Metabolism & Metabolomics

How and why cells reprogram their machinery for energy and biomass production
In this Review, we discuss the emerging links between metabolism and the signalling metabolites, and the cognate targets and transducers than vice versa. This important shift in perspective places common signals within the range of physiological fluctuations of the concentration abundance. Regardless of the manner in which nutrient sensing is context dependent. In healthy people, the importance of nutrient homeostasis for all living organisms, and application of new technologies over the last few decades has not only allowed for tumour growth in the organism. Despite the morbidity caused by high levels of lipid storage, embryonic development, cell survival and di...
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Why study metabolism?

Old view: Metabolism is a servant for cell biosynthetic demands

New view: Metabolism is a driver of biology

Normal processes
• Proliferation
• Cell death
• Differentiation
• Gene expression
• Response to stress
• Aging

Pathology
• Cancer
• Inflammation
• Obesity
• Diabetes
• Neurodegeneration
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**What are the mechanisms?**
What is the difference with Biochemistry I, II, III, ....?
What is the difference with Biochemistry I, II, III, ....?
What is the difference between Biochemistry I, II, III, ...?
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What is the difference with Biochemistry I, II, III, ...?

While the biochemistry of the metabolic network is well characterized, its regulatory nodes and implications for signaling are being continuously discovered.
Metabolism is DYNAMIC.

Cells need to reprogram their metabolism in order to:

- Produce more biomass (cell division; cell growth)
- Produce more nucleotides (cell division; meiosis)
- Preserve energy (storage; response to nutrient scarcity)
- Cope with (oxidative) stress (replication and nutrient stress)
- Compartmentalize toxic metabolites (iron overload)
- Adapt to different environments (mobility, 3D growth)
- Secrete immunomodulatory molecules (immune response)
- Adjust availability of “signaling metabolites” (support signals)
- Support epigenetic rewiring (differentiation)

...NOT to “produce” more energy
Class layout:

Part 1: Basics of integrated metabolism (AC)
Part 2: Impact of metabolism on biological processes (MS)
Part 3: Contribution of metabolism to pathophysiology (AC/MS)
Part 4: Metabolism across scales (AC)
Part 5: Journal clubs (AC/MS)
Part 6: Methods in metabolic research (hybrid)
What is metabolism?

**GOES IN**
- Oxygen
- Water
- Dry Food
- Chemical Energy

**GOES OUT**
- Carbon Dioxide
- Sweat and Breath
- Urine
- Faeces
- Heat
What is metabolism?

Humans ingest, metabolize or encounter more than 200,000 metabolites#. Metabolite classes include peptides, lipids, amino acids, nucleic acids, carbohydrates and minerals found in the diet, as well as food additives, drugs, cosmetics, contaminants and pollutants incorporated from our modern life.

METABOLISM removes unwanted or toxic substances and ensures adequate levels of energy and building blocks in a dynamic environment.

Metabolism is linked to body health and performance.
Systemic Metabolism
Systemic Metabolism
Tissue Metabolism
Cellular Metabolism

- Glucose Metabolism
- Lipid Metabolism
- Amino Acid Metabolism
- Energy Metabolism
- Nucleotide Metabolism
- TCA Cycle
- Urea Cycle
What is Systemic Metabolism?

- Gut Lumen
- Gut Wall
- Intestinal metabolism e.g. CYP3A4
- Hepatic metabolism
- Liver
- Hepatic portal vein
- To systemic circulation
- To systemic

pre-systemic
Systemic metabolism is a multi-organ affair

Inter-organ communication contributes significantly to nutrient uptake and metabolite availability.

: discussed in this article as therapeutic targets
Inter-organ metabolism provides nutrient supply to satisfy tissue-specific demands
Systemic metabolism tends to homeostasis

In the 19th century, Claude Bernard articulated the need to maintain a stable internal environment that would allow biological processes to proceed despite variations in the external environment. Bernard's concept was further explored, developed, and popularized by Walter Cannon, who coined the term “homeostasis” in describing how key physiological variables are maintained within a predefined range by feedback mechanisms.

In 1954, James Hardy proposed a model in which homeostatic mechanisms maintain physiological variables within an acceptable range.

