Measurement of breath acetone in patients referred for an oral glucose tolerance test

To cite this article before publication: Brian Terence Andrews *et al* 2018 *J. Breath Res.* in press https://doi.org/10.1088/1752-7163/aabd88

Measurement of breath acetone in patients referred for an oral glucose tolerance test.

BTE Andrews[3], W Denzer[1,5], G Hancock[1], AD Lunn[2], R Peverall[1‡], GAD Ritchie[1], K Williams[4]

[1] Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, Oxford, OX1 3QZ, UK

[2] Department of Statistics, University of Oxford, OX1 2JD, UK

[3] Department of Vascular Surgery, Medway Maritime Hospital, Windmill Rd, Gillingham, ME7 5NY, UK

[4] Department of Pathology, Medway Maritime Hospital, Windmill Rd, Gillingham, ME7 5NY, UK

[5] Current address: Wolfden Scientific Consulting, Calle Rio Segura 26, Archena, Murcia 30600, Spain

[‡] corresponding author, email: Robert.Peverall@chem.ox.ac.uk

Abstract

Breath acetone concentrations were measured in 141 subjects (aged 19 – 91 yrs, mean = 59.11 yrs standard deviation = 12.99 yrs), male and female, undergoing an oral glucose tolerance test (OGTT), having been referred to clinic on suspicion of type 2 diabetes. Breath samples were measured using an ion-molecule-reaction mass spectrometer, at the commencement of the OGTT, and after 1 and 2 hrs. Subjects were asked to observe the normal routine before and during the OGTT, which includes an overnight fast and ingestion of 75 g glucose at the beginning of the routine. Several groups of diagnosis were identified: type 2 Diabetes Mellitus positive (T2DM), *n* = 22; impaired glucose intolerance (IGT), *n* = 33; impaired fasting glucose (IFG), *n* = 14; and reactive hypoglycaemia (RHG), *n* = 5. The subjects with no diagnosis (i.e. normoglycaemia) were used as a control group, *n* = 67. Distributions of breath acetone are presented for the different groups. There was no evidence of a direct relationship between blood glucose and acetone measurements at any time during the study (0hr: p = 0.4482; 1hr: p = 0.6854; and 2hr: p = 0.1858). Nor were there significant differences between the measurements of breath acetone for the control group and the T2DM group (0hr: p = 0.1759; 1hr: p = 0.4521; and 2hr: p = 0.7343). However, the ratio of breath acetone at 1hr to the initial breath acetone was found to be significantly different for the T2DM group compared to both the control and IGT groups (*p* = 0.0189 and 0.011, respectively). The T2DM group was also found to be different in terms of ratio of breath acetone after 1hr to that at 2hrs during the OGTT. And was distinctive in that it showed a significant dependence upon the level of blood glucose at 2hrs (p = 0.0146). We conclude that single measurements of the concentrations of breath acetone cannot be used as a potential screening diagnostic for T2DM diabetes in this cohort, but monitoring the evolution of breath acetone could open a non-invasive window to aid in the diagnosis of metabolic conditions.

Introduction

It is known that there are strong associations with breath acetone and the glycaemic state, a fact that has led many researchers to suggest that breath acetone can be used to help gauge or even directly indicate blood glucose levels in sufferers of diabetes, or at least act as a screening diagnostic for both type 1 and type 2 diabetes (see [1] and references therein). Here we seek to build on the

scientific consensus by reporting on a trial measuring breath acetone in a relatively large cohort of undiagnosed subjects referred to a clinic for an OGTT (oral glucose tolerance test) to test for type 2 diabetes.

