by reducing the amount of values on the screen.

5.4 DISCUSSION

In this study the PuppyNose was tested on a group of healthy adults who were not in a state of ketosis. The usability and comfort of the device was also assessed.

When comparing the time series of the two PuppyNose devices worn by the same participant with each other (figure 5.2 and 5.1), long term trends seem to show good correspondence with each other. This might be attributed to slowly changing environmental variables such as the humidity and the temperature, which have on equal effect on both outputs. On the other hand, as figure 5.2 has shown, on the short term a

PuppyNose device can become prone to sudden, temporarily lasting spikes that affect the output for significant amounts of time. These noisy events can have a disturbing effect on 20 minute average of the VOC output signal. As these events were not measured by the other sensor, this is most likely an erroneous measurement from within the sensor itself. These errors put additional question marks on the reliability of the PuppyNose device and its potential use in clinical situations.

When measuring in-vivo, a paired t-test showed that wearing the device again or applying perturbations to the device (moving the device, shaking arm or pulling the device from the skin) did not produce a significantly different output than wearing the PuppyNose normally. Secondly, there was no statistical difference in the output of two PuppyNose devices (PN6 and PN8) during the measurement of each of the events. This seems have been in part caused by the compensation algorithm, which corrected for baseline drift in the resistance values. Although these results could be interpreted as good stability of the devices, it must be taken with consideration. The variation of the results among the participants was large, indicated by a standard deviation in most cases as high as the output itself. This suggests that the disturbances in the output might not have caused any significant differences, not because these disturbances had no effect, but because the variation in the data measured by the device is large when measuring on multiple occasions. When extending this to relevant signals in DKA detection, this becomes problematic as the true signal must be much higher than the normal variation in the values that the PuppyNose measures. As the fasting experiment has indicated, this is not likely to be the case.

The results from the usability questionnaire was a SUS score of 80 points. This indicates a good usability, but still leaves room for improvement. The usability score of the PuppyNose was compared with another device that is worn on the arm and is used for healthcare measurements. This device was a watch that could monitor blood pressure in Glaucoma patients [85]. For a multiple of sessions, participants had to put on the watch, do measurements with it and transfer the measurements to a mobile application. These actions were very comparable to this sub-study. This device received a median SUS of 80 among 53 participants. This demonstrates that the usability of the PuppyNose is on par with similar technology that is already introduced in the marketplace. The PuppyNose was also compared with the usability of normal smart watches. In a study seven smart watches were assessed by 388 participants in total [86]. The watches received System Usability Scores between 61.36 and 65.97. Even though the smartwatches offer many more functionalities and hence are not directly comparable to the PuppyNose, these results show that the PuppyNose is already a very usable device, compared to other wearable devices that are already used by millions of people.
In the PuppyNose study, the question that most deviated from a perfect score was whether participants would want to use the device again. This problem was reflected in the remarks that many participants made. Namely, they often found the device not to be appropriate when wearing it in combination with clothes due to its size. In order to improve usability a decrease in size, readability of the screen and a better

working arm band might be a solution. This way end-user need less time to operate the device and can use it more easily.

Regarding the comfort assessment, the device scored relatively high results in two areas as compared to the other categories. Participants indicated that they felt their appearance had changed ('Perceived change') and that they could clearly feel the device on the arm when wearing it ('Attachment'). The subject 'Attachment' seems to be linked to the tightness of the arm band. For proper functioning a tight fit needs to be ensured. A possible solution might lie in the size of the device. Namely, when the device is smaller a less broad arm band could be used, which would improve the pressure of the device on the arm. This change in size can also be a solution for the 'Perceived change' category, as this would improve how prominently aware the participants are of the PuppyNose. No other studies using the CRS could be found that were about devices that are worn on the upper arm and are kept in place with a tightening band. Hence, the scores obtained from the CRS in this study could not be linked with comparable devices from the literature.

5.5 CONCLUSION

The aim of this study was to assess the performance of the PuppyNose in an in-vivo population of healthy non-ketotic adults. A second objective was to evaluate how these participants perceived the usability and comfort of the device.

1. The test-retest analysis after a 30 minute break showed on average good, stable outcomes based on statistical results and visual interpretation of the data. However, spontaneously occurring fluctuations are concerning and can have a significant effects in the output of single measurement.

2. Disturbing the device by moving it on the skin, pulling it from the skin or shaking with the arm had no significant effect on the averaged VOC output of the device and did not affect the reliability of the device. Visual inspection of the time graphs showed the same results.

3. There was no statistically significant difference between the VOC outputs of the two different PuppyNose devices. On average devices showed good correspondence, but there was a large spread in the measurements of each of the devices.

4. A SUS score of 80 revealed that usability of the PuppyNose was indicated as 'good', while usability could still be improved for optimal device design. The comfort assessment and suggestions made by the participants revealed that the categories of 'Perceived change' and 'Anxiety' leave room for improvement as compared to the other categories. A smaller device with less values on the LCD screen that are larger in size could possibly improve the comfort and usability assessment.

All in all, the output of the in-vivo measurements in non-ketotic participants were in often stable during different events and two devices showed often good agreement. However, there was a large variation in the measurements between the participants and in one case unexpected, long-lasting fluctuations were present in the data. Due to the observed variation in baseline values among participants and the expected amount of acetone emanating from the skin reported in literature, it seems unlikely that the PuppyNose device will be able to accurately measure relevant acetone concentrations in-vivo.

6 SUB-STUDY 4: INDIVIDUALS IN AN ELEVATED STATE OF KETOSIS

6.1 RESEARCH QUESTIONS

OBJECTIVE 4 Testing the technical feasibility of the device in healthy individuals who are in an elevated state of ketosis.

1. Can the PuppyNose device detect ketone levels in a clinically relevant range of > 0.5 mmol/L that are induced by a multiple days fast?
2. Is there a statistically significant difference in the output of the device between when individuals are in an elevated state of ketosis and when individuals are not?

6.2 METHODS

6.2.1 Study design

Sub-study 4 investigates if the device is able to measure in-vivo ketones within a clinically relevant level with multiple participants. This is done by measuring in participants who voluntarily partake in an already existing 40-hour fasting challenge organised by a professional dietician. A week later, the same participants use the device when they are on a normal diet.
This study is designed to measure during an already existing fasting event. Therefore, participants do not have to follow additional rules of behavior imposed by the researchers. The research should be observational and not subjected to the Medical Research Involving Human Subjects (non-WMO).

6.2.2 Study population

For sub-study 4, participants are recruited who undertake a 40-hour fasting challenge. This 40-hours fasting challenge is an already planned event, organized by a professional dietician for her client group. Possible candidates are approached by the researcher together with the dietician. For this sub-study four to eight participants should be selected.
Participants should be included based on the criteria that they are older than 18 and that they will not consume alcohol the day before the event and while wearing the PuppyNose. The participation for all participants should be based on the informed consent.

6.2.3 Study procedures

During this observational study, participants are measured for two 48 hours periods when they undertake a 40-hour fasting challenge and a week later when they are on a normal diet. Studies have shown that a two-day fast increases ketones to levels between 1.5 and 2.5 mmol/L β-HB, which are comparable to levels of diabetic patients with an increased DKA risk [18, 20]. Measuring skin acetone during this fasting challenge shows if these relevant ketone levels fall in the sensor's measurement range and if the sensor can make reliable distinctions in levels between certain time points.

