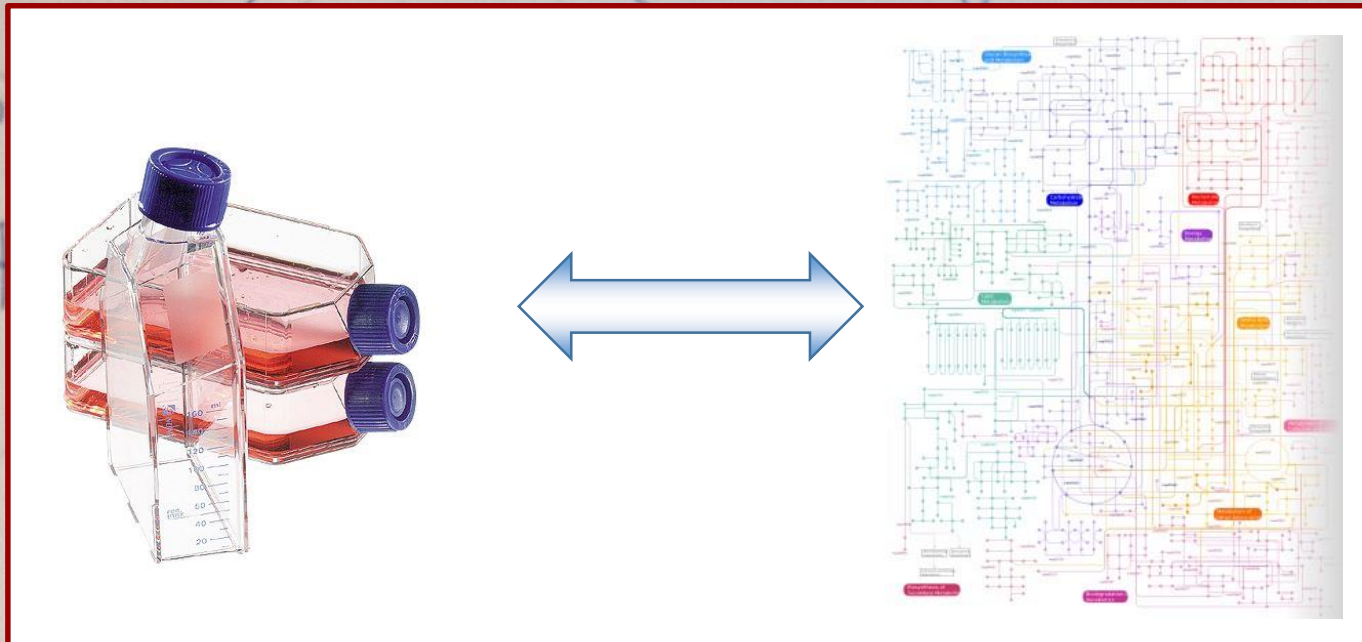


## Flux analysis with stable isotopes





# The Menu

## *Kinetics of stable-isotope labelling*

- *Experimental analysis (Barbara)*
- *Computational analysis (Marvin)*

# Fluxes versus concentrations

A

Metabolomics  
→ measures concentration

Isotope tracing  
→ probes flux



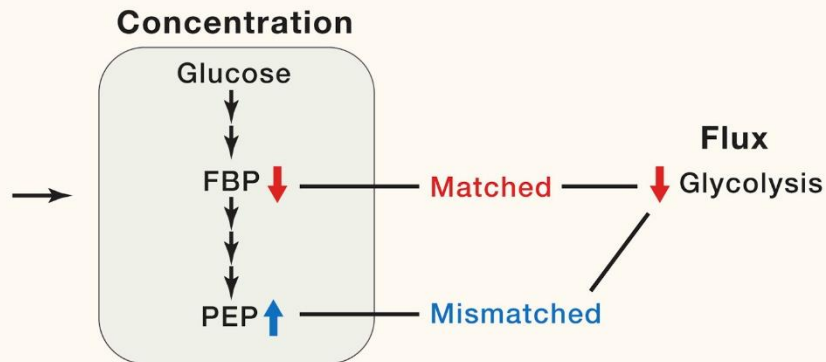
Flux increases with car density (concentration) until traffic slows



