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biochemistry sheet

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Biochemistry of Cardiac Muscle 2

The PC-CK system in cardiomyocytes

- **CK synthesizes PC from creatine and group from ATP in a reversible reaction, acting as a functional ATP reserve.**

- **CK associated with myofilaments catalyzes the transference of the phosphate from phosphocreatine towards ADP, replenishing**

ATP in ATPase active sites, such as myosin heads.

-CK: creatine kinase

PC: phosphocreatine

-CK participate in 2 opposite reactions :

1- CK take phosphate from ATP and give it to creatine to form phosphocreatine

2- CK and myofilaments take phosphate from PC and give it to ADP

- PC is a stored form of ATP because the lifespan of ATP is short.

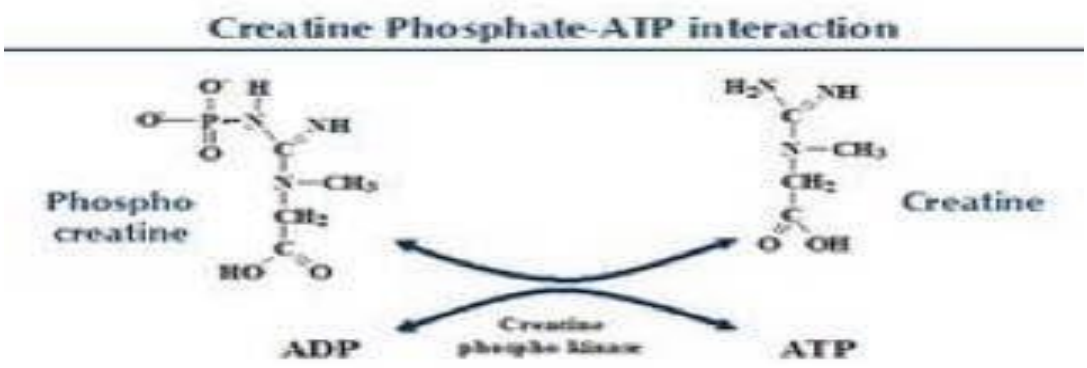
- It is a fast one-step reaction that produces 1 ATP.

- **The PC-CK system represents the first line of energetic reserves in cardiomyocytes, providing a quick source of ATP and favoring its transportation to its utilization sites, especially myofilaments.**

- **The disruptions in the PC-CK system have been linked to impaired myocardial contractility and increased risk for arrhythmias. Moreover, alterations in the functionality of CK have been identified as an independent risk factor for heart failure.**

CK is composed of dimers, which consist of subunits M and B, and originate three isoenzymes: CKMM, -MB and -BB.

- **A fourth isoenzyme is found in mitochondria (mi-CK) and accounts for 20%-40% of all CK activity in the heart.**



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- **-CK-MM: found in skeletal muscles and partially in cardiac muscles**
- **-CK-MB :found in cardiac muscles and we use it to diagnose suspected patients with myocardial infraction**
- **-CK-BB: found in the brain with unknown function**
- **-mi-CK (mitochondrial CK):found near the ATP-ADP translocase**
- **The ATP produced from the electron transport chain should transport out of the mitochondria and ADP enters by this translocase ,so the mi-CK is located near the ATP- ADP translocase to capture some of the ATP before it goes outside and store it in a form of ADP and pc**

- **The mi-CK isoform is coupled to the external surface of the internal mitochondrial membrane, near the ATP/ADP translocases.**

- **During oxidative phosphorylation, the ATP generated in the mitochondrial matrix is exported by ANT (Adenine Nucleotide Translocase) to the inter-membrane space and transphosphorylated by mi-CK to PC and ADP, with the latter being immediately available for oxidative phosphorylation, stimulating cellular respiration.**

- **In a healthy heart, approximately two thirds of all creatine is phosphorylated by CK to yield PC.**

- **In heart failure, the level of PC is lower in relation to the concentrations of ATP, with a lower PC/ATP index.**

- **The PC/ATP index normally is 4:1, and if it decreases, then**

there is a decrease in the production of PC. This is of high mortality rate due to insufficient amounts of energy.