Regulated variables: physiological parameters that are maintained at stable levels

Controlled variables: activities (or rates) of the processes that contribute to the stability of the RV

Kotas & Medzhitov, Cell, 2015
Systemic metabolism tends to homeostasis
Metabolites mediate homeostatic mechanisms

Adapted from: Ye & Medzhitov, *Nat Metab*, 2019
Homeostatic mechanisms are subject to regulation

In addition to being subject to well-appreciated homeostatic control, metabolism is subject to supply-driven and demand-driven controls, each operated by a dedicated set of signals throughout various physiological states.

Ye & Medzhitov, Nat Metab, 2019
Overnutrition (or excessive fat intake) initiates a series of events that lead to systemic dyshomoeostasis.
Some nutrients bypass homeostatic regulation

Hedonic or reward-based regulation can override the homeostatic pathway during periods of relative energy abundance by increasing the desire to consume foods that are highly palatable.
Some **micronutrients** bypass homeostatic regulation.
Nutrient uptake (and bioavailability) is regulated by the microbiome

Mavros & Ronda, *Curr Opin Endocr Metab*, 2022
Systemic Metabolism
Systemic Metabolism
Tissue Metabolism
What is Tissue Metabolism?

Every tissue is composed by a mixture of different cell types (and states), each having specific metabolic demands/activities.

Balance dictates LOCAL nutrient availability.
Tissue metabolism is multifactorial

1

Dietary intake dictates local abundance of metabolites in peripheral tissues

SYSTEMIC/TISSUE RELATIONSHIP
Tissue metabolism is multifactorial

**1**
Dietary intake dictates local abundance of metabolites in peripheral tissues

**2**
Different cell types often compete for the same nutrients.

**SYSTEMIC/TISSUE RELATIONSHIP**

**METABOLIC COMPETITION**
Tissue metabolism is multifactorial

**1** Dietary intake dictates local abundance of metabolites in peripheral tissues

**2** Different cell types often compete for the same nutrients.

**3** Nutrients can be provided by a different cell type in the tissue
Hepatic glutamine synthetase controls $N^\delta$-methylglutamine in homeostasis and cancer

**Diabetic glutamine synthetase controls $N^\delta$-methylglutamine in homeostasis and cancer**

**Nature Chemical Biology**

https://doi.org/10.1038/s41589-022-01154-9

Hepatic glutamine synthetase controls $N^\delta$-methylglutamine in homeostasis and cancer

Glutamate $+\text{NH}_3$ + $+\text{NH}_4$

GS

Glutamine

$H_2\text{N}$

ATP

ADP

$\text{O}$

$\text{O}$

$\text{O}$

$\text{O}$

$\text{O}$

$\text{O}$
Hepatic glutamine synthetase controls $\text{N}^\text{6}$-methylglutamine in homeostasis and cancer

Nature Chemical Biology

Glutamate + NH$_3$ + NH$_4^+$

Glutamine

GS

Glutamine synthetase controls $\text{N}^\text{6}$-methylglutamine in homeostasis and cancer.

We applied orthogonal pharmacological and genetic approaches to determine the role of hepatic GS in the regulation of glutamine levels and $\text{N}^\text{6}$-methylglutamine production. Glutamine synthetase controls $\text{N}^\text{6}$-methylglutamine production, as well as glutamine levels and $\text{N}^\text{6}$-methylglutamine production.

Figure 3 | Untargeted metabolomics reveals $\text{N}^\text{6}$-methylglutamine production.

(a) Volcano plot showing enriched metabolic pathways that correlate with $\text{N}^\text{6}$-methylglutamine production.

Glutamine

Glutamate

C$_6$H$_2$N$_2$O$_3$

percentage of maximum values

log$_2$(Δ/Δwt/wt)

-log(P-value)

The volcano plot shows that glutamine synthetase controls $\text{N}^\text{6}$-methylglutamine production, as well as glutamine levels and $\text{N}^\text{6}$-methylglutamine production.
Hepatic glutamine synthetase controls N°-methylglutamine in homeostasis and cancer
OVERVIEW

• In mammals, nutrients (carbohydrates, lipids, and proteins) are enzymatically hydrolyzed.

• Intestine epithelial cells absorb relatively small molecules that reach the liver through blood circulation, mainly through the portal vein.