The human body can directly digest fat, resulting in the production of triacylglycerol which in turn can be hydrolysed to produce fatty acids. Triacylglycerol is also stored in adipose tissue, and released at a later stage, being directly converted into free fatty acids by hormone sensitive lipase. Fatty acids can diffuse into cells and undergo β-oxidation to produce acetyl coenzyme A (acetyl CoA), which enters the Krebs cycle. In the liver, some of the acetyl CoA is always diverted for ketogenesis; this produces acetoacetate and β-hydroxybutyrate both of which can be used as energy substrates, the latter by enzymatic conversion back into acetoacetate and the eventual generation of acetyl CoA. The acetone that is present in blood, and therefore breath, is a direct result of the decarboxylation of acetoacetate and given the reported very long lifetime of acetoacetate against spontaneous decarboxylation [2], the decarboxylation is likely to occur through the action of acetoacetate decarboxylase, although the presence of this has only been shown thus far in rat tissues and human plasma (see e.g. [3,4,5]). β-hydroxybutyrate and acetoacetate exist in the blood as the anions of the fully dissociated β-hydroxybutyric (pK_a = 4.7) and acetoacetic (pK_a = 3.58) acids, and together with acetone are collectively known as the '*ketone bodies*'. When the body requires more energy than is available from normal dietary sources, its own fat stores can be utilised which increases the amount of ketogenesis. Such ketogenesis can also be enhanced by shifting the balance of diet away from carbohydrates as in a ketogenic diet [6]. It has been hypothesised that the increased production of ketone bodies in times of starvation acts as an important source of fuel for the brain (as well as other vital organs), as most energy substrates other than glucose cannot pass the blood-brain barrier [7]. In addition, in type 1 diabetes it is well known that the concentration of ketone bodies can increase as a response to a lack of insulin which can result in ketoacidosis, and which usually occurs with a concomitant increase in blood glucose. A review of normal and diabetic physiology with respect to ketone bodies can be read in reference [7].

Since acetone is intrinsically related to metabolism, and can be readily measured in breath (see e.g. [8-14]), thereby constituting a possible non-invasive marker, it is a logical progression to hypothesise that breath acetone measurements could form part or all of a diagnostic or screening tool for metabolic conditions. This work seeks to determine if this is the case in previously undiagnosed sufferers of type 2 diabetes.

Methods

The OGTT and breath tests were carried out at the Medway Maritime Hospital, Medway & Kent NHS Trust, UK. Ethical permission was sought and granted from the national research ethics committee (SE coast, UK, application 10/H1101/86). Patients were referred to the hospital by their GP because of a high blood glucose level found during a health check or a combination of other potential indicators of type 2 diabetes including: family history of Type 2 diabetes; over 40 years of age and overweight (*BMI* > 27); sedentary lifestyle; and high waist circumference. No selection has been made based on gender or ethnicity. Prior to these tests, T2DM-positive patients were previously undiagnosed and were receiving no treatment for T2DM. People with severe respiratory disease (COAD/COPD), those unable to give informed consent and those under 16 years old were excluded.

Symptoms of type 2 diabetes usually include a combination of insufficient insulin secretion and insulin resistance, which can lead to difficulties in controlling blood glucose. The purpose of the

OGTT is to test the body's response to a glucose stimulus (75 g) following a 10 hr fasting period, usually overnight. If there is impaired control of blood glucose, the blood glucose levels will rise. The WHO classifies subjects undergoing OGTT according to the following responses during a 2 hour OGTT (table 1) [15]:

Diagnosis	**Normal**		**Impaired fasting glycaemia (IFG)**		**Impaired glucose tolerance (IGT)**		**Type 2 Diabetes Mellitus (T2DM)**	
Glucose levels (*mmol/l*)	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs	Fasting	2hrs
	<6.1 **AND**	<7.8	≥ 6.1 <7.0 **AND**	<7.8	<7.0 **AND**	≥7.8	≥7.0 **OR**	≥11.1

Table 1. Recommendations for diagnosis of diabetes and intermediate hyperglycaemia from the World Health Organisation guidelines.

A summary of the patient characteristics is given in table 2; all but 5 of the 141 participants declared their ethnicity to be white British. The OGTT will classify patients as being normal, impaired fasting glycaemia (IFG), impaired glucose tolerance (IGT), or diabetic (T2DM). A further classification of reactive hypoglycaemia (RHG) was included for 5 participants, where their blood glucose fell during the OGTT to < ~3 mmol/l. Prior to the test the patients were asked to maintain an adequate and normal diet (carbohydrate intake > 150g/day) for a minimum of three days and fast overnight for a minimum of 10 hours (only water is permitted). For the duration of the test (approx. 2.5 hours) the patients were asked to remain seated (or as inactive as possible) and refrain from smoking, eating or drinking.