This 40-hours fasting challenge is an already planned event, organized by a professional dietician for her client group. The dietician has asked for participants in her monthly newsletter. Before the start of the observations, participants are selected from the pool of candidates based on inclusion criteria and participants are asked what their age and gender is. During the observational periods, each participant is measured for two 48h periods. The first measurement period is during the 40h fasting challenge and the second one is a week later when participants are on a normal diet. The 40h fasting challenge starts on Friday at 20:00 hours and end on Sunday at 12:00 hours. Before the start of the fasting challenge participants receive a PuppyNose device, a logbook and instructions on how to use those and when to measure. Participants are asked to wear the device and wear the device for two hours at five predefined time points during the weekend. The measurement time points will be each day at 10:00 hours and 20:00 hours, starting on Friday at 20:00 hours and ending on Sunday at 20:00 hours. At each time point, after the PuppyNose has initialized, the participant is asked to write the data from the device for two hours at 30 minute intervals in the logbook. The data which should be written down are the VOC acetone value, the measured temperature and relative humidity. If participants are not able to sustain their fasting, they should continue with the measurements, but make a note in the logbook what they consumed and when. For the second part of the study, the same participants are asked to measure with the PuppyNose device again after a wash-out period of one week. Again, participants wear the device for a two-hour period each day at 10:00 hours and 20:00 hours, starting on Friday at 20:00 hours and ending on Sunday at 20:00 hours. If participants drink alcohol, they are asked to make a note of how much and when. This is done because alcohol, which diffuses through the skin after consumption, interferes with the measurement of acetone. At the end of both parts, the PuppyNose devices and the data from the device will be collected.

6.2.4 Outcome measures and analysis

The VOC concentration (parts per million) data measured by the PuppyNose device is the primary outcome measure. Besides that, the temperature and relative humidity should also be recorded by the participants. For sake of clarity, the first measurement on Friday 20h will be indicated with t = 0h and the final measurement with t = 48h. To evaluate whether and when a significant difference has been measured, the paired student t-test will be calculated at each time point between t = 0h and t = 38h of the fasting challenge.

Moreover, another analysis is done to evaluate if the differences measured during the fasting challenge are not caused by other influences such as sensor drift. To this end, the fasting arm (intervention) is compared with the normal diet arm (control). Using 2 (groups) x 5 (timepoints) ANOVA, the total statistical difference between the control and intervention moments is determined.

When participants also use other measuring methods to determine their ketones voluntarily, for instance via finger prick tests, they are asked if they want to share this data. However this is strictly voluntarily and on the participants own accord as this is a non-WMO subjected study. If sufficient data points for blood β-HB are collected on the same time points as the PuppyNose measurements, the two are correlated with Pearson's correlation test. For this analysis, the data points from different time points and groups can be pooled as long as each SkAce value has a corresponding β-HB value.

6.3 DISCUSSION

In the end, it was not possible to conduct this sub-study within the planning of this project. This was caused by the combination of reasons. First of all due to restrictions outlined by the WMO, participants for this study could only be recruited who would partake in multiple days of fasting on their own account. The only possibility to do this was via the earlier mentioned fasting challenge. Within the time frame of this project there was only one date to this. Unfortunately, two weeks prior to this event, the device short-circuited and broke down due to sweat entering the device during the exercise experiment. Thereafter, it was not deemed safe to knowingly provide multiple devices with this design risk to possible participants.

7 GENERAL CONCLUSION AND RECOMMENDATIONS

It was the goal of this report to reach Technology Readiness Level 4 and make efforts in reaching TRL5. For this a set of objectives had been formulated that divided the report in four sub-studies. Three of these four sub-studies have been completed, while for the last one, research into the technological feasibility of the PuppyNose in detecting ketosis in a population of people in ketosis, only a research protocol was constructed, but no results were obtained.
Sub-study 1 has validated the PuppyNose in a laboratory environment and matured this level to TRL4. This first sub-study has shown that the device can measure acetone in a range of 1 to 100 ppm accurately and linearly when measuring during a single session. However, the sensor response and the baseline resistance of the device varied strongly between measurement sessions, which had a negative effect on the inter-observer and test-retest reliabilities. This was caused by aggressive short-term drift, which seemed to occur due to instabilities in the sensor material itself. Moreover, humidity affected the sensor response to acetone. All of these disturbances were not corrected by the compensation algorithm, which was designed for this purpose.
Sub-study 2 and 3 were conducted to see how the device would perform in a setting of TRL5: in a controlled, in-vivo environment. Sub-study 2 showed that it was not possible to measure ketone bodies with the device when levels were in a clinically relevant range for the detection of DKA. It was also demonstrated that the output of the PuppyNose was sensitive to humidity and unpredictable fluctuations, which could occur as a result of moving the device or originated spontaneously and could last for tens of minutes to hours.

The results of sub-study 2 showed that the PuppyNose is not able to detect an increased risk of DKA. There is little indication that either the acetone concentration is high enough or the PuppyNose is accurate enough to differentiate relevant signals on the skin from baseline VOC readings of 1 to 5 ppm, that were always found to be present on the skin (sub-study 3). As sub-study 1 indicates, it is not likely that simple design changes will solve these problems, because the BME680 sensor appears to have inherent instabilities that make reproducible measurements under varying in-vivo conditions difficult. It is therefore the conclusion of this report that the current BME680 sensor and the corresponding device design of the PuppyNose will not be able to reach TRL5. It is likely, that a new sensor chip needs to be adopted for this technology to be successful. It is also advisable to do research with more accurate technology, such as mass-spectrometry, to find how much acetone is really present in the sensor chamber of the PuppyNose. This can provide more information on whether the concentration of acetone emanating from the skin is high enough to fall in the measurement range of MOx sensors.

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A APPENDIX

A.1 ADDITIONAL FIGURES

A.1.1 Sensor dynamics

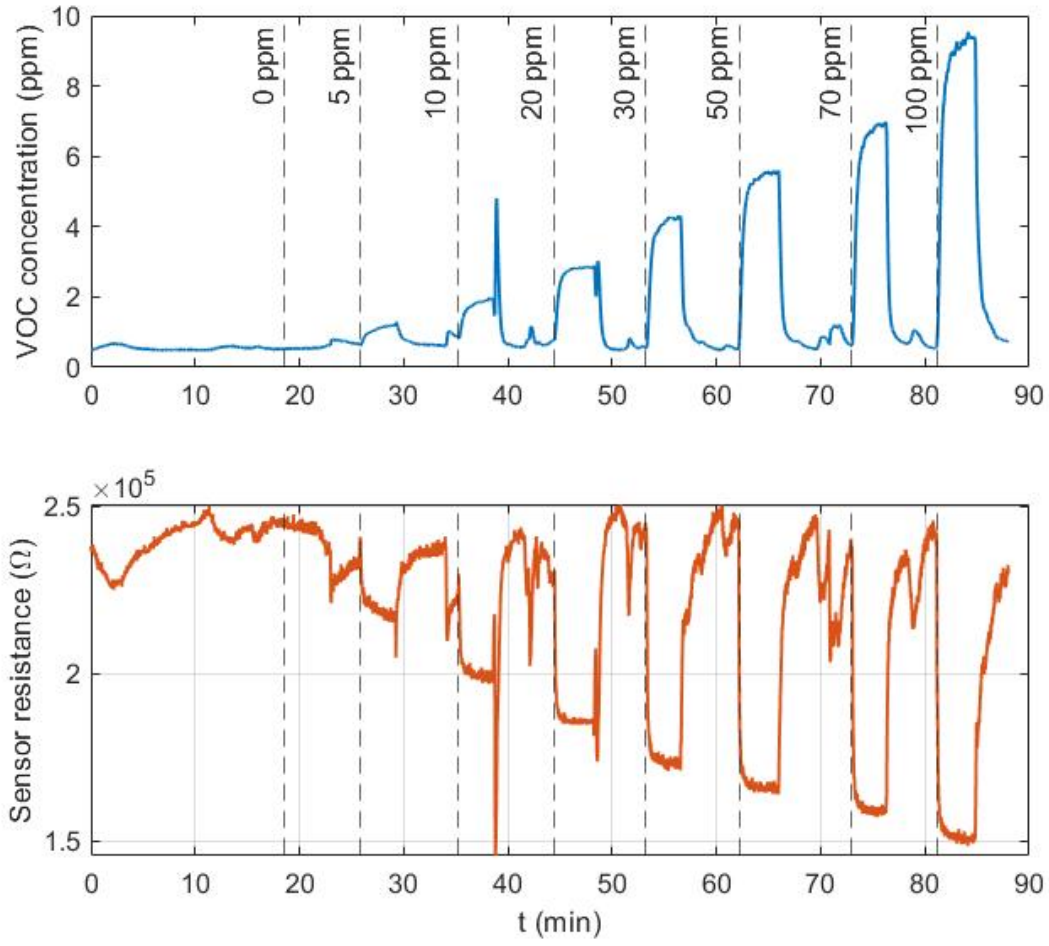


Figure A.1: Time series graph of VOC and sensor resistance outputs (PN3) after injection of acetone in concentrations ranging from 1 to 100 ppm.

