



# Exhaled Breath Isoprene Rises During Hypoglycemia in Type 1 Diabetes

Diabetes Care 2016;39:e97–e98 | DOI: 10.2337/dc16-0461

Sankalpa Neupane,<sup>1</sup>  
Robert Peverall,<sup>2,3</sup>  
Graham Richmond,<sup>2</sup> Tom P.J. Blaikie,<sup>2</sup>  
David Taylor,<sup>2</sup> Gus Hancock,<sup>2,3</sup> and  
Mark L. Evans<sup>1</sup>

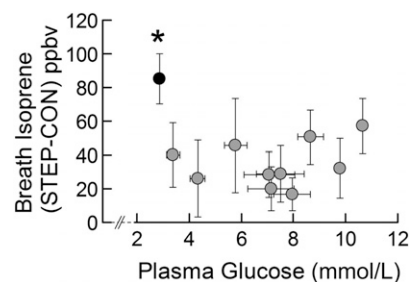
Hypoglycemia and/or fear of hypoglycemia are major challenges for many with type 1 diabetes (T1D), limiting ability to lower glycemia. Given anecdotal reports of domestic pets alerting owners to blood glucose changes, especially hypoglycemia (1), we hypothesized that volatile organic compounds (VOCs)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

We studied eight female nonsmoking participants with T1D (aged  $46 \pm 5$  years, diabetes duration  $23 \pm 7$  years, none treated with statins) twice using a single-blinded, computer code–randomized crossover design. An independent research ethics committee approved studies in advance, and subjects provided written consent. Using a stepped insulin clamp (Actrapid; Novo Nordisk, Crawley, U.K.; 0.3 mU/kg/min increasing to 1.5 mU/kg/min), on one occasion (STEP), arterialized plasma glucose (Yellow Springs Instrument 2300 STAT Plus Analyzer) was raised sequentially ( $7.1 \pm 0.8$ ,  $8.7 \pm 0.4$ , and  $10.7 \pm 0.1$  mmol/L) then lowered with higher insulin infusion to  $4.3 \pm 0.3$  and  $2.8 \pm 0.1$  mmol/L. On control days (CON), procedures were identical except that plasma glucose was maintained at  $6.2 \pm 0.1$  mmol/L (Fig. 1).

For breath collection, subjects held their breath for 3 s, partially exhaled, and then breathed into a 1.1-L breath bag

(Fischer Analysen Instrumente GmbH). VOCs were measured by soft-ionization 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<sub>2</sub>. To look specifically for a biomarker of low blood glucose, we compared VOC values (two-sample Student *t* test; SPSS Statistics 21) during hypoglycemia (2.8 mmol/L STEP) with values from nonhypoglycemia. 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 ( $275 \pm 109$  vs.  $268 \pm 95$  pmol/L at 120 min and  $1,001 \pm 194$  vs.  $978 \pm 171$  pmol/L at 220 min; STEP vs. CON). Strikingly, exhaled breath isoprene rose significantly at hypoglycemia (220-min values) compared with nonhypoglycemia (Fig. 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).



**Figure 1**—Exhaled breath isoprene during studies. \* $P < 0.01$  compared with nonhypoglycemia.

It is unclear how hypoglycemia could increase isoprene. Despite being one of the most common 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, 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

<sup>1</sup>Wellcome Trust–MRC Institute of Metabolic Science, University of Cambridge, Institute of Metabolic Science–Metabolic Research Laboratories, Addenbrookes Hospital, Cambridge, U.K.

<sup>2</sup>Oxford Medical Diagnostics, Centre for Innovation & Enterprise, Begbroke Science Park, Begbroke, U.K.

<sup>3</sup>Physical & Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, Oxford, U.K.

Corresponding author: Mark L. Evans, mle24@cam.ac.uk.

Received 2 March 2016 and accepted 16 April 2016.

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glucose, although hypoglycemia was not examined (5).

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

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**Acknowledgments.** Hormonal assays were performed by Keith Burling and colleagues in the National Institute of Health Research Cambridge Biomedical Research Centre Core Biochemical Assay Laboratory. Clamp studies were performed in the Cambridge National Institute of Health Research Wellcome Trust Clinical Research Facility.

**Funding.** This work was supported by the National Institute of Health Research Cambridge Biomedical Research Centre, including salary support for S.N.

**Duality of Interest.** R.P., G.R., T.P.J.B., and D.T. are employees and G.H. is a director of Oxford

Medical Diagnostics, a developer and supplier of breath analysis technology. Studies were funded in part by Oxford Medical Diagnostics. In addition, M.L.E. has received speaker/writer fees and/or served on advisory boards for Abbott Diabetes Care, Medtronic, and Roche (manufacturers of glucose-sensing technology). No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** S.N. and M.L.E. designed the studies and performed insulin clamps. R.P., G.R., T.P.J.B., D.T., and G.H. analyzed breath samples. All authors interpreted data and contributed to, reviewed, and approved the manuscript. M.L.E. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Prior Presentation.** An abstract containing the data was presented at the Diabetes UK Professional Conference 2015, London, U.K., 11–13 March 2015.

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