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MOLECULAR MECHANISMS INVOLVED IN PHYSICAL EXERCISE AND FACTORS THAT MAY INFLUENCE THEM. PARTICULARITIES IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Abstract

Phisical activity, regularly performed, give us a lot of health benefit, especially in preventing cardiovascular disease, diabetes mellitus (DM) and obesity. Physical exercise, defined as a controlled, progressive, supervised, requires muscular activity, involving energy consumption through metabolic and thermoregulatory processes. It can be classified as aerobic and anaerobic, according to the metabolic processes that take place. The metabolic equivalent (MET) represents the body's energy consumption during rest and it is used for quantifying fhisical activity (for example, a MET value of 3 would require 3 times the energy that is consumed at rest). Muscle contraction has two different phases: the isometric one (usually during the first part of the contraction) and the isotonic one. This article presents the interrelation of phisical activity with with the complexity of metabolic patwais, bringing the arguments for the necessity of performing regular and controlled phisical activity.

key words: *phisical activity, type 2 diabetes mellitus, metabolic pathwais*

Introduction

Physical activity (PA) regularly performed is seen as a healthy lifestyle component. Recently its importance has been underlined by studies that have shown the link between constantly and regularly performed PA and the overall health benefit, especially in preventing cardiovascular disease (CD), diabetes mellitus (DM) and obesity.

Definition of terms

The notion of physical exercise (PE) is defined as a controlled, progressive, supervised PA. This action requires muscular activity, involving energy consumption through metabolic and thermoregulatory

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processes [1]. PE can be classified as follows: aerobic PE and anaerobic PE according to the metabolic processes that take place.

Aerobic PE consists of rhythmic, repeated and continuous movements of the same muscle groups for a minimum of 10 minutes (brisk walking, jogging, swimming**,** cycling).

Anaerobic PE is characterized by short, intense periods of muscle activity (sprinting, swimming vigorously for short periods, isometric PE-lifting, pushing, pulling weights). Anaerobic PE is accompanied by an increase in blood pressure and heart rate due to sympathetic nervous system stimulation, with secondary catecholamine hypersecretion.

Muscular fitness refers to muscular strength and muscular endurance [5].

Resistance PE: PA that uses muscular strength to lift a weight [5].

The metabolic equivalent (MET) represents the body's energy consumption during rest and it is used for quantifying PA (for example, a MET value of 3 would require 3 times the energy that is consumed at rest) [5]. Muscle contraction has two different phases: the isometric one (usually during the first part of the contraction) and the isotonic one.

Isometric contraction *increases muscle tension, although the length remains constant.* It is a characteristic of postural muscles. It doesn't produce mechanical work but instead produces heat. The joints angle also does not change. For example, when a person pushes a wall or tries to lift a very heavy object, the muscles are involved in isometric contraction. The tension developed in the muscles during these types of contractions is higher than the one obtained during isotonic contractions.

Isotonic contraction, considered to be a dynamic contraction, causes the muscle to change its length while the tension remains constant. It is a characteristic of the skeletal muscles. Isotonic contraction produces mechanical work and movement.

Elements of anatomy and physiology of the skeletal muscle fiber [2]

There are two types of muscle fibers: type I – slow twitch fibres and type II – rapid twitch fibres.

Type I muscle fibres or slow twitch-oxidative fibres have the following characteristics:

- small amount of glycogen;
- large amount of myoglobin (red fibres) and mitochondria ;
- small calibre;
- extensive network of capillaries providing an important oxygen supply;
- preferentially use of aerobic reactions;
- resistant to fatigue;
- increased lipoprotein lipase (LPL) activity;
- they slowly develope muscle strength and maintain it for a long period, being used for intense PA.

They are found in the postural muscles: psoas, soleus muscle.

Type II muscle fibres differentiate in two subtypes:

1. Type IIb or slow twitch fibres have the following features:

- increased amount of glycogen;
- decresed amount of myoglobin and mytochondria;
- limited aerobic metabolism;
- glycogenolysis and glycolysis are important metabolic processes;
- large calibre;
- increased sensitivity to fatigue due to ATP synthesis mainly through glycolysis with an increased

production of lactic acid followed by low pH and downregulation of this macroergic compound;
- decreased lipoprotein lipase (LPL) activity;
- activated in sprint and resistance PE.

2. Type IIa fibres are a combination of type I and type IIb fibres, sharing their functional characteristics.

Fast twitch fibres may rapidly produce an increased force of contraction. They are found in muscles of the lower and upper extremities, which are responsible for high intense movements of short duration. The percentage of the fibres varies: the triceps muscle has 32.6 % type I fibres, while the soleus muscle has 87.7% type I fibres.

