Exhaled Breath Isoprene Rises During Hypoglycemia in Type 1 Diabetes

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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 ± 5 years, diabetes duration 23 ± 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 ± 0.8, 8.7 ± 0.4, and 10.7 ± 0.1 mmol/L) then lowered with higher insulin infusion to 4.3 ± 0.3 and 2.8 ± 0.1 mmol/L. On control days (CON), procedures were identical except that plasma glucose was maintained at 6.2 ± 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_2. 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 ± SEM.

Plasma insulin (DiaSorin LIAISON XL chemiluminescence immunoassay) was similar on study days (275 ± 109 vs. 268 ± 95 pmol/L at 120 min and 1,001 ± 194 vs. 978 ± 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).

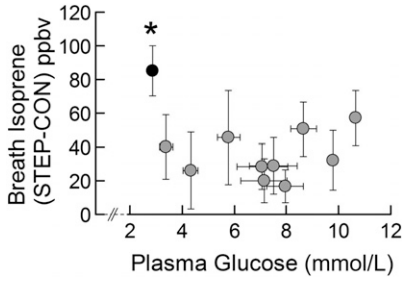

Figure 1—Exhaled breath isoprene during studies. *$P < 0.01$ compared with nonhypoglycemia.

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

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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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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.

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