

TITLE: Exhaled Breath Isoprene rises during Hypoglycemia in Type 1 Diabetes

RUNNING TITLE: Breath Isoprene and Hypoglycemia

AUTHORS:

Sankalpa Neupane MB BS¹

Robert Peverall PhD^{2,3}

Graham Richmond PhD²

Tom PJ Blaikie MChem²

David Taylor MChem²

Gus Hancock PhD^{2,3}

Mark L. Evans MD¹

1. Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, IMS-MRL, Box 289 Addenbrookes Hospital, Cambridge, UK

2. Oxford Medical Diagnostics Ltd, Centre for Innovation and Enterprise

Begbroke Science Park, Begbroke Hill, Begbroke OX5 1PF UK

3. Department of Chemistry, Physical and Theoretical Chemistry Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QZ UK

Correspondance to

Dr Mark Evans

University of Cambridge,

Wellcome Trust-MRC Institute of Metabolic Science

IMS Metabolic Research Laboratories,

Box 289 Addenbrookes Hospital,

Cambridge, UK CB2 0QQ

Mle24@cam.ac.uk

Tel +44 (0)1223 336994

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Hypoglycemia is a major fear for many with type 1 diabetes (T1D), limiting ability to lower glycemia. Given anecdotal reports of domestic pets alerting owners of blood glucose changes, especially hypoglycemia (1), we hypothesized that volatile organic compounds (VOCs) in exhaled breath might change at low glucose.

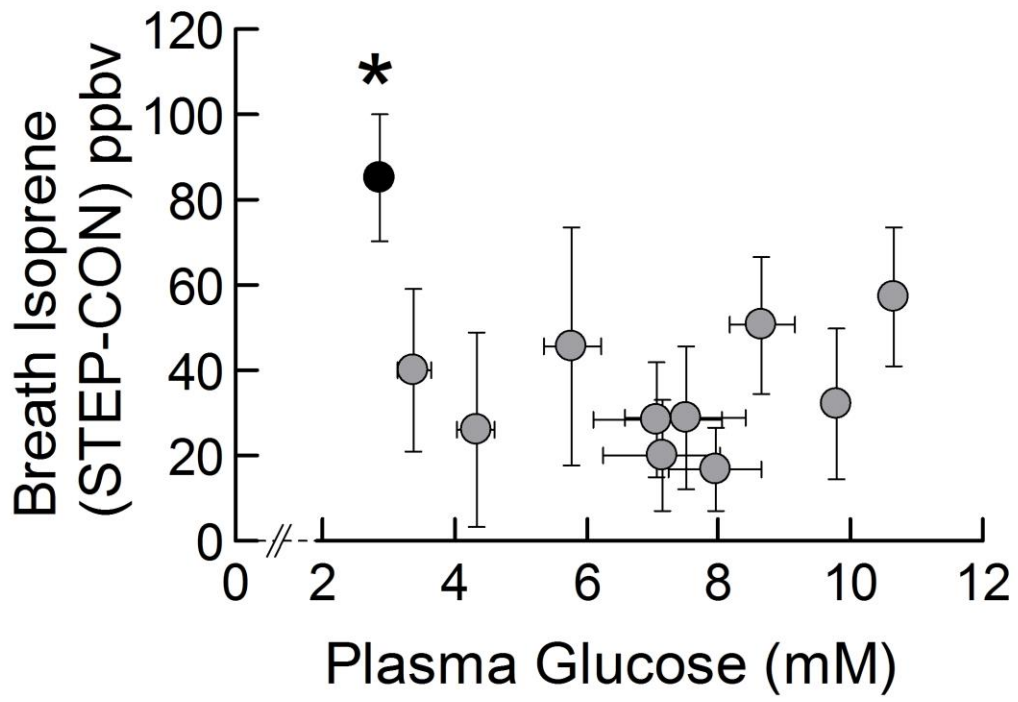
We studied 8 female non-smoking participants with type 1 diabetes (T1D, age 46 ± 5 years, diabetes duration 23 ± 7 years, none treated with statins) twice using a single blinded, computer-code randomized cross over design. An independent research ethics committee approved studies in advance and subjects provided written consent. Using a stepped insulin clamp (actrapid, NovoNordisk, Crawley UK; 0.3 mU/kg/min increasing to 1.5 mU/kg/min), on one occasion, arterialized plasma glucose (Yellow Springs Instrument 2300 STAT Plus™ Analyzer) was raised sequentially (7.1 ± 0.8 , 8.7 ± 0.4 , $10.7 \pm 0.1 \text{ mmol/l}$) then lowered with higher insulin infusion to 4.3 ± 0.3 and $2.8 \pm 0.1 \text{ mmol/l}$. On control days (CON), procedures were identical, except that plasma glucose was maintained at $6.2 \pm 0.1 \text{ mmol/l}$ (Figure 1A).