Gender	*N*	Mean age (yr)	Age min (yr)	Age max (yr)	Age sd (yr)	Mean girth (cm)	Girth sd (cm)	Mean BMI (kg/m^2)	BMI sd (kg/m^2)
All	141	59.1	19	91	13.0	105	13.9	31.3	6.4
F	66	59.4	26	91	14.3	102	14.5	31.7	6.7
M	75	58.9	19	78	11.8	108	12.6	30.9	6.1

Table 2. Cross-section of the patients' details; sd denotes standard deviation.

At the beginning of the test a blood sample and a triplicate breath sample were taken to determine the baseline levels of blood glucose (*BG0*) and breath acetone (*AC0*). Subsequently, 394ml of a glucose drink (equivalent to 75g glucose) was consumed and triplicate breath samples taken every hour throughout the duration of the OGTT (i.e. at time = 0 hr, 1 hr and 2 hr; *AC0*, *AC60* and *AC120*), and blood glucose at 0 and 2 hr (*BG0* and *BG120*).

For this study we employed an Ion-Molecule Reaction Mass Spectrometer (IMR-MS, V&F Analysen and Messtechnik GmbH, Austria) capable of chemically ionising analytes using several reactant ions, Hg^+, Xe^+, Kr^+, or by electron impact. The instrument is configured to sample from atmospheric pressure via a heated capillary, and directly shows data as a function of time during exhalation. In this study, the mass analyser sits on mass channels for CO_2 (m/z = 44) and acetone (m/z = 58), using electron impact to ionise the former, and Hg^+ the latter. In order to provide an adequate breath sample, the patients exhaled through a straw into the analyser until a 4.7% CO_2 level was reached to ensure that end-tidal breath is sampled. In all cases the breath acetone was recorded as the average of three separate measurements. At regular intervals the mass spectrometer was calibrated for CO_2

and acetone using bottled gas mixtures of 1 ppmv acetone in air and 5 % CO_2 in air (BOC special gases, UK); methods that have been well tried and tested [13,16]. Periodically, clinic air was sampled and tested, but showed no significant levels of acetone. The repeatability of *AC* measurements taken with the methods used in this study was assessed and found to be acceptable, with any variance due to measurement and method likely to be small: for example, repeated *AC* measurements from the same healthy subject taken over 20 minutes (number of measurements = 17) yielded a standard deviation of 15 ppb for a mean *AC* of 825 ppb, which is typical for the performance of this method. Clearly, with all measurements of the response variables being averages of an identical number of measurements (3 replicates), there is no requirement to apply variance weighting factors.

Statistical analyses were carried out using the R statistical package [17]; in particular functions from the Modern Applied Statistics (MASS) and linear and non-linear mixed effects (nlme) libraries were used. Regression models were fitted to suitably transformed response variables and model residuals were checked for normality and homoscedasticity. Restricted maximum likelihood (REML) methods were used throughout as a precaution against under-estimation of variance parameters.

The analyses were aimed at answering three specific questions.

(i) Does breath acetone (*AC*) depend in any way upon blood glucose (*BG*), classification (i.e. *Type* or the diagnosis), *age*, *sex* and body mass index (*BMI*)? In particular, is there any relationship between *AC* and *BG*?

(ii) During OGTT does breath acetone depend in any way upon *BG*, *Type*, *Age*, *Sex* and *BMI*?

(iii) Can measurement of breath acetone during OGTT distinguish between *Type*?

There is a causative element here in the sense that the investigation is to determine whether breath acetone measurements respond to levels of the other variables. Therefore regression models were fitted in which *AC* (or rather suitable functions of *AC*) were the responses and all of the other variables were treated as explanatory.

Results and statistical analysis

The outcomes for blood glucose and breath acetone are summarised in table 3. Distributions for breath acetone separated into classification are presented in figure 1 at time 0 (pane A) and time = 1 hr (pane B) during the OGTT.