Very high car density but low flux

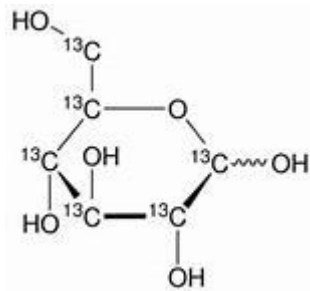
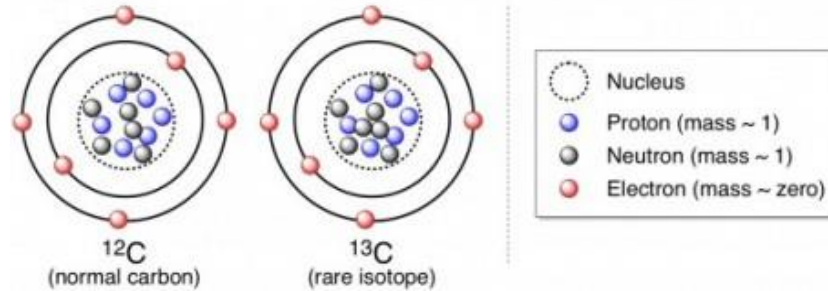
B

**Experiment**  
Remove glucose from growing yeast

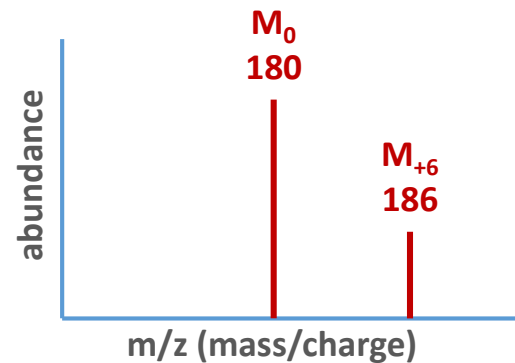


# Stable isotope tracers

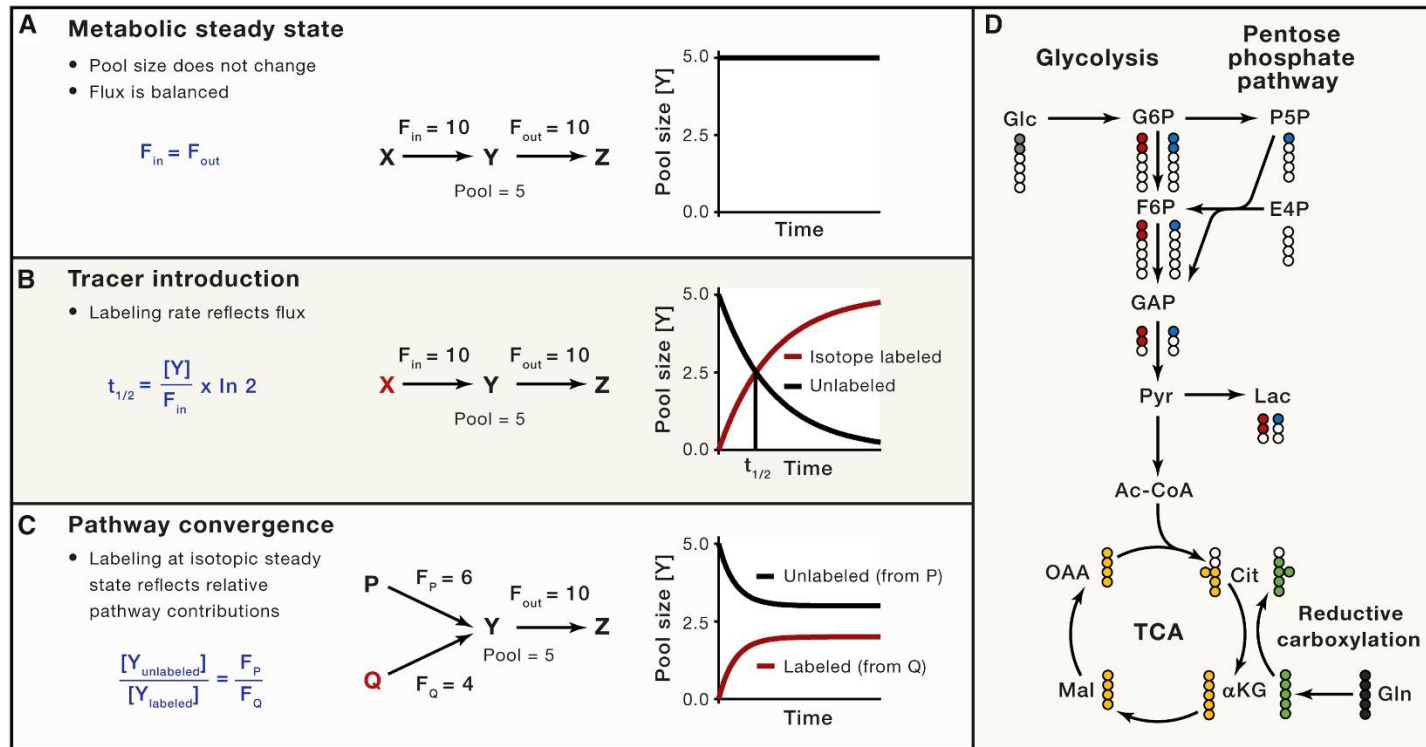