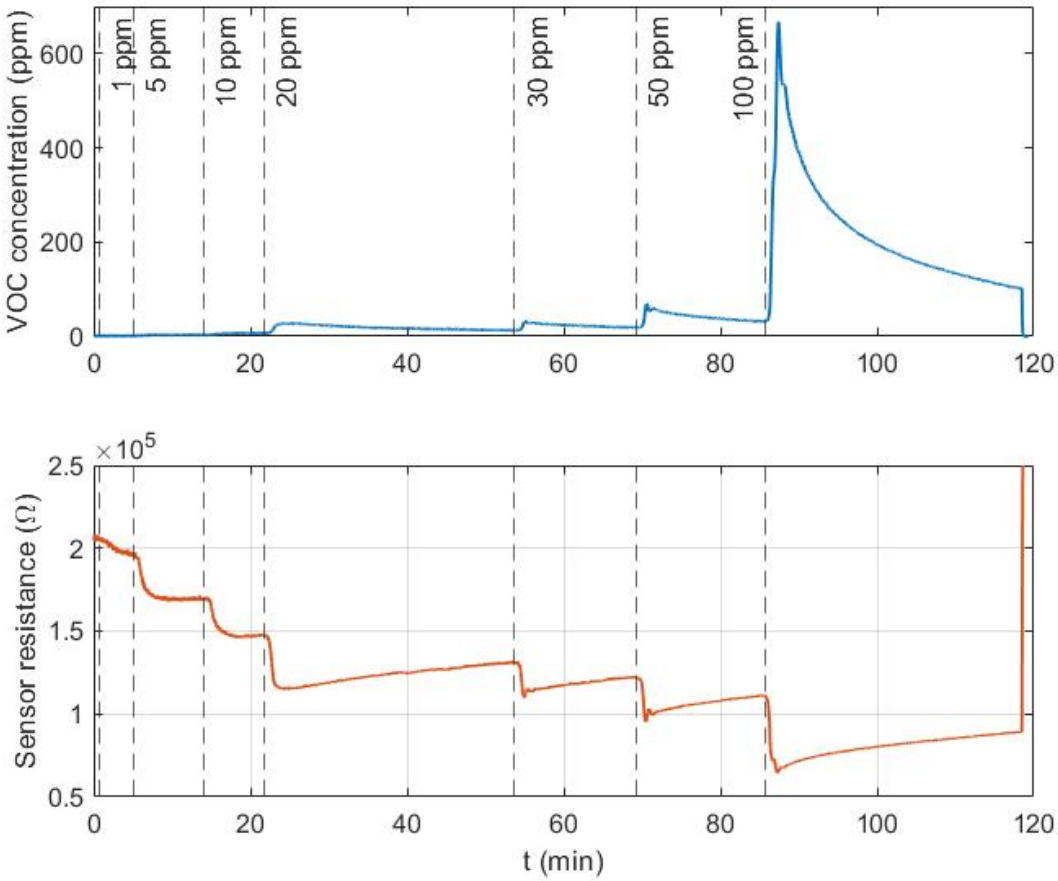


Figure A.2: Time series graph of VOC and sensor resistance outputs after injection of ethanol (PN3) in concentrations ranging from 5 to 100 ppm. The difference with acetone was that the ethanol concentration was increase in gradual steps and the sensor was not taken out of the measurement container during the experiment. Therefore, the sensor response and recovery times could only be estimated.

A.1.2 Exercise experiment

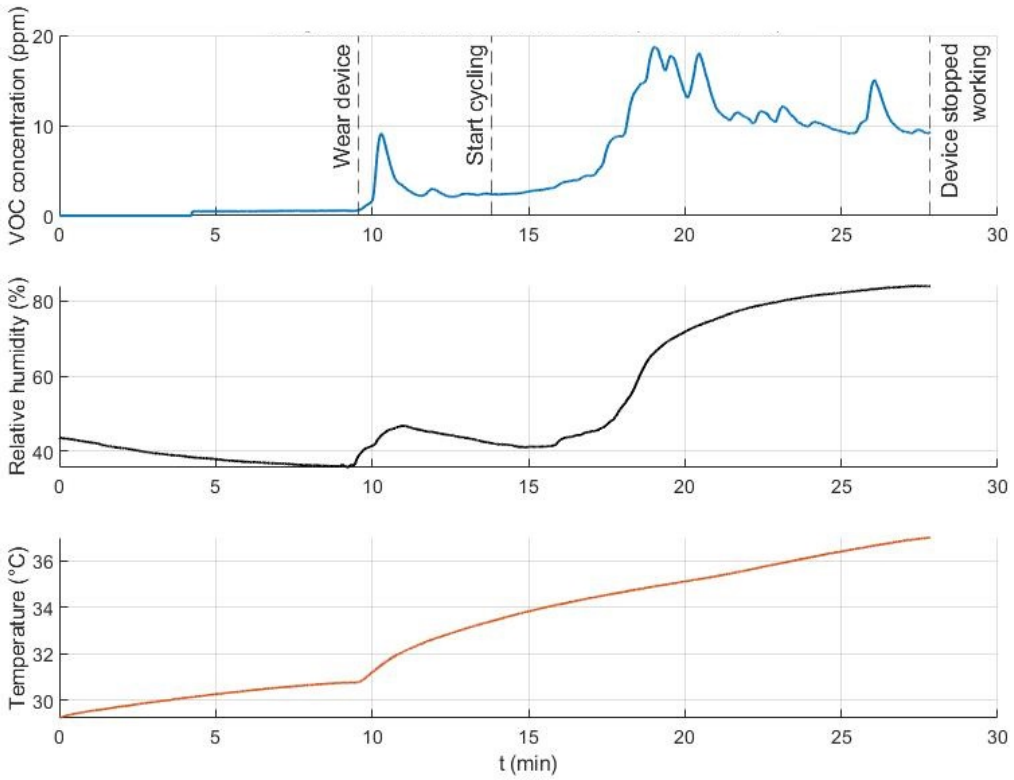


Figure A.3: Time series graph by the PN5 during exercise. At the t = 27 min. the sensor stopped working.

