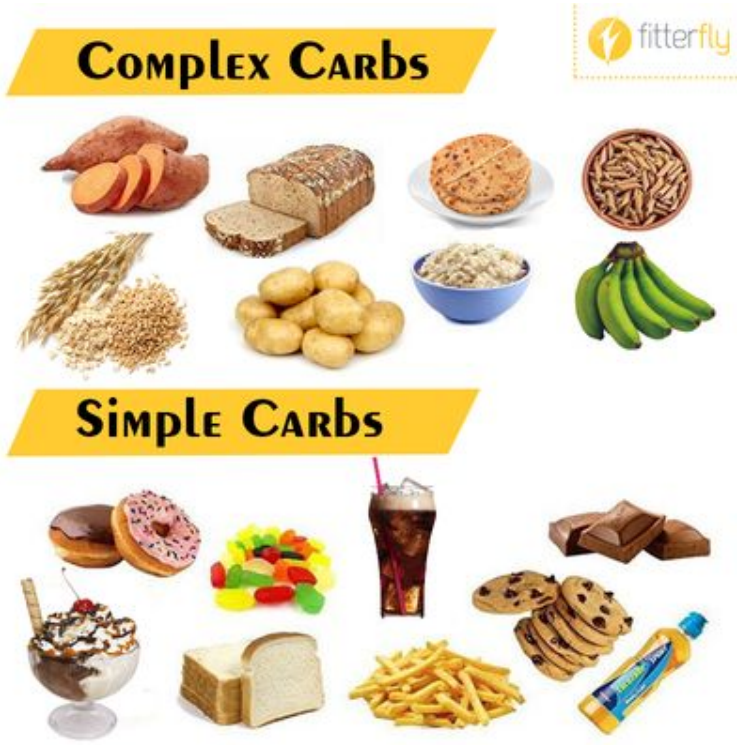
كلية الطب | جامعة القادسية

University of Al-Qadisiyah

College Of Medicine

Clinical Chemistry Department

Carbohydrates



Overview of Metabolism

At the end of the lecture, you will be able to answer questions on the following topics:

1. Study on six levels of organizations.
2. Metabolic pathways and control mechanisms
3. Blood glucose homeostasis
4. Metabolic profile of organs
5. Metabolic adaptations during starvation

Alexis Carrel
NP, 1912
1873-1944

Friedrich
Wohler
1800-1882

EXPERIMENTAL STUDY OF METABOLISM

The study of metabolic sequences may be conducted at six levels of organisations, each at deeper levels of cellular architecture, and each giving different perspectives to the same phenomenon.

Level 1: The Intact Organism

The essential nature of amino acids and vitamins, etc. could be understood by feeding animals with diets lacking in one of the ingredients of food. In 1842, Friedrich Wohler showed that benzoic acid when injected is excreted as **hippuric acid** (benzoyl glycine); this was the starting point of metabolic study in animals. Radiolabelled iron (^{59}Fe) is given, and incorporation of the radioactivity in bone marrow and erythrocyte precursors are studied, which provides information regarding the life span of **RBCs** and rate at which heme is degraded. The studies on inborn errors have been of great help in understanding normal processes inside the body. It is easy to study individual enzyme systems in microorganisms. By utilizing mutant strains of bacteria, metabolic defects may be elucidated

.Level 2: Organ Perfusion

The organ can be isolated preserving its blood vessels. The organ is canulated and perfused with Ringer solution. To the perfusion fluid any compound may be added and the fluid emerging from the organ is analyzed for the metabolites of the compound.

Level 3: Organ Slices

The next lower level of study is by using the slices of organs, about 50 micrometer thick. Otto Warburg (Nobel prize 1931) was the first scientist to study metabolic pathways using organ slices. (The instrument for study of tissue respiration is known as Warburg apparatus). The advantage of this procedure is that the cellular organelle were preserved intact. Metabolic transformations of nutrients could be studied in detail. If rat liver slices are incubated with medium containing glucose, carbon dioxide is evolved.