## Carbon Isotopes



[U- $^{13}\text{C}$ ]-glucose



# Dynamic analysis of isotope dilution

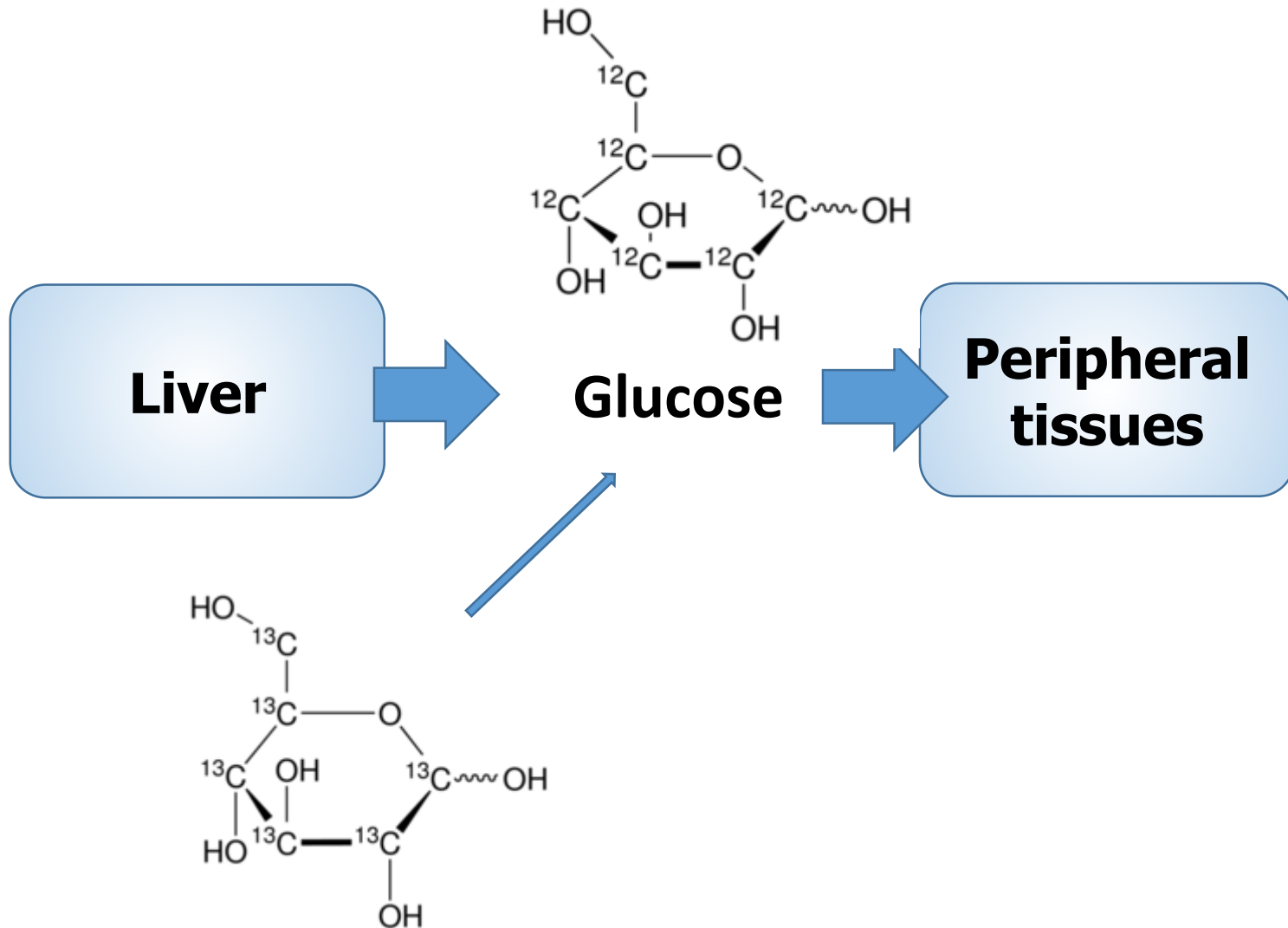


Models become more complex when we look simultaneously at the labelling of multiple metabolites in a branched pathway.