- **Lower values of this index have been related to increased mortality.**

Ketone bodies

- Liver synthesizes ketone bodies as it has the required enzymes for ketogenic reactions, but it doesn't have any of the enzymes needed for ketolytic pathways. The liver synthesizes them for the rest of the organs.

- **A special source of fuel and energy for certain tissues.**

- **Some of the acetyl-CoA produced by FAs oxidation in liver mitochondria is converted to acetone, acetoacetate and β- hydroxybutyrate.** Is formed as a result of hydroxylation reaction

- **Source of fuel for brain, heart and muscle.**

- **Major energy source for brain during starvation.**

- **ketogenesis:**

- **Large amounts of acetyl CoA accumulate.**

-Two acetyl CoA molecules combine to form acetoacetyl CoA.

- **Acetoacetyl CoA hydrolyzes to acetoacetate, a ketone body. - Acetoacetate reduces to β-hydroxybutyrate or loses CO2 to form acetone, both ketone bodies**

Reactions of Ketogenesis

Production

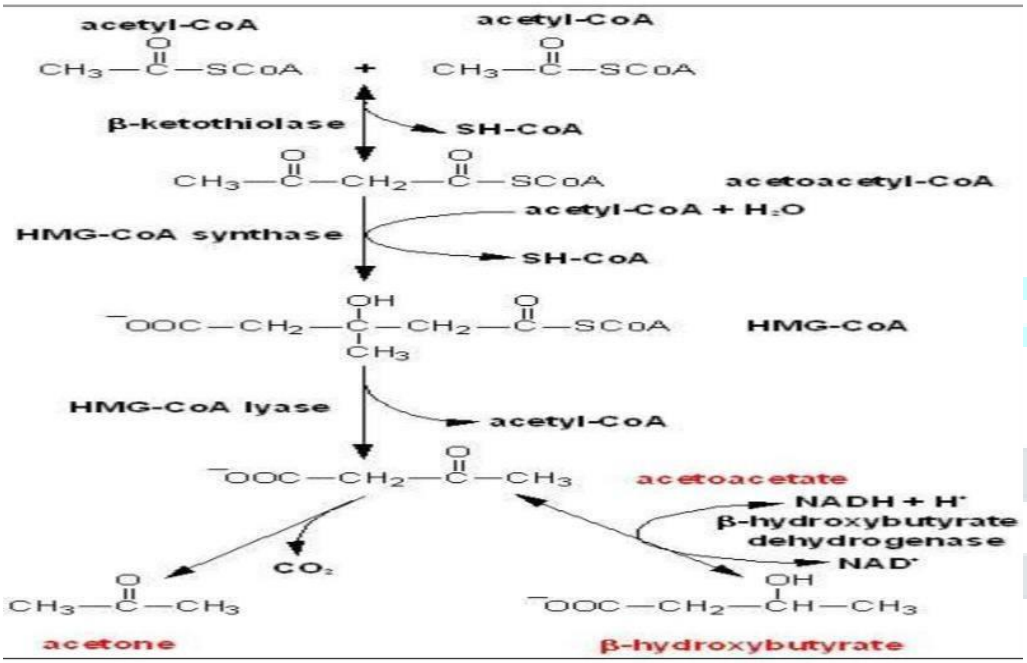
- **Ketone bodies are produced mainly in the mitochondria of liver cells.**

- **-Their synthesis occurs in response to low glucose levels in the blood and after exhaustion of cellular carbohydrate stores, such as glycogen.**

Fatty acid are enzymatically broken down in B-oxidation to form acetyl-CoA

- **Under normal conditions, acetyl-CoA is further oxidized and its energy transferred as electrons to NADH, FADH2, and GTP in TCA cycle.**