Level 4: Intact Cells and Tissue Culture set up Tissues or cells can be kept in defined culture medium for a few days for metabolic studies. The medium contains nucleotides, carbohydrates, amino acids, vitamins and growth factors. The pH of the medium should be kept around 7.2. If **labelled nucleotides** are added in the culture, cells take them up for DNA synthesis and the uptake of radioactivity will be proportional to the cell division. Activities of **drugs** can be studied in cell culture system. Biologically useful substances can be harvested from tissue culture set up. For example, specific **monoclonal antibodies** could be obtained from the supernatant of cultured **hybridoma** cells.

Level 5: Homogenates

The tissue is homogenized in an isotonic medium and cell wall is broken by ultrasonic vibration and cellular organelle are separated. For example, isolated mitochondrial preparation will show enzymes of electron transport chain.

Level 6-A: Purified Enzymes

Enzyme preparations may be used to study individual metabolic reactions, their regulation, cofactors, etc.

Level 6-B: DNA or Genomics

Present day research work mainly involves, the studies at genetic level (molecular biology). For example, phenyl ketonuria is due to a mutation in the gene coding for the enzyme phenyl alanine hydroxylase. The full complement of genes within the cells (**genomics**), their expression and regulation (**transcriptomics**) and the gene products (**proteomics**) can be studied. The cells from cancer tissues have indefinite capacity to grow into any number of passages. This immortalization is characteristic of cancer tissues. A good example is the HeLa cell line from cervical cancer tissue, now growing in laboratories all over the world. This cell line was originally started in 1938 from a patient, Henrietta Lacks whose first and last names were abbreviated to name the culture. A pioneer in tissue culture work was Alexis Carrel (Nobel prize, 1912).

Use of Radioisotope Tracers

The isotope studies provide valuable information regarding precursor–product relationship, rate of metabolism and anatomical distribution. When **^{14}C-labelled glucose** is administered, the metabolites can be traced to different organs. Administration of **^{15}N-labelled glycine** was followed by appearance of the label in different compounds like hemoproteins, nucleic acids, and creatinine.

METABOLISM

Thousands of chemical reactions are taking place inside a cell in an organized, well co-ordinated, and purposeful manner; all these reactions are collectively called as **Metabolism**. The metabolism serves the following purposes:

1. Chemical energy is obtained from the degradation of energy rich nutrients.

2. Food materials are converted into the building block precursors of cellular macromolecules. These building blocks are later made into macromolecules, such as proteins, nucleic acids, polysaccharides, etc. Biomolecules required for specialized functions of the cell are synthesized.

3. Metabolic pathways are taking place with the help of sequential enzyme systems. These pathways are regulated at three levels:

a. Regulation through the action of allosteric enzymes, which increase or decrease the activity under the influence of effector molecules.

b. Hormonal regulation. Hormones are chemical messengers secreted by different endocrine glands.

3c. Regulation at the DNA level; the concentration of the enzyme is changed by regulation at the level of synthesis of the enzyme.

Types of Metabolic pathways

A. Catabolic (degradation) pathways, where energy rich complex macromolecules are degraded into smaller molecules.

Energy released during this process is trapped as chemical energy, usually as ATP

B. Anabolic (biosynthesis) pathways. The cells synthesize complex molecules from simple precursors. This needs energy.

C. Amphibolic pathways are seen at cross-roads of metabolism, where both anabolic and catabolic pathways are linked.

Stages or Phases of Metabolism

The degradation of food stuffs occurs in three stages.

i. In the first stage, digestion in the gastrointestinal tract converts the macromolecules into small units. For example, proteins are digested to amino acids. This is called **primary metabolism.**

ii. Then these products are absorbed, catabolized to smaller components, and ultimately oxidized to CO_2. The reducing equivalents are mainly generated in the mitochondria by the final common oxidative pathway, citric acid cycle. In this process, NADH or $FADH_2$ are generated. This is called **secondary or intermediary metabolism**.

iii. Then these reduced equivalents enter into the **electron transport** chain (ETC, or Respiratory chain), where energy is released. This is the **tertiary metabolism** or Internal respiration or cellular respiration

METABOLISM

The metabolic pattern or metabolic profile of different organs is different depending on its function. Moreover, the organs are able to adapt to metabolic alterations in fed state and starvation.