• Hepatocytes transform diet compounds into compounds that generate energy or to precursors necessary for other tissues.

• These molecules reach their destination through blood circulation.

• To answer the organism's needs, the liver has a high metabolic flexibility.

• This flexibility derives primarily from the 5-10 folds higher rate of modulation of the expression of hepatic enzymes involved in biosynthesis and degradation.

Tissue metabolism is heterogeneous.

[Diagram showing the metabolic pathways of various tissues and the circulation of nutrients.]
In mammals, nutrients (carbohydrates, lipids, and proteins) are enzymatically hydrolyzed. Intestine epithelial cells absorb relatively small molecules that reach the liver through blood circulation, mainly through the portal vein. Hepatocytes transform diet compounds into compounds that generate energy or to precursors necessary for other tissues. These molecules reach their destination through blood circulation. To answer the organism's needs, the liver has a high metabolic flexibility, which derives primarily from the 5-10 times higher rate of modulation of the expression of hepatic enzymes involved in biosynthesis and degradation.

Tissue metabolism is heterogeneous.
Systemic Metabolism
Systemic Metabolism
What is Cellular Metabolism?

Cellular metabolism is a collective term that denotes a wide set of biochemical processes whereby small molecules (called “metabolites”) change in abundance over time and in the steady states that characterize various physiologic conditions.

Metabolites are small molecules that supply the cell with energy, structural constituents and the materials to enable the synthesis of other macromolecules such as DNA or proteins.
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Underscoring the importance of metabolic reactions in cellular and organismal fidelity, it is estimated that more than 30% of human genes are involved in metabolism (Human Metabolome Database 5.0), accounting for ~3,000 possible chemical reactions. Defects in these pathways or their regulation can result in human disease, with inborn errors of metabolism thought to underlie over 1,300 disorders. The vast complexity of human metabolism necessitates a high degree of organization.
What is Cellular Metabolism?
What is Cellular Metabolism?
What is Cellular Metabolism?
Energy “production”

(ATP, GTP, NAD(P)H)
"Break down" nutrients to produce biomolecules for energy exchange

CATABOLISM

Energy “production”

(ATP, GTP, NAD(P)H)
Synthesis of Lipids (Fatty Acids & Sterols)
Synthesis of Glycans

Synthesis of Lipids (Fatty Acids & Sterols)
Synthesis of Glycans

Synthesis of Lipids (Fatty Acids & Sterols)

Synthesis of Nucleotides

Synthesis of Non-Essential Amino Acids (NEAA)
Synthesis of Glycans

Synthesis of Lipids (Fatty Acids & Sterols)

Synthesis of Nucleotides

Synthesis of Non-Essential Amino Acids (NEAA)

Metabolism of Amino Acids
Synthesis of Glycans
Synthesis of Lipids (Fatty Acids & Sterols)
Synthesis of Nucleotides
Synthesis of Non-Essential Amino Acids (NEAA)
Redox Balance
Waste
Waste
Waste
Metabolism of Amino Acids

“Build up” components of cell dry mass
ANABOLISM
Synthesis of Glycans
Synthesis of Lipids (Fatty Acids & Sterols)
Synthesis of Nucleotides
Synthesis of Non-Essential Amino Acids (NEAA)
Redox Balance
Waste
Metabolism of Amino Acids

“Build up” components of cell dry mass
ANABOLISM
Macromolecules compose 70/80%* of the cell mass

* different in quiescent/proliferating

Palm & Thompson, *Nature*, 2017
Catabolism and Anabolism determine energy balance

CATABOLIC PATHWAYS

ANABOLIC PATHWAYS

food molecules
the many molecules that form the cell
useful forms of energy
lost heat
the many building blocks for biosynthesis
Catabolism and Anabolism determine energy balance
Catabolism and Anabolism determine energy balance

which can be stored in high-energy molecules.
Catabolism transfers energy to ATP and electron donors
Catabolism transfers energy to ATP and electron donors

NADH and FADH$_2$ donate electrons to ETC to generate ATP \((\text{mitochondria})\)

NADPH donates electrons for reductive biosynthesis \((\text{e.g.: lipid synthesis})\)
NADH and NAD cannot cross the mitochondrial membrane