The production of ketone bodies is then initiated to make available energy that is stored as fatty acids



2 molecules of acetyl-CoA >> to form acetoacetyl-CoA >> enzyme called HMG-CoA synthase add one more molecule of acetyl- CoA>>to form HMG-CoA >> enzyme called HMG-CoA lyase take one molecule of acetyl-CoA>>to form the first product of ketone bodies "acetoacetate" and it can get spontaneously decarboxylated without any enzymes to form acetone that can be discharged by exhaling and sweating or it can be reduced " reduction reaction by beta-hydroxybutyrate dehydrogenase with the presence of NADH + H+) to give beta-hydroxybutyrate and NAD+

The amount of ATP consumed is ---20 ATP consumed from the 2 acetyl-CoA in first step

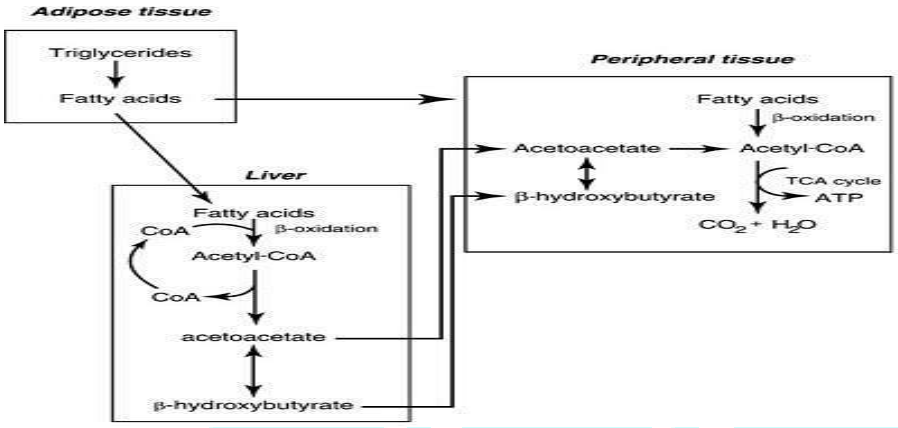
10 ATP consumed from acetyl-CoA from the second step 10

ATP produced due to the release of acetyl-CoA in step 3 And

2.5 consumed from dehydrogenases of NADH

So the total consumed amount of ATP is 20+2.5=22.5 ATP

- However, if the amounts of acetyl-CoA generated in fatty acids β-oxidation exceeds the capacity of the TCA cycle or if activity in



the TCA cycle is low due to low amount of intermediate such as oxaloacetate, acetyl-CoA is then used instead in biosynthesis of ketone bodies via acetoacyl - CoA ß-hydroxy-ß- methylglutaryl - CoA (HMG-CoA), which is also an intermediate in the synthesis of cholesterol

There is a similar reaction happens in cytoplasm has the first 2 steps of ketogenesis which is cholesterol synthesis so the HMG- CoA is an intermediate in both ketogenesis and cholesterol synthesis

Ketosis or keto-Acidosis

- A large accumulation of ketone bodies is dangerous, because it leads to profound metabolic acidosis.

-Ketone bodies are acids so they will shift the physiological PH

Intracellular PH is slightly acidic in normal condition due to the intermediate metabolic pathways that produce acids (citric cycle, glycolysis) and the extracellular is slightly basic

-The physiologic ketogenesis of fasting and the adaptive ketosis in starvation never progress to acidosis.

Ketonemia

-It is increased concentration of ketone bodies in blood due to increased their production by the liver rather than to a deficiency in their utilization by extra hepatic tissues.

-The production of ketone bodies occurs at a relatively low rate during normal feeding and under conditions of normal physiological status.