Calories are stored in the body as fat and glycogen. The approximate percentage of storage form of energy (total fuel reserve) present in a normal human body is, fat 85%, glycogen 1%, and proteins 14%.

Fat stores are mobilized actively only on prolonged fasting, even though adipose tissue fat is undergoing turnover on a daily basis. Caloric homeostasis is maintained regardless of whether a person is well fed, fasting, or in a state of starvation. Similarly metabolic profile of various organs and tissues change to adapt to physiological and pathological states, so that caloric homeostasis is maintained unless extreme conditions set in. The reciprocal regulation of glycolysis and gluconeogenesis is the major deciding factor in the flux of metabolic intermediates through these pathways.

1. Brain

i. **Although brain represents only 2% of adult body weight, it needs 10–20% cardiac output**. About 750 ml of blood circulates through the brain per minute. Neurons can survive only a few minutes without blood supply. Occlusion of blood supply to brain causes unconsciousness within 10 seconds.

ii. There is no stored fuel in the brain. Glucose, the preferred fuel for the brain, should be in continuous supply. Glucose can freely enter the brain cells.

iii. The total consumption of glucose by brain is about 120 g/day (480 kcal). Thus, about 60% of the total carbohydrate intake by the body is metabolized by the brain. Moreover, about 25% of the oxygen consumed by the adult body is due to glucose oxidation in brain. In children, this may be as high as 50%.

iv. Brain under conditions of anoxia: In anoxia the rate of lactate production by glycolysis rises to 5 or 8 times within one minute. The Pasteur effect is the brain's protection against conditions of anoxia. Blood glucose level below 30 mg/dl is fatal.

v. Brain and acetoacetate: The brain is unable to utilize fatty acids as a source of fuel since the fatty acids complexed to albumin are unable to traverse the blood brain barrier. But, brain can effectively utilize acetoacetate. This is again a survival technique in diabetic and starvation ketosis.

vi. Brain and starvation: During starvation, a significant part (60-70%) of the energy requirement of the brain is then met by ketone bodies few minutes without blood supply. Occlusion of blood supply to brain causes unconsciousness within 10 seconds. meal, the level of the glucose and insulin are high. So glycogen synthesis is enhanced (Fig. 2).

Figure 1. Energy Utilization of Average Person

The energy consumption varies based on life style in adults. Approximately 300 g of carbohydrates (1200 Kcal or 4800 kJ), 70 g of proteins (294 Kcal or 1190 kJ) and 80 g of fats (720 kcal or 2960 kJ) are consumed by a person with a sedentary life style. Therefore, about 60% calories are derived from carbohydrates, 15% from proteins and rest from fats. The energy reserves provide energy in between meals and after overnight fasting (glycogenolysis and gluconeogenesis).

