MEDICAL EDUCATION RESOURCE

Overview of Metabolism

Experimental Approach to Study of Metabolism

Metabolism can be investigated at different biological levels, each giving a unique window into how pathways function in living systems.

1. Intact Organism

Whole-animal studies reveal integrated physiology.

Key examples:
• Feeding experiments identified essential amino acids and vitamins.
• Benzoic acid ? hippuric acid conversion discovered by Wohler (1842).
• Radiolabelled iron (??Fe) used to study RBC turnover and heme metabolism.
• Inborn errors of metabolism help uncover normal biochemical pathways.

2. Isolated Organ Perfusion

Organ is removed with intact vessels and perfused with Ringer solution.
• Test substrates added to perfusion fluid
• Outflow analyzed to determine metabolic changes
Ideal for **organ-specific** metabolism.

3. Organ Slices (Warburg Method)

Thin slices (~50 µm) preserve internal organelles.
• Introduced by Otto Warburg
• Used for measuring respiration and glucose oxidation
• Liver slices can be used to assess CO? evolution
Warburg apparatus measures tissue respiration.

4. Intact Cells & Tissue Culture

Cells are cultured in a nutrient-rich medium (pH ~7.2).
Used to study:
• Radiolabelled glucose metabolism
• DNA synthesis using labelled nucleotides
• Drug toxicity and drug response
• Biological product synthesis (e.g., monoclonal antibodies)
HeLa cells are classic immortal human cell lines.

5. Tissue Homogenates

Tissues are homogenized and organelles separated.
Used to study:
• Mitochondrial function
• Electron transport chain activity
• Subcellular enzyme function

6A. Purified Enzymes

Isolated enzyme systems help determine:
• Reaction mechanism
• Cofactor requirement
• Regulation of enzyme activity

6B. Genetic (DNA) Approach

Modern metabolic research focuses on gene-level study.
• Mutation analysis (e.g., PAH mutation in PKU)
• Genomics – entire set of genes
• Transcriptomics – gene expression
• Proteomics – protein profile patterns

Radioisotope Tracer Technique

Radiolabelled compounds track metabolism.

Examples:

• ^{1}?C-glucose ? traced to metabolic end-products in organs

• ^{1}?N-glycine ? incorporated into hemoproteins, nucleic acids, creatinine

Tracing helps identify:

• Metabolic pathways

• Precursor–product relationships

• Turnover rates

• Organ-specific metabolism

Important Points to Remember

• Metabolism is studied at **six levels**: whole organism ? perfused organ ? organ slices ? intact cells ? tissue homogenates ? purified enzymes/DNA.

• Warburg apparatus measures **oxygen consumption**.

• Tissue culture allows drug testing, metabolic flux studies, DNA synthesis studies.

• Radioisotopes map metabolic pathways and turnover.

• HeLa cells are immortal human cancer cells widely used in metabolic experiments.

• Gene mutations help explain metabolic disorders and enzyme regulation.

FAQs

1. Why study metabolism at multiple levels?

Each level provides different insight — from whole-body physiology to molecular mechanisms.

2. What is the advantage of organ perfusion?

Allows metabolic study of a single organ without systemic interference.

3. Why are organ slices used instead of isolated cells?

They maintain organelle integrity and preserve native tissue architecture.

4. Why use radioisotopes in metabolism?

To identify the path and rate at which substrates flow through metabolic pathways.

5. What does HeLa cell culture demonstrate?

Immortal cells enable consistent and reproducible metabolic studies.

6. How do genomics and transcriptomics help?

They reveal gene defects responsible for metabolic disorders and pathway regulation.

Tissue Culture

Definition

Tissue culture is the maintenance and growth of **isolated cells, tissues, or organs** outside the organism in a controlled, nutrient-rich environment.

Basic Requirements

- Appropriate culture medium (amino acids, glucose, vitamins, salts)
- Physiological pH (~7.2)
- Temperature control (usually 37°C)
- Sterile conditions
- CO? incubator to maintain buffering
- Aseptic handling environment (laminar airflow)

Types of Tissue Culture

1. Primary Cell Culture

Cells obtained directly from fresh tissues.

