

An automatic workflow for analyzing untargeted stable isotope tracking experiments by GC-TOF MS

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INTRODUCTION

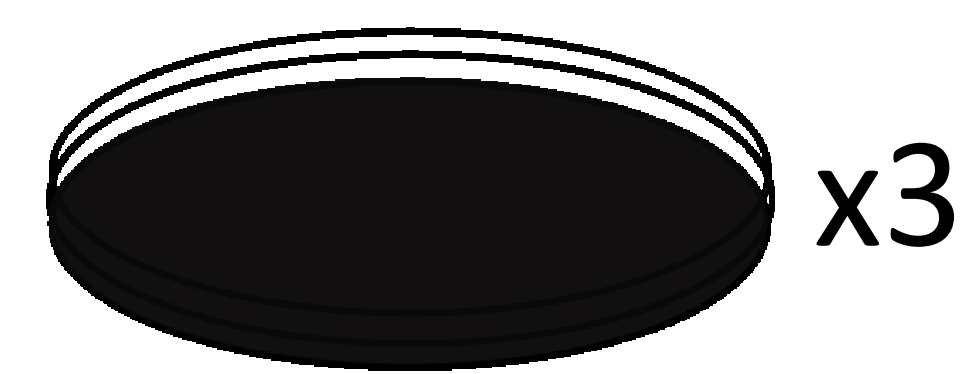
The use of GC-(EI)MS to study metabolic fluxes is hampered by the difficulty of analyzing the datasets derived.

eRah is a computational tool that allows the unbiased tracing of labelled atoms

We have analyzed an *in vitro* model of diabetic retinopathy that simulates hyperglycaemic conditions on the metabolism of a human retinal pigment epithelial cell line (ARPE-19)

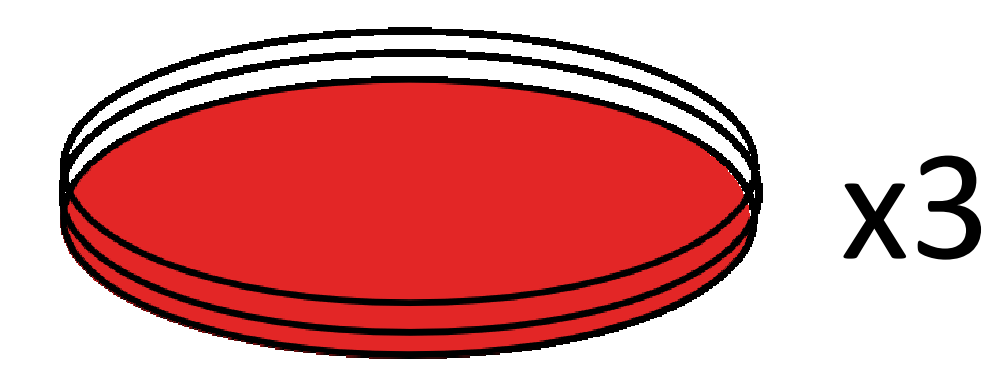
MATERIALS AND METHODS

Unlabelled Samples



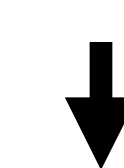
5.5 or 25 mM Glucose (N5 or N25)

Labelled Samples



5.5 or 25 mM [U-¹³C]Glucose (N5 or N25)

Extraction and derivatization of metabolites



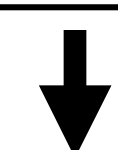
GC-qTOF MS

Electron Ionization and Chemical Ionization (using isobutane gas)