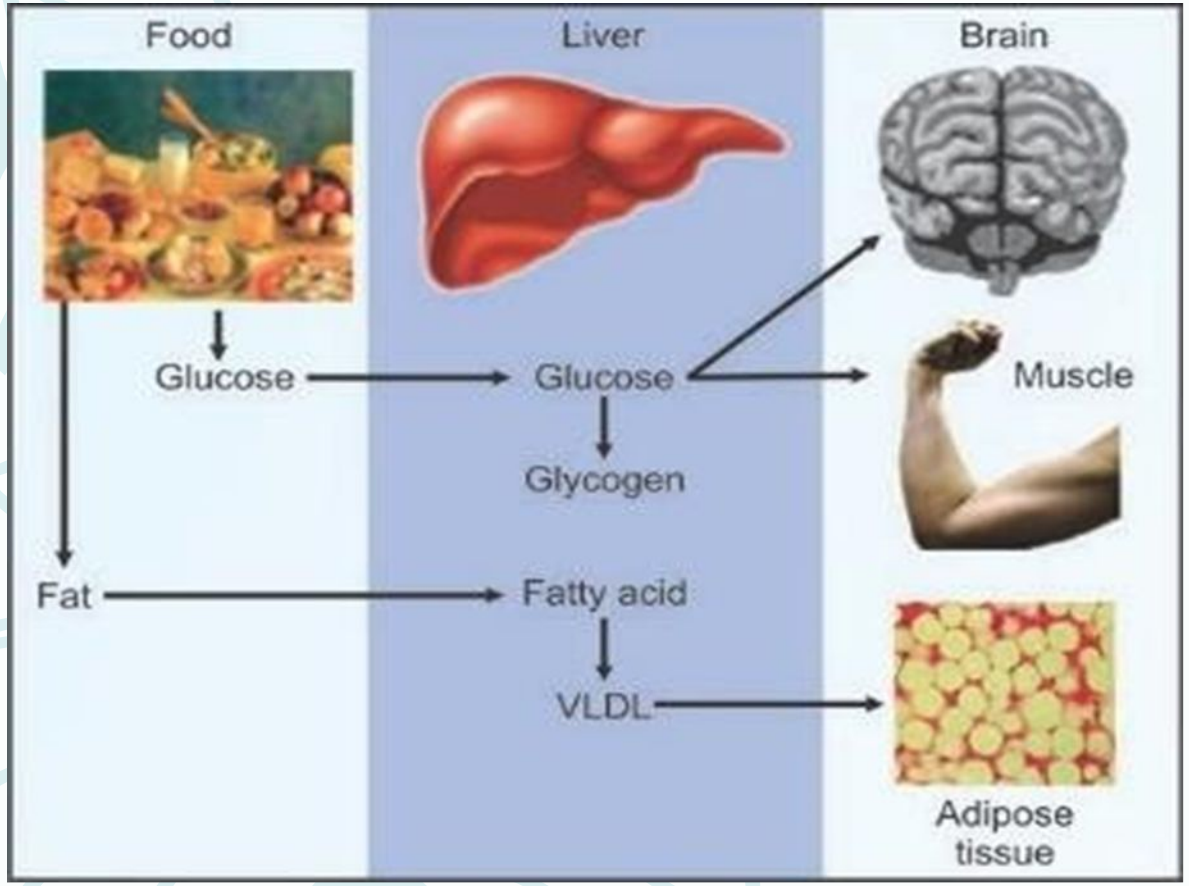


Fig. 2. Metabolism in well fed state

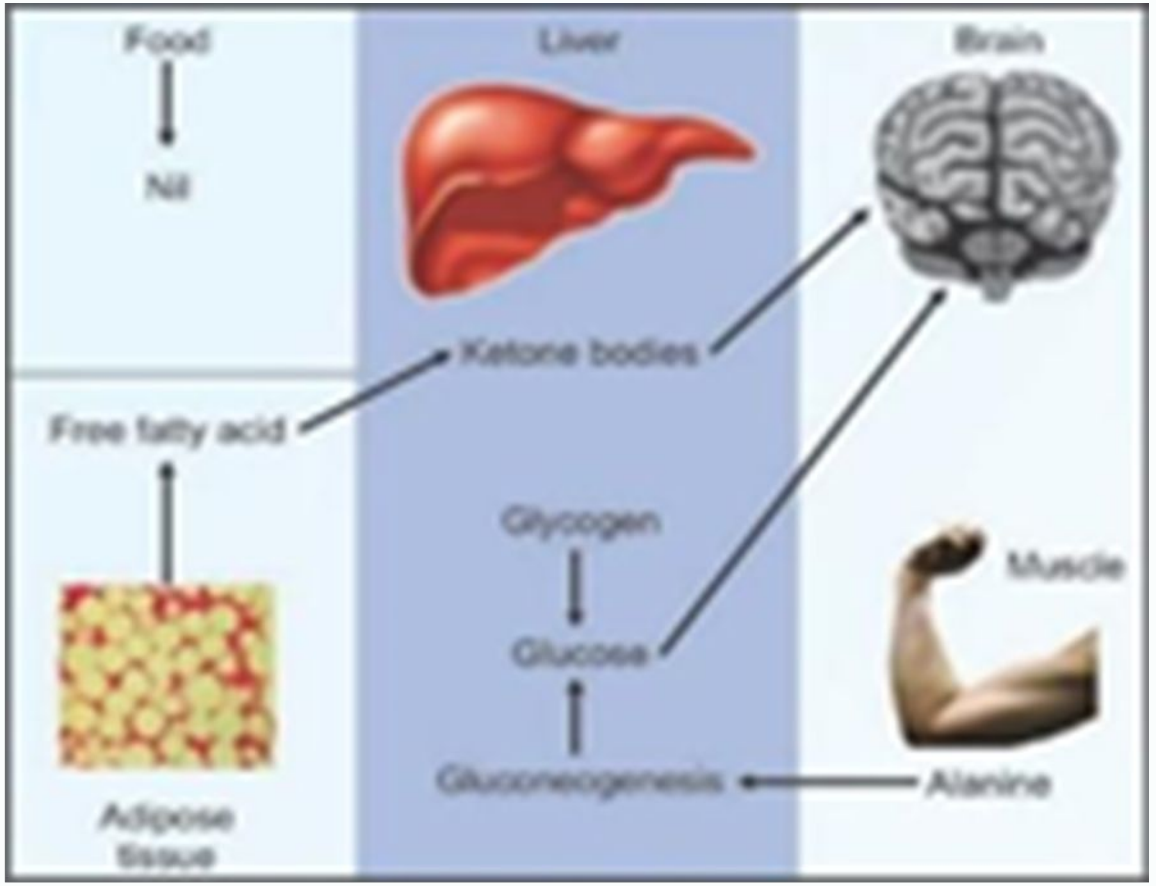


Fig. 3. Metabolism in fasting state

iii. Muscle metabolism during exercise: Muscle uses glycogen for short active spurts of activity. Glycogen is rapidly broken down to form lactate. The lactate has to be transported to liver to undergo gluconeogenesis (Cori's cycle). Muscle however uses fatty acid as fuel for aerobic exercise and long distance running.

Muscle metabolism during starvation: During starvation, maximum glucose is spared for the brain. The free fatty acid (FFA) mobilized from adipose tissue is the preferred fuel for muscle during starvation. FFA does not require insulin, and during fasting insulin level is low (Fig.2)

During prolonged starvation, muscle protein breakdown occurs and alanine is released to the blood stream. It is transported to liver to provide substrate for gluconeogenesis. The metabolic fuel during prolonged fasting is ketone bodies. Branched chain amino acids are utilized by the skeletal muscle (Fig.3)

Table 4. Key enzymes under well fed conditions, fasting and starvation

Enzyme	Fed	Fasting	Starvation	Activator	Inhibitor
Glucokinase	Increase	Decrease	Decrease	Insulin, Glucose	F-6-P
Phosphofructokinase1	Increase	Decrease	Decrease	F-2,6-bisP, AMP	ATP, Citrate
Fructose 1,6 bisphosphatase	Decrease	Increase	Increase	ATP, Citrate	F-2,6-bisP, AMP
Pyruvate carboxylase	Decrease	Increase	Increase	AcetylCoA	
PEPCK	Decrease	Increase	Increase	Glucocorticoids	Insulin