Classification *Type*	*n*	*sex*		group mean						
		f	m	*BMI* (kg/m^2)	*age* (yr)	*BG0* (mmol/l)	*BG120* (mmol/l)	*AC0* ppb	*AC60* ppb	*AC120* ppb
Normal	67	37	30	31.2	59	5.4	5.65	951	838	773
IFG	14	5	9	27.6	64	6.4	5.75	711	623	588
IGT	33	14	19	32.8	58	6.1	8.9	859	739	695
RHG	5	1	4	26.6	48	5	2.4	877	722	615
T2DM	22	9	13	32.5	59	6.5	11.85	887	852	752

Table 3. Blood glucose and breath acetone results from the OGTT and division of test results into sub-groups. *BG* = blood glucose, *BMI* = body mass index, *AC* = breath acetone.

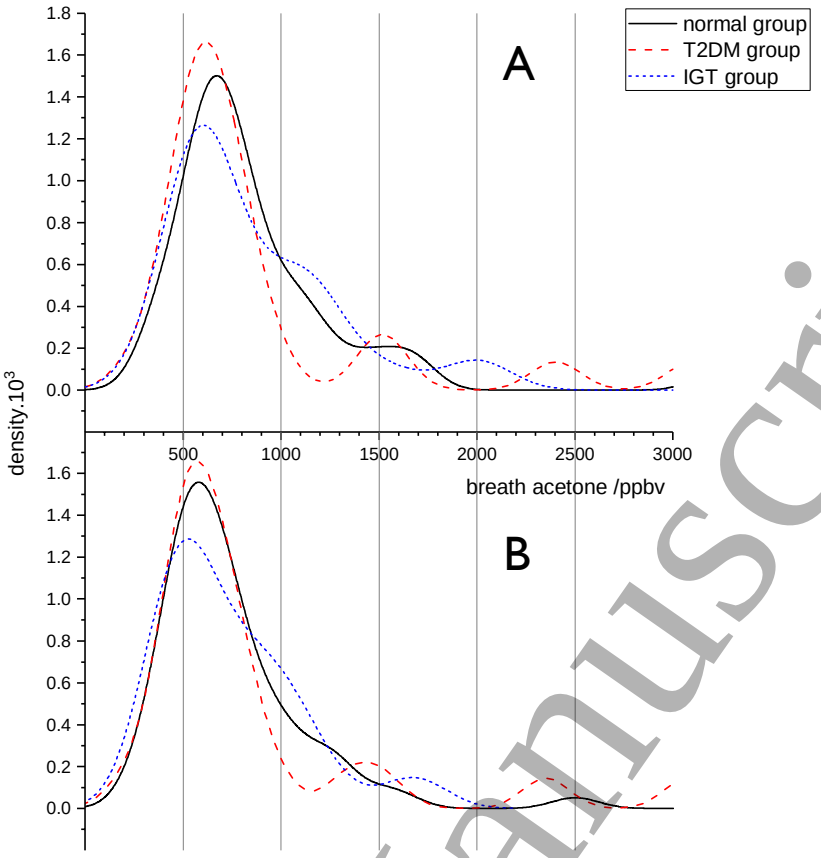

Figure 1. Density plots for the group distributions of breath acetone for the normal (black solid), T2DM (red dashed), and IGT (blue dotted) groups. Pane A at time = 0; pane B at time = 60 minutes during the OGTT.

Exploratory analyses of the proposed explanatory variables revealed that both *BMI* and *BG* were right skewed and needed to be transformed to remove excessive leverage from the tails; thus $\sqrt{BMI}$ and log(*BG*) were used throughout. *AC* turned out to be non-normal and heteroscedastic, therefore requiring suitable transformations before valid models could be fitted. When *AC* at different time points was to be the response variable, it was found that fitting linear models to $1/\sqrt{AC}$ resulted in normally distributed, homoscedastic model residuals.

When investigating changes in *AC* during *OGTT*, ratios of *AC* at time 0 with *AC* at the two subsequent measuring times were found to require log transformations. Only the ratio *AC*60/*AC*120, where *AC*60 and *AC*120 are measurements taken after 1 hour and 2 hours respectively, was found to require no transformation, although variance weighting for the different diagnostic groups had to be included.