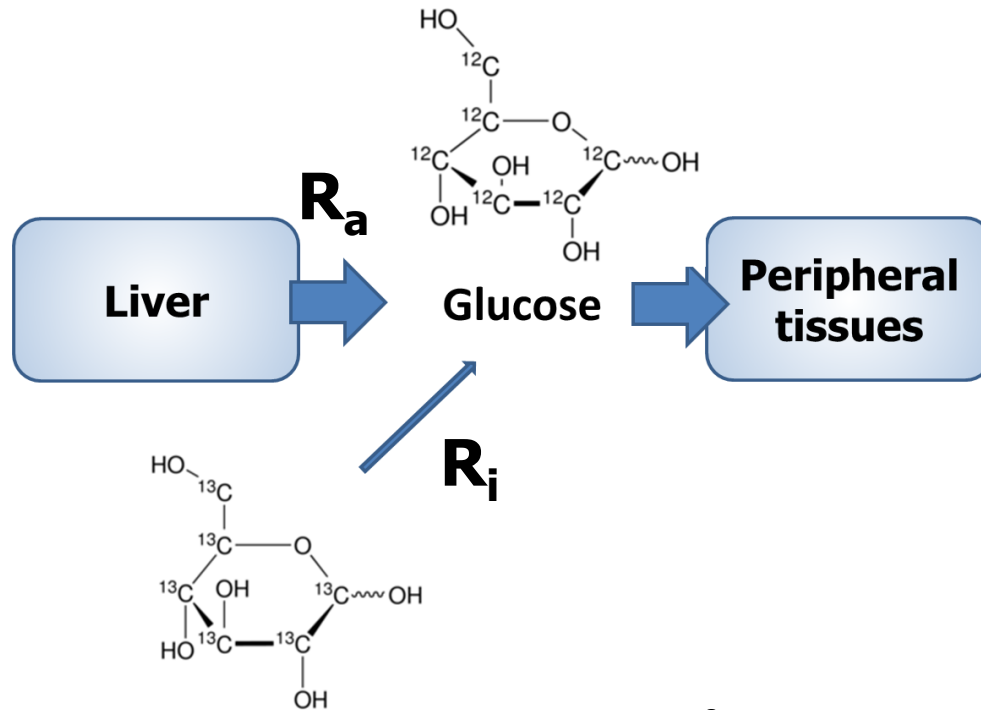
# Metabolite flow between organs



# Metabolite flow between organs



# Metabolite flow between organs



$R_a$  = rate of appearance

$R_i$  = rate of infusion

At steady state:

$$\frac{[\text{C}^{12}\text{-glucose}]}{[\text{C}^{13}\text{-glucose}]} = \frac{R_a}{R_i}$$

