From the biochemical pieces to the nutritional puzzle: using meta-reactions in teaching and research

Jaap J. van Milgen

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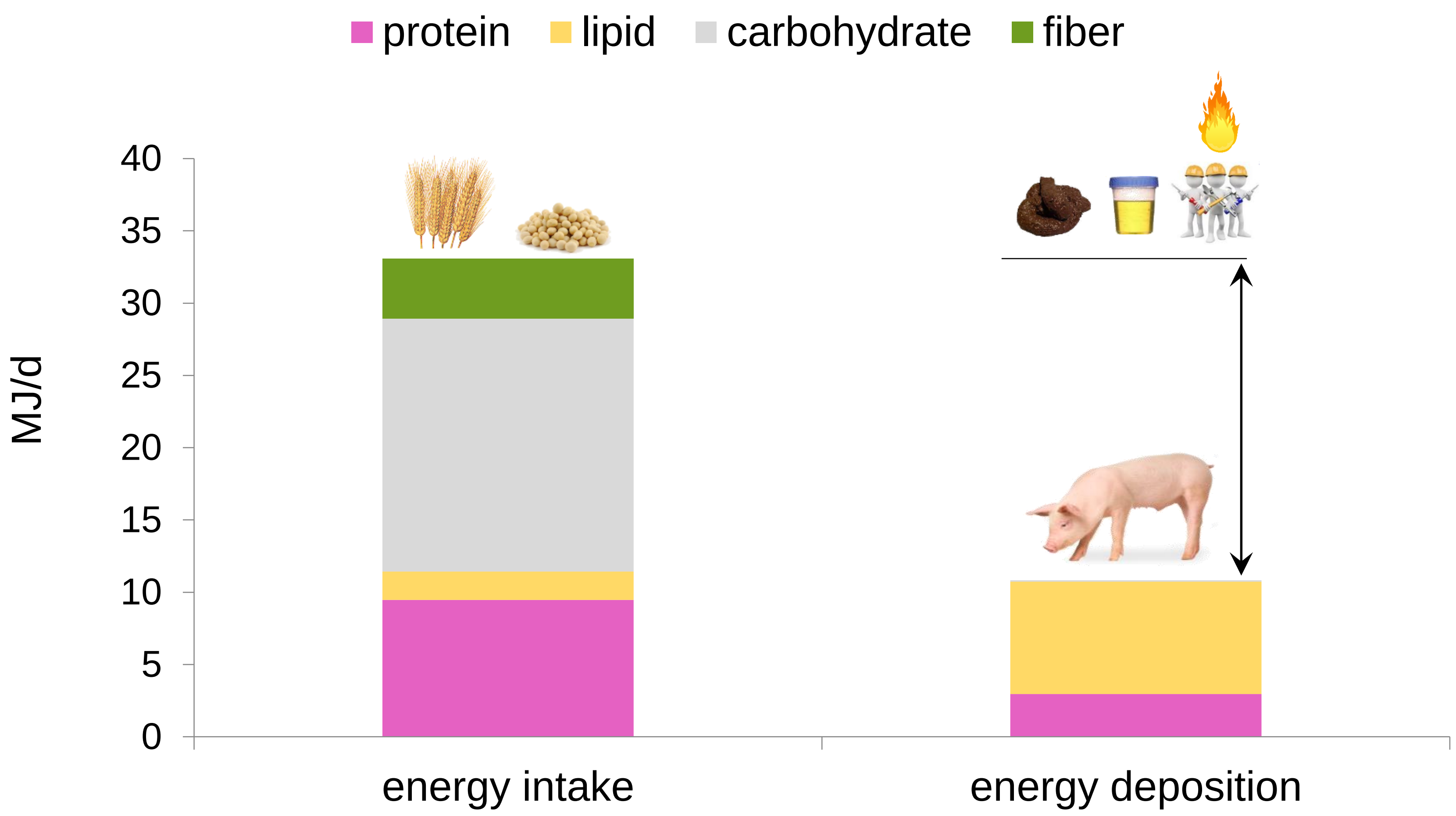
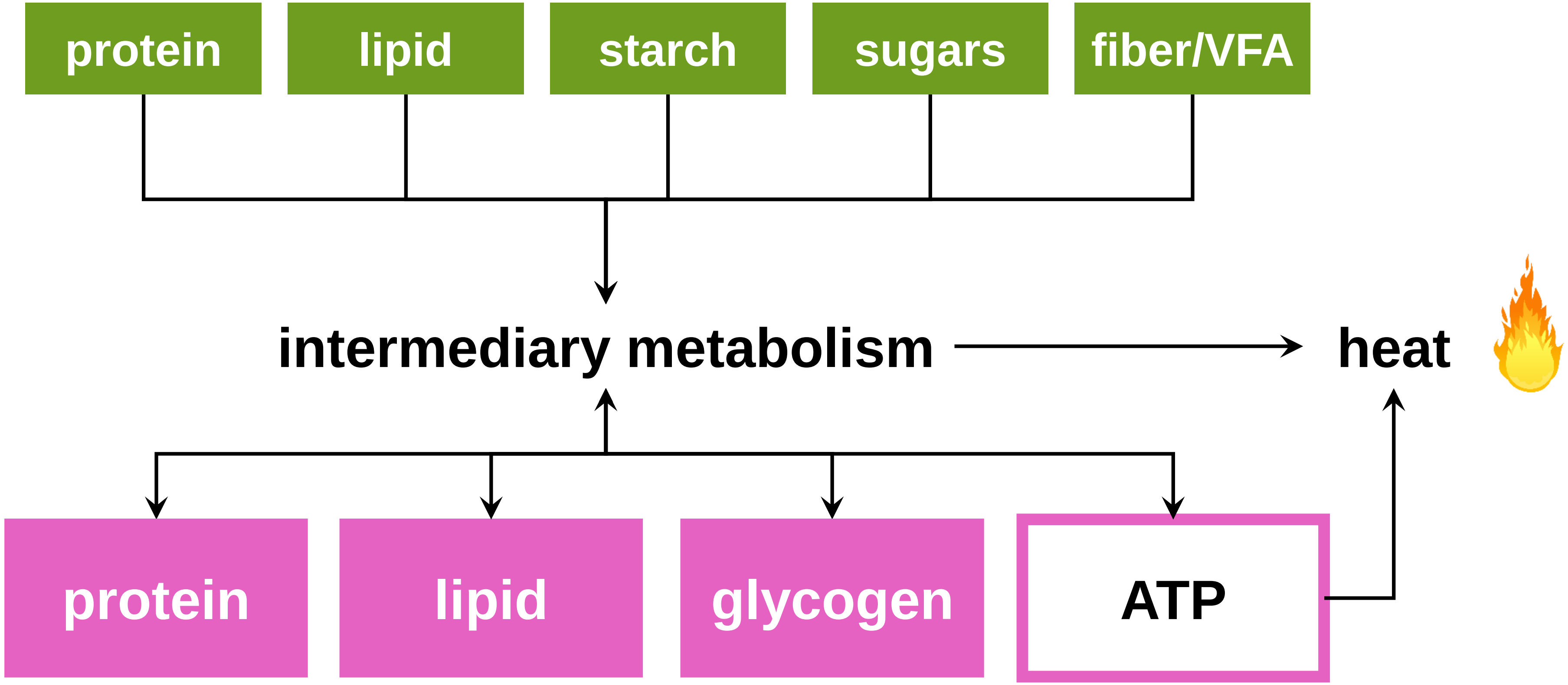
Objectives of the training

- Become more familiar with the biochemical background of the main metabolic pathways in animals (and humans)
- Be able to calculate the stoichiometric balance of complete pathways
- Understand the relationship between nutritional biochemistry and energy systems
- Be(come) aware that the energy "value" of a nutrient depends on its use

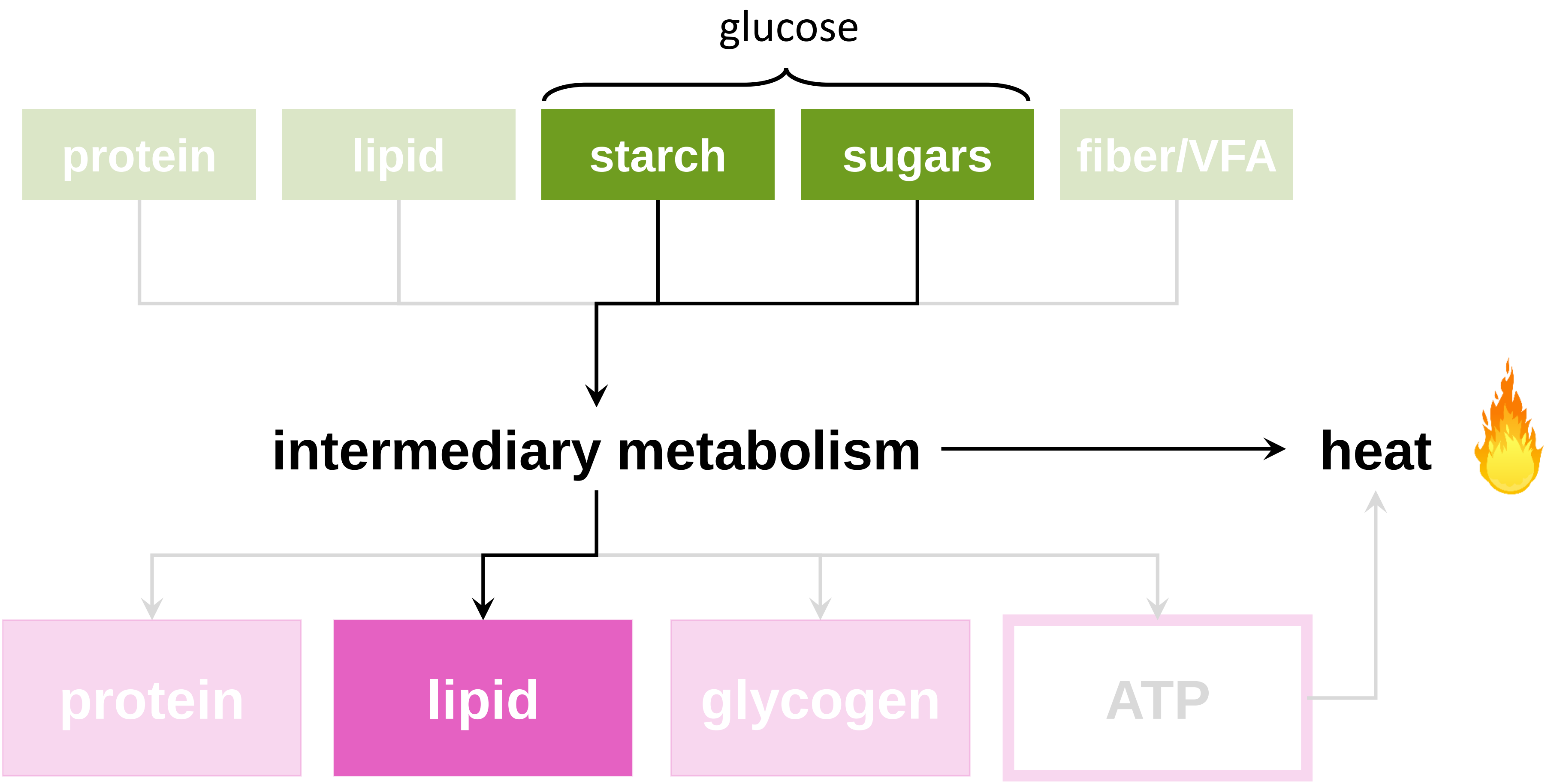
The energy efficiency of a growing pig



From nutrient intake to nutrient utilization



What is the energy efficiency of lipid deposition from glucose?

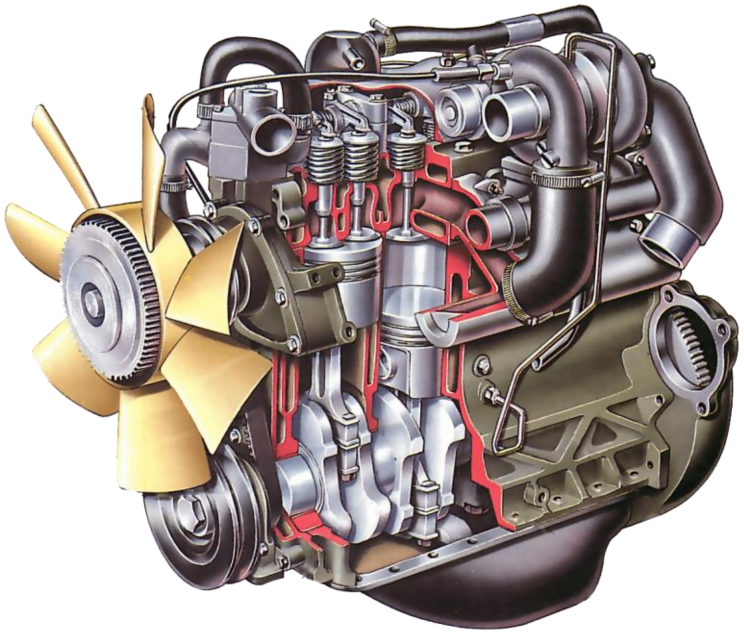


What is energy?

- Energy is not a nutrient
- Energy is a property of nutrients to “perform work”
- Energy exists in different forms, e.g.:
  - Chemical energy
  - Thermal energy
  - Kinetic energy
  - Radiant energy
- The chemical energy (heat of combustion) can be measured in a bomb calorimeter

ATP: the molecular currency of cellular energy

Glucose and other fuels will go in …



… but it is ATP that drives most of the mechanical, electrical, and chemical systems

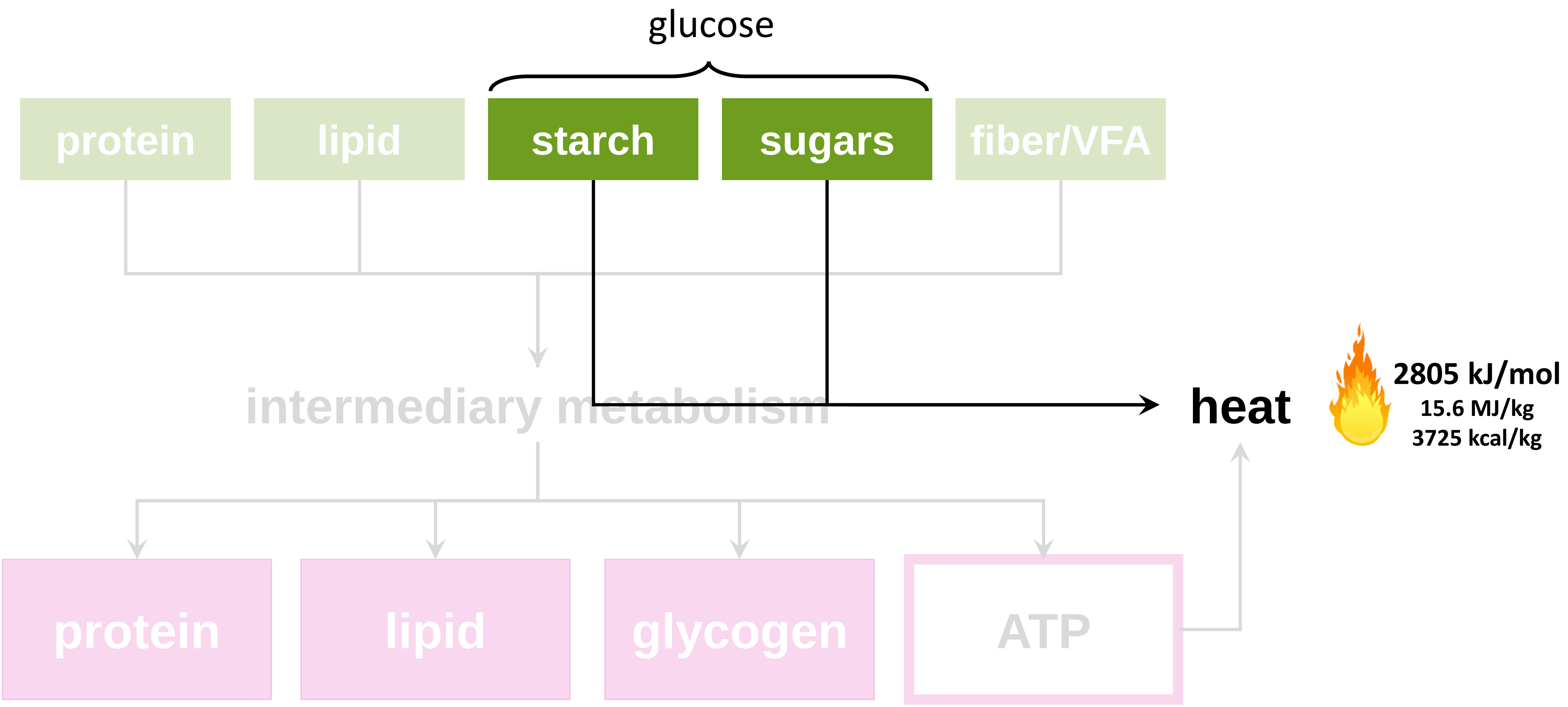
The catabolism of glucose (or: the catabolism of glucose via ATP)

$$1 \text{ glucose} + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{heat}$$

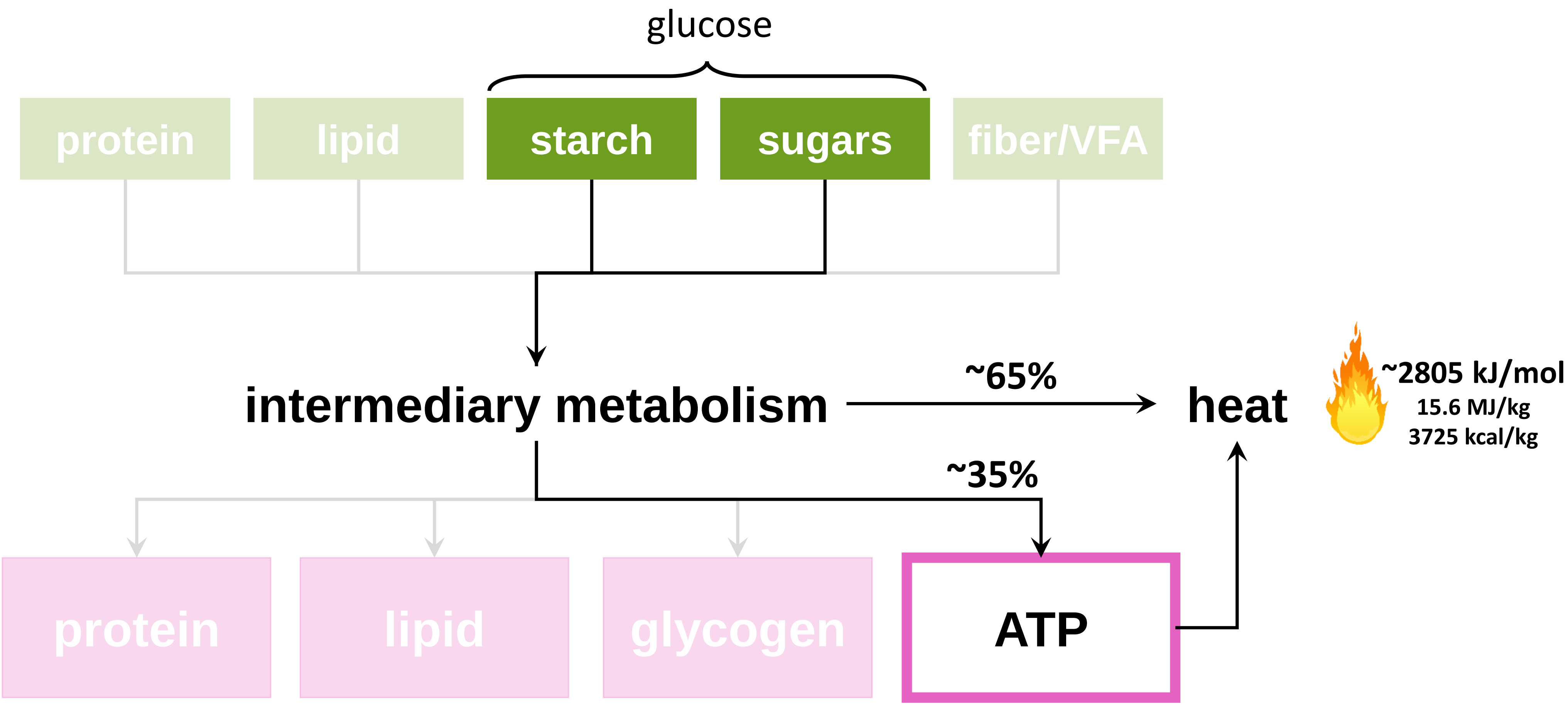
$$1 \text{ glucose} + 6\ O_2 + 32\ (ADP + P_i) \rightarrow 32\ ATP + 6\ CO_2 + 6\ H_2O + \text{heat}$$

$$\text{“work”} \rightarrow \text{heat}$$

How much energy is in glucose?



How much energy is in ATP when derived from glucose?



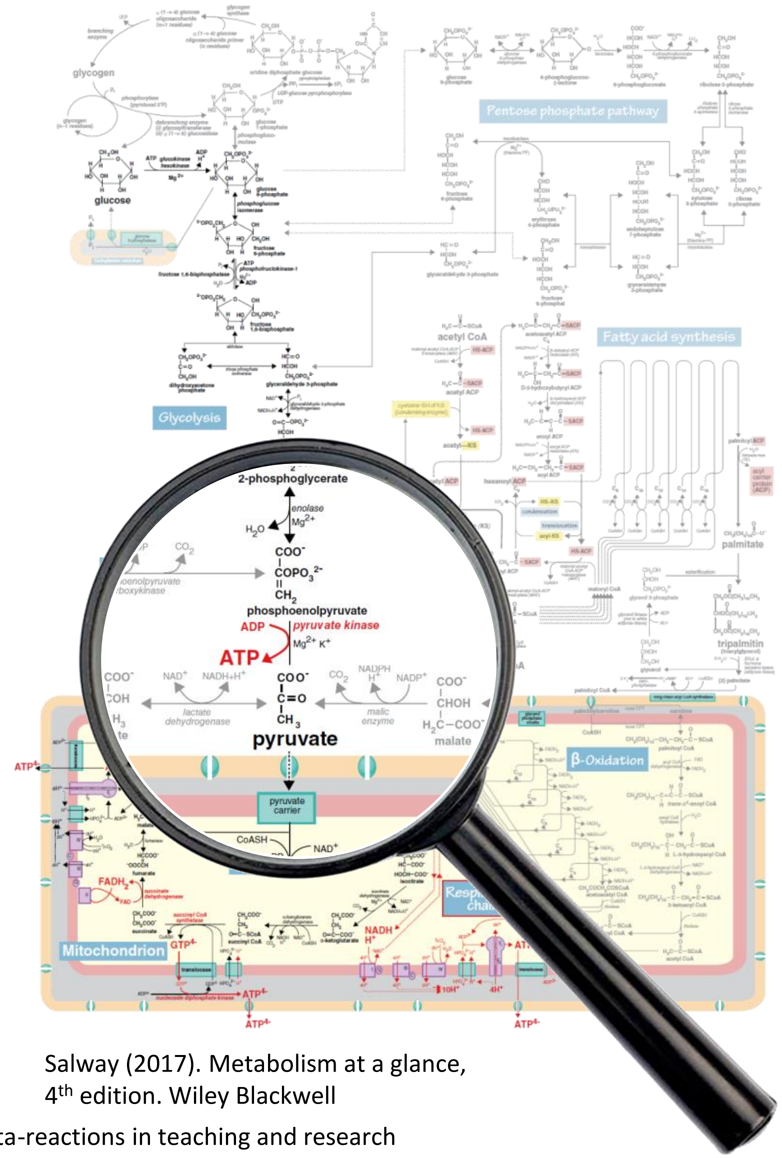
Have you heard of and what do you know about these metabolites?

- (ATP)
- NADH
- $FADH_2$
- NADPH

Have you heard of and what do you know about these metabolites?

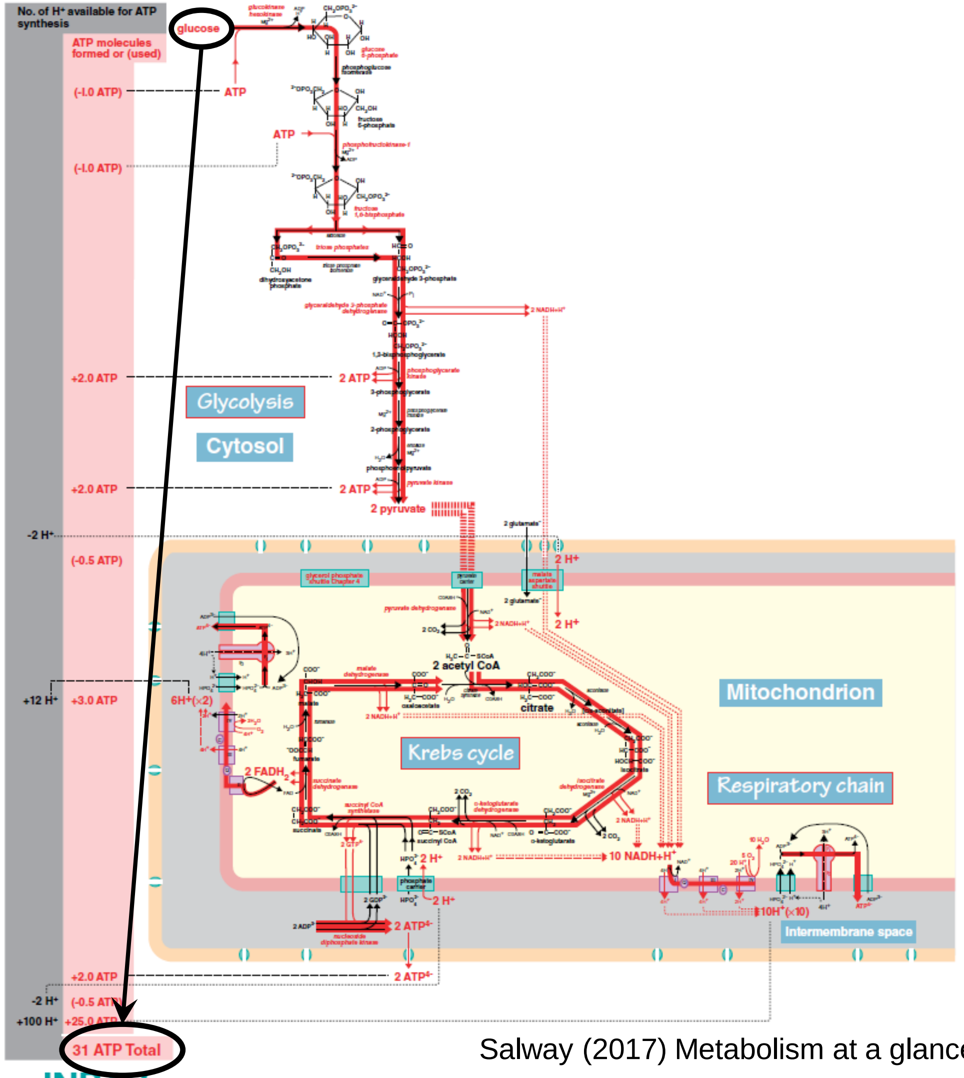
- Glucose 6-phosphate
- Pyruvate
- Acetyl-CoA
- α-Ketoglutarate
- Oxaloacetate

Here is pyruvate



Salway (2017). Metabolism at a glance, 4th edition. Wiley Blackwell

Finding your path in metabolism



Salway (2017) Metabolism at a glance

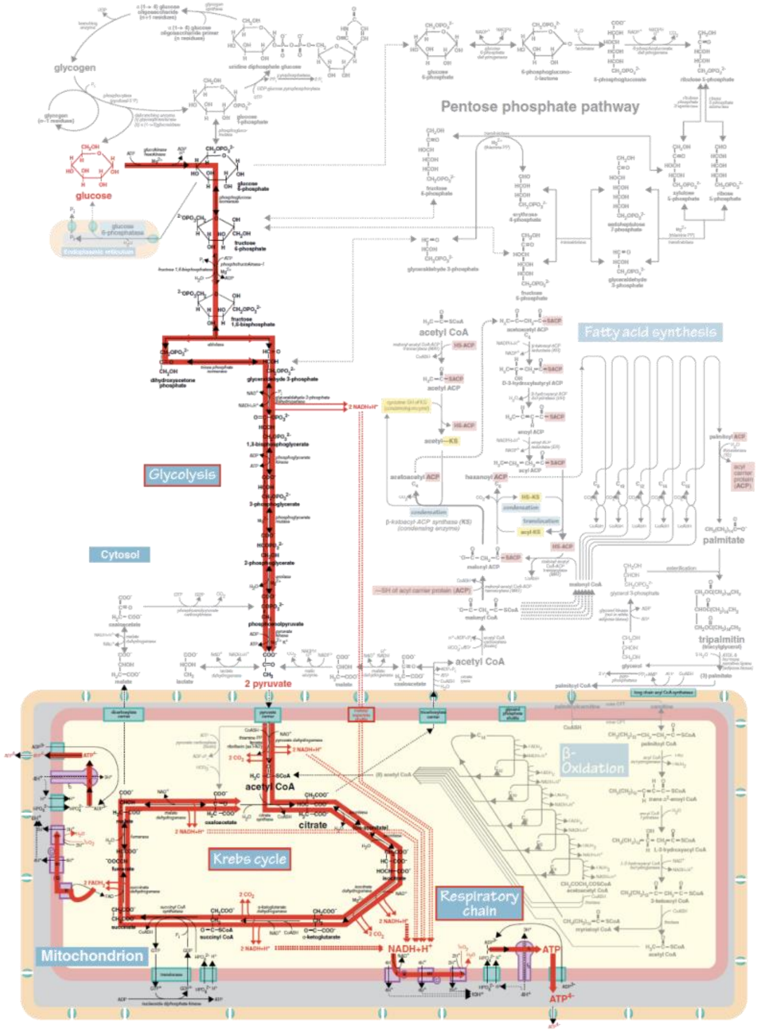
- 20 “carbon” metabolites involved:

  glucose, glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, dihydroxyacetone phosphate, glyceraldehyde 3-phosphate, 1,3-bisphosphoglycerate, 3-phosphoglycerate, 2-phosphoglycerate, phosphoenolpyruvate, pyruvate, acetyl-CoA, citrate, isocitrate, α-ketoglutarate, succinyl CoA, succinate, fumarate, malate, oxaloacetate