"Fuels" utilization

Skeletal muscles use different "fuels" to generate adenosine triphosphate (ATP), each with a unique and special role in the mechanism of contraction. ***ATP*** is a macroergic compound responsible for energy transfer and not for energy storing [4]. Aerobic and anaerobic glycolysis lead to ATP generation**.**

All skeletal muscles contain mitochondria that are capable of fatty acids (FA) and ketone bodies oxidation (beta-hydroxybutyric acid, acetoacetic acid). They are also able to completely oxidize the carbon skeleton of alanine, aspartate, glutamate, valine, leucine and isoleucine. ATP level allosterically inhibits or activates energy producing reactions such as oxidation or hydrolysis [2, 4].

Creatine phosphate (CP), also known to be an important macroergic compound, is an immediate source of high energy which can be used to replenish ATP from adenosine diphosphate (ADP). CP plays a particularly important role in muscles during PE [2]. Creatine synthesis begins in the kidneys and is completed in the liver. In the kidney, glycine combines with arginine to form guanidine acetate. This product is next methylated in the liver by S-adenosyl methionine forming creatine (Figure 1). Hence creatine is provided to the skeletal muscles, heart and brain. Under creatine phosphokinase action and in the presence of ATP, CP is synthesized (Figure 2). CP is a stable product which during a non-enzymatic reaction is converted to creatinine, which in turn is excreted by the kidney (Figure 3).

For up to 40 minutes of mild PE, glucose is obtained initially during muscle glycogenolysis and next during hepatic glycogenolysis. During PE, muscle glycogen phosphorylase is activated by increased levels of adenosine monophosphate (AMP), an allosteric activator of this enzyme and also by phosphorylation, which is stimulated by $Ca2^{+}$ release during contraction. Glycogen phosphorylase converts glycogen to glucose-6-phosphate (G-6-P) in the muscles, while phosphofructokinase-1 (PFK-1) converts G-6-P to pyruvic acid. Next, there are two possibilities: the first one consists in conversion of the pyruvic acid to acetyl-CoA (that will enter the Krebs cycle) due to pyruvate dehydrogenase intervention and the second one is represented by lactic acid synthesis from pyruvate under lactic dehydrogenase action**.** Both pathways generate ATP.

In the liver, glycogen is transformed to glucose-1-phosphate (G-1-P), which in turn is converted to G-6-P. Under the action of glucose-6-phosphatase, an enzyme found in

the liver and kidney, lacking in the muscles, G-6-P is converted to glucose [4].