known  
measured  
calculated



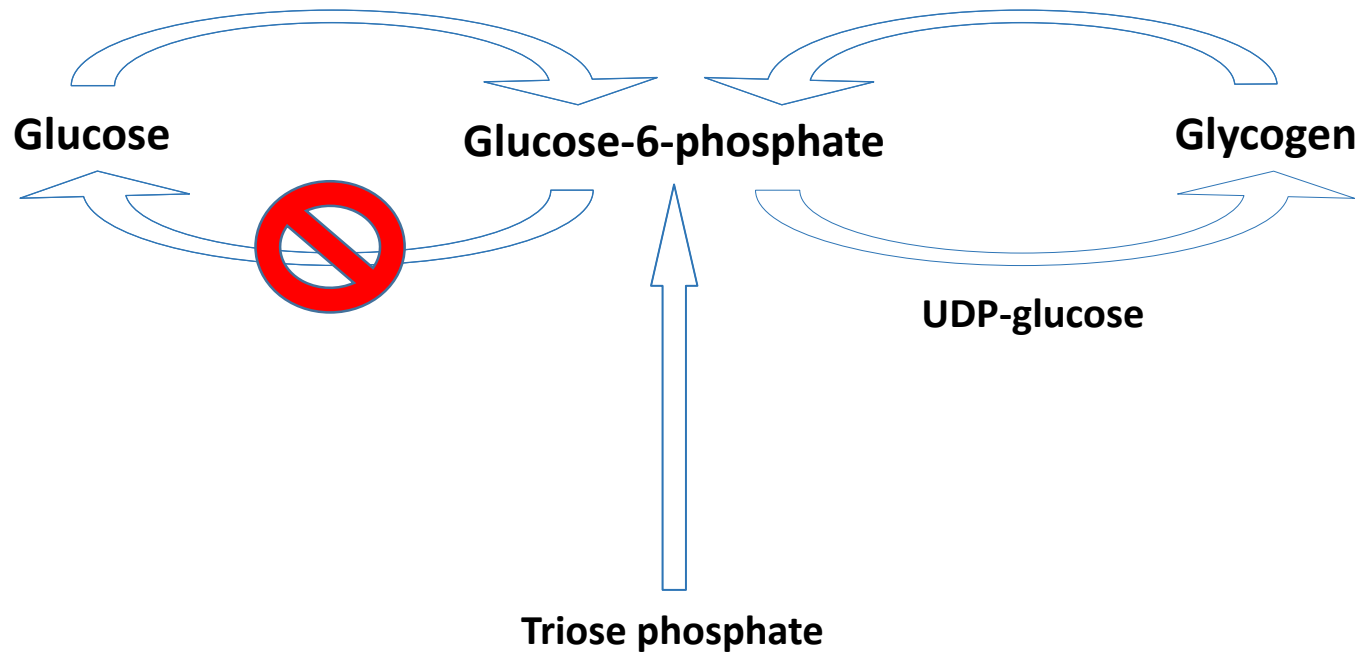
# Fluxes of circulating metabolites

Table 1 | Turnover fluxes for different circulating carbon metabolites

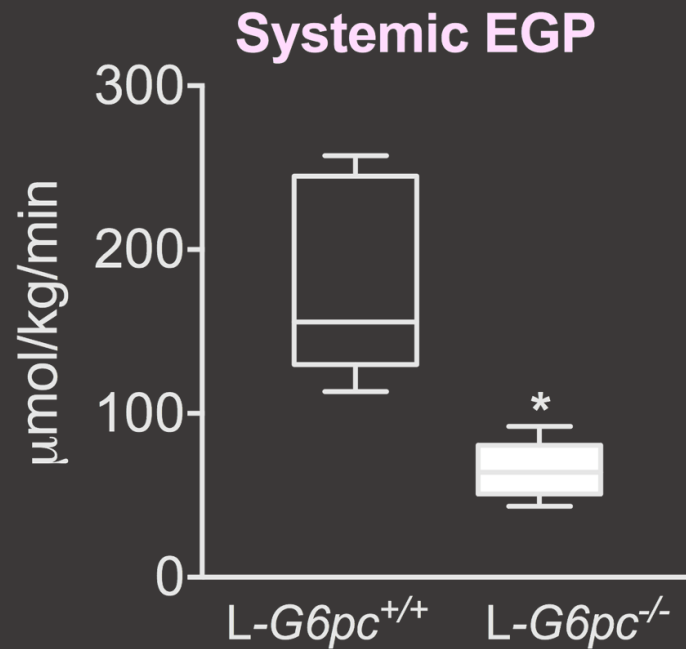
Metabolite	$F_{\text{circ}}$ (nmol g <sup>-1</sup> min <sup>-1</sup> )	Metabolite	$F_{\text{circ}}$ (nmol g <sup>-1</sup> min <sup>-1</sup> )
Lactate	374.4 ± 112.4	Arginine	9.0 ± 2.6
Glucose	150.9 ± 46.7	Tyrosine	8.0 ± 2.2
Acetate	72.7 ± 17.5	Threonine	7.6 ± 0.8
Alanine	70.2 ± 5.4	Proline	7.3 ± 2.9
Pyruvate	57.3 ± 14.2	Isoleucine	6.5 ± 0.7
Glycerol	53.3 ± 2.1	Asparagine	6.5 ± 0.8
Glutamine	45.6 ± 4.7	Phenylalanine	5.9 ± 0.8
3-Hydroxybutyrate	43.3 ± 17.1	2-Oxoglutarate	5.8 ± 0.8
Palmitic acid	24.6 ± 4.2	Histidine	5.0 ± 0.4
Glycine	21.9 ± 4.2	Methionine	3.9 ± 1.6
Taurine	19.4 ± 0.9	Succinate	3.1 ± 1.1
Serine	19.3 ± 4.2	Creatine	2.6 ± 0.5
Citrate	16.2 ± 6.6	Tryptophan	2.3 ± 0.3
Leucine	11.5 ± 1.2	Malate	2.0 ± 0.4
Valine	9.6 ± 0.4	Betaine	1.6 ± 0.2
Lysine	9.3 ± 1.8		

*n* = 24 for lactate; *n* = 22 for glucose; *n* = 5 for glutamine; *n* = 4 for 3-hydroxybutyrate; *n* = 5 for palmitic acid; *n* = 4 for essential amino acids; *n* = 3 for others; mean ± s.d.

