Metabolomic Analysis of Type 1 Diabetic Primary T Cells Using Isotopic Ratio Outlier Analysis (IROA) by LC-HRMS

Candice Z. Ulmer,[1] Christopher Beecher,[2] Timothy J. Garrett,[3] Jing Chen,[3] Clayton Matthews,[3] Richard A. Yost[1,3]
[1]Department of Chemistry, University of Florida, Gainesville, FL; [2]IROA Technologies, Ann Arbor, MI
[3]Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, FL

Objectives

- **Objective**: Investigate the metabolomic differences of Type 1 Diabetic clinical T cell samples using phenotypical Isotopic Ratio Outlier Analysis (IROA).
- **Methods**: Jurkat T-human leukemia cells were grown in 95% U-^{13}C IROA media for 2-3 passages. Primary T cells were collected from Type 1 diabetics, 1st degree relatives, and control patients. Equal aliquots of 95% ^{13}C labeled Jurkat cells were spiked into the patient samples using the protein content for normalization. Samples were analyzed using LC-HRMS.
- **Results**: The ratio of the C12/C13 peak intensity was calculated for each sample within all three groups using the ClusterFinder software. Many metabolites such as valine and arginine show a higher percent increase in the T1D samples compared to the control.

Introduction

Type 1 Diabetes (T1D) is an incurable, auto-immune disease that results from the destruction of insulin-producing pancreatic beta cells by pathogenic T lymphocytes. These defective T cells can differentiate into CD4$^+$ T cells that correlate with T1D progression. Of the few experimental designs targeted to identifying the metabolic profile of solely T1D, many incorporate animal models that fail to account for pathophysiological differences in humans. There is a need to better understand the metabolic and lipidomic signature of this disease using human samples. This work employs isotopic labeling LC-HRMS methodologies to identify the metabolic and lipidomic trends of immune dysregulation using primary T cells obtained from T1D patients compared to 1st degree relatives and healthy controls.

Methods

Sample Preparation

- Jurkat cells were grown and labeled on 95% ^{13}C-IROA media. Once confluent, Jurkat cells were pelleted via centrifugation, rinsed 2x with 40 mM ammonium formate, and reconstituted in the ammoniated cell washing buffer.
- Equal aliquots of the 95% ^{13}C-labeled Jurkat cells were spiked into approximately 1x10^6 CD4$^+$ T cells obtained from T1D patients, 1st-degree relatives, and healthy controls.
- The metabolites were isolated using ice cold 80% MeOH (2 mL) and the protein content was used for normalization.
- Samples were dried under nitrogen. The metabolites were reconstituted in 30 µL of 0.1% formic acid in water.

Mass Spectrometry

Instrument	Thermo Scientific Q Exactive
Source	Ion Max Source with HESI II Probe
Polarity	Positive Ion Mode
Scan Range	*m/z* 70-1000
Resolution	70,000 @ *m/z* 200
Capillary Temperature	320 °C
Spray Voltage	3.5 kV
S-lens RF level	30

Liquid Chromatography

Pump System	Thermo Dionex UltiMate 3000 RS
Column Temperature	30 °C

METABOLITES	
Column	ACE Excel 2 C18-PFP column (2.1 x 100 mm, 2.1 µm particle size)
Solvent A	0.1% formic acid in water
Solvent B	acetonitrile
Flow Rate	350 µL/min
Gradient	0-3 min hold at 0% B, a linear ramp to 80% B at 13 min, a hold at 80% B until 16 min
Injection Volume	5 µL

