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Metabolic Exploration of Muscle Biopsy

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1. Introduction

It has been well established that in several chronic diseases such as in chronic obstructive pulmonary disease (COPD), diabetes, cancer or congestive heart failure patients, next to central dysfunctions, the patients develop some systemic consequences that can lead to peripheral muscle dysfunction. Some important clinical implications such as reduced exercise capacity, reduced quality of life and lower survival in these patients are related to changes in muscle structure (mass) and function (power and endurance) (Maltais et al., 1996; 1999; Mettauer et al., 2006). In chronic disease, peripheral perturbations generally include neurohormonal and inflammatory changes, microvascular dysfunction, endothelial abnormalities, tissue wasting, apoptosis and energetic imbalance in skeletal muscle cells, causing reduced exercise capacity. These multisystem abnormalities contribute to the progressive worsening of the disease, and ultimately, lead to premature death. Among the numerous skeletal muscle cell changes that occur in chronic disease, energetic dysfunction has received renewed attention in the last decade and is increasingly considered to be a possible unifying mechanism in the development of muscle failure (Mettauer et al., 2006).

The mitochondrial impairments seem to be central in the development of energy dysfunction. Indeed, morphometric analysis of vastus lateralis from chronic heart failure patients revealed a decreased volume density of the mitochondria and decreased surface of the cristae in proportion with the decrease in VO2peak (Drexler, 1992). The percentage of the mitochondria stained for cytochrome oxidase (COX) is also reduced but improves with training (Hambrecht et al., 1995). Muscle enzymatic analysis as citrate synthase activity was decreased (De Sousa et al., 2000; Mettauer et al., 2001; Williams et al., 2004). Mitochondrial dysfunction has also been implicated in the pathology of chronic metabolic disease characterized by insulin resistance such as obesity, type 2 diabetes mellitus, and aging (Johannsen & Ravussin, 2009). In some chronic diseases as heart failure, skeletal muscle abnormalities resemble those induced by physical deconditioning, but some features argue for a generalized metabolic myopathy.

These observations shown in several studies were obtained using various experimental techniques such as nuclear magnetic resonance (NMR) spectroscopy, measurement of mitochondrial function in situ or in vitro by oxygraphy, proteomics and genomics in human or animal models, which have all revealed muscular energetic perturbations. Indeed, mitochondria play a central role in hereditary mitochondrial diseases, ischemia reperfusion injury, heart failure, metabolic syndrome, neurodegenerative diseases and cancer. Thus, comprehension of mitochondrial function regulation is fundamental in order to enlarge the knowledge in the field of mitochondrial physiology, and above all in order to better

diagnose the implication of mitochondria in many diseases. Direct assessment of mitochondrial function by measuring coupled respiration and ATP synthesis provides more full information, and the study of oxidative phosphorylation in skeletal muscles is an important initial screening procedure for the potential presence of mitochondrial diseases. At the fundamental level, comprehension of the mechanisms governing mitochondrial function as well as mitochondrial biogenesis remain to be explored in details with more and more molecular and cellular approaches.

2. Different types of skeletal muscle are available

2.1 Different muscle types

In function of pathologies, clinical symptoms, experimental conditions or physical exercise type, the choice of skeletal muscle could be different. Deltoid and Vastus Lateralis muscles are the skeletal muscles classically explored. But muscular biopsy could also be carried out in Tibialis anterior and Gastrocnemius muscles. During chirurgical intervention, some other striated muscles such as pectoral, respiratory or backbone muscles could also be explored. For that, researchers need to obtain the informed consent from all patients and the study has to be approved by the institutional ethical review board.

2.2 Different muscle fibres

In function of muscle types, the composition and the properties of muscle fibres will be different. Indeed, an abundance of literature shows that human skeletal muscles are made of a mixed nature, depending on its function. Muscles are composed of various proportions of the three fibre types I, IIa and IIx (or IIb depending on the species), each having specific contractile and metabolic characteristics. Fibre type composition exhibits great plasticity that depends on activity, mechanical load, hormonal status and age (Baldwin et al., 1975; Schiaffino & Reggiani, 1996; Fluck & Hoppeler, 2003). Moreover, quantitative differences between muscles in terms of mitochondrial and capillary density, enzymatic profile and content of high-energy phosphates have been widely reported (Table 1, figure1).

	Skeletal muscle fibres			Cardiac fibres
fibers type	Slow-oxidative	Fast-oxidative	Fast-glycolytic	
	Type I	Type IIa	Type IIx, Type IIb	
Myosin ATPase activity	Low	High	High	Low
Speed of contraction	Slow	Fast	Fast	Slow
Resistance to fatigue	High	Intermediate	Low	Very high
Oxidative capacity	High	High	Low	Very high
Mitochondria density	High	High	Low	Very high
Myoglobin content	High	High	Low	Very high
Glycogen content	Low	Intermediate	High	Very low
Citrate synthase	High activity	High activity	Low activity	Very high activity
miCK	High activity	High activity	Low activity	Very high activity

Table 1. The different characteristics of muscle fibres. For the sake of simplicity fibre types have been separated in slow oxidative type I, fast oxidative type IIa and fast glycolytic type IIb. miCK: mitochondrial isoenzyme of creatine kinase. (Mettauer et al., 2006)
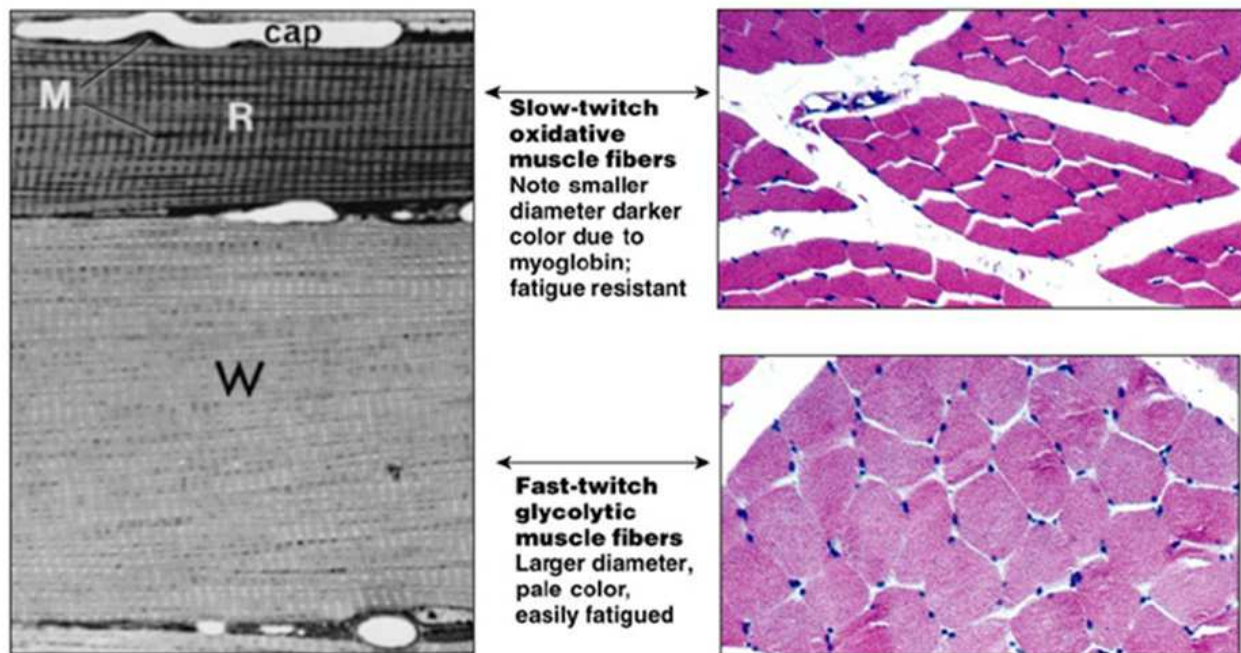
Fig. 1. Representative photographies to compare oxidative fibres to glycolytic fibres. On the left, there is a representative electron photomicrographs. On the right, muscles are stained with Haematoxylin-eosin (*source: Pearson education, Inc., publishing as Benjamin Cummings*).