In cells with functioning mitochondria and oxygen available, NADH is shuttled into the mitochondria via the malate-aspartate shuttle with electrons transferred to the electron transport chain (this is relatively slow)

Rates of NADH usage and compartmentalization are dictated by multiple conditions (i.e.: hypoxia, differentiation stage, etc)
Mitochondrial DNA
(many copies, maternally inherited, 37 genes, 13 in OXPHOS)
Catabolism and Anabolism determine energy balance

food molecules → the many molecules that form the cell

CATABOLIC PATHWAYS → useful forms of energy

→ ANABOLIC PATHWAYS + lost heat

→ the many building blocks for biosynthesis
Catabolism and Anabolism determine energy balance

...and levels of intracellular metabolic intermediates!!
Glycolytic flux provides substrates for biosynthetic pathways

1. Glucose
2. Glucose 6-phosphate
3. Fructose 6-phosphate
4. Fructose 1,6-bisphosphate
5. Aldolase
   - Glyceraldehyde 3-phosphate (G3P)
   - Dehydroxyacetone phosphate (DHAP)
6. Isomerase
   - 1,3-bisphosphoglycerate
7. Phosphoglycerokinase
   - 3-phosphoglycerate
8. Phosphoglyceromutase
   - 2-phosphoglycerate
9. Enolase
   - Phosphoenolpyruvate (PEP)
10. Pyruvate kinase
    - Pyruvate
Glycolytic flux provides substrates for biosynthetic pathways

1. glucose → ATP → ADP
2. glucose 6-phosphate
3. fructose 6-phosphate
4. fructose 1,6-bisphosphate
5. glyceraldehyde 3-phosphate (G3P)
6. dehydroxyacetone phosphate (DHAP)
7. glyceraldehyde 3-phosphate (G3P)
8. dehydroxyacetone phosphate (DHAP)
9. glyceraldehyde 3-phosphate (G3P)
10. fructose 1,6-bisphosphate

Nucleotides → Glycans → Phospho-Lipids → Glycine, Serine → Folates
Glycolytic flux provides substrates for biosynthetic pathways

1. Glucose
   - Hexokinase
   - ATP -> ADP

2. Glucose 6-phosphate
   - Phosphoglucomutase

3. Fructose 6-phosphate
   - Phosphofructokinase
   - ATP -> ADP

4. Fructose 1,6-bisphosphate
   - Aldolase
   - Glyceraldehyde 3-phosphate (G3P)
   - Dehydroxyacetone phosphate (DHAP)

5. Glyceraldehyde 3-phosphate isomerase

6. Triose phosphate dehydrogenase
   - $2 \text{NAD}^+ \rightarrow 2 \text{NADH} + 2 \text{H}^+$

7. 1,3-bisphosphoglycerate
   - Phosphoglycerokinase
   - 2 ATP -> 2 ADP

8. 3-phosphoglycerate
   - Phosphoglyceromutase

9. 2-phosphoglycerate
   - Enolase
   - $2 \text{H}_2\text{O}$

10. Phosphoenolpyruvate (PEP)
    - Pyruvate kinase
    - 2 ATP -> 2 ADP

Nucleotides
Glycans
Phospho-Lipids
Glycine, Serine
Folates
Glycolytic flux provides substrates for biosynthetic pathways

1. Glucose → ATP
2. Glucose 6-phosphate
3. Fructose 6-phosphate
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5. Glyceraldehyde 3-phosphate (G3P)
6. Triose phosphate dehydrogenase
7. 1,3-bisphosphoglycerate
8. 3-phosphoglycerate
9. 2-phosphoglycerate
10. Phosphoenolpyruvate (PEP)

ATP, TCA cycle intermediates

Nucleotides
Glycans
Phospho-Lipids
Glycine, Serine
Folates
Glycolytic flux provides substrates for biosynthetic pathways

1. Glucose (G6P) → ATP, ADP

2. Glucose 6-phosphate (G6P) → Fructose 1,6-bisphosphate (F1,6BP)

3. Fructose 1,6-bisphosphate (F1,6BP) → Glyceraldehyde 3-phosphate (G3P), Dehydroxyacetone phosphate (DHAP)

4. G3P, DHAP

5. G3P → 1,3-Bisphosphoglycerate (1,3-BPG)

6. 1,3-BPG → Triose phosphate dehydrogenase (NAD^+), NADH + 2H^+

7. 1,3-BPG → 3-phosphoglycerate (3-PG)