Normality of residuals was checked using the Shapiro-Wilk test of normality (*p*-values for all final fitted models lay in the range $0.193 < p < 0.784$); homoscedasticity was checked by plotting model residuals against fitted values. The possibility of different diagnosis groups exhibiting different variance was checked by fitting variance unweighted and weighted models with the generalised least squares (*gls)* function from the *nlme* library and comparing them via likelihood-ratio tests. Significant differences in the variance across *Type* were only found for the ratio *AC60*/*AC120*. (For example, weighting for different variances between the *Types* in *Outcomes iii* below resulted in a better model fit, the likelihood-ratio test (against a model without weighting) resulting in p = 0.0337.

Similar weighting did not produce a significant difference in the model fit in *Outcomes ii(a)* (p = 0.59).)

No potential outliers were detected. Significant dependence of the ratio AC60/AC120 upon blood glucose level measured at the 2hr point was found for the T2DM group; there was no evidence of dependence on blood glucose measured at earlier times or in other groups or with other responses.

Outcomes

(i) Does breath acetone depend in any way upon blood glucose, glycaemia classification (Type), Age, Sex and body mass index (BMI)? In particular, is there any relationship between breath acetone and blood glucose?

Little evidence of dependence of baseline breath acetone on *Type*, *Age*, *Sex*, *BMI* was found when measured prior to *OGTT*, with only *Sex* having any effect (p = 0.0063); a likelihood-ratio test of models with and without the other variables resulted in p = 0.5765. The result of this was therefore, at baseline level, females were found to have 83% of the amount of the males' breath acetone.

There was no evidence of a relationship between breath acetone and blood glucose (p = 0.4901) at baseline level, or of *Type* (p = 0.4875).

(ii) During OGTT, does breath acetone depend in any way upon blood glucose, Type, Age, Sex and BMI?

(a) Measurements at 60 minutes

Linear models were fitted to log(*AC0*/*AC60*). *Sex* and baseline blood glucose were not found to be significant predictors (p = 0.5237), but there was weak evidence that *Age* and *BMI* adjustments should be retained (p = 0.1010). For *Type* T2DM the ratio *AC60*/*AC0* was found to be higher than that for Controls by a factor of 8.1% (p = 0.0189) and 9.9% higher than *Type* IGT (p = 0.0110). No other significant differences were found.

(b) Measurements at 120 minutes

Linear models were fitted to log(*AC0*/*AC120*). The only significant predictor was *Age* (p = 0.0223), which perhaps is not surprising. The variables *Type*, *Sex*, *BMI*, *BG*0 made no significant contribution (p = 0.2101); inclusion of *BG*120 did not improve the model (p = 0.2957).

(iii) Can measurement of breath acetone during OGTT distinguish between diagnoses (Type)?

It seems reasonable to surmise that the glucose meal won't have an immediate effect. With this in mind a model was fitted to the ratio *AC60*/*AC120*. As expected, there was significant age dependence (p = 0.0063, consistent with (ii)b) but this model also produced a significant interaction of *Type* with *BG120*, entirely due to the rate of change of the ratio being significantly different for *Type* T2DM. This rate of change was not significantly different from zero for all other categories of *Type*, but for the T2DM group there was a steady rise (p = 0.0140). The relative relationships are best illustrated by figure 2, which has been plotted over the restricted range of *BG120* values measured for T2DM subjects. The quality of the relationship between the age-adjusted *AC60*/*AC120* acetone ratio and *BG120* for *Type* T2DM can be judged from a graph of fitted values from the model against measured values, as shown in figure 3.

