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

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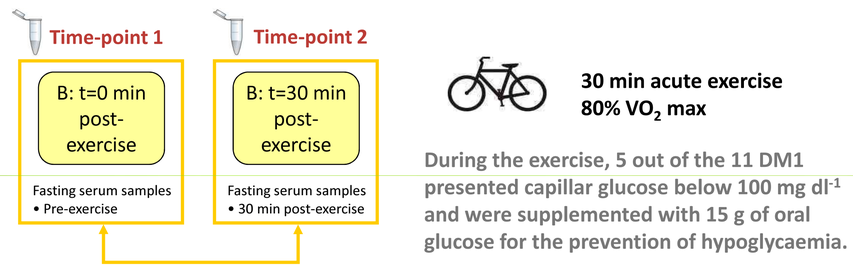
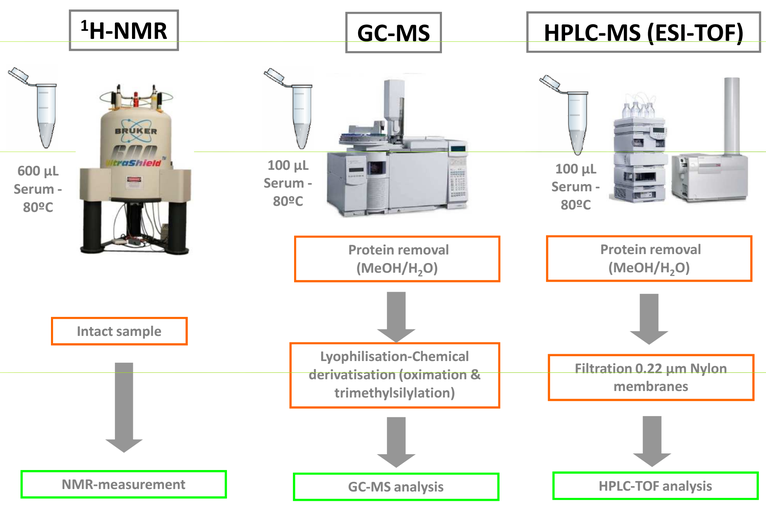
INTRODUCTION & GOALS

There is an increasing concern about the different response and effect that acute exercise induces on diabetic people and a novel insight into this effect can be performed using a metabolomic approach. Metabolomic is becoming widely spread used as a new and powerful tool for discerning significant changes at metabolic level. In this study we aimed to perform a non-targeted analysis and to identify metabolic differences arisen after 30 minutes of acute exercise in both young men suffering from Type 1 Diabetes Mellitus (T1DM) and control individuals by using three different analytical platforms. A first overlook using ^{1}H NMR to identify the largest compounds presents in the sample and a second stage using both gas (GC) and liquid (LC) chromatography coupled to mass spectrometry to identify the minority compounds.

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

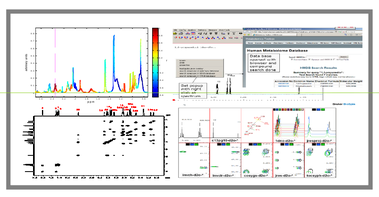


MATERIAL & METHODS



DATA ANALYSIS

NMR identification



Bucketing of NMR regions

Bbioref AMIX database identification

GC-MS identification

XCMS automatic GC-MS peak detection & alignment

MSCA to split-up variation induced either by exercise or by diabetic status

Univariate analysis (Wilcoxon rank-sum test) of MSCA metabolites + FDR

Fiehn / NIST identification

LC-MS identification

MassProfiler Pro® automatic LC-MS peak detection & alignment

Univariate analysis (Wilcoxon rank-sum test)

Metlin / HMDB identification

RESULTS

All the data produced, regardless of the analytical technique used, was clearly separated by using multilevel two-component PCA model [1]. This model was selected in order to discard the biological variation and focus only on the effect produced by the exercise.

