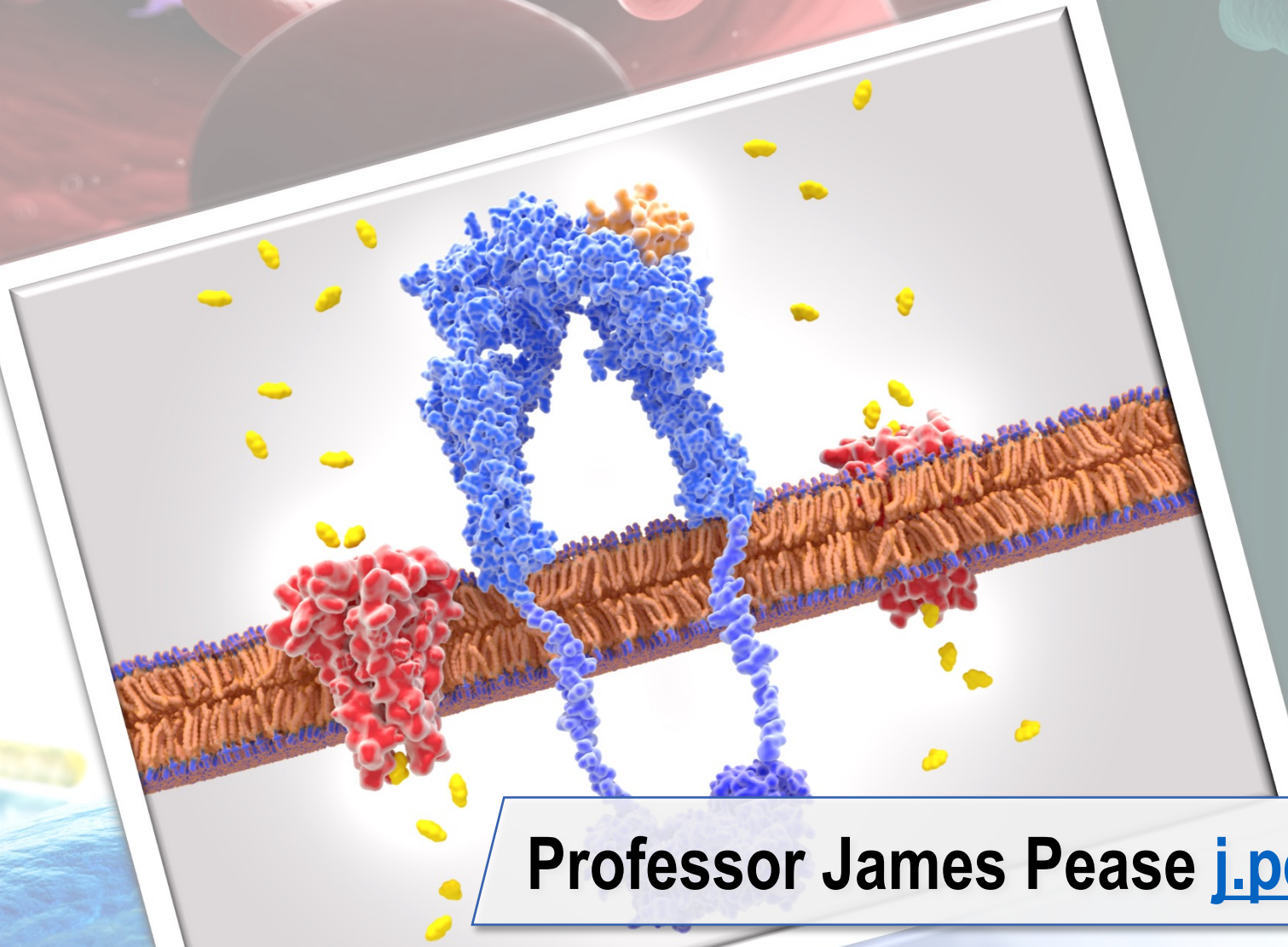
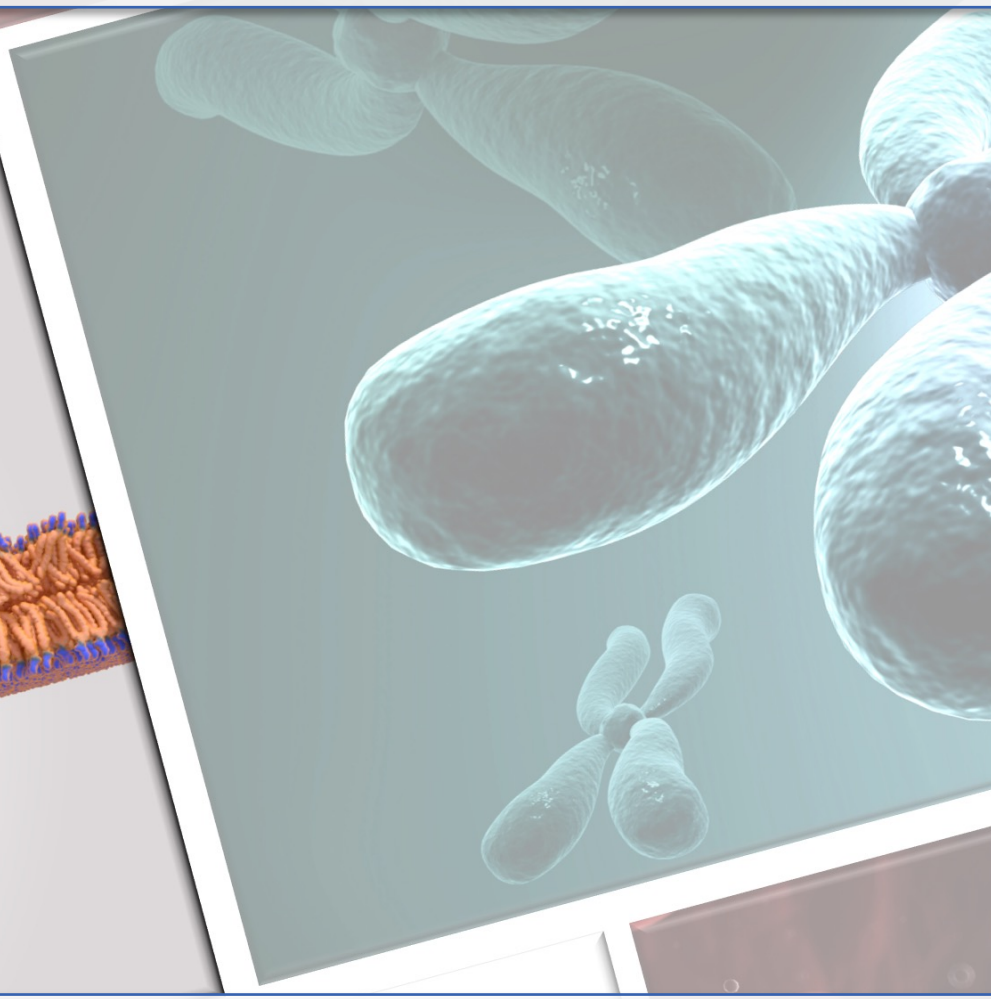
IMPERIAL

Cell metabolism 2





Professor James Pease j.pease@imperial.ac.uk

Session Plan

Part 1

The Tricarboxylic acid cycle

- Acetyl CoA entry
- Protein metabolism
- NADH transportation
- TCA cycle defects in cancer

