AN INTEGRATED ^{1}H NMR, HPLC-(ESI/TOF) AND GC-MS BASED METABOLOMICS APPROACH TO THE STUDY OF ACUTE EXERCISE IN HUMAN SERUM METABOLOME

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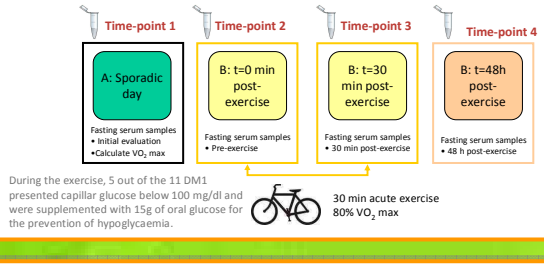
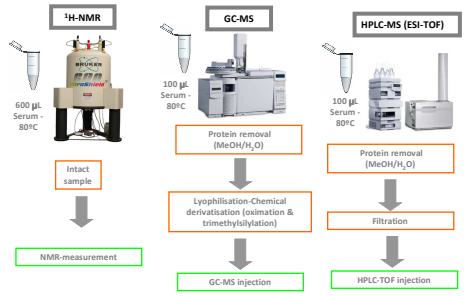
INTRODUCTION & GOALS

It is well known that exercise affects substrate utilisation and insulin sensitivity, which in turn improve blood glucose and lipid levels in subjects with type 2 diabetes. However, these evidences are not so clearly stated in subjects with type 1 diabetes (DM1). In DM1 there are several hazards that make exercise difficult to manage: risks of hypoglycemia during or after exercise or of worsening metabolic control if insulin deficiency is present. Thus, A combined GC-MS, HPLC-MS (ESI/TOF) and NMR-based metabolomics approach was used to the assessment of the global metabolic rearrangement produced by the impact of acute short-term exercise in DM1 subjects.

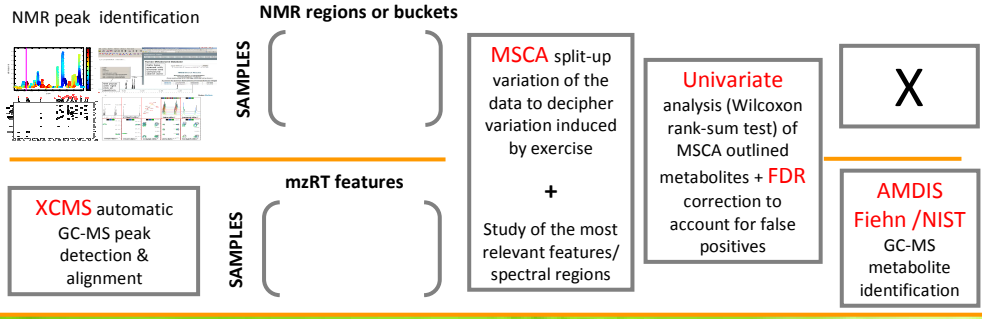
EXPERIMENTAL DESIGN: CTR (N=11), DM1 (N=10)



MATERIAL & METHODS



DATA ANALYSIS



RESULTS

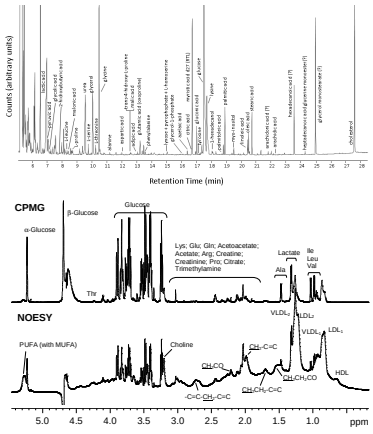


Figure 1: Example of a total ion chromatogram and CPMG and NOESY spectra of the same control serum sample with their corresponding metabolite assignment

