BIOGENIC AMINES AND THEIR FUNCTION IN CARDIOVASCULAR DISEASE: MULTIPLEXED ANALYTICAL METHOD FOR DEEPER MOLECULAR PHENOTYPING

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INTRODUCTION

Biogenic amines are important molecules involved in a number of different biological processes. Specially, branched chain amino acids (BCAA) which are comprised of; Leucine, Isoleucine and Valine. It has been previously reported[1] than an increased level of BCAA is associated with insulin resistance and Type 2 Diabetes. BCAA regulate glucose homeostasis and aid insulin secretion. In 2012 the number of individuals in the USA with Type 2 Diabetes was 9.3% of the population. Type 2 diabetes and obesity continues to grow with no sign of slow down due to life style and other genetic predispositions. Active biomedical research for the metabolic syndrome is on going to identify different therapeutic modalities that can improve the lifestyle and ameliorate further metabolic complications. In biomedical research it is important to have fast and reliable analytical biomarker assays that can help to understand mechanism of action and biomarker/s of efficacy. In this poster, we show a highly multiplexed and fit for purpose analytical strategy (Figure 1) used in biomedical research using LC/MS that focuses on biogenic amine phenotyping using stable labeled isotopic (SLI) internal standards to quantify endonegeous levels of biogenic amines from plasma of Lean and T2D diabetic subjects.

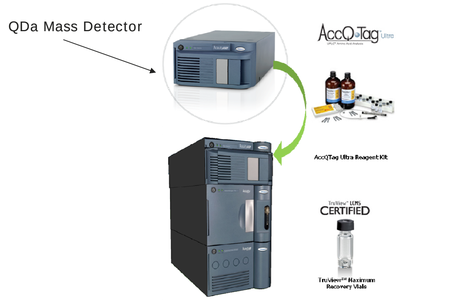


Figure 1. Acquity I-Class, QDa Mass detector and AccQTag Ultra reagents for Biogenic Amine profiling and quantitation

METHODS

Samples:

Human plasma samples were obtained from Innovative research ; n=20 T2D and n=17 Lean. QCs were generated from pooled plasma samples n=6.

Biogenic amine extraction:

To 5µL of plasma samples 10uL of heavy labeled internal standards were added at the desired concentration (Cambridge isotopes, MA). Then, samples were deproteinased using 50µL 75% Acetonitrile/ 25% Water. Following this step the samples were centrifuged at 10,000 rpm for 5 minutes at 5 ° C. 10µL of the supernatant was used for the derivatisation step.
The supernatant was derivatized as described below in figure 2;

The resulting reaction was derivatized using the AccQTag Ultra reagent as shown below;

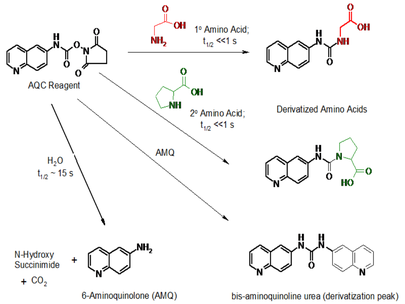


Figure 2. Derivatisation steps for 1 ° and 2 ° Amino acids

Derivatization step:

10µL of the supernatant was added to 70µL buffer from the AccQTag Ultra kit. Then, 20µL of reagent was added and heated for 10 minutes at 55 ° C as shown in Figure 3

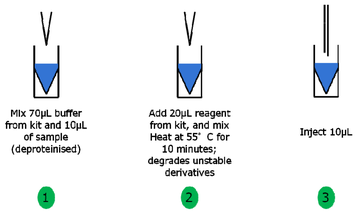


Figure 3. Derivatization steps diagram following protein precipitation of samples

LC conditions:

LC system: Waters H-Class Acquity
Column: AccQTag Ultra 2.1x100 mm, 1.7µm
Column Temperature: 43 ° C
Sample Organizer temperature:20 ° C
Mobile Phase A: Eluent A
Mobile Phase B: 10:90 waters/AAA eluent B
Mobile Phase C: Water
Mobile Phase D: AAA eluent B