8. 3-PG → 2-phosphoglycerate (2-PG)

9. 2-PG → Phosphoenolpyruvate (PEP)

10. PEP → Pyruvate (Lactate + NAD+)

ATP, TCA cycle intermediates

Nucleotides

Glycans

Phospho-Lipids

Glycine, Serine

Folates
Catabolism and Anabolism coexist in each cell

- **Energy delivering nutrients**
  - carbohydrates
  - lipids
  - proteins

- **Low-energy endproducts**
  - CO₂
  - H₂O
  - NH₃

- **Macromolecules of the cell**
  - Proteins
  - Polysaccharides
  - Lipids
  - Nucleic acids

- **Precursors**
  - Amino acids
  - Sugars
  - Fatty acids
  - Nitrogenous bases
Catabolism and Anabolism coexist in each cell

...and their equilibrium is tightly regulated by sensing mechanisms!!
What is Cellular Metabolism?

**Metabolic pathways**

**Anabolic:** Small molecules are assembled into large ones. *Energy is required.*

**Catabolic:** Large molecules are broken down into small ones. *Energy is released.*
In eukaryotes, catabolic pathways converge to generate acetyl-CoA - a pivotal metabolite.
In eukaryotes, catabolic pathways converge to generate acetyl-CoA - a pivotal metabolite.
Main catabolic pathways

Generation of Cytosolic Acetyl-CoA

In physiological and normoxic conditions, glycolysis- or β-oxidation-derived mitochondrial acetyl-CoA represents the major source of cytosolic acetyl-CoA upon transportation (see below). That said, there are at least two relatively ubiquitous metabolic circuitries through which cells actually produce acetyl-CoA in the cytosol (Figure 1).

First, cytosolic acetyl-CoA can originate from glutamine reductive carboxylation, especially when glycolysis is blocked (Yang et al., 2014), in hypoxic conditions (Metallo et al., 2012),

Figure 1. Mitochondrial and Nucleo-Cytosolic Bioenergetic Metabolism of Acetyl-CoA in Mammalian Cells

(A–D) Acetyl coenzyme A (acetyl-CoA) is a key metabolic intermediate. Generally, the majority of cellular acetyl-CoA is generated (A) and consumed (C) in the mitochondrial matrix, in the context of the oxidative metabolism of glycolytic pyruvate (Pyr), free fatty acids (FFAs), branched-chain amino acids (BCAAs), or ketone bodies within the tricarboxylic acid (TCA) cycle. Acetyl-CoA can also be generated in the cytosolic compartment (B), where it supports several anabolic reactions, including lipogenesis, steroidogenesis, and the synthesis of specific amino acids (D). In some malignant cells, the pyruvate dehydrogenase complex (PDC), ATP citrate lyase (ACLY), and acyl-CoA synthetase short-chain family, member 2 (ACSS2) are also found in the nucleus and produce acetyl-CoA therein.

Of note, both mitochondrial and nucleo-cytosolic acetyl-CoA pools are critically involved in protein acetylation reactions. ACAA2, acetyl-CoA acyltransferase 2; ACAC, acetyl-CoA carboxylase; ACAD, acyl-CoA dehydrogenase; ACAT1, acetyl-CoA carboxylase 1; ACAT2, acetyl-CoA acetyltransferase 2; Ace, acetate; Ach, acetaldehyde; ACO1, aconitase 1, soluble; ACSS1, acyl-CoA synthetase short-chain family, member 1; ADH1B, alcohol dehydrogenase IB (class I), beta polypeptide; ALDH1A1, aldehyde dehydrogenase 1 family, member A1; ALDH2, aldehyde dehydrogenase 2 family; BCAT1, branched chain amino-acid transaminase 1, cytosolic; BCAT2, branched chain amino-acid transaminase 2, mitochondrial; BCKD, branched-chain α-ketoacid dehydrogenase; BDH1, 3-hydroxybutyrate dehydrogenase, type 1; β-OHB, D-β-hydroxybutyrate; Cit, citrate; CPT1, carnitine palmitoyltransferase 1; CPT2, carnitine palmitoyltransferase 2; ECH, enoyl-CoA hydratase; ER, endoplasmic reticulum; Eth, ethanol; FASN, fatty acid synthase; Gln, glutamine; GLS, glutaminase; GLUD1, glutamate dehydrogenase 1; GOT1, glutamic-oxaloacetic transaminase 1, soluble; HADH, hydroxyacyl-CoA dehydrogenase; HAT, histone acetyltransferase; HMGCL, 3-hydroxymethyl-3-methylglutaryl-CoA lyase; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; HMGCS1, 3-hydroxy-3-methylglutaryl-CoA synthase 1; HMGCS2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; IDH1, isocitrate dehydrogenase 1; KAT, lysine acetyltransferase; MPC1, mitochondrial pyruvate carrier 1; MPC2, mitochondrial pyruvate carrier 2; NAT, Nα-acetyltransferase; NEA, non-enzymatic acetylation; OXCT, 3-oxoacid CoA transferase.
Acetyl-CoA is a central metabolite because:

• It is at the cross-road of all catabolic pathways
• It is the building block for the synthesis of several macromolecules (fatty acids, sterols, glycans)
• It regulates protein acetylation
• It is compartmentalized
• Its levels fluctuate constantly
• Highly regulated / controlled / monitored
Main catabolic pathways: carbohydrates

Glycolysis:
- Glucose → Glucose 6-phosphate → Fructose 6-phosphate
- PFK (Phosphofructokinase)
- Fructose 1,6-bisphosphate → ATP and citrate
- ATP or citrate

ATP or citrate dehydrogenase (KHK)

Fructose 1-phosphate → Fructose metabolism

Diet pathways:
- Glucose → AR (Allosteric regulation)
- Fructose

Polyol pathway:
- Fructose 1-phosphate

Glycogen metabolism:
- Glucose 1-phosphate ↔ Glucose 6-phosphate

Acetyl-CoA
Central carbon metabolism: **glycolysis**
Central carbon metabolism: **glycolysis**

- **Glucose** → ATP + ADP
- Unstable → Fructose-1,6-bisphosphate
- Fructose-1,6-bisphosphate → 2 DHAP
- DHAP ↔ glyceraldehyde-3-phosphate
- Glyceraldehyde-3-phosphate → NAD⁺ + ADP
  - Happens 2x
- NAD⁺ + ADP → NADH + ATP
- Lactate → NAD⁺ + ADP
- Reduction

**Pyruvate**
Central carbon metabolism: **glycolysis**
Central carbon metabolism: **glycolysis**

- **Glucose** → **ATP** → **ADP**
- Fructose-1,6-bisphosphate → Unstable
- **DHAP** ↔ **glyceraldehyde-3-phosphate**
- **NAD^+** → **NADH**
- All DHAP will be converted into glyceraldehyde-3-phosphate
- ADP → ATP
- Oxidation: **O_2** → ATP
- Reduction: **Lactate** → **Pyruvate**
- **CO_2**
Main catabolic pathways: lipids

Diet comprises a large set of lipid species, but two primarily enter the circulation:
- Fatty acids (as TAGs)
- Cholesterol (as LDL/HDL particles)
Main catabolic pathways: lipids

Diet comprises a large set of lipid species, but two primarily enter the circulation:
Fatty acids (as TAGs)
Cholesterol (as LDL/HDL particles)
Main catabolic pathways: fatty acids oxidation (FAO)

- Fatty acids are incorporated into the cell by dedicated transporters (e.g.: CD36)
- Fatty acids are activated by CoA ligation
- An acyl-carnitine shuttle brings them into the mitochondria
- Beta-ox of FA occurs in the mitochondrial matrix
- Beta-ox is a cyclic reaction that breaks FAs into multiple acetyl-CoA molecules (ANAPPLEROSIS)
- Palmitoyl-CoA + 7CoA + 7NAD⁺ + 7FAD + 7H₂O → 8Acetyl-CoA + 7NADH + 7FADH₂ + 7H⁺
- Ton of ATP
Main catabolic pathways: cholesterol is metabolically inert
Main catabolic pathways: proteins and amino acids

8 Top Dietary Protein Sources

- Meat
- Poultry
- Fish
- Eggs
- Dairy products
- Whey protein
- Soy products
- Quinoa

If you're thinking about adding an amino acid supplement to your current diet, be sure to look for a balanced formula that includes all nine essential amino acids.
Main catabolic pathways: proteins and amino acids

Transaminases swap nitrogen to and from different amino acid carbon backbones.