Part 2

β-oxidation of fatty acids

- carnitine shuttle
- acetyl CoA production
- ketone body formation

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Lipogenesis

- comparison with beta oxidation
- lipogenesis in cancer
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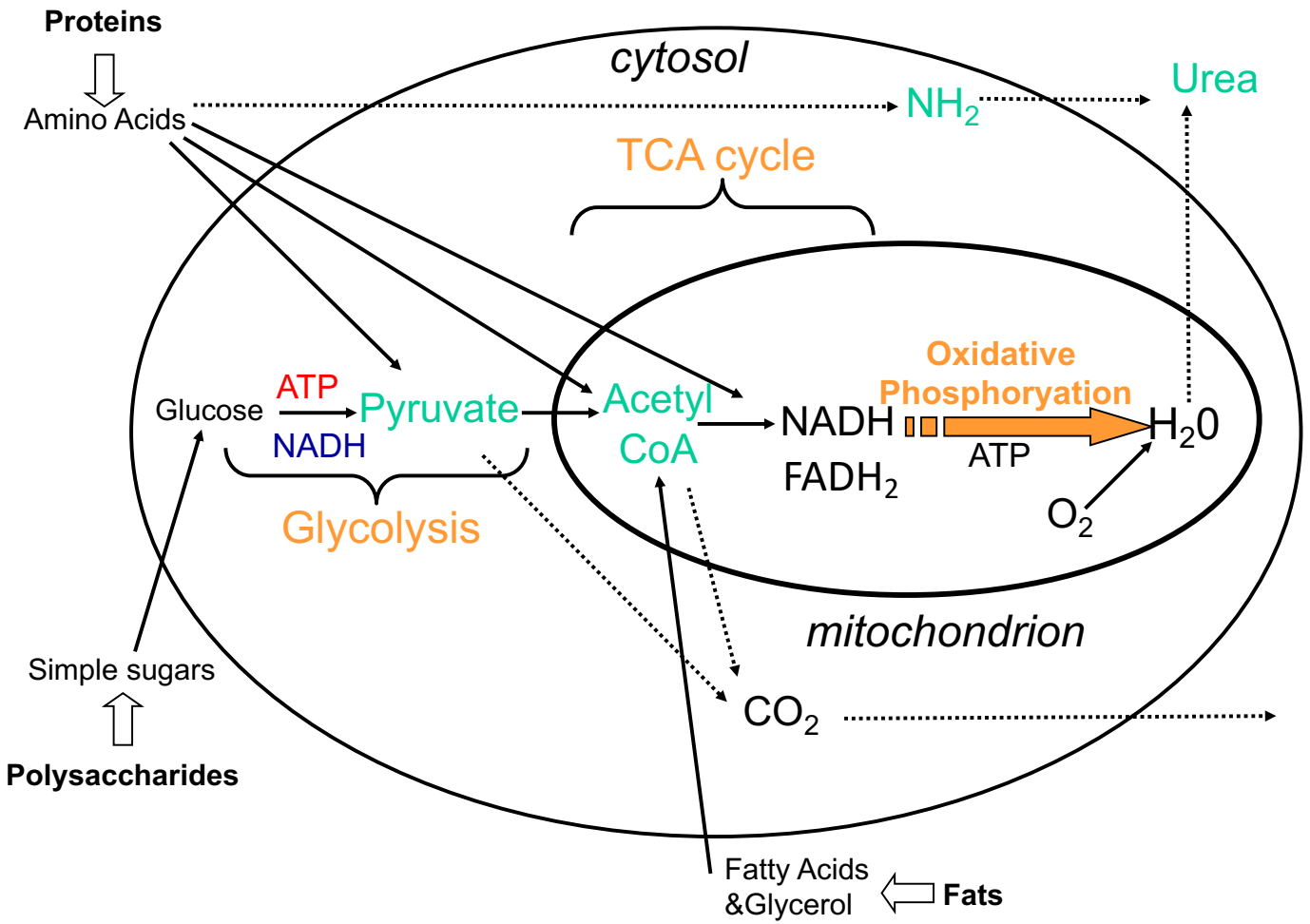
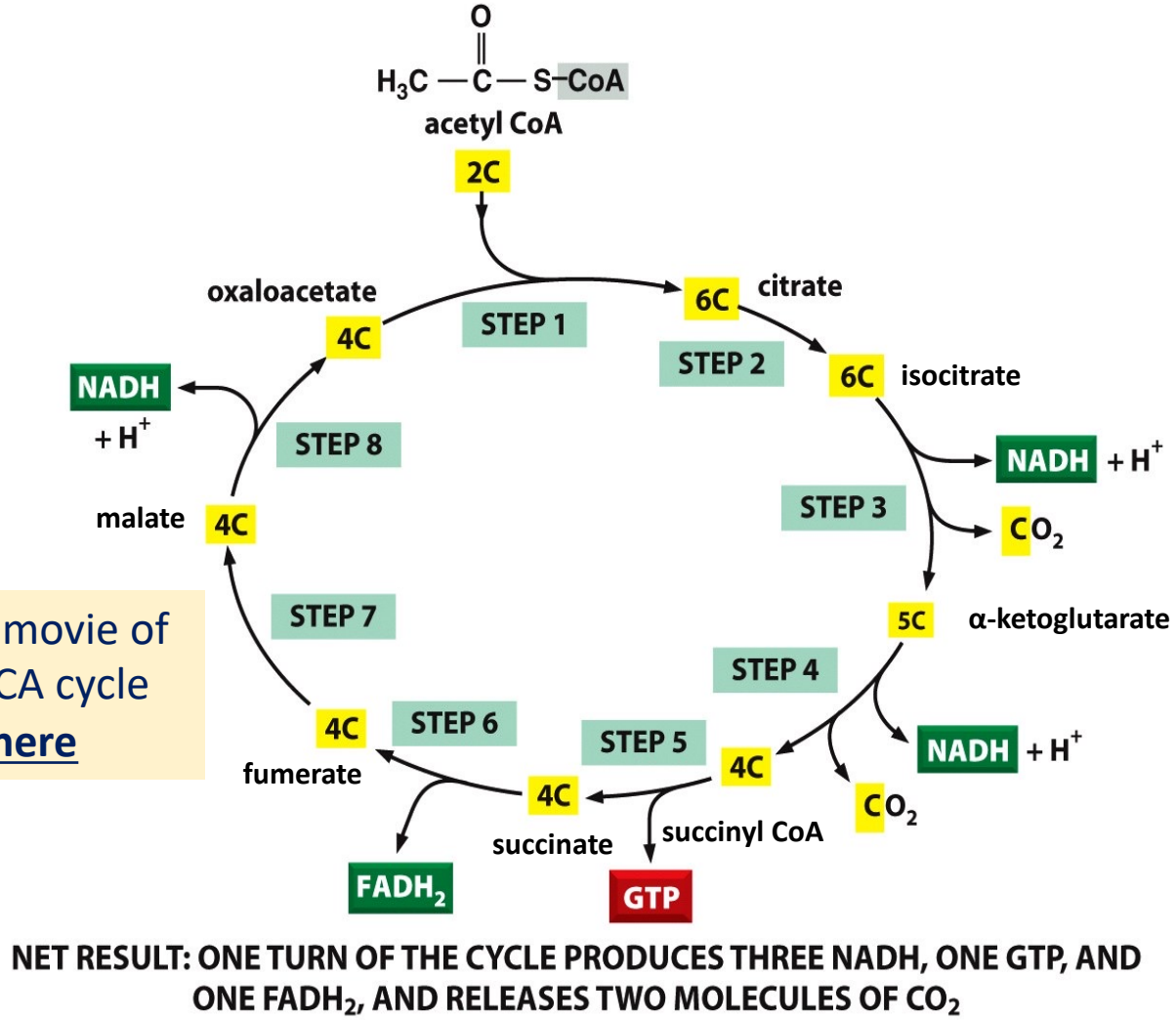
An overview of cellular metabolism



1-POM-1-1: Cell metabolism: Summarise the metabolism of carbohydrates, fats and proteins in health, exercise, illness and disease.

The Krebs or TCA (Tricarboxylic Acid) cycle



NET RESULT: ONE TURN OF THE CYCLE PRODUCES THREE NADH, ONE GTP, AND ONE $FADH_2$, AND RELEASES TWO MOLECULES OF CO_2

For a movie of the TCA cycle click **here**

- Each turn of the cycle produces two molecules of CO_2 (waste) plus three molecules of **NADH**, one molecule of **GTP** and one molecule of **$FADH_2$**.
- The Krebs cycle enzymes (with one notable exception) are soluble proteins located in the mitochondrial matrix space.
- The bulk of **ATP** is generated when the reduced coenzymes are re-oxidised with the help of oxygen (**oxidative phosphorylation)**.
- This re-oxidation means that the TCA cycle only operates under **aerobic** conditions.