Normal physiological responses to carbohydrate shortages cause the liver to increase the production of ketone bodies from the acetyl-CoA generated from fatty acid oxidation

Causes of ketosis:

- **Uncontrolled diabetes mellitus pathological**
- **Starvation physiological**
- **Chronic alcoholism patological because it is chronic**
- **Von Gierke's disease (Glycogenosis due to deficiency of G6P) pathological**
- **Heavy exercise physiological**
- **Low carbohydrate diet physiological**
- **Phosphorylase kinase deficiency physiological**
- **Pyruvate carboxylase deficiency physiological**
- **Prolonged ether anesthesia**
- **Toxemia of pregnancy**
- **Nonpathologic due to high-fat feeding and**
- **After severe exercise in the post absorptive state**
- **Starvation induced ketosis**

In prolonged fasting:

-There will be drop in the levels of plasma glucose, amino

acids, triacylglycerols and decline in insulin level and an

-increase in glucagon release (and the anti-insulin hormones) (decreased insulin /glucagon ratio), makes this period of nutritional deprivation a catabolic state, characterized by glycogenolysis, triacylglycerol and protein degradation.

-There will be an exchange of substrates between liver, adipose tissue, muscle and brain that is guided by two priorities:

1- The need to maintain glucose level to sustain the energy metabolism of brain, red blood cells and other glucose requiring cells, and;

2- To supply energy to other tissues by mobilizing fatty acids from adipose tissues and converting them to ketone bodies to supply energy to other cells of the body.

3- In early stages of starvation , heart and skeletal muscle consume primarily ketone bodies to preserve glucose for use by the brain.

- After several weeks of starvation, ketone bodies become the major fuel of the brain

- In starving :organs will defend on ketone bodes to leave the glucose for erythrocytes and brain

- After months of starvation when there is uncontrolled lipolysis and production of ketone bodies, this might advance to DKA

- Normally we have low concentrations of ketone bodies in the blood ((Not O))

Diabetic ketoacidosis (DKA)

-It is a state of inadequate insulin levels resulting in high blood sugar and accumulation of ketone bodies in the blood.

-It is a potentially life-threatening complication.

-It happens predominantly in type 1 diabetes mellitus, but can

-also occur in type 2 under certain circumstances, which may be due to intercurrent illness (pneumonia, influenza, gastroenteritis, a urinary tract infection), pregnancy, inadequate insulin intake , myocardial infarction, eating disorder , stroke or the use of drugs.'Precipitation factors of keto acidosis'

-DKA results from relative or absolute insulin deficiency combined with counter regulatory hormone excess (glucagon, catecholamines, cortisol, and GH).'anti-insulin drugs'

- In 5% of cases, no cause for the DKA is found.
- Normally the upper hand in the post-absorptive state is for insulin and insulin keeps blood glucose level in a normal level
- Insulin actions (again):
- -increase cellular uptake by increasing GLUT 4 expression
- -stimulates regulatory steps in glycolysis to break glucose
- - initiate glycogenesis
- -convert extra glucose to TAG
- -stimulates glycoprotein synthesis

- Insulin inhibits any mechanism that could lead to increase glucose in blood
- Insulin inhibits : glycogenolysis +liolysis +gluconeogenesis

$** Acetyl-CoA participates in 4 major biochemical pathways:

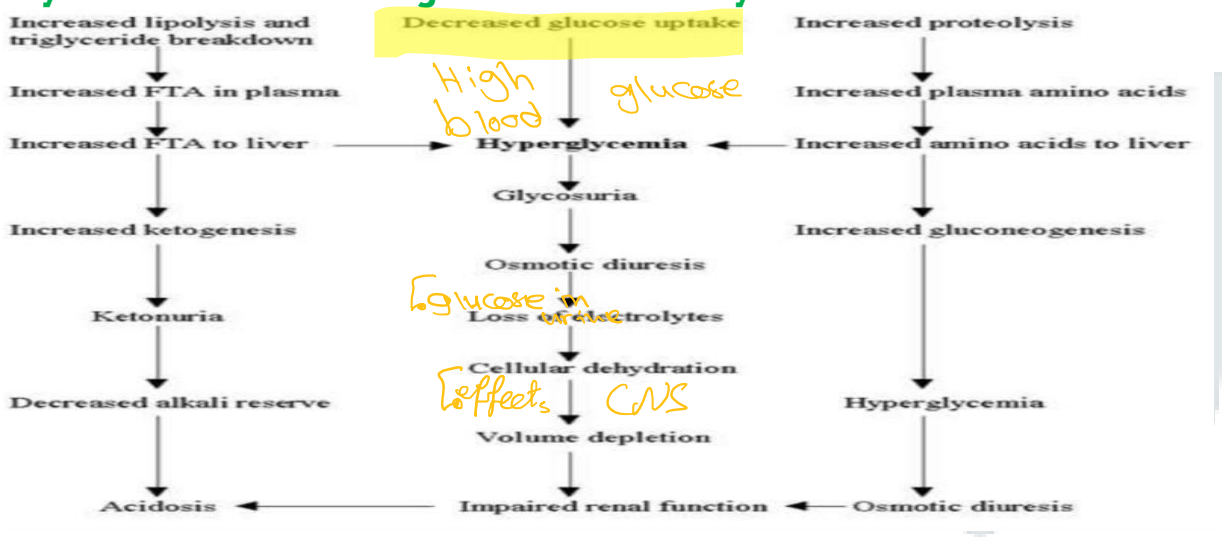
1) Fatty acid biosynthesis
2) Cholesterol synthesis
3) Ketone bodies synthesis
4) Any acetylation reaction

- Decreased ratio of insulin/glucagon ratio promotes gluconeogenesis, glycogenolysis, and ketone bodies formation in the liver, as well as increases in substrate delivery from adipose and muscle tissues (free fatty acids, amino acids) to the liver 'physiological pH is 7,35 to 7,45'

-The ketone bodies have a low pH → metabolic acidosis. -The body initially buffers these with the bicarbonate

buffering system ($which is at a limited volume of 55-70 mmol/L), and other mechanisms to compensate for the acidosis, such as hyperventilation (Kussmaul respiration) to ↓ blood CO2 levels. And $phosphate buffering which is found mainly at the kidney.

- **Ketone bodies lead to electrolyte losses due to osmotic diuresis.**
- **Diabetic ketoacidosis may be diagnosed when the combination of hyperglycemia, ketones on urinalysis and acidosis are demonstrated**
- **We have 3 compensatory mechanism for the pH drop :**
- **1. Bicarbonate buffer (alkaly reserve): this buffer has limited volume (55-70 mmol /L)which is considered as a disadvantage because if we had sever DKA it might lead to depletion of Alkaly reserve**
- **2. Respiratory hyperventilation:CO2 wash**
- **3. Phosphate buffer : this is the main buffer in the kidneys , this mechanism will result in increased urine acidity which could lead to crystal urine which might lead to urinary stones**



-$The problem od DBK is shifting of the hormonal regulation

-Under normal conditions ,the hormone that has the upper hand is insulin but in DM they are anti insulin hormones and top of them is glucagon (does the opposite of insulin functions) and other hormones like growth hormones and thyroxin

-in DM all the pathways that insulin activates get inhibited and the pathways that insulin inhibits will get activated and on top of that is glycogenolysis (degradation of glycogen), gluconeogenesis(forming of glucose from non-carbohydrates source), lipolysis and ketogenesis and as we said the cells don't need to do beta oxidation to FAs to produce energy but the availability of FAs will push the cells to do beta oxidation reaction and produce a huge amounts of

acetyl-CoA which can not be utilized by the citric cycle because it has a limited need (capacity of citric cycle to utilize acetyl-CoA) so there will be extra amount of acetyl-CoA beyond the capacity of citric cycle thus it will go to produce (الدكتور قولة على مصيبتين) which are cholesterol and ketone bodies and those bodies are acids ,so the first step to prevent the dangerous effect of metabolic acidosis is the buffer and the main physiological buffer is bicarbonate ,it neutralize the acidic effect of ketone bodies but it has a

$problem which is off limited volume , we have 50-70 mmol/L of bicarbonate so with the continuous production of ketone bodies , there will be depletion of alkali reserve , so the bicarbonate amount is not sufficient for all the ketone bodies thus the compensatory mechanism starts through kidney and respiratory system .