- Limited lifespan
- Most closely resemble in-vivo cells

2. Continuous / Immortal Cell Lines

Cells that can proliferate indefinitely.

Examples: **HeLa cells**, hybridoma cells

• Used extensively in biochemical and drug studies

3. Organ Culture

Small tissue fragments cultured while preserving architecture.

• Useful for studying **organ-specific metabolism** and histological effects.

Applications of Tissue Culture

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1. Study of Metabolic Pathways

Cells metabolize labelled substrates

• e.g., radiolabelled glucose ? CO?, glycogen, lipids

Useful to map pathways like glycolysis, TCA, biosynthesis.

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2. DNA and RNA Synthesis Studies

Cells incorporate radiolabelled precursors

• e.g., labelled thymidine ? DNA

• labelled uridine ? RNA

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3. Drug Testing and Toxicology

Evaluate:

• Drug metabolism

• Cytotoxicity

• Growth inhibitory effects

• Anticancer screening

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4. Production of Biological Molecules

• Monoclonal antibodies (using **hybridoma technology**)

• Therapeutic proteins

• Vaccine production

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5. Virology

Viruses must grow inside living cells ? tissue culture is essential for:

- Viral multiplication
- Testing antiviral drugs
- Vaccine development

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6. Genetic Engineering & Recombinant DNA Technology

- Gene transfection
- Gene expression studies
- Production of recombinant proteins
- CRISPR editing studies

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7. Cancer Research

Cancer cell lines help study:

- Uncontrolled cell division
- Metastasis
- Gene mutations
- Chemotherapeutic responses

Advantages

- Highly controlled environment
- Reproducible results
- Easy manipulation of nutrients, hormones, drugs
- Direct observation of cell behavior
- Enables growth of viruses (cannot be grown in cell-free systems)

Limitations

- High skill and aseptic technique required
- Expensive equipment
- Contamination risk

• Cells may behave differently from whole tissue environment

Exam-Oriented Points to Remember

• Tissue culture = **growth of cells in artificial medium**.
• HeLa cell line = classic immortal cancer cell line.
• Used for **metabolism studies, drug testing, DNA synthesis, virology**.
• Hybridoma cells ? monoclonal antibodies.
• Culture medium must maintain pH ~7.2 and provide amino acids, glucose, vitamins.
• Used in genetic engineering and recombinant protein production.
• Offers excellent control but limited ability to mimic whole-organ physiology.

FAQs

1. Why is tissue culture important in studying metabolism?

Because it allows direct measurement of substrate uptake, metabolic flux, and enzyme activity in living cells.

2. What is the difference between primary cells and cell lines?

Primary cells have limited lifespan; continuous cell lines can proliferate indefinitely.

3. Why is HeLa cell line famous?

It is the first immortal human cell line, widely used for metabolic and drug studies.

4. Why is pH control critical in tissue culture?

Most cells require **pH ~7.2**; even small deviations affect enzyme function and survival.

5. What is hybridoma technology?

Fusion of B-cells with myeloma cells to produce **monoclonal antibodies**.

6. Why are tissue cultures used in virology?

Viruses require living cells to replicate, so tissue culture is essential for their study.

Radioisotope Tracers

Definition

Radioisotope tracers are **radiolabelled atoms or molecules** used to follow the path, rate, and fate of metabolic processes inside living cells, organs, or organisms.
They allow direct tracking of **precursor ? product** relationships.

Why Radioisotopes Are Useful in Metabolism

• Incorporate naturally into metabolic pathways
• Emit detectable radiation ? easy to track
• Reveal metabolic **pathways, turnover, and organ distribution**
• Help measure **synthesis and degradation rates**
• Identify **metabolic blocks** in disease

Radioisotopes act as “metabolic markers.”