Glycogen phosphorylase	Decrease	Increase		Glucagon, AMP	Insulin
Glycogen synthase	Increase	Decrease	Decrease	Insulin, G-6-P	Glucagon
Carnitine acyl transferase	Increase		Increase	Glucagon	Malonyl CoA
Acetyl CoA carboxylase	Increase	Decrease	Decrease	Insulin, Citrate	Fatty acylCoA
Hormone sensitive lipase	Decrease	Increase	Increase	Glucagon	Insulin

3. Adipose Tissue

It is the storehouse of energy in the body (about 1,35,000 kcal) (Table 1). The energy is stored in the concentrated form, triacyl glycerol. The chylomicrons and VLDL are hydrolysed by lipoprotein lipase present on capillary walls. It is activated by insulin. The fatty acids are re-esterified to form triacyl glycerol. The glycerol is derived from dihydroxy acetone phosphate (DHAP), an intermediate of glycolysis. Therefore, for storage of triacyl glycerol, both fatty acid synthesis and glycolysis should operate. The uptake of glucose, glycolysis and lipogenesis are all favored by insulin.

About 25% of glucose taken up by adipose tissue is metabolized by the HMP shunt pathway, and the rest by glycolysis. The NADPH generated from the shunt pathway is used for the synthesis of fatty acids. The NADH produced during glycolysis is used to reduce the DHAP to glycerol-3-phosphate

During fasting, triglycerides in the adipose tissue are hydrolysed. Cyclic AMP mediated activation of hormone sensitive lipase occurs in response to the high glucagon-insulin ratio. Glucocorticoids also have a stimulant lipolytic effect during fasting.