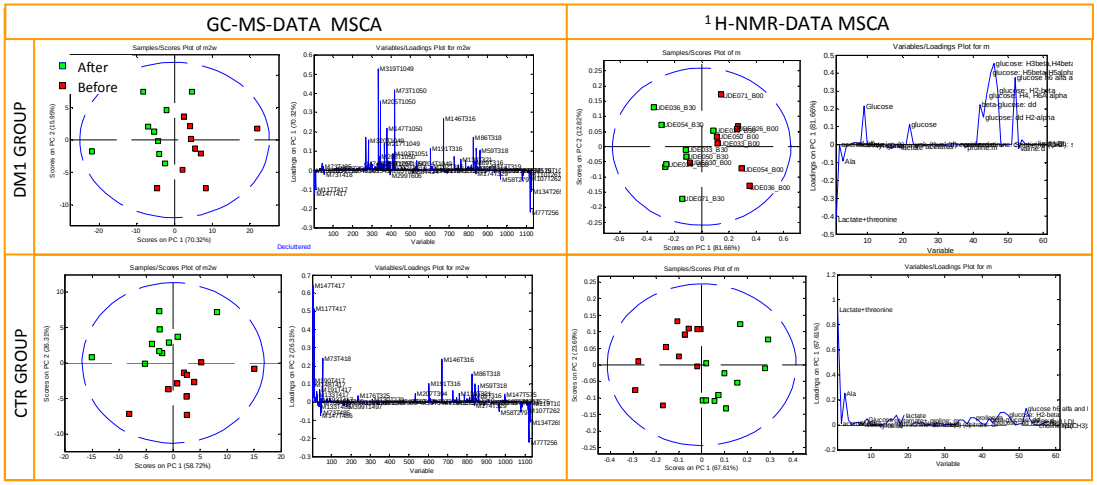


Figure 2: A multilevel two-component PCA model fitted either on 1D-CPMG spectra or on GC-MS m/zRT features showed two clearly separated clusters corresponding to individuals prior and post-acute exercise. Loadings plot holding exercise effect revealed a common metabolic pattern variation in DM1 and CTR groups showing increased levels of lactate (NMR, GC-MS) and alanine (NMR). Conversely only DM1 group revealed clear changes in glucose levels after acute exercise.

			^{1}H-NMR		GC-MS		
	Assignment	multiplicity	δ shift (ppm)	corrected p-values	retention time (min)	m/z	corrected p-value
CTR	Alanine	d	1.46	0.01			
	Lactic acid	d	1.33	0.04	6.95	147	0.03
	Glycerol	dd	3.65	0.02			
	Pyruvic acid				6.80	174	0.03
	2-ketoisocaproic acid				9.14	200	0.04
DM1 no supplemented	X	-	-	-	-	-	-
DM1 supplemented	X	-	-	-	-	-	-

Table 1: Assignment of metabolites found to be varied significantly with the exercise (paired sample Wilcoxon signed rank test, p-corrected value<0.05). CTR group was the only one which showed significant metabolic changes with the exercise.

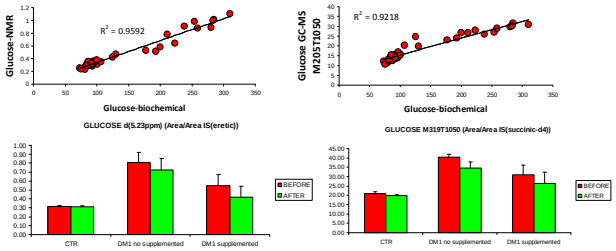


Figure 3: Glucose levels correlation of biochemical measurements with GC-MS and NMR measurements. GC-MS and NMR glucose levels comparison between DM1, DM1-supplemented and CTR group before and after exercise. In spite of lacking for statistical significance DM1 group showed decreased levels of glucose after exercise. This effect was not observed within CTR group.

CONCLUSIONS

- Since serum closely reflects muscle cellular changes in lactate and pyruvate, their increased levels might be indicative of an accumulation of such glycolisis metabolites in muscular cells after short-term intensive exercise.
- Serum alanine levels appeared to be increased since it is transported to the liver where it acts as a gluconeogenesis substrate.
- Raised levels of glycerol together with elevated levels of some fatty acids suggest mobilization of triglycerides with acute exercise.
- Increased levels of KIC might be indicating leucine catabolism.

van Velzen EJJ, Westerhuis JA et al (2008). Journal of Proteome Research 7: 4483-4491

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