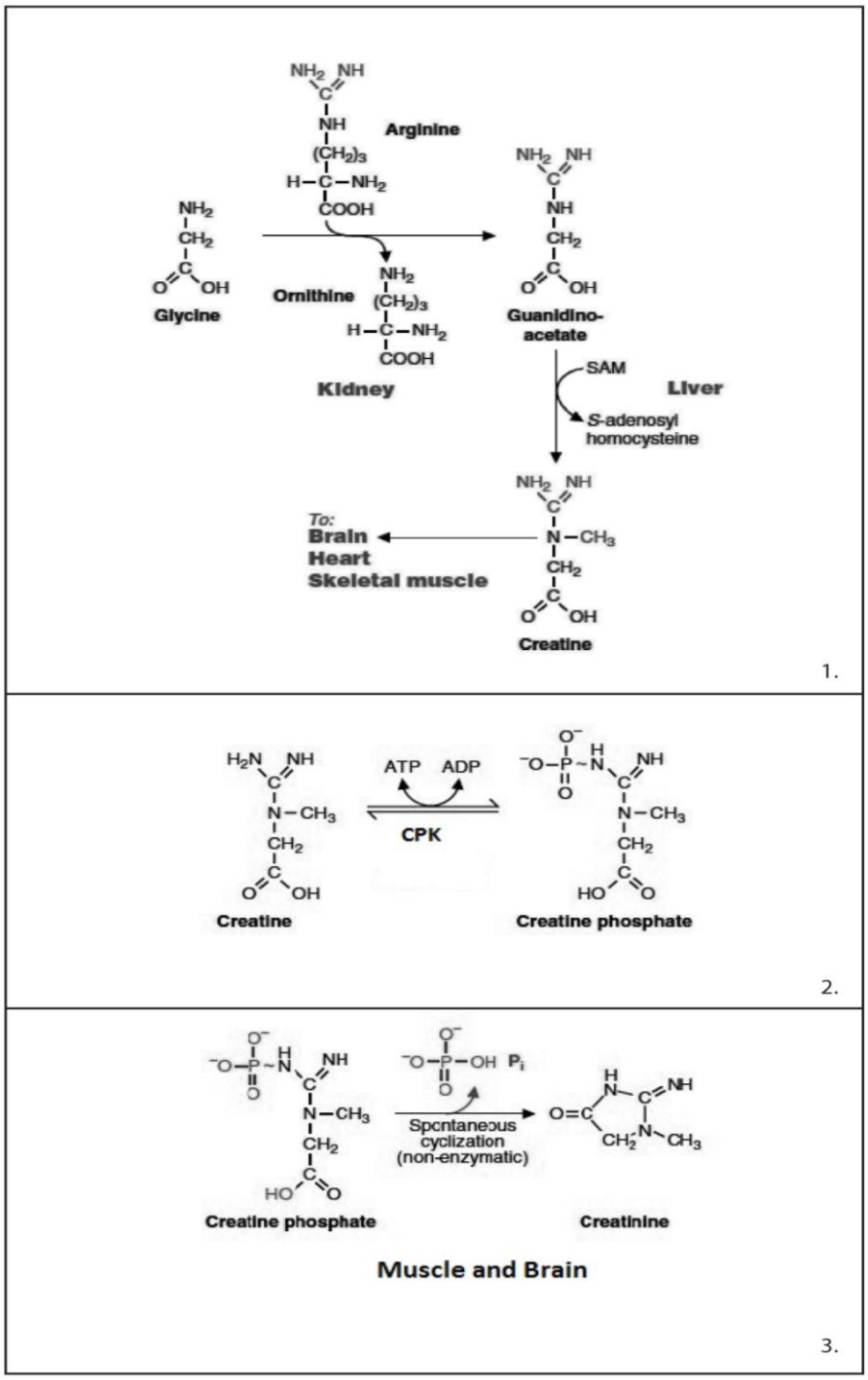

Figure 1. Sinthesys of creatine, creatine phosphate and creatinine (2, *modified*).
CPK- creatine phosphokinase.

After 40 to 240 minute from the PE onset, there is an increased release of catecholamines, glucagon, cortisol, and STH, thus leading to adipose tissue lipolysis activation and production of triglycerides (TG). TG are formed by combining 3 fatty

acids (palmitic, oleic and stearic acids) with glycerol phosphate. They travel bound to albumin to various tissues. One of these tissues is the muscular tissue where they are exposed to β oxidation in the mytochondria resulting:

- acetyl CoA that will enter the Krebs cycle;
- ketone bodies (in the liver) that will be converted to acetyl CoA in the muscles, entering also the Krebs cycle.