- And also:

  ATP, GTP, NADH, $FADH_2$, H^+ (proton motive force), O_2, CO_2
  Other cofactors
  Enzymes

Finding your path in metabolism



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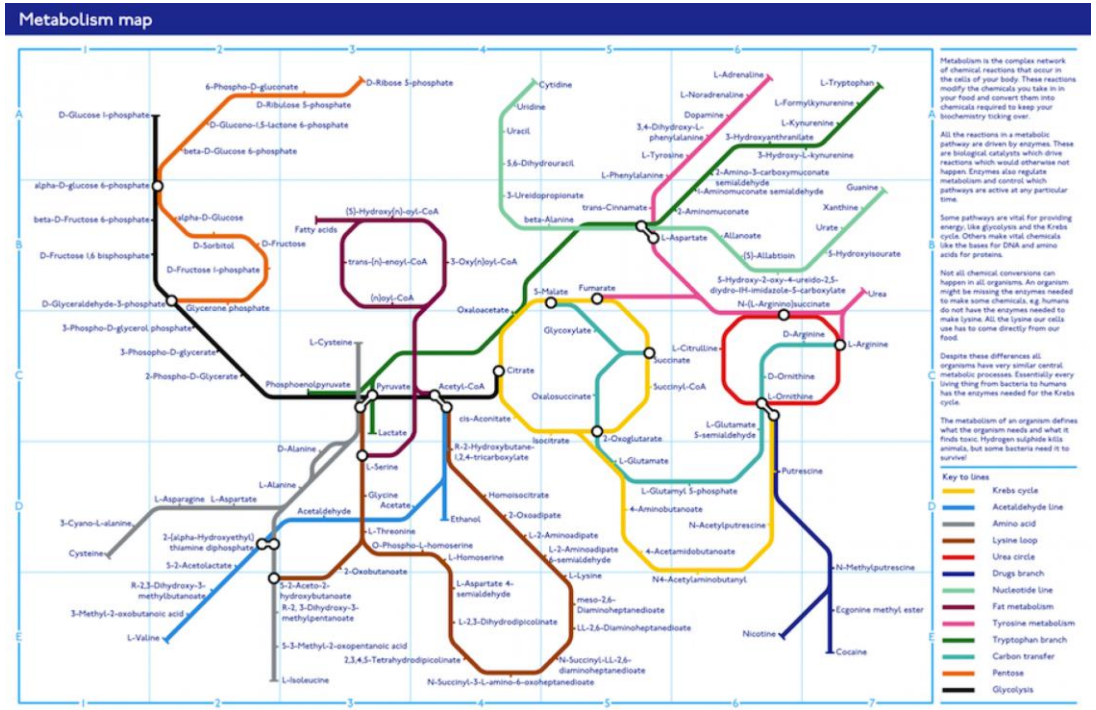
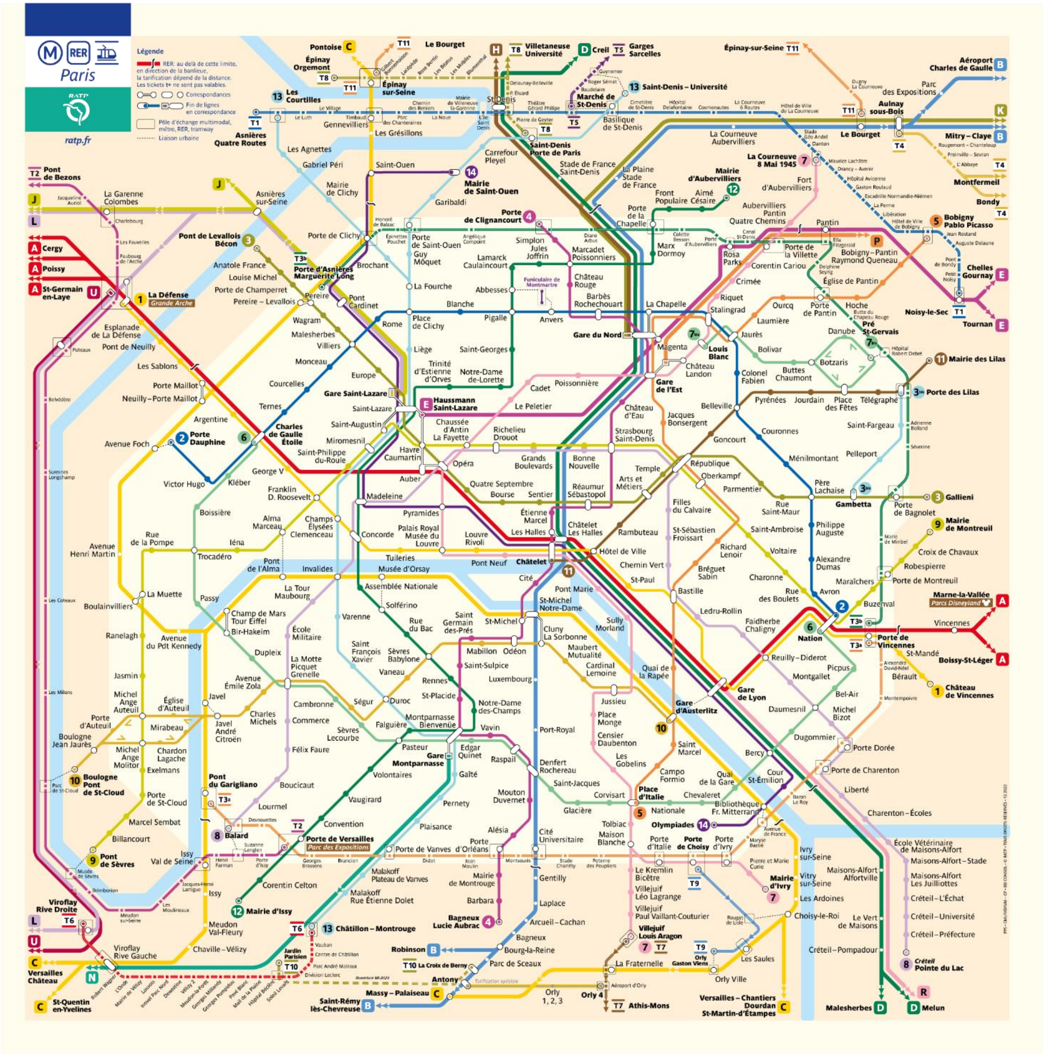
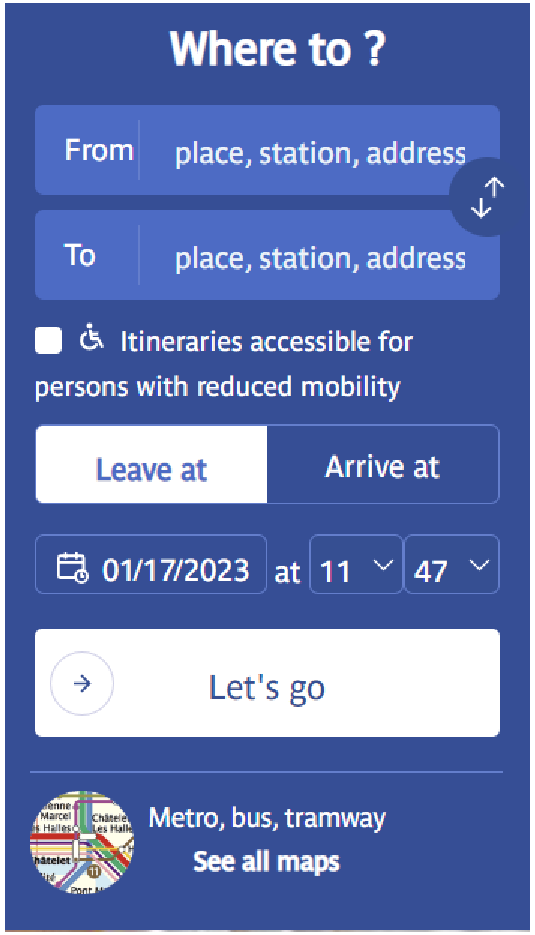


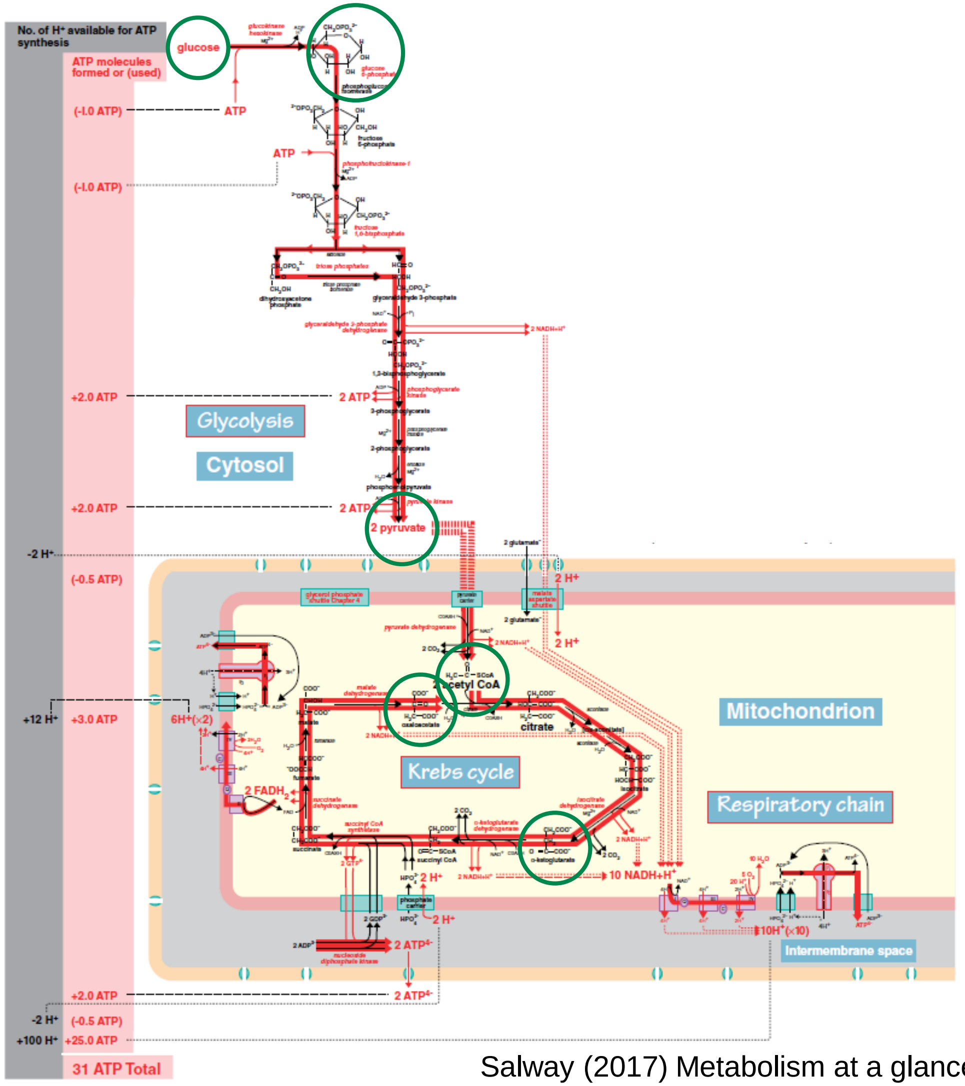
Richard Wheeler

Finding your path in Paris





Finding your path in metabolism



Salway (2017) Metabolism at a glance

Nutritional Models

Modeling Biochemical Aspects of Energy Metabolism in Mammals[1]

Jaap van Milgen[2]

INRA, Unité Mixte de Recherches sur le Veau et le Porc, 35590 Saint-Gilles, France

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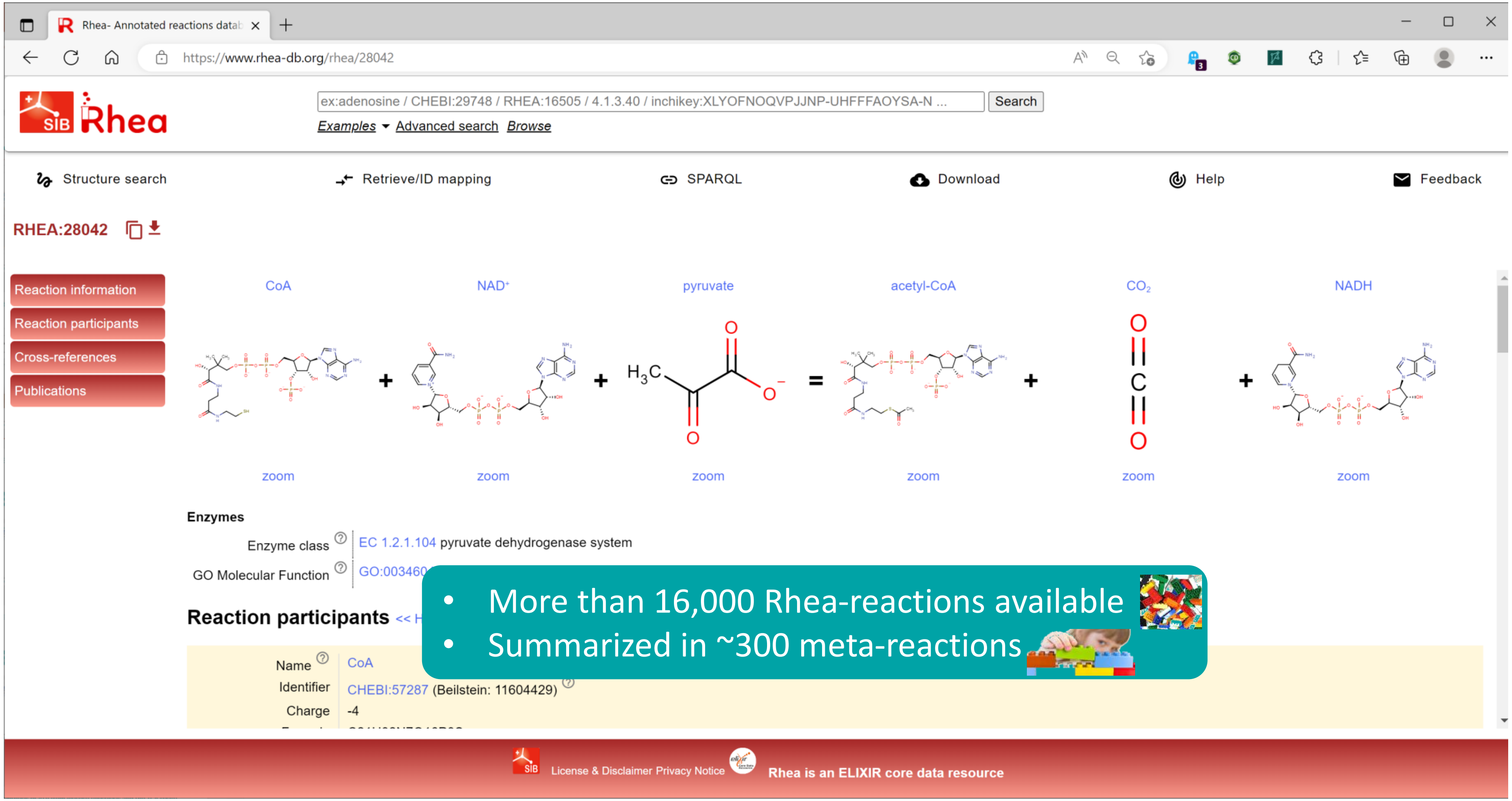
- Identification of “key pivots” of metabolism:
  - carbon-chain pivots
  - co-factors
- A stoichiometric matrix of “meta-reactions”:
  - substrate → intermediate
  - intermediate → intermediate
  - intermediate → product

- Allows constructing complete pathways from intermediate meta-reactions
- It quantifies <u>how</u> it happens, but not <u>if</u> it happens
- Biochemical bookkeeping

Rhea, a database with curated chemical and transport reactions



- More than 16,000 Rhea-reactions available
- Summarized in ~300 meta-reactions

An example of a meta-reaction (glucose 6-phosphate → pyruvate)

Meta-reaction ID

Rhea reaction IDs

Multiplyer

Meta-reaction	ReactionID	Flux	*Reaction*
2	11816	1	*glucose 6-phosphate → beta-D-fructose 6-phosphate*
2	16109	1	*ATP + beta-D-fructose 6-phosphate → ADP + H^+ + beta-D-fructose 1,6-bisphosphate*
2	14729	1	*beta-D-fructose 1,6-bisphosphate → dihydroxyacetone phosphate + D-glyceraldehyde 3-phosphate*
2	18585	-1	*D-glyceraldehyde 3-phosphate ← dihydroxyacetone phosphate*
2	10300	2	*P_i + NAD^+ + D-glyceraldehyde 3-phosphate → NADH + H^+ + (2R)-3-phospho-glyceroyl phosphate*
2	14801	-2	*ATP + (2R)-3-phosphoglycerate ← ADP + (2R)-3-phospho-glyceroyl phosphate*
2	15901	-2	*(2R)-2-phosphoglycerate ← (2R)-3-phosphoglycerate*
2	10164	2	*(2R)-2-phosphoglycerate → phosphoenolpyruvate + H_2O*
2	18157	-2	*pyruvate + ATP ← ADP + H^+ + phosphoenolpyruvate*

Meta-reaction: glucose 6-phosphate → pyruvate

1 glucose 6-phosphate + 2 NAD^+ + 3 ADP + 2 P_i → 2 pyruvate + 3 ATP + 2 NADH + 1 H^+ + 2 H_2O

Meta-reactions, based on Rhea, to construct complete pathways

The pathway and heat production (if available)

Drop-down lists to select the meta-reactions

The (full) meta-reactions and reaction IDs are shown

glucose + 2 ADP + 2 P_i + 2 NAD^+ → 2 pyruvate + 2 ATP + 2 NADH + 2 H^+ + 2 H_2O (Q not available)

Detail level	**Intermediate**
Energy unit	**kJ**

ID	Type	Metabolite	As a …	To/From …	Meta-reaction	Flux	Step	ID met
	carbohydrates	glucose	substrate	glucose 6-phosphate	glucose + ATP → glucose 6-phosphate + ADP + H^+	1	1	1
	intermediary metabolites	glucose 6-phosphate	substrate	pyruvate	glucose 6-phosphate + 3 ADP + 2 P_i + 2 NAD^+ → 2 pyruvate + 3 ATP + 2 NADH + H^+ + 2 H_2O	1	2	2

Rhea reactions are balanced (e.g., for C, N, O, H, S)

Meta-reactions and pathways are therefore also balanced

Meta-reactions, based on Rhea, to construct complete pathways

List the meta-reactions in the (logical) order you want

The pathway and heat production (if available)

Multiply the meta-reactions to balance the pathway

Indicate the detail level to see fewer or more items in the lists

glucose + 2 ADP + 2 P_i + 2 NAD^+ → 2 pyruvate + 2 ATP + 2 NADH + 2 H^+ + 2 H_2O (Q not available)

	Detail level	**Intermediate**
	Energy unit	**kJ**

ID	Type	Metabolite	As a …	To/From …	Meta-reaction	Flux	Step	ID met
	carbohydrates	glucose	substrate	glucose 6-phosphate	glucose + ATP → glucose 6-phosphate + ADP + H^+	1	1	1
	intermediary metabolites	glucose 6-phosphate	substrate	pyruvate	glucose 6-phosphate + 3 ADP + 2 P_i + 2 NAD^+ → 2 pyruvate + 3 ATP + 2 NADH + H^+ + 2 H_2O	1	2	2

Rhea reactions are balanced (e.g., for C, N, O, H, S)

Meta-reactions and pathways are therefore also balanced

The fate of the metabolites

Metabolites that are substrates or products

Intermediates that you have to metabolize further

Metabolites that you can ignore

$$1\ \text{glucose} + 2\ NAD^+ + 2\ ADP + 2\ P_i \rightarrow 2\ \text{pyruvate} + 2\ ATP + 2\ NADH + 2\ H^+ + 2\ H_2O$$

glucose + 2 ADP + 2 P_i + 2 NAD^+ → 2 pyruvate + 2 ATP + 2 NADH + 2 H^+ + 2 H_2O (Q not available)								
	Detail level	**Intermediate**						
	Energy unit	**kJ**						
ID	**Type**	**Metabolite**	**As a …**	**To/From …**	**Meta-reaction**	**Flux**	**Step**	**ID met**
	carbohydrates	glucose	substrate	glucose 6-phosphate	glucose + ATP → glucose 6-phosphate + ADP + H^+	1	1	1
	intermediary metabolites	glucose 6-phosphate	substrate	pyruvate	glucose 6-phosphate + 3 ADP + 2 P_i + 2 NAD^+ → 2 pyruvate + 3 ATP + 2 NADH + H^+ + 2 H_2O	1	2	2

Rhea reactions are balanced (e.g., for C, N, O, H, S)
The meta-reactions and the pathway are therefore also balanced

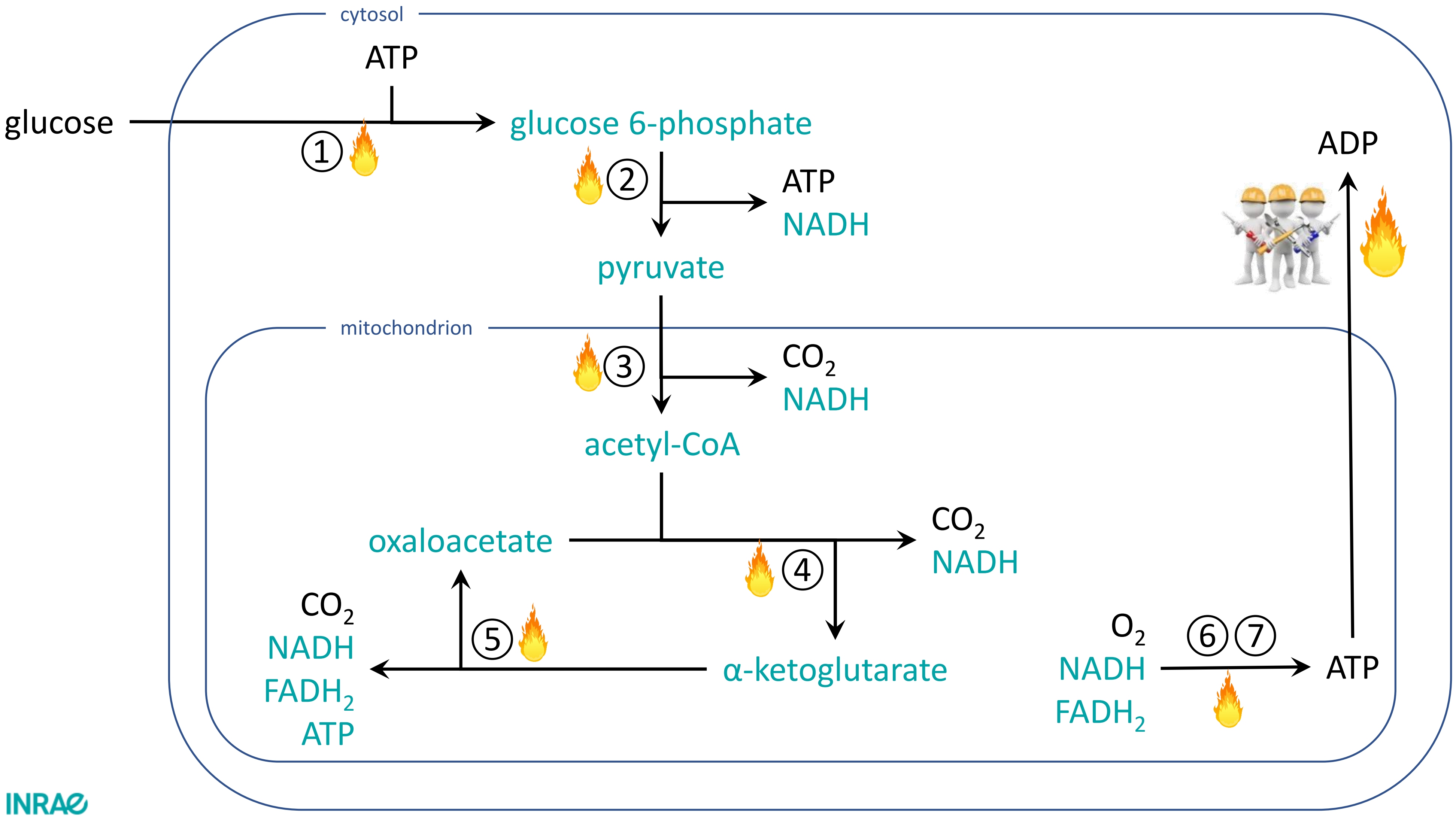
The fate of the metabolites (examples)

Substrates and products	Intermediate metabolites	Metabolites to be ignored
carbohydrates	glucose 6-phosphate	
amino acids	pyruvate	
fatty acids and fats	acetyl-CoA	
NH_4^+, urea, uric acid	α-ketoglutarate	
O_2, CO_2, H_2O	oxaloacetate	
ATP		ADP, P_i
	GTP, NADH, NADPH, $FADH_2$	GDP, NAD^+, $NADP^+$, FAD
	N^5,N^{10}-methylene THF	THF

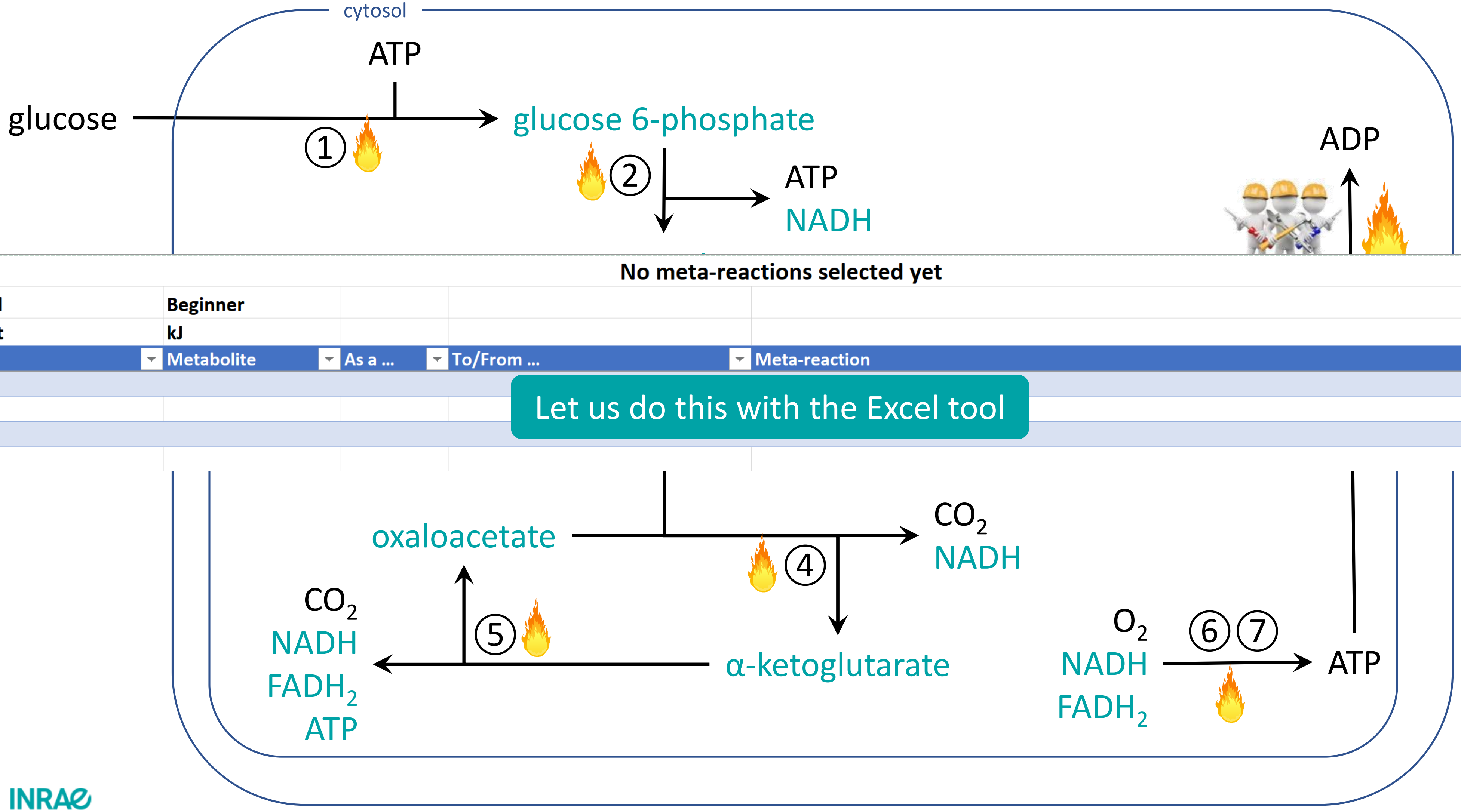
Five sessions

1. Familiarization with the Excel tool and the ATP synthesis from glucose and palmitate (C16:0)
2. ATP synthesis from amino acids, urea cycle
3. Fatty acid and fat synthesis
4. Energy storage, mobilization and transfer
5. Exploring further or 1-carbon metabolism (Met, Ser, Gly) and the uric acid cycle

The synthesis of ATP from glucose (glycolysis and the TCA cycle)