Amino Acids can also enter the TCA cycle

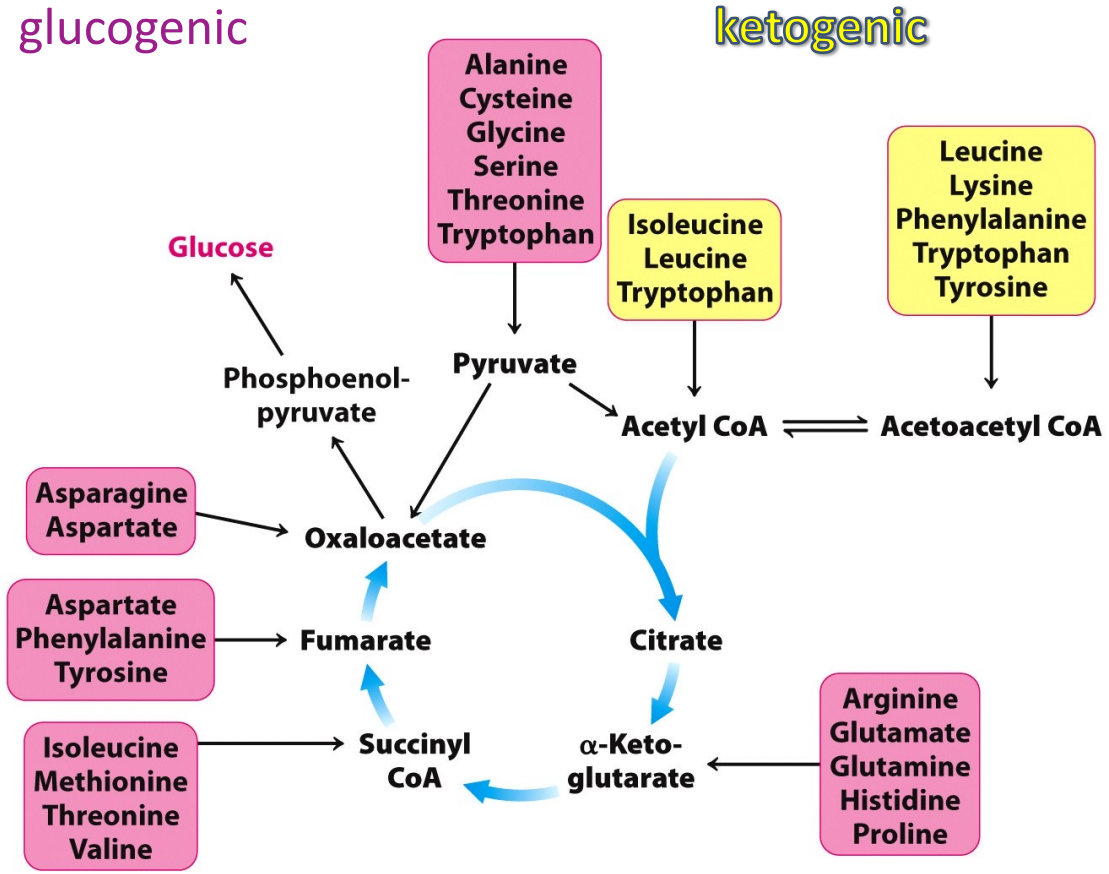


Figure 23.22
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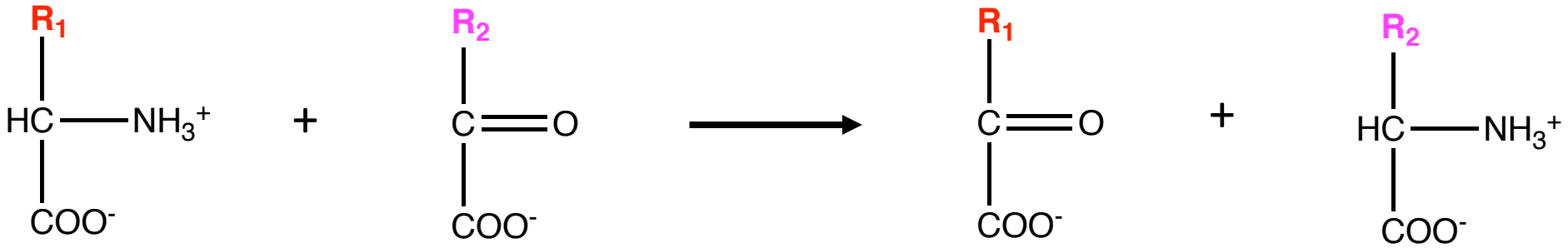
- The general strategy of amino acid degradation is to remove the amino group (which is eventually excreted as urea) whilst the carbon skeleton is either funnelled into the production of glucose (**MBC-Integration of metabolism**) or fed into the TCA cycle.
- Degradation of all twenty amino acids gives rise to only seven molecules: pyruvate, acetyl CoA, acetoacetyl CoA, a-ketoglutarate, succinyl CoA, fumarate and oxaloacetate.

Protein metabolism involves transamination reactions

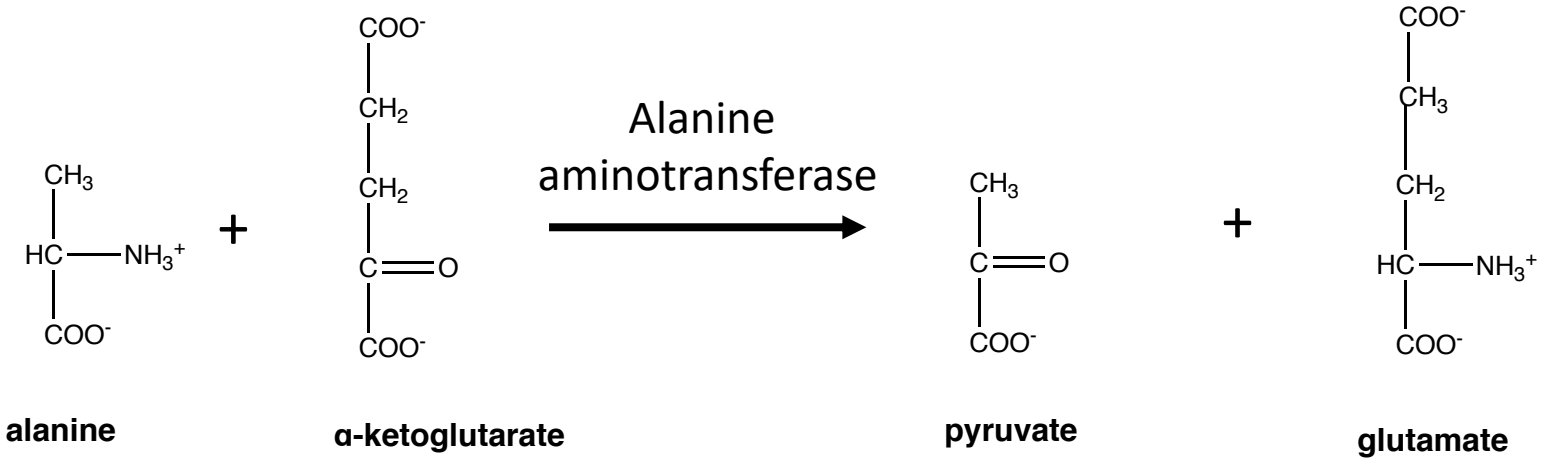


Q. What type of reaction is this?

GROUP TRANSFER

An amine group is transferred from one **amino** acid to a **keto** acid forming a new pair of amino and keto acids.

Alanine metabolism as an exemplar



Alanine (C3) undergoes transamination by the action of the enzyme alanine aminotransferase.

- Pyruvate can undergo decarboxylation and the acetyl CoA generated can enter the TCA cycle.
- Glutamate is re-converted to α-ketoglutarate by glutamate dehydrogenase.
- This generates NH_4^+ which is ultimately converted to urea.

NADH transportation – a problem….

- NADH produced in glycolysis needs to enter the mitochondria to be utilised by the process of oxidative phosphorylation and to regenerate NAD^+.
- In addition, there is only a finite amount of NAD^+ and unless it is regenerated, glycolysis (MBC – Cell metabolism 1) will very quickly grind to a halt.
- How does NADH, or more accurately, its high-energy electrons, cross from the cytosol into the matrix of the mitochondria ?
- The Glycerol phosphate shuttle – skeletal muscle, brain
- The Malate-aspartate shuttle - liver, kidney and heart