Common Radioisotopes Used

Carbon (^{1}?C)

Used for:
• Carbohydrate metabolism
• Lipid synthesis
• Amino acid incorporation
• Following fate of labelled glucose

Nitrogen (^{1}?N)

Used for:
• Protein turnover
• Nucleotide and nucleic acid synthesis
• Heme synthesis

Phosphorus (^{32}P)

Used for:

• ATP production

• Phosphorylation studies

• DNA/RNA synthesis

• Kinase activity

Iodine (^{131}I)

Used for:

• Thyroid metabolism and hormone uptake

Tritium (^{3}H)

Used for:

• DNA replication

• Lipid membranes

• Hormone binding studies

Applications in Metabolic Study

1. Tracing Pathways

Example:

• ¹?C–glucose ? measure labelled CO? ? map glycolysis & TCA

• ¹?C–acetate ? trace lipid biosynthesis

2. Measuring Turnover Rates

Used to calculate rates of synthesis and breakdown:

• Proteins

• Nucleic acids

• Glycogen

• Lipids

3. Studying Organ-Specific Metabolism

Feeding, infusion, or injection of labelled compounds:

• Track where they accumulate

• Identify which organ metabolizes them fastest

(example: **liver ? highest glucose metabolism**)

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4. Diagnosing Metabolic Blocks

Inborn errors of metabolism show:

• Failure to convert labelled substrate to expected product

• Accumulation of certain intermediates

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5. Cell Culture Studies

Labelling cells with:

• ^{3}H-thymidine ? DNA replication

• ^{3}H-uridine ? RNA synthesis

• ^{1}?C-amino acids ? protein synthesis

Used to study cell cycle and drug effects.

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6. Measuring Hemoglobin & RBC Turnover

??Fe-labeled iron allows measurement of:

• RBC lifespan

• Heme turnover

• Iron storage disease evaluation

Advantages of Radioisotope Tracer Technique

• Highly sensitive

• Very small quantities needed

• Allows real-time metabolic tracking

• Can be used **in vivo** and **in vitro**

• Quantitative measurements possible

Limitations

- Radioactive waste handling
- Potential radiation hazard
- Expensive detectors
- Requires specialized laboratory

Important Points to Remember

- Radiolabels help trace **metabolic pathways** and **reaction sequences**.
- ^{1}?C ? carbohydrate + lipid metabolism.
- ^{1}?N ? protein and nucleic acid studies.
- ^{32}P ? ATP turnover, kinases, DNA/RNA synthesis.
- ^{3}H-thymidine ? DNA replication marker.
- ??Fe ? RBC lifespan and heme metabolism.
- Radioisotopes help identify **metabolic blocks** in genetic diseases.
- Critical for research in **cell cycle, oncology, endocrinology, hematology**.

FAQs

1. Why are radioisotopes used in metabolism studies?

Because they allow direct tracking of molecules through metabolic pathways.

2. What is the advantage of ^{1}?C-glucose?

It helps trace carbohydrate metabolism all the way to CO?.

3. Why is ^{32}P important?

It labels ATP and phosphorylated intermediates ? used to study energy metabolism.

4. How is ^{3}H-thymidine used?

As a marker for **DNA synthesis** and cell proliferation.

5. How do radioisotopes help diagnose metabolic errors?

A defective pathway shows **failure of conversion** of labelled precursor to expected product.

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6. Why is ??Fe used?

To study RBC lifespan and iron turnover.

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7. Can radioisotope studies be done on living humans?

Some can (like iodide uptake in thyroid), but most are done in **cells, animals, and isolated tissues** due to safety concerns.

Metabolic Profile of Organs

Different organs have distinct metabolic preferences depending on their function and physiological state. Fuel choice changes in fed, fasting, and starvation periods to maintain energy homeostasis.

1. Brain

• Requires **10–20% of cardiac output** despite being only 2% of body weight.
• Depends almost entirely on **glucose** in the fed state.
• Cannot utilize fatty acids because they do not cross the blood–brain barrier.
• During prolonged starvation, uses **ketone bodies**, reducing glucose requirement.
• Extremely sensitive to hypoglycemia.

2. Liver

• Central organ for **carbohydrate, fat, and protein metabolism**.
• Stores **glycogen** post-meal.
• Performs **fatty acid synthesis** and secretes **VLDL**.
• Major site of **amino acid degradation** and **urea cycle**.
• Fasting state: performs **glycogenolysis** followed by **gluconeogenesis**.

