**Please look at the graphs in the slides while studying this sheet

Metabolism of cardiac muscle

In order to understand anything in medicine whether a physiological process or a pathological mechanism you need to get all the way down to the biochemistry of molecules; if you are going to develop a drug, a molecule that target a disease, or if you discover a marker that can help you out to identify whether a person is at risk of a disease or not and what kind of treatment you can give that person.
In the past they knew that cancer is just cells out of control that divide and divide, then they tried to understand what caused cancer, and they were able to identify certain molecules; oncogenes, tumor suppressor genes, apoptotic genes and so on. Then they were able to develop certain drugs, they gave agents that kills any cell that proliferate and divide, which caused many side effects. Then when they understood how cancer is formed, they were able to give certain agents that target the defective molecule so the patient will not suffer any more from side effects. Like treating leukemia with drug gleevec ; specific target and no side effects. Also like Herceptin for breast cancer.

*What we will do is to understand the metabolism of cardiac muscle; specifically related to ischemia.
**What happens when there is low oxygen in cardiac muscle? Cardiac muscle must be functioning all time and must have ATP. If there is deficiency of oxygen in tissues there will be changes in metabolic pathways.

Heart failure: is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood. So it is the Inability of cardiac muscle to function.

This topic is really important because heart failure is the leading cause of death all over the world, so we need to understand heart failure because eventually if there is heart failure we will have myocardial infarction or any other cardiac disease.
Heart failure is associated with changes in metabolic profile. Patients with diabetes have different metabolic mechanisms all over their bodies and they may have heart failure. Is diabetes is a cause for these alterations in metabolism? Yes, and it is a cause of heart failure. 20-30% of heart failure patients are diabetic indicating a connection between both .

They noticed that when we optimize substrate metabolism we improve cardiac function, so we can help a patient for example by giving right diet.

So in this lecture we will cover the following subjects:

1- Normal Metabolic profile in cardiomyocytes.
2- Alteration in metabolic profile in failing heart and reperfusion*(look next page).
3- Therapeutic targets.
4- Biomarkers of heart failure.

*[Reperfusion: is when there is oxygen deficiency and then you fix it and there is sudden high oxygen in heart muscle. This can be fatal; it kills the cells when u all of a sudden supply oxygen to heart.]

Pathways

1- glycolysis:

Glucose is converted to pyruvate which may have different fates: can be converted to amino acids like alanine by transamination, or carboxylated to oxaloacetate, or converted to acetyl coA (by oxidative decarboxylation) which is a central metabolite, or converted to lactate in anaerobic metabolism (reduction) .

Acetyl coA can enter CAC (Krebs) cycle, or it can be converted to ketone bodies or steroids.

Following the CAC cycle we have the electron transport chain then generation of lot of ATP.

**Acetyl coA is produced from glucose but also can be produced from FAs and proteins as well; (central metabolite).

Metabolic profile of cardiac myocyte:

- About 60% to 90 % of acetyl coA comes from fatty acids (beta-oxidation) , so they really depend on FAs, and 10% to 40% from glucose.
- 95 % of ATP comes from oxidative phosphorylation (acetyl CoA pathway)

Cardiac muscle really depends on presence of ATP so it needs acetyl coA.

- There is quick turnover of ATP molecules so they last 10 seconds, then new ATP molecules are generated.
- 60% to 70% of ATP hydrolysis is fuel for contractile power, the rest is used to maintain ionic hemostasis in cardiac cell.

***Important part*:** Even though you learned that FAs produce more ATP than glucose; <u>they need more oxygen than glucose.</u>
People who want to lose weight they burn their fat by exercise and get into anaerobic exercise, they get really tired and not getting oxygen into their body. So instead of burning fat they burn glucose into lactic acid, so they are not benefiting from this exercise. So you need to do aerobic exercise when you are relaxed and have oxygen in your system.

**The substrates that cardiac tissue depend on when there is enough oxygen: <u>Fatty acids (50-70%) and glucose (30%)</u>. [In glycolysis: glucose to pyruvate produces 5% of ATP].
**When there is increased muscular activity or ischemia: there is a lot of dependency on <u>glucose</u> and more formation of <u>lactate</u>.
**Under pathological conditions: the cardiac muscle depends on <u>ketone bodies and amino acids</u>.