Oxidative capacity of a given muscle can be linked to quantitative characteristics, especially mitochondrial and enzymatic contents. According to the relative importance of glycolysis and mitochondria, preferred substrate utilization can also vary from mainly carbohydrate to high-lipid utilization in highly oxidative muscles (Jackman & Willis, 1996; Ponsot et al., 2005).

Two families of muscles have been described in function of their metabolic activities. In oxidative muscles, composed essentially with slow oxidative fibres, the energy supplied continuously by mitochondria can sustain contractile activity for long periods of time without fatigue. By contrast, the glycolytic muscle composed with fast glycolytic fibres, has high levels of phosphocreatine and a high sensitivity to cytosolic ADP, which is, thus, the likely metabolic signal driving mitochondrial respiration (Mettauer et al., 2006).

Among these systems, there is the family of creatine kinase (CK) that catalyzes the reversible transfer of a phosphate moiety between creatine and adenosine diphosphate (ADP) (Mettauer et al., 2006). Four different isoforms of CK are expressed in a tissue specific and developmentally regulated manner. Among these isoforms, there is the mitochondrial isoenzyme (mi-CK), which is functionally coupled to oxidative phosphorylation and controls respiration in oxidative muscles (Wyss et al., 1992; Saks et al., 1994).

Mechanisms of high-energy phosphate transfer from mitochondria to local ATPases in the oxidative muscle fibres (type I skeletal muscle fibres, cardiac myocyte) can be described as "pay as you go" energy production, whereby, production is finely tuned to the needs of local ATPases within subcellular energetic units (figure 2).
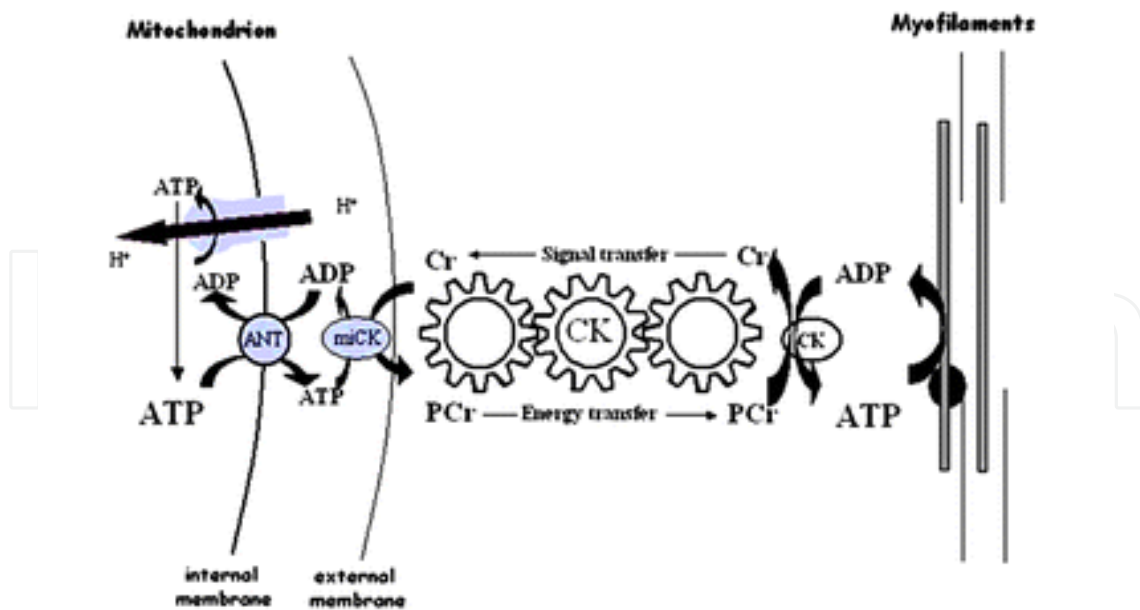
Fig. 2. The mechanisms of energy production in oxidative muscles, from. (Mettauer et al., 2006)

In glycolytic muscle fibres (type IIX/B), the mitochondria, together with glycolytic complexes, are concerned with replenishing intracellular phosphocreatine (PCr) stores which are immediately available for the ATPases-bound creatine kinases. This can be described as a "twitch now pay later" mode of operation (Kaasik et al., 2003; Ventura-Clapier et al., 2004; Mettauer et al., 2006)(figure 3).
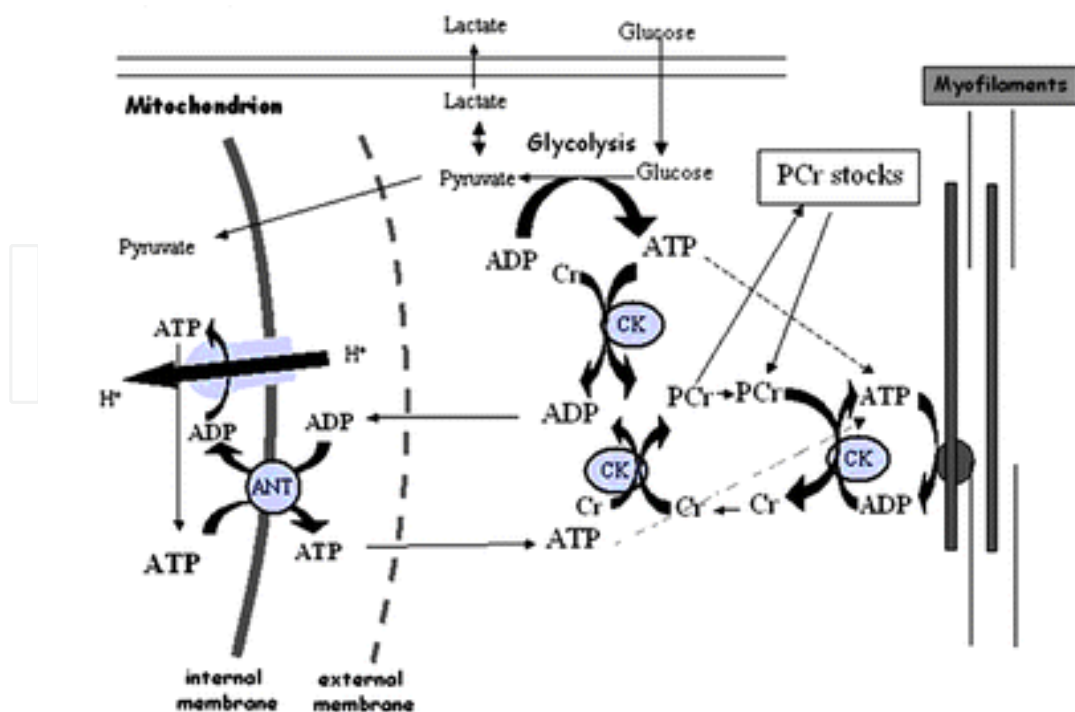