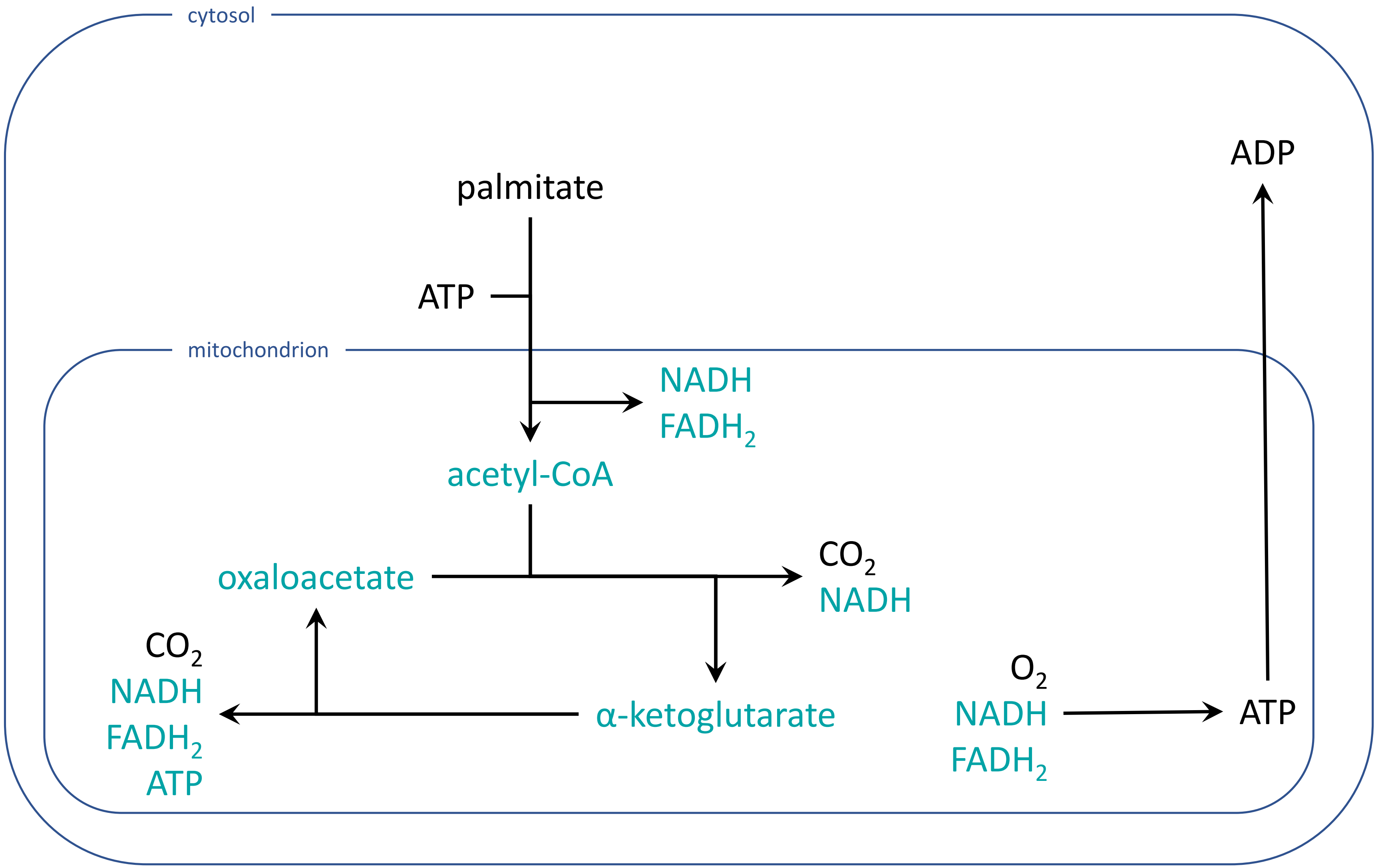
The synthesis of ATP from glucose (glycolysis and the TCA cycle)



			No meta-reactions selected yet				
	Detail level	**Beginner**					
	Energy unit	**kJ**					
ID	**Type**	**Metabolite**	**As a ...**	**To/From ...**	**Meta-reaction**	**Flux**	**Step**

Let us do this with the Excel tool

The synthesis of ATP from palmitate (C16:0)



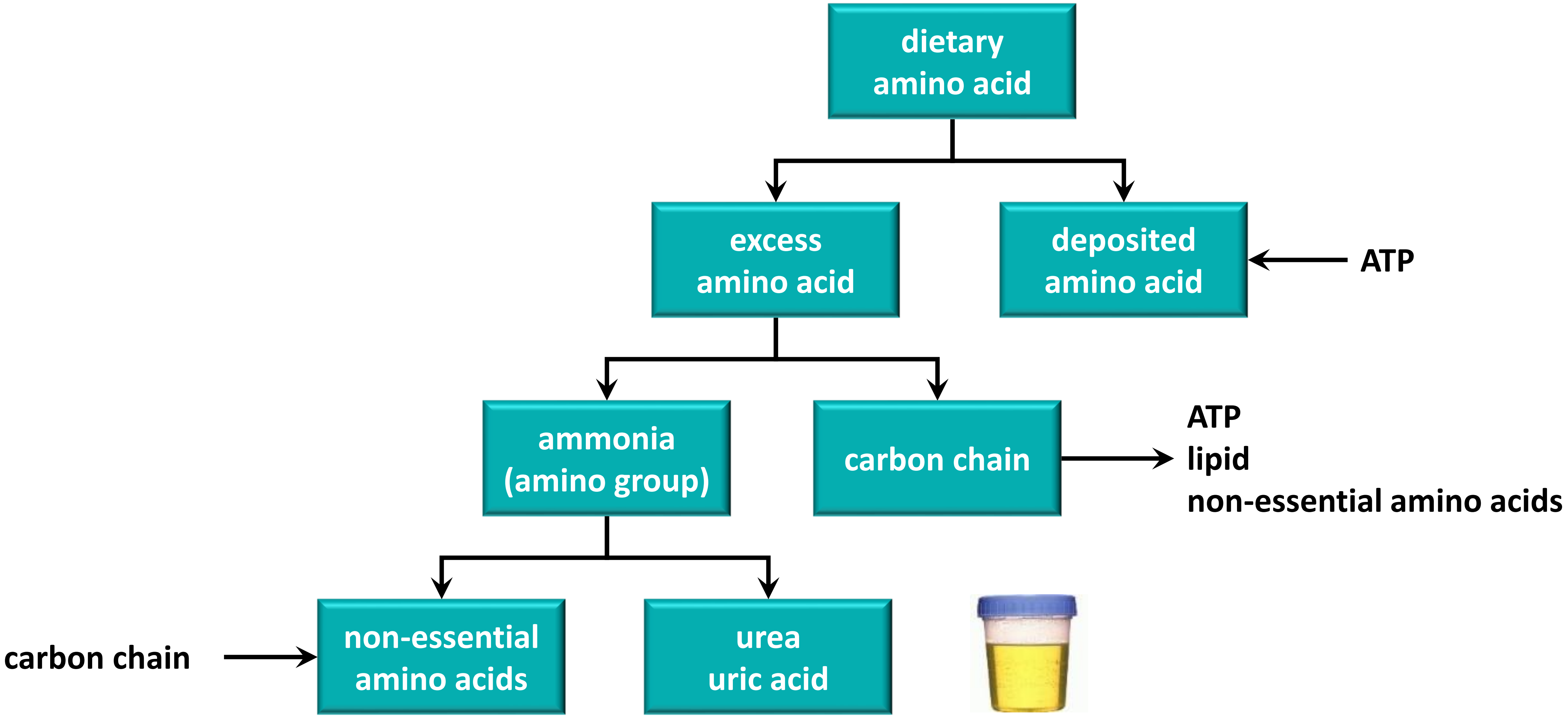
Exercises session 1

- Familiarize yourself with the Excel tool:
- Recalculate the ATP production from glucose (as we have done today)
- Calculate how much ATP can be made from 1 palmitate (C16:0)
- Calculate how much energy is required to make 1 ATP from glucose and palmitate
- Calculate the respiratory quotients of these pathways
- Calculate the energy efficiencies of these pathways

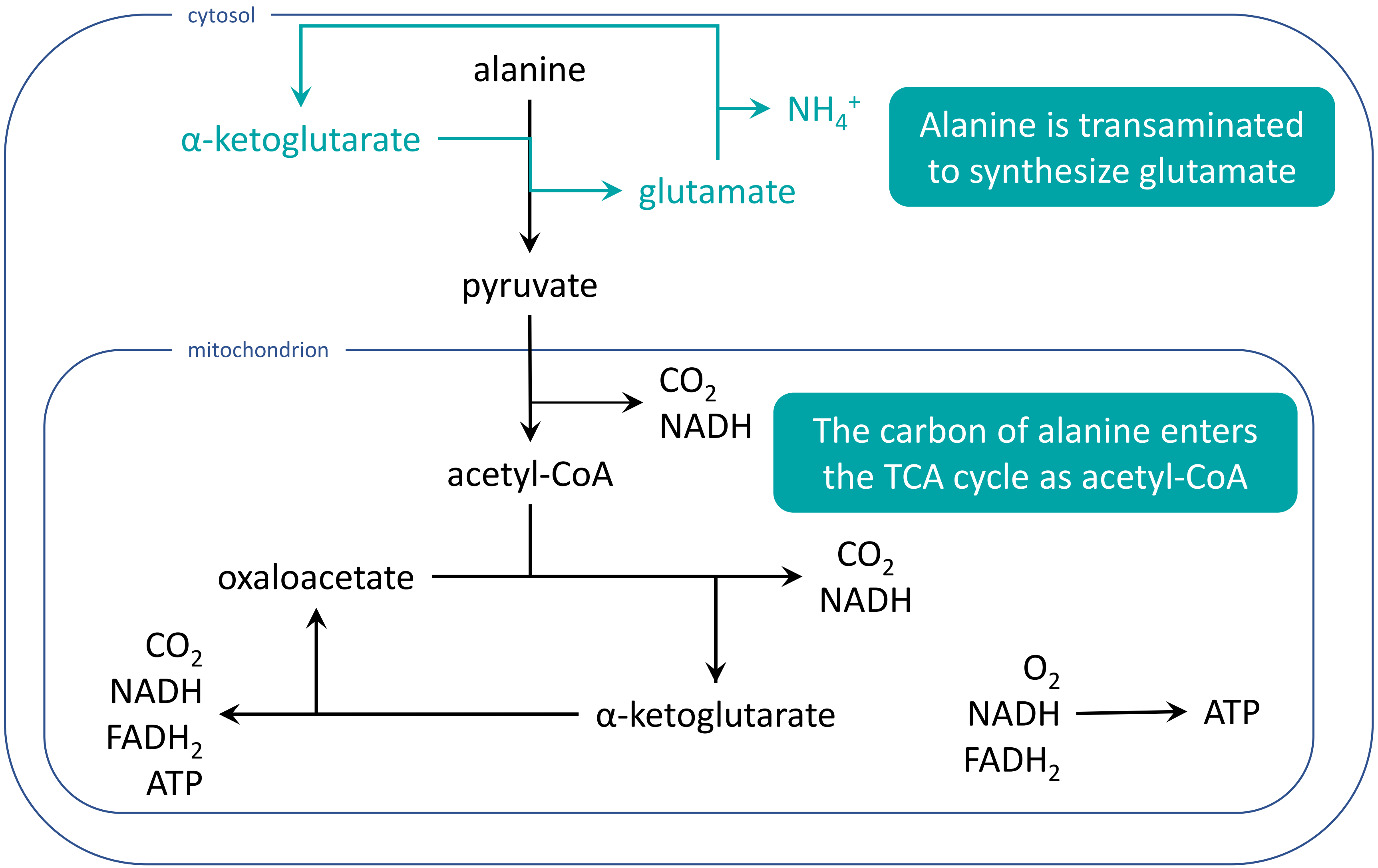
Program session 2

- Previous exercises
- ATP synthesis from amino acids
- The cost of urea synthesis
- Glucose synthesis from amino acids (gluconeogenesis)

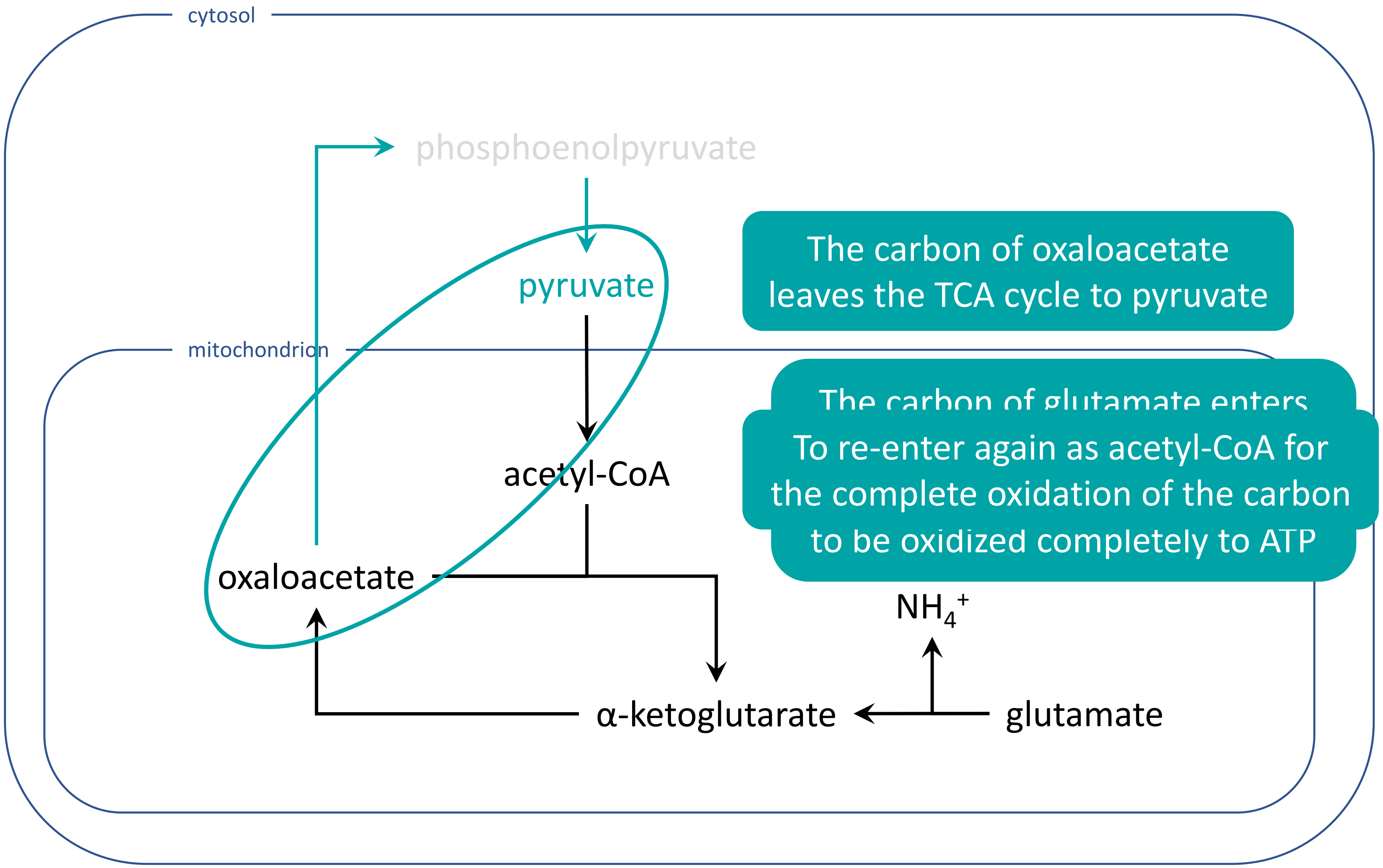
The energy value of an amino acid depends on its utilization



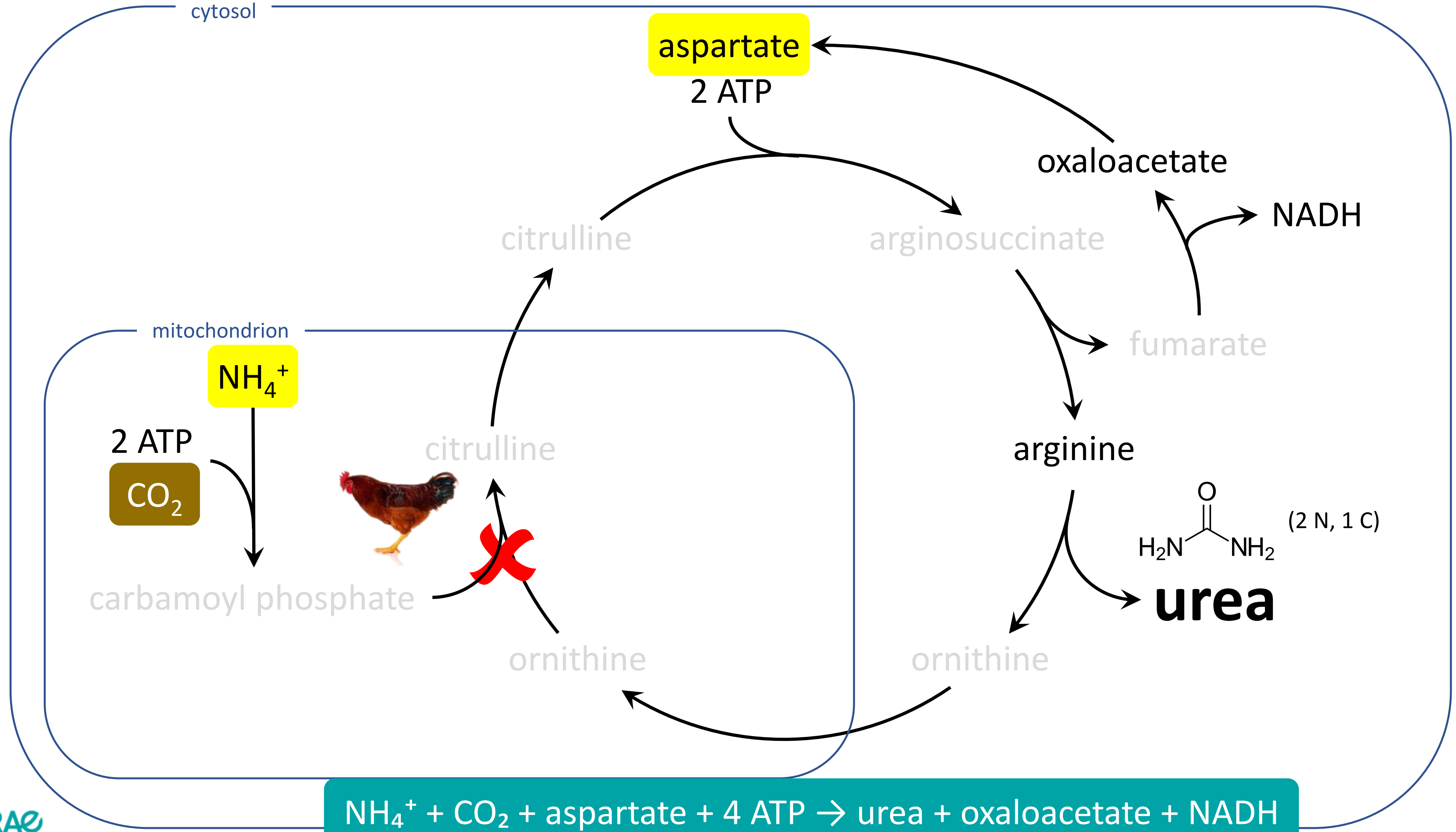
The synthesis of ATP from alanine



The synthesis of ATP from glutamate

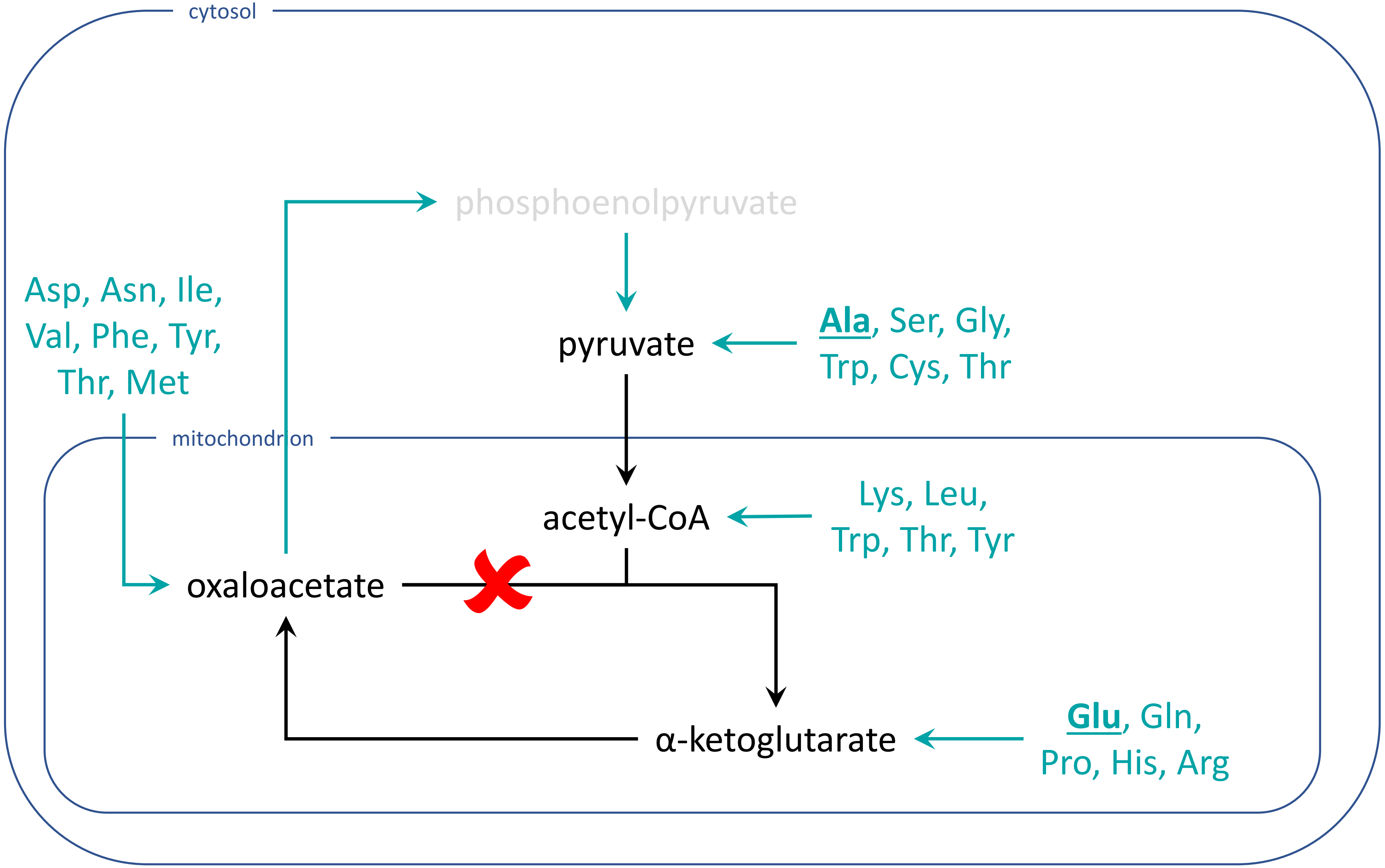


Urea cycle (Krebs and Henseleit, 1932)