Gradient:

Step	Time (min)	Flow (mL/min)	%A	%B	%C	%D	Curve
1	0.00	0.7	2.0	0.0	98.0	0.0	N/A
2	0.29	0.7	2.0	0.0	98.0	0.0	11
3	5.49	0.7	9.0	80.0	11.0	0.0	7
4	7.10	0.7	8.0	15.6	57.9	18.5	6
5	7.3	0.7	8.0	15.6	57.9	18.5	6
6	7.69	0.7	7.8	0.0	70.9	21.3	6
7	7.99	0.7	4.0	0.0	36.3	59.7	6
8	8.59	0.7	4.0	0.0	36.3	59.7	6
9	8.68	0.7	2.0	0.0	98.0	0.0	6
10	10.20	0.7	2.0	0.0	98.0	0.0	6

MS Conditions:

Mass Spectrometer: QDa Mass Detector

Ionization mode: Electrospray Positive ion mode, 1.4 kV capillary voltage, 25 V cone voltage

SIR mode was used for each amino acid and its SIL internal standard

RESULTS

Good chromatographic separation for all amino acids of interest was achieved in under 10 minutes (figure 4)

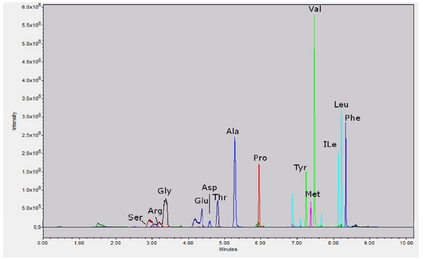
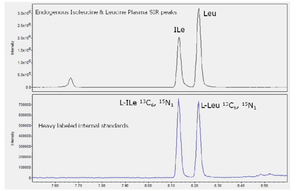


Figure 4. Single reaction monitoring combined chromatogram for amino acids of interest

BCAA corresponding to Isoleucine, Leucine and Valine were all detected with good levels of analytical sensitivity as shown below in figures 5A and 5B with their corresponding SIL amino acid which was used as a single point calibrator

A)



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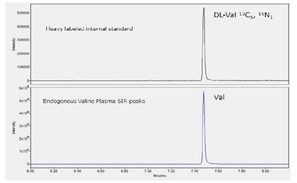

Figure 5. Chromatograms for Ile, Leu and their SILs (A) and Valine and its SIL (B)

The resulting plasma QCs (n=6) gave confidence in the reproducibility of the LC/MS amino acid assay with coefficient of variations <6% as described in Figure 6.

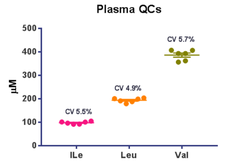

Figure 6. Plasma QCs reproducibility for described LC/MS assay in relation to BCAAs

Of interest was to measure the concentrations of the BCCAs in these samples to determine trends associated with insulin resistance.
And as shown below in Figure 7. It can be observed an upward trend for all BCAAs in the T2D samples when compared with the Control group. These results correlates with other research reports in the literature[1]

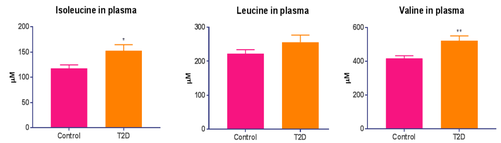

Figure 7. Plasma BCAAs concentrations in plasma, T2D vs. Control.
* p<0.05, ** p<0.001, student t-test, unpaired, two-tails

CONCLUSION

- **Fast runtimes <10 min per samples is a major improvement over ion-exchange chromatography methods in which typical separations are ~2 hours per sample, in general the method in this poster is cheaper, cleaner and faster than ion-exchange chromatography methods**
- **Derivatization of plasma samples for biogenic amines improves selectivity (removes matrix interferences) and specificity (only detects derivatized metabolites)**
- **BCAAs were increased in the T2D samples vs. controls which correlates with other published research reports**

References

1. Newgard, C. B. *Interplay between lipids and branched-chain amino acids in development of insulin resistance. Cell Metab* **15**, 606–614 (2012)

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