4. Liver

The liver plays a central role in metabolism by providing adequate quantities of metabolic fuel for other organs. Almost all the metabolic pathways operate in the liver; a notable exception being ketolysis.

Liver metabolism in fed state: Under well fed conditions, the liver takes up glucose from

circulation and stores it as glycogen. Similarly the fatty acids synthesized by the liver are incorporated into VLDL and secreted into blood stream. Liver is the major site of degradation of amino acids and detoxification of ammonia into urea.

iii. During starvation, liver provides glucose by glycogenolysis and later by gluconeogenesis so that the obligatory requirements of the brain are met. Moreover, liver also produces the ketone bodies, an alternate source of fuel. But the liver cannot use ketone bodies as its own fuel.

5. Cardiac Muscle

Heart consumes more energy than any other organ. It utilizes about 6 kg of ATP per day, 20-30 times of its own weight. Cardiac muscle derives its energy by oxidative metabolism of fatty acids (60-90%) and glucose 10-40%. Ketone bodies are also normally metabolized.

In addition, energy transfer to heart's myofibrils occurs by **creatine kinase** catalyzed energy shuttle. Phosphocreatine being a smaller molecule than ATP can easily diffuse into the myofibrils from mitochondria. The myofibrillar creatine kinase catalyses the reformation of ATP. The free creatine diffuses back. The creatine kinase system acts as an energy buffer, by keeping ATP level constant. When ADP level increases