2- Fatty acid metabolism:

First we have circulating free fatty acids (FFA) bound to albumin and enter to cardiac myocyte by diffusion or by fatty acid transporter (all these steps are under control). Once in, they are

acetylated (conjugated) with Co-enzyme A. Now we have fatty acyl Co-A.
This FA Co-A have two different fates; either stored in triglycerides (30%) or mainly most of it enters the mitochondria by a transporter CPT1 (carnitine palmitoyltransferase) that takes the FA Co-A, combines it with carnitine and transports it into the mitochondria. Then there is another molecule CPT2 that takes FA Co-A from the intermembranous space to the mitochondrial matrix.
Now In mitochondria, the FA Co-A is oxidized by a mechanism of degrading the fatty acid two units at a time, with each time there is production of acetyl Co-A (e.g. if we have 18 unit fatty acid it will become 16, 14, 12,… with production of acetyl Co-A in each cycle). This is known as Beta oxidation of fatty acids. Then acetyl Co-A goes to Krebs cycle.

Regulation:
There is a molecule known as malonyl Co-A (3-carbon molecule) produced from acetyl Co-A (2-carbon molecule) by carboxylase enzyme (ACC) which adds a carbon to acetyl Co-A converting it to malonyl Co-A.

Malonyl Co-A if present in high levels it's an indication that we don't need more acetyl Co-A, so it **inhibits CPT1** so no more FAs goes into the mitochondria, so no more oxidation of FAs.

The opposite is true: no acetyl Co-A → no malonyl Co-A → no inhibition of CPT1→ FAs goes into the mitochondria → oxidation.

*Decarboxylase enzyme (MCD) converts malonyl Co-A back to acetyl Co-A.

*The activity of carboxylase enzyme is regulated by AMP kinase, it's a sensor that tells the cells whether there is enough acetyl Co-A (energy) or not (low energy (ATP) means high AMP), so when activated by high AMP it will inhibit the carboxylase, there will be low levels of malonyl Co-A, so no inhibition of CPT1.

Glucose and glycogen:

There is little bit of glycogen in cardiac myocyte that is converted to glucose-6-phosphate, which enters the glycolysis and converted to pyruvate.

Also there is glucose that comes from outside and transported by glucose transporter GLUT, then glucose is converted to pyruvate which is converted by pyruvate dehydrogenase To acetyl Co-A which enters Krebs cycle.

If you look at GLUTs, there are 7 of them in the body, 1 and 4 are present in cardiac myocyte. GLUT 4 is the most important with highest abundance, and it is insulin responsive and has a high affinity for glucose (medium Km) .

This GLUT is present in the cytosol, so whenever you have insulin or ischemia, you will have activation of a signaling pathway that will result in translocation of the vesicle that contains the transporter to the plasma membrane of the cell, so more glucose goes in.

So ischemia, workload, insulin can all stimulate the translocation of the transporter to the cell membrane.

Lactate metabolism:

**In previous lecture we talked about lactate dehydrogenase. LDH have 5 different isozymes. LDH 1 is present in high abundance in cardiac myocyte and it has high affinity for lactate and converts it to pyruvate . LDH 5 which is present in muscles converts pyruvate to lactate which is released to blood to cardiac tissue which takes lactate and converts it again to pyruvate which goes to Krebs cycle.

So LDH 1 functions aerobically, it converts lactate to pyruvate, has low km (high affinity) for lactate, and is inhibited by pyruvate (high pyruvate inhibits the enzyme).

Lactate enters the cells by a mono-carboxylate transporter (which is specific not only for lactate but for other molecules as well).

Ketone bodies:

***Under starvation** we have production of ketone bodies; acetoacetate, acetone and hydroxybutyrate. Acetoacetate and hydroxybutyrate are produced in the liver, they go to blood stream, then in the cardiac tissue they are converted back to acetyl Co-A which can be used to produce energy.

How all of these pathways interact with each other to make hemostasis?

They noticed that ketone bodies metabolism:

1- Increases acetyl Co-A that when at high level activates *pyruvate dehydrogenase kinase* which phosphorylates (inhibits) pyruvate dehydrogenase which after being phosphorylated becomes inactive . So ketone bodies prevent the oxidation of glucose by preventing the conversion of pyruvate to acetyl Co-A .
2- Increases the level of citrate which inhibits phosphofructokinase (PFK; an early enzyme in glycolysis pathway). So there will be no more conversion of glucose to pyruvate.