• Produces ketone bodies for peripheral tissues but **does not use ketones** itself.

3. Skeletal Muscle

• At rest ? uses **fatty acids** predominantly.
• During intense activity ? uses **muscle glycogen**, later shifts back to fatty acids as exercise continues.
• During fasting ? uses **fatty acids and ketone bodies**.
• During prolonged starvation ? metabolizes **branched-chain amino acids**.
• Contains the **phosphocreatine** system for rapid ATP generation.

4. Heart (Cardiac Muscle)

• Extremely energy-dependent; consumes **large amounts of ATP daily**.
• Primarily uses **fatty acids (60–90%)** for energy.
• Also utilizes **glucose and ketone bodies**.
• Relies on the **creatine kinase shuttle** to deliver ATP to myofibrils.
• In heart failure, shifts toward higher glucose use.

5. Adipose Tissue

• Stores **triglycerides**, which form nearly **85% of the body's total fuel reserve**.
• Releases **free fatty acids** during fasting through hormone-regulated lipolysis.
• Insulin inhibits lipolysis; glucagon and catecholamines stimulate it.
• Continuous turnover occurs even in fed state.

6. Kidney

• Performs significant **gluconeogenesis** during prolonged fasting.
• Utilizes **fatty acids** and **glutamine** for energy.
• Produces ammonia to maintain **acid–base balance**.

7. Intestine

• Absorbs dietary glucose, amino acids, and fatty acids.
• Converts a portion of glucose to **lactate**, which enters the liver for the Cori cycle.
• Enterocytes use **glutamine** as their primary fuel.

8. Red Blood Cells (RBCs)

• Lack mitochondria ? rely exclusively on **anaerobic glycolysis** for ATP.
• Produce **lactate** as end product.
• Require **NADPH** from the HMP shunt to protect against oxidative stress.

9. Starvation: Organ Adaptations

Skeletal muscle: shifts to fatty acids, ketone bodies, and branched-chain amino acids.
Heart: continues using fatty acids and ketone bodies.
Brain: begins using ketone bodies after ~3 days.
Liver: increases gluconeogenesis and ketogenesis.
Adipose tissue: increases lipolysis.

Important Points to Remember

• Each organ has a unique metabolic preference based on physiology.
• Brain uses glucose; shifts to ketones only during prolonged fasting.
• Heart relies heavily on fatty acids.
• Skeletal muscle uses glycogen during exercise; fatty acids during rest and fasting.
• Liver is the main site for gluconeogenesis, ketogenesis, and urea synthesis.
• RBCs depend only on glycolysis.
• Kidney contributes to gluconeogenesis in starvation.
• Adipose tissue is the major storage site of body fuel.

FAQs

1. Why does the brain not use fatty acids?

Fatty acids cannot cross the blood–brain barrier.

2. Why can’t liver use ketone bodies?

Liver lacks the enzyme **thiophorase**, required to utilize ketones.

3. Why does heart prefer fatty acids?

Fatty acids provide the highest ATP yield and support continuous contractile activity.

4. Why do RBCs depend only on glycolysis?

They lack mitochondria, so oxidative pathways are not possible.

5. What fuel does skeletal muscle use during prolonged starvation?

Fatty acids, ketone bodies, and branched-chain amino acids.

6. What is the key fuel for enterocytes?

Glutamine, not glucose.

Important Points to Remember

• Each organ has a characteristic **fuel preference** based on its function.
• **Brain** relies almost entirely on glucose; shifts to ketone bodies only after prolonged fasting.
• **Heart** uses fatty acids as its major energy source; ketone use increases in fasting.
• **Skeletal muscle** uses glycogen during exercise; fatty acids during rest and fasting; BCAA oxidation increases in starvation.
• **Liver** regulates blood glucose via glycogen storage, glycogenolysis, and gluconeogenesis.
• Liver produces **ketone bodies** but can’t use them because it lacks thiophorase.
• **RBCs** use only anaerobic glycolysis (no mitochondria) and rely on NADPH to prevent oxidative stress.
• **Kidney** contributes significantly to gluconeogenesis in prolonged fasting and helps regulate acid–base balance via ammonia production.
• **Adipose tissue** stores triglycerides and releases fatty acids during fasting through hormone-sensitive lipolysis.
• **Intestine** uses glutamine as its primary fuel and releases lactate into portal circulation.
• During starvation, the body shifts from glucose to **fatty acids and ketone bodies** to spare

muscle protein.

