WTC-LI on curated data. Random Forest (RF) out-of-bag estimated success rates were used to measure classification utility of the refined biomarker profile. Principal components analysis (PCA) was used to visualize class separation produced by the refined profile. RESULTS/ANTICIPATED RESULTS: Of the 765 metabolites detected, 580 metabolites were quantified in more than 80% of subjects/group with relative standard deviation $\geq$15%. Relevant chemokines, cytokines, and clinical biomarkers were included based on previously established clinical importance. Initial PCA explained 34.7% of the variance in the first 3 components. RF was used to identify the top 5% of biomarkers important to class separation. RF of the refined biomarker profile correctly classified cases and controls with a 96.7% estimated success rate. A PCA of the refined metabolic profile now explained 46.2% of the variance in components 1–3, demonstrating improved class separation. Differentiators between cases of WTC-LI and controls included elevated sphingolipids in cases of WTC-LI. The metabolic-inflammatory serum biomarkers MDC, Apo AI, GM-CSF, and heart rate play an important role in class separation. Phospholipids and lysolipids also appeared to differentiate cases of WTC-LI from controls. Specifically, several glycero-phosphatidylcholines (GPC) were elevated in cases of WTC-LI. DISCUSSION/SIGNIFICANCE OF IMPACT: High-dimensional data analysis on the metabolic fingerprints, serum, and clinical biomarker data of a subset of WTC-exposed 9/11 rescue workers has identified pathways associated with the loss of lung function. Sphingolipids, known to function as inflammatory signaling mediators, are thought to play important roles in lung function under both physiological and pathological conditions. Changes in sphingolipid metabolism have been linked to several pulmonary disorders, including asthma, COPD, and acute lung injury. Interestingly, a relation between sphingolipid metabolism and the metabolic-inflammatory pathway is suggested by similarities observed in PCA. Findings of elevated GPCs are similar to COPD literature. Higher levels of GPCs could correspond to elevated levels of lysophosphatidic acid (LPA), a ligand of RAGE. RAGE is a known proinflammatory mediator; LPA species have well-described roles as lipid signaling molecules, function as synthetic intermediates in other metabolic pathways, and were found to be predictive of WTC-LI. Since metabolites are more proximal markers of disease processes, metabolites could capture the complexity of past exposures and, therefore, may better inform treatment. These pathways warrant further investigation into their mechanisms and therapeutic importance.

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Electrical stimulation to the subcallosal cingulate and amygdala drive shifts in affective bias across patient populations

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OBJECTIVES/SPECIFIC AIMS: Deep brain stimulation is currently being evaluated as an experimental therapy for various psychiatric disorders, as well as being investigated as a method for mapping emotional brain functions. This growing area of research requires sensitive measures to quantify effects of stimulation on emotional processing. The current study examined the effects of acute stimulation to 2 limbic regions—the subcallosal cingulate (SCC) and the amygdala—on bias in the perception and evaluation of emotional facial expressions. We hypothesized that transient electrical stimulation to the limbic system would produce acute reductions in negative bias, consistent with its antidepressant effects in patients with severe depression. METHODS/STUDY POPULATION: The current study uses a novel affective bias task, developed to rapidly and covertly quantify emotional state. Over 4–6 minutes, patients rate the intensity and valence of static images of emotional facial expressions. We examined effects of electrical brain stimulation in 2 groups: patients with treatment-refractory depression undergoing SCC DBS therapy, and epilepsy patients undergoing amygdala stimulation via stereo-EEG electrodes during inpatient intracranial monitoring. DBS patients completed the task under stimulation and sham conditions during monthly visits over the first 6 months of therapy, as well as daily during a 1 week, blinded period of DBS discontinuation at the 6-month time point. Epilepsy patients completed the task under stimulation and sham conditions at a single visit. Mixed linear models and paired-samples *t*-test were used to investigate effects of stimulation as well as depression scale scores on affective bias ratings. RESULTS/ANTICIPATED RESULTS: Four SCC DBS patients showed significant effects of stimulation ($p < 0.0001$) and depressive state ($p < 0.0001$) on affective bias scores across 6 months of chronic DBS therapy, where emotional faces were perceived as less sad with stimulation ON, as well as during visits in which patients were nondepressed (typically later in the treatment course). Furthermore, 2 DBS patients showed rapid negative shifts in bias following acute blinded discontinuation of chronic stimulation, an effect which persisted over the 1-week period of discontinuation ($t_{29} = -2.58$, $p = 0.015$), in the absence of any self-reported change in mood. Likewise, 6 epilepsy patients showed significant positive shifts in affective bias with acute amygdala stimulation ($t_5 = -4.75$, $p = 0.005$). Current analyses are investigating electrophysiological, autonomic and facial motor correlates to affective bias in these patients. DISCUSSION/SIGNIFICANCE OF IMPACT: Affective bias has revealed rapid, significant changes with stimulation at 2 limbic targets—one a white matter hub and one a nuclear subcortical structure—suggesting the task's utility as an emotional outcome measure in brain stimulation studies. These stimulation-sensitive measures may provide a new metric to track treatment response to deep brain stimulation therapy for affective disorders. Future studies will determine whether affective bias can predict neuropsychiatric complications in patients undergoing stimulation mapping of brain circuitry ahead of resection surgery for epilepsy.