A.1.3 Individuals not in ketosis

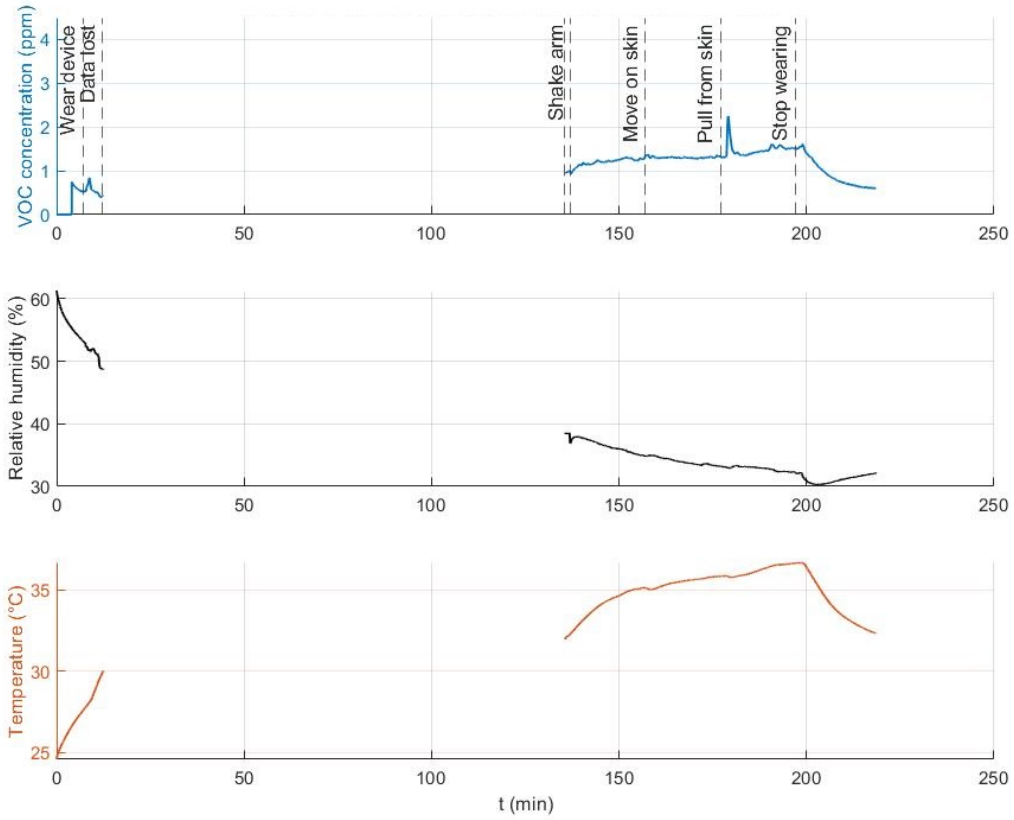


Figure A.4: The time series graphs for participant 6. Only one device (PN8) was used. Data was lost from 12 until 136 minutes due to a Bluetooth error.

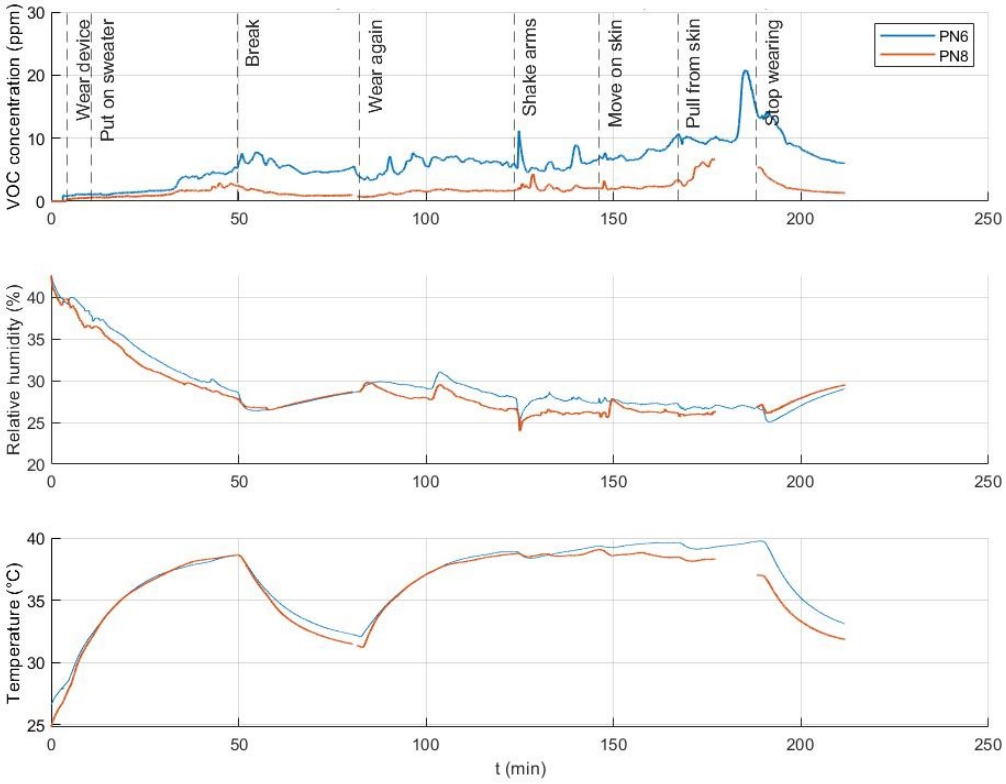


Figure A.5: The time series graphs of participant 2. Data was lost for PN8 between 177 and 188 minutes.

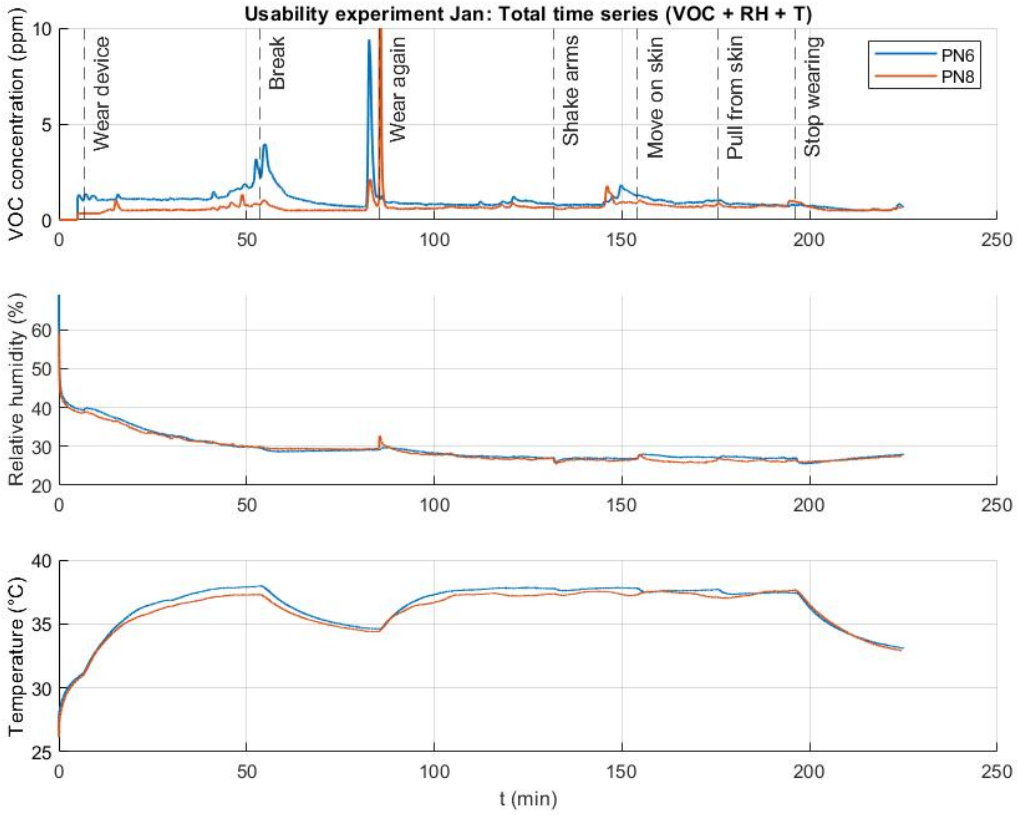


Figure A.6: The time series graphs of the for participant 4.