For breath collection, subjects held their breath for 3 seconds, partially exhaled then breathed into a 1.1 litre breath bag (Fischer Analysen Instrumente GmbH). VOCs were measured by soft-ionisation mass spectrometry (V&F Airsense Compact Ion-Molecule-Reaction Mass Spectrometer) by a researcher blinded to clamp glucose values (2). VOC values were adjusted to 5% exhaled CO_2 . To look specifically for a biomarker of low blood glucose, we compared VOC values (2-sample t test; SPSS Statistics 21) during hypoglycemia (2.8 mmol/l step) with values from non-hypoglycemia. We also examined the correlation between plasma glucose and VOCs (STEP – CON values) across the range of experimental glucose values (Spearman correlation). Data are presented as mean \pm SEM.

Plasma insulin (Diasorin Liaison XL chemiluminescence immunoassay) was similar on study days (275 ± 109 vs 268 ± 95 and 1001 ± 194 vs 978 ± 171 pmol/l; STEP vs EU 120 and 220 min respectively). Strikingly, exhaled breath isoprene rose significantly at hypoglycemia (220 minute values) compared to non-hypoglycemia (Figure 1). Outside hypoglycemia, there was no correlation between exhaled isoprene and plasma glucose across the broader range of experimental plasma glucose values and no significant associations with other measured VOCs (acetone, methyl nitrate, ethanol, ethyl benzene and propane).

It is unclear how hypoglycemia could increase isoprene. Despite being one of the commonest VOCs in human breath, the source of endogenous isoprene remains undetermined. At least in part, isoprene may be a by-product of cholesterol biosynthesis (3). Although glucose can alter fatty acid formation via carbohydrate response element binding protein (CHREBP), this has not been described for cholesterol biosynthesis. (4). Alternatively/ additionally, during hypoglycemia, tachycardia and increased blood flow could increase pulmonary delivery of isoprene. Against this, we saw no changes in other VOCs. Of note, a previous study using insulin clamps in T1D reported that clusters of VOCs rather than an individual VOC correlated with plasma glucose, although hypoglycemia was not examined (5).

In summary, our data suggest that breath VOCs such as isoprene offer a non-invasive alternative for monitoring changes in blood glucose in diabetes, including detection of hypoglycemia.



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AUTHOR CONTRIBUTIONS

SN and ME designed studies and performed insulin clamps. RP, GR, TB, DT and GH analysed breath samples. All authors interpreted data, contributed to, reviewed and approved manuscript. ME is the guarantor of the manuscript.

PRIOR PRESENTATION

An abstract containing the data was presented at Diabetes UK Professional Conference in 2015.

DUALITY OF INTEREST

RP, GR, TB, DT are employees and GH a director of Oxford Medical Diagnostics (OMD); a developer and supplier of breath analysis technology. Studies were part funded by OMD. In addition, ME has also received speaker's/-writer's fees and/or served on advisory Boards for Abbott Diabetes Care, Medtronic and Roche (manufacturers of glucose-sensing technology).

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FIGURE LEGEND

Exhaled breath isoprene during studies. * $p < 0.01$ compared to non-hypoglycemia