Fatty acids metabolism:

1- (long chain fatty acids) Inhibits hexokinase (which phosphorylates glucose) so glucose is not phosphorylated to glucose-6-phosphate.
2- Also you will have high level of NADH (due to beta-oxidation) which inhibits pyruvate dehydrogenase.
3- Increases the amount of acetyl Co-A and citrate.

**You either have glucose oxidation or FAs oxidation, difficult to oxidize both.

What if the cardiac myocyte is metabolizing glucose? There will be no need to metabolize FAs. So when we have high glycolysis we will have high citrate which is transported from the mitochondria to the cytosol then it can activate carboxylase enzymes, which converts acetyl Co-A to malonyl Co-A which inhibits fatty acid oxidation by inhibiting CPT 1.

[[If you noticed that citrate can be produced from fatty acids metabolism and glucose metabolism, and wondered how the cell knows where this citrate came from; the answer it's not just one molecule that do one thing, there is inhibition on different levels]]

Randle cycle:

- Describes the reciprocal relationship between fatty acid and glucose metabolism.
- The increased generation of acetyl CoA derived from fatty acid-oxidation decreases glucose (pyruvate) oxidation.
- The increased generation of acetyl CoA derived from glucose (pyruvate) oxidation inhibits fatty acid –oxidation.

So again there is a balance and it's not just at one enzyme, it's at multi-level.

*In the heart, the inhibition of glucose utilization by fatty acids is a form of glucose intolerance. So this is the connection between diabetes and heart failure; if we have more FAs in the heart it will prevent oxidation of glucose so heart muscle is not responding to glucose any more as if the person is diabetic.
[[The diabetic person is suffering that cells don't respond to insulin, so a person with failing heart is a person dependent a lot on fatty acid metabolism so he is not metabolizing glucose and not transporting it inside the myocyte; as if the person is diabetic. And this brings us back to where we said there is a relation between diabetes and heart failure (diabetic heart is not depending a lot on glucose metabolism, and is dependent on FAs metabolism. Now fatty acid metabolism needs more oxygen, so if the heart gets ischemic and the person is not depending on glucose metabolism, the heart of this person will be consuming a lot of oxygen which kills heart cells)]].

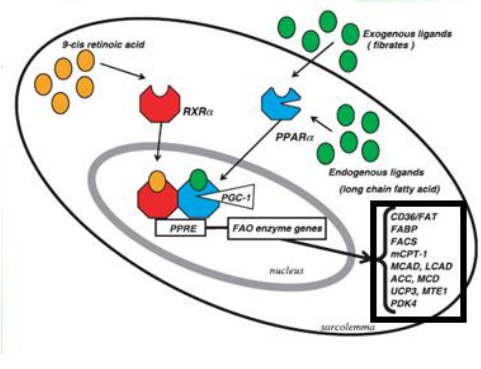
Two regulatory molecules:

1- AMPK

It is a sensor that controls metabolism in almost all cells of the body.
When we have high AMP; AMP Kinase will be stimulated to stop all ATP consuming pathways (FAs synthesis, protein synthesis, cholesterol synthesis, glycogen synthesis) and stimulates ATP producing pathways (glycolysis, beta oxidation, glucose uptake).
**AMPK activates GLUT-4 translocation into the membrane so more glucose gets inside the cell , also activates hexokinase + PFK + glycogenolysis and inhibits glycogenesis .
**AMPK activates CPT1 directly or by inhibiting the carboxylase, so more FAs enter the mitochondria and we will have more beta oxidation .

2- Peroxisome proliferator activated receptor PPAR

It is a transcription factor, it regulates gene expression. Mainly it controls expression of genes related to enzymes, transporters, regulatory proteins in the beta oxidation pathway.
[Don't memorize the list of enzymes in the slides, but understand the logic behind it. If the doctor asks about them in the exam one of the options will be wrong because it doesn't't make sense].



There are three isoforms of this transcription factor: alpha, beta, and gamma.

- Alpha is present in cardiac myocytes, skeletal muscles, and liver cells.
- Beta in skeletal muscles.
- Gamma in adipose tissue.

When these are activated they increase beta oxidation of FAs. So the more of this transcription factor in cells; the more beta oxidation will take place.
They will Increase uptake of FAs, and increase oxidation.

If we increase gamma transcription factor in adipose tissue how it will affect the metabolism in the cardiac myocytes?
There will be less FAs released from adipose tissue to become free circulating FAs for cardiac muscle to take up, so we reduce beta oxidation in the heart.