Nitrogen groups can be funneled into nucleotide biosynthesis, synthesis of other amino acids, synthesis of bioactive amines, or the urea cycle.
Main catabolic pathways: amino acids
Main catabolic pathways: amino acids

Glutaminolysis

Glutamine is the most abundant EAA in the circulation
Multi-layer view of cell catabolism

- **Carbohydrates**
  - Some amino acids
  - Glycerol

- **Fatty acids**
  - Some amino acids

- **Some amino acids**

**Pathway**

- **Glycolysis** → **Pyruvate oxidation** → **Citric acid cycle** → **Oxidative phosphorylation**
Unconventional catabolic pathway: AUTOPHAGY

- Self degradation of cellular proteins/structures within dedicated acidic compartments (lysosomes)
- Specific (targets exhausted proteins/organelles, or specific proteins)
- Inhibited in nutrient-replete conditions
- Triggered by nutrient sensors through the recruitment of ULK1 initiation complex
- Requires autophagy-related genes/proteins (ATGs)
- Marker: lipoylation of LC3
Catabolism can be opportunistic
Multi-layer view of cell catabolism

Figure 4.8: Overall Metabolism of Protein, Carbohydrates and Lipids
Cells can utilize non-canonical nutrients

Ketone bodies are small, water-soluble lipids (containing ketone group) that are produced in excess during fed state and can be mobilized as alternative energy source.

Also: lactate, uridine, inosine, SCFA, formate, vitamins, still growing………
Cells can utilize non-canonical nutrients

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Also: lactate, uridine, inosine, SCFA, formate, vitamins, still growing………

CARBON SOURCE: molecule that can provide carbon units to living cells for biosynthetic purposes
Main anabolic pathways

Typically linked to catabolic pathways (ex: glycolysis branching pathways)
Pentose Phosphate Pathway (PPP)

**Oxidative Phase**

- **Glucose (HK)** → **Glucose 6P**
- **G6PDH** → **6-Phosphogluconolactone**
- **6PGL** → **6-Phosphogluconate**
- **6PGDH** (with NADPH and CO₂) → **Ribulose 5P**

**Non-Oxidative Phase**

- **Fructose 6P** → **Glyceraldehyde 3P** → **Phosphoenolpyruvate (PK)** → **Pyruvate**
- **Fructose 6P** → **Xylulose 5P** → **Ribose 5P**
- **GA3P** → **Erythrose 4P** → **Fructose 6P**

**Generate pentose phosphates**
Serine/glycine pathway is a branch of glycolysis.

Serine/glycine pathway is branch off glycolysis at 3-phosphoglycerate.

3-phosphoglycerate dehydrogenase (PHGDH) requires NAD$^+$ (must have functional ETC).

Conversion of serine to glycine generate one-carbon folate units for methylation (DNA/RNA/protein) and nucleotide biosynthesis.

PHGDH is amplified in several cancers.

Chandel “Navigating Metabolism”
Mitochondria are major metabolic hubs

Main anabolic pathways

DeBerardinis RJ, Thompson CB, Cell Metab, 2008
Main anabolic pathways
Mitochondria are major metabolic hubs

DeBerardinis RJ, Thompson CB, Cell Metab, 2008
The TCA cycle at the crossroad of catabolism and anabolism
The TCA cycle at the crossroad of catabolism and anabolism
Fatty acid synthesis

Fatty acid synthesis is an iterative elongation by 2-carbon acetyl-CoA units and reduction by NADPH

Acetyl-CoA carboxylase is key enzyme regulating fatty acid synthesis
- ACC uses ATP to carboxylate acetyl-CoA and make 3-carbon malonyl-CoA
- Malonyl-CoA condenses with first with acetyl-CoA, then repeatedly with elongating fatty acid chain, each time undergoing decarboxylation, in effect adding acetyl-CoA units (coupling elongation to decarboxylation of malonyl-CoA is energetically favorable)