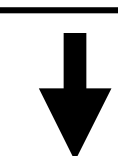


eRah workflow**

¹²C & ¹³C Samples



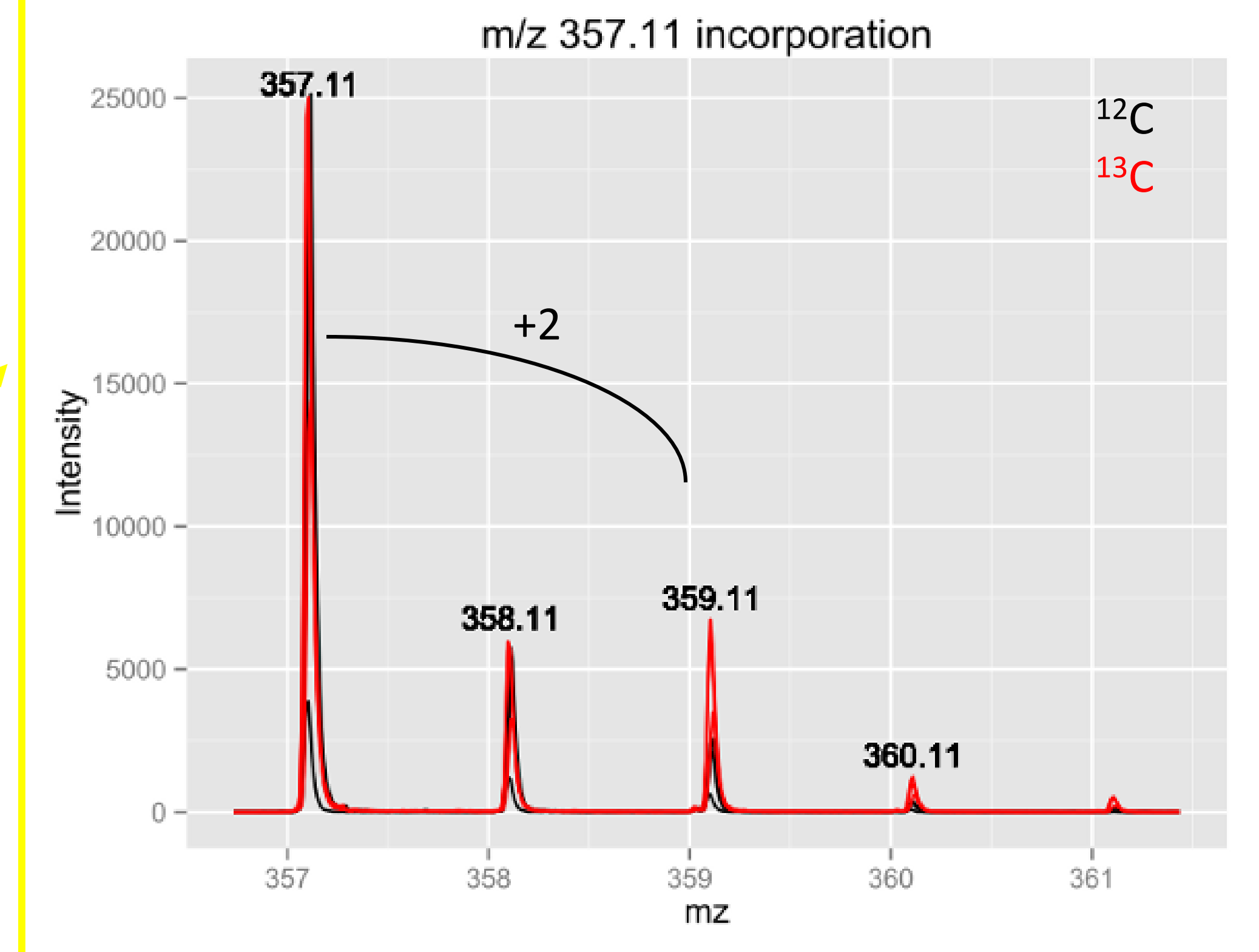