***$خليه Kidney will excrete ketone bodies and this will cause polyuria why ? because ketone bodies has osmotic activity , so while it gets excreted it will drag water with it and that will cause dehydration , not only water but also electrolytes which will cause electrolytes imbalance that will mainly affect the CNS because it depends on Na+ and K +, so if there is no Na+ and K+ it will affect the CNS and also the transportation of molecules across the cell membrane , also proteins may be excreted like vit.B1 which will cause numbness of the legs , however ketone bodies are still found in the body which will cause the respiratory system to do hyperventilation(Kussmaul respiration) to wash maximum amount of CO2 in blood**

-$glucagon cause lipolysis

- ketonuria : loss of ketone bodies in urea

-Glycosuria: loss of sugar in urea

Glycose is high in the blood but its unable to be uptaken by the cells so cell starvation will happen and it will cause polyphagia

- **3 polys of DM:**

1- **Polydipsia**
2- **Polyphagia**
3- **Polyuria**

***Uncontrolled DKA might lead to skeletal muscle wasting the reason for that is Cathepin**

***Cathepsin : enzyme which is in normal pH and gets activated after a pH drop to start working as a proteolytic enzyme**

Glucagon : stimulates lipolysis by HSL enzyme, uncontrolled diabetic patient will show loss of adipose tissue.

*we don't defend on urine to diagnose DM because there is a certain threshold for plasma Glucose to appear high in urine (threshold is 180)

Example: if we took urine sample from diabetic patient with lower plasma glucose than 180 you would notice a normal urine .

For the following up of DM its better not to use fasting blood glucose but use HbA1C (glycosylated Hb)

Regulation

- **Ketogenesis may or may not occur, depending on levels of available carbohydrates in the cell or body.**

-This is closely related to the paths of acetyl-CoA:

$-regulation undergoes hormonal activity

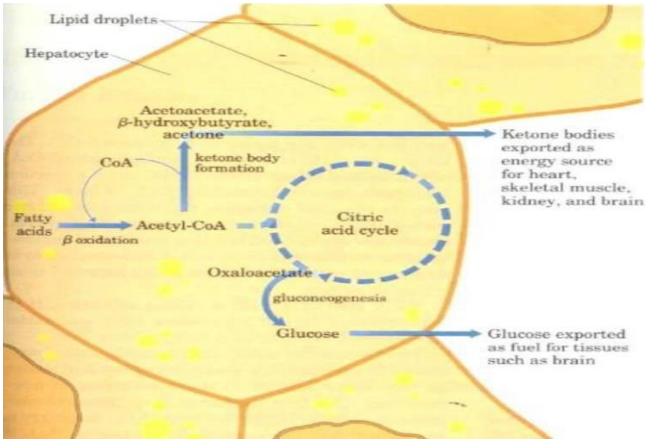
$In well fed state insulin will work and cause inhibition of ketogenesis and activation of glycolysis and it will produce Glyceraldehyde 3-phosphate and Dihydroxyacetone phosphate turn by dehydrogenase enzyme to glycerol-3-phosphate (storage of FAs) this will leave no FAs available for beta oxidation also insulin activates lipogenesis and protein synthesis to store amino acids in a protein form so it wont be available for gluconeogenesis