The Glycerol phosphate shuttle

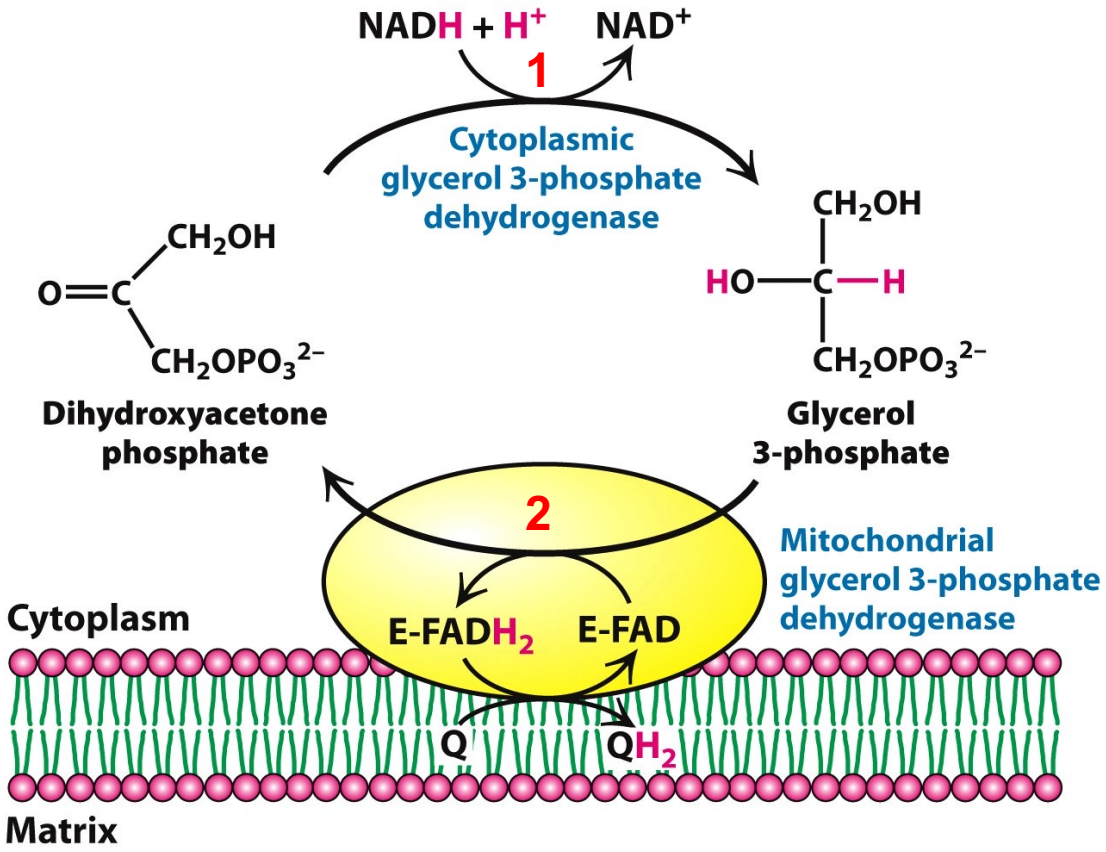


Figure 18.34
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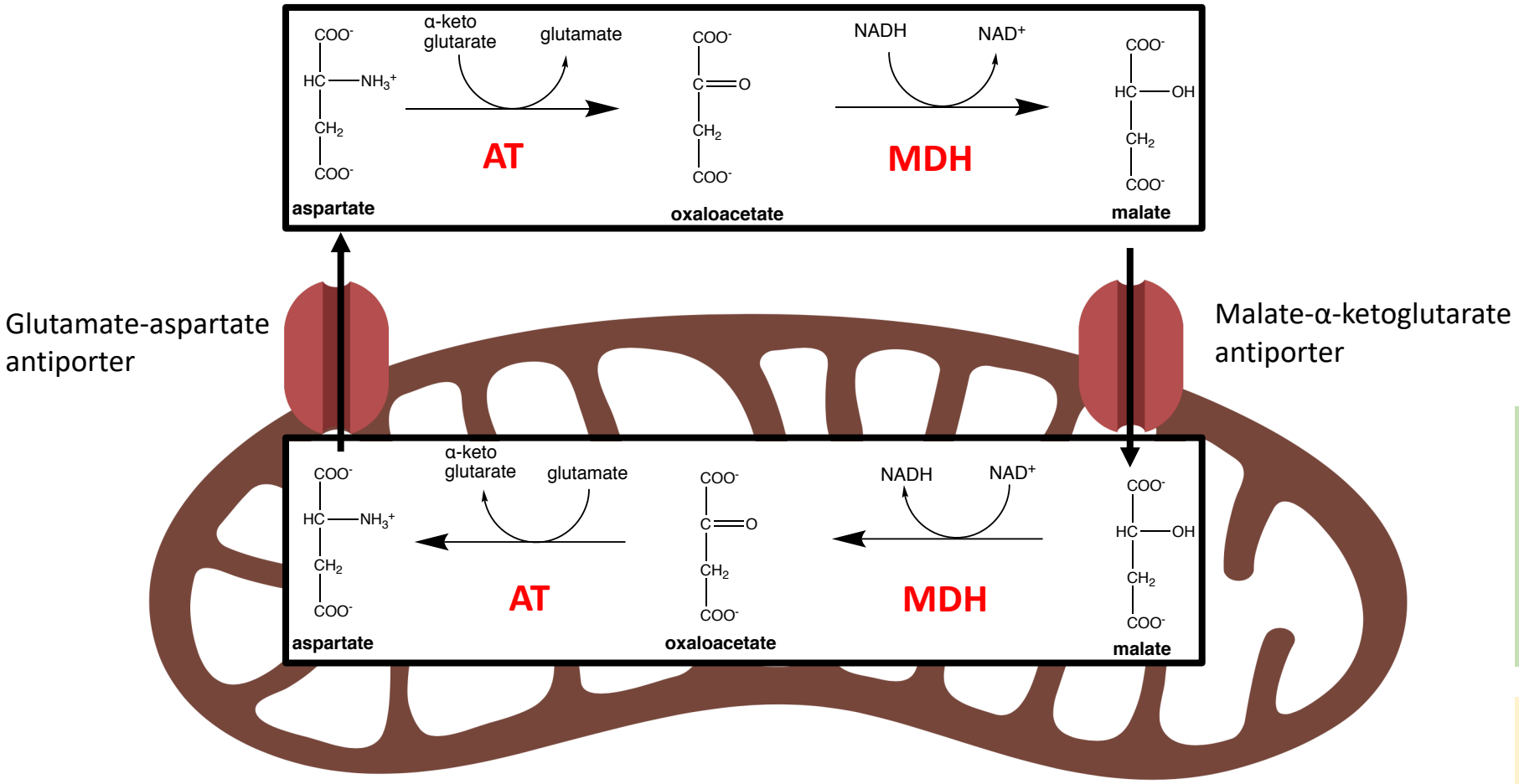
Electrons from NADH, rather than NADH itself are carried across the mitochondrial membrane via a **shuttle**.

1. Cytosolic glycerol 3-phosphate dehydrogenase transfers electrons from NADH to DHAP to generate glycerol 3-phosphate.

2. A membrane bound form of the same enzyme transfers the electrons to FAD. These then get passed to co-enzyme Q, part of the electron transport chain.
MBC - Cell integrity

