Breath biomarkers of insulin resistance in pre-diabetic Hispanic adolescents with obesity

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Background:

- A quarter of the world's population are Insulin Resistance (IR)[1], a metabolic condition associated with cellular imbalance between insulin and blood glucose metabolism.
- On average 312 million adolescence have IR, without diabetics.
- Approximately 70% of individuals with IR develop type-2 diabetes within 15-20 years[2].
- IR is strongly association with cardiometabolic disease[3] and nonalcoholic fatty liver disease even without diabetic[4].
- Previous studies by our group showed exhaled breath VOC signatures discriminated between nonhuman primates with and without cardiometabolic dysfunction[5].
- Despite progress on diabetic management, there is a lack of a non-invasive and point-of-care diagnostic tool to track the development of IR prior to the onset of clinical type-2 diabetes.

Hypothesis:

- Exhaled breath volatiles will correlate and predict cardiometabolic status in humans
  - Identify a small set of VOCs biomarkers that are important for cardiometabolic disease

Method:

- Participants were recruited by Children's Hospital of San Antonio, TX.
- Inclusion requirements: Ages 13-17, BMI% ≥ 95, Hispanic.
- Breath samples were collected using ReCIVA® device and the medical grade air was supplied to remove any background contamination.
- Comprehensive two-dimensional gas chromatography time of flight (GC × GC-TOF) was used for the breath analysis using TD100 ® thermal desorption unit.
- Data was analyzed using the ChromaTOF ® software and statistical test was performed in R statistical program.

Schematic of sample collection

Fig 1. A total 28 Hispanic adolescence (Male = 18 and Female = 18) were consented for breath collection. The blood, breath, medical record, clinical exam were collected during the visit to the Health and Weight Management Clinic at Children's Hospital of San Antonio, TX. Total 4 breath samples were collected from each participant. The serum samples were analyzed for total sugar, lipid, and liver profile. The IR was calculated by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR)[6].

Result:

Breath samples clustered separately from medical air supply

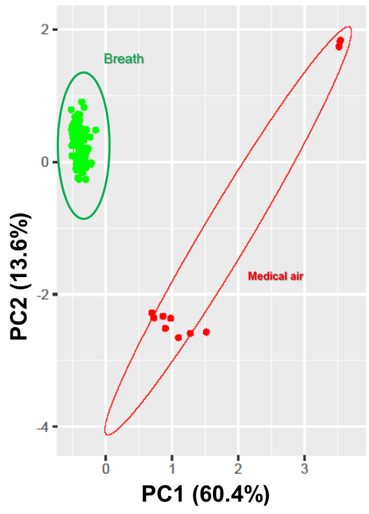

Fig 2. Following the removal of known contaminates and features containing missing data the data processing pipeline was able to remove unwanted variation of breath dataset clustering breath samples from medical air.

Random Forest regression machine learning method selected breath features important for IR measurement

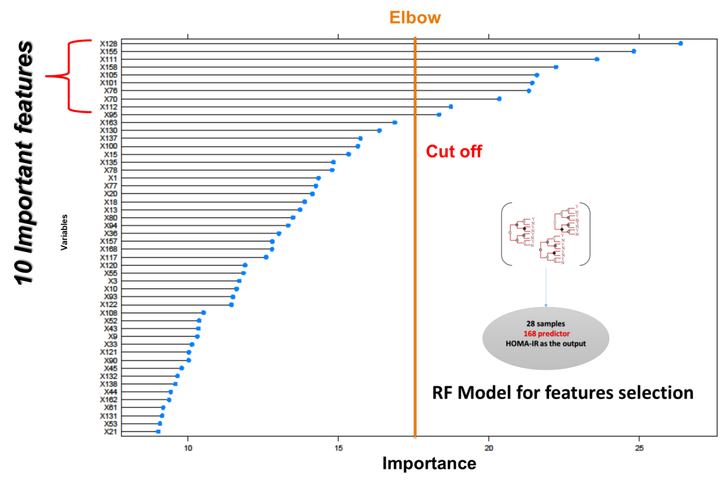

Fig 3. A training model was developed with 2/3 of the study samples with the remaining samples being used for validation. The top 10 features based on importance measures were selected by an elbow cutoff as and used for the prediction of HOMA-IR.

Breath signatures for IR strongly correlates with experimental IR

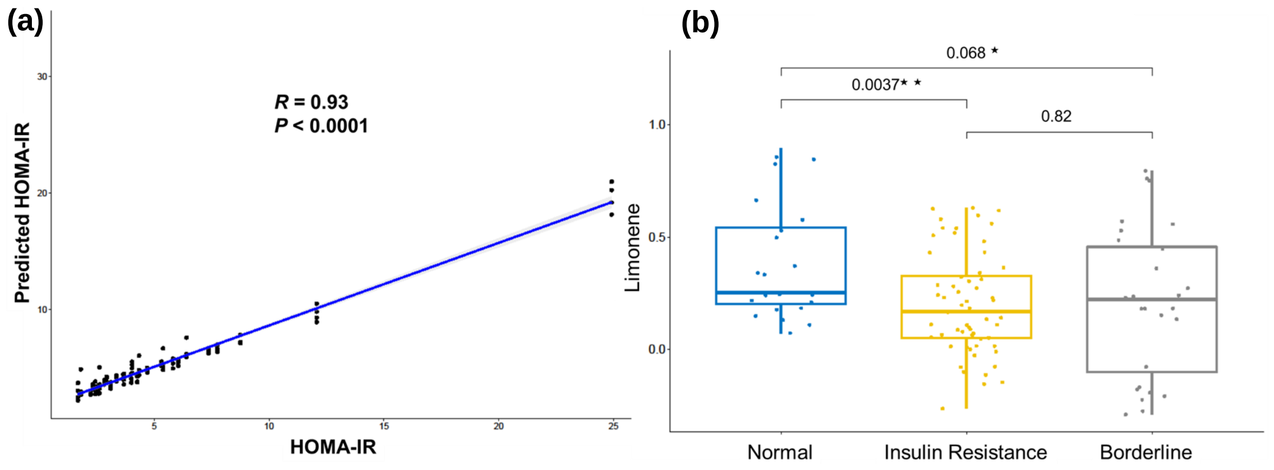

Fig 4. (a) A high correlation was observed with breath model predicted IR and experimental HOMA-IR (R = 0.93, p <0.0001). A strong correlation was also observed between the breath-based IR both Fasting Blood Insulin (mU/L) and Fasting bold glucose (mg/DL) level (R=-0.91, p <0.001 and R=-0.61, p <0.001, respectively). (b) Identified metabolite, limonene clusters individuals with and without insulin resistance, without and borderline insulin resistance significantly (p =0.0037 and p =0.068, respectively). The cut off is based on published result on Mexican Americans: HOMA-IR<2.60 as the "Normal", HOMA-IR 2.60–3.80 as "Borderline" HOMA-IR>3.80 as "Insulin Resistance"[7].

Conclusion and future perspectives:

- This study shows feasibility of a metabolic disease breath signature in adolescents at high risk of developing diabetes.
- Identified breath signature including limonene, previously reported in breath of liver cirrhosis patients[8], shows a strong correlation for identifying individuals with IR
- Development of a IR breath test could impact cardiovascular health by providing a non-invasive point of care tool for monitoring and tracking disease progression
- Future studies will test this breath signature in a larger cohort of at-risk adolescents taking into consideration all ethnicities

References:

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