2 NADPH are used to reduce each acetyl-CoA unit
Mevalonate Pathway

- Occurs in the ER
- Generates sterols (cholesterol) and isoprenoids/terpenoids (several anti-oxidants or redox-stabilizing prosthetic groups)
- Targeted by statins
Nucleotide synthesis

Critically different for **purines** (double ring: 6C+5C) and **pyrimidines** (one ring: 5C)
Nucleotide synthesis

Different for purines and pyrimidines

Purines nucleotide synthesis begins with 5-phosphoribosyl-1-pyrophosphate (PRPP) which ultimately is converted to inosine-5’-monophosphate (IMP)

Requires glutamine, glycine, aspartate (NAD\(^+\)), one carbon folate units, and lots of ATP

IMP can be converted to AMP->ADP or GMP->GDP (IMP->GMP directly requires NAD\(^+\), while IMP->AMP requires aspartate)

Humans cannot catabolize purine rings; partial catabolism produces uric acid

Pyrimidine synthesis begins with carbamoylphosphate and aspartate generating the pyrimidine base orotate

Requires glutamine, aspartate (NAD\(^+\)) and ATP

Dihydroorotate dehydrogenase (DHODH) is located in the mitochondria (interesting);

Pyrimidine rings can be completely catabolized
Nucleotide synthesis is targeted in cancer therapy

MTX was the first drug used (approved) to treat cancer (chemotherapy)
Metabolic waste (or sinking) pathways
Co-PEG in combination with pembrolizumab augments the synthesis of hydrophobic neopeptides due to a detectable increase in transversion mutations, induction of PD1 and/or PDL1 antagonists in tumor cells, increases in the expression of two or more UC enzymes, and increases in T cell activity. The results of this study, another promising therapeutic approach, explored the benefit of combinational therapies, and increase T cell activity and increase T cell activity and increase T cell activity and increase T cell activity. The immediate metabolic consequence of UC dysfunction is the increased availability of UC substrates for the UC and CAD in cancer. This novel feature of cancers with UC dysfunction could be exploited for immunotherapy with checkpoint inhibitors. Lastly, UC intermediates provide substrates essential for neoantigen presentation, leading to the generation of neoantigens in tumor cells.

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Nucleotide pool imbalance promotes a specific mutagenic signature, manifesting as an increase in the ratio of pyrimidines to purines. Elevated pyrimidine to purine ratio will increase the rate of transversion mutations, perturbed through the upregulation (yellow) or downregulation (blue) of UC enzymes.

Increased availability of nitrogenous metabolites leads to exacerbation of pyrimidine synthesis, which results in an increase in the production of pyrimidine nucleotides and purines. Elevated pyrimidine to purine ratio will increase the rate of transversion mutations, perturbed through the upregulation (yellow) or downregulation (blue) of UC enzymes.

Glutamine is assimilated into glutamate through the urea cycle, which recycle ammonia by the urea cycle. The immediate metabolic consequence of UC dysfunction is the increased availability of UC substrates for the UC and CAD in cancer. This novel feature of cancers with UC dysfunction could be exploited for immunotherapy with checkpoint inhibitors. Lastly, UC intermediates provide substrates essential for neoantigen presentation, leading to the generation of neoantigens in tumor cells.

Keshet et al, Nat Rev Cancer, 2020
Metabolic recycling of ammonia via glutamate dehydrogenase supports breast cancer biomass

Jessica B. Spinelli,¹,² Haejin Yoon,¹ Alison E. Ringel,¹ Sarah Jeanfavre,² Clary B. Clish,² Marcia C. Haigis¹*

A

Assimilation

Waste

NH₃

Glutamine

Glutamate

Direct Pathways

Nucleotides

H₂¹⁷N₂

GLS

Glutamine

Glutamate

Proline

Ammonia Recycling

Asparagine

Glutamate

Aspartate

BCAAs

α-Ketoglutarate

OTC

UC gene expression
dysregulation

ASS1

CPS1

ORNT1

Citrin

Increased availability of nitrogenous metabolites

Aspartate

CP

Exacerbated pyrimidine synthesis

Keshek et al, Nat Rev Cancer, 2020

Spinelli et al, Science, 2017
Metabolic waste (or sinking) pathways