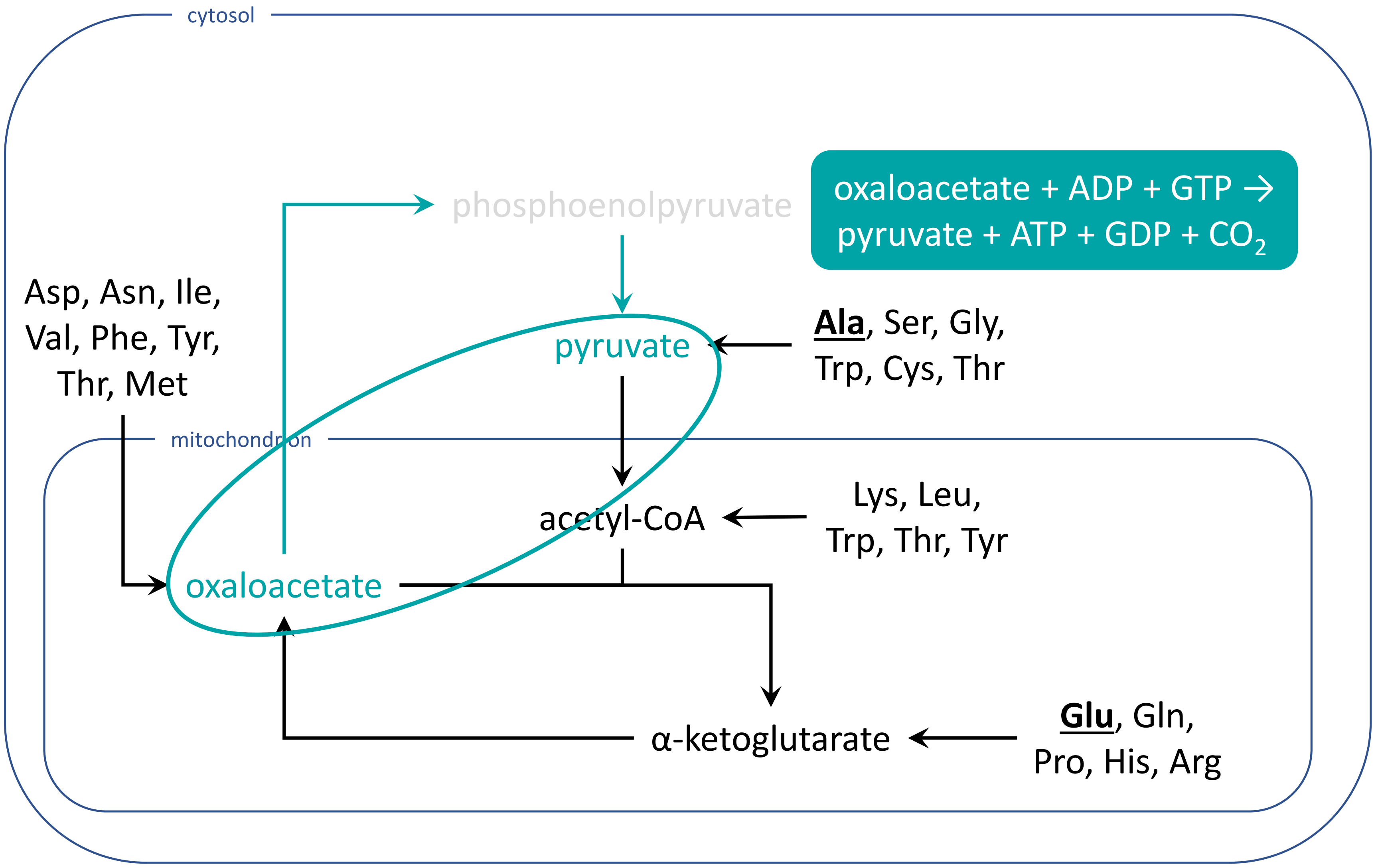


$NH_4^+ + CO_2 +$ aspartate + 4 ATP → urea + oxaloacetate + NADH

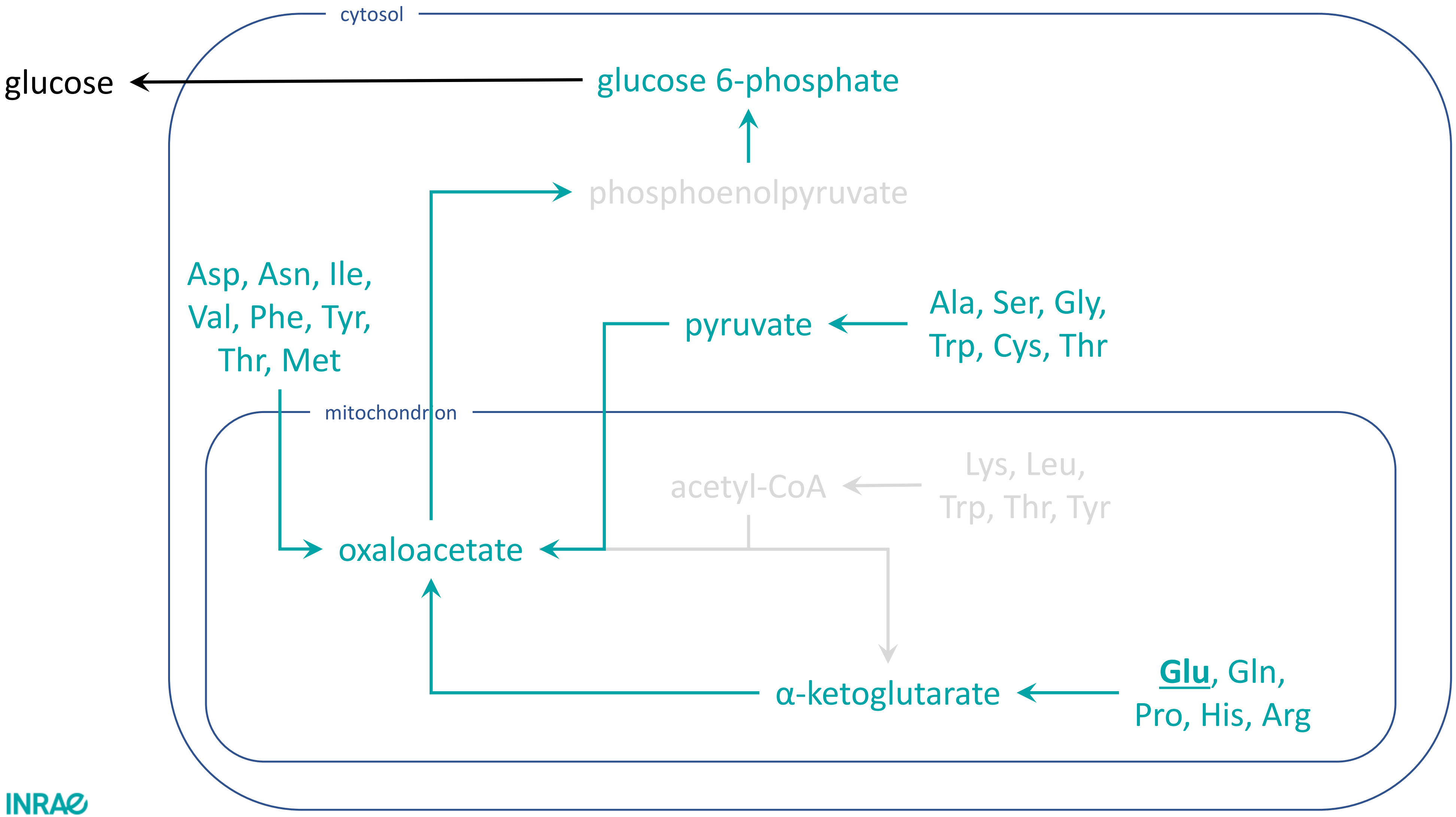
ATP synthesis from amino acids



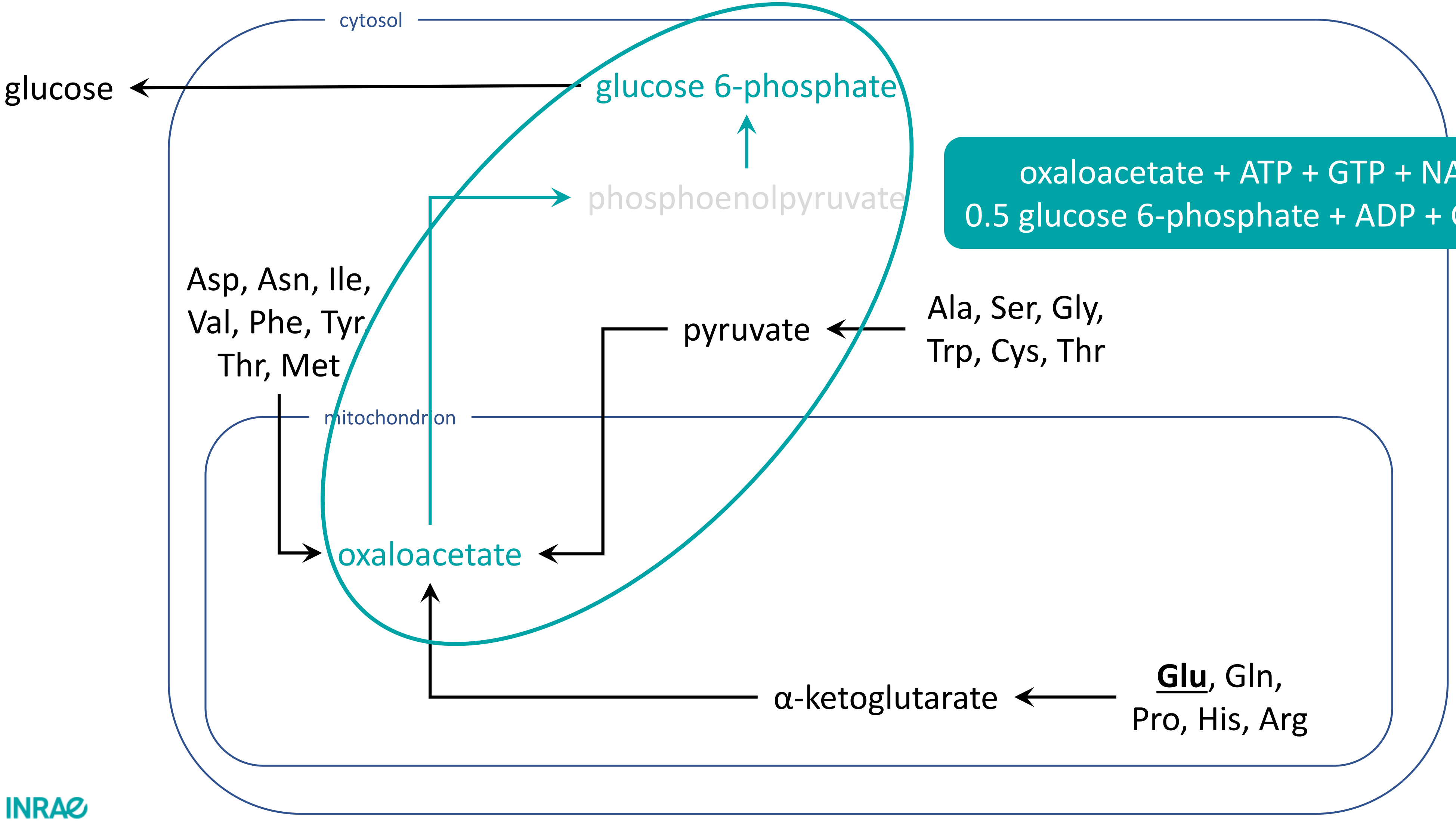
ATP synthesis from amino acids



Gluconeogenesis from amino acids



Gluconeogenesis from amino acids



oxaloacetate + ATP + GTP + NADH → 0.5 glucose 6-phosphate + ADP + GDP + CO_2

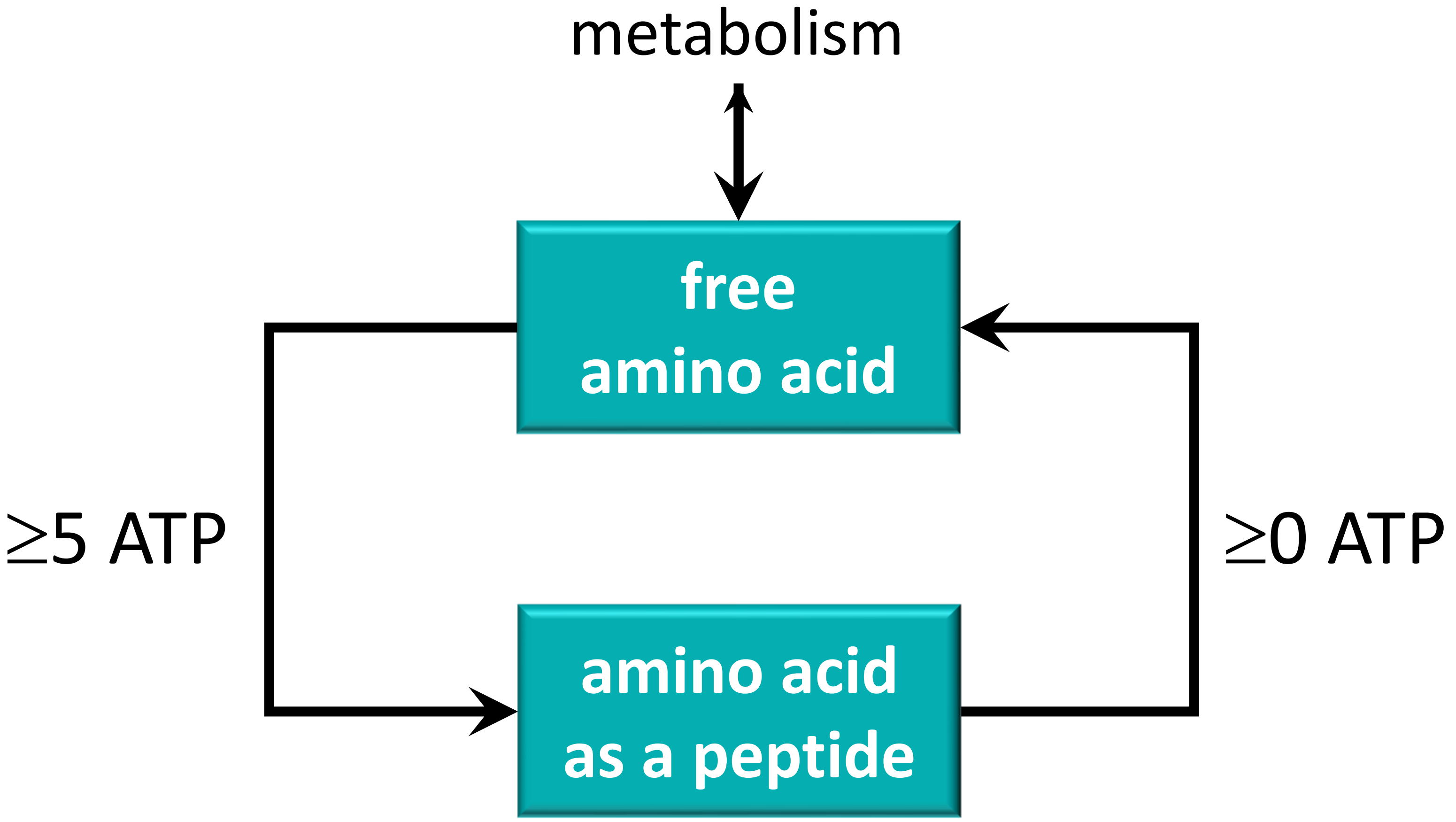
Exercises session 2

- ATP synthesis from alanine and glutamate with NH_4^+ as end-product
- Urea synthesis
- ATP synthesis from glutamate with urea as end-product
- Glucose synthesis from glutamate (gluconeogenesis)

Program session 3

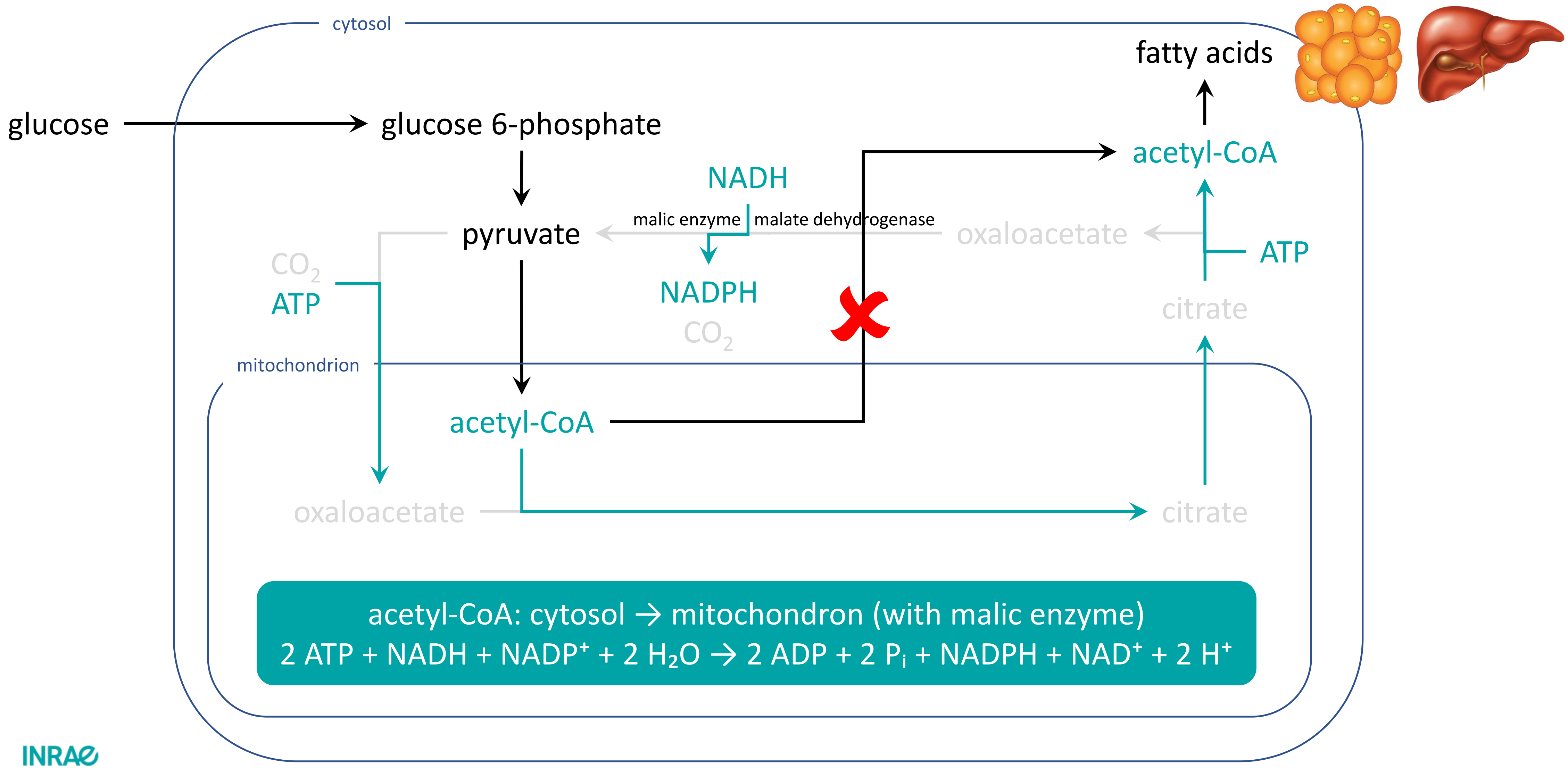
- Exercises of the previous session
- Fatty acid and fat synthesis from glucose
- Fat synthesis from dietary fat

The energy cost of peptide synthesis and turnover



The fractional protein synthesis rates vary from 4%/day in muscle to more than 100%/day for splanchnic tissues

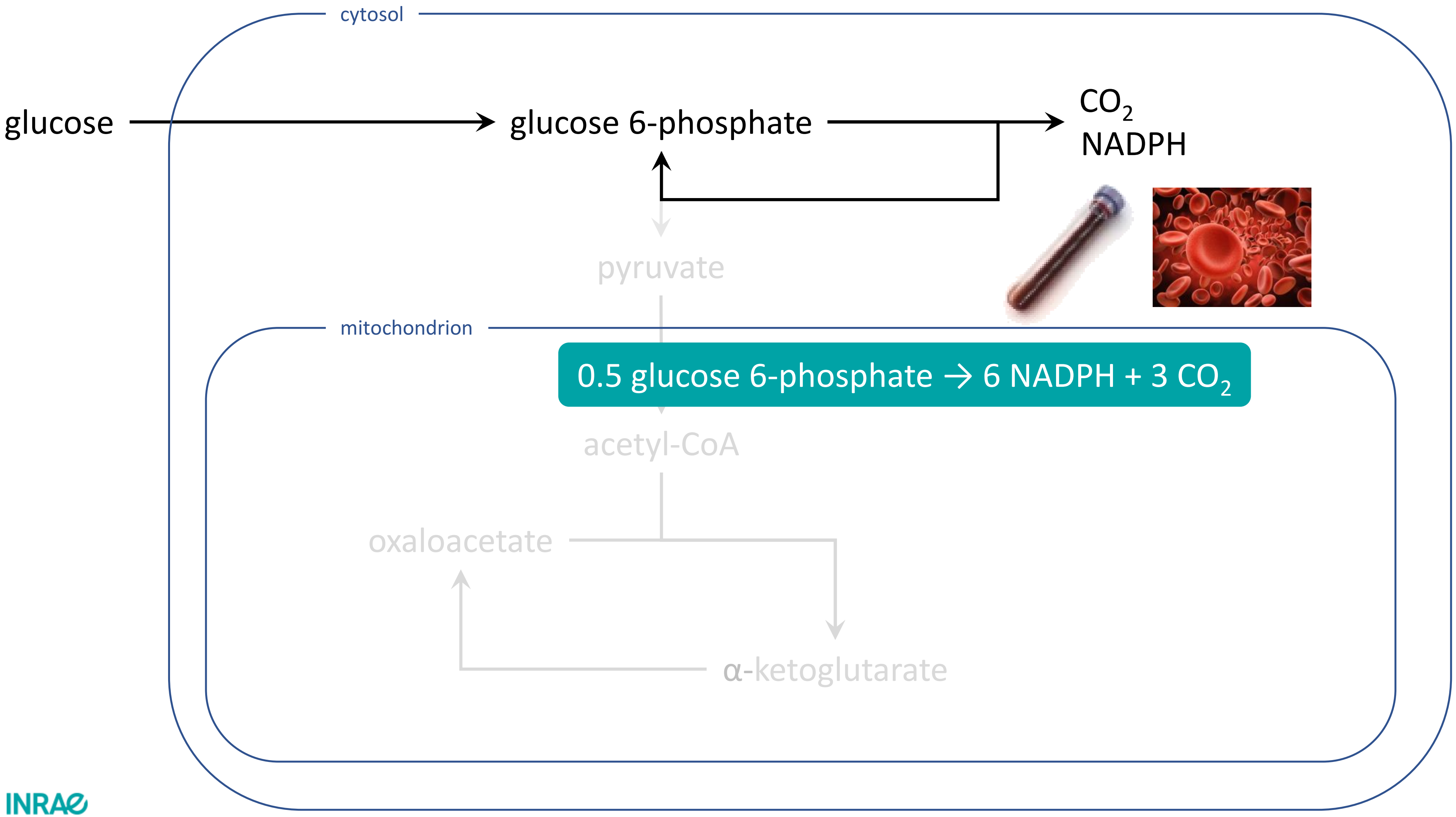
The synthesis of fatty acids from glucose



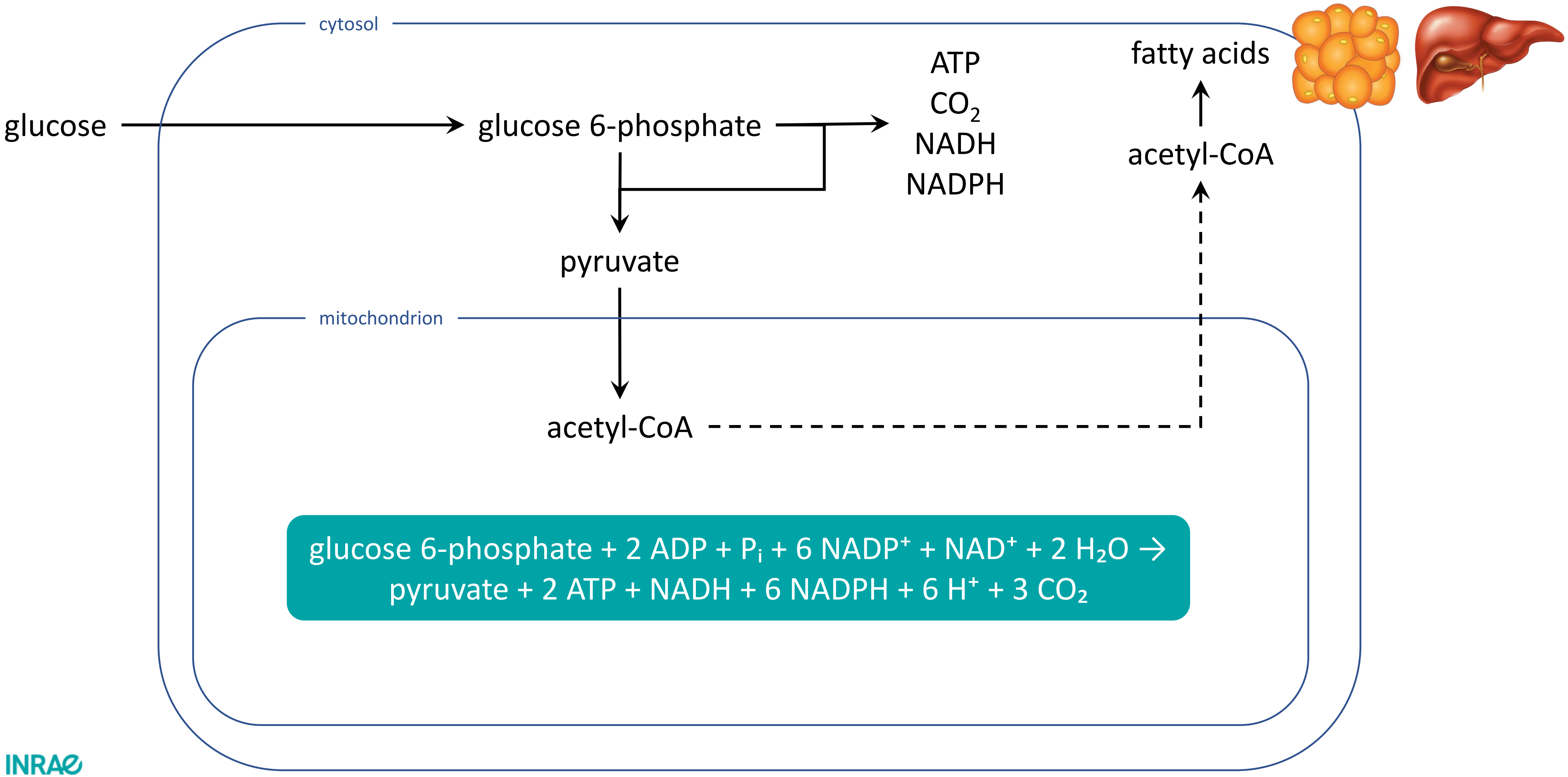
acetyl-CoA: cytosol → mitochondron (with malic enzyme)

$2\ ATP + NADH + NADP^+ + 2\ H_2O \rightarrow 2\ ADP + 2\ P_i + NADPH + NAD^+ + 2\ H^+$

The synthesis of NADPH from glucose (pentose-phosphate pathway)



The synthesis of NADPH from glucose (pentose-phosphate pathway)



glucose 6-phosphate + 2 ADP + P_i + 6 $NADP^+$ + NAD^+ + 2 H_2O → pyruvate + 2 ATP + NADH + 6 NADPH + 6 H^+ + 3 CO_2

Today's exercises

- Synthesis of non-essential amino acids
- Protein synthesis and turnover
- Fatty acid and fat synthesis from glucose
- Fat synthesis from dietary fat

Today's program

- Yesterday's exercises
- Energy storage, mobilization, and transfer

Spatial and temporal aspects of energy metabolism

Where is the energy of your breakfast right now?

Spatial and temporal aspects of energy metabolism

Spatial and temporal aspects of energy metabolism

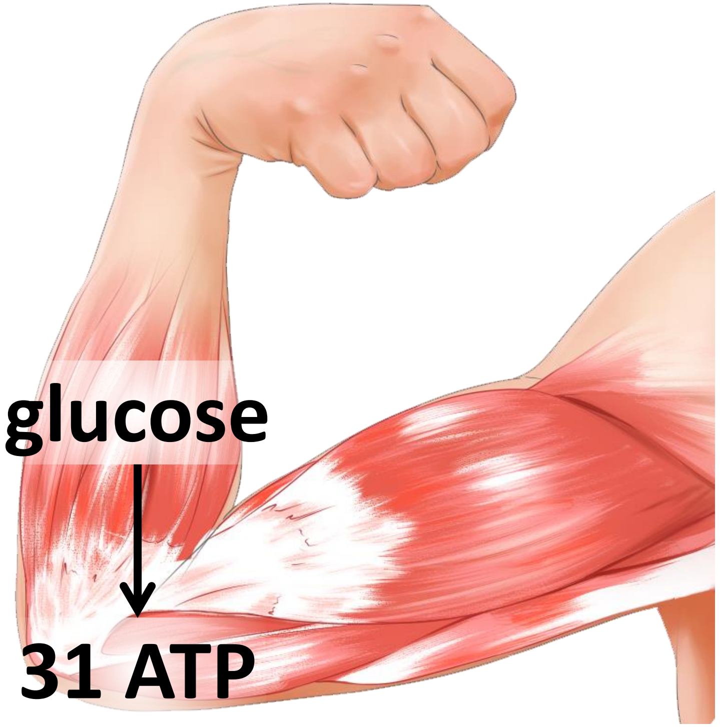
We have some control, …

… are rather robust,
and able to self-repair.

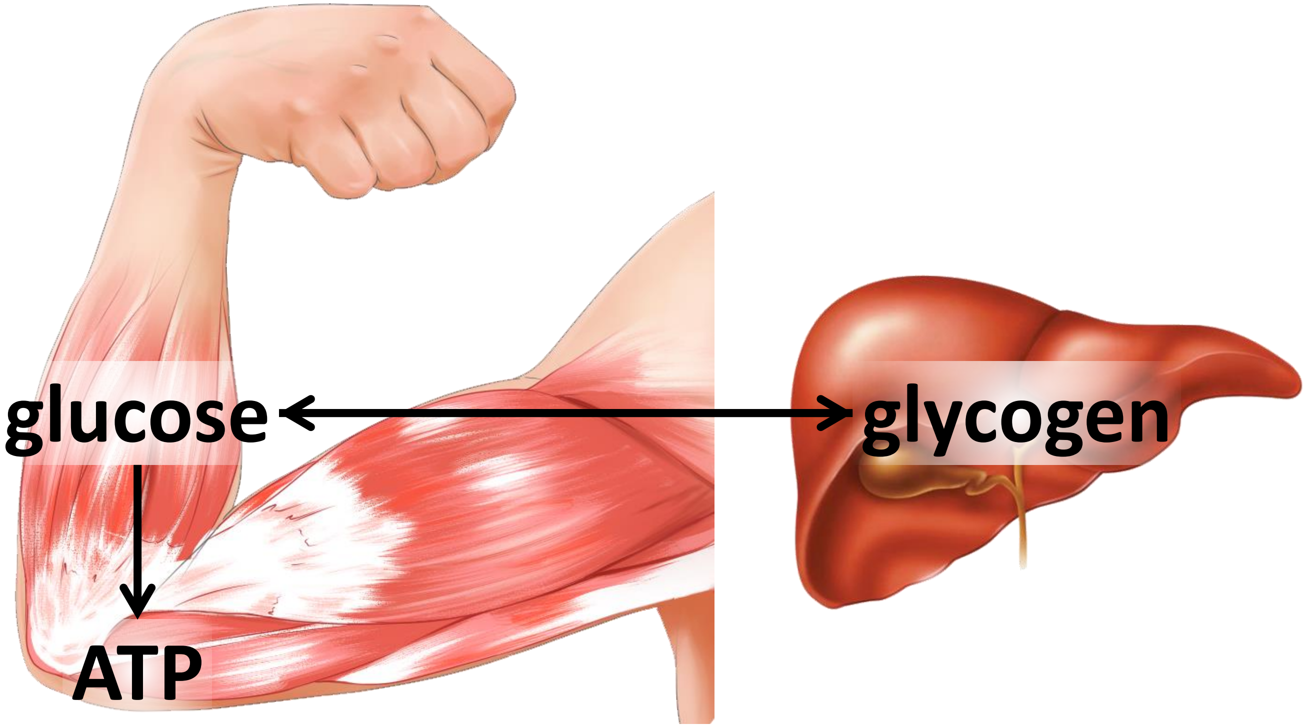
We can convert
Regular into Premium, …

… and put 100 liter of fuel
in a 50 liter tank.

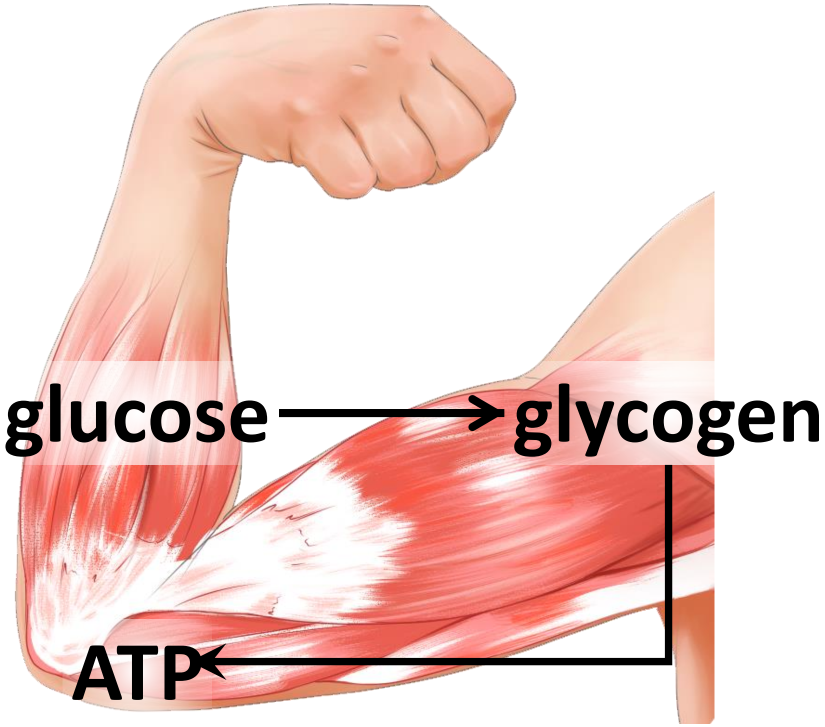
The metabolism of glucose to ATP



The metabolism of glucose to ATP



The metabolism of glucose to ATP



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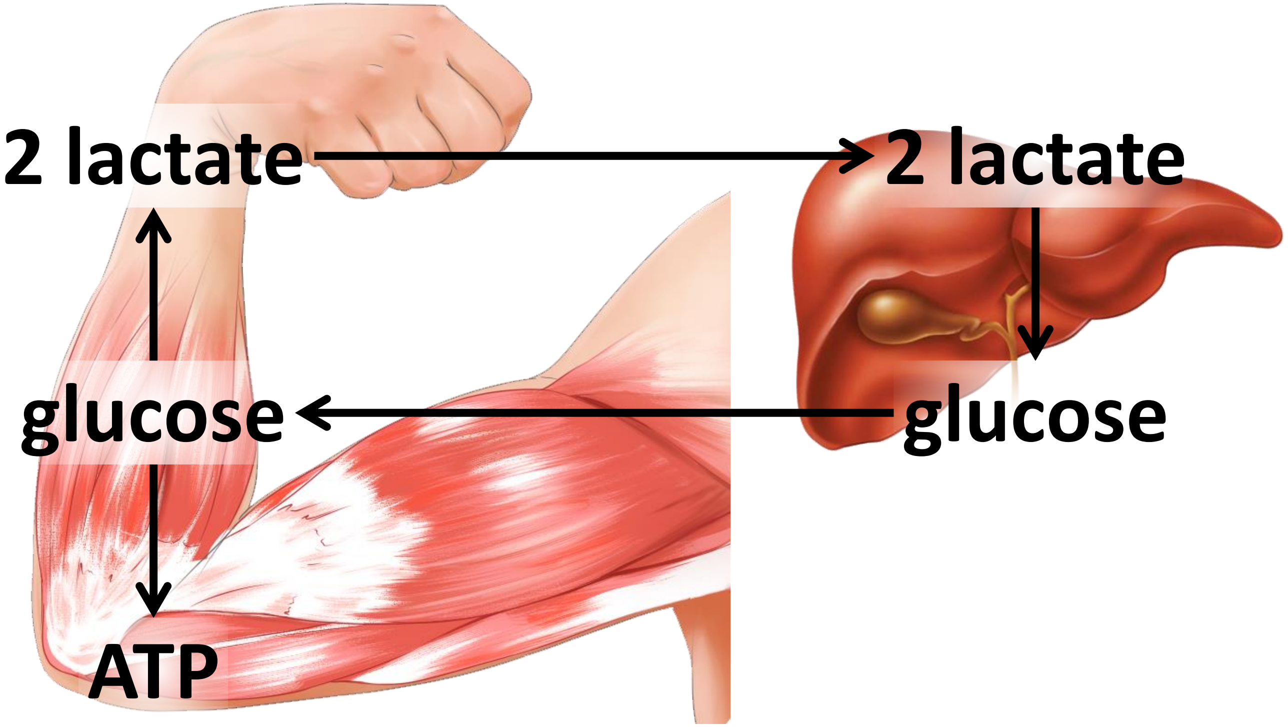
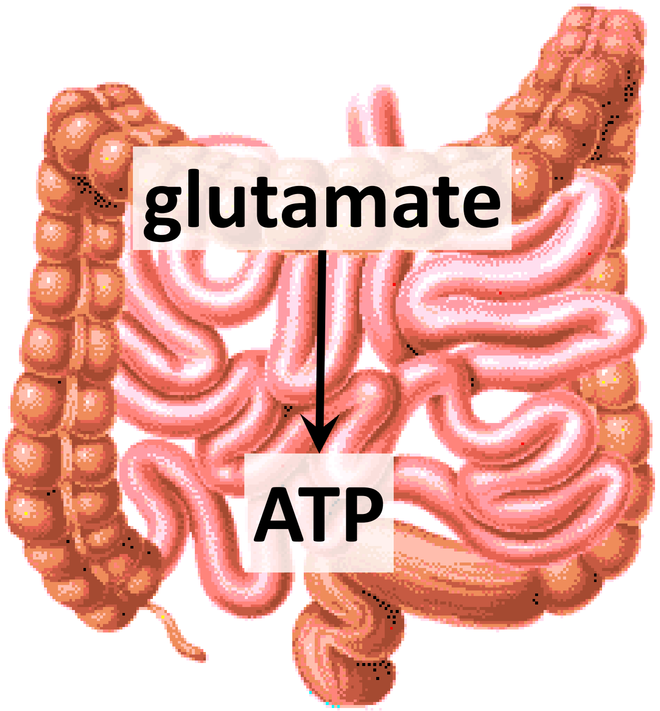
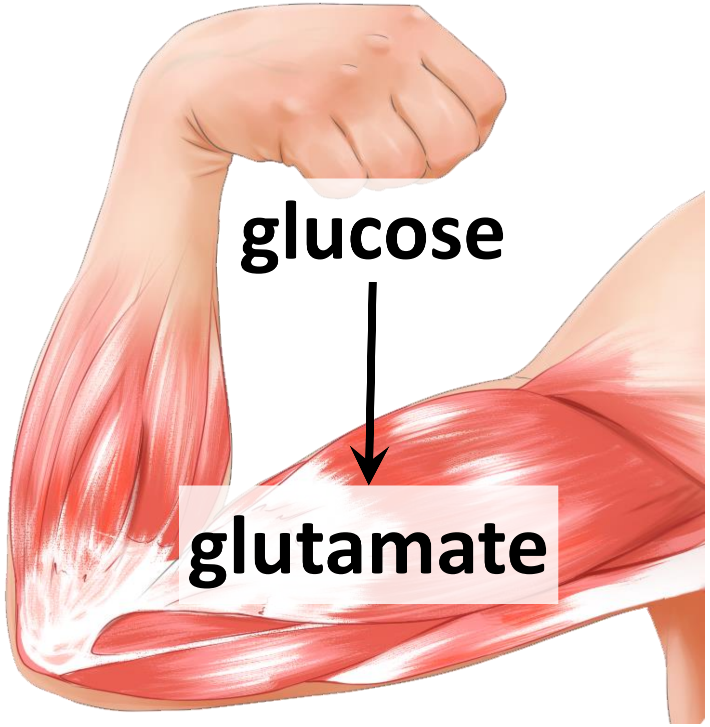
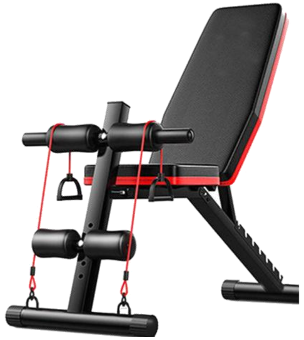