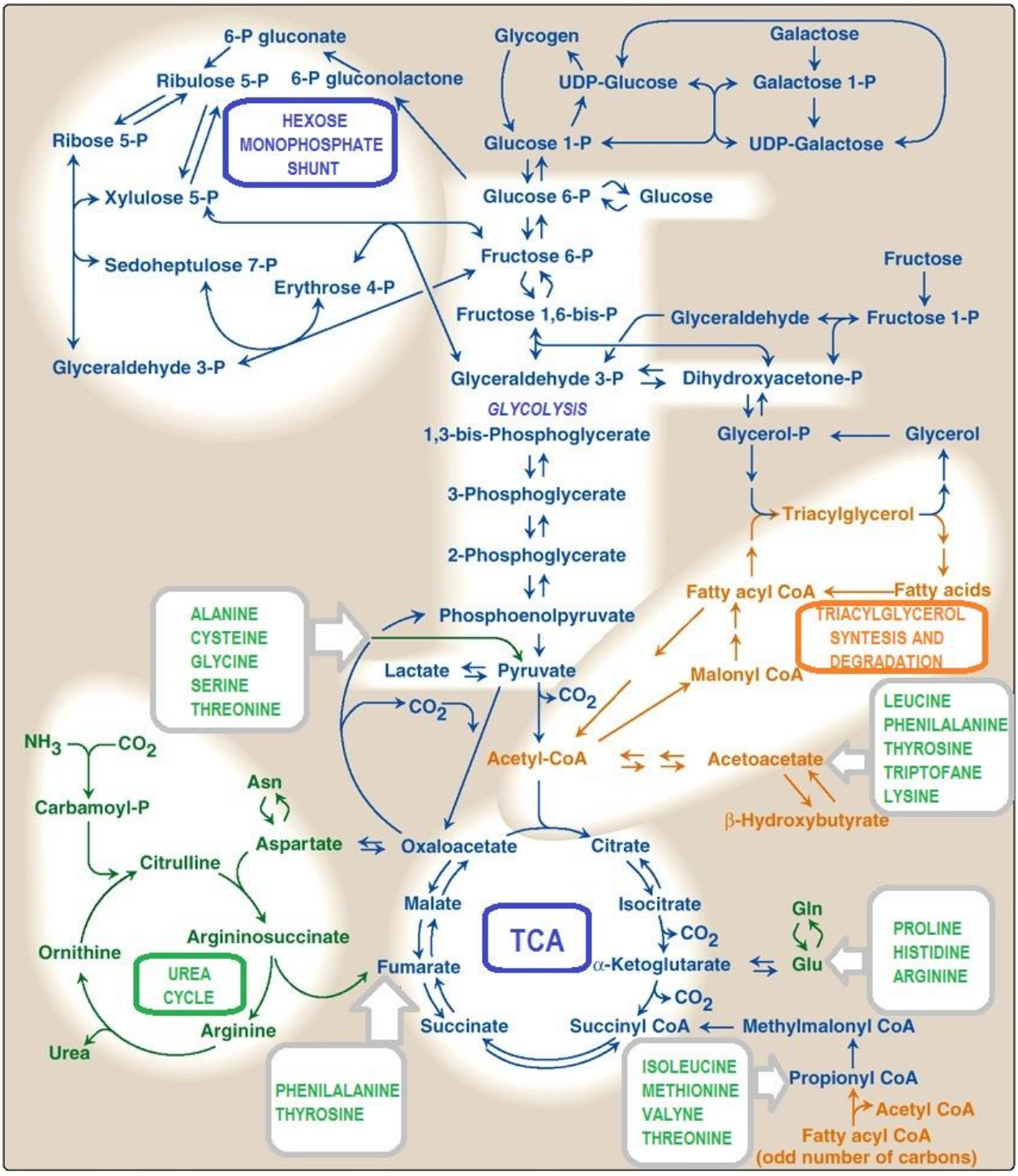

Figure 2. The most important reactions of intermediary metabolism (3, modified).

The liver produces glucose during hepatic glycogenolysis and gluconeogenesis which uses as substrates: lactic acid, glucogenic amino acids, glycerol, citric cycle intermediates (oxaloacetate, α-ketoglutarate, succinyl-CoA and fumarate) [4].

The liver is the only organ capable of amino acids (AA) synthesis and degradation through all the possible pathways. During AA degradation, the carbon skeleton may be reduced to:

– CO_2;
– Subtrates for gluconeogenesis;
– Ketone bodies or their precursors (acetoacetat and acetyl-CoA).

There are ketogenic AA (if the carbon skeleton is finally reduced to acetoacetate and acetyl CoA), glucogenic AA (if the carbon skeleton is degraded to a glucose precursor) and also ketogenic-glucogenic AA [2].

"Fuels" utilization during rest

The level of fuel used at rest by the muscular tissue depends on the serum levels of glucose, AA and FA. Glucose has several metabolic functions: it represents the primary source of energy for the human body; converted to glycogen it represents a form of energy storage; glucose precursors may give rise to different substrates for other metabolic pathways:

– *Glycerol and acetyl CoA:* used for synthetising FA, TG, cholesterol;
– *Pentoses: used for nucleotides and acids synthesis*;
– *NADPH: used in reductive biosynthesis*;
– *The carbon skeleton:* necessary for AA biosynthesis [5].

There are three mechanisms of glucose uptake: simple diffusion, facilitated diffusion, active transport. In case of simple diffusion, the transport rate for glucose correlates with the its concentration gradient. As for glucose entering the cell through passive transport it is possible only with the help of a class of glucose transporters (GLUT) and it doesn't require energy. Glucose active transport is realized against its concentration gradient and it consumes energy (ATP). Sodium glucose transporters (SGLT) are an important class of glucose transporters. They act through a sodium-glucose cotransport and are found in the intestinal mucosa, in the kidney and choroid plexus. They are tissue-specific [5].

GLUT family has 13 members. GLUT 1, GLUT 4, GLUT 11, GLUT 12 [8, 9, 10, 11] are found in the muscles. GLUT 4 is insulin-sensitive and its action depends on the PE. These transporters are stored in intracellular vesicles. Insulin binding to its receptor activates the vesicles containing GLUT 4 transporters. Furthermore the vesicles fuse with the membrane increasing the number of GLUT4 transporters expressed at the cell surface and finally increasing glucose uptake [6, 11]. During some studies carried out in 2009, it was found that insulin causes GLUT 12 translocation to the cell surface [8].