• Brain ketone use reduces glucose demand and slows muscle proteolysis.

• Heart failure alters fuel preference—shifts from fatty acids to more glucose utilization.

Clinical Problems

1. Hypoglycemia and Brain Dysfunction

Since the brain depends heavily on glucose, prolonged hypoglycemia can cause confusion, seizures, coma, and irreversible neuronal injury.

Conditions: insulin overdose, alcoholism, liver failure.

2. Diabetic Ketoacidosis (DKA)

Excess fatty acid oxidation ? massive ketone production.

Brain adapts but acidosis produces dehydration, altered sensorium, and rapid respiration.

Occurs in Type 1 diabetes due to insulin deficiency.

3. Myocardial Ischemia (Heart Metabolism Failure)

When oxygen is low, the heart cannot use fatty acids and switches abruptly to anaerobic glycolysis ? lactate accumulation ? pain and reduced contractility.

Seen in angina, heart attacks.

4. Exercise-Induced Muscle Fatigue

Intense exercise uses glycogen; depletion leads to fatigue.

Lactic acid accumulation temporarily lowers pH ? muscle ache.

In prolonged endurance exercise, muscle shifts to fatty acid oxidation.

5. McArdle Disease (Glycogen Storage Disease V)

Skeletal muscle cannot break down glycogen due to **myophosphorylase deficiency**.

Causes exercise intolerance, muscle cramps, and “second wind” phenomenon.

6. Liver Failure — Hypoglycemia & Hyperammonemia

Damaged liver cannot perform gluconeogenesis ? hypoglycemia.
Impaired urea cycle ? ammonia accumulation ? hepatic encephalopathy.

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7. Starvation and Muscle Wasting

During prolonged starvation, the body increases ketone production.
If starvation persists, skeletal muscle proteolysis increases to supply amino acids for gluconeogenesis.

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8. Chronic Alcoholism

Alcohol metabolism increases NADH ? inhibits gluconeogenesis.
Leads to hypoglycemia, fatty liver, lactic acidosis.

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9. Renal Tubular Acidosis (RTA)

Kidney metabolism fails to generate enough ammonia for acid–base balance ? metabolic acidosis.
Seen in Type 2 (proximal) and Type 1 (distal) RTA.

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10. Impaired RBC Metabolism

G6PD deficiency ? reduced NADPH ? hemolysis after oxidative stress (drugs, fava beans, infections).
Symptoms: jaundice, anemia, dark urine.

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11. Intestinal Mucosal Atrophy

In malnutrition or prolonged fasting, reduced glutamine supply weakens enterocytes ? impaired absorption, diarrhea, increased infection risk.

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12. Heart Failure Metabolic Shift

Heart reduces fatty acid oxidation and uses more glucose due to insulin resistance.
Patients show fatigue, reduced exercise capacity.

MCQs — Metabolic Profile of Organs

1. The primary fuel for the brain in the fed state is:

A. Fatty acids
B. Glucose
C. Ketone bodies
D. Amino acids

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2. During prolonged fasting, the brain predominantly uses:

A. Lactate
B. Glutamine
C. Ketone bodies
D. Propionate

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3. The liver cannot utilize ketone bodies because it lacks:

A. HMG-CoA synthase
B. Thiophorase
C. Carnitine acyltransferase
D. Pyruvate carboxylase

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4. The preferred fuel of cardiac muscle at rest is:

A. Glucose
B. Ketone bodies
C. Fatty acids
D. Pyruvate

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5. Which tissue relies exclusively on anaerobic glycolysis?