- but in starvation , glucagon will activates Proteolysis to produce amino acids

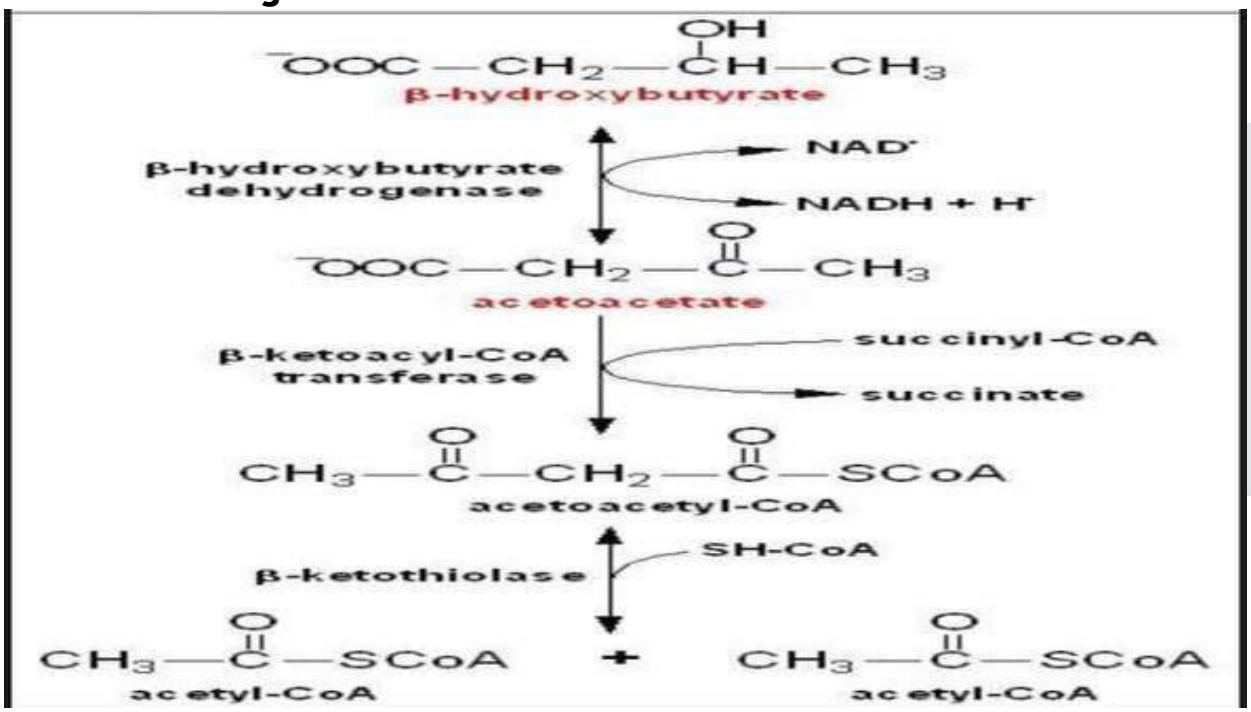
-insulin inhibits ketogenesis and glucagon activates ketogenesis

1- **When the body has ample carbohydrates available as energy source, glucose is completely oxidized to CO_2; acetyl-CoA is formed as an intermediate in this process, first entering the citric acid cycle followed by complete conversion of its chemical energy to ATP in oxidative phosphorylation.**

2- **When the body has excess carbohydrates available, some glucose is fully metabolized, and some of it is stored by using acetyl-CoA to create fatty acids. (CoA is also recycled here).**



3- **When the body has no free carbohydrates available, fat must be broken down into acetyl-CoA in order to get energy. Acetyl-CoA is not being recycled through the citric acid cycle because the citric acid cycle intermediates (mainly oxaloacetate) have been depleted to feed the gluconeogenesis pathway, and the resulting accumulation of acetyl-CoA activates ketogenesis.**



***under physiological conditions glucagon stimulates ketogenesis but insulin keeps ketone bodies within a very small range because it inhibits lipolysis which means no AcetylCoA.**

$The amount of ATP produced in this reaction :

20 ATP produced from 2 molecules of acetyl-CoA

2.5 ATP produced from NADH

1 ATP consumed from the formation of succinate

4 ATP produced from the oxidation of succinate (1,5 ATP from FAD, 2.5 ATP from NADH)

Total amount is 25.5 ATP

Ketolysis (ketone body utilization)

-Tissues that can use fatty acids can generally use ketone bodies in addition to other energy sources.

