Breath Ketone Measurement

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Oxford AHSN Diabetes Clinical Network Annual Meeting
Friday, 24th April 2015
• Founded in 2010
  • Spin-out from Department of Chemistry, University of Oxford
  • Based on Begbroke Science Park, Oxford

• Develop optical techniques for non-invasive measurement of clinically relevant Volatile Organic Compounds (VOCs)
  • Measure concentration of certain molecules in exhaled breath as indicators of disease and physiology
  • More affordable approach than conventional analytical equipment e.g. mass spectrometry

• Exhaled breath acetone (BA) – first application
  • Correlates with circulating blood ketone (BK) levels
  • Early detection of diabetic ketoacidosis
  • Potential tool for identifying individuals with undiagnosed diabetes
Diabetic Ketoacidosis (DKA)

- Potentially fatal complication of diabetes
  - Caused by insulin deficiency
  - Mortality rates of 2-10%

- In absence of insulin:
  - Body cannot use glucose as energy source so burns fats to compensate – leads to ketosis
  - Excess ketone bodies cause metabolic acidosis
  - 3 principal ketone bodies: β-OHB, AcAc and acetone
  - Low insulin leads to elevated glucagon to produce glucose from glycogen – rapid onset of DKA

- £17.5m per annum in associated acute admissions costs in NHS in England
  - 16,158 admissions in England in 2012/13
  - Excludes expenditure on ambulances, A&E etc.
  - Present in ~25% diagnoses of type 1 diabetes (~35% for children <5 years)
Current Methods for Ketone Testing

• BK testing advised every 4-6 hours on sick days or when blood glucose >16.7 mmol/L

• Urine testing
  • Cheap (£0.06 per test)
  • Urine ketones lag levels in blood by ~2-3 hours
  • Varies with hydration levels and interfering molecules

• BK with electrochemical strips
  • Expensive (£2.06 per test strip)
  • Direct measure of BK levels
  • Influenced by blood pH

• Lifetime of test strips only 6-9 months – high wastage

<table>
<thead>
<tr>
<th></th>
<th>Mild DKA</th>
<th>Moderate DKA</th>
<th>Severe DKA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Glucose (mmol/L)</td>
<td>&gt;13.9</td>
<td>&gt;13.9</td>
<td>&gt;13.9</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.25 - 7.30</td>
<td>7.00 - 7.24</td>
<td>&lt;7.00</td>
</tr>
<tr>
<td>Sodium Bicarbonate (mEq/L)</td>
<td>15 - 18</td>
<td>10 - 14</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Urine Ketones</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Serum Ketones</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Serum Osmolality (mOsm/kg)</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Anion Gap</td>
<td>&gt;10</td>
<td>&gt;12</td>
<td>&gt;12</td>
</tr>
<tr>
<td>Mental Status</td>
<td>Alert</td>
<td>Drowsy</td>
<td>Stupor/Coma</td>
</tr>
</tbody>
</table>

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Breath Testing Alternative for BK

- Acetone (a BK) is highly volatile
  - Produced through spontaneous and enzymatic decarboxylation of AcAc
  - Readily diffuses out of blood into air in the lungs
  - Exhaled BA provides a reliable indication of circulating BK levels

- Instantaneous measurement
  - No lag time between exhaled BA and levels in blood
  - Effectively a large “blood” sample

- Less prone to interference than existing methods

<table>
<thead>
<tr>
<th>Blood β-hydroxybutyrate (mmol/l)</th>
<th>Exhaled Breath Acetone (ppmv)</th>
<th>Action required</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.6</td>
<td>&lt;3.0</td>
<td>Normal – recheck every 1-2 hours if blood glucose &gt;13.9 mmol/l</td>
</tr>
<tr>
<td>0.6 - 1.5</td>
<td>3.0 - 7.0</td>
<td>Insulin required – follow sick day rules, checking ketone and glucose levels every 1-2 hours</td>
</tr>
<tr>
<td>1.5 - 3.0</td>
<td>&gt;7.0</td>
<td>Risk of DKA – seek urgent medical attention</td>
</tr>
</tbody>
</table>
Technology: Cavity Enhanced Absorption Spectroscopy

- All molecules absorb light at characteristic frequencies
- Spectroscopy exploits this effect to identify molecules and measure them quantitatively
- CEAS amplifies the absorption by enclosing the sample between two high-reflectivity mirrors
- Preconcentration method used to treat breath sample prior to measurement – increases sensitivity in small device
- Sub-ppm measurements of acetone readily achieved using this approach
CEAS vs. Mass Spectrometry

- Mass spectrometry (MS) is the gold-standard for breath analysis
- 1:1 correlation between OMD’s CEAS technology and MS
- Robust technology benefits from investment in telecoms sector
- MS costs ~£250k; with high-volume manufacture, CEAS could cost ~£100.

Data comparing OMD’s CEAS technology, obtained using the current prototype device, and a gold-standard mass spectrometric method. All of these data are for breath samples acquired in 1 L breath bags for normal, healthy subjects without diabetes. BA levels were elevated through fasting (up to 36 hours for BA >20 ppm).
Clinical Data

Study Objective

- Conducted at Oxford John Radcliffe Hospital, sponsored by Oxford University
- Establish relationship between breath acetone, blood ketones and blood glucose
- 113 juveniles with type 1 diabetes
- Published in the Journal of Breath Research

Study Protocol

- Blood ketone, blood glucose and breath samples taken during regular clinic visit
- Breath tested for acetone content using mass spectrometry and compared with standard finger prick measurements of ketones and glucose
- OMD sub-contracted to analyse breath samples using mass spectrometry

Blood ketone vs. exhaled breath acetone levels (measured using mass spectrometry) from 113 juveniles with type 1 diabetes under tight glycaemic control at routine clinical appointments. Calibration plot from an inverse regression analysis in which a Gamma Generalised Linear Model was successfully fitted ($\chi^2 = 99.49$). Dashed red lines indicate 95% confidence limits.
Product Development

Dec 2011

- Alpha device
- Bench top instrument
- 45 cm cavity
- Expensive, complex componentry

Current

- Prototype
- iPad Footprint
- Portable
- 7 cm cavity
- Simplified – reduced number of components
- Direct breath sampling demonstrated

Q1 2017

- LiteAir
- Industrial design for mass manufacture
- Disposable items included to aid breath sampling
- Image is of a conceptualised block model

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Summary

- New breath test for the non-invasive measurement of blood ketone levels
- Robust, reliable technology addresses key problems with existing ketone testing methods
- Strong clinical support for BA as an indicator of BK levels
- Currently in product development phase, producing 20 prototypes for clinical evaluation in late 2015
- Device to be suitable for clinical and home-use settings
  - Clinical – rapid identification of DKA and potentially undiagnosed people with diabetes
  - Home use – routine monitoring on sick days and during hyperglycaemic episodes
- Market launch anticipated in 2017
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