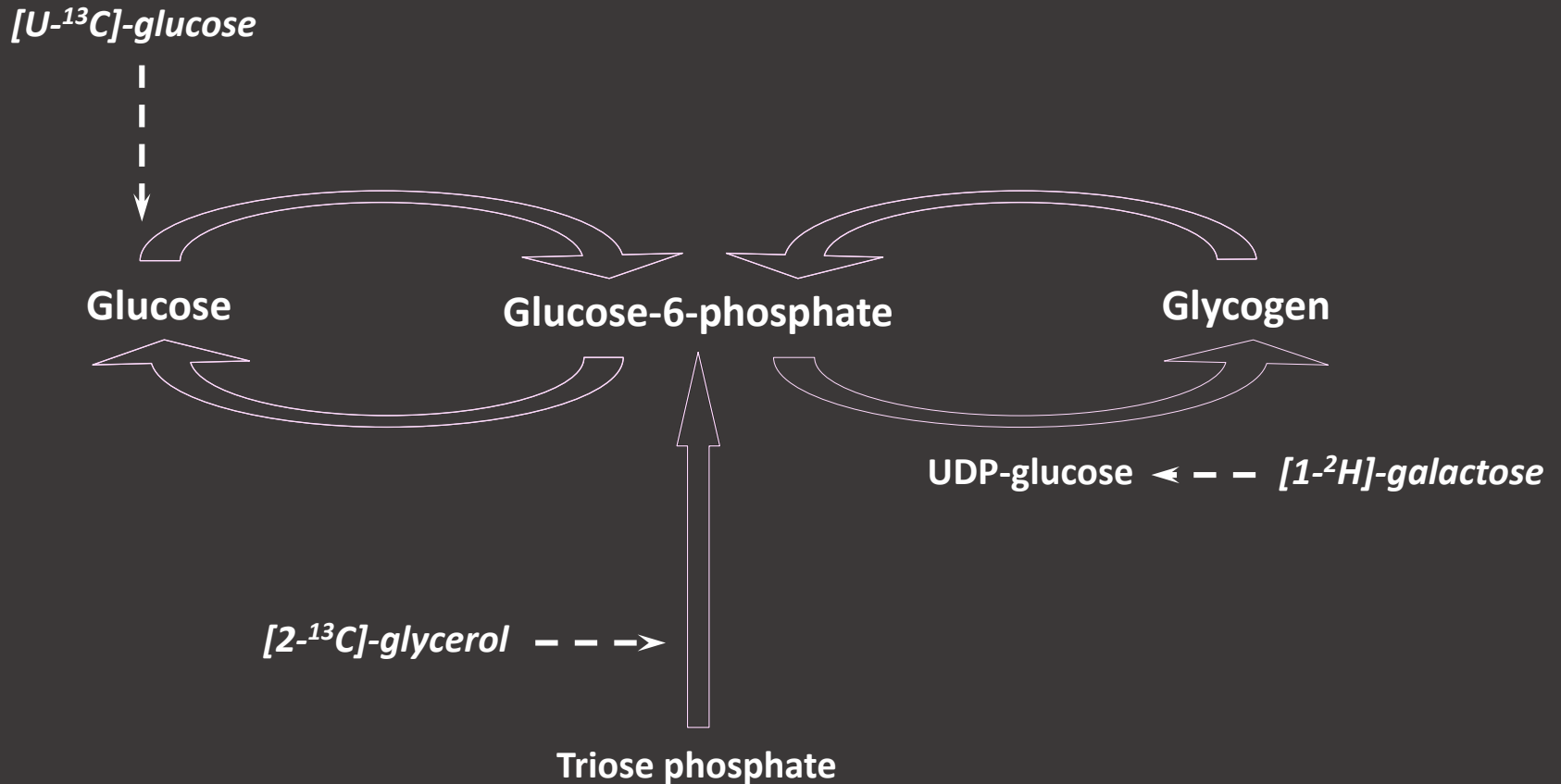
# Glycogen storage disease type I



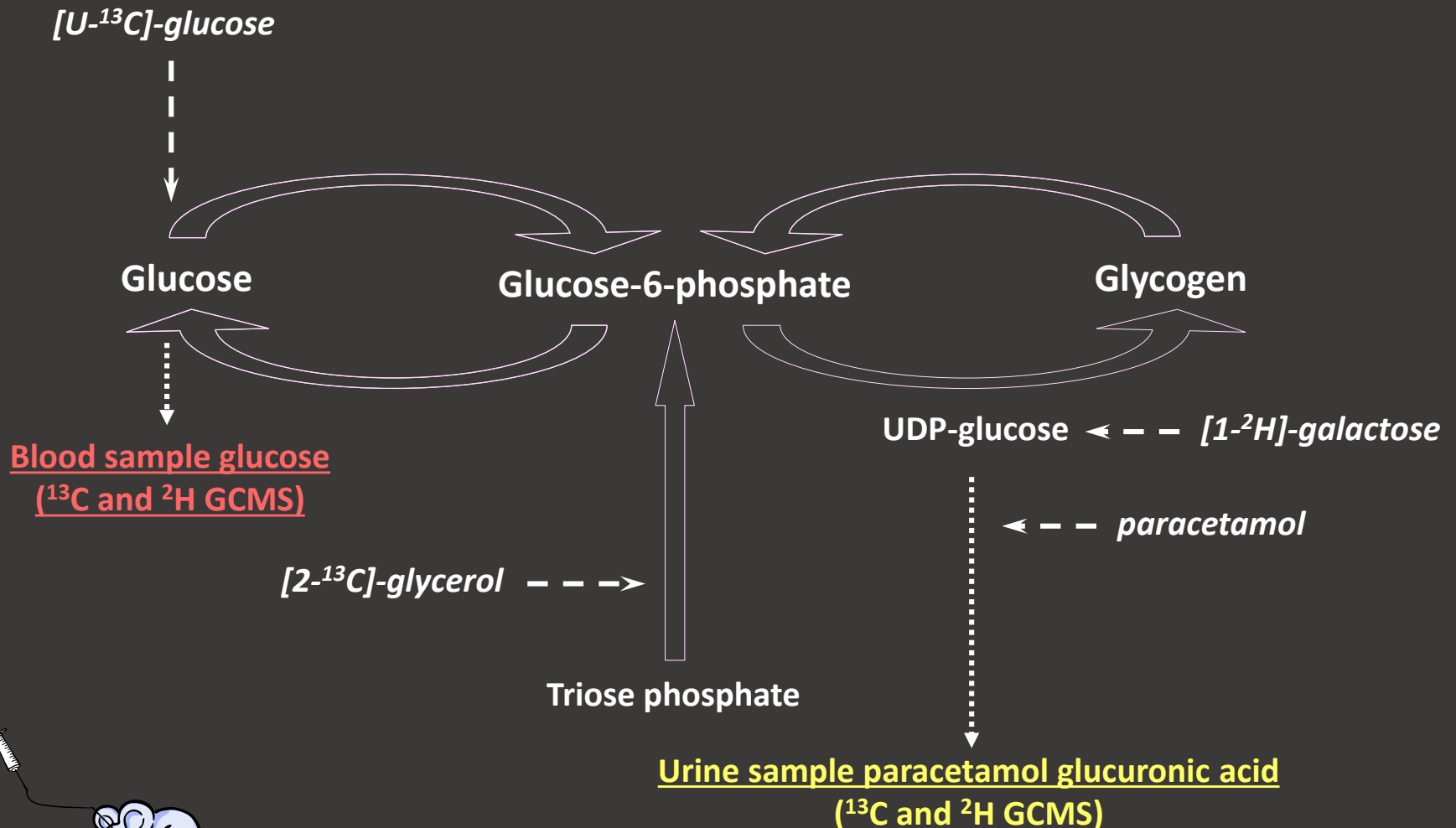
# Residual glucose production in L-G6pc<sup>-/-</sup> mice



# Quantification of hepatic glucose fluxes *in vivo*



# Quantification of hepatic glucose fluxes *in vivo*



# Increased glycogen synthase and -phosphorylase fluxes in L-G6pc<sup>-/-</sup> mice