Fig. 3. The mechanisms of energy production in glycolytic muscles described before. (Mettauer et al., 2006)
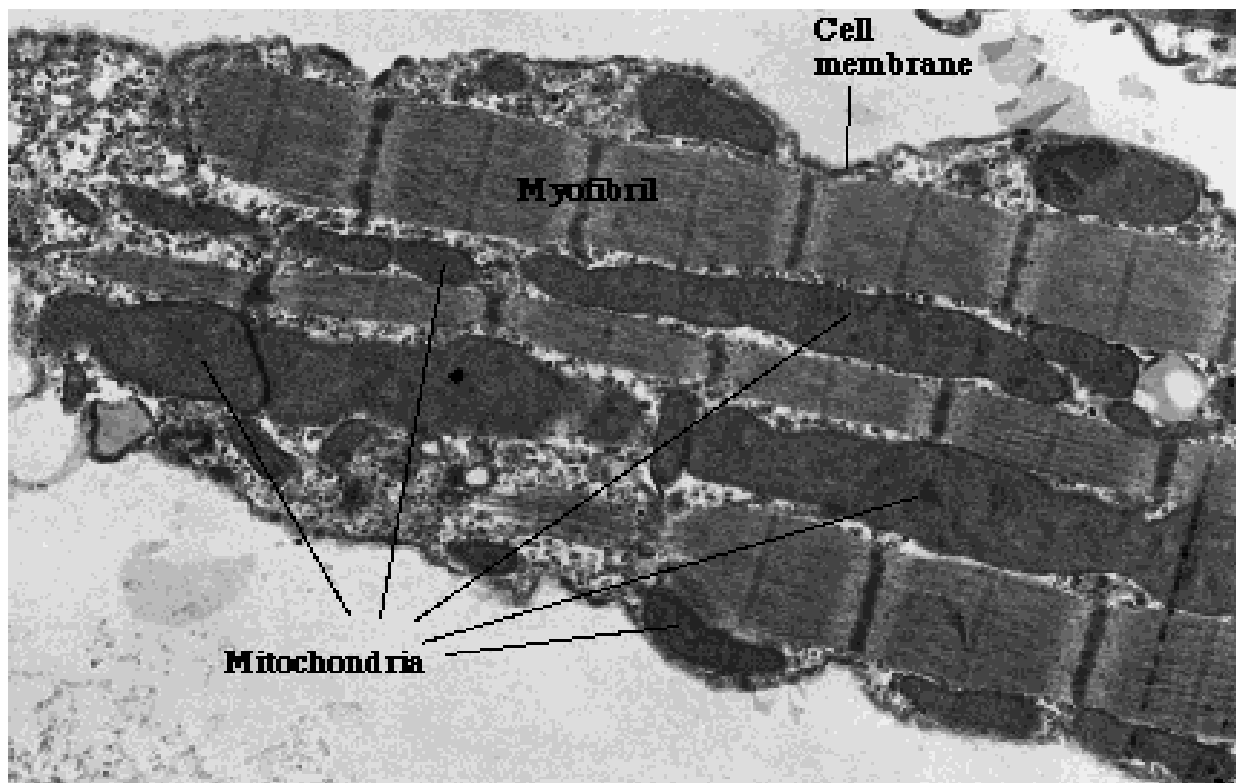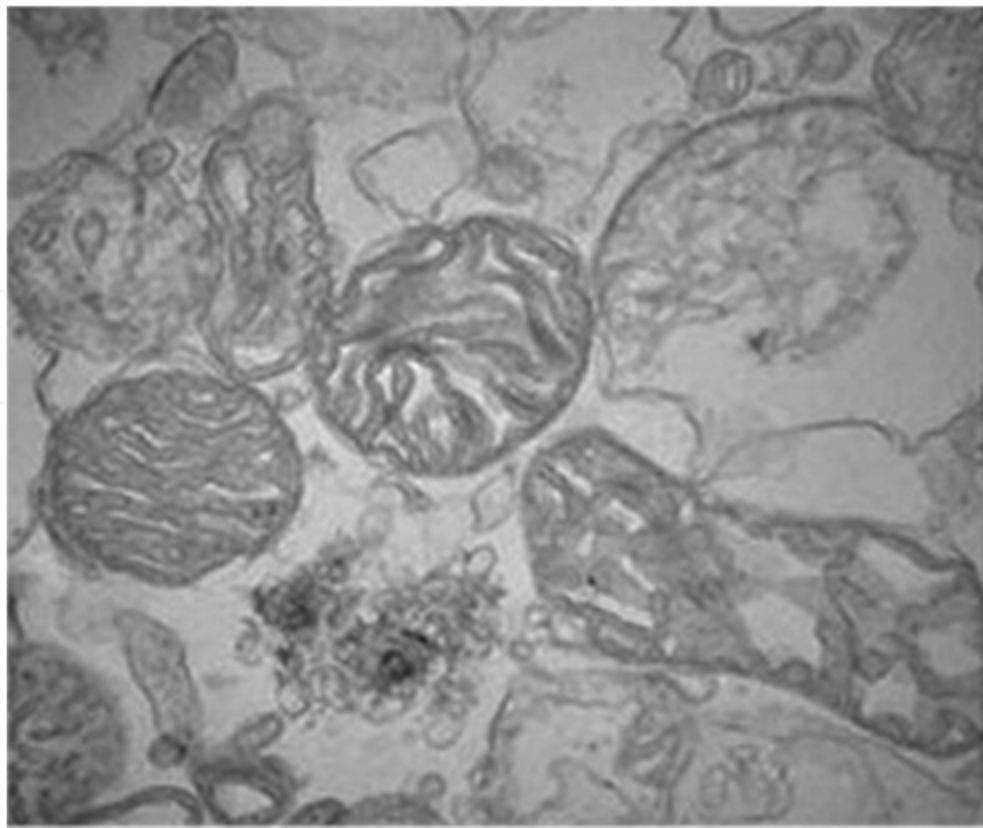
Fig. 4. Representative electron photomicrographs of mitochondrial ultrastructure in muscle fibres control (Mariappan et al., 2007). The second photo represents a muscle fibre with myofibrils and mitochondria (source: David R. Caprette, Rice University)

3. Mitochondrial analysis of muscle biopsy

3.1 Methods

Techniques and protocols of assessment of mitochondrial properties are of important physiological and physiopathological significance. 31P NMR spectroscopy has given the opportunity to study in vivo the intracellular metabolism under various conditions, including exercise in normal subjects and patients. However this approach did not reveal the intrinsic mitochondrial properties but rather the mitochondrial function under an uncontrolled intracellular medium. In the past, muscle mitochondrial properties were more closely explored either:

- By morphometric methods that give access to the mitochondria volume density and surface of cristae. The technique used the transmission electronic microscopy (figure 4) (Veksler et al., 1987),
- By biochemical methods determining the activity of intramitochondrial enzymes like citrate synthase (CS) and cytochrome oxidase (Cox). Actually, we can measure, directly the enzymatic activity of citrate synthase, which can be a good marker for quantifying mitochondria. Moreover, there are more and more different enzyme immunoassays to detect the consequence of the activities of metabolic enzymes. In addition, the different complexes implicated in the mitochondrial respiration can be explored (Birch-Machin & Turnbull, 2001).
- By polarographic methods, measuring O2 uptake of isolated mitochondria. These studies, on isolated mitochondria, required large amounts of tissue (approximately 500 mg), above the yield of routine human biopsy technique (10–50 mg), although efforts have been made to improve their sensitivity. More than two decades ago, Veksler et al. (Veksler et al., 1987) reported a method to assess the mitochondrial function of animal as well as human muscles. This new technique was based on the selective permeabilization of the sarcolemma by a low concentration of saponin (Kuznetsov et al., 2008). This approach allows the analysis of mitochondria within an integrated cellular system, preserving essential interactions with the cytoskeleton (Saks et al., 1998; Milner et al., 2000), nucleus (Dzeja et al., 2002) and endoplasmic reticulum (Rizzuto et al., 1998; Csordas et al., 2006).