SGLT family is represented in the muscular tissue by 3 transporters: SGLT 1, SGLT 2, SGLT 3 [5].

During rest FA are the main energetic sources for the muscle fibres.

There is a balance maintained by the level of citrate between oxidation of glucose and FA. If the muscle cell has sufficient energy resources, citrate leaves the mitochondria and activates acetyl CoA carboxylase 2 (ACC-2), which mediates the synthesis of malonyl CoA. This will inhibit carnitine palmitoyl transferase I (CPT-1), thereby adjusting the FA oxidation rate by blocking their entry into

the mitochondria. Muscles also contain malonyl-CoA decarboxylase that catalyzes the conversion of malonyl-CoA to acetyl-CoA and CO2 [2]. Branched-chain AA give 20 % energy during rest. Their oxidation generates ATP and synthesizes glutamine. Under conditions of acidosis, there is an increased demand for glutamine, necessary for transferring ammonia to the kidney and tamponating urine as ammonium during protons excretion.

"Fuel" utilization during exercise

The usage rate of ATP in skeletal muscles during PE is about 100 times more intense than the rate used at rest. ATP and CP would be quickly exhausted if they were not continuously regenerate. ATP comes from aerobic/anaerobic glycolysis and oxidative phosphorylation. Anaerobic glycolysis is an important source of ATP in the following three situations:

- during onset of PE, when the blood flow with high oxygen supply increases allowing aerobic processes to take place;
- in muscles with fast twitch contracting fibres, with a high reserve of glycogen;
- during intense PE, when the demand for ATP exceeds the aerobic capacity of tissue.

Anaerobic glycolysis at the onset of PE

ATP at rest is provided to the muscular tissue through aerobic reactions. Once the PE starts the energy required increases. ATP reserves in the muscles are sufficient to support FE only for 1-2 seconds and the amount of CP for 9 seconds (considering they would not be continuously regenerated). More than 1 minute is required for the muscle blood supply to increase significantly during contraction secondary to vasodilatation. That is why glycogen conversion to lactic acid will be the main source of ATP until the glucose and FA oxidation increase.

Anaerobic glycolysis in type IIb muscle fibres (fast twitch fibres)

These muscles contract quickly and vigorously only for short periods of time, being used to lift weights. At this point, glycolytic capacity is increased by the presence of specific enzymes.

The tissues rely on the endogenous reserves of glycogen and CP to produce energy. Glycogen is catabolized to glucose-1-phosphate, which is converted to glucose-6-phosphate and finally lactic acid is obtained.

Glucose-6-phosphate resulted from glycogenolysis, inhibits hexokinase II, thereby permitting to a small amount of circulatind glucose to be used for ATP synthesis, preventing hypoglycemia.

It is confirmed that an increase in hexokinase II gene transcription during PE, could explain the persistence of insulin action after PA stops and also the adaptation which occurs through practice [5].

During PE, glycogenolysis and glycolysis are both activated, as AMP alloesterically upregulates phosphofructokinase-1 (PFK-1) and glycogen phosphorylase b (Figure 3). AMP is an ideal activator since its concentrations are kept low by adenylate kinase activity (2ADP → ATP + AMP). Thus, whenever ATP levels are low, AMP concentration proportionally increases. Increased AMP stimulates AMP-kinase activation (signaling pathway involved in stimulation of transcription and protein synthesis), which in turn causes translocation of GLUT4 to the cell surface and increases

insulin-independent transmembrane glucose transport.

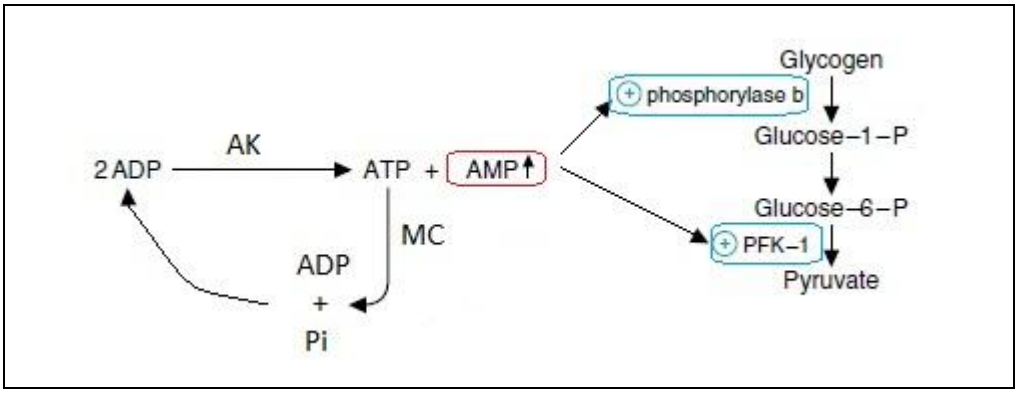

Figure 3. Activation of muscle glycogenolysis and glycolysis by AMP (2, modified). PFK- 1 – phosphofructokinase- 1, MC – muscle contraction, AK – adenylate kinase.