Malate-aspartate shuttle

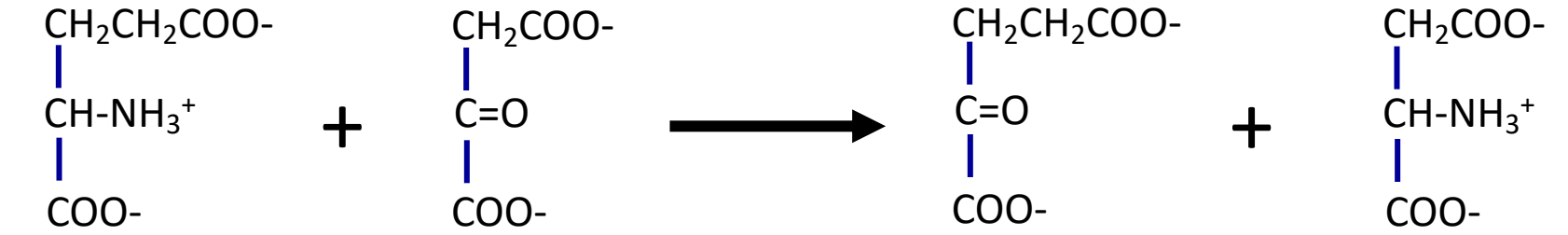


Q. What reactions are happening in the shuttle?

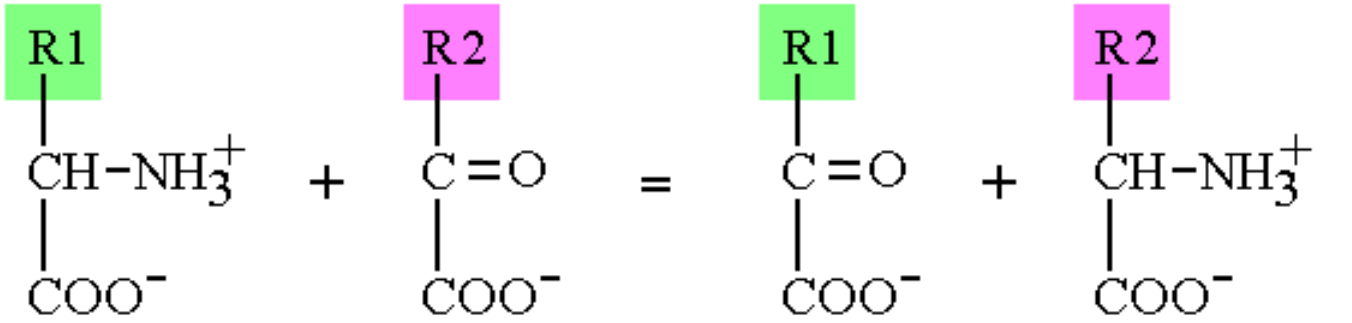
REDOX

AT – aspartate transaminase
MDH – malate dehydrogenase

Transamination in the Malate-aspartate shuttle



glutamate oxaloacetate α-ketoglutarate aspartate



amino acid 1 keto acid 2 keto acid 1 amino acid 2

Q. Any other reactions?

TRANSAMINATION

ATP Production by glycolysis and the Krebs cycle is only a prelude to oxidative phosphorylation

- Re-oxidation of the reduced co-factors NADH and $FADH_2$ by the process of oxidative phosphorylation MBC- Cell integrity yields the following:
- ~ three ATP molecules are formed by the re-oxidation of each NADH molecule.
- ~ two ATP molecules are formed by the re-oxidation of each $FADH_2$ molecule.
- Therefore, from the TCA cycle:
- Oxidation of 1 X acetyl CoA molecule gives 3 x NADH + 1 x $FADH_2$ + 1 x GTP = 12 ATP

Glycolysis and the Krebs cycle provide the starting point for many biosynthetic reactions

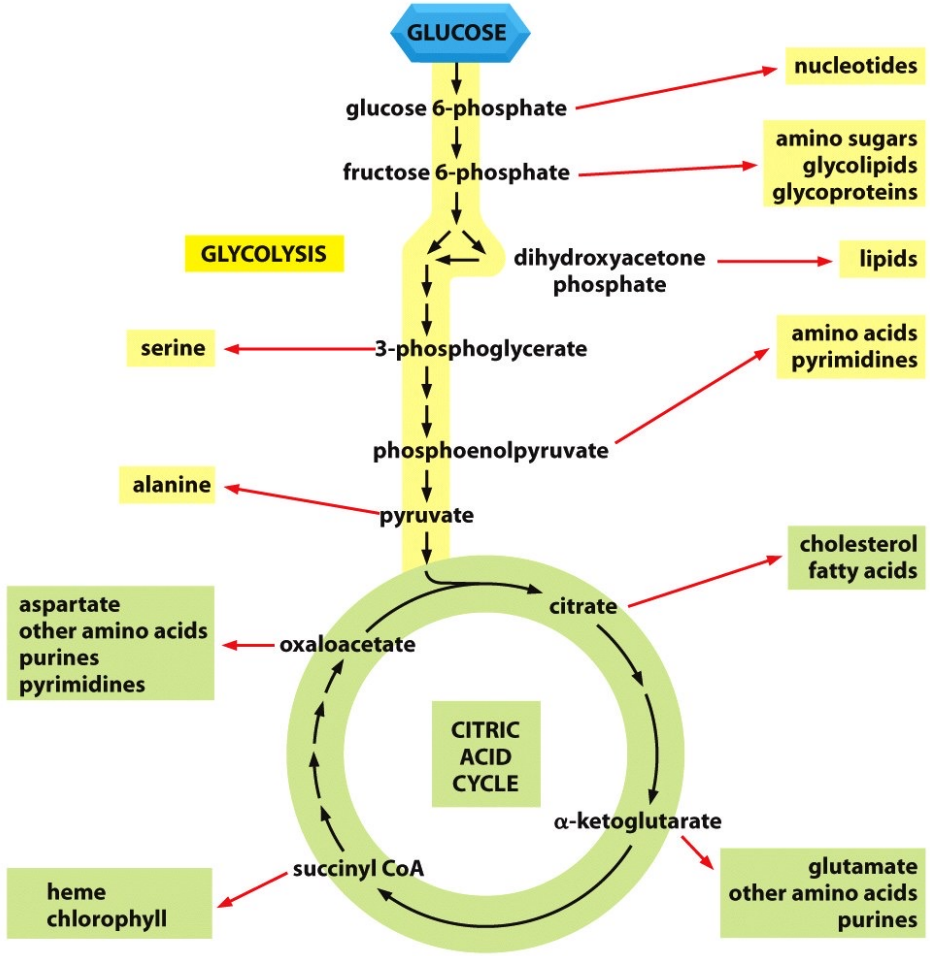
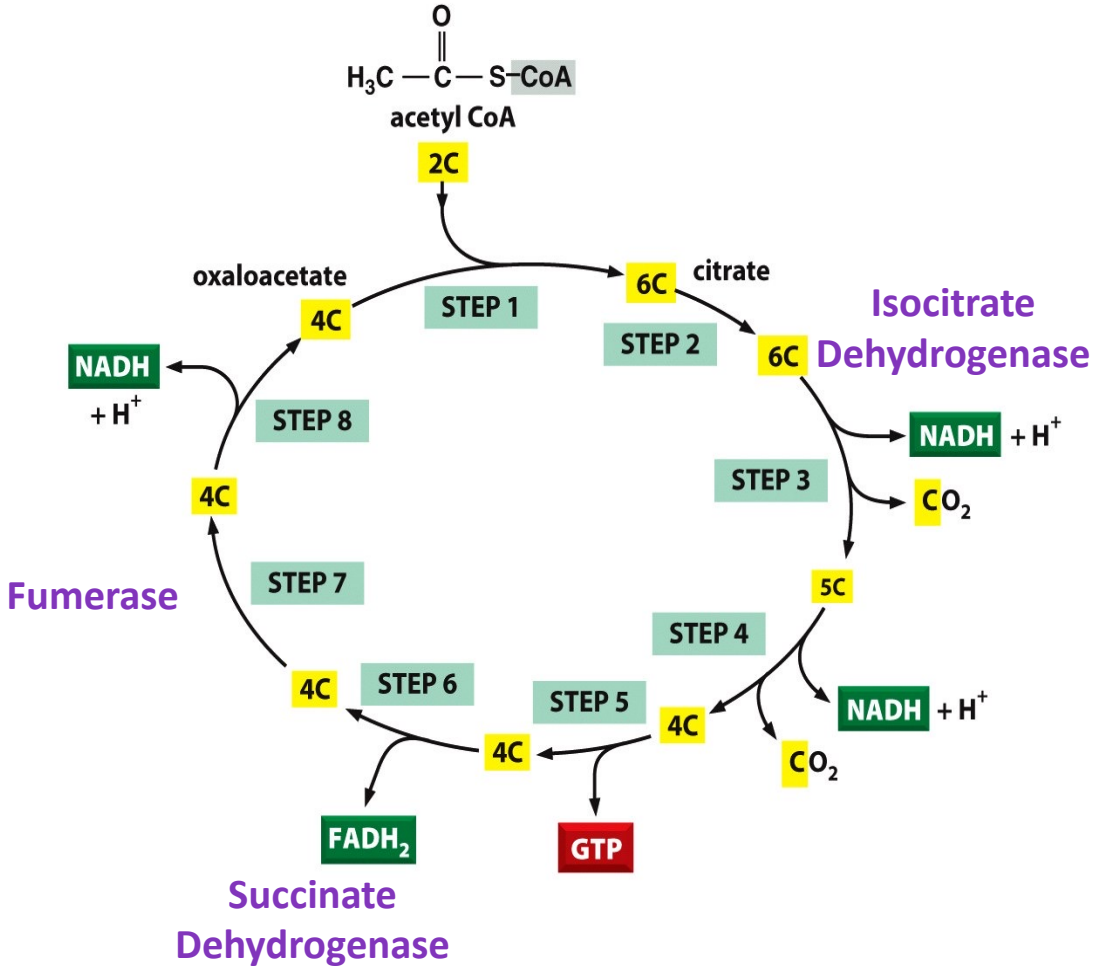


Figure 13-17 Essential Cell Biology (© Garland Science 2010)

- The amino acids, nucleotides, lipids, sugars, and other molecules shown here as products, in turn, become the precursors for the many of the macromolecules of the cell.
- Each black arrow in this diagram denotes a single enzyme-catalysed reaction. Red arrows generally represent multi-step pathways.

