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Cellular ATP levels do not reflect mitochondrial defects associated with Barth syndrome

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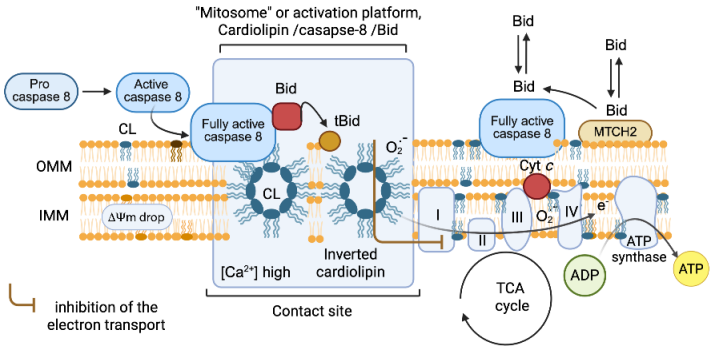
Summary



Barth syndrome (BTHS) is a rare X-linked genetic disease characterized by cardioskeletal myopathy, growth retardation, neutropenia, and abnormally high urinary 3-methylglutaconic acid. BTHS is known to result mutations in the TAFAZZIN gene that encodes an enzyme that is essential for the biosynthesis of mature cardiolipin, an inner membrane phosholipid required for