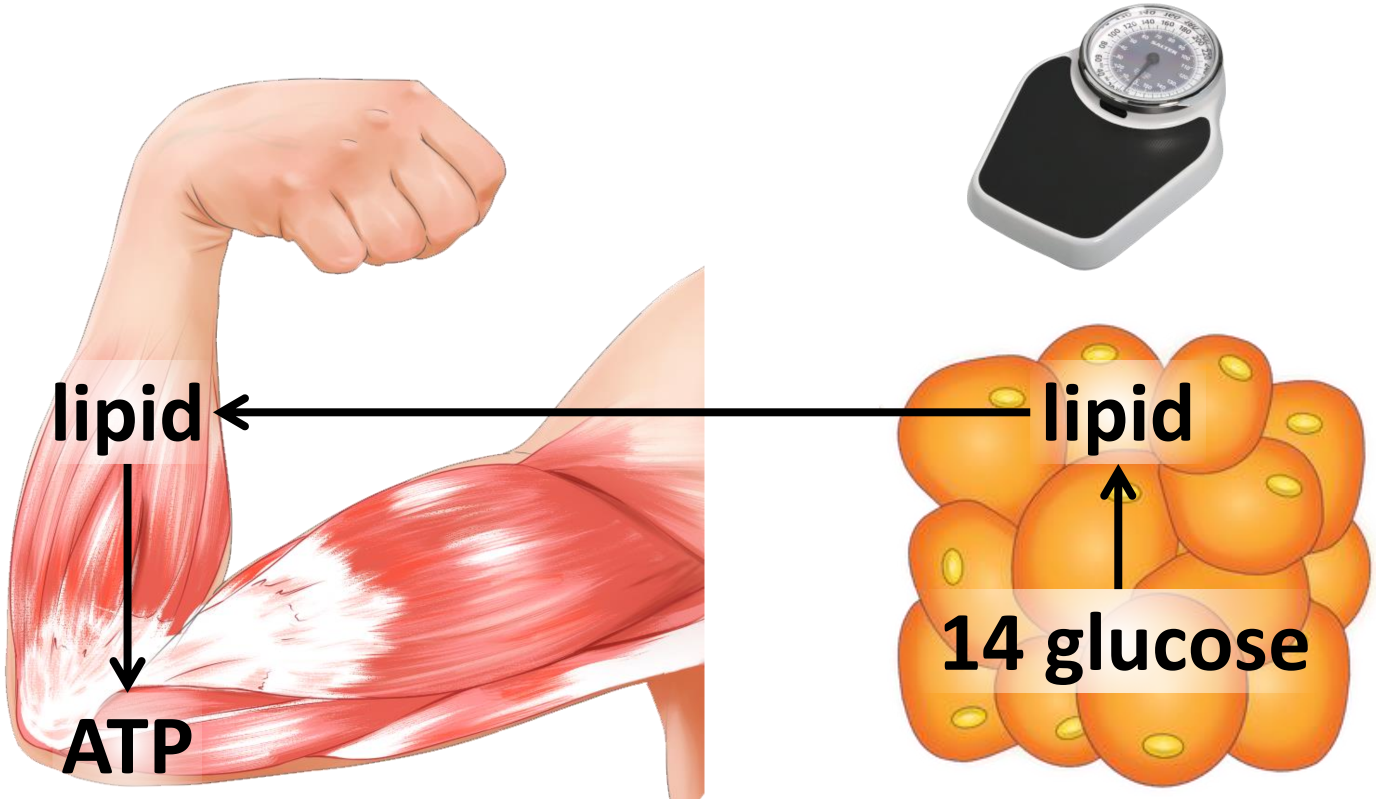
The metabolism of glucose to ATP



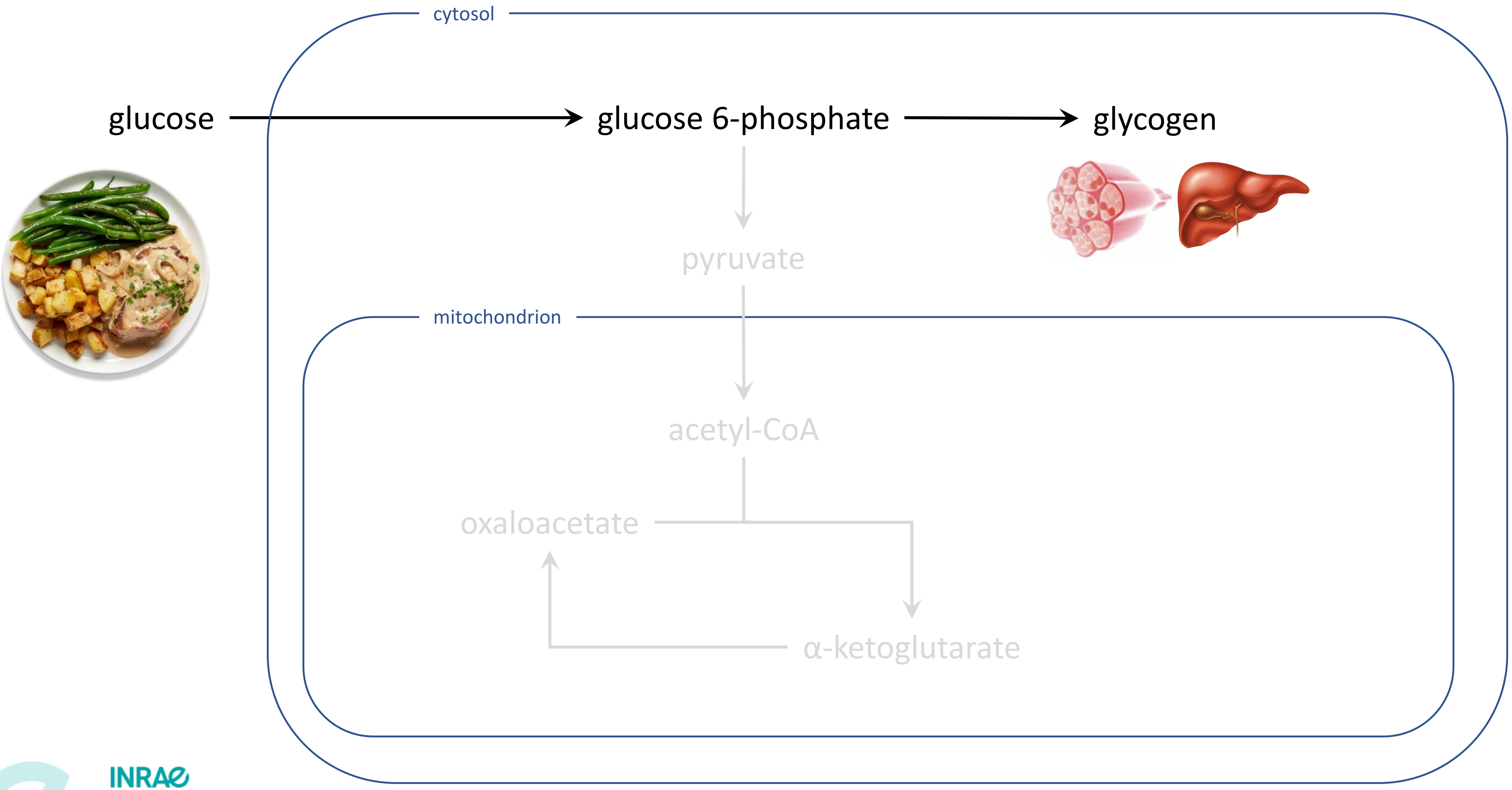


The metabolism of glucose to ATP





The cost of storing glucose as glycogen



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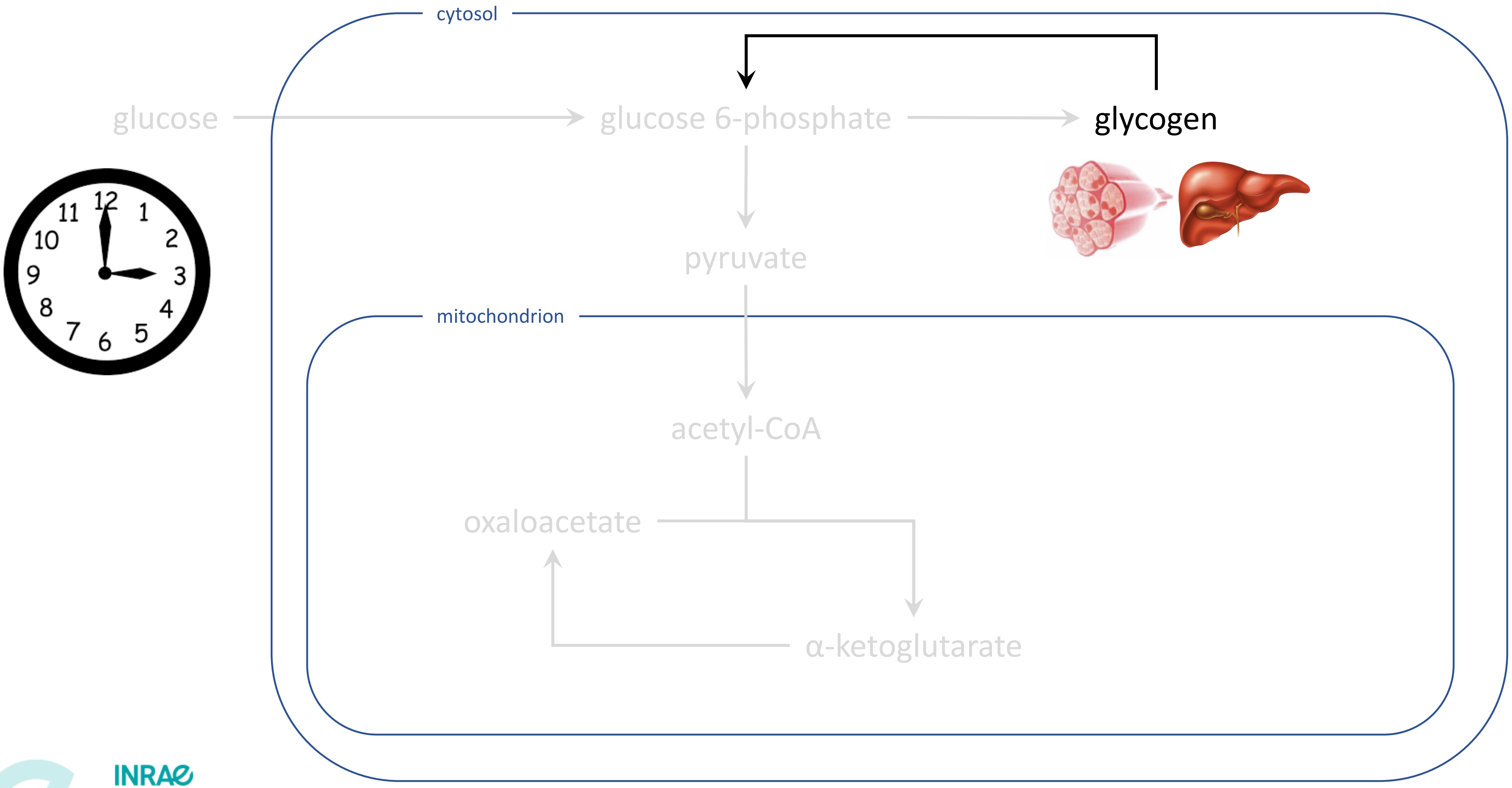
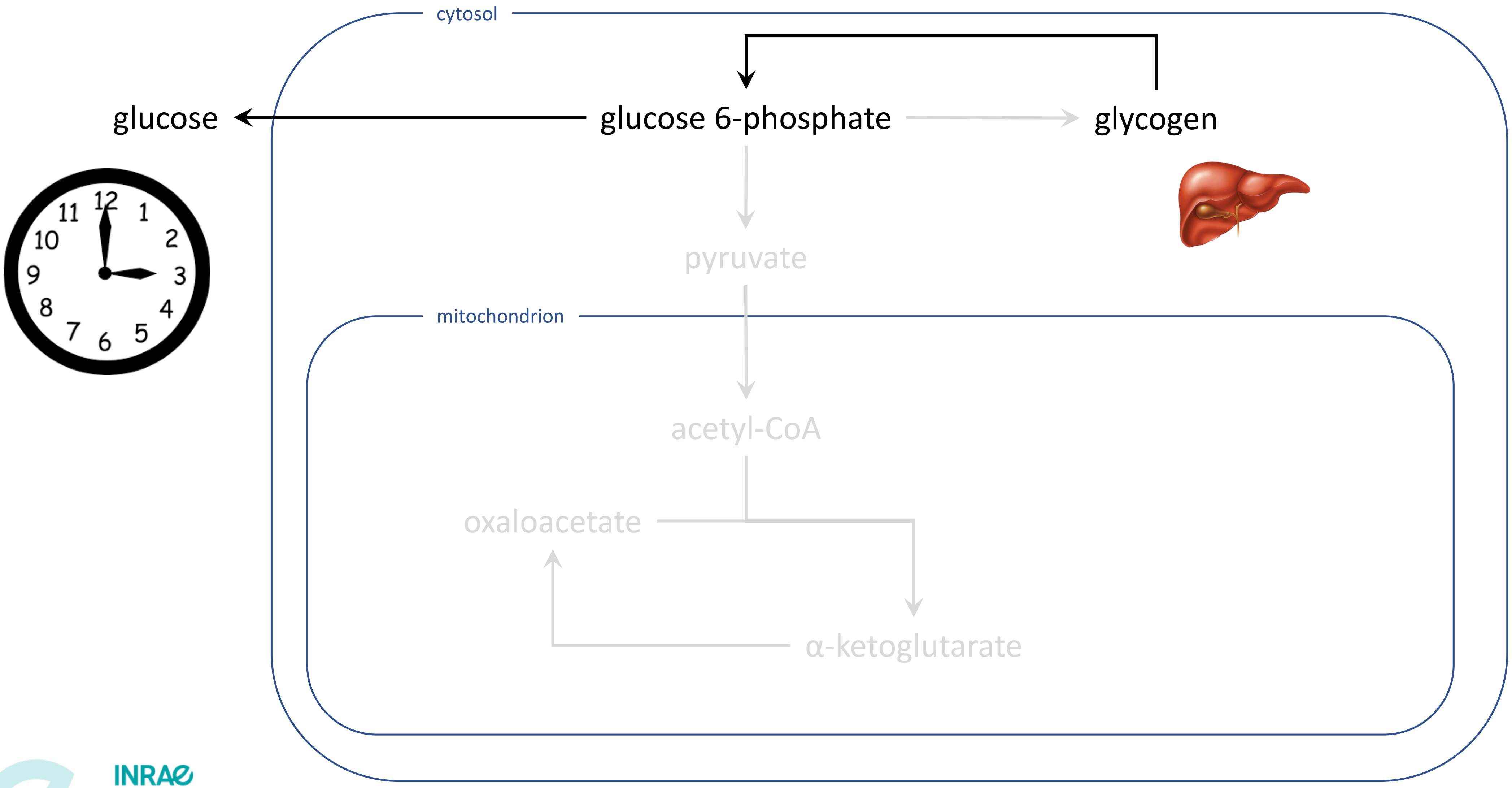
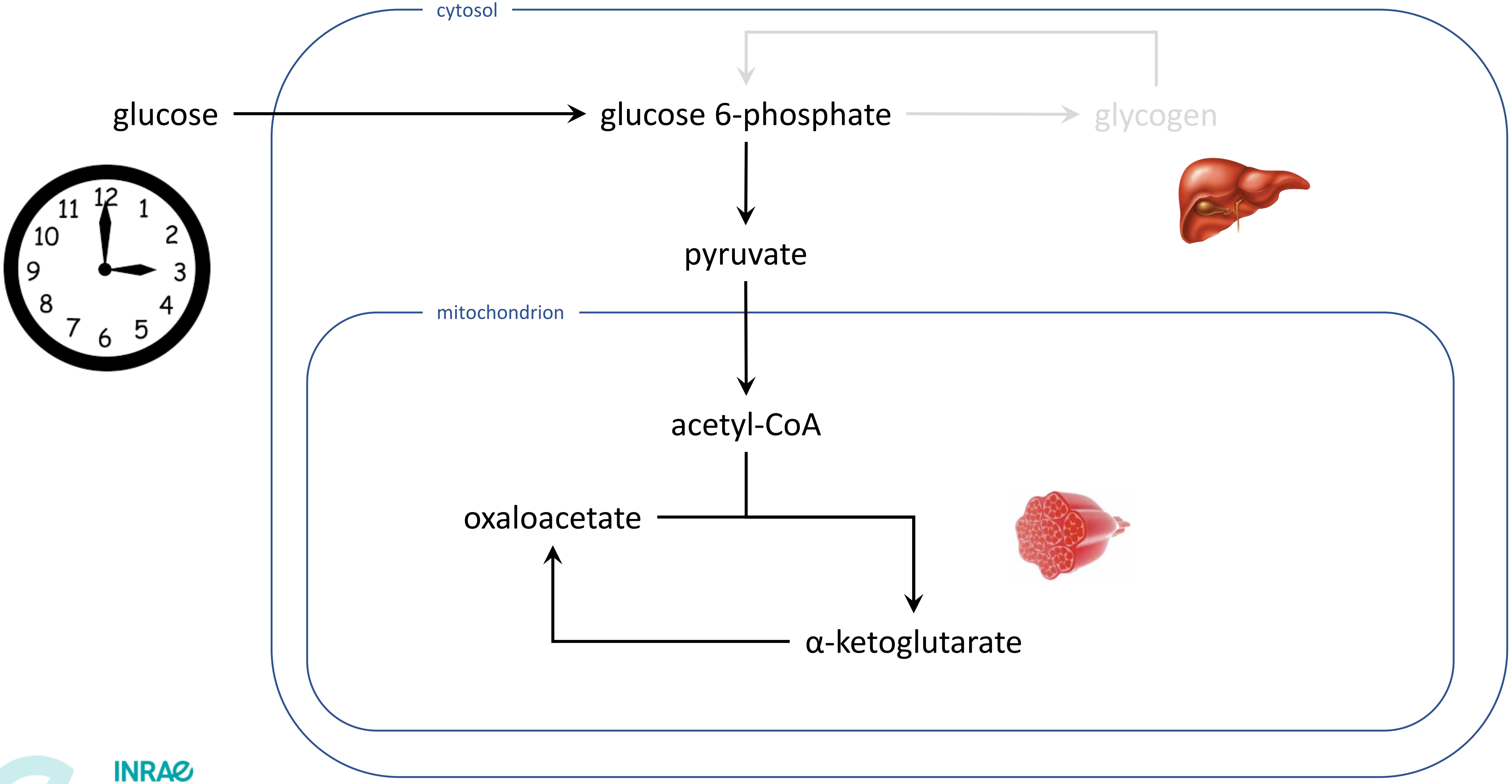


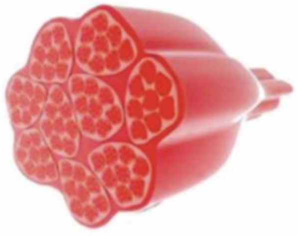
The cost of storing glucose as glycogen

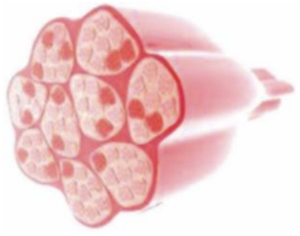


The cost of storing glucose as glycogen



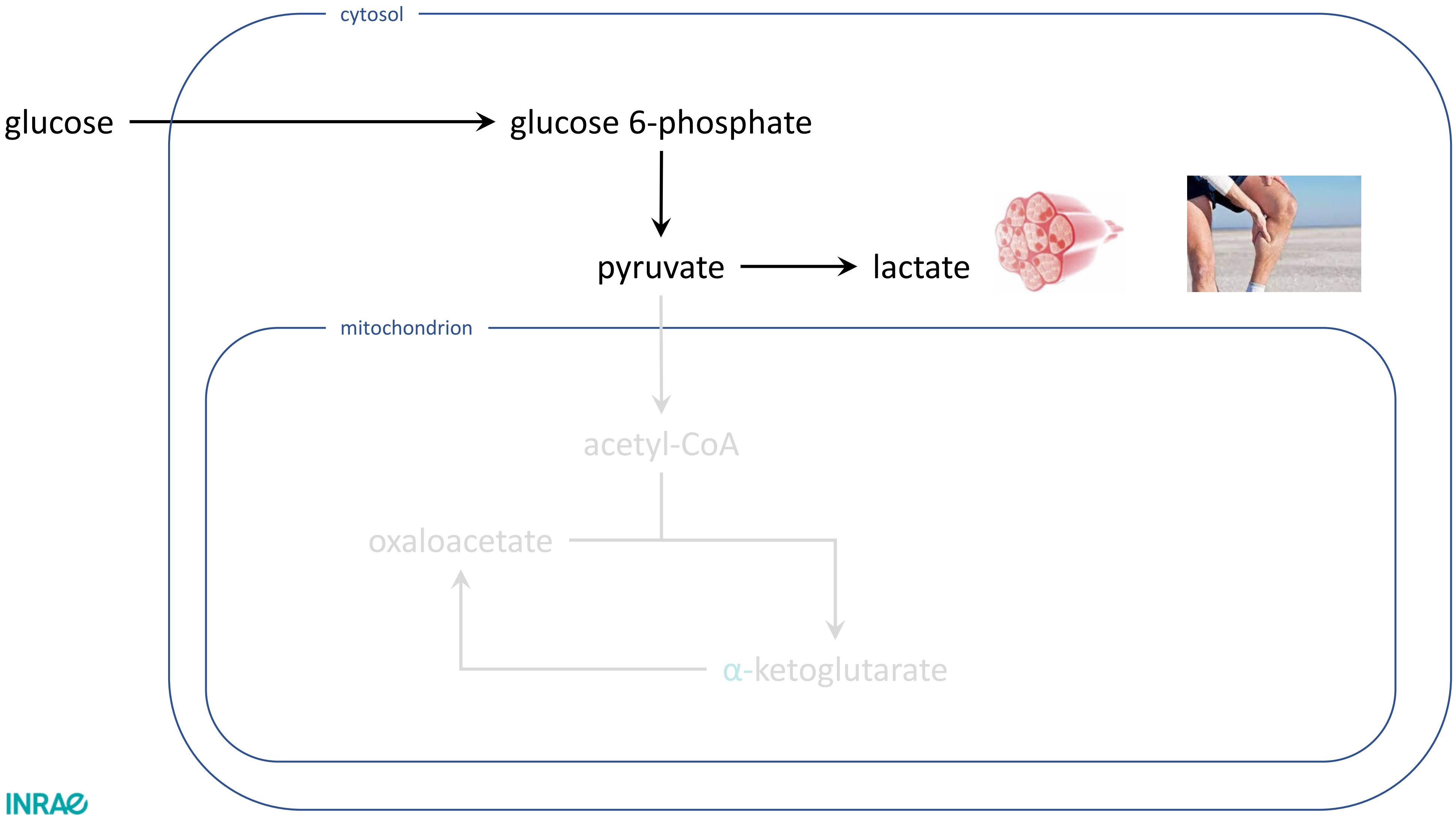
Metabolism of skeletal muscle



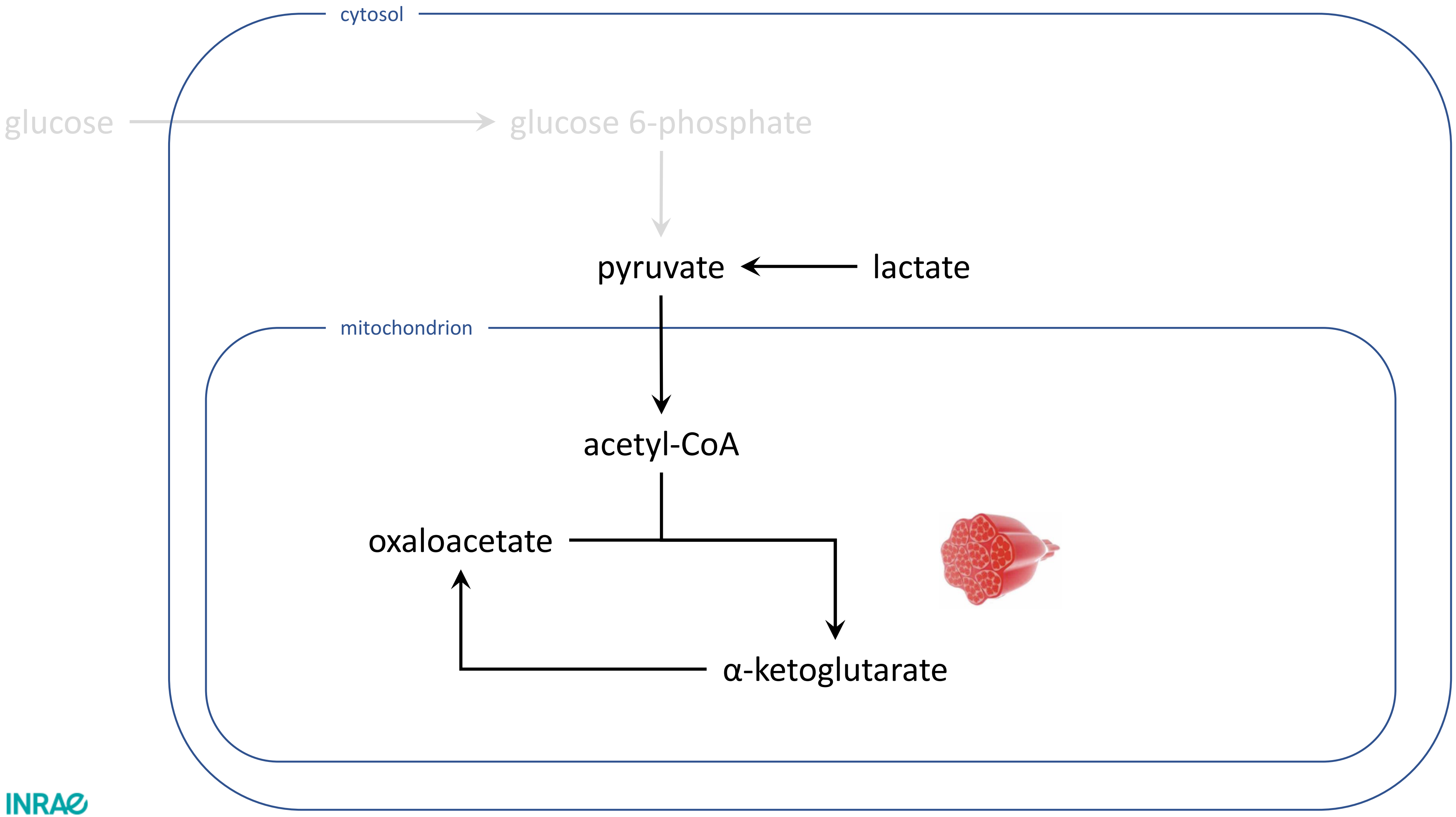


	Red muscle fiber	White muscle fibers
Blood supply	rich	poor
Oxygen storage (as myoglobin)	present	little or none
Mitochondria	abundant	fewer and smaller
ATP production	aerobic	anaerobic
Muscle contraction	slow and for prolonged periods	fast and for short periods
Fatigue	little	very rapidly
Glycogen storage	low	abundant