3.2 Mitochondrial respiration

Oxidative phosphorylation has to be studied in intact mitochondria, which can be achieved by measuring the oxygen consumption of isolated mitochondria or muscle fibres from a tissue.

The skinned muscle fibre technique is adapted by Veksler and Saks for cardiac muscle fibres and also for skeletal muscle fibres (Veksler et al., 1987; Kuznetsov et al., 2008). This approach applies the ability of several chemical agents to specifically interact with the cholesterol in plasma membranes of cells or muscle fibres. These agents, for example saponin, have a high affinity to cholesterol and thus preferentially interact with cholesterol from membranes. Since, plasma membranes contain more cholesterol than the membrane of endoplasmic reticulum (ER) as well as the mitochondrial outer and inner membranes (Comte et al., 1976; Kuznetsov et al., 2008), there are no lesions on the intracellular

membrane structures (mitochondria and ER) (Saks et al., 1998). Importantly, functionally intact mitochondria, myofilaments or sarcoplasmic reticulum (SR) of permeabilized muscle fibres respond quickly to changes in concentrations of ions, adenine nucleotides, substrates, inhibitors (Kuznetsov et al., 2008). So the intracellular space of permeabilized muscle fibres is equilibrated with the external medium (figure 5) (Veksler et al., 1987; Kunz et al., 1993; Kuznetsov et al., 1997; Kuznetsov et al., 1998; Kuznetsov et al., 2004), and mitochondria are able to use substrates added in the extracellular medium.
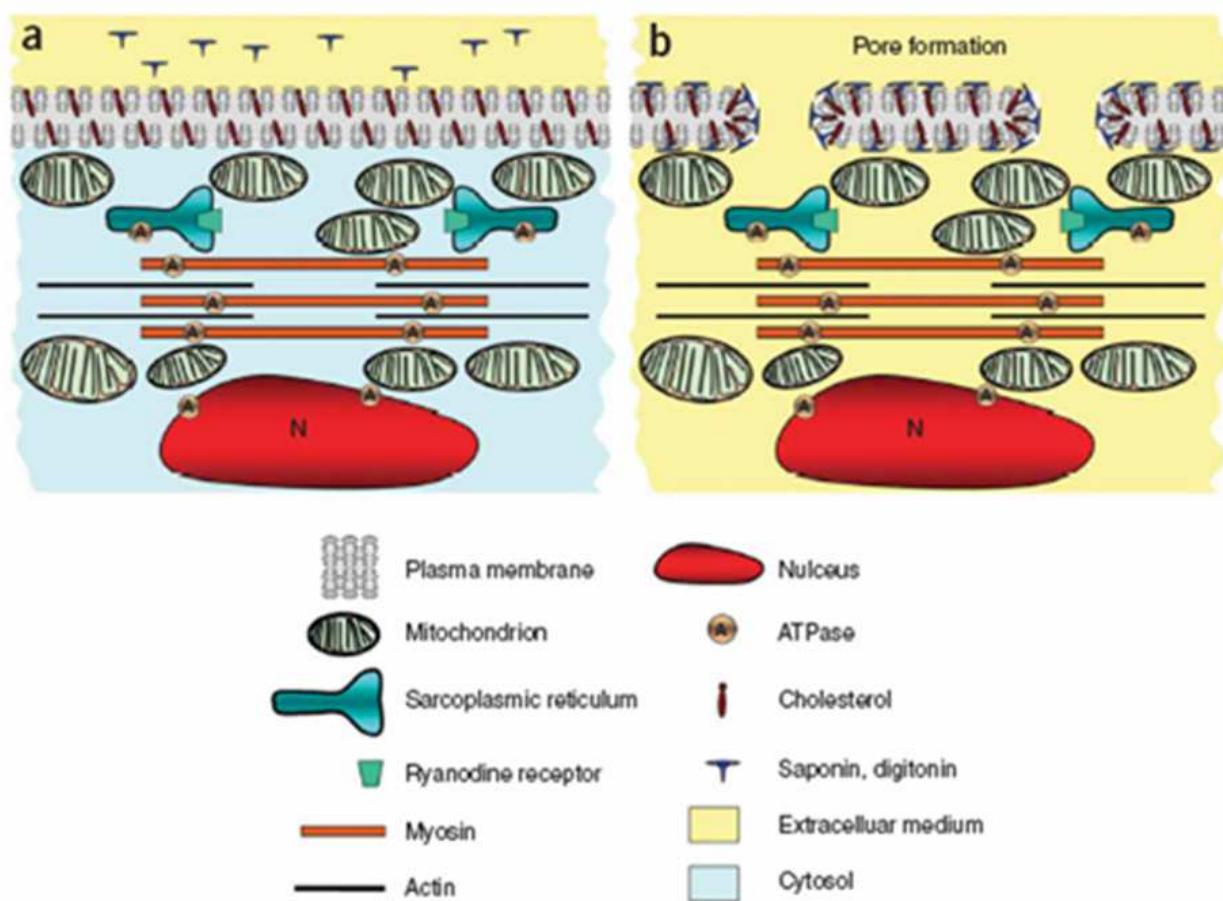

Fig. 5. Scheme explaining the effect of saponin on muscle fibres. Panel A represents the intracellular compartiment, with mitochondria, sarcoplasmic reticulum, nucleus (N). Panel B, when saponin acted; It appears a loss of plasmic membrane integrity. The mitochondrial sarcoplasmic membranes stayed intact. (Kuznetsov et al., 2008)

The addition of substrates allows us to analyze separately the different complexes I, II, III and IV of the mitochondrial respiratory chain. The measure consists in measuring the oxygen consumption polarographically with a Clark-type electrode. The substrates used are in function of the complex activity observed. The experiment started, all the time, by a measure of the basal respiration in non-phosphorylated condition but in the presence of glutamate-malate substrate, and is named V0. Then, for observing the maximal mitochondrial respiration by activating complexes I, III and IV, the addition of ADP (in