-The exceptions are the liver and the brain.

-The liver synthesizes ketone bodies, but has little α-ketoacyl- CoA transferase, and therefore little ability to convert acetoacetate into acetyl-CoA.

The brain does not normally use fatty acids, which do not cross the blood-brain barrier; under ordinary circumstances, the brain uses glucose as its sole energy.

-The metabolic rate of the brain is essentially constant.

-While other tissues reduce their metabolic requirements during starvation, the brain is unable to do so.

-After a few days of fasting, the brain undergoes metabolic changes to adapt to the decreased availability of glucose.

-One major change is increased amounts of the enzymes necessary to metabolize ketone bodies.

-The utilization of ketone bodies requires one enzyme not present in the ketone body biosynthetic pathway, β - ketoacyl-CoA transferase (thiophorase), converts acetoacetate to acetoacetyl- CoA.

-The lack of thiophorase in the liver prevents the futile cycle of synthesis and breakdown of acetoacetate.

-The β- ketoacyl-CoA transferase uses succinyl-CoA as the CoA donor, forming succinate and acetoacetyl-CoA

-This reaction bypasses the succinyl-CoA synthetase step of the TCA cycle, although it does not alter the amount of carbon in the cycle.

-This implies that the TCA cycle must be running to allow ketone body utilization; a fact which is necessarily true, because the TCA cycle is necessary to allow generation of energy from acetyl- CoA.

- The other enzymes of the ketone body utilization pathway, β hydroxybutyrate dehydrogenase and thiolase , are identical to the enzymes used for ketone body synthesis.

DHAP is intermediate in glycolysis that could work in TAG formation.

Some aspects of myocardial biochemistry of heart failure

Left ventricular hypertrophy

Left ventricular HF

- Heart failure reduces the capacity to transduce the energy from foodstuff into ATP.

HF results in lesser blood pumping

- In the advanced stage of HF→ → →

- Down regulation in F.A.s oxidation;

- Increased glycolysis and glucose oxidation

- Reduced respiratory chain activity.

****most dangerous MI is inferior MI**

Cardiac muscle and Ischemia

- Coronary artery occlusion → ischemia → significant change in cell structure, chemistry and function

- Loss of contractile function

- Arrhythmias

- Cell death

- **Depletion of ATP**

Increase in AMP and ADP

- مشكلة الـ **MI** انه الجزء اللي صارله **occlusion** تحول من **muscular** لـ **fibrosis**

-The decrease of the ATP / ADP, the accumulation of AMP, inorganic phosphate, metabolic products are removed (lactate).

-The rapid decline in creatine phosphate (only short-term mechanism to compensate for reduced ATP production in mitochondria)

- Even mild ischemia reduces the concentration of ATP and creatinephosphate, increases the level of inorganic phosphate

→ activation of glycolysis (glucose needed from the bloodstream into the heart cells) → increase in the concentration of pyruvate

→ conversion by LDH to lactate.

- Prolonged ischemia - the accumulation of substrates (lactate, NADH+ and H+) → inhibition of glycolysis at the level of PFK-1 and glyceraldehyde-3-dehydrogenase. **ADP & AMP are stimulators**
Lactate & NADH+& H+ are inhibitors

- **To solve the problem we should balance between inhibitory and stimulatory factors to save the cardiac muscle.**
  $-HF >> less blood supply to different organs including cardiomyocytes >> less O2 >> shifting of the metabolic pathways from aerobic to anaerobic

$so some parts of cardiac muscles will go under glucose oxidation with aerobic conditions and produce pyruvate and energy and other parts will not get sufficient amount of O2 so it will go under anaerobic conditions and produce lactic acid

- $electron transport chain will be reduced because it depends on O2