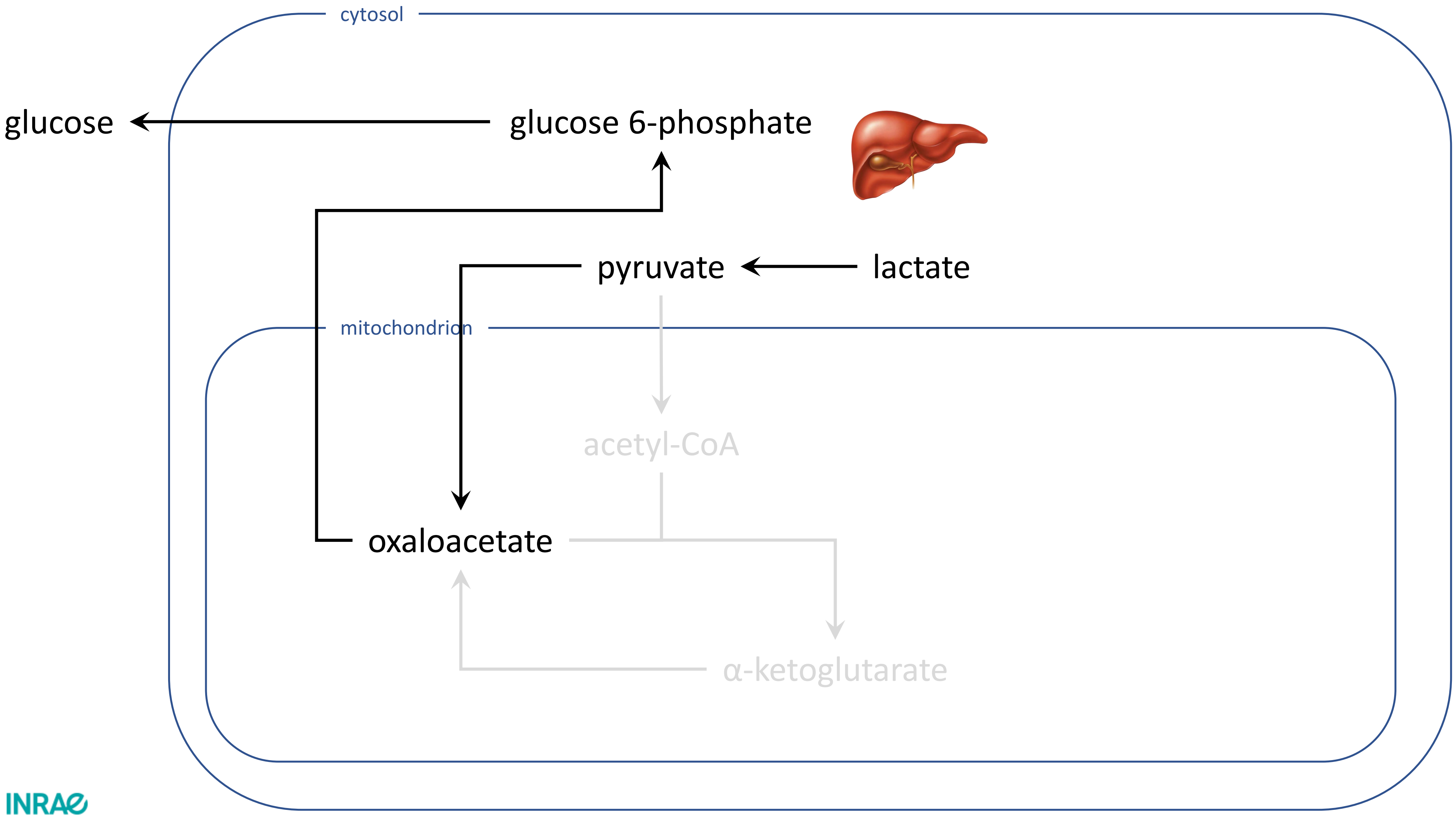
ATP synthesis in (fast and glycolytic) white muscles



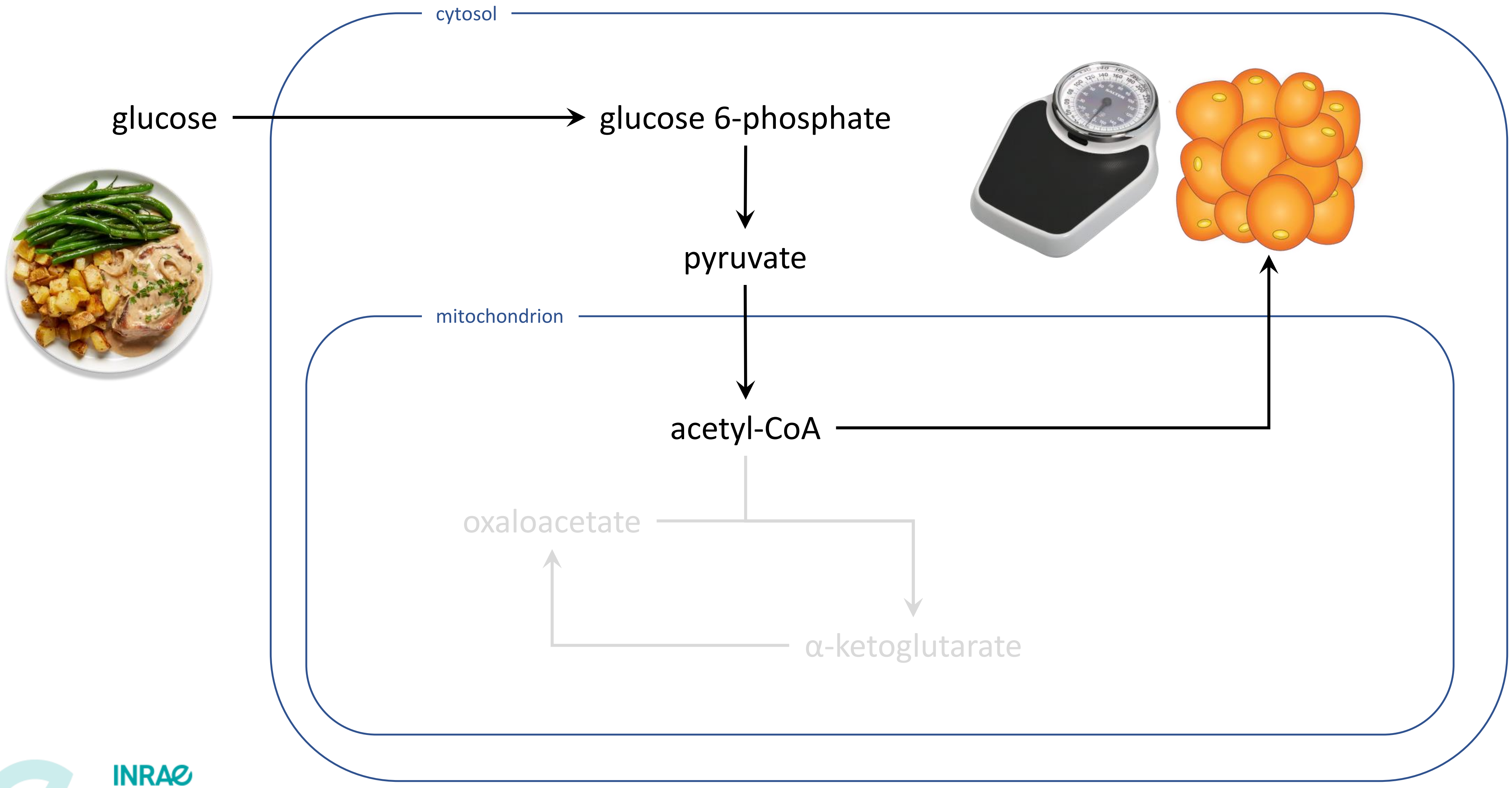
ATP synthesis in (slow and oxidative) red muscles



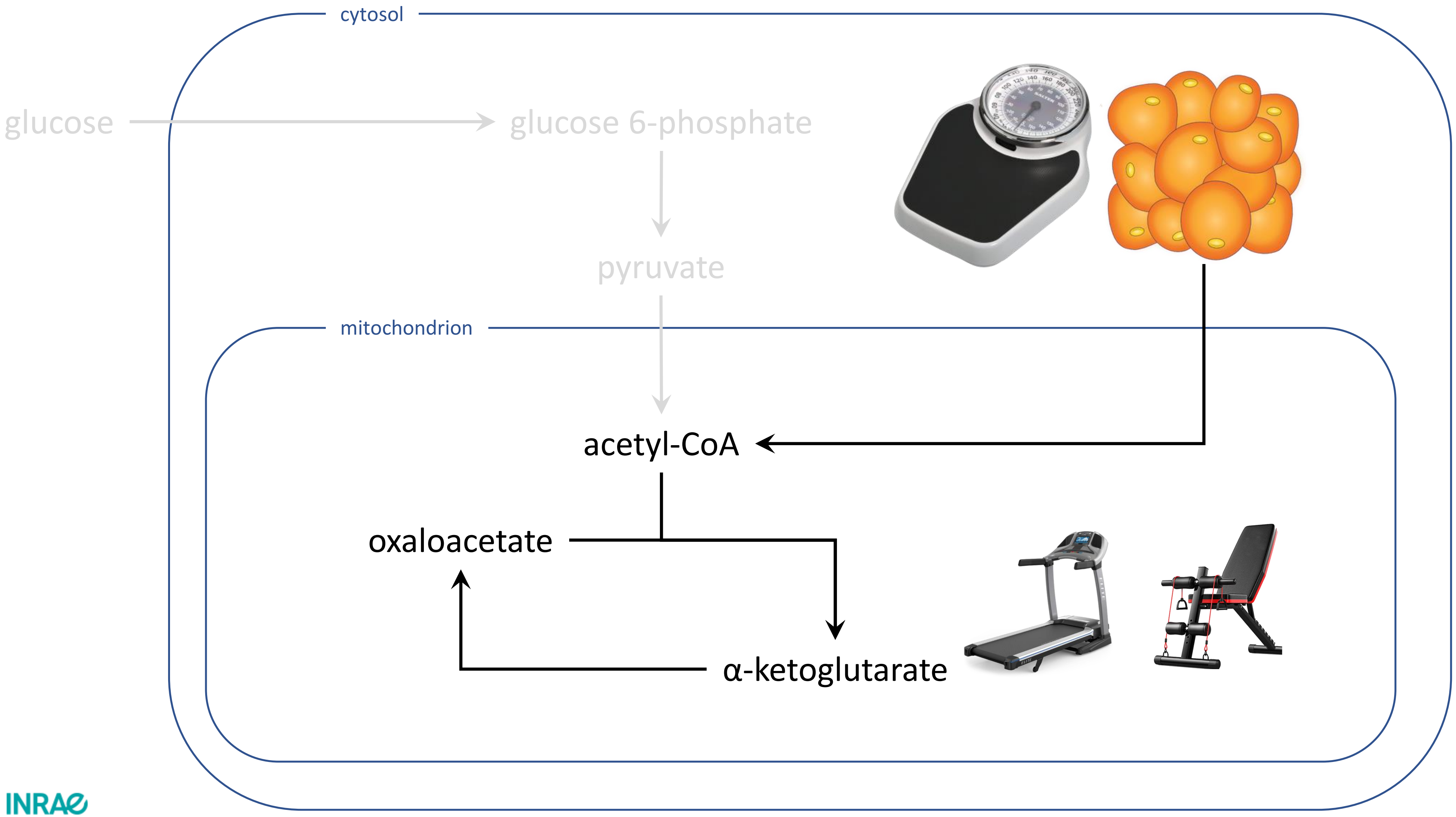
Regeneration of glucose from lactate through the Cori cycle



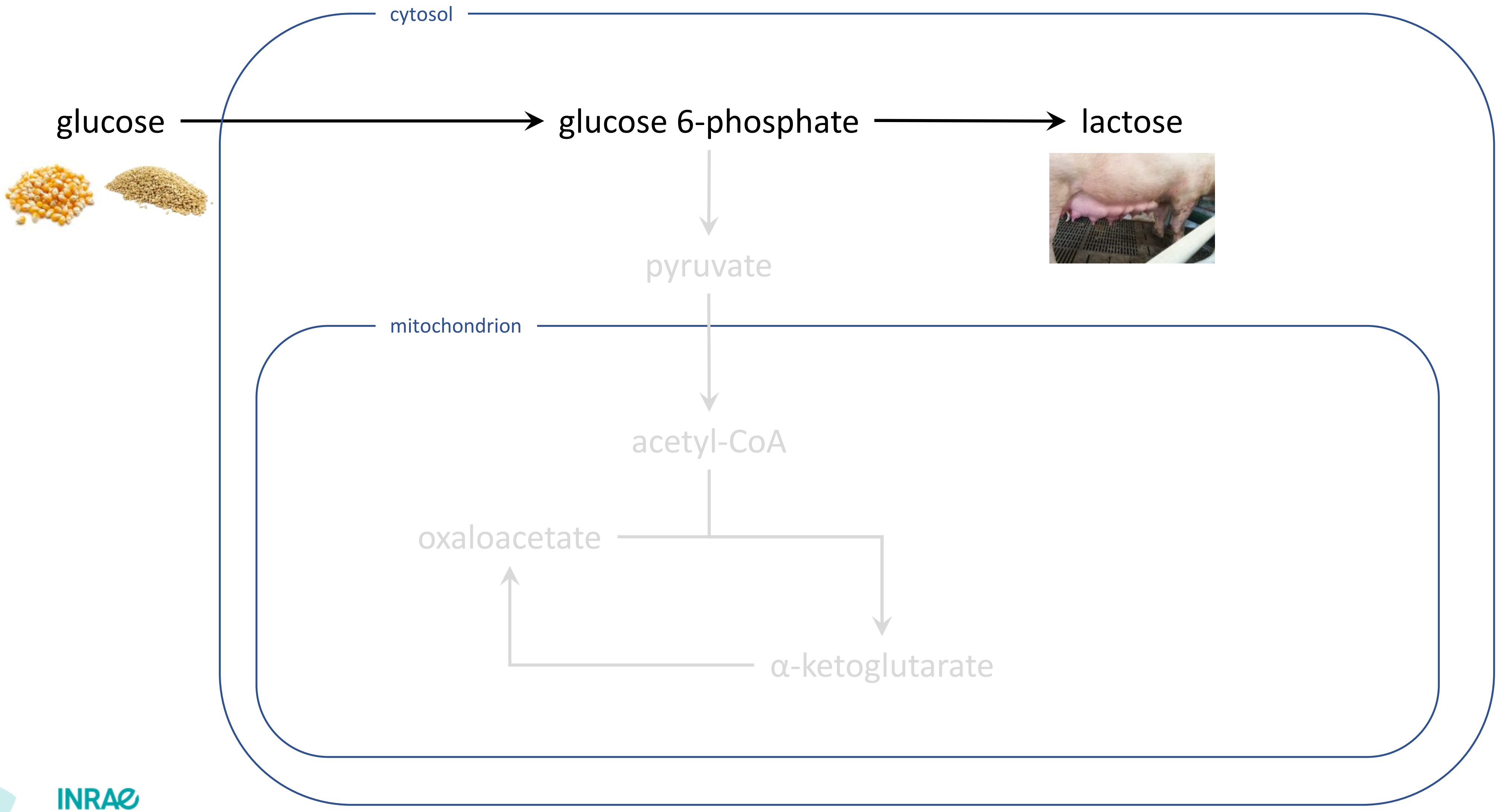
The cost of storing glucose as lipid



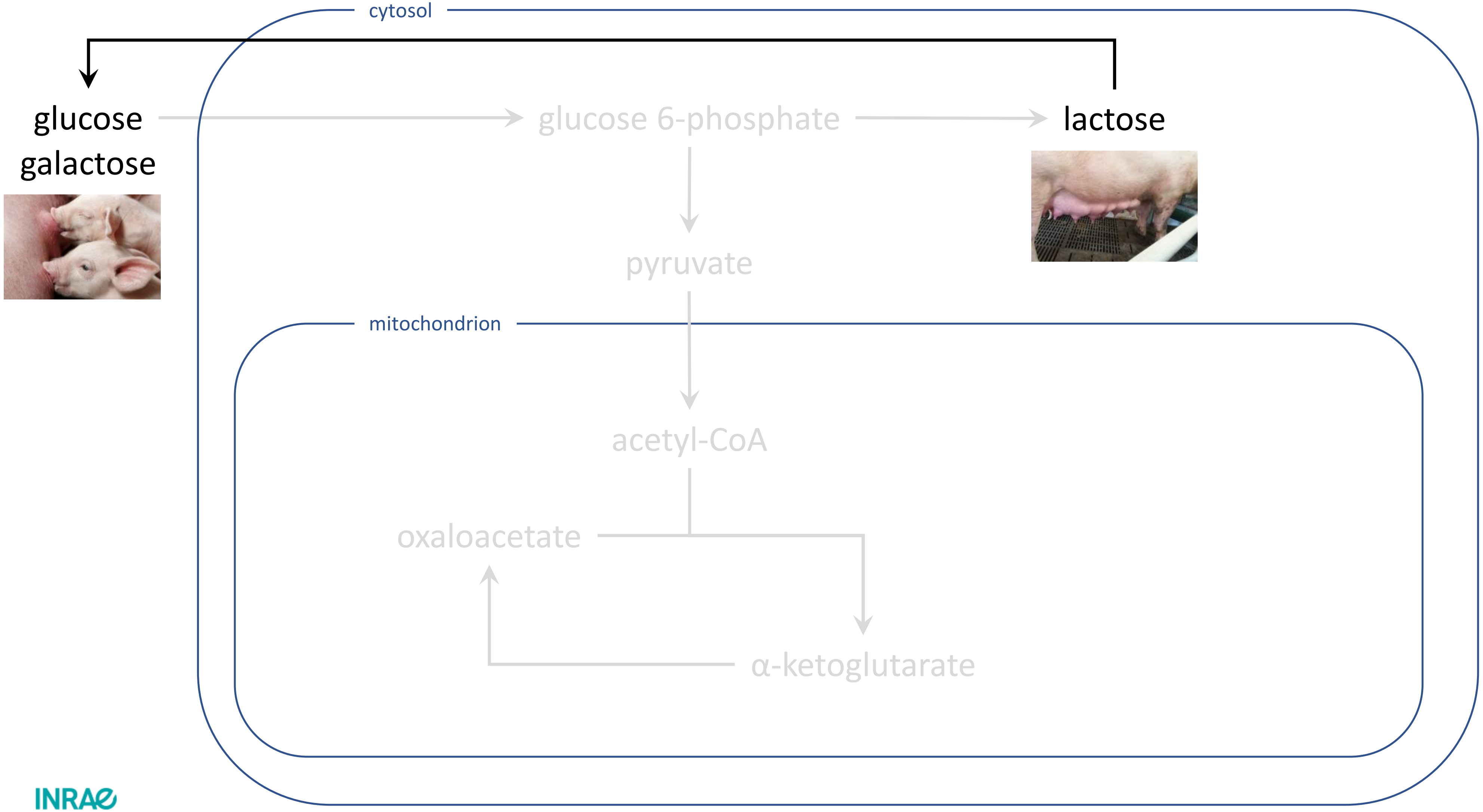
The cost of storing glucose as lipid



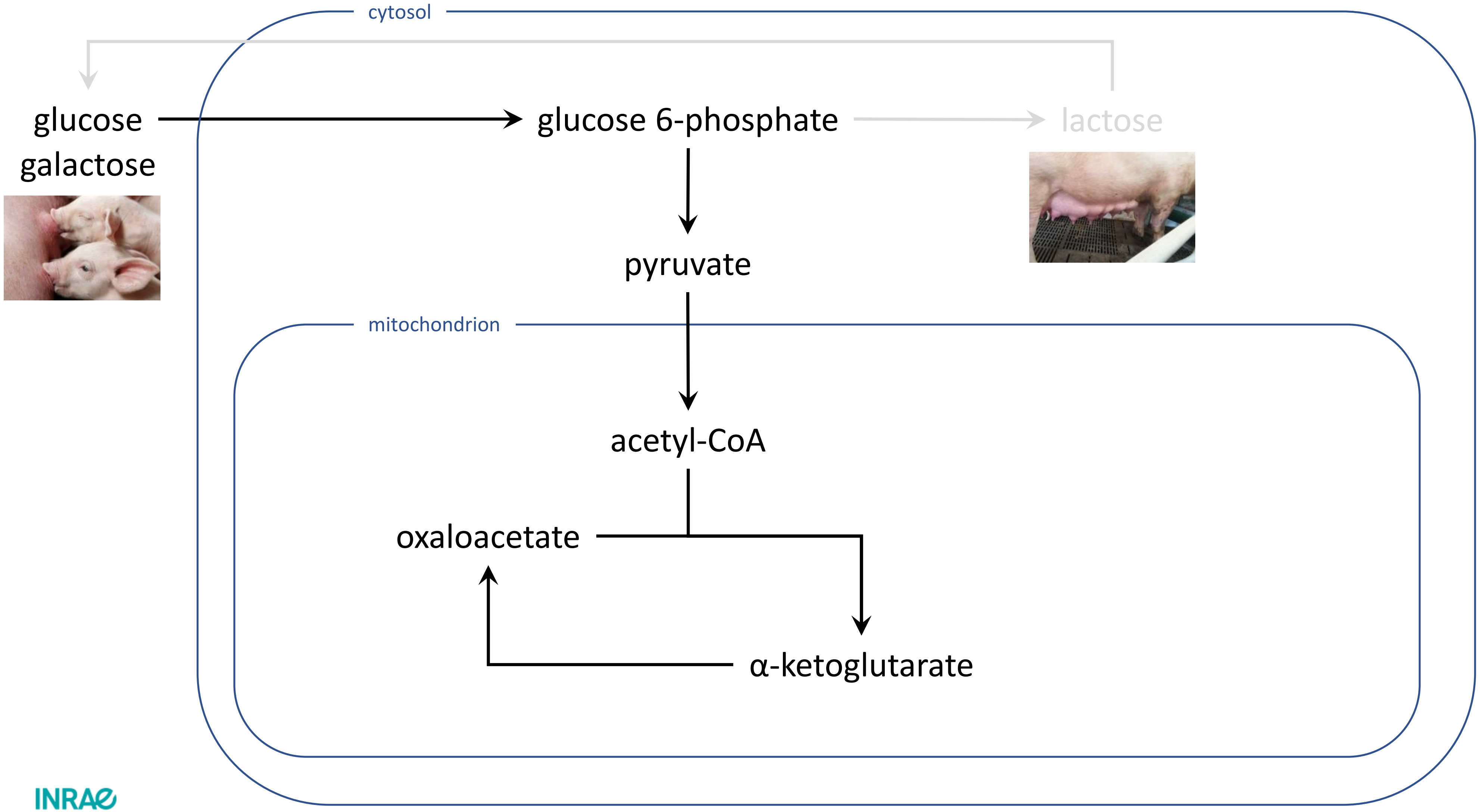
The cost of transporting glucose (via lactose) from the sow to piglets



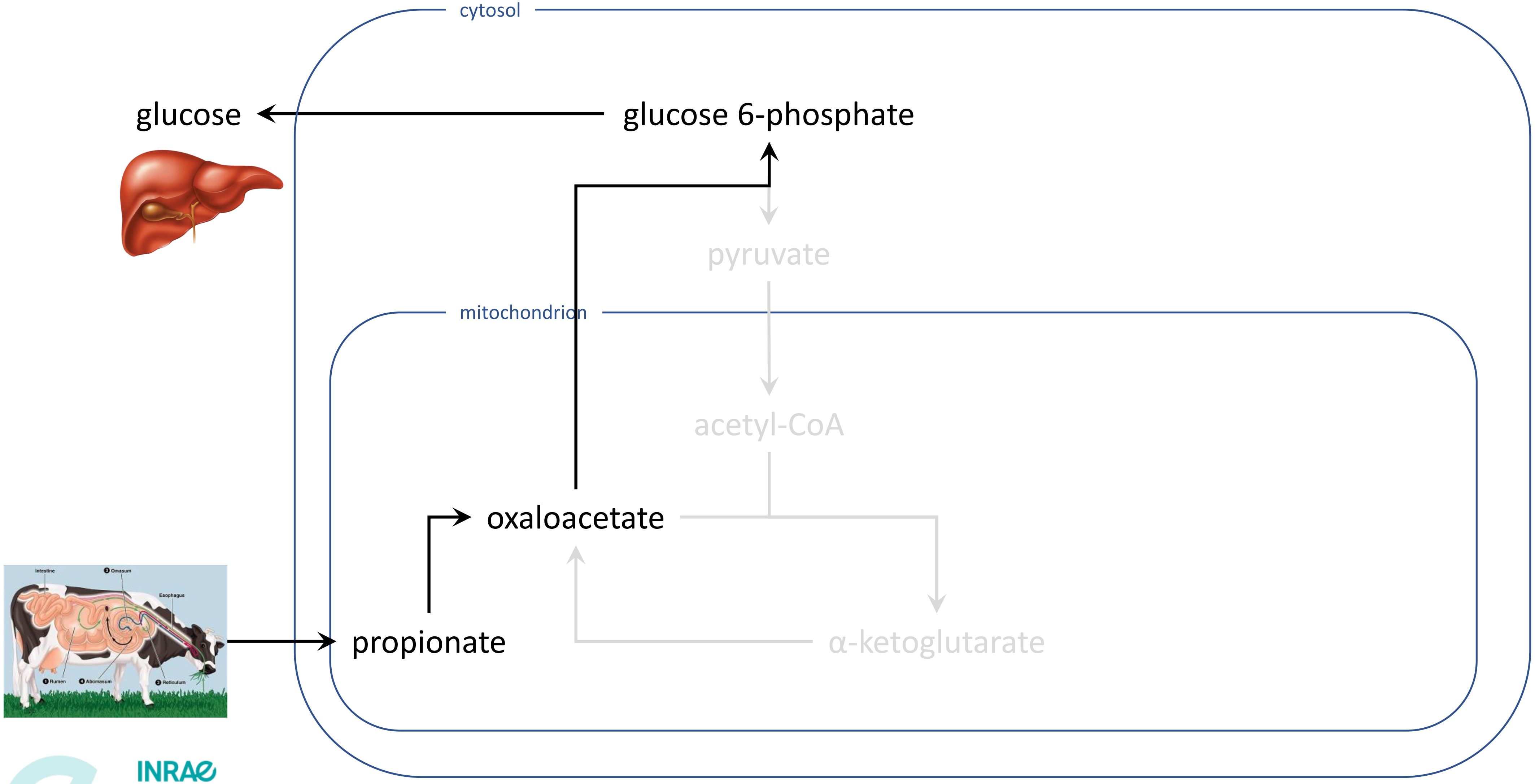
The cost of transporting glucose (via lactose) from the sow to piglets



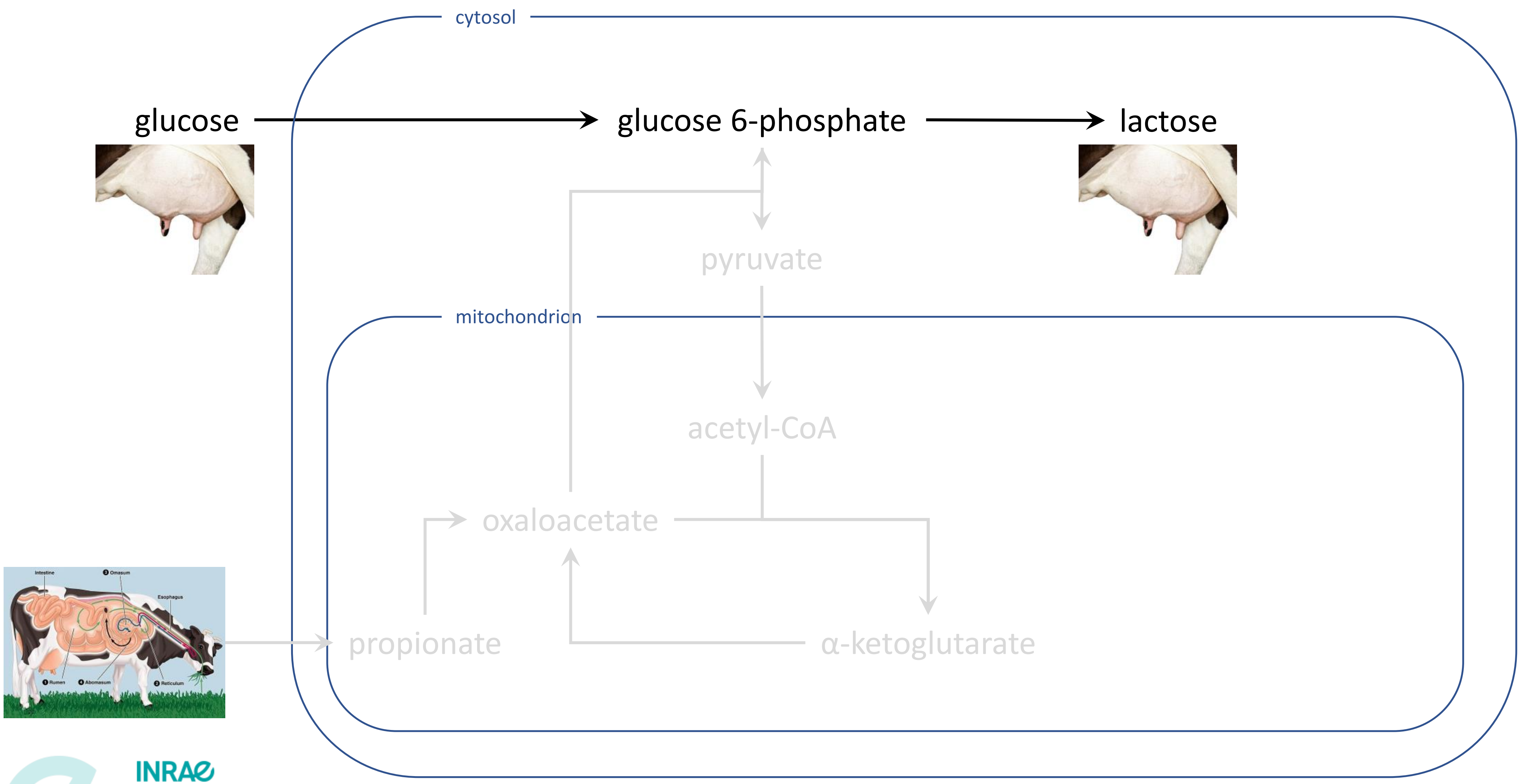
The cost of transporting glucose (via lactose) from the sow to piglets