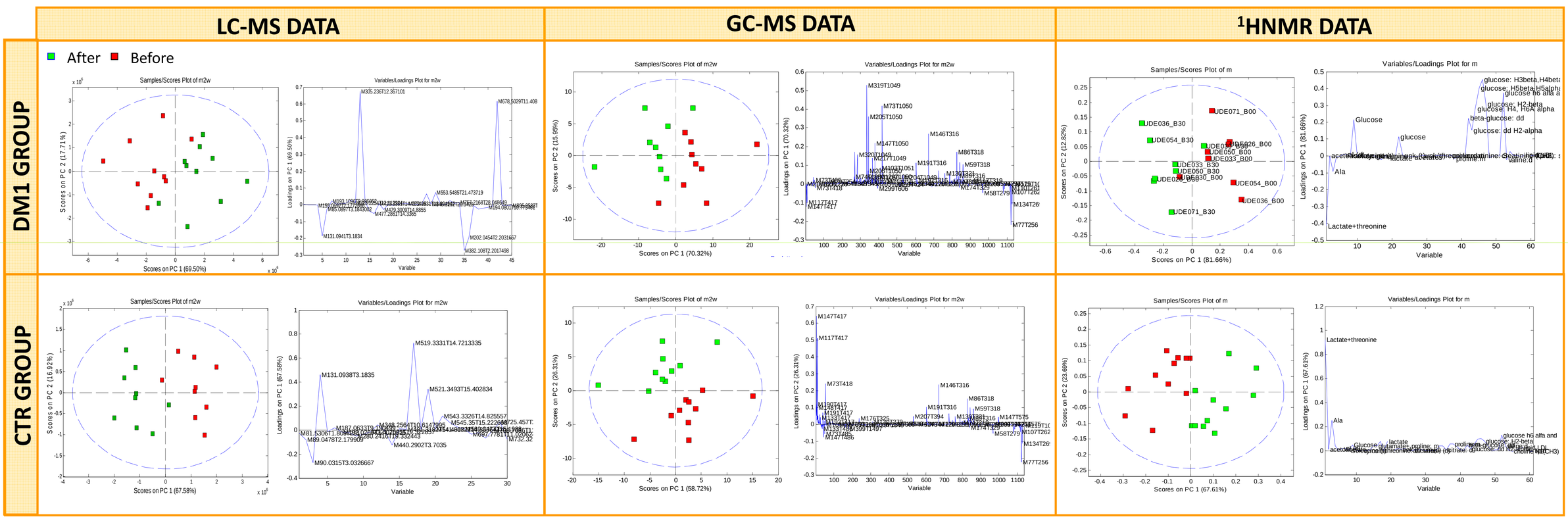


Figure 2: Scores and loading plots obtained from all the analytical techniques used.

Tables 1-3 summarize the metabolites found to be significantly varied after the exercise (paired Wilcoxon signed rank test, *p*-corrected value < 0.05).

Table 1

LC-MS*

Metabolite	Diabetic	Control
Glutamine	↓	—
Isoleucine	↓	↓
Pyrrolidone	↓	—
Palmitoylcarnitine	↑	—
GPCho	↓	—
Ethanolamine	↑	↑
Fatty acids	—	↑

** For LC-MS data, the suggested compounds were confirmed by MS/MS spectrum.*

Table 2

GC-MS

Metabolite	Diabetic	Control
Pyruvic acid	—	↑
Lactic acid	—	↑
2-Ketoisocaproic	—	↑

Table 3

^{1}H-NMR

Metabolite	Diabetic	Control
Alanine	↑	↑
Lactic acid	↑	↑
Glucose	↑	—
Free Glycerol	↑	—

CONCLUSIONS

- Since serum closely reflects muscle cellular changes, their increased levels of lactate and pyruvate might be indicative of an accumulation of such glycolisis metabolites in muscular cells after short-term intensive exercise.
- Decreased levels of isoleucine combined with increased levels 2-ketoisocaproic is indicative of branched chain aminoacids metabolism.
- Raised levels of glycerol together with increased levels of palmitoylcarnitine as well as some fatty acids is suggestive of mobilization of triglycerides with acute exercise.

[1] van Velzen E.J.J., Westerhuis J.A. *et al*. J. Prot. Res. 7 (2008) 4483-4491

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