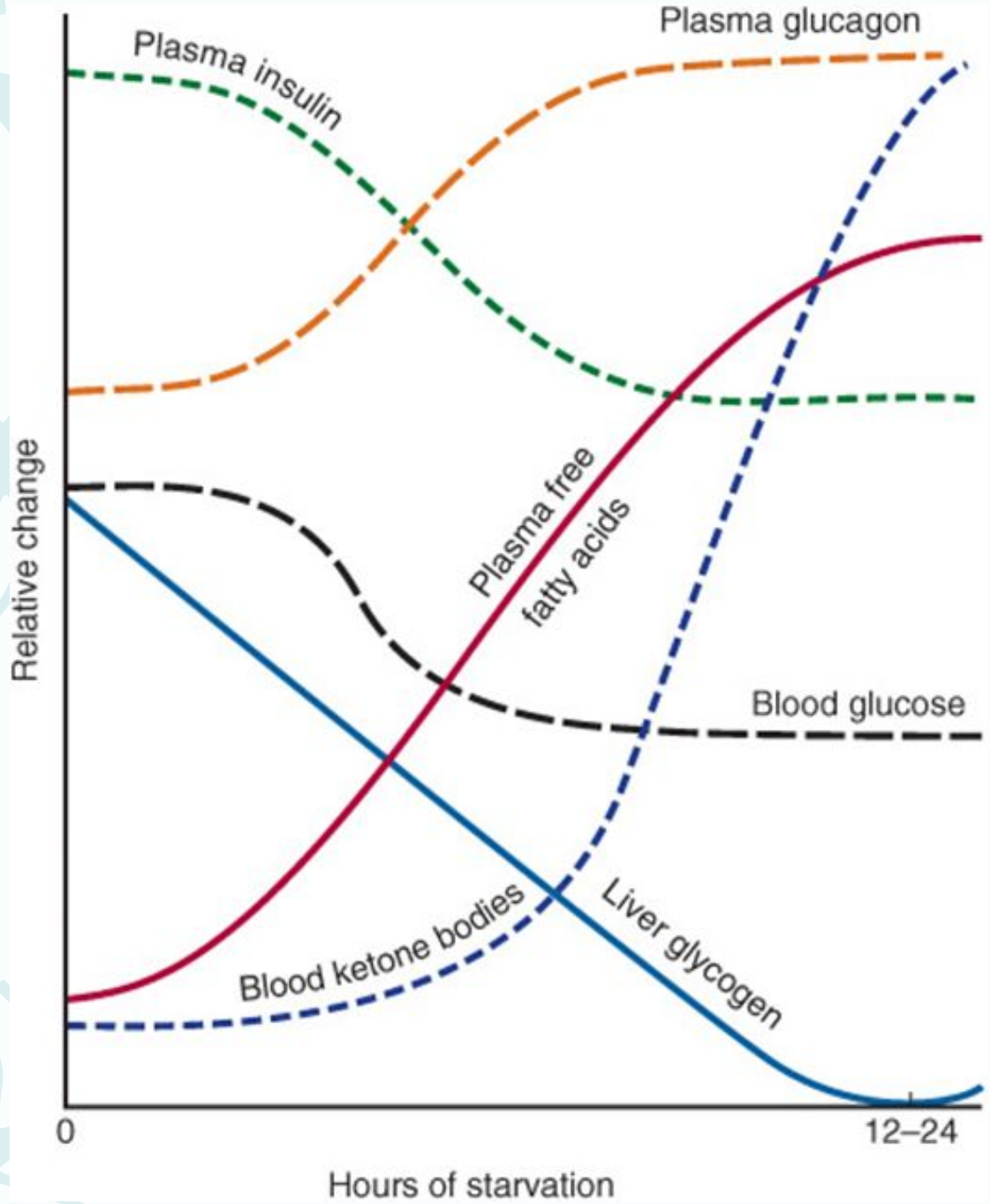


Fig. 4. Relative changes of important parameters during starvation

due to a fall in phosphocreatine, it inhibits intracellular enzymes causing failure of the heart's contracting mechanism. In a failing heart, the uptake and utilization of fatty acids and glucose occurs. In advanced heart failure, insulin resistance also develops, further decreasing the glucose utilization. At the same time, the metabolism of a hypertrophied heart switches from fatty acid utilization to glucose.

Long distance running is the typical example of aerobic exercise, where as sprinting or weight lifting exemplifies anaerobic exercise. During anaerobic exercise, the major organ involved is the skeletal muscle with very little involvement of other organs. The relative ischemia created by the
compression of blood vessels in the muscle will necessitate the use of glycogen and phosphocreatine available in the muscle to supply the required energy.

During moderate aerobic exercise, the muscular stores of glycogen are used, but in a normal individual this is not sufficient to provide a continuous supply of ATP for exercise like long distance running. The RQ falls during long distance running since there is a progressive change from glycogenolysis to fatty acienergy demands. Muscles start oxidizing fatty acids and the high AMP level which activates AMP kinase and low malonyl CoA that activates CAT will favor fatty acid oxidationd oxidation to meet the. The training for athletes is different depending on whether they are sprinters or long distance runners since the energy sources are different. Rest after a vigorous muscular activity often results in repletion of the exhausted glycogen stores.
In muscle developed by exercise and training, the size and number of mitochondria are more as well as the level of enzymes for fatty acid oxidation and ketone body utilization. Hence, the trained muscle can better utilize noncarbohydrate sources of energy. So exhaustion is delayed

Metabolic Adaptations During Starvation

In early fasting the effect of short term regulation by altering the activity of existing enzymes (fine control) is more significant. When starvation is prolonged (>3 days), long term adaptation sets in, e.g. brain starts metabolising ketone bodies deriving about 30% energy from ketone bodies.

First Stage: Glycogenolysis

Table 2 and Figure 3 show the changes in activities during starvation. During fasting, at first, blood glucose level is maintained by hepatic glycogenolysis. The glycogen stores are sufficient for about 18 hours. The primary requirement for glucose is to meet the demands of the brain.

Second Stage: Gluconeogenesis

Even before the glycogen stores are depleted, gluconeogenesis is accelerated (Fig.8.3). The amino acids released from muscle form the major substrate for gluconeogenesis. The amino nitrogen is transferred from other amino acids to pyruvate to form alanine. Thus the amino group reaches the liver as alanine where it is transaminated to give pyruvate for gluconeogenesis. This glucose alanine cycle serves to transport the amino nitrogen of other amino acids to liver in a harmless form. Glutamic acid also serves as an important mode of transport of amino acids to liver.

Thank you for your attention!

Question and comments are welcome