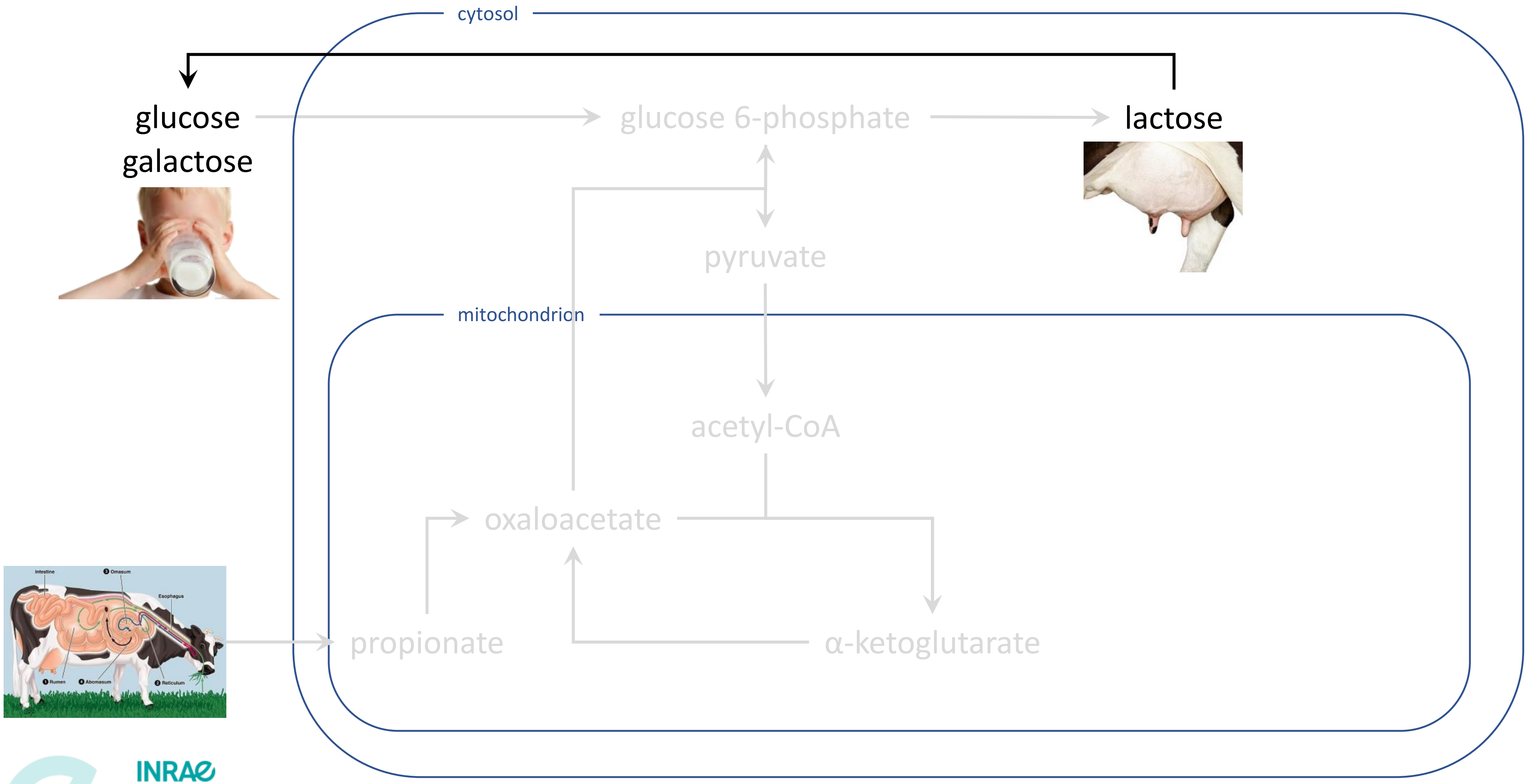
The cost of transporting glucose (via lactose) from a cow to a child



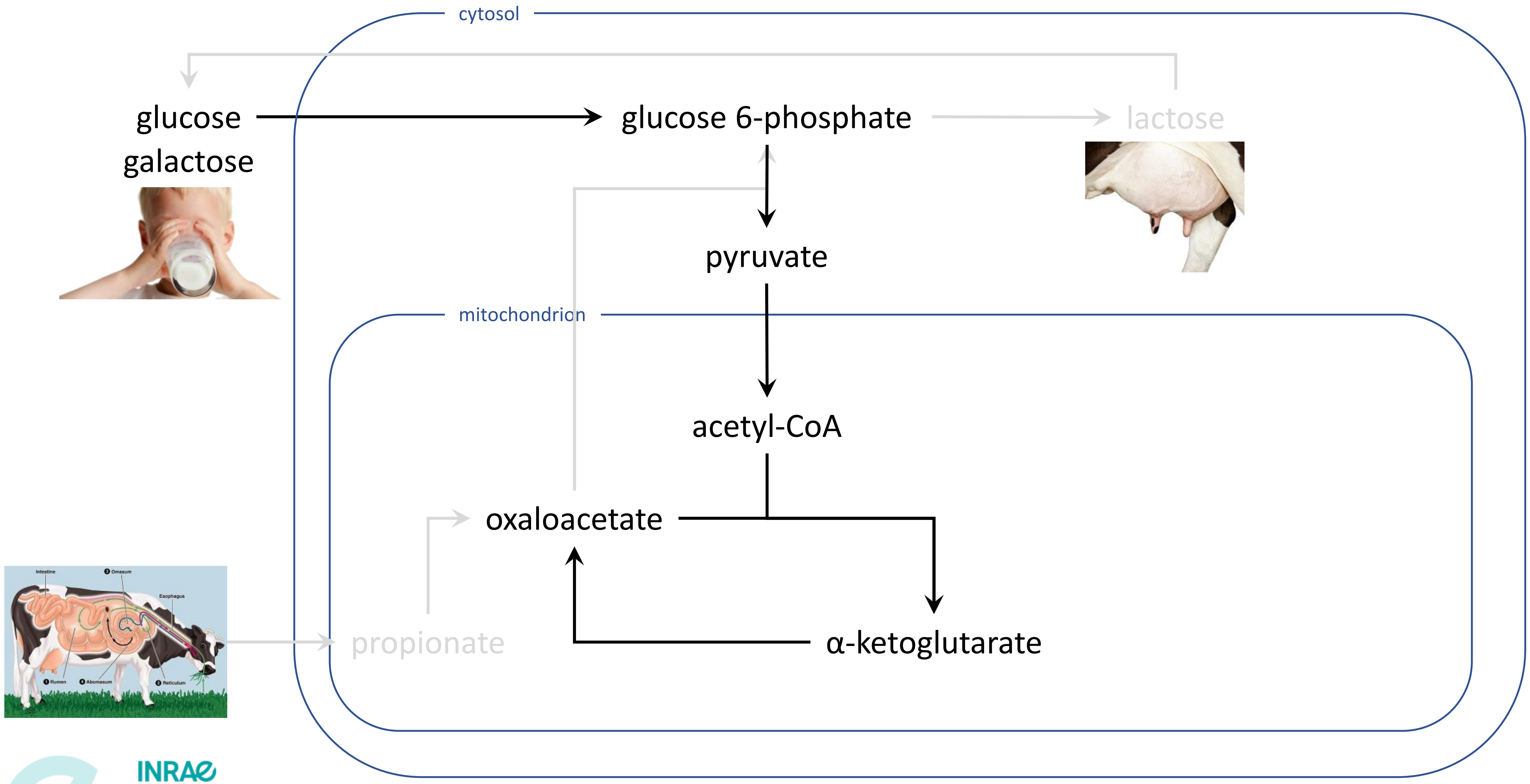
The cost of transporting glucose (via lactose) from a cow to a child



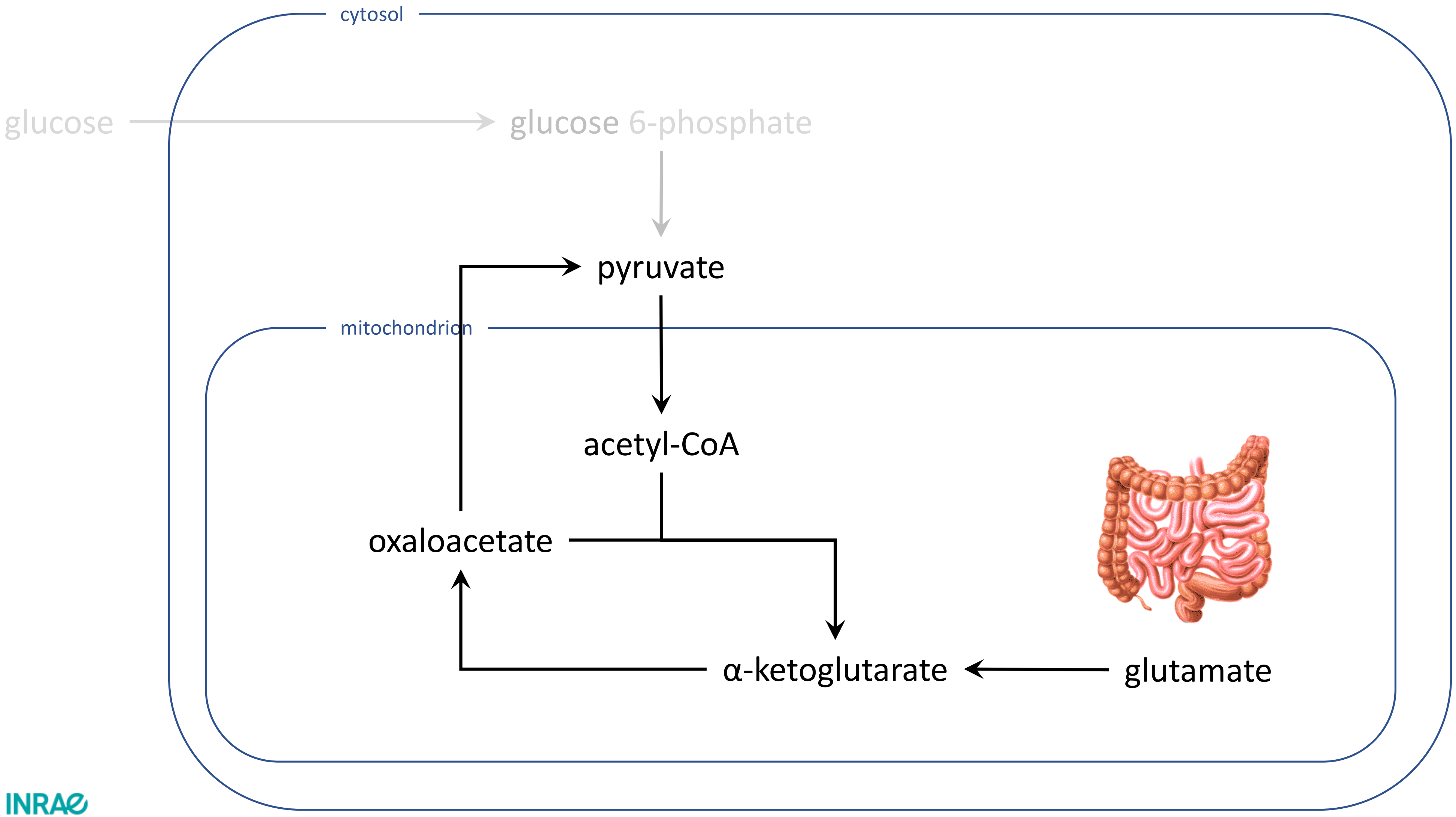
The cost of transporting glucose (via lactose) from a cow to a child



The cost of transporting glucose (via lactose) from a cow to a child



The GI-tract uses glutamate as fuel, but muscle needs it for protein



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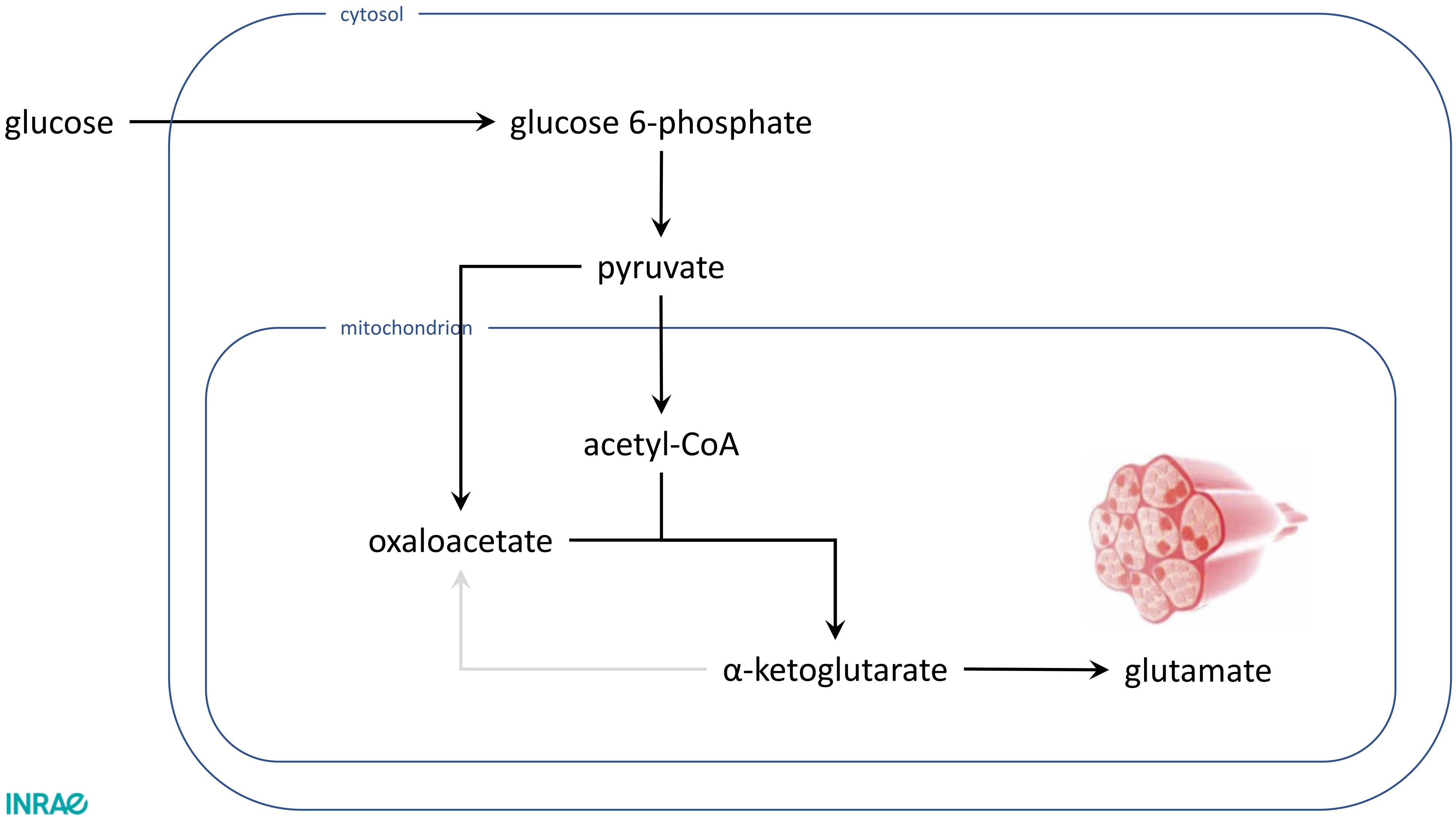
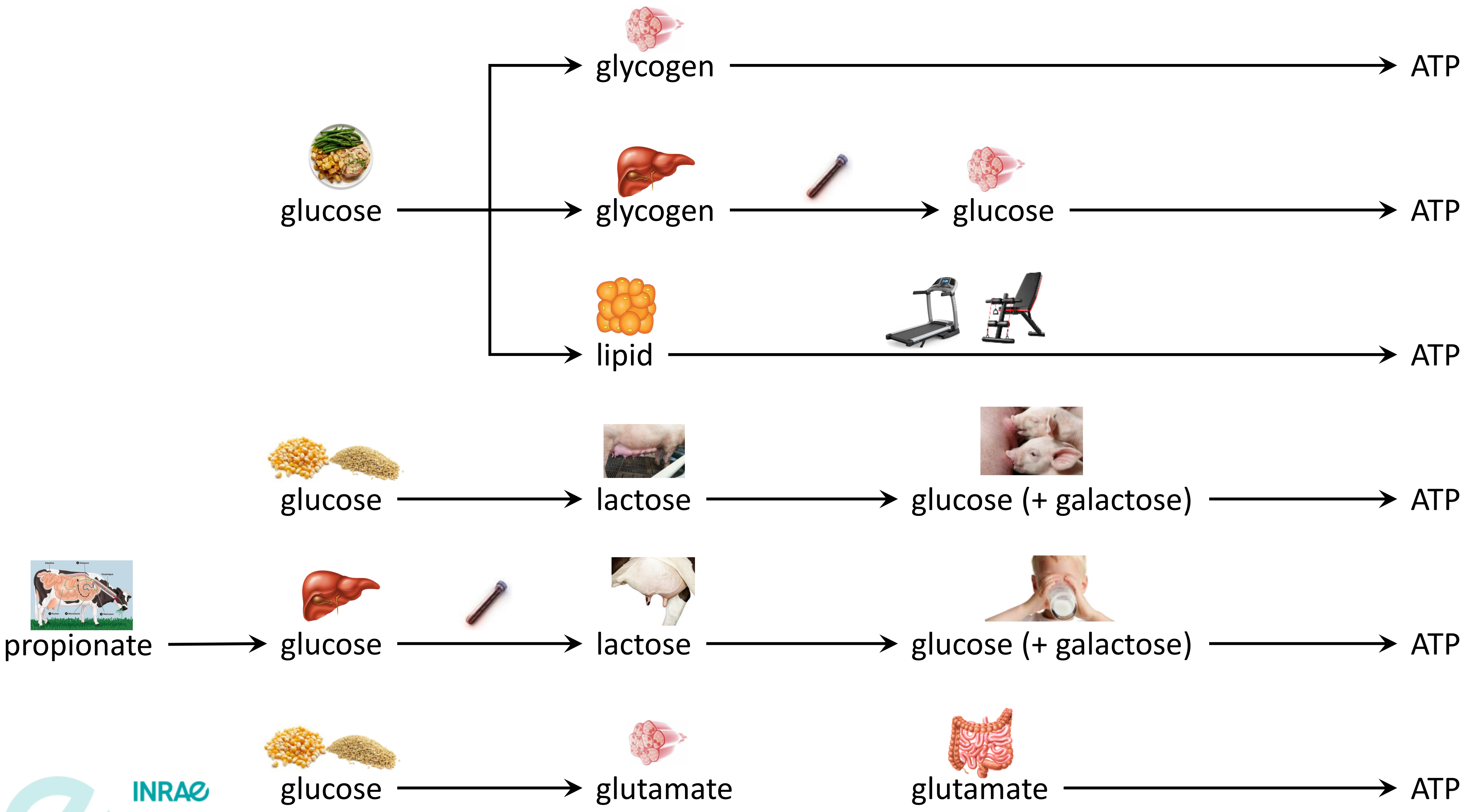


Today's exercises



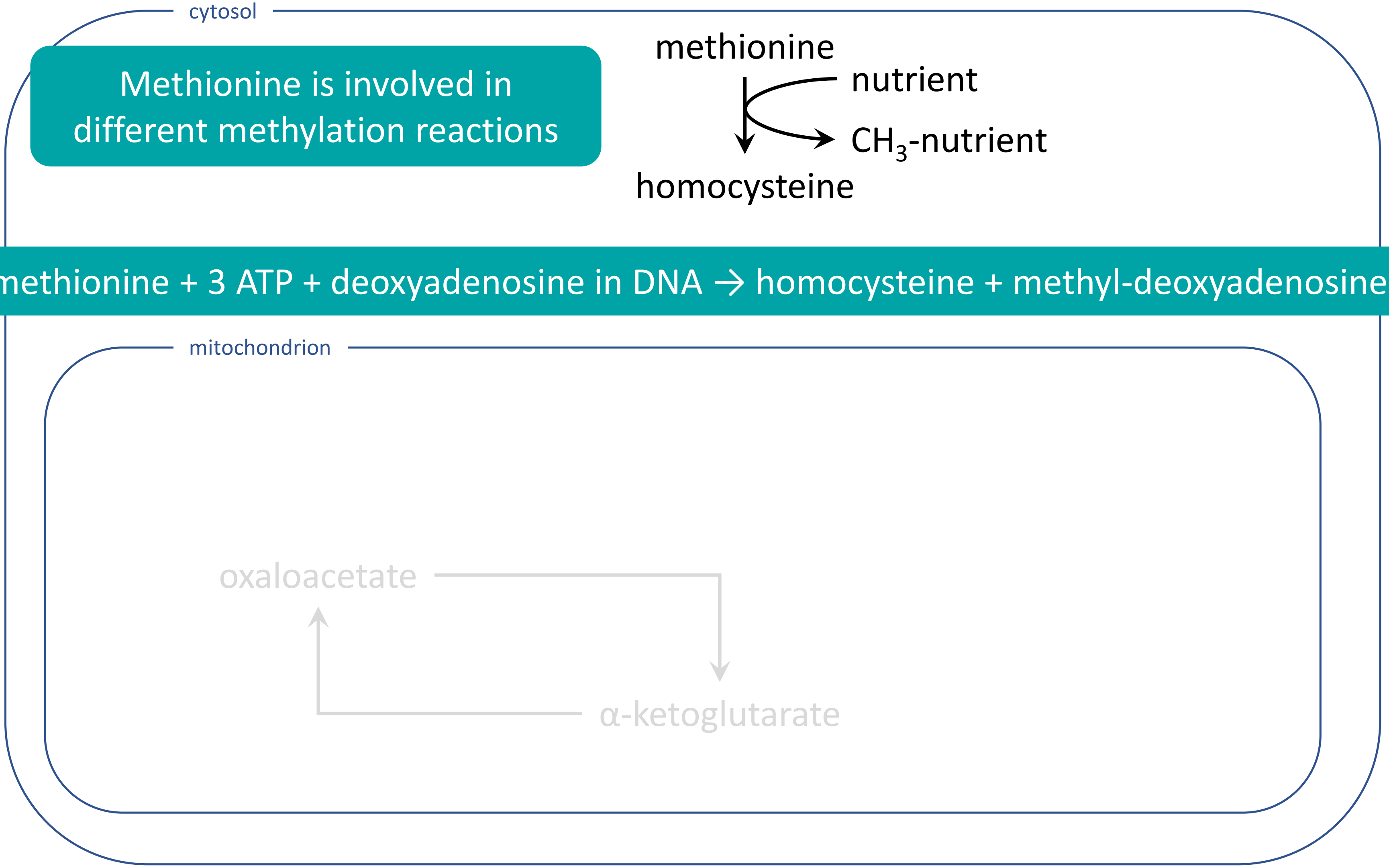
Today's program

- Yesterday's exercises
- What (do you know) about …?
  - methionine
  - uric acid
  - 1-carbon

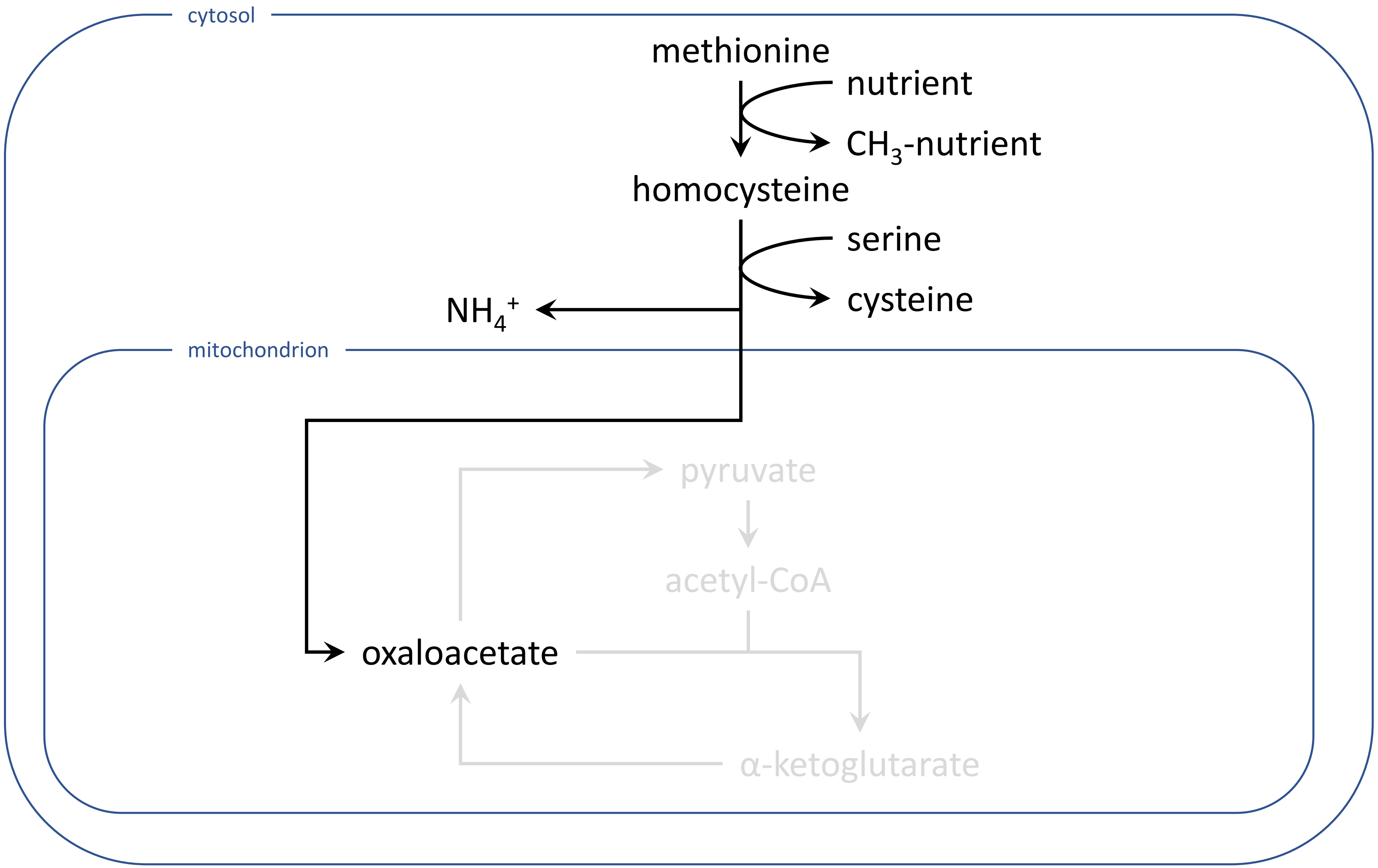
Methionine, cysteine, and 1-carbon metabolism

Methionine is involved in different methylation reactions

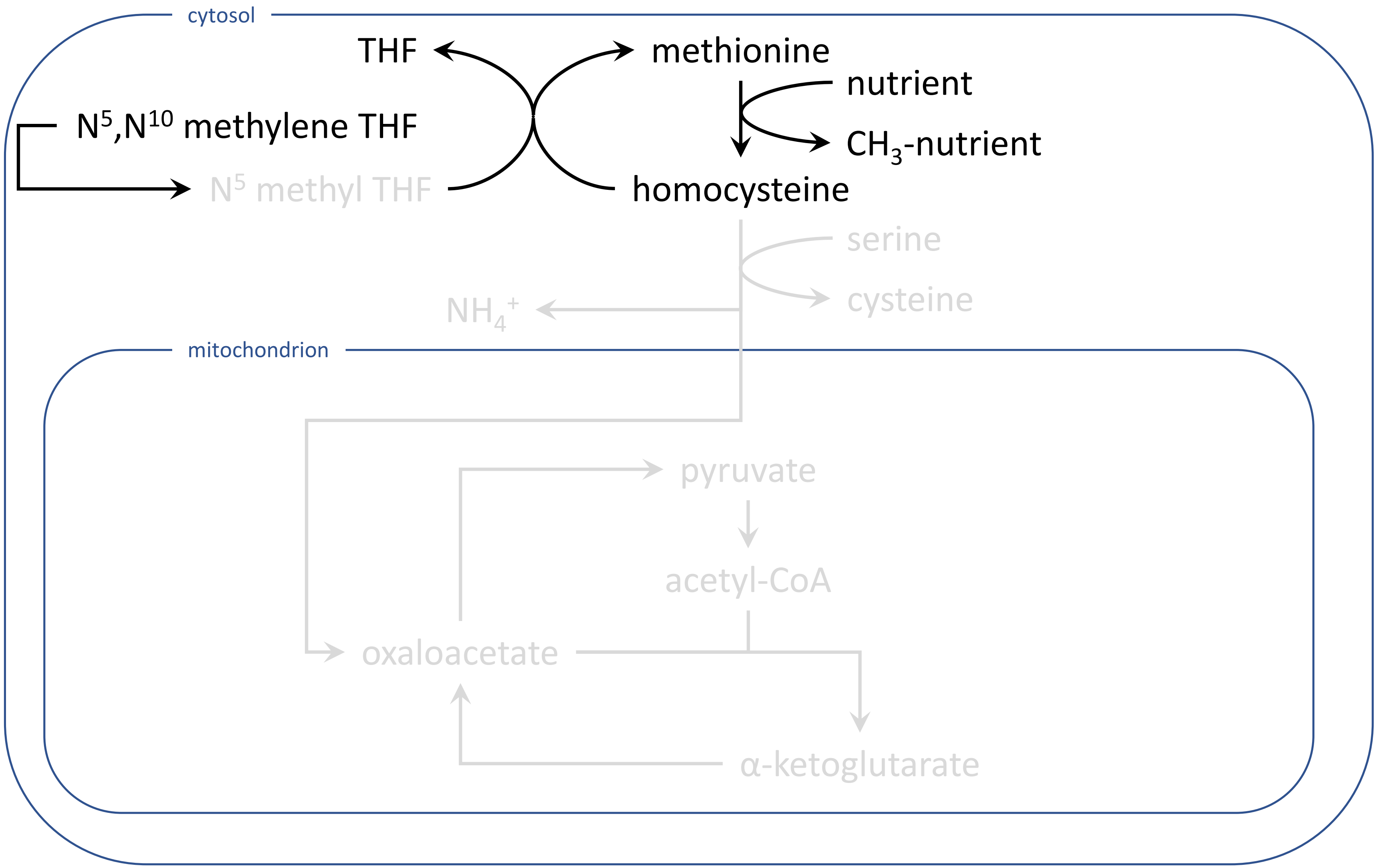
methionine + 3 ATP + deoxyadenosine in DNA → homocysteine + methyl-deoxyadenosine in DNA