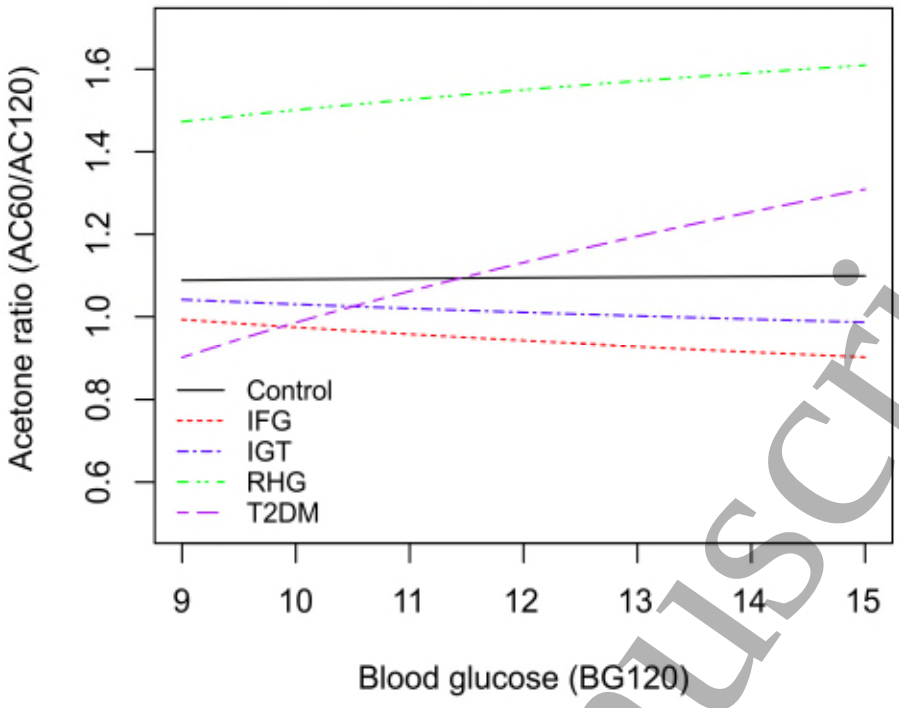

Figure 2. The model relationship between the breath acetone ratio (*AC60*/*AC120*) and blood glucose level after 2 hrs (*BG120*) for different categories showing the significantly different behaviour for the T2DM group.

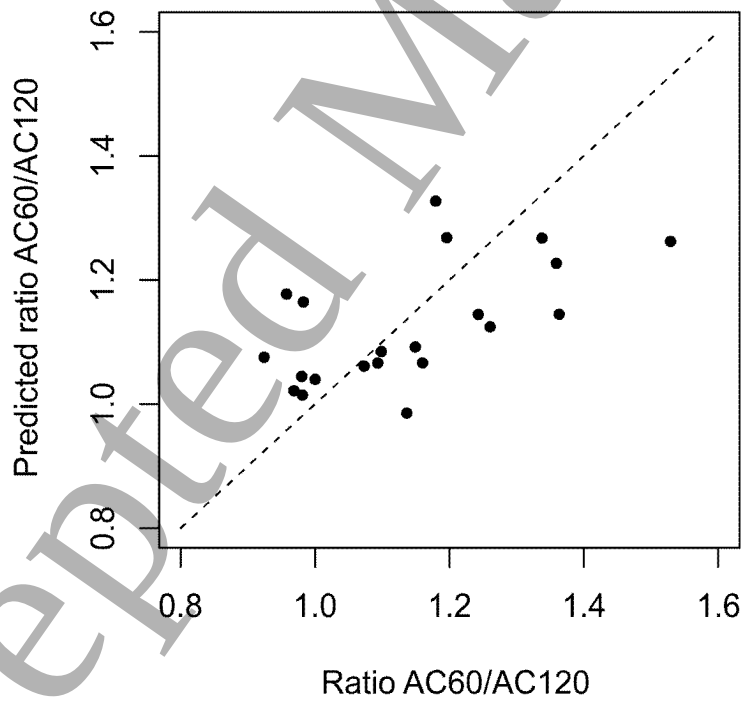

Figure 3. The breath acetone ratio predicted by the model compared to the measured ratio for the T2DM group (RMS error = 0.142).