saturating concentration) is sufficient, and the respiration rate is named Vmax. The next step is to activate the complex II in adding amytal or rotenone, (specific inhibitors of complex I), followed by the addition of succinate, this respiration rate is named Vsucc. The complex III can be inhibited by the addition of antimycin A. Finally, to measure the specific activity of complex IV, N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD) and ascorbate were added as an artificial electron donor to complex IV, the mitochondrial rate is named VTMPD/asc (Charles et al., 2011.; Kuznetsov et al., 2008)(figure 6).
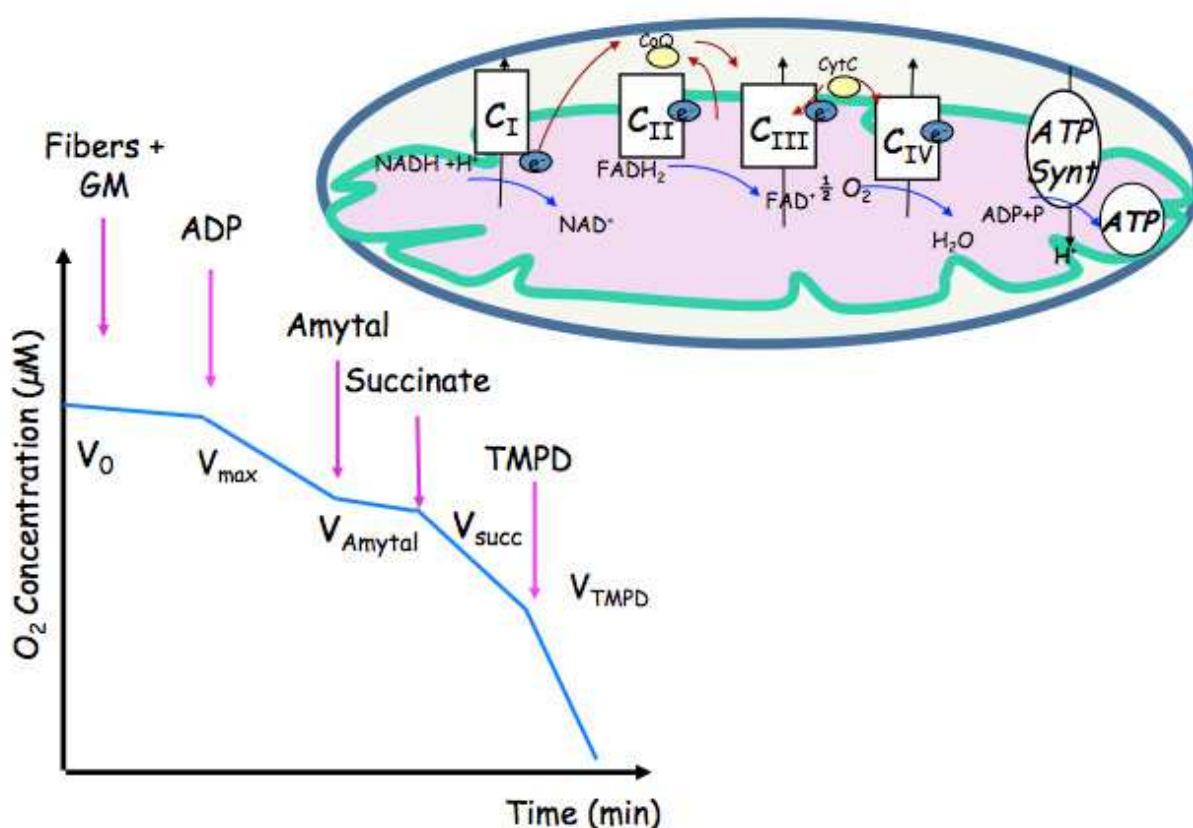

Fig. 6. Mitochondrial respiratory chain complexes activities using saponin skinned fibres. GM : glutamate/ malate. C_I: complex I; C_{II}: complex II; C_{III}: complex III ; C_{IV}: complex IV ; ATP Synt : ATP synthase ; cytC : cytochrome c; CoQ : coenzyme Q.

It is important to indicate that substrate utilization differ among muscle types (Baldwin et al., 1972; Holloszy & Booth, 1976; Jackman & Willis, 1996; Dyck et al., 1997). This means that muscle tissue has developed specific adaptations in terms of respiration control and intracellular energy distribution depending on its specific needs (Saks et al., 2001).

The Glycerol-3-Phosphate (G3-P) has a key role in the transfer of reducing equivalents from the cytosol to the mitochondrial matrix. This substrate is more used by the glycolytic muscles (Jackman & Willis, 1996).

Pyruvate is the substrate preferentially oxidized by all the different muscles (Ponsot et al., 2005). While the fatty acids like palmitoyl-carnitin are predominantly used by the cardiac and more generally by the oxidative skeletal muscles (Ponsot et al., 2005). The mechanisms of these different substrates are summarized in the figure 7.
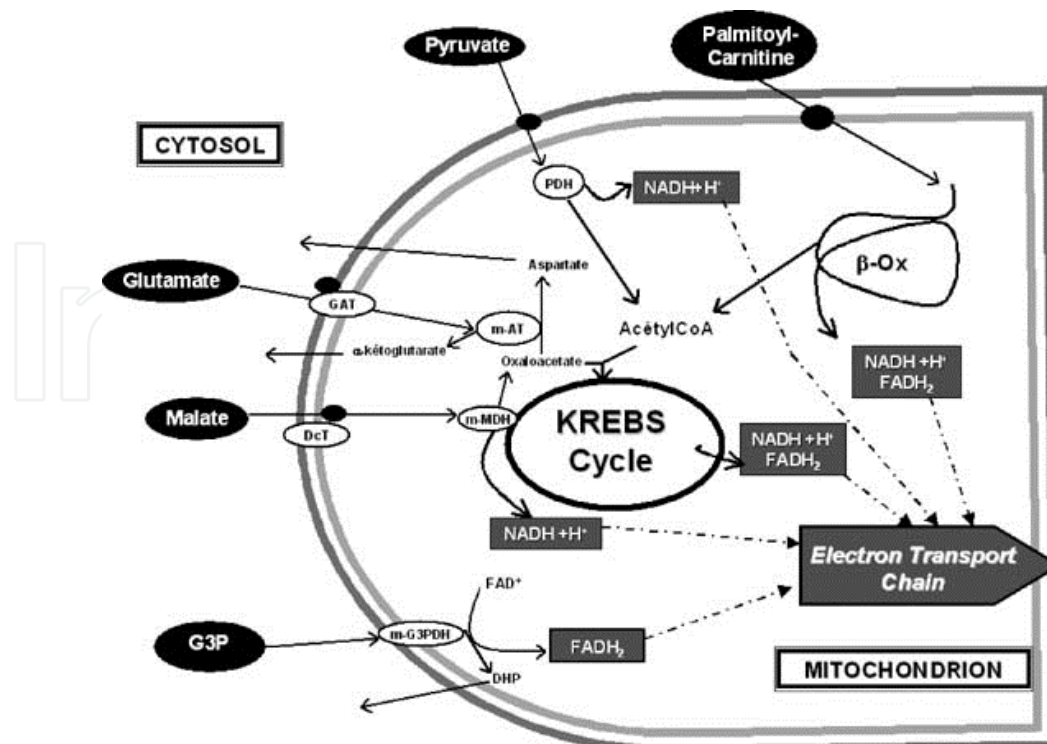
Fig. 7. The intervention of different substrates depending on their transport mechanism
(Ponsot et al., 2005).
(1) pyruvate (Pyr), which activates the pyruvate dehydrogenase complex (PDH) localized in
the mitochondrial matrix; (2) palmitoyl-carnitine (Palm-C), which is transferred into the
matrix by the inner membrane-localized carnitine translocase (CT) and carnitine palmitoyl
transferase II (CPTII), and activates the b-oxidation (b-ox); (3) G3-P, which diffuses to the
intermembrane space and activates the mitochondrial G3-P dehydrogenase; (4) lactate
(Lact), which could be converted into Pyr by mitochondrial LDH if it is present and
functional. dicarboxylate translocase (DcT); dihydroxyacetone- phosphate (DHP); glutamate
aspartate translocase (GAT); glycerol-3-phosphate (G3P); mitochondrial aspartate
transaminase (m-AT); mitochondrial glycerol-3-phosphate dehydrogenase (m-G3PDH);
mitochondrial malate dehydrogenase (m-MDH); pyruvate dehydrogenase complex (PDH).

3.3 Adenosine-5'-triphosphate (ATP) production

3.3.1 Definition

Adenosine-5'-triphosphate (ATP) is a multifunctional nucleotide used in cells as a
coenzyme. ATP transports chemical energy within cells for metabolism. It is produced from
Adenosine-5'-triphosphate (ADP) during glycolysis and the oxidative phosphorylation via
the mitochondrial electron transport chain, which is the principal source of ATP in aerobic
condition in mammals. ATP is used by enzymes and structural proteins in many cellular
processes. It is used as a substrate in signal transduction pathways by kinases that
phosphorylate proteins and lipids, as well as by Adenylate cyclase, which uses ATP to
produce the second messenger molecule cyclic AMP. Apart from its roles in energy
metabolism and signaling, ATP is also incorporated into nucleic acids by polymerases in the
processes of DNA replication and transcription.