A. Brain
B. Intestinal mucosa
C. RBC
D. Heart

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6. During intense exercise, skeletal muscle first uses:

A. Fatty acids
B. Glycogen
C. Ketone bodies
D. Amino acids

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7. Which of the following is the major fuel for enterocytes (intestinal mucosal cells)?

A. Glucose
B. Glutamine
C. Ketone bodies
D. Palmitate

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8. The major fuel released from adipose tissue during fasting is:

A. Lactate
B. Amino acids
C. Free fatty acids
D. Glycerol only

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9. Which organ performs the majority of gluconeogenesis during prolonged fasting?

A. Heart
B. Intestine
C. Kidney
D. Brain

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10. Heart muscle uses the creatine kinase shuttle primarily to:

A. Transport glucose
B. Facilitate ATP delivery to myofibrils
C. Increase lactate production
D. Store glycogen

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11. Which organ produces lactate even under aerobic conditions?

A. Liver
B. Brain
C. Intestine
D. Heart

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12. The major fuel for resting skeletal muscle is:

A. Glucose
B. Ketone bodies
C. Fatty acids
D. Glycogen

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13. Muscle proteolysis during starvation primarily provides:

A. Ketone bodies
B. Glycerol
C. Branched-chain amino acids
D. Cholesterol

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14. The biggest energy reserve in the body is found in:

A. Liver glycogen
B. Skeletal muscle glycogen
C. Plasma glucose
D. Adipose triglycerides

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15. Which organ converts glucose to lactate and sends it to the liver via the Cori cycle?

A. Kidney

B. Intestine

C. Adipose tissue

D. Brain

Answers

1-B

2-C

3-B

4-C

5-C

6-B

7-B

8-C

9-C

10-B

11-C

12-C

13-C

14-D

15-B

Viva Voce — Metabolic Profile of Organs

1. What is the primary fuel for the brain in the fed state?

Glucose.

2. Why can’t the brain use fatty acids?

Fatty acids cannot cross the blood–brain barrier.

3. What fuel does the brain use during prolonged starvation?

Ketone bodies.

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4. Why does ketone utilization by the brain help during starvation?

It reduces glucose demand and **spares muscle protein**.

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5. What is the main fuel for cardiac muscle?

Fatty acids.

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6. Can the heart use ketone bodies?

Yes — especially during fasting and prolonged exercise.

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7. Why is the heart highly dependent on fatty acids?

Fatty acid oxidation yields **maximum ATP**, needed for continuous contraction.

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8. Which enzyme is missing in the liver that prevents ketone body utilization?

Thiophorase (succinyl-CoA:acetoacetate CoA transferase).

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9. What metabolic roles does the liver play during fasting?

Glycogenolysis ? gluconeogenesis ? ketogenesis.

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10. What is the preferred fuel for resting skeletal muscle?

Fatty acids.

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11. What fuel does skeletal muscle use during intense exercise?

Muscle glycogen (via anaerobic glycolysis).

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12. What amino acids are predominantly oxidized by skeletal muscle in starvation?

Branched-chain amino acids (leucine, isoleucine, valine).

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13. What is the ATP buffer system in muscle?

Phosphocreatine system.

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14. What is the major metabolic fuel of enterocytes?

Glutamine.

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15. Which organ converts glucose to lactate during normal feeding?

Intestine.

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16. What metabolic pathway do RBCs exclusively rely on?

Anaerobic glycolysis.

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17. Why can’t RBCs use the TCA cycle?

They lack mitochondria.

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18. What important pathway provides NADPH in RBCs?

Hexose monophosphate (HMP) shunt.

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19. What is the main storage form of body energy?

Adipose triglycerides.

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20. What fuel is released from adipose tissue during fasting?

Free fatty acids (and glycerol).

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21. Which organ contributes to gluconeogenesis during prolonged fasting besides the liver?

Kidney.

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22. What substrate does the kidney preferentially use during fasting?

Glutamine.

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23. What is the metabolic role of kidney ammonia production?

Helps maintain **acid–base balance**.

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24. How does the heart supply ATP to contracting myofibrils?

Through the **creatine kinase shuttle**.

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25. When does the brain begin to significantly use ketone bodies?

Around **3 days** of starvation.