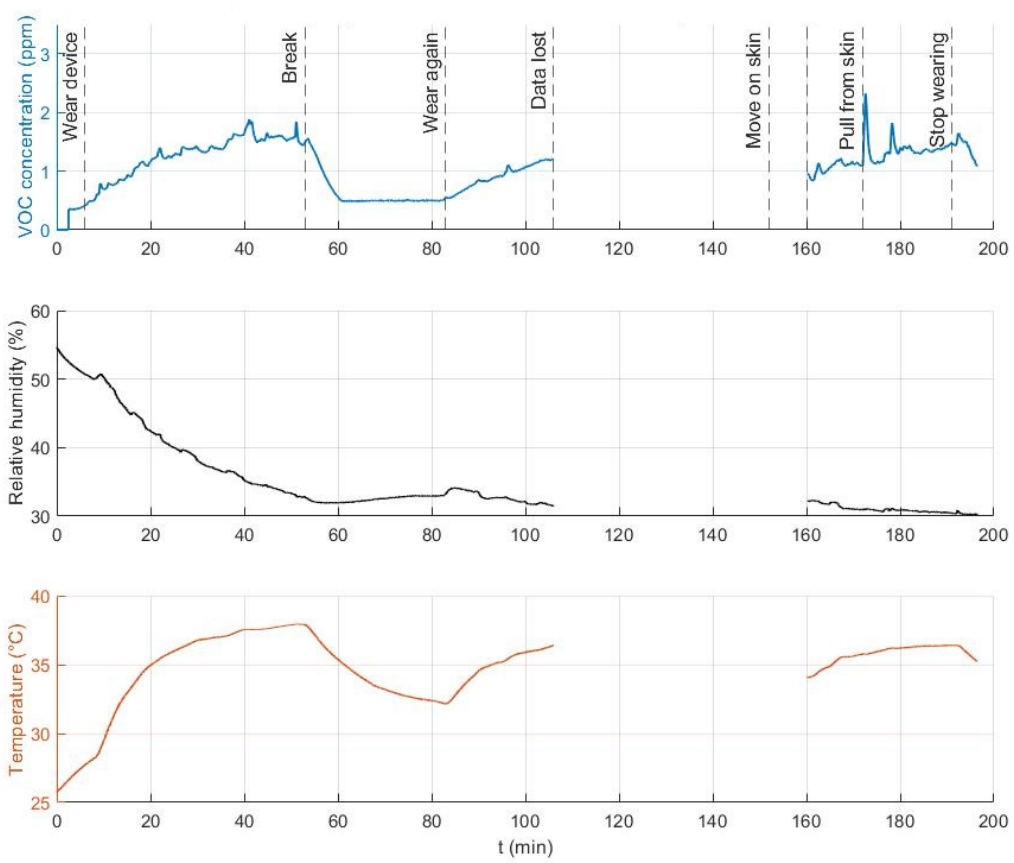


Figure A.7: The time series graphs for participant 6. Only one device (PN6) was used. Data was lost from 105 until 160 minutes due to a Bluetooth error.

A.2 QUESTIONNAIRE

Vragenlijst

Bedankt voor uw deelname aan het onderzoek naar het meten van ketonen met de PuppyNose-sensor. In de vragenlijst op de volgende pagina wordt uw ervaring tijdens het gebruik van de PuppyNose-sensor gevraagd. De gebruiksvriendelijkheid de PuppyNose-sensor wordt uitgevraagd aan de hand van tien stellingen. Deze stellingen kunt u beantwoorden door middel van puntenschaal, hierbij dient u één cijfer te omcirkelen. De comfort wordt beoordeeld met zes stellingen. Hierbij geldt ook een puntenschaal, echter een stuk uitgebreider. U kunt uw mate van overeenkomst aanduiden door een cijfer tussen 0 en 20 te omcirkelen. Hier staat 0 voor de laagste overeenkomst en 20 voor de hoogste mate van overeenkomst. Verdere verbeterpunten of opmerkingen die u mogelijk heeft, kunt u kwijt op de laatste pagina.

Datum: ...../...../.....

Gebruiksvriendelijkheid van de PuppyNose-sensor	Sterk mee oneens	Oneens	Neutraal	Eens	Sterk mee eens
1. Ik denk dat ik de PuppyNose sensor vaak zou willen gebruiken.	1	2	3	4	5
2. Ik vond het onnodig ingewikkeld.	1	2	3	4	5
3. Ik vond de PuppyNose makkelijk te gebruiken.	1	2	3	4	5
4. Ik denk dat ik technische ondersteuning nodig heb om de PuppyNose te gebruiken.	1	2	3	4	5
5. Ik vond de verschillende functies van de PuppyNose goed met elkaar geïntegreerd.	1	2	3	4	5
6. Ik vond dat er te veel tegenstrijdigheden in de PuppyNose zaten.	1	2	3	4	5
7. Ik kan me voorstellen dat de meeste mensen snel met de PuppyNose overweg kunnen.	1	2	3	4	5
8. Ik vond de PuppyNose omslachtig in het gebruik.	1	2	3	4	5
9. Ik voelde me zelfverzekerd tijdens het gebruik van de PuppyNose.	1	2	3	4	5
10. Ik moest veel over de PuppyNose leren voordat ik het goed kon gebruiken.	1	2	3	4	5

Comfort van de PuppyNose-sensor	← Laag					Mate van overeenkomst															Hoog →
1. Ik maak me druk om hoe ik eruit zie als ik de PuppyNose om mijn arm draag.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2. De PuppyNose beperkt mijn bewegingsvrijheid of heeft effect op hoe ik beweeg.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
3. Het dragen van de PuppyNose was pijnlijk of zorgde voor irritaties op de huid.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
4. Ik voel de PuppyNose heel duidelijk op mijn arm als ik hem draag.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
5. Ik heb het gevoel dat ik er fysiek anders uitzie nadat ik de PuppyNose ben gaan dragen.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
6. Ik voel me niet veilig als ik de PuppyNose op mijn arm draag.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

Verbeterpunten en opmerkingen
Heeft u opmerkingen op het gebied van comfort na het dragen van de PuppyNose?
Zijn er bepaalde dingen die u in het ontwerp van de PuppyNose anders zou willen om de sensor gebruiksvriendelijker te maken?
Heeft u nog verdere op- of aanmerkingen over uw ervaring met het gebruik van de PuppyNose die u hier kwijt zou willen?

A.3 CONSENT FORM

Toestemmingsformulier deelnemer

Behorende bij: *Ketonen meten aan de huid met een nieuwe sensor*

- Ik heb de informatiebrief gelezen. Ook kon ik vragen stellen. Mijn vragen zijn goed genoeg beantwoord. Ik had genoeg tijd om te beslissen of ik meedoe.
- Ik weet dat meedoen vrijwillig is. Ook weet ik dat ik op ieder moment kan beslissen om toch niet mee te doen met het onderzoek. Of om ermee te stoppen. Ik hoef dan niet te zeggen waarom ik wil stoppen.
- Ik weet dat gegevens worden verzameld voor dit onderzoek door middel van metingen van de PuppyNose-sensor en een beoordelingsformulier over deze sensor.
- Ik geef de onderzoekers toestemming om mijn gegevens te verzamelen en gebruiken. De onderzoekers doen dit alleen om de onderzoeksvraag van dit onderzoek te beantwoorden.
- Ik weet dat voor de controle van het onderzoek sommige mensen al mijn gegevens kunnen inzien. Die mensen staan in deze informatiebrief. Ik geef deze mensen toestemming om mijn gegevens in te zien voor deze controle.

Wilt u in de tabel hieronder ja of nee aankruisen?

Ik geef toestemming om mijn gegevens te bewaren om dit te gebruiken voor ander onderzoek, zoals in de informatiebrief staat.	Ja ☐	Nee ☐
Ik geef toestemming om de onderzoeker contact met mij te laten opnemen voor een online interview over het comfort en de gebruiksvriendelijkheid van de sensor.	Ja ☐	Nee ☐

Indien u wilt meedoen aan het onderzoek:

Mijn naam is (deelnemer): ……

Handtekening: ..……

Datum : __ / __ / __

01-08-2022

In te vullen door de onderzoeker:

Ik verklaar dat ik deze deelnemer volledig heb geïnformeerd over het genoemde onderzoek.

Wordt er tijdens het onderzoek informatie bekend die die de toestemming van de proefpersoon kan beïnvloeden? Dan laat ik dit op tijd weten aan deze deelnemer.

Naam onderzoeker: ……..

Handtekening: ……..

Datum: __ / __ / __

De deelnemer krijgt een volledige informatiebrief mee, samen met een getekende versie van het toestemmingsformulier.