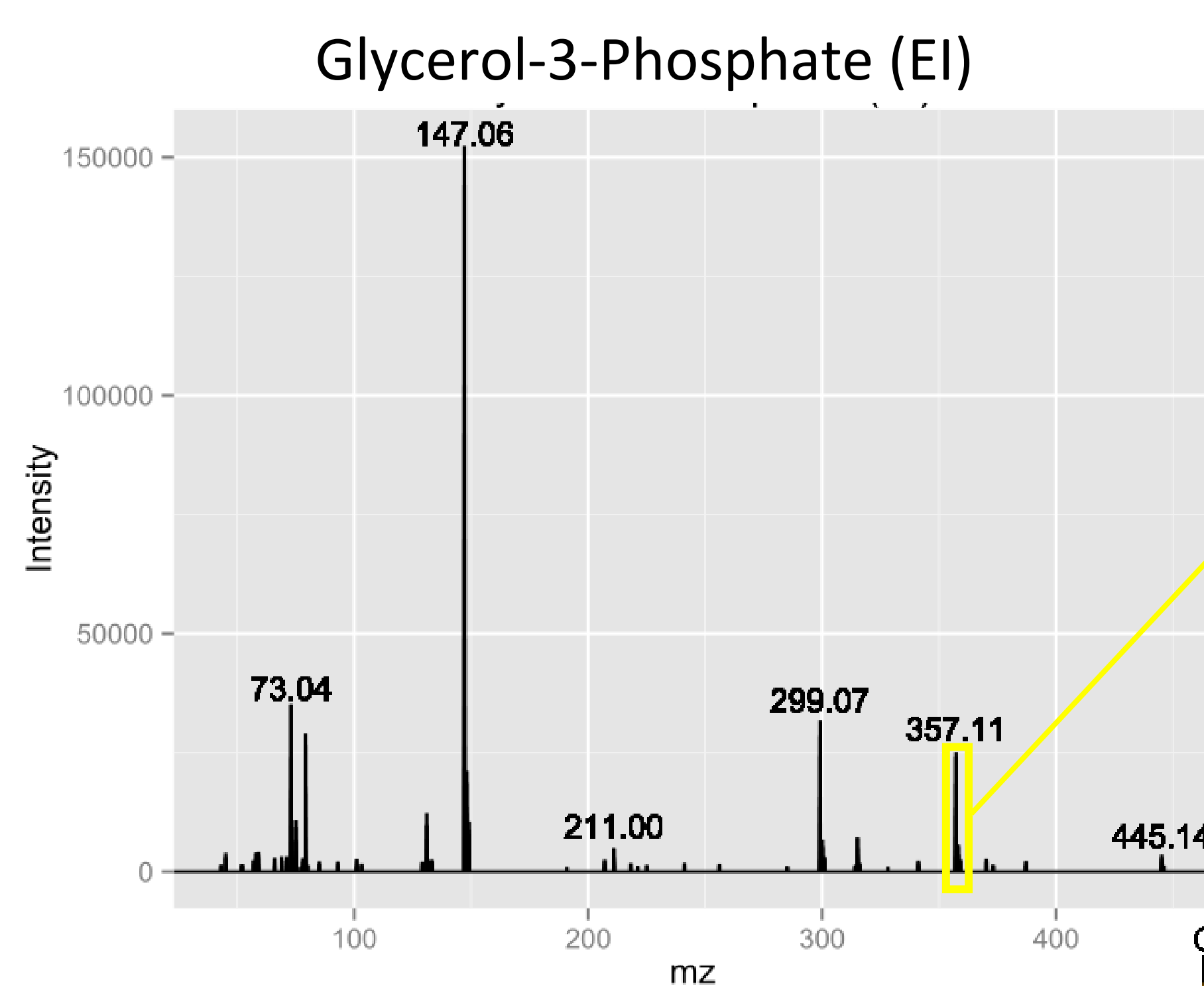
Deconvolution (ODS)
Alignment
Identification
Compound recovery (PLS)