TCA cycle defects in cancer



- Mutations in the TCA genes shown in **purple** have been shown to decrease TCA activity and enhance aerobic glycolysis: the preferential generation of lactate from glucose even under conditions of ample O_2. (The Warburg Effect).
- If we can force the cells to utilise oxidative phosphorylation instead, can we turn them into non-malignant cells?
- Sullivan et al, (2016). Nature Rev. Cancer. doi:10.1038/nrc.2016.85

Progress Check

Part 1

- Acetyl Co enters the TCA cycle under aerobic conditions to generate NADH, $FADH_2$, GTP and CO_2
- The carbon skeletons of amino acids can enter the TCA cycle after transamination.
- Two shuttles exist to help electrons from NADH enter the mitochondria.
- Glycolysis and the TCA cycle provide building blocks for further biosynthesis reactions.

Part 2

- Fatty acids are transported into the mitochondria where they undergo β-oxidation.
- Acetyl CoA produced can either enter the TCA cycle or form ketone bodies.
- The complete oxidation of palmitate generates significant quantities of ATP.

Part 3

- Just two enzymes drive lipogenesis which is essentially a reversal of the reactions seen in β-oxidation.
- MCADD is a disorder of fatty acid metabolism which can be treated with lifestyle adaptations.

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Fatty acid metabolism

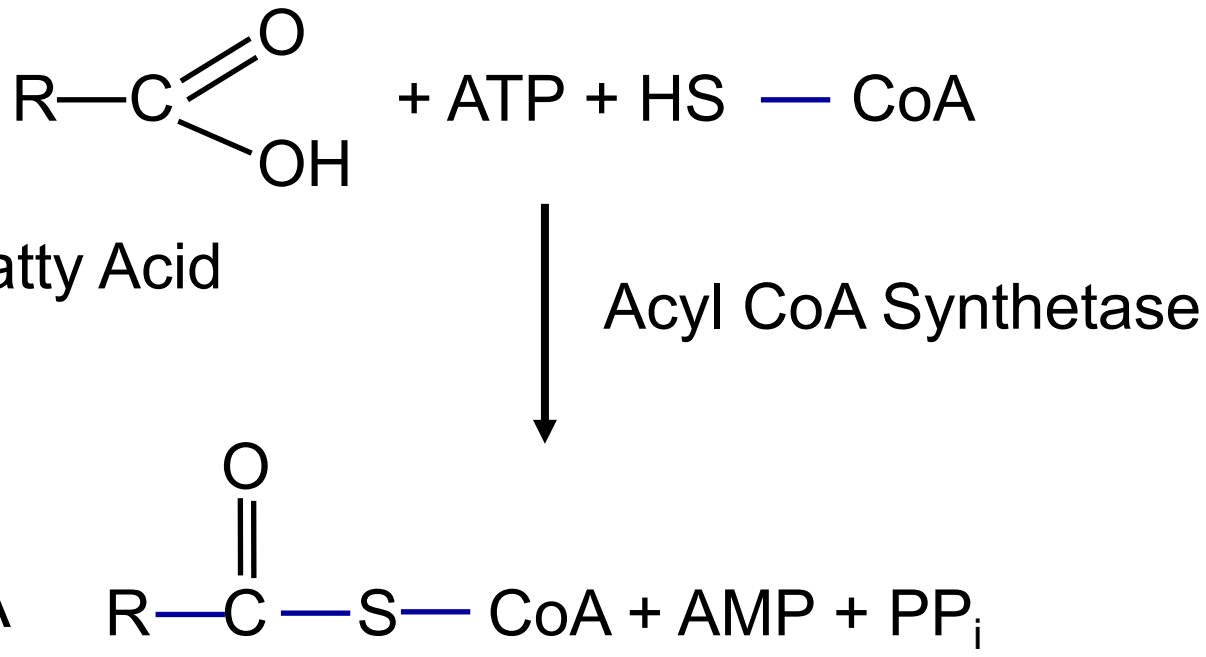
Image: Shutterstock

On a weight basis, the caloric yield from fatty acids is about **double** that from carbohydrates.

More than half of the body's energy needs including the liver, but not the brain, comes from fatty acid oxidation and this is enhanced during fasting over long periods of time.

This process is known collectively as **β-oxidation** and occurs in the mitochondria in several stages, resulting in the generation of acetyl CoA.

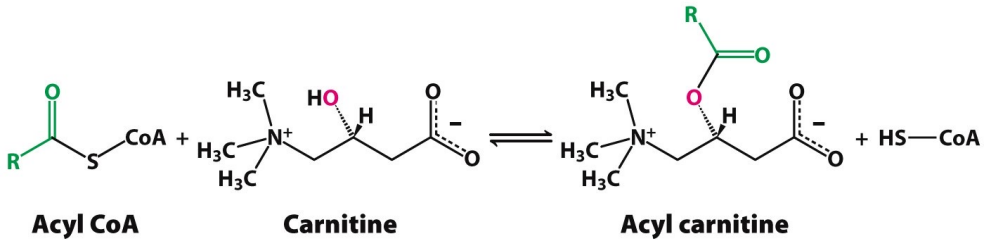
β-oxidation of fatty acids



Firstly, fatty acids are converted into an acyl CoA species.

i.e. **ATP** ⟶ **AMP**, 2 high energy bonds are used.

The carnitine shuttle



- Generation of the Acyl CoA species occurs on the outer mitochondrial membrane.
- To transport the species into the matrix it is coupled to the molecule carnitine to form acyl carnitine.
- Carnitine and Acyl carnitine are moved to and from the matrix by a translocase.

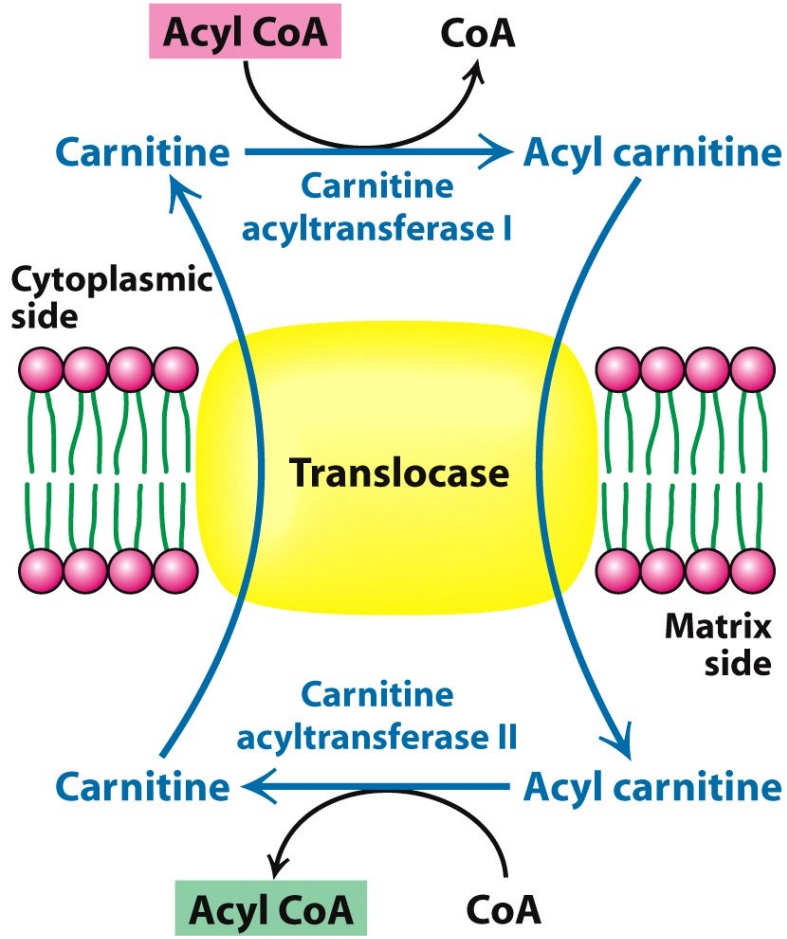


Figure 22.8
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Primary carnitine deficiency