A.4 INFORMATION BROCHURE FOR THE PARTICIPANTS

Deelnemersinformatie

Informatie voor deelname aan wetenschappelijk onderzoek

Het testen van een nieuwe sensor die ketonen kan meten aan de huid

De technische validatie van een nieuw, non-invasief apparaat dat ketonen kan meten aan de huid

Inleiding

Geachte heer/mevrouw,

Met deze informatiebrief willen we u vragen of u wilt meedoen aan wetenschappelijk onderzoek. Meedoen is vrijwillig. U krijgt deze brief omdat u aangaf interesse te hebben in dit onderzoek. U leest hier om wat voor onderzoek het gaat, wat het voor u betekent, en wat de voordelen en nadelen zijn. Het is veel informatie. Wilt u de informatie doorlezen en beslissen of u wilt meedoen? Als u wilt meedoen, kunt u het formulier invullen dat u vindt in bijlage B.

Stel uw vragen

U kunt uw beslissing nemen met de informatie die u in deze informatiebrief vindt. Daarnaast raden we u aan om dit te doen:

- Stel vragen aan de onderzoeker die u deze informatie geeft.
- Praat met uw partner, familie of vrienden over dit onderzoek.

1. Algemene informatie

De Universiteit Twente, ZGT Almelo en Diabeter Oost hebben dit onderzoek opgezet. Hieronder noemen we de Universiteit Twente steeds de ‘opdrachtgever’. Onderzoekers verbonden aan de Universiteit Twente voeren dit onderzoek uit.

Voor dit onderzoek zijn 4 tot 6 deelnemers nodig. De Ethische Commissie van de Universiteit Twente heeft dit onderzoek goedgekeurd.

2. Wat is het doel van het onderzoek?

In dit onderzoek bekijken we hoe betrouwbaar een nieuwe sensor is die in staat is om ketonen te meten. Deze nieuwe sensor kan ketonen meten op de arm zonder dat een naald (deels) door de huid moet worden gestoken. Ketonen zijn stoffen die door het lichaam aangemaakt worden als er langere tijd geen suikers of koolhydraten gegeten worden. Door te meten bij mensen die normale dagelijkse activiteiten uitvoeren of sporten, kunnen we kijken of de sensor ook betrouwbaar is als er geen ketonen aangemaakt worden door het lichaam.

3. Wat is de achtergrond van het onderzoek?

Als mensen met diabetes problemen hebben met hun medicatie, kunnen zij grote hoeveelheden ketonen aanmaken. Als hier niets aan gedaan wordt, kan dit leiden tot serieuze complicaties voor deze patiënten. In de laatste jaren is er steeds meer aandacht gekomen om ketonen bij deze patiënten te meten, zodat op tijd ingegrepen kan worden. Er bestaan al een aantal testen om ketonen te meten, zoals vingerpriktesten, ademtesten en urinetesten. Het probleem is dat deze testen vaak onnauwkeurig zijn of vervelend zijn voor de gebruiker. Daarom is een nieuwe, draagbare sensor ontwikkeld, genaamd de PuppyNose, die ketonen kan meten op de huid. Deze sensor is nu nauwkeurig getest in het laboratorium, maar nog niet op echte mensen. Voordat onderzoek met de sensor gedaan wordt met patiënten met diabetes, willen we de sensor testen in gezonde mensen. Voor dit eerste onderzoek kijken we naar de betrouwbaarheid van de sensor in normale situaties. In volgende onderzoeken gaan we kijken hoe goed de sensor ketonen kan meten wanneer die worden aangemaakt door het lichaam.

4. Hoe verloopt het onderzoek?

Hoelang duurt het onderzoek?
Doet u mee met het onderzoek? Dan duurt dat in totaal een ochtend. Er wordt bij u gemeten terwijl u uw normale, dagelijkse activiteiten onderneemt. Dit deel duurt in totaal drie tot vier uur. Gedurende het onderzoek hoeft u op sommige momenten alleen de sensor om te doen en kunt u gewoon de dingen blijven doen die u anders ook zou doen.

Stap 1: bent u geschikt om mee te doen?
We willen eerst weten of u geschikt bent om mee te doen. Daarom stelt de onderzoeker een aantal vragen:

- Bent u van plan om tijdens de dagen dat er gemeten wordt alcohol te nuttigen?

Stap 2: onderzoeken en metingen
Voordat het meten begint vragen wij u naar een aantal gegevens:

- Uw naam

<u>Metingen tijdens dagelijkse activiteiten</u>
Tijdens dit deel van het onderzoek worden metingen gedaan, terwijl u uw normale, dagelijkse activiteiten uitvoert. Deze metingen zullen plaatsvinden op u normale werkplek op de Universiteit Twente. Tijdens dit onderzoek kunt u dan ook gewoon uw werk uitvoeren, ontspannen of eten. Wij vragen u op bepaalde momenten de PuppyNose-sensor te dragen en er op bepaalde momenten kleine handelingen mee uit te voeren. Het kan ook zijn dat wij u vragen twee sensoren te dragen, op elke arm één. Dit deel van het onderzoek zal onder het toezicht van de onderzoekers gebeuren en zij zullen op het juiste moment uitleggen wat u met de sensor moet doen.

Dit zal er als volgt uitzien:

1. Wij geven u een PuppyNose-sensor en uitleg hoe deze te gebruiken.

2. U zet de sensor aan. De onderzoekers verbinden nu de sensor via een Bluetooth-verbinding met een computer.
3. U start een meting, wacht tot de sensor is geijkt en doet hem dan om.
4. Voor de komende drie uur vragen wij van u om de sensor op de volgende manier te gebruiken:
   0-45 min: U draagt de sensor.
   45 min - 1u 15 min: U doet de sensor af en laat hem ergens anders rusten. Het is belangrijk dat u niet op de start-stopknop drukt en de meting gewoon laat doorlopen.
   1u 15min – 2u: U doet de sensor weer om en draagt hem.
   2 – 3u: Tijdens het laatste uur vragen wij u elke 20 minuten een handeling uit te voeren met de sensor (30s de sensor heen en weer te schuiven op de arm, 30s de sensor van de huid optillen en er mee bewegen en 30s met de arm schudden waarop de sensor zit).
5. Na in totaal drie uur mag u de sensor afdoen en is het onderzoek afgelopen.

Stap 2: Na het meten met de sensor
Nadat u klaar bent met de metingen, vragen wij u een vragenlijst in te vullen. Deze vragenlijst is gericht op hoe voor u de gebruikservaring was met de sensor en of deze comfortabel was voor u om te dragen. De vragenlijst zal maximaal tien minuten kosten om in te vullen.

Stap 3: Interview met de onderzoeker
Het kan zijn dat de onderzoeker u nog een aantal vragen wil stellen naar aanleiding van de vragenlijst. Dit zal dan gaan over uw ervaringen met de sensor en of er dingen zijn die u veranderd zou willen hebben aan de sensor. Het interview zal afgenomen worden via een online videogesprek. De informatie van het interview kan gebruikt worden om de sensor te verbeteren in de toekomst. De onderzoeker zal via de e-mail contact met u opnemen. Mocht u hier geen interesse in hebben dan kunt u dat aangeven op het toestemmingsformulier in bijlage B.

5. Welke afspraken maken we met u?

We willen graag dat het onderzoek goed verloopt. Daarom maken we de volgende afspraken met u:

- U neemt contact op met de onderzoeker als u niet meer wilt meedoen met het onderzoek.

6. Van welke bijwerkingen, nadelige effecten of ongemakken kunt u last krijgen?

Wat zijn de mogelijke ongemakken van metingen tijdens het onderzoek?

- Bij het wat langer dragen, kan de huid onder het kastje van de sensor wat warmer of zweteriger worden. .

7. Wat zijn de voordelen en de nadelen als u meedoet aan het onderzoek?

Meedoen aan het onderzoek kan voordelen en nadelen hebben. Hieronder zetten we ze op een rij.

Meedoen aan het onderzoek kan deze nadelen hebben:

- Metingen doen met de sensor kost u extra tijd.