¹²C vs ¹³C comparison
Statistical analysis + FC

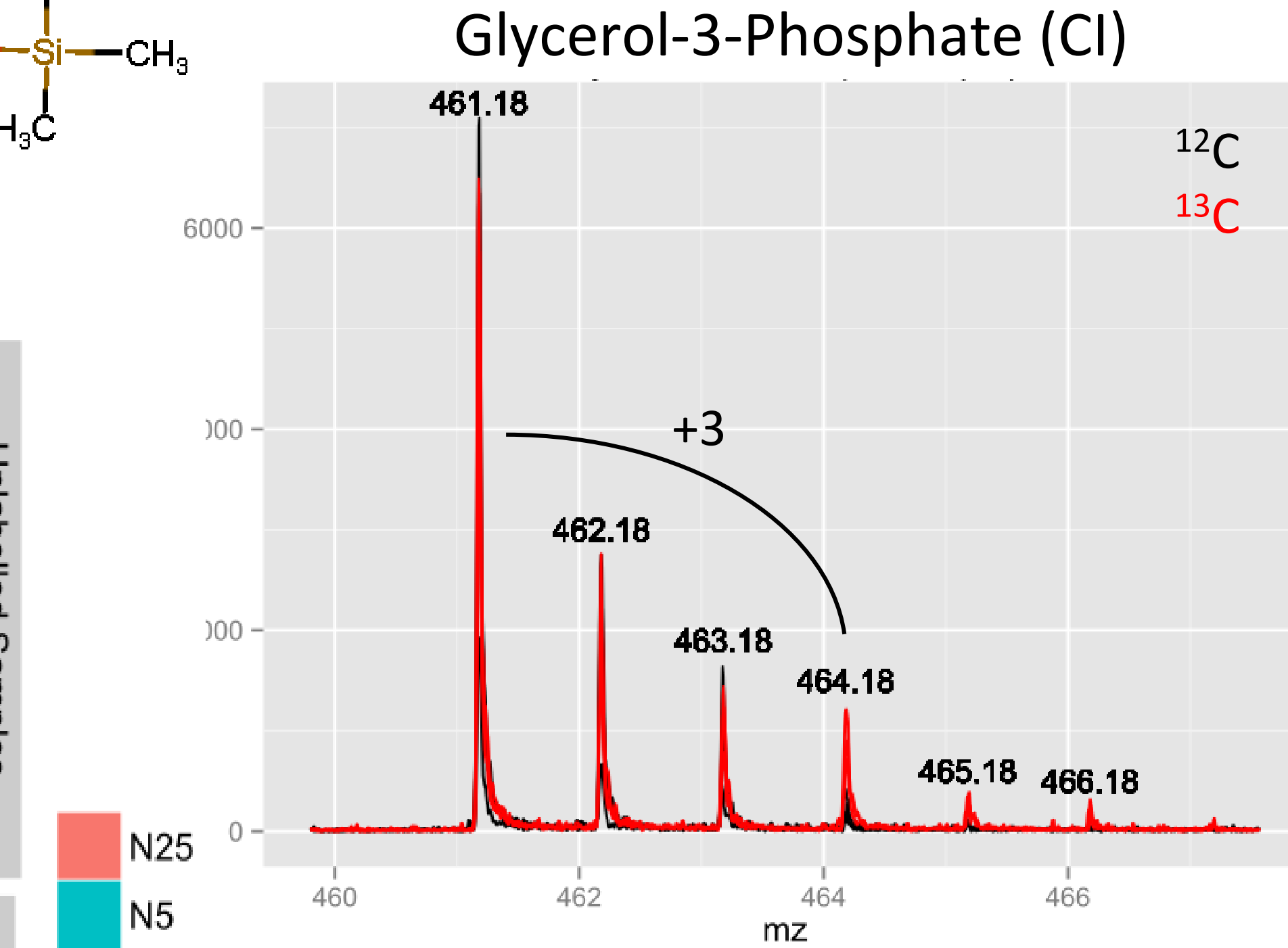