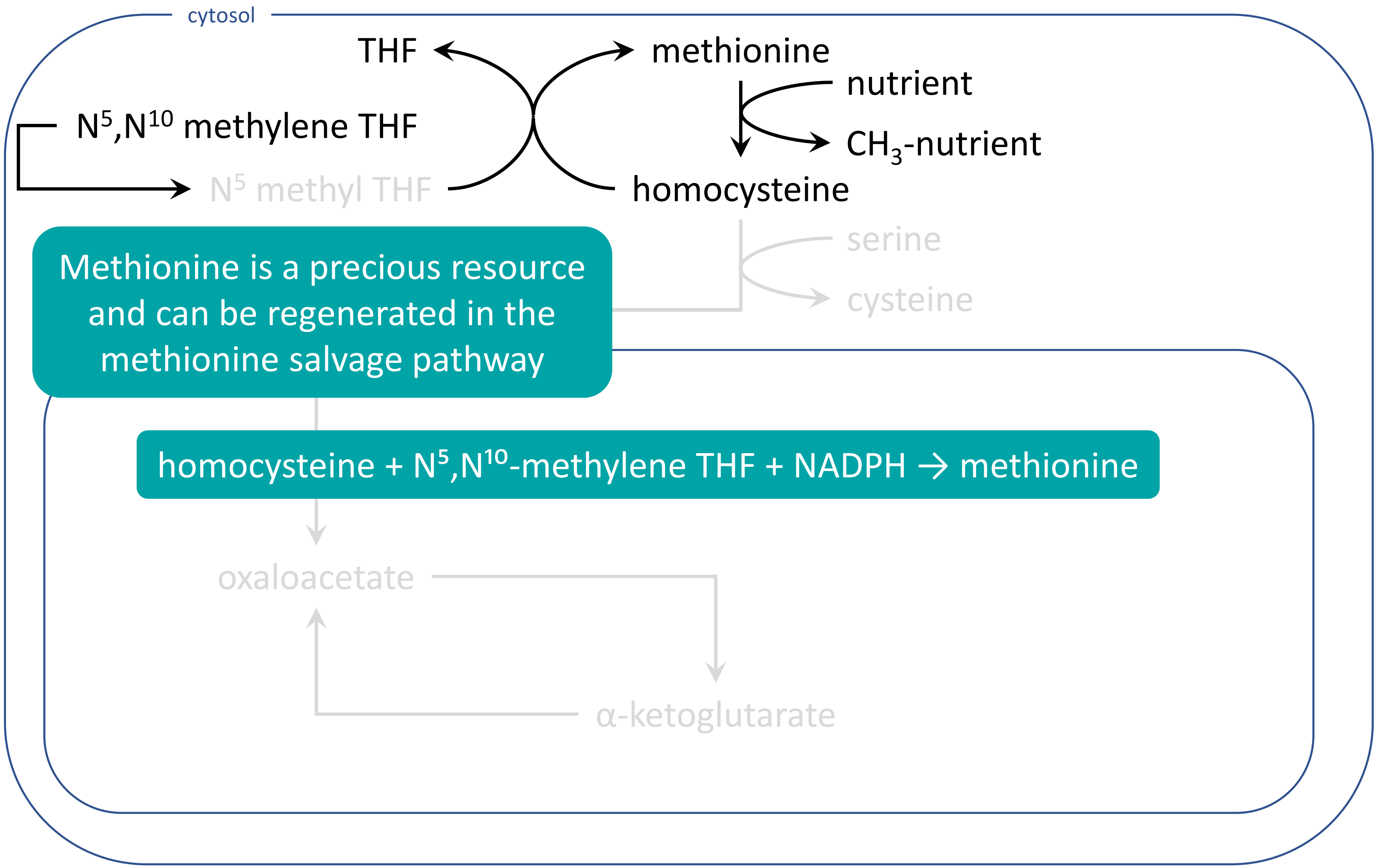
Methionine, cysteine, and 1-carbon metabolism



Methionine, cysteine, and 1-carbon metabolism



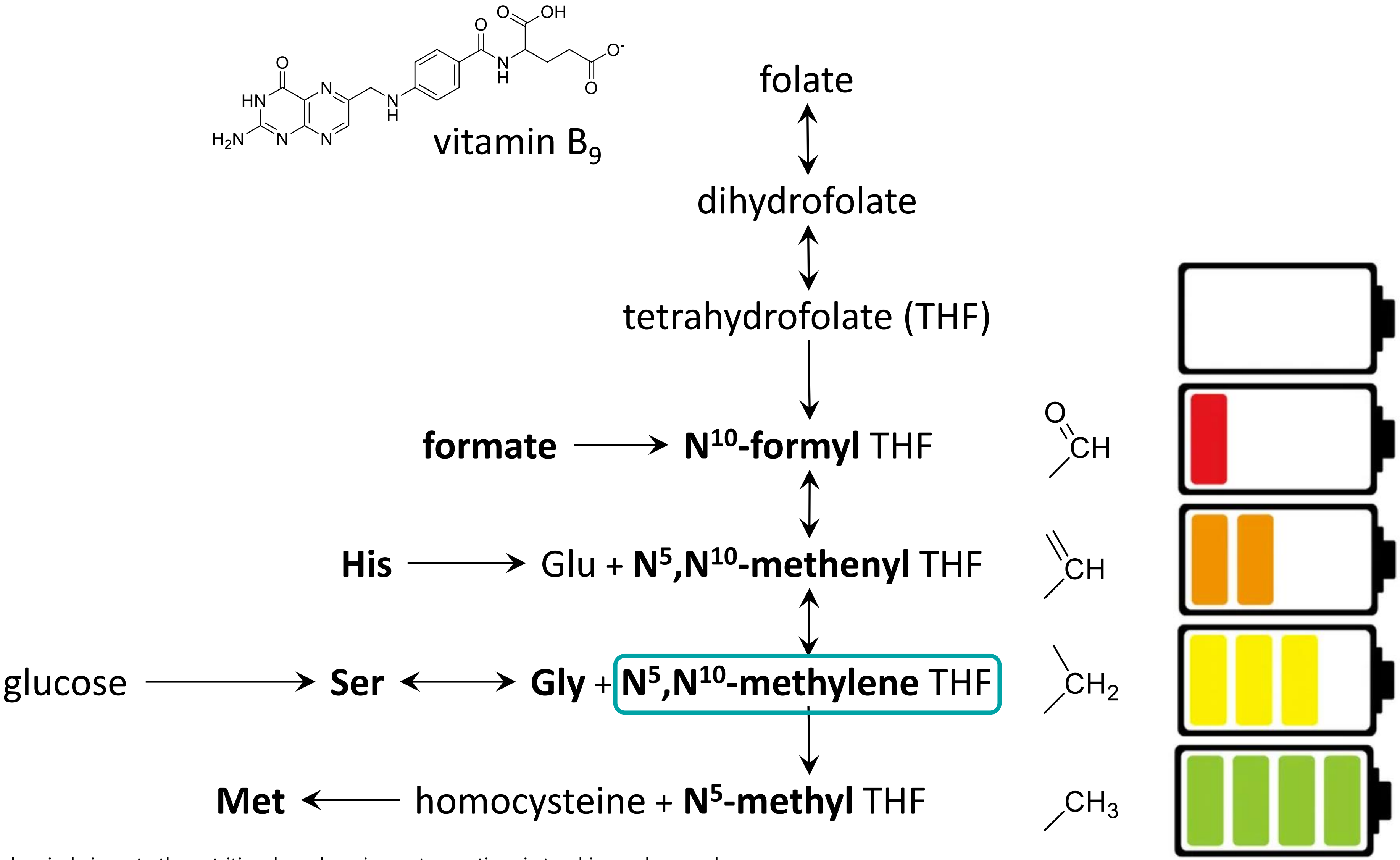
Methionine, cysteine, and 1-carbon metabolism



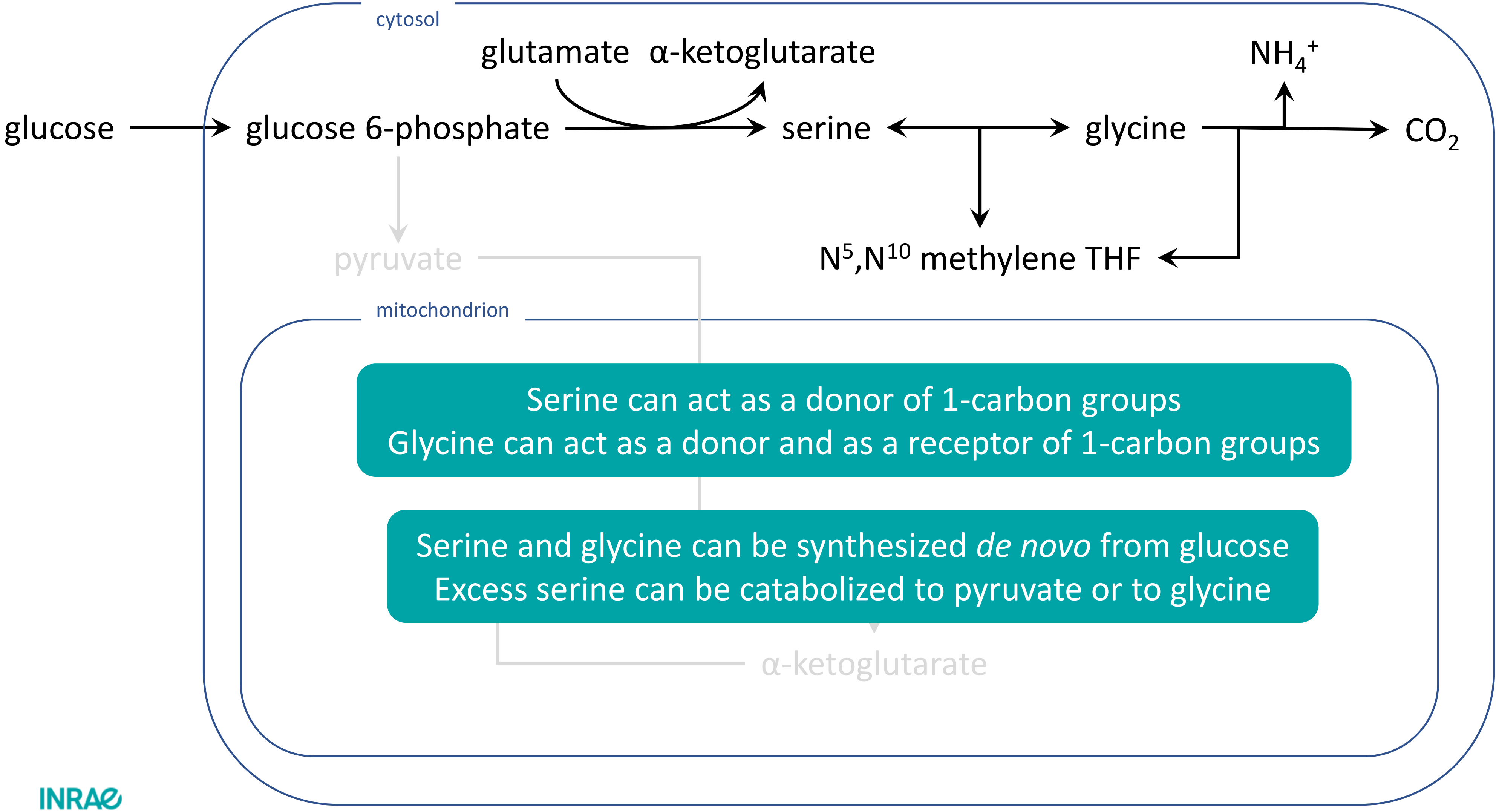
Methionine is a precious resource and can be regenerated in the methionine salvage pathway

homocysteine + N^5,N^{10}-methylene THF + NADPH → methionine

1-carbon metabolites attached to THF come in different flavors



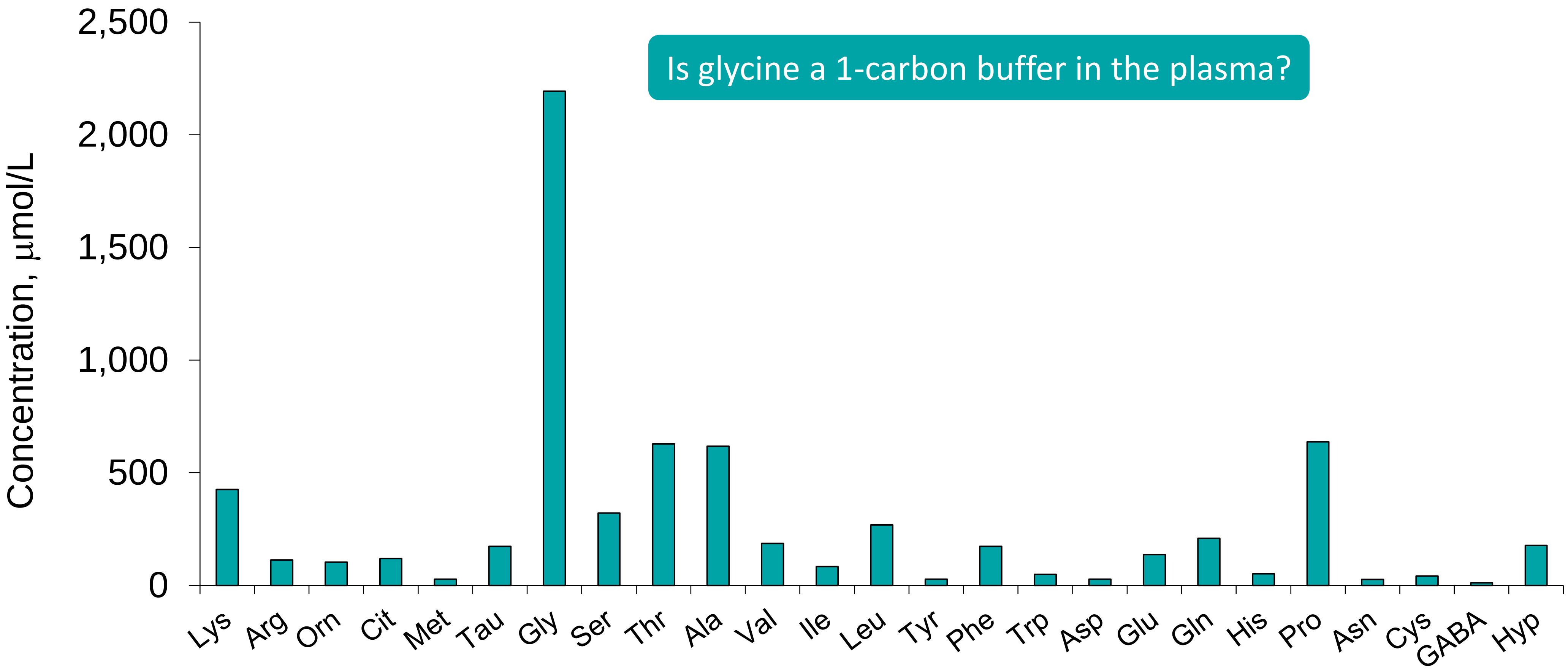
The *de novo* synthesis of 1-carbon from glucose



Serine can act as a donor of 1-carbon groups
Glycine can act as a donor and as a receptor of 1-carbon groups

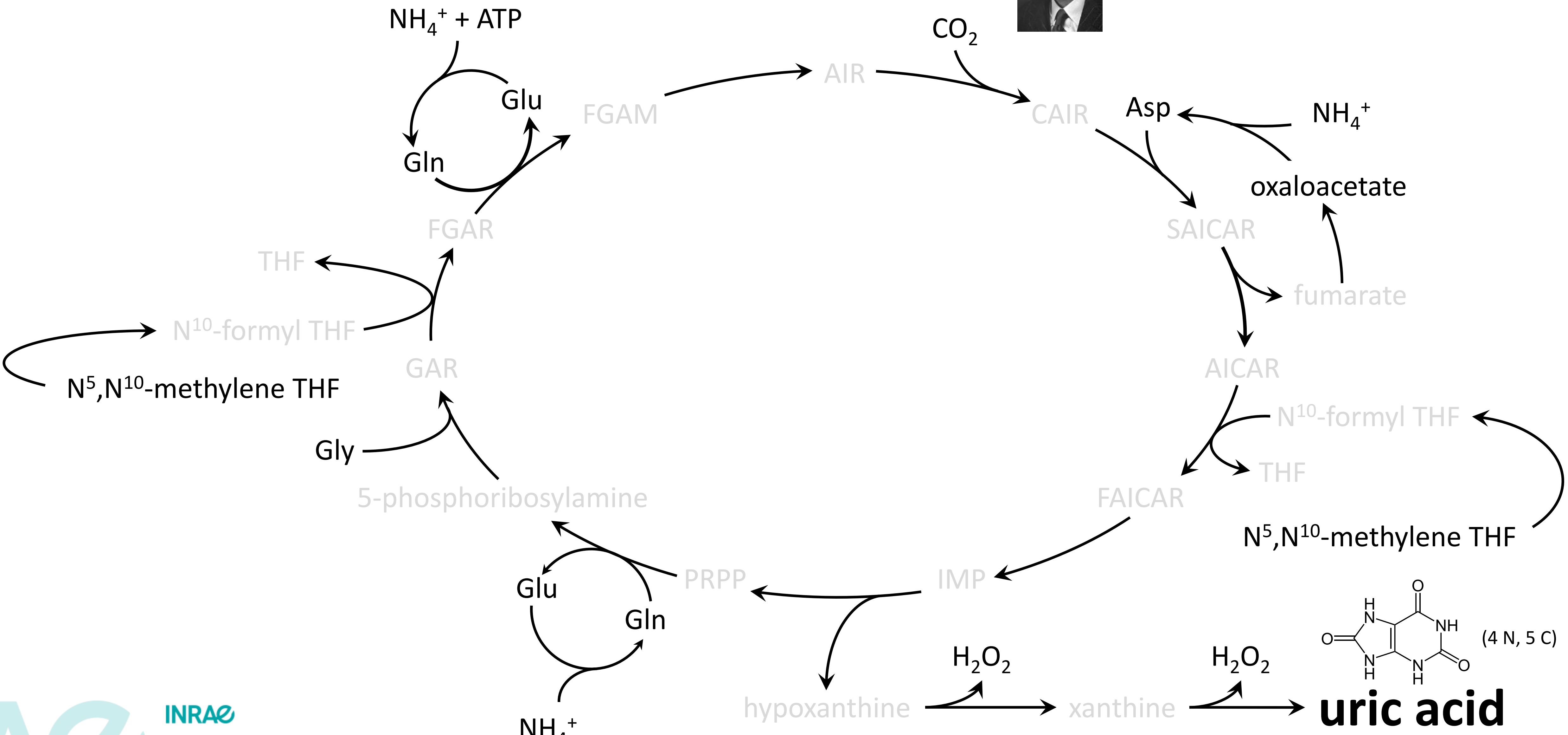
Serine and glycine can be synthesized *de novo* from glucose
Excess serine can be catabolized to pyruvate or to glycine

Amino acid concentration in the plasma

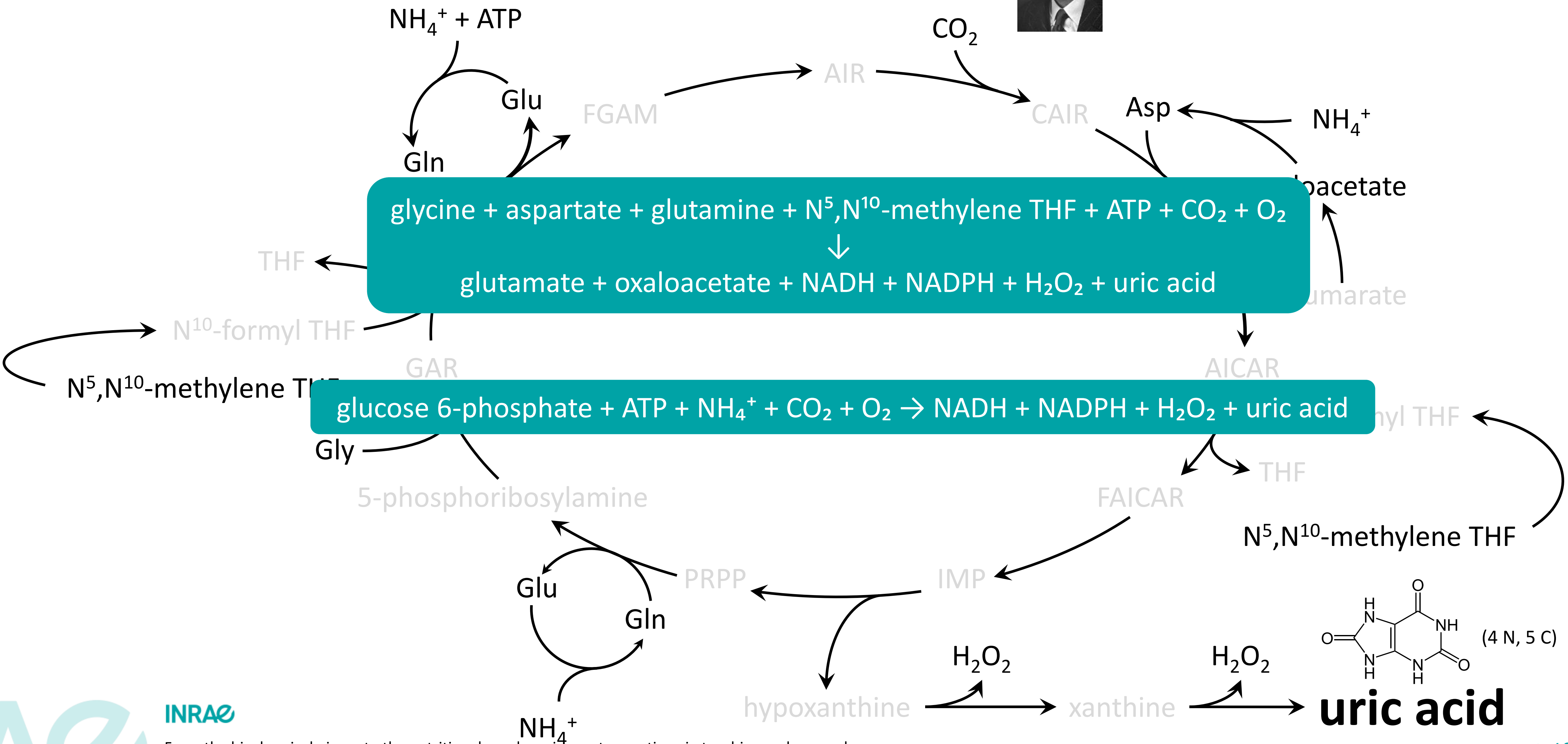


Bertolo *et al.* (2000)

Uric acid cycle (Mapes and Krebs, 1978)



Uric acid cycle (Mapes and Krebs, 1978)



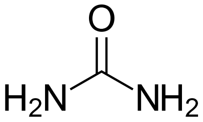
glycine + aspartate + glutamine + N^5,N^{10}-methylene THF + ATP + CO_2 + O_2

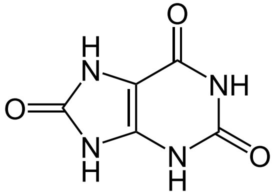
↓

glutamate + oxaloacetate + NADH + NADPH + H_2O_2 + uric acid

glucose 6-phosphate + ATP + NH_4^+ + CO_2 + O_2 → NADH + NADPH + H_2O_2 + uric acid

The energy cost of nitrogen excretion

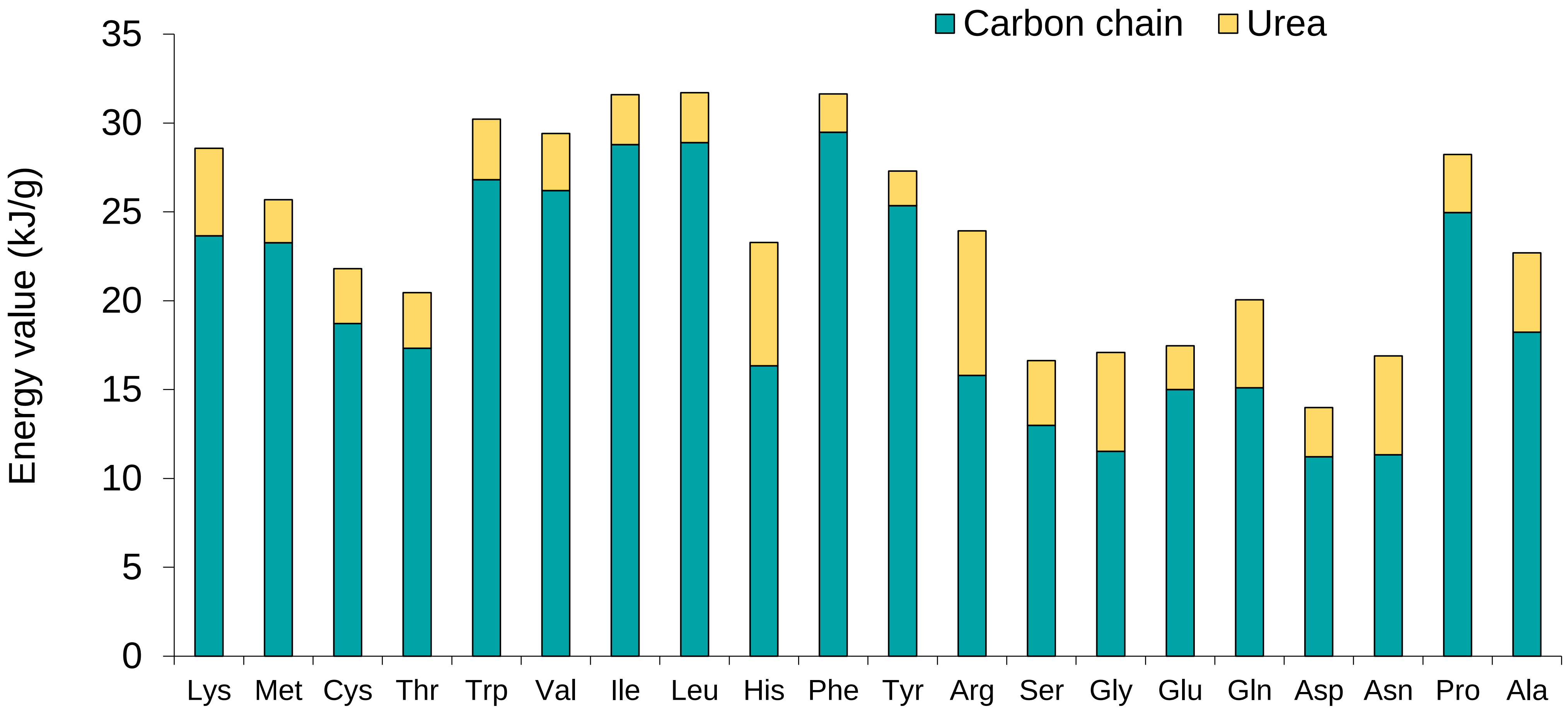




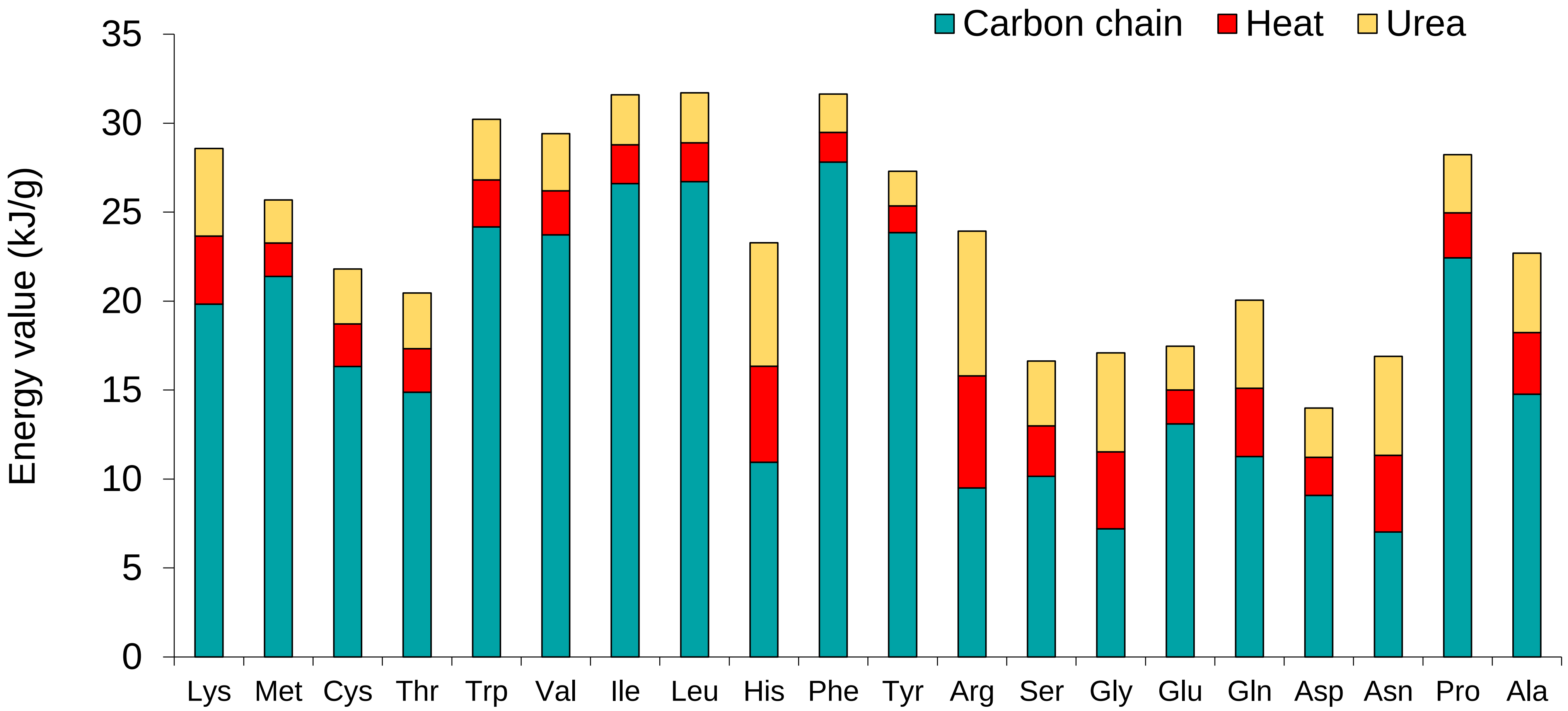
	Urea	Uric acid*
Cost to produce, kJ/mol N	564	850
Energy in product, kJ/mol N	318	480
Heat, kJ/mol N	247	370
DE, kJ/g digestible protein	23.8	23.8
ME*, kJ/g excess protein	20.2	18.3
NE*, kJ/g excess protein	17.4	14.1

* Maximum values based on stoichiometry

Metabolizable energy values of excess amino acids (in mammals)



Net energy values of excess amino acids (in mammals)



Net energy values of excess amino acids (in birds)