In muscle, it plays a crucial role for the contraction. Indeed, ATP is the direct energy source for muscle contraction (Rayment et al., 1993).

3.3.2 The interest of ATP measurement

In living cells, the distribution of ATP is ubiquitous, and is lost rapidly in dead cells. It is an appropriate marker for cell viability (Petty et al., 1995). Moreover ATP is extracted and measured easily.

3.3.3 Methods

Several methods exist allowing to quantify ATP concentration in myocytes:

-   High-performance liquid chromatography (HPLC) with phosphate buffer as the mobile phase and UV detection (Lazzarino et al., 2003),
-   Ion exchange chromatography, also with UV detection (Ally & Park, 1992; Maguire et al., 1992).
-   However, the firefly luciferin-luciferase bioluminescence method is the most rapid, sensitive, and reproducible assay.

The bioluminescence assay is based on the reaction of ATP with recombinant firefly luciferase and its substrate luciferin. The stabilities of the reaction mixture as well as relevant ATP standards were quantified (Wibom & Hultman, 1990; Wibom et al., 1990).

It is a reagent based upon firefly luciferase, which emits light proportional to the ATP concentration.

The production of light is caused by the reaction of ATP with added luciferase and D-luciferin. This is illustrated in the following reaction scheme:

$$\text{ATP + D-Luciferin + O2} \xrightarrow[\text{Mg2+}]{\text{LUCIFERASE}} \text{Oxyluciferin + AMP + PPi + CO2 + Light}$$
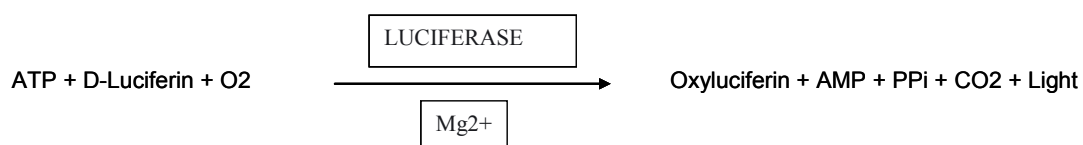
So, the ATP measurement permits to explain the importance of understanding the energy capacity of mitochondria in biology, physiology, cellular dysfunction, and ultimately, disease pathologies and aging (Drew & Leeuwenburgh, 2003; Aas et al., 2010).

3.4 Uncoupling of mitochondria

To phosphorylate ADP into ATP, the mitochondrion uses a coupling of oxidative phosphorylation across the mitochondrial inner membrane. But there is a phenomenon called mild uncoupling which allows the return of protons into the matrix without ATP production. This proton leak lowers the membrane potential across the inner membrane and increases the mitochondrial respiration rate (Brand, 1990). This leak goes by the uncoupling proteins (UCP). Discovered in 1978 (Nicholls et al., 1978), the first one, UCP1, localized in brown adipose tissue, is involved in cold-induced thermogenesis. The role of the other UCP, principally UCP2 (expressed ubiquitously) and UCP3 (expressed almost exclusively in skeletal muscle), is more controversial. As they are activated in extreme conditions (fasting, intensive exercise, high fat diet…) they could be a protective mechanism against oxidative

stress. Indeed, when uncoupling is activated, mitochondrial respiration has to increase in order to maintain the membrane potential and ATP production. It seems that this mechanism reduces mitochondrial ROS production (Starkov, 1997).

Visualization of this phenomenon is indirect in vivo (calorimetric approaches), and only observed in vitro in specific conditions. The proton leak is shown from the measurement of the membrane potential together with the respiration rate (in non-phosphorylating state) (Brand, 1995; Cadenas et al., 2002).

3.5 ROS production

Reactive oxygen species (ROS) are involved in the regulation of many physiological processes. However, overproduction of ROS under various cellular stresses results in cell death and organ injury and thus contributes to a broad spectrum of diseases and pathological conditions. ROS are formed preferentially in mitochondria also under normal conditions and may participate in many signaling and regulation pathways. However, under various cell stresses, such as ischemia–reperfusion, hypoxia–reoxygenation, and treatment with toxic agents, mitochondrial ROS are produced in excess and are rapidly released into cytoplasm, where they may have damaging effects, leading to oxidative stress and cell injury. Different methods exist allowing to characterise ROS formation at the level of muscle tissue (see the chapter from Lejay et al. for much more explanations and the description of the methods).

3.6 Mitochondrial biogenesis and genes expression

Gene expression profiling is considered as a key technology for understanding the biology of tissue plasticity as well as pathological disorders. A growing body of evidence is accumulating that implies muscular gene expressional alterations to be involved to a significant extent in the unique response of cells and tissues to external stressors. Transcriptional profiling evolves as a powerful tool to explore the molecular mechanisms underlying such adaptation. Real time RT-PCR (reverse transcription-polymerase chain reation) is the basic but efficient technique allowing to explore mitochondrial gene expression in muscle.

Advances in molecular biology have started to elucidate the transcriptional events governing mitochondrial biogenesis. Peroxisome proliferator-activated receptor gamma co-activator (PGC-1α) is considered to be the major regulator of mitochondrial biogenesis (Ventura-Clapier et al., 2008). Mitochondrial biogenesis can be defined as the growth and division of pre-existing mitochondria. According to the accepted endosymbiotic theory, mitochondria are the direct descendants of a-proteobacteria endosymbiont that became established in a host cell. Due to their ancient bacterial origin, mitochondria have their own genome and a capacity for auto-replication. Mitochondrial proteins are encoded by the nuclear and the mitochondrial genomes. The double-strand circular mitochondrial DNA (mtDNA) is ≈16.5 kb in vertebrates and contains 37 genes encoding 13 subunits of the electron transport chain (ETC) complexes I, III, IV, and V, 22 transfer RNAs, and 2 ribosomal RNAs necessary for the translation. Correct mitochondrial biogenesis relies on the spatiotemporally coordinated synthesis and import of ≈1000 proteins encoded by the nuclear genome, of which some are assembled with proteins encoded by mitochondrial DNA within newly synthesized phospholipid membranes of the inner and outer mitochondrial membranes. All of these processes have to be tightly regulated in order to meet the tissue requirements. Mitochondrial biogenesis is triggered by environmental stresses such as exercise, cold exposure, caloric restriction, oxidative stress, cell division and