8. Wanneer stopt het onderzoek?

De onderzoeker laat het u weten als er nieuwe informatie over het onderzoek komt die belangrijk voor u is. De onderzoeker vraagt u daarna of u blijft meedoen.

In deze situaties stopt voor u het onderzoek:

- Alle onderzoeken volgens het schema zijn voorbij.
- U wilt zelf stoppen met het onderzoek. Dat mag op ieder moment. Meld dit dan meteen bij de onderzoeker. U hoeft er niet bij te vertellen waarom u stopt.

Wat gebeurt er als u stopt met het onderzoek?
De onderzoekers gebruiken de gegevens die tot het moment van stoppen zijn verzameld.

9. Wat gebeurt er na het onderzoek?

Krijgt u de resultaten van het onderzoek?
Ongeveer twee maanden nadat het onderzoek is afgerond laat de onderzoeker u weten wat de belangrijkste uitkomsten zijn van het onderzoek.

10. Wat doen we met uw gegevens?

Doet u mee met het onderzoek? Dan geeft u ook toestemming om uw gegevens te verzamelen, gebruiken en bewaren.

Welke gegevens bewaren we?
We bewaren deze gegevens
- uw naam
- gegevens die we tijdens het onderzoek verzamelen

Waarom verzamelen, gebruiken en bewaren we uw gegevens?
We verzamelen, gebruiken en bewaren uw gegevens om de vragen van dit onderzoek te kunnen beantwoorden. En om de resultaten te kunnen publiceren.

Hoe beschermen we uw privacy?

Om uw privacy te beschermen geven wij uw gegevens een code. Op al uw zetten we alleen deze code. De sleutel van de code bewaren we op een beveiligde plek op de Universiteit Twente. Als we uw gegevens verwerken, gebruiken we steeds alleen die code. Ook in rapporten en publicaties over het onderzoek kan niemand terughalen dat het over u ging.

Wie kunnen uw gegevens zien?

Sommige personen kunnen wel uw naam en andere persoonlijke gegevens zonder code inzien. Dit kunnen gegevens zijn die speciaal voor dit onderzoek zijn verzameld, maar ook gegevens uit uw medisch dossier. Dit zijn mensen die controleren of de onderzoekers het onderzoek goed en betrouwbaar uitvoeren. Deze personen kunnen bij uw gegevens komen:

- Leden van de commissie die de veiligheid van het onderzoek in de gaten houdt.
- Een controleur die voor de Universiteit Twente werkt.
- Nationale en internationale toezichthoudende autoriteiten.

Deze personen houden uw gegevens geheim. Voor inzage door deze personen vragen wij u toestemming te geven. De Inspectie Gezondheidszorg en Jeugd kan zonder uw toestemming uw gegevens inzien.

Hoelang bewaren we uw gegevens en lichaamsmateriaal?

We bewaren uw gegevens 10 jaar bij de Universiteit Twente. De gegevens worden 10 jaar bewaard om in de loop van dit project nog nieuwe bepalingen te kunnen doen die te maken hebben met dit onderzoek.

Mogen we uw gegevens gebruiken voor ander onderzoek?

Uw verzamelde gegevens kunnen ook van belang zijn voor ander wetenschappelijk onderzoek op het gebied van het meten van ketonen met dit type sensor. Daarvoor zullen uw gegevens 10 jaar worden bewaard bij de Universiteit Twente. In het toestemmingformulier geeft u aan of u dit goed vindt. Geeft u geen toestemming? Dan kunt u nog steeds meedoen met dit onderzoek.

Kunt u uw toestemming voor het gebruik van uw gegevens weer intrekken?

U kunt uw toestemming voor het gebruik van uw gegevens op ieder moment intrekken. Zeg dat dan tegen de onderzoeker. Dit geldt voor het gebruik in dit onderzoek en voor het gebruik in ander onderzoek. Maar let op: trekt u uw toestemming in, en hebben onderzoekers dan al gegevens verzameld voor een onderzoek? Dan mogen zij deze gegevens nog wel gebruiken.

Wilt u meer weten over uw privacy?

- Wilt u meer weten over uw rechten bij de verwerking van persoonsgegevens? Kijk dan op www.autoriteitpersoonsgegevens.nl.
- Heeft u vragen over uw rechten? Of heeft u een klacht over de verwerking van uw persoonsgegevens? Neem dan contact op met degene die verantwoordelijk is voor de verwerking van uw persoonsgegevens. Voor uw onderzoek is dat:
    - Universiteit Twente. Zie bijlage A voor contactgegevens, en website.

- Als u klachten heeft over de verwerking van uw persoonsgegevens, raden we u aan om deze eerst te bespreken met het onderzoeksteam. U kunt ook naar de Functionaris Gegevensbescherming van de Universiteit Twente gaan (zie de contactgegevens in bijlage A).

11. Krijgt u een vergoeding als u meedoet aan het onderzoek?

U wordt niet betaald voor het meedoen aan dit onderzoek

12. Bent u verzekerd tijdens dit onderzoek?

U bent niet extra verzekerd voor dit onderzoek. Want meedoen aan het onderzoek heeft geen extra risico's. Daarom hoeft de UT van de Centrale Commissie Mensgebonden Onderzoek geen extra verzekering af te sluiten.

13. Heeft u vragen?

Vragen over het onderzoek kunt u stellen aan de onderzoekers van de Universiteit Twente. In bijlage A vindt u de contactgegevens. Wilt u dit liever niet? Ga dan naar de klachtenfunctionaris van de Universiteit Twente, C. D. Lammertink. In bijlage A staat waar u die kunt vinden.

14. Hoe geeft u toestemming voor het onderzoek?

U kunt eerst rustig nadenken over dit onderzoek. Daarna vertelt u de onderzoeker of u de informatie begrijpt en of u wel of niet wilt meedoen. Wilt u meedoen? Dan vult u het toestemmingsformulier in dat u bij deze informatiebrief vindt. U en de onderzoeker krijgen allebei een getekende versie van deze toestemmingsverklaring.

Dank voor uw tijd.

15. Bijlagen bij deze informatie

A. Contactgegevens Universiteit Twente
B. Toestemmingsformulier

Bijlage A: contactgegevens voor Universiteit Twente

Onderzoeksteam Universiteit Twente

• Onderzoeker: D. M. Bos BSc., d.m.bos@student.utwente.nl

• Hoofdonderzoeker: dr. ir. B. J. F. van Beijnum, b.j.f.vanbeijnum@utwente.nl

Klachtenbemiddeling UT (voor afhandeling klachten)

U kunt op een klacht indienen door te mailen naar C. D. Lammertink, te bereiken via c.d.lammertink@utwente.nl

Meer informatie hierover vindt u op de website van de Universiteit Twente.

Functionaris gegevensbescherming Universiteit Twente

M. Davids

E-mailadres: dpo@utwente.nl

Functionaris klachten Universiteit Twente

C. D. Lammertink

E-mailadres: c.d.lammertink@utwente.nl

A.5 USER MANUAL

Gebruikshandleiding van de PuppyNose-sensor

Algemene beschrijving

De PuppyNose-sensor is ontwikkeld om op een niet-pijnlijk manier en zonder het gebruik van naalden, ketonen te kunnen meten op de huid. De PuppyNose is weergegeven in afbeelding 1. De PuppyNose bestaat uit een kastje dat met een band vastgemaakt kan worden op de huid. Aan de onderkant van het kastje zit de sensor die de ketonen meet. Naast ketonen, meet deze sensor ook de luchtvochtigheid op uw huid en de huidtemperatuur. De computer in de PuppyNose gebruikt deze metingen om de bepaling van de ketonen nauwkeuriger te kunnen bepalen. Aan de bovenkant van de PuppyNose zit een scherm, waarop de waarden zichtbaar worden, en twee knoppen: een aan-knop en een start-stopknop. De PuppyNose werkt op een accu en de gebruiker zijn normale dagelijkse activiteiten doen, terwijl het apparaat gebruikt wordt. De PuppyNose heeft een batterijduur van ongeveer 4 tot 6 uur. Aan de zijkant van de PuppyNose is een USB-C ingang, waarmee deze opgeladen kan worden (zie afbeelding 2). De waardes die gemeten worden op het scherm weergegeven, maar kunnen ook via Bluetooth naar een computer verzonden worden.