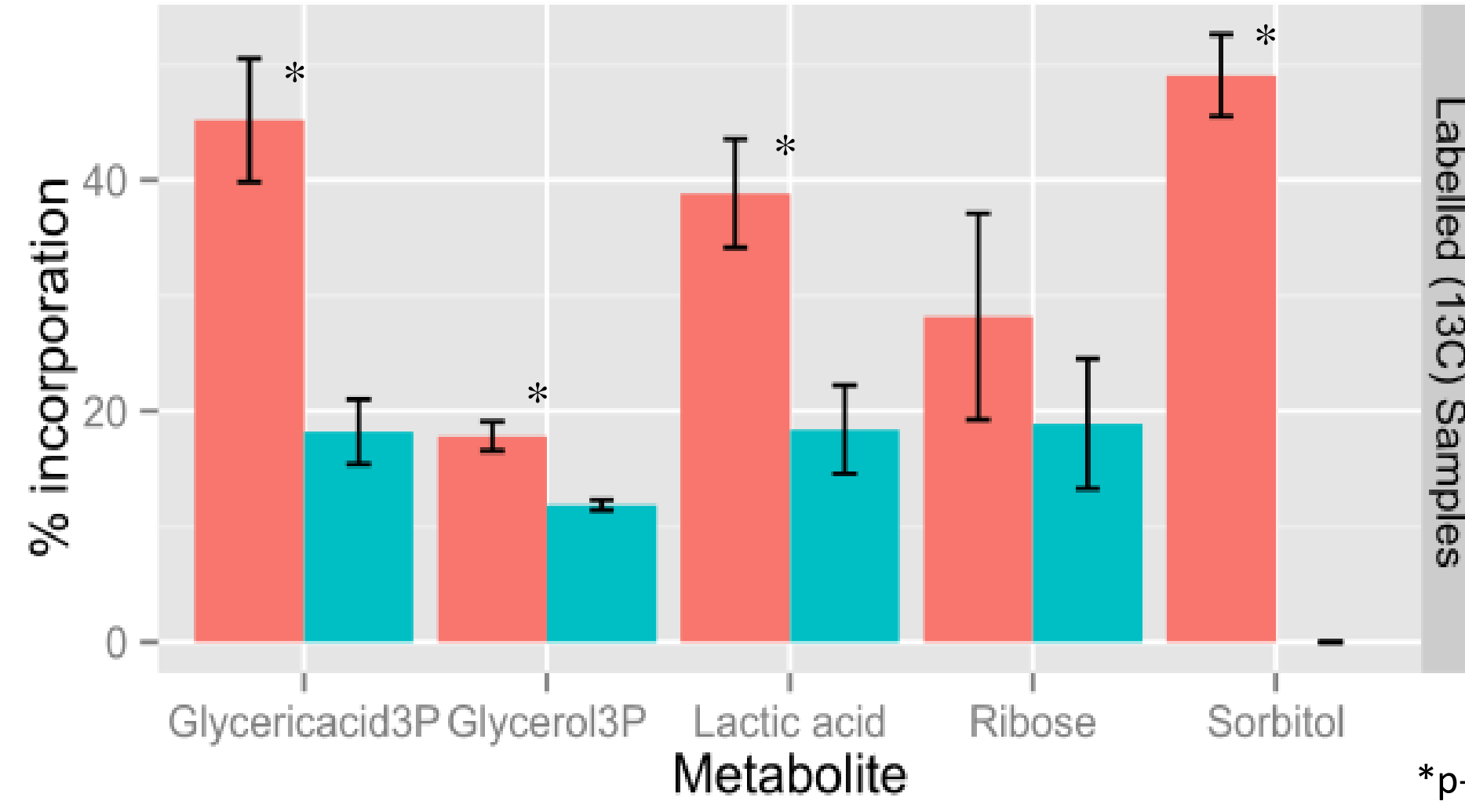