renewal, and differentiation. The biogenesis of mitochondria is accompanied by variations in mitochondrial size, number, and mass. The discovery that alterations in mitochondrial biogenesis contribute to some chronic pathologies have increased the interest of the scientific community in this process and its regulation (Ventura-Clapier et al., 2008). Mitochondrial biogenesis is induced as followed: Peroxisome proliferator-activated receptor gamma co-activator (PGC-1a) activates nuclear transcription factors (NTFs) leading to transcription of nuclear-encoded proteins and of the mitochondrial transcription factor Tfam. Tfam activates transcription and replication of the mitochondrial genome. Nuclear-encoded proteins are imported into mitochondria through the outer- (TOM) or inner (TIM) membrane transport machinery. Nuclear- and mitochondria-encoded subunits of the respiratory chain are then assembled. Mitochondria in the cells of most tissues are tubular, and dynamic changes in morphology are driven by fission, fusion, and translocation (Bereiter-Hahn, 1990). The ability to undergo fission/fusion enables mitochondria to divide and helps ensure proper organization of the mitochondrial network during biogenesis. Mitochondrial fission is driven by dynamin-related proteins (DRP1 and OPA1), while mitochondrial fusion is controlled by mitofusins (Mfn1 and 2) (figure 8).
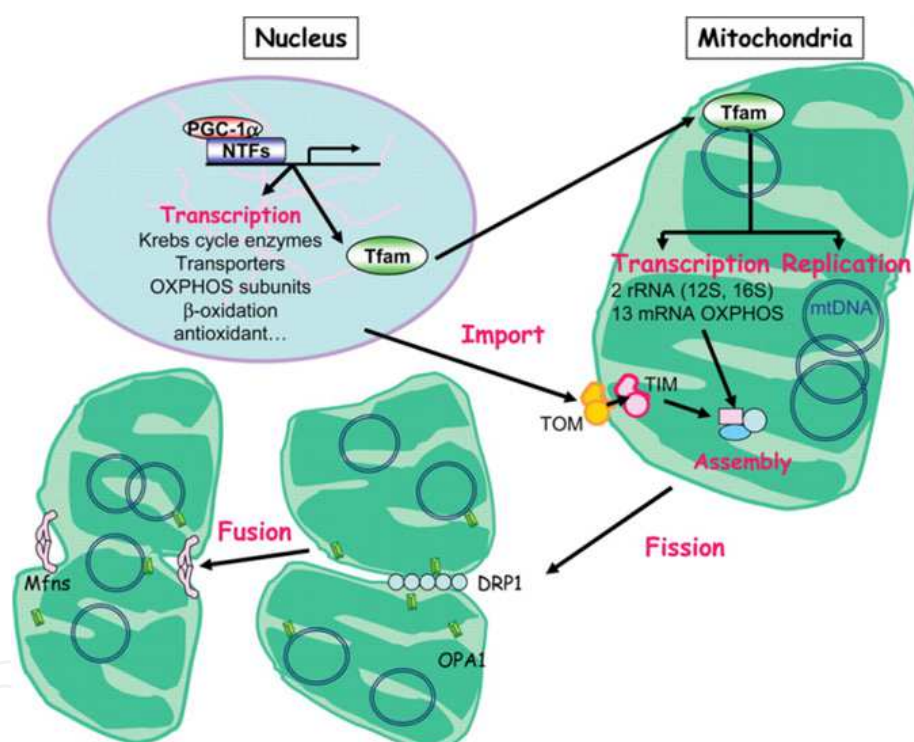

Fig. 8. Schematic representation of mitochondrial biogenesis. Peroxisome proliferator-activated receptor gamma co-activator (PGC-1α) activates nuclear transcription factors (NTFs) leading to transcription of nuclear- encoded proteins and of the mitochondrial transcription factor Tfam. Tfam activates transcription and replication of the mitochondrial genome. Nuclear-encoded proteins are imported into mitochondria through the outer- (TOM) or inner (TIM) membrane transport machinery. Nuclear- and mitochondria-encoded subunits of the respiratory chain are then assembled. Mitochondrial fission through the dynamin-related protein 1 (DRP1) for the outer membrane and OPA1 for the inner membrane of mitochondria allow mitochondrial division while mitofusins (Mfn) control mitochondrial fusion. Processes of fusion/fission lead to proper organization of the mitochondrial network. OXPHOS: oxidative phosphorylation (Ventura-Clapier *et al.*, 2008).

Measurement of mRNA expression of all theses proteins by RT-PCR technique could be a good means in order to show activation or deactivation of the mechanisms of mitochondrial biogenesis as well as mitochondrial fission/fusion. For details see the review of Ventura-Clapier et al (Ventura-Clapier et al., 2008).

In skeletal muscles, the consequences of a dysregulation of the mitochondrial biogenesis mechanisms could induce some important energetic changes including:

- a reduction of oxidative capacity and energy production;
- a decrease of energy transfer by the phosphotransfer kinases,
- a reduction of antioxidant buffering capacity;
- a global decrease of energy consumption efficiency. On the other hand, the signalling and molecular origins of these defects are unknown.

4. Analysis of muscle biopsy for detection of mitochondrial defects

Metabolic myopathies are inborn errors of metabolism that result in impaired energy production due to defects in glycogen, lipid, mitochondria, and possibly adenine nucleotide metabolism. Mitochondrial myopathies, fatty acid oxidation defects, and glycogen storage disease represent the three main groups of disorders (Burr et al., 2008; van Adel & Tarnopolsky, 2009). The mitochondrial myopathies manifest predominantly during endurance-type activity, under fasted or other metabolically stressful conditions. The clinical examination is often normal, and testing requires various combinations of exercise stress testing, serum creatine kinase activity and lactate concentration determination, urine organic acids, muscle biopsy, neuroimaging, and specific genetic testing for the diagnosis of a specific metabolic myopathy. Mitochondrial diseases are often disorders caused by an impairment of the mitochondrial respiratory chain function. They are usually progressive, isolated or multi-system diseases and have variable times of onset. Because mitochondria have their own DNA (mtDNA), mitochondrial diseases can be caused by mutations in both mtDNA and nuclear DNA (nDNA). The complexity of genetic control of mitochondrial function is in part responsible for the intra- and inter-familiar clinical heterogeneity of this class of diseases (Scarpelli et al., 2010).

Many forms of mitochondrial defects require a muscle biopsy to determine if any impairment exists. Unfortunately, not all mitochondrial defects are fully known, and so cannot be tested. Therefore, the detection of ragged red fibres by histological technique is looked for indications of a mitochondrial defect. A high lactic acid level is also often an indication. Moreover if a patient has three or more body systems affected, (for example circulatory, respiratory, and digestive systems), there is a suspicion of mitochondrial defect.

5. Skeletal muscle responses to metabolic and mechanical stimulations (i.e. physical exercise)

The introduction of the skeletal muscle biopsy procedure in the 1860's (Duchennes, 1864) has led to a tremendous step forward in our understanding of skeletal muscle physiology in humans. One of the fields that has most benefited from this new technique is the area of exercise physiology. There are major advances in the cellular and molecular mechanisms underlying the skeletal muscle responses to acute and chronic exercise, either with or

without the combined effect of additional environmental stressors such as altitude or hypo/hyperthermia. Then, the skeletal muscle biopsy is considered as a masterpiece in the ongoing development of the integrative approach of exercise physiology. This links the molecular and cellular events occurring in individual skeletal muscle fibres to cellular, tissue and whole body structures and functions. Thanks to the insights provided by skeletal muscle biopsies, skeletal muscle plasticity to exercise training is currently believed to be driven by metabolic (i.e increased energy demand) or mechanical (i.e increased muscle tension) stimuli generated during the training sessions (Coyle, 2000; Dufour et al., 2007). The paragraphs below present some examples of scientific advances obtained through the use of skeletal muscle biopsies in the area of exercise physiology.

5.1 Skeletal muscle responses to metabolic stimulation

One way to selectively increase the metabolic stimulation on skeletal muscle is to compare normoxic vs hypoxic exercise training (Dufour, 2005). The lowered partial pressure for oxygen (PO2) in the inspired air translates into lowered muscle intracellular PO2 (Richardson et al., 1995), thereby triggering skeletal muscle adaptations to cope with the enhanced metabolic load. After 6 weeks of intermittent hypoxic vs normoxic treadmill training, our laboratory has shown specific and significant improvement in whole body aerobic performance capacity in endurance athletes (VO2max, time to exhaustion,...) (Dufour et al., 2006). Using biopsies of the vastus lateralis, we observed that the enhanced performance capacity was concomitant to an improved skeletal muscle mitochondrial function (Ponsot et al., 2006) and an up-regulated transcription of selected genes involved in oxygen sensing, mitochondrial biogenesis, mitochondrial metabolism, carbohydrate metabolism, pH regulation and oxidative stress (Zoll et al., 2006a). In this series of studies, muscle biopsies proved useful in highlighting the role of metabolic stimulations in the regulation of the metabolic component of skeletal muscle plasticity to exercise training.