Discussion and conclusions

In some other studies it has been found that sufferers of type 2 diabetes generally have higher breath acetone levels than controls, but this is not so in the present study. Indeed there is a range of different relationships published in the literature, some have reported breath acetone from sufferers of T2DM to be greater on average than controls [14,18-23], some not [24-26]. Others have reported gender differences and relationships between blood glucose and breath acetone [23]. Here, we observe no such relationships for absolute breath acetone with blood glucose. And thus conclude that in our cohorts, breath acetone cannot be used as a screen for T2DM from a single measurement. It is not totally transparent as to the cause of these differences in reported relationships with breath acetone, but usually, nevertheless, differences can be found in the cohort selection procedures, and so we can speculate that the different outcomes are caused by different aspects of control or different demographic in the cohorts, a small number of study participants, or that the subjects in the studies are already being treated for T2DM, or some combination of these. In studies that have been performed during an OGTT e.g. [22], the number of participants is limited (a total of 16 in [20], with only 5 T2DM). In this study it is possible that there is some pre-selection because all of the subjects have been referred on suspicion of T2DM. We note that for the groups defined here, there is evidence of a difference in *BMI* when compared with the national average: the median for the control group is above the threshold for clinical obesity (*BMI* = 30 kg/m^2), but there is no evidence of difference in *Sex*-proportion from the national average (Fisher's Exact Test; p = 0.35). As expected the age distribution is skewed towards old-age (median = 61 yrs). However there is no evidence of a difference between the experimental groups in *Sex* and *Age* and only weak evidence that *BMI* is different from the control group (median = 30.93 kg/m^2) for the IFG and RHG groups (medians 27.52 kg/m^2 (p = 0.048), and 26.45 kg/m^2 (p = 0.096), respectively). We stress that despite these differences in *BMI*, we have found that *BMI* is not a significant predictor in any of the statistical models fitted here. Nevertheless, it would have been interesting to take another non-referred control group undergoing an OGTT and a group already diagnosed with T2DM, to make further comparisons.

The breath acetone behaviour in the different cohorts identified in this study can be rationalised by considering the likely response of the metabolism to the OGTT with respect to the endocrinology and the likely differences between T2DM and normal subjects. In T2DM patients, generally insulin resistance and insulin deficiency go hand in hand, and unsurprisingly it is this which ultimately controls the metabolism effects with regards to breath acetone production. Plasma insulin response during an OGTT is shown in reference [27,28]. Markedly, for subjects where fasting plasma glucose is above approximately 8.3 mmol/l, the insulin response is a fraction of that for normal subjects. In addition, after two hours of an OGTT, the plasma insulin of normal subjects is already falling, while that of T2DM patients is still potentially increasing.

Insulin, glucagon and adrenaline mediate the release of triacylglycerol from adipose tissue via the action of hormone sensitive lipase to form free fatty acids. During periods of relative starvation and low circulating insulin, the release of fatty acids from adipose tissue is enhanced and more acetyl CoA becomes available for ketogenesis. (Glucagon plays an important role in the mediation of gluconeogenesis by increasing the synthesis of several important enzymes which favour the process. Incidentally, however, glucagon also restricts glycolysis, promoting glucose preservation.) Glucagon is released by the pancreas, as is insulin, and it is important to note that insulin acts as an inhibitor for glucagon release, possibly acting via an 'intra-islet' mechanism [29,30]. During an OGTT, for

normal subjects, we would expect the increase in insulin to put a restriction on lipolysis and the release of glucagon, both of which should lead ultimately to a reduction in breath acetone. For the T2DM group, after one hour of the OGTT, clearly these influences are suboptimal. It is perhaps surprising that the different breath acetone response of the T2DM group isn't distinct on a more individual level, and we suspect that this is a reflection of the spectrum within the IGT and T2DM groups of the different levels of insulin resistance (IGT group) through to insulin resistance and insulin deficiency (T2DM group, potentially approaching insulin dependent T2DM). IGT is considered a precursor to T2DM, but the circulating insulin can be even higher than normal [27,28]. It seems, therefore that perhaps because of the variations in insulin deficiency and resistance in the T2DM and 'pre-diabetes' IGT groups, in the context of an OGTT, or referral to an OGTT, breath acetone alone is an impractical indicator for the purpose of any sort of individual diagnosis or screening for T2DM. However, because of the relationship between breath acetone and metabolism there could well be merit in trend measurements for individuals over a much longer timescale.

Acknowledgements

We would like to thank the staff and nurses of the phlebotomy unit of the Medway Hospital for their work and support during this study.