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Detecting cardiometabolic disease through breath analysis: A metabolomic approach

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OBJECTIVES/SPECIFIC AIMS: The purpose of this study is to use the baboon as a novel animal model for breath research and to identify and characterize baboon breath metabolites that reflect cardiometabolic function to inform us in the development of a noninvasive, cost-effective, and repeatable point-of-care diagnostic breath test. METHODS/STUDY POPULATION: Blood and urine was collected from control and IUGR at the approximate age of 3.5 years. Both groups were then placed on a high fat, high sugar, high salt diet for 7 weeks, after which blood, urine, and breath were collected. The breath samples were then subjected to comprehensive, 2-dimensional gas chromatography coupled with time-of-flight mass spectrometry. Using ChromaTOF software, breath VOCs were identified with at least an 80% spectral match against the National Institute of Standards and Technology (NIST) chemical reference library. The raw data were then statistically analyzed using MetaboAnalyst. We then interrogated multiple online databases to characterize and identify the role of VOCs that were present in both control and IUGR groups. RESULTS/ANTICIPATED RESULTS: Preliminary analyses of the breath VOCs indicate differences in expression between sexes and in control Versus IUGR groups. These results indicate unique "breath signatures." Further analysis of the breath VOCs reveals the presence of metabolites that are involved in β-oxidation and oxidative stress pathways. DISCUSSION/SIGNIFICANCE OF IMPACT: This breath study, a first of its kind, will develop the baboon as a superior animal model for breath biomarker research. Our observed unique "breath signatures" indicate changes in lipid metabolism and oxidative stress pathways, which we hypothesize are the early metabolic changes at the cellular level that are not yet reflected in clinical lab measures. Future directions include analyzing breath VOCs that did not meet 80% spectral match, validation using SPME technology and commercial standards, and initiating a human pilot study in clinically obese, at-risk children in collaboration with physicians at the Children's Hospital of San Antonio to develop a noninvasive, cost-effective, rapid, and repeatable point-of-care diagnostic breath test.

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Protective immunity to live vaccines among children with solid tumors

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OBJECTIVES/SPECIFIC AIMS: Determine whether children with solid tumors maintain intact protective immunity to live vaccines during cancer therapy and after completing cancer therapy (postTx). METHODS/STUDY POPULATION: We will perform a prospective cohort study of children with solid tumors (Hodgkin lymphoma, brain, Wilms, and germ cell tumors) followed at the Puerto Rico's University Pediatric Hospital. Protective immunity will be measured with antibody titers against live vaccines (Measles, Mumps, Rubella, and Varicella) at diagnosis, during cancer therapy, upon completion and

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