Results and Discussion

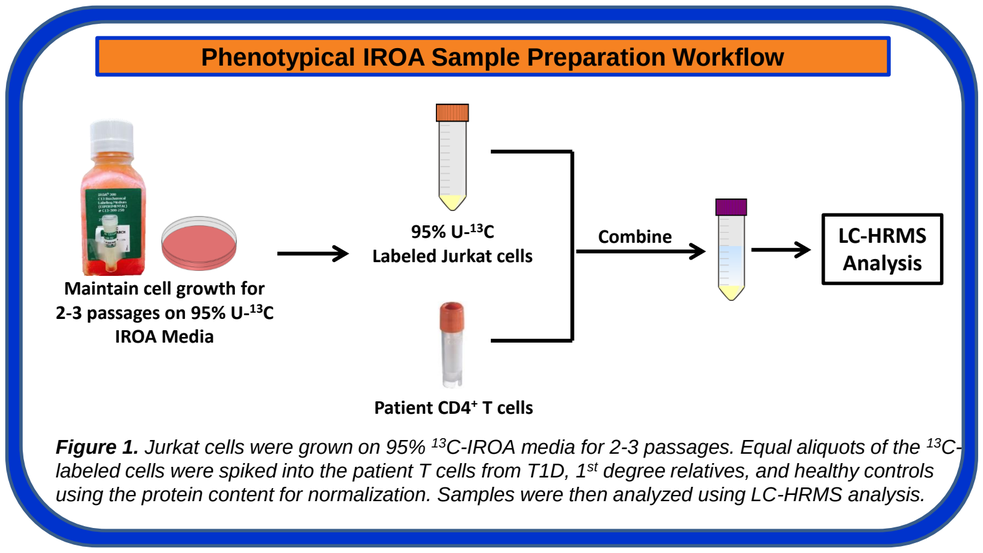


Figure 1. *Jurkat cells were grown on 95% ^{13}C-IROA media for 2-3 passages. Equal aliquots of the ^{13}C-labeled cells were spiked into the patient T cells from T1D, 1st degree relatives, and healthy controls using the protein content for normalization. Samples were then analyzed using LC-HRMS analysis.*

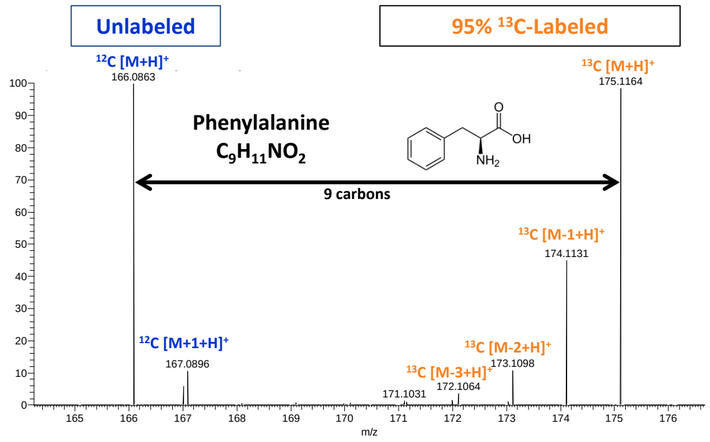


Figure 2. *Extracted ion chromatogram for **phenylalanine**. The IROA signal is composed of two halves, an unlabeled section corresponding to the patient T cells and a 95% ^{13}C labeled envelope from the labeled Jurkat cells. Biologically relevant peaks are distinguished from the noise based on the predictable relative intensity shown above and the mass spectral peak spacing of 1.0034 u.*

Patient History

	Control	Relative	T1D
Age (yr)	7-39	10-56	14-36
Glucose Level (mg/dL)	-	64.9-82.2	46.05-102
T1D Duration (yr)	-	-	1.25-27.5
Gender	4F/1M	3F/1M	3F/3M
Total # of samples	5	4	6

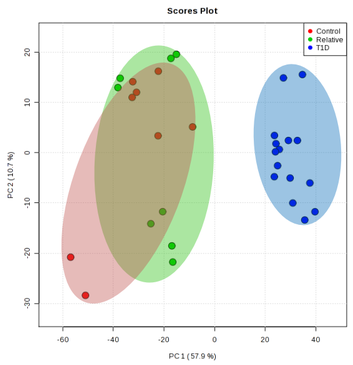


Figure 3. *Principal component analysis (PCA) scores plot for **T1D**, **1st degree relatives**, and **healthy controls** displays first component separation between the disease and control groups. As expected, the 1st degree relatives and the healthy controls group together and load negatively in component space because the 1st degree relatives were not diagnosed with the disease.*