If we activate alpha transcription factor we will activate beta oxidation in heart but also at the same time there will be no more FAs coming from the liver cells as well. So also there will be reduction in FAs coming to the heart.

So to get all the things together:
Let's start with a metabolic profile of ischemic heart:
If we have ischemia, it means there will be low pressure of oxygen in the cardiac cells, AMPK is activated, increased glucose uptake, increased glycolysis, but glucose oxidation is reduced because there is no oxygen for oxidation, so glycolysis will produce ATP enough to maintain some functions of heart muscles and ionic hemostasis, and also inhibits FAs oxidation. So the heart mainly depends on ATP coming from glycolysis.

If suddenly there is increase in the amount of oxygen in the heart (perfusion). What we will have is increase in circulating FAs, that increase FAs oxidation, and as a result there will be inhibition of glucose oxidation, and AMP Kinase still activated which activates glycolysis that produces a lot of lactic acid meaning that there will be a lot of protons and the pH inside cells will go down (acidosis). So there will be oxygen wasting because FAs oxidation require more oxygen than glucose oxidation.
The other thing is that when we have a lot of FAs oxidation there will be synthesis of uncouplers (discussed in the next paragraph).

[in electron transport chain protons are transported from matrix to intermembranous space so we will have electrochemical gradient; high protons in intermembranous space relative to the matrix. Then these protons enter back to matrix via ATP synthase which converts ADP to ATP. What uncouplers do is that they transport the protons back to the matrix without passing through the ATP synthase, so there is a lot of burning and O_2 converted to H_2O and no ATP is produced, so the oxygen is wasted by beta oxidation of FAs and synthesis of uncouplers].

So to sum up; we have increased FAs metabolism, oxygen wasting, production of free radicals from the electron transport chain that will do damage to molecules and to mitochondria, so cells will die.

We have high glycolysis which is instead of producing acetyl Co-A from pyruvate; it is producing lactate and there will be increased production of protons, so this causes acidosis inside cells. So cells must handle this high level of protons by pumping these protons via proton-sodium transporter. So sodium gets in and protons gets out. Now sodium must go out by sodium-calcium transporter, so sodium goes out and calcium comes in. Now calcium is high inside the cell which causes toxicity to cells; because eventually instead of using ATP for the contractile power; this ATP is mainly consumed for pumping protons and sodium out and calcium in, so we have ATP wasting also.

So we have oxygen wasting, ATP wasting, acidosis, and mitochondrial damage. Eventually cardiac cells die.

How we treat this?

There are 4 different levels by which drugs can target this whole thing we talked about:

1- Target the transporters, So FAs don't get into cells so no oxidation of FAs. and targeting CPT1 so its inactivated so FAs are not getting into mitochondria , or by Malonyl-CoA decarboxylase (MCD) inhibitors

2- Target the transcription factor PPAR by agonist to alpha, beta, or gamma. Remember if we increase the expression of PPAR that will increase beta oxidation of FAs in the peripheral tissues like liver and adipose tissue and there will be low circulating FAs for the heart, so there will be less beta oxidation inside the heart. And this is the reason of using the beta adrenoceptor antagonist so there will be no release (lipolysis) of FAs into the blood.
   *Note : Glucose–insulin–potassium (GIK) (reduce circulating fatty acid concentrations, while maintaining circulating glucose concentration)

3- Targeting enzymes that inhibit beta oxidation so cardiac myocytes metabolism switches to glucose oxidation which consumes less oxygen releasing the pressure on the heart.

4- Inactivating the *pyruvate dehydrogenase kinase* enzyme, so pyruvate dehydrogenase is active so pyruvate is converted to to acetyl Co-A, so we have glucose oxidation consuming less oxygen in the cardiac tissue and producing less lactic acid.

*So this is the reason behind treating current or future failing or ischemic heart, by regulating the utilization of substrates in the heart tissue.

**People are trying to study and discover biomarkers for heart failure and ischemic heart. We talked in the previous lecture about one of them which is ischemia induced albumin.
There are other markers and so many markers, but one of the most promising markers is VNP (V type natriuretic peptide), so people with high VNP have high pressure on the heart and this is an indication that the person has heart failure.

***Note: in the exam if a question comes with a name of an enzyme, the doctor will write the full name and the abbreviation.

~Every accomplishment starts with the decision to try~