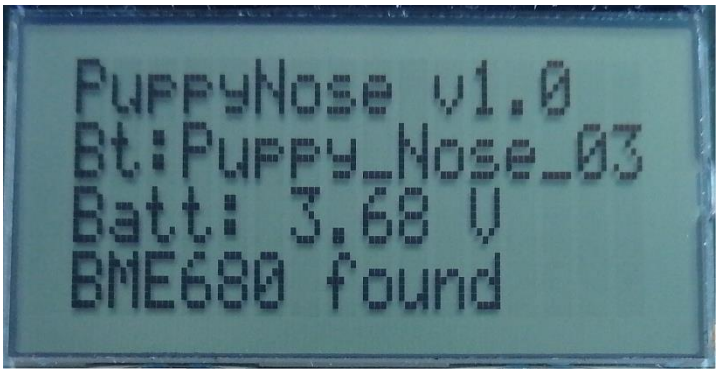
Afbeelding 1 - De PuppyNose-sensor

Afbeelding 2 - De verschillende knoppen op de sensor.

Hoe moet u de PuppyNose gebruiken?

Hier volgt een korte beschrijving in chronologische volgorde over hoe de PuppyNose gebruikt moet worden. Het is belangrijk om het apparaat nog niet om te doen, voordat u het apparaat opgestart heeft en een meting succesvol heeft gestart.

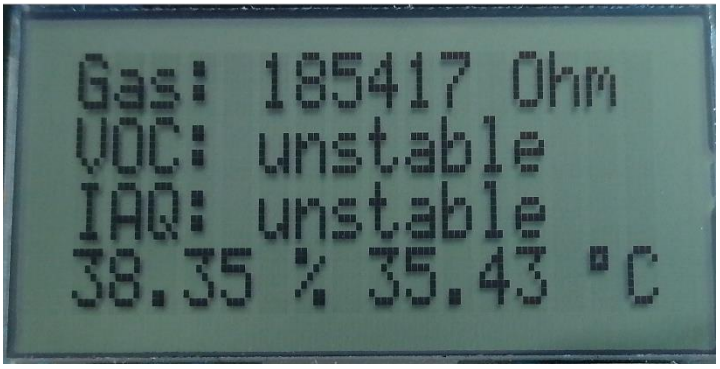
De PuppyNose aanzetten
Als u de PuppyNose wilt gebruiken, drukt u eerst op de aan-knop. Er verschijnt nu een startscherm (zie afbeelding 3) met de status van de batterij en algemene informatie over de sensor. Als u dit scherm krijgt, weet u dat het apparaat aanstaat, maar dat nog geen meting is gestart. Op het startscherm is voornamelijk de derde regel belangrijk, waarop de batterijstatus is aangegeven (zie afbeelding 3).



Afbeelding 3 - Het startscherm, nadat u op de aan-knop heeft gedrukt.

De batterijstatus varieert tussen 4.2 en 3.4 Volt. Als de batterij lager dan 3.6V, geeft het scherm een waarschuwing af met ‘battery low’. Omdat de PuppyNose volledig opgeladen is aan het begin van dit onderzoek, zal dit niet voorkomen.

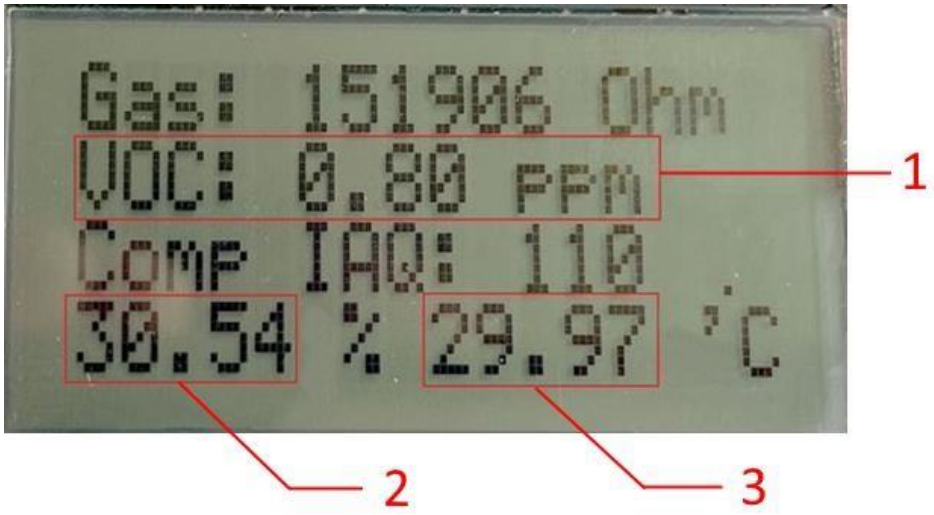
Een meting starten
Vanuit het eerste startscherm kunt u een meting starten. Als u dit wilt doen drukt u op startstopknop. Er verschijnt nu scherm zoals op afbeelding 4. Meteen na het drukken op de startstopknop verschijnt er achter VOC op de tweede regel de waarde ‘unstable’. Dit betekent dat de PuppyNose zichzelf aan het ijken is aan de omgeving en nog geen betrouwbare waarden kan weergeven. Als dit scherm wordt getoond, kan de PuppyNose ook met Bluetooth worden verbonden met de computer. Dit zal u niet zelf hoeven te doen, maar zal de onderzoeker doen. Het is belangrijk om de PuppyNose nog niet om te doen en in de omgevingslucht te laten totdat ‘unstable’ verdwijnt. Anders wordt het ijkingsproces verstoort. Na ongeveer vijf minuten, verdwijnt dit en zal er normale waardes weergegeven worden (zie afbeelding 5). U kunt nu de PuppyNose omdoen.



Afbeelding 4 - Het scherm nadat u op de start-stopknop heeft gedrukt. Na enkele minuten verdwijnt 'unstable'.

De PuppyNose omdoen

De PuppyNose is ontworpen om op de bovenarm gedragen te worden. Het is het handigst om de PuppyNose op uw niet-dominante arm te plaatsen. Maak deze vast met de band om uw arm. Hierbij moeten de knoppen op het apparaat naar uw borst toewijzen. Op deze manier kunt u zelf de waarden lezen die op het scherm verschijnen. Als u de band vastmaakt, zorg ervoor dat het kastje niet kan bewegen, maar dat de band niet te strak zit.



- *Afbeelding 5 - Het scherm nadat 'unstable' is verdwenen. 1 - Hoeveelheid ketonen op de huid. 2 - Luchtvochtigheid op de huid. 3 - Temperatuur op de huid*

Uitleg van de waarden op het scherm

Zodra een meting is begonnen, verschijnen er waarden zoals op afbeelding 5. Voor dit onderzoek zijn alleen de tweede en de vierde regel belangrijk. Deze waarden hebben de volgende betekenis: • VOC – deze waarde geeft aan hoeveel ketonen er op de huid gemeten worden (zie het rood omlijnde gedeelte aangeduid met een rode '1' in afbeelding 5).

- … % – dit percentage is de luchtvochtigheid op uw huid
  (zie het rood omlijnde gedeelte aangeduid met een rode '2' in afbeelding 5).

- … ^{o}C – dit is de temperatuur op uw huid
  (zie het rood omlijnde gedeelte aangeduid met een rode '3' in afbeelding 5).

U hoeft verder de waarden niet op te schrijven, omdat dit automatisch via de computer zal gebeuren.

De meting stopzetten

Wanneer u wilt stoppen met de meting, drukt u nogmaals op de start-stopknop. De metingen worden gestopt en het apparaat schakelt zich automatisch uit na 5 minuten. Zodra u op de stopknop heeft gedrukt, kunt u de PuppyNose-sensor afdoen.

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