C12 m/z	C13 m/z	RT (min)	Carbon #	Formula	Potential ID	Percent Change	
						Relative	T1D
118.087	123.103	1.02	5	$C_6H_9NO_2$	valine	2.6	16.8
116.071	121.088	0.88	5	$C_5H_9NO_2$	proline	33.8	34.6
150.059	155.075	1.40	5	$C_5H_{11}NO_2$	methionine	41.3	34.9
132.122	138.122	2.28	6	$C_6H_{13}NO_2$	leucine	18.4	20.9
147.113	153.134	0.66	6	$C_6H_{14}N_2O_2$	lysine	31.5	22.4
182.082	191.111	3.01	9	$C_9H_{11}NO_3$	tyrosine	21.1	31.8
156.077	162.098	0.79	6	$C_6H_9N_3O_2$	histidine	-15.2	-17.0
175.119	181.139	0.73	6	$C_6H_{14}N_4O_2$	arginine	5.83	70.7
166.087	175.116	6.00	9	$C_9H_{11}NO_2$	phenylalanine	27.5	40.5
123.056	129.076	2.83	6	$C_6H_6N_2O$	nicotinamide	19.3	39.0
145.050	151.071	0.78	6	$C_6H_8O_4$	dimethylmaleate	-32.5	-27.6
136.062	141.079	1.44	5	$C_5H_5N_5$	adenine	15.8	22.6
139.051	145.071	1.85	6	$C_6H_6N_2O_2$	urocanate	-24.3	6.5
127.039	133.061	0.82	6	$C_6H_6O_3$	1,2,3-trihydrobenzene	-71.8	-62.6
104.071	108.084	0.80	4	$C4H_9NO_2$	4-aminobutanoate	-12.9	16.6
112.077	118.096	0.88	6	C_6H_9NO	2-acetyl-1-pyrroline	-28.5	-34.7
128.071	134.091	0.77	6	$C_6H_9NO_2$	2,3,4,5-tetrahydropyridine-2-carboxylate	33.3	76.8

***Table 1. The p**ercent change (increase or decrease) for metabolites was calculated using the C12/C13 median fold change for **1st degree relatives** and **T1D** compared to **healthy controls**. Compounds were searched against the ClusterFinder library for identifications using the carbon number, and the percent labeling for each distribution.*

Conclusion and Future Work

- The phenotypical IROA pattern can be predicted for metabolites of interest using the formula below.

$$Intensity(n,p,k) = \left(\frac{n!}{k!(n-k)!}\right) p^k (1-p)^{n-k}$$

$n = \#\ of\ carbons$
$k = peak\ \#\ (0 = unlabeled\ compound)$
$p = \%\ incorporation$

- Jurkat cells incorporated the 95% ^{13}C IROA media into the metabolites after only 1 passage.
- While principal component analysis groups the healthy controls and the relatives in component space, the phenotypical IROA methodology allowed for the identification of many metabolites that differed amongst those two groups.
- Compared to the healthy control group, 4-aminobutanoate and urocanate were lower in the relatives and upregulated in the T1D samples.
- Metabolites such as arginine and valine were of a higher intensity in the T1D samples compared to the controls and relatives.

Future investigations will use phenotypical IROA to compare the differences between the lipid content in positive and negative ion mode.

References

1. Balderas, C.; Ruperez, F. J.; Ibanez, E.; Senorans, J.; Guerrero-Fernandez, J.; Casado, I. G.; Gracia-Bouthelier, R.; Garcia, A.; Barbas, C. Plasma and urine metabolic fingerprinting of type 1 diabetic children. *Electrophoresis* **2013**, *34*, 2882-2890.
2. Stupp, G. S.; Clendinen, C. S.; Ajredini, R.; Szewc, M. A.; Garrett, T.; Menger, R. F.; Yost, R. A.; Beecher, C.; Edison, A. S. Isotopic ratio outlier analysis global metabolomics of Caenorhabditis elegans. *Anal. Chem.* **2013**, *85*, 11858-11865.
3. Lanza, I. R.; Zhang, S.; Ward, L., E.; Karakelides, H.; Raftery, D.; Nair, K. S. Quantitative Metabolomics by 1H-NMR and LC-MS/MS Confirms Altered Metabolic Pathways in Diabetes. **2010**, *5*, e10538-1-e10538-10.
4. Wagner, D. In *The Role of T Cells in Type 1 Diabetes;* Type 1 Diabetes - Pathogenesis, Genetics, and Immunotherapy; InTech: Rijeka, Croatia, **2011**; pp 83-92.

Acknowledgements

- Department of Chemistry
- Yost Group Members
- McKnight Doctoral Fellowship
- SECIM (NIH Grant #U24 DK097209
- JDRF Research Grant #17-2012-595