The anaerobic glycolysis, considered a primitive mechanism for obtaining energy [4], generates 3 molecules of ATP, compared to 32-34 molecules of ATP provided during Krebs cycle. Rapid contractile fibers have a high concentration of glycolytic enzymes thus compensating for this energetic difference. The usage rate of G-6-P is 12 times faster than in type I fibers. Muscle wasting during PE occurs by lowering the *p*H to about 6.4. Depletion of glycogen reserve occurs in less than two minutes of anaerobic exercise.

Muscle glycogen degradation is not influenced by glucagon as there are no specific receptors at this level. Glycogen synthesis is inhibited during the PE, but can be activated at rest after ingestion of high amounts of carbohydrates.

Unlike the liver isoform of glycogen phosphorylase, the muscle isoform contains an AMP allosteric site. When AMP binds to this site the enzyme is activated although it is not phosphorylated. As PE begins myosin ATPase hydrolysis the existing ATP to ADP. AMP begins to accumulate and glycogen degradation is stimulated. The glycogen phosphorylase b activation is stimulated additionaly by $Ca2^{+}$ release from sarcoplasmic reticulum once muscle contraction starts [2].

During vigorous PE, the catecholamines stimulate adenylate cyclase in muscle cells by activating AMP-dependent protein kinase. The protein kinase A phosphorylates and activates the glycogen phosphoryl kinase, so that a continuous activation of glycogen phosphorylase can be possible.

Anaerobic glycolysis during vigorous exercise

Once PE starts, electron transport chain, Krebs cycle and FA oxidation are activated by an increased level of ADP associated with a decreased level of ATP. Pyruvate dehydrogenase remains active in a non-phosphorylated form as long as NADH can be reoxidised in the electron transport chain and acetyl CoA can enter the Krebs cycle. Although mitochondrial metabolism is working at full capacity, additional ATP is needed when AMP begins to accumulate during intense PE. The increased level of AMP activates PFK-1 and glycogenolysis, thus providing the necessary ATP through anaerobic glycolysis [2].

Pyruvic acid resulted from anaerobic glycolysis, crosses mitochondrial membrane through active transport. At this level, under the pyruvate dehydrogenase action, it is

converted to acetyl-CoA, further being oxidised in the Krebs cycle [4].

The lactate released during PE can be used by muscle at rest and also by heart (a muscle rich in mitochondria and with an increased oxidative activity). In this type of muscle the NADH / NAD^+ ratio is lower than the one during FE.

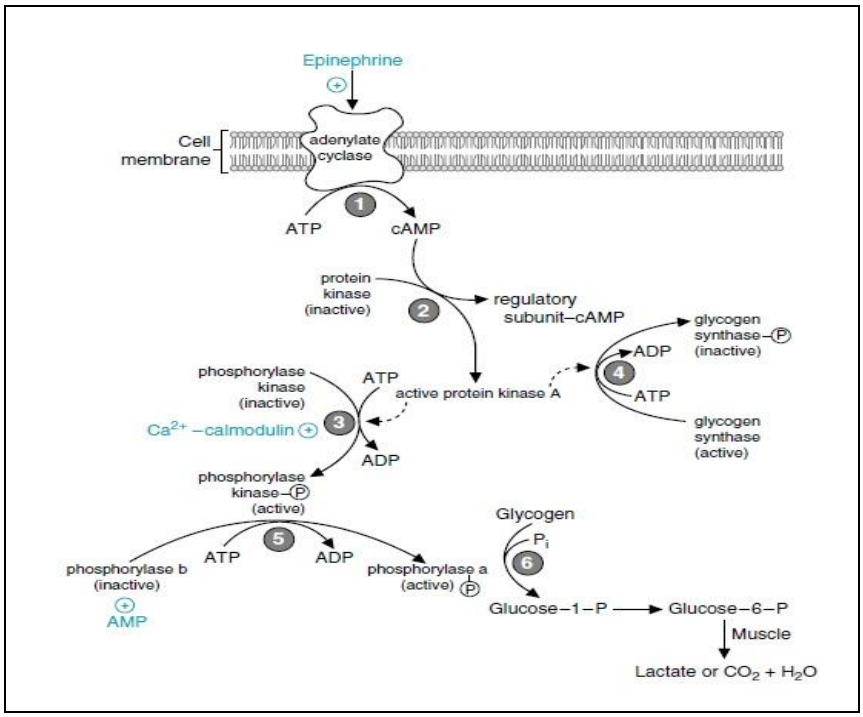

Figure 4. Stimulation of glycogenolysis in muscle by epinephrine (2, modified).