1 Wang Z and Wang C, J. Breath Res. (2013), 7, art.037109
2 Wolfenden R, Lewis Jr CA., Yuan Y, J. Am. Chem. Soc. (2011), 133, p5683
3 Koorevaar G, Van Stekelenburg GJ, Clin. Chim. Acta (1976), 71(2) p173
4 Kalapos MP, Biochimica et Biophysica Acta (2003), 1621, p122
5 Ruzsanyi V, Kalapos MP, J. Breath Res. (2017),11(2), art.024002
6 Musa-veloso K, Likhodii SS and Cunnane SC, Am. J. Clin. Nutr. (2002), 76, p65
7 Laffel L, Diabetes Metab. Res. Rev. (1999), 15(6), p412
8 Diskin AM, Spanel P, Smith D, Physiol. Measure. (2003), 24(1), p107
9 King J, Kupferthaler A, Unterkofler K, Koc H, Teschl S, Teschl G, Miekisch W, Schubert J, Hinterhuber H, Amann A, J. Breath Res. (2009), 3(2), art.027006
10 Sun MX, Chen ZY, Gong ZY, Zhao XM, Jiang CY, Yuan Y, Wang ZN, Li YX, Wang CJ, Anal. and Bionanal. Chem. (2015), 407(6), p1641
11 Ciaffoni L, Hancock G, Harrison JJ, van Helden JPH, Langley CE, Peverall R, Ritchie GAD, Wood S, Anal. Chem. (2013), 85(2), p846
12 Blaikie TPJ, Couper J, Hancock G, Hurst PL, Peverall R, Richmond G, Ritchie GAD, Taylor D, Valentine K, Anal. Chem. (2016), 88(22), p11016
13 Hancock G, Langley CE, Peverall R, Ritchie GAD, Taylor D, Anal. Chem. (2014), 86(12), p5838
14 Jiang C, Sun M, Wang Z, Chen Z, Zhao X, Yuan Y, Li Y, Wang C, Sensors (2016), 16, p1199
15 'Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia' WHO (2006) ISBN 92 4 159493 4
16 Blaikie TPJ, Edge JA, Hancock G, Lunn D, Megson C, Peverall R, Richmond G, Ritchie GAD, Taylor D, J. Breath Res. (2014), 8(4), art.046010
17 R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0
18 Greiter MB, Keck L, Siegmund T, Hoeschen C, Oeh U, Paretzke HG, Diabetes Technol. Ther. (2010), 12,p455
19 Deng C, Zhang J, Yu X, Zhang W and Zhang X, J. Chromatogr. B (2004), 810(2), p269
20 Wang C, Mbi A and Shepherd M, IEEE Sensors (2010), 10, p54
21 Li WW, Liu Y, Lu XY, Huang YP, Liu Y, Cheng SQ, Duan YX, J. Breath Res. (2015), 9(1), art.016005
22 Ghimenti S, Tabucchi S, Lomonaco T, Di Francesco F, Fuoco R, Onor M, Lenzi S,Trivella MG, J. Breath Res. (2013), 7, art.017115
23 Chen ZY, Sun MX, Gong ZY, Yuan Y, Zhao XM, Liu WC, Jiang CY, Wang ZN, Li XY, Prog. In Biochem. And Biophys. (2015), 42(5), p483
24 Ueta I, Saito Y, Hosoe M, Okamoto M, Ohkita H, Shirai S, Tamura H and Jinno K, J. Chromatogr. B (2009), 877, p2551

25 Storer M, Dummer J, Lunt H, Scotter J, McCartin F, Cook J, Swanney M, Kendall D, Logan F and Epton M, J. Breath Res. (2011), 5, art.046011
26 Spanel P, Dryahina K and Smith D, J. Breath Res. (2007), 1, art.011001
27 Reaven GM, Bernstein R, Davis B, Olefsky JM, The Am. J. Med. (1976), 60, p80
28 Taylor SI, Accili D, Imai Y, Diabetes (1994), 43, p735
29 Xu E, Kumar M, Zhang Y, Ju W, Obata T, Zhang N, Liu SY, Wendt A, Deng SP, Ebina Y, Wheeler MB, Braun M, Wang QH, Cell Metab. (2006), 3(1), p47
30 Wang QH, Liang XY, Wang S, Front. in Physiol. (2013), 3, art. UNSP 485