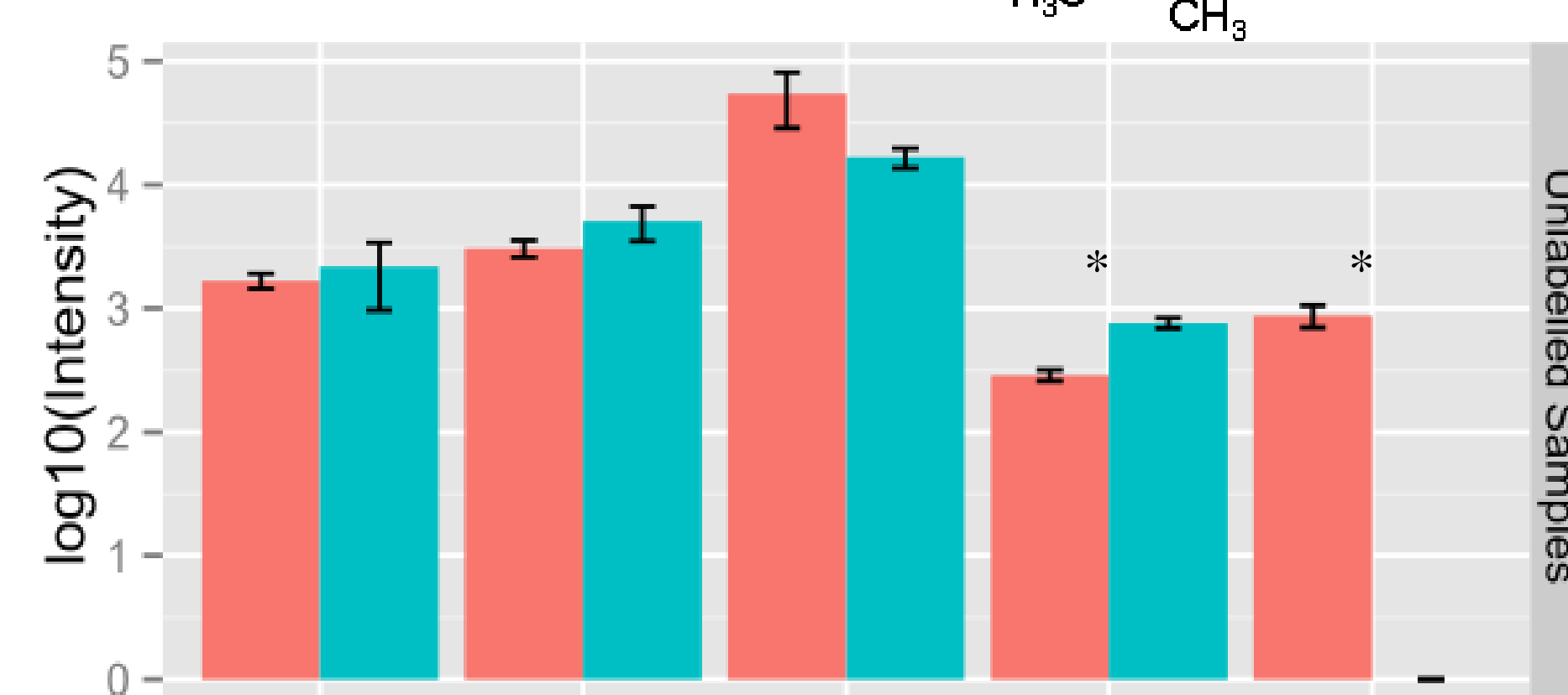
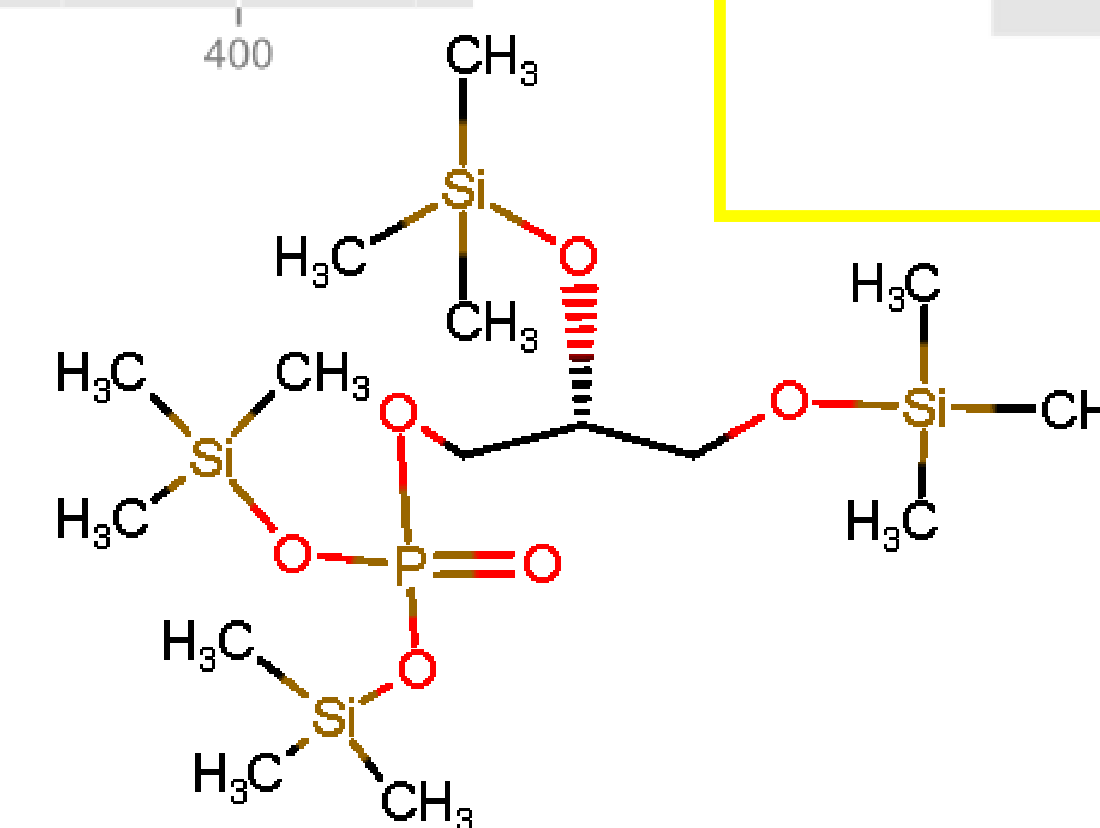
RESULTS

