

## Plasma Metabolomics in Childhood Obesity using $^1\text{H}$ NMR

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**Introduction:** Over the last two decades, the prevalence of both obesity and type 2 diabetes has reached epidemic proportions, not only in adults, but also in children. Metabolomics, the quantification of small molecules in plasma and tissue fluids, allows for a comprehensive view of the changes in several metabolic and signaling pathways and their interactions. NMR-based metabolomic analysis of plasma samples provides a complimentary non-destructive quantification method that requires minimal sample preparation. Previous studies in adult and adolescent populations, performing plasma metabolomics with liquid chromatography-mass spectrometry, have shown a significant association between changes in specific plasma amino acids and both obesity and insulin resistance [1,2]. Interestingly, while this association was positive in the adult cohort, a negative association was observed in the adolescent cohort, underscoring the need for further characterization of patients at risk for complications. Obesity has been associated with the development of insulin resistance, progression to type 2 diabetes, development of fatty liver, and progression to liver cirrhosis. However, not all obese subjects develop these complications. Therefore, we are still in need for sensitive biomarkers that allow identification of the particularly at risk individuals amenable to early and intensive interventions. Here we correlate clinical markers of obesity-related complications with plasma metabolite levels in obese insulin-sensitive and insulin-resistant youth, using a novel workflow for acquisition and processing of metabolomic  $^1\text{H}$  NMR data.

**Methods:** Plasma metabolomic analyses were performed on a group of 22 young obese subjects, consisting of 11 insulin-sensitive and 11 gender-, age-, and BMI-matched insulin-resistant subjects. Oral glucose tolerance tests (OGTT) were performed and subjects underwent an MRI to determine hepatic fat content and abdominal fat distribution [3]. Plasma samples were 1:1 diluted (250 mM phosphate buffer, 5 mM formate, and 10%  $\text{D}_2\text{O}$ ) and metabolites were measured using  $^1\text{H}$  NMR [4]. Experiments were performed on a Bruker 500 MHz magnet. The MR pulse sequence consisted of an adiabatic double-spin echo with 4 ms magnetic field gradients on all three axes, giving a maximum  $b$ -value of 15 ms/ $\text{um}^2$  at an echo-time TE of 17.28 ms. All samples were measured at 298 K with 96 averages and a TR of 6,000/3,000 ms for low/high  $b$ -value acquisitions. Quantification of the metabolites was done with custom-built LCModel [5] and plasma glucose levels determined during OGTT were used to normalize metabolite levels. Statistical analyses were performed with SPSS. Two-tailed unpaired Student's  $t$  tests were executed to compare differences between groups. Correlations of clinical parameters with plasma metabolite concentrations were calculated using the non-parametric Spearman's rho test.

**Results:** Subject characteristics are presented in Table 1. In the comparison of metabolite concentrations between insulin-sensitive and insulin-resistant obese subjects ( $n=11$  per group), creatinine was significantly higher in insulin-sensitive ( $0.066 \pm 0.02$  mM) than in insulin-resistant ( $0.050 \pm 0.01$  mM) obese subjects ( $p=0.036$ ). A correlation matrix of clinical outcome parameters and plasma metabolite concentrations of the total group of obese subjects ( $n=22$ ) is presented in Figure 1. WBISI, DI, and BMI show correlations with specific plasma metabolites. Among the strongest metabolite-metabolite correlations are the branched-chain amino acids isoleucine, leucine, and valine.

**Table 1. Subject characteristics.**

Obese (n=22)	Insulin-sensitive (n=11)	Insulin-resistant (n=11)	p-value
Gender	n=5 male, n=6 female	n=5 male, n=6 female	1.000
Age (years)	$16.4 \pm 3.0$	$15.9 \pm 3.1$	0.726
BMI ( $\text{kg}/\text{m}^2$ )	$34.4 \pm 3.5$	$35.5 \pm 3.2$	0.443
WBISI	$2.97 \pm 1.05$	$1.13 \pm 0.55$	<0.001
DI	$7.08 \pm 3.08$	$4.65 \pm 1.62$	0.033
HFC (%)	$4.54 \pm 10.86$	$12.36 \pm 13.43$	0.177
VSR	$0.07 \pm 0.04$	$0.09 \pm 0.04$	0.171
LBM (kg)	$57.1 \pm 12.8$	$51.6 \pm 12.7$	0.352

Abbreviations: WBISI: whole body insulin sensitivity index, DI: disposition index; HFC: hepatic fat content; VSR: visceral versus subcutaneous fat ratio; LBM: lean body mass.

**Discussion:** A novel workflow for the acquisition and processing of metabolomic  $^1\text{H}$  NMR data from human blood plasma was used in this study. We observed a significant difference in creatinine concentrations between obese insulin-sensitive and insulin-resistant subjects. In addition, we identified specific blood plasma metabolites that showed correlations with WBISI, DI, and BMI. Correlating individual metabolites with each other, we were also able to show that certain metabolic pathways such as those of the branched-chain amino acids, arginine metabolism, and the urea cycle, were correlated with each other. These results may guide future investigations of underlying pathophysiological mechanisms of obesity and its complications.

This research was supported by K08-DK082618 from NIDDK (RIH), R01-HD028016 from NICHD (SC), and R01-HD40787 from NICHD (SC).

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**Figure 1. Correlation matrix showing color-encoded correlation coefficients. Red colors correspond to positive correlations, blue colors correspond to negative correlations, and green equals zero.**