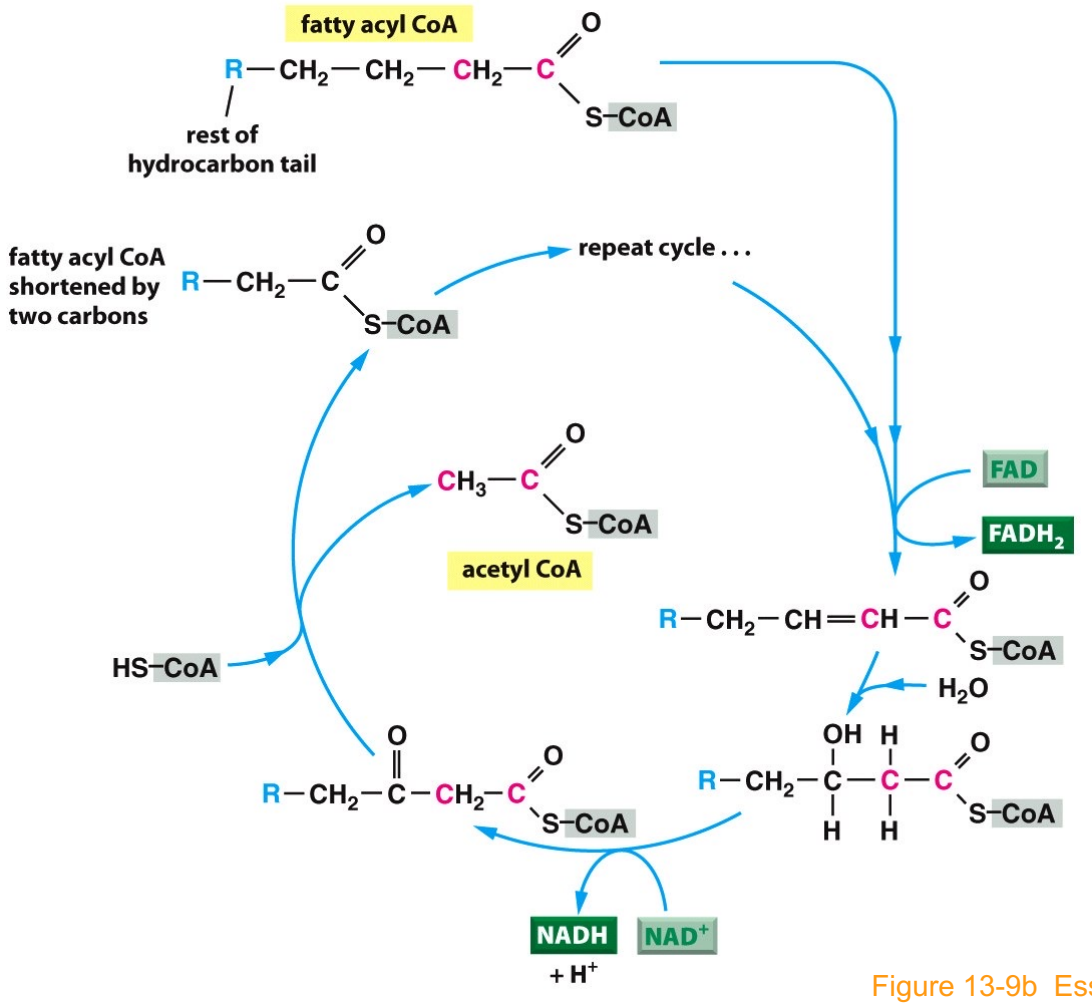
Carnitor® /Levocarnitine
Used as a supplement

- Autosomal recessive disorder. 1-POM-4-1
- Occurs 1 in 100,000 live births in the USA per year (1 in 40,000 live births in Japan; 1 in 500 in the Faroe Islands).
- Symptoms appear during infancy or early childhood and include encephalopathies, (cardiomyopathies, muscle weakness; and hypoglycaemia).

Mutations in a gene known as SLC22A5 which encodes a carnitine transporter result in reduced ability of cells to take up carnitine, needed for the β-oxidation of fatty acids.

1-POM-1-1: Cell metabolism: Summarise the metabolism of carbohydrates, fats and proteins in health, exercise, illness and disease.

The β–oxidation cycle



- The acyl CoA undergoes a sequence of oxidation, hydration, oxidation and thiolysis reactions (collectively called β-oxidation).
- This results in the production of one molecule of acetyl CoA and an acyl CoA species which is 2 carbons shorter than the original.

Figure 13-9b Essential Cell Biology (© Garland Science 2010)

β–oxidation of palmitic acid generates Acetyl CoA

- The β-oxidation reactions continue to consecutively remove 2-carbon units from the acyl CoA thereby producing acetyl CoA.
- On the final cycle (4-carbon fatty acyl CoA intermediate), two acetyl CoA molecules are formed.
- From just 7 β-oxidation reactions, the 16-carbon palmitoyl CoA molecule produces 8 molecules of acetyl CoA.

During each cycle one molecule each of $FADH_2$ and NADH are produced. The overall reaction of β-oxidation of palmitoyl CoA is:

palmitoyl CoA + 7 FAD + 7 NAD^+ + 7 H_2O + 7 CoA → 8 acetyl CoA + 7 $FADH_2$ + 7 NADH

Ketone body formation

Enzymes:
1=3-ketothiolase
2=hydroxymethylglutaryl CoA synthase
3=hydroxymethylglutaryl CoA cleavage enzyme,
4=D-3-hydroxybutyrate dehydrogenase

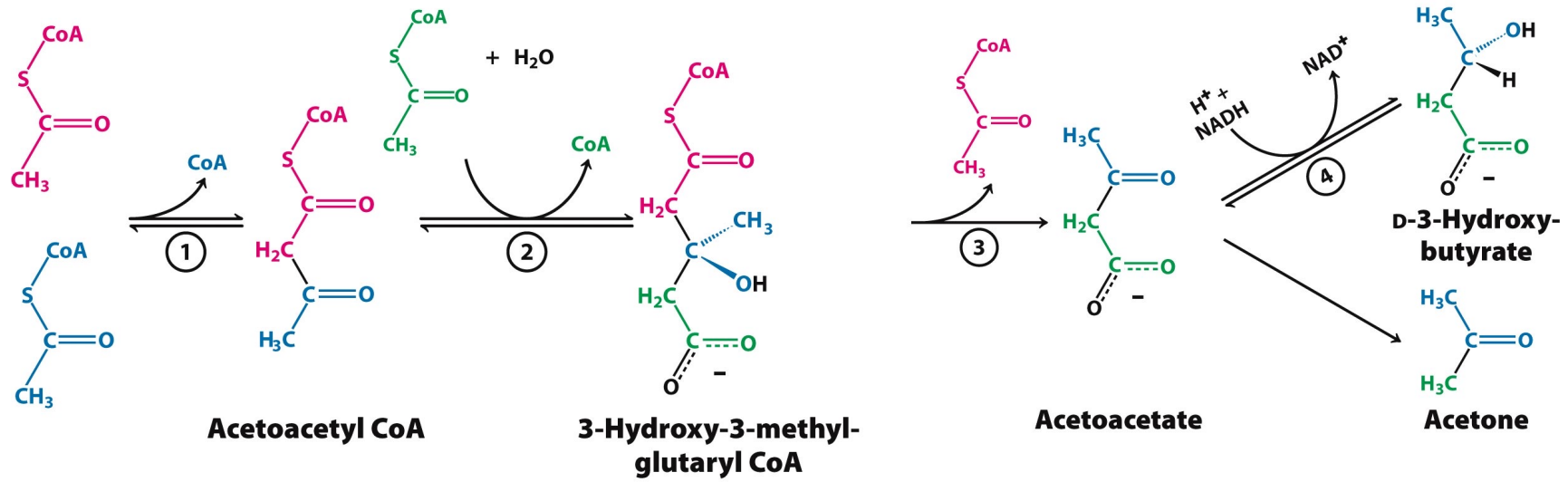


Figure 22.21 part 1
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- Acetyl CoA generated by β-oxidation enters the TCA cycle only if β-oxidation and carbohydrate metabolism are balanced, since oxaloacetate is needed for entry. Hence the adage “Fat burns in the flame of carbohydrate”.
- When fat breakdown predominates e.g. during fasting, acetyl CoA forms acetoacetate, D-3-hydroxybutyrate and acetone known collectively as **ketone bodies**.