Incorporation of ¹³C in downstream metabolites

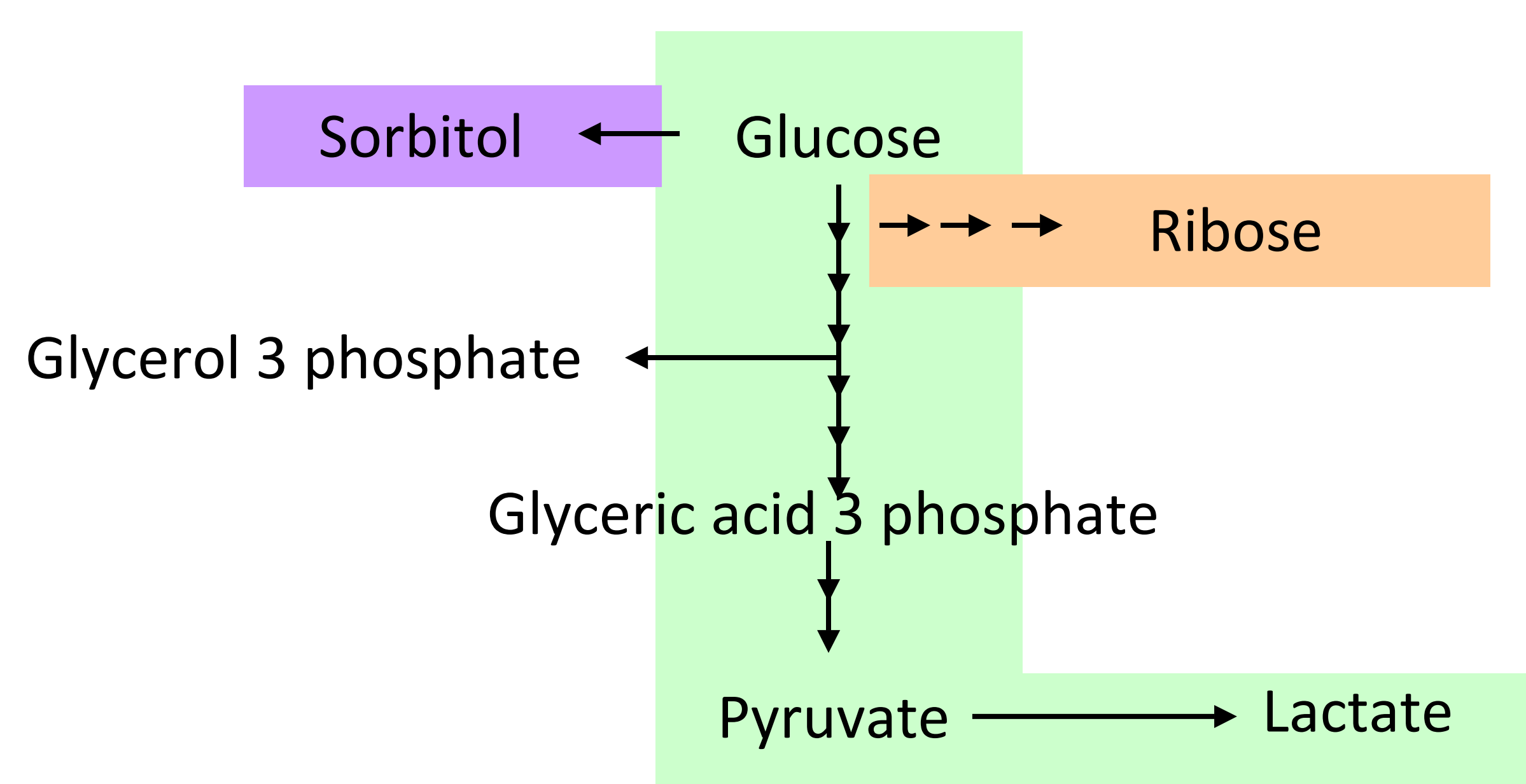


CI exact mass validation

Glycerol-3-phosphate (4TMS)
MW: 460.17



Glucose incorporation: N5 vs N25



Pentose phosphate pathway
Polyol pathway
Glycolysis pathway

**Please, see poster 308 for *eRah* details

*p-value < 0.05