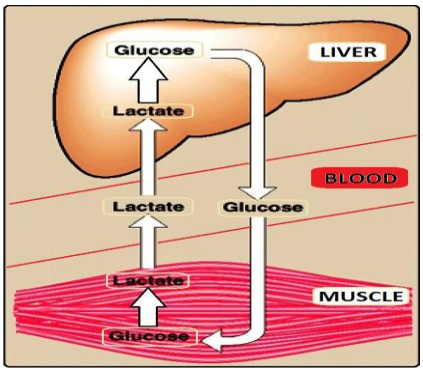

Figure 5. The Cori Cycle (3, modified).

The reaction catalyzed by lactate dehydrogenase will lead to pyruvate synthesis. Hence, pyruvate is converted to acetyl CoA which will enter the Krebs cycle producing energy through oxidative phosphorylation. Another possibility is that lactate, as an end product of the anaerobic glycolysis, to re-enter the Cori cycle (Figure 5) in the liver, where it is converted to glucose so that the body recovers a large amount of energy [4]. Mild or moderate PE is possible for a longer period of time than vigorous PE, due to aerobic degradation of glucose and FA, which produces more energy than anaerobic reactions, and also due to lactic acid production at a slower rate compared to anaerobic metabolism. Thus, the lactate production decreases if the energy is obtained

mainly through the aerobic metabolism of glucose and AG.

Particularities in patients with type 2 DM

Progression of DM may associate skeletal muscle atrophy, weakness, reduced neural activity and skeletal muscle hypoperfusion. Skeletal muscle atrophy in patients with type 2 DM is mediated by an ubiquitin proteasome system [12].

Diabetic neuropathy affects both the motor and the sensory nerves, with hypoxia and hyperglycemia being the main causes. Hypoxia is the result of reduction in capillary density on one hand and of the vascular luminal diameter on the other. Decreased muscle contractility can be explained by several phenomena: altered neuromuscular junction, altered muscle architecture, modified contractile properties, defective excitation-contraction coupling [12].

Obesity and type 2 DM are associated with increased levels of FA and TG, with reduced muscle oxidative capacity of the FA, leading to TG accumulation in the muscle [12].

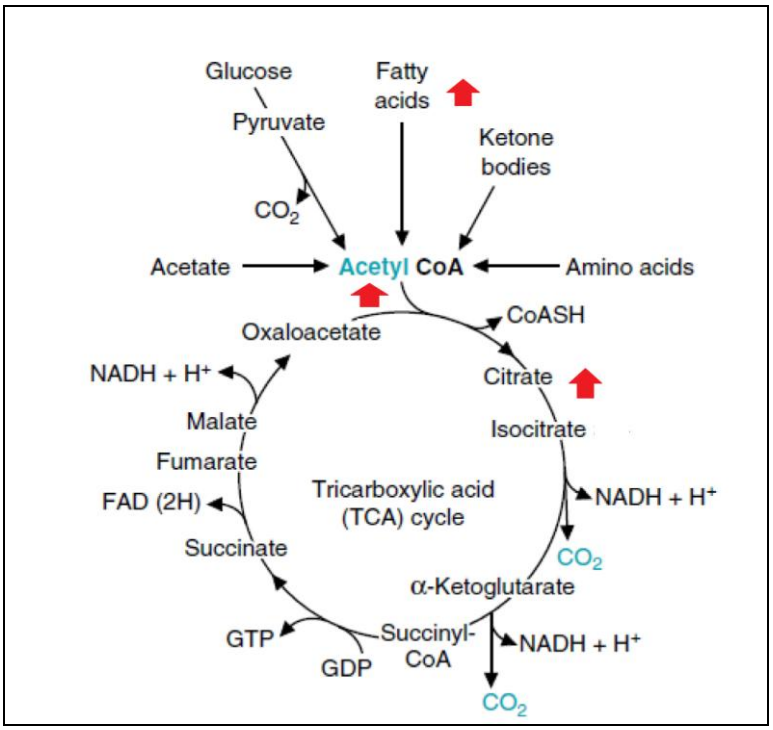

Figure 6. The TCA Cycle at patients with type 2 diabetes mellitus (2, *modified*).