5.2 Skeletal muscle responses to mechanical stimulation

Similarly to the metabolic stimuli, it is also possible to selectively increase the mechanical stimuli generated during the training sessions using concentric vs eccentric cycle ergometry (Dufour, 2005). Eccentric muscle actions are characterized by high forces and low energy expenditure emphasizing muscle mechanical tension with very little energy demand (Lastayo et al., 1999; Lastayo et al., 2000; Lindstedt et al., 2001; LaStayo et al., 2003b). Currently taken as a promising tool to develop skeletal muscle force in order to improve performance in athletes (Gross et al., 2010), eccentric cycle ergometry is also increasingly considered as a valuable method to counteract the impairment of skeletal muscle function observed in various populations including elderly (Lastayo et al., 2002; LaStayo et al., 2003a; LaStayo et al., 2007), chronic obstructive pulmonary disease (Rooyackers et al., 2003), coronary artery disease (Steiner et al., 2004), type 2 diabetes mellitus (Marcus et al., 2008; Marcus et al., 2009), Parkinson disease (Dibble et al., 2006a; Dibble et al., 2006b; Dibble et al., 2009), multiple sclerosis patients (Hayes et al., 2011) and cancer survivors (Hansen et al., 2009; Lastayo et al., 2010; LaStayo et al., 2011). After 8 weeks of eccentric vs concentric cycle ergometry with coronary patients, significant improvement in knee extensor muscle force has been observed (Steiner et al., 2004). Biopsies of vastus lateralis demonstrated an increased volume of myofibrils, an increased proportion of type IIa muscle fibres and an

enhanced transcription of IGF-1 in the eccentric group (Zoll et al., 2006b). For the elderly patients (mean age = 80 yr old), eccentric cycle ergometry induced a greater gain in isometric strength of the knee extensors (Mueller et al., 2009), as compared with a conventional resistance training program. In this study, biopsies of vastus lateralis showed an enhanced expression of transcripts encoding factors involved in muscle growth, repair and remodeling (i.e. IGF-1, HGF, MYOG, MYH3) (Mueller et al., 2011). Of note, eccentric cycle ergometry was observed to depress genes encoding mitochondrial and metabolic transcripts. Taken together, the above experiments using muscle biopsies in human subjects show that mechanical stimulation of skeletal muscle trigger beneficial responses of the mechanical but not the metabolic component of skeletal muscle plasticity to exercise training.

5.3 Future developments in the muscle biopsy procedure

In human subjects, the withdrawal of muscle samples to perform biochemical, histochemical and histomorphometric muscle analyses has evolved from open air to semi open procedures (Henriksson, 1979), including "forceps" and the nowadays "gold standard" percutaneous Bergstrom needle procedure (Bergstrom, 1962). A suction system through the cutting trocard was introduced in 1982 in order to augment the size of the muscle tissue withdrawn at each insertion of the needle. These techniques do all need skin, subcutaneous and deep fascia anesthesia as well as a 5-10mm incision to access the muscle tissue with a 4 to 6 mm diameter Bergstrom needle (Hennessey et al., 1997). With these techniques, muscle samples of 77-170 mg can be obtained for each sample and doubling the sampling by rotating the needle 90° clockwise increased the size of the muscle sample to 172-271 mg in one pass (Hennessey et al., 1997). However, limitations exist for these procedures as their invasive character makes difficult the realization of serial sampling for studies examining the time course of intracellular physiological events (Hayot et al., 2005). Moreover, the procedure is sometimes difficult to get accepted by local ethics committee when applied to healthy normal subjects or athletes. Finally, some reservations should be made about the sterilization process and particularly the risk associated to Prion-contaminated medical instruments (sterilization of a hollow needle) (Weber & Rutala, 2002). As a less invasive alternative, microbiopsy procedures have been developed using fine disposable needles to obtain muscle samples in human subjects (Cote et al., 1992; Hayot et al., 2005). Although local anaesthesia is still required, skin incision is not always necessary. The skin is directly punctured with an insertion cannula perpendicular to the muscle until the fascia is pierced. The biopsy needle is subsequently inserted through the cannula and the muscle sample is obtained by the activation of a trigger button, which unloads the spring of the microbiopsy system and activates the needle to collect the muscle sample. Given the smaller size of the cannula and biopsy needles ranging from 11 to 18 gauges (i.e. 3.2 to 1.2 mm), the muscle samples obtained with microbiopsy procedures are much smaller. Despite the reduced muscle volumes, these developing microbiopsy procedures greatly facilitate serial muscle sampling either to increase the total size of the biopsy sample and/or to investigate the time course of intracellular physiological process of interest. An additional strength of the microbiopsy is that the procedure has been reported to be much more comfortable for the subjects and easier to perform compared to open air or percutaneous Bergstrom needle procedure (Cote et al., 1992; Hayot et al., 2005), allowing its wider use in the future of many areas of skeletal muscle physiology, including exercise physiology.

6. Conclusion

Exploration of energetic metabolism with skeletal muscle biopsy is central in order to characterise and to better understand the mitochondrial function and the mechanisms of cell death and pathophysiology of a variety of human diseases, including myopathies, neurodegenerative diseases, heart failure, diabetes and cancer. Indeed, clinical implications such as reduced exercise capacity, reduced quality of life are related to changes in muscle mitochondrial function. In the last decade, new experimental approaches with new biological techniques were applied to human biopsies allowing to help to diagnose several metabolic impairments in skeletal muscle. A lot of mitochondrial dysfunctions developed in chronic disease may be reversible, and then, improvement of the comprehension of mitochondrial physiology and pathophysiology could help to find new therapeutic avenues in the future.

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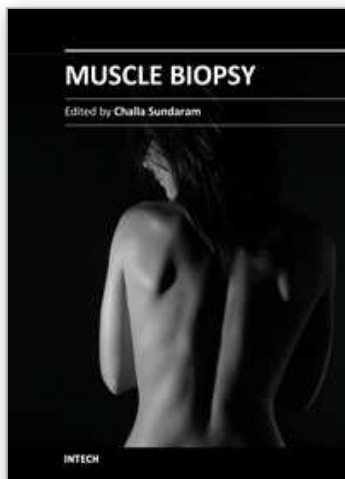
Muscle Biopsy

Edited by Dr. Challa Sundaram

Investigation of muscle diseases has changed dramatically with the understanding of genetic basis of a wide range of muscle diseases. Muscle biopsy has become a powerful tool not only to provide diagnosis but to make tissue available for genetic studies and to basic scientists for biomedical research. Accurate interpretation of muscle biopsy to detect cell dysfunction/ damage/death or absence / abnormality of a protein or genetic defect by the sophisticated technologies is important to guide treatment of various muscle diseases. In this book on muscle biopsy various chapters deal with the procedure and interpretation of muscle biopsy, its use in the culture of myotubes and membrane transport studies.Muscle biopsy is an important technique to investigate mitochondrial dysfunction and the mitochondrial DNA integrity in oxidation. Phosphorylation in various metabolic diseases like obesity, type 2 diabetes mellitus and peripheral vascular disease is explored in the other chapters with detailed descriptions on methodology. This book provides the advances in the basic techniques of muscle biopsy for a neuroscientist.

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