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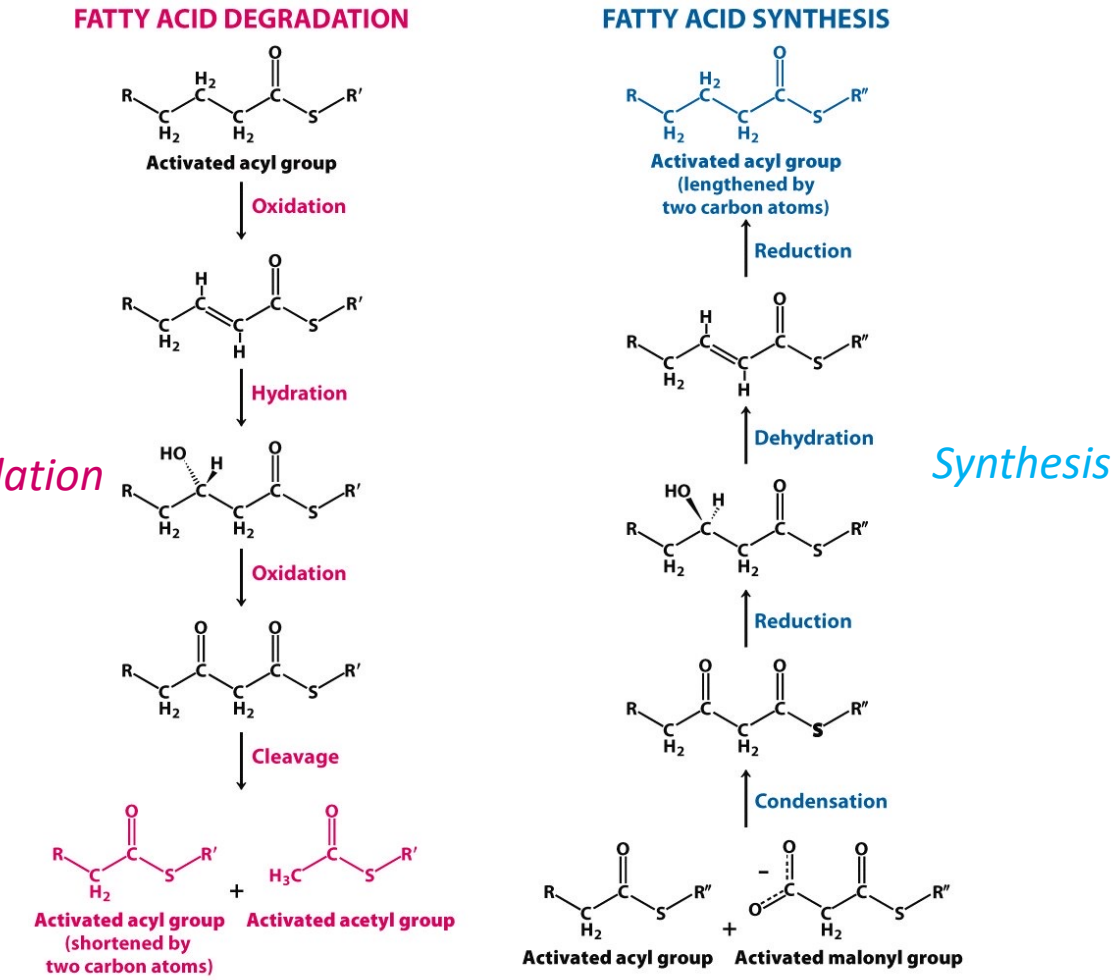
Fatty acid biosynthesis a.k.a. lipogenesis

- In contrast to β–oxidation, **Fatty acid biosynthesis** involves just **two** enzymes:
- Acetyl CoA Carboxylase and Fatty acid synthase.
- Fatty acids are formed sequentially by decarboxylative condensation reactions involving the molecules acetyl-CoA and malonyl-CoA.
- Following each round of elongation, the fatty acid undergoes reduction and dehydration by the sequential action of a ketoreductase (KR), dehydratase (DH), and enol reductase (ER) activity.
- The growing fatty acyl group is linked to an acyl carrier protein (ACP).

Similarities between synthesis and degradation



β-oxidation

Synthesis

Figure 22.2
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Distinctions:

- Carriers: ACP vs CoA
- Reducing Power: NADPH v FAD/NAD^+
- Locations: Cytoplasm v Mitochondrial Matrix

Summary of lipogenesis

Overall reaction:

Acetyl CoA (C2) + 7 Malonyl CoA (C3) + 14 NADPH +14 H^+

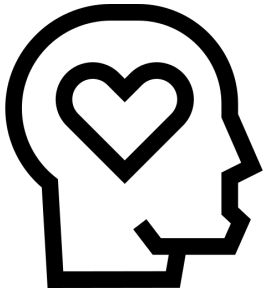
⟶ Palmitate (C16) + 7 CO_2+ 6 H_2O + 8 CoA-SH + 14 $NADP^+$

- **Elongation** of the acyl group to make fatty acids longer than 16 carbons occurs separately from palmitate synthesis in the mitochondria and endoplasmic reticulum (ER).
- **Desaturation** of fatty acids requires the action of **fatty acyl-CoA desaturases**
- The enzyme that creates oleic acid and palmitoleic acid from stearate and palmitate, respectively, is called a Δ-9 desaturase, as it generates a double bond nine carbons from the terminal carboxyl group.

Fatty acid synthesis in cancer

In adults, de novo FA biosynthesis is restricted mainly to the liver, adipose tissue and lactating breast.

Evidence suggests that reactivation of FA synthesis also occurs in certain cancer cells.
Energy source?

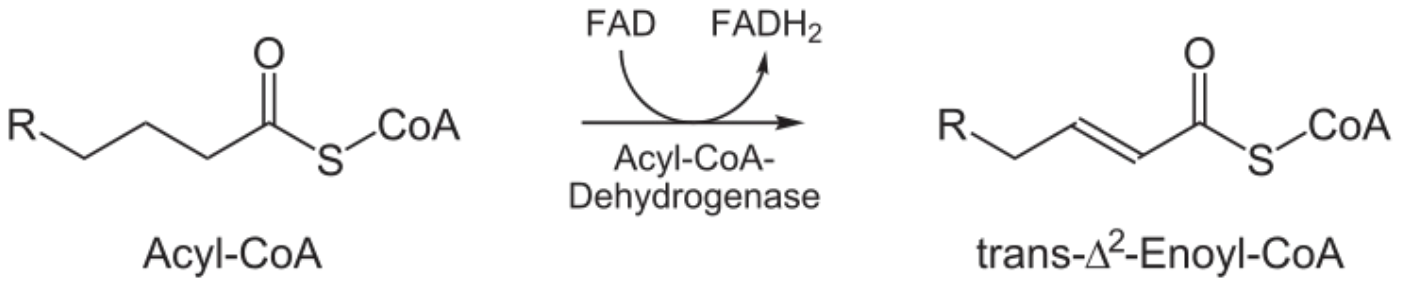


Can we selectively target FA synthetase (FASN) in cancer?

Inhibition of FASN by cerulenin (an antifungal antibiotic) shown to reduce tumour growth of ovarian cancer cells.

Röhrig and Schulze (2016) The multifaceted roles of fatty acid synthesis in cancer. Nat. Rev. Cancer **16**:732-749 doi:10.1038/nrc.2016.89

Disorders of β-oxidation



Acyl-CoA

trans-Δ^2-Enoyl-CoA

A family of different Acyl-CoA-dehydrogenases catalyse the initial step in each cycle of fatty acid β -oxidation within the mitochondria matrix.

Each Acyl-CoA-dehydrogenase can bind a fatty acid chain of varying lengths:

- Short-chain acyl-Co enzyme A dehydrogenase (<6C)
- Medium-chain acyl-Co enzyme A dehydrogenase (C6-C12)
- Long-chain 3-hydroxyacyl-Co enzyme A dehydrogenase (C13-C21)
- Very long-chain acyl-Co enzyme A dehydrogenase (>C22)

Medium chain acyl-coenzyme A dehydrogenase deficiency (MCADD)

MCADD screening via a heel prick test
Image: Shutterstock

- Autosomal recessive. Predominantly occurring in Caucasians. Genetics: Modes of inheritance
- Occurs 1 in 10,000 live births in the UK per year.
- If undiagnosed, can be fatal. Thought to account for 1 in 100 deaths from Sudden Infant Death Syndrome (SIDS).

- If diagnosed, patients should never go without food for longer than 10–12 hours (a typical overnight fast). Adhere to a high carbohydrate diet.
- Patients with an illness resulting in appetite loss or severe vomiting may need i.v. glucose to make sure that the body is not dependent on fatty acids for energy.

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