Lipid accumulation in the muscle and liver is responsible for:

- poor insulin signaling;
- enzymatic equipment malfunction;
- insulin resistance development.

An accelerated FA oxidation leads to an increased amount of mitochondrial acetyl CoA, inhibition of pyruvate dehydrogenase activity and high level of cellular citrate, that inhibit phosphofructokinase resulting in decreased glycolysis with G-6-P upregulation and intracellular hexokinase II downregulation. Hexokinase II inhibition causes accumulation of glucose and reduced glucose uptake.

The insulin resistance induced by lipid accumulation decreases muscle glycogen synthesis due to reduction in insulin receptor

signaling and glucose transport. The increased level of products resulted from FA metabolism activates serine/threonine kinases responsible for phosphorylation of the insulin receptor substrate 1 (IRS-1) and insulin receptor substrate 2 (IRS-2), thereby altering insulin signaling through phosphatidyl-inositol-3-kinase (PI3K) pathway and glucose transport. Insulin-dependent stimulation of PI3K is altered in patients with type 2 DM.

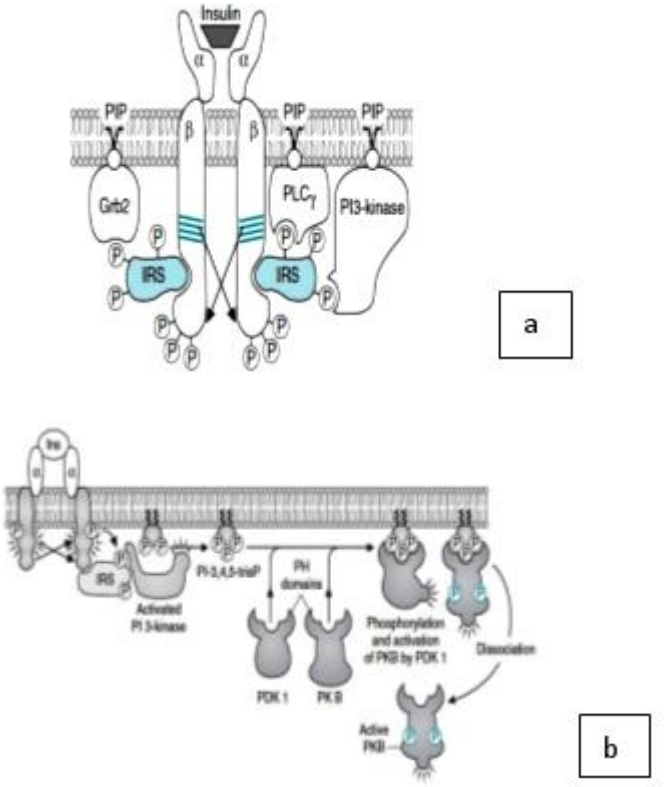

Figure 7. Insulin receptor signaling (a).
The insulin receptor- protein kinase B signaling pathway (b) (2- *modified*).
Ins – insulin; IRS – insulin receptor substrate; PDK 1 – Phosphoinositide-dependent protein kinase 1;
PKB – protein kinase B.

People with type 2 DM have normal levels of GLUT 4 in skeletal muscles, although insulin-dependent glucose uptake is defective. Glucose uptake and GLUT 4 translocation that depend on the PE are normal. Moderate PE is associated with 10 times increase in lipid oxidation due to rised energy costs and increased FA availability. Accelerated lipolysis in adipose tissue and reduced TG re-esterification occur under the action of catecholamines and insulin. It was also found the TG are an important source of energy in the muscle [5]. In patients with type 2 DM and obesity, FA metabolism is reduced during PE while intramuscular TG utilization is increased [5].

Characterstically, these patients have altered glycogen storage. Numerous studies have shown a 60% decrease in glycogen synthesis caused by alterations of glucose transport and hexokinase II activity and not by the compromised glycogen synthase action [12].

Insulin-independent glucose disposal is sustained by the lack of IRS-1and IRS-2 or PI3K phosphorylation in response to muscle

contraction, explaining thus the increased glucose use as a response to the PE in patients with type 2 DM and IR [5]. Increased insulin action in response to PE is explained by the following mechanisms: increased blood flow with increased capillary surface and insulin bioavailability and also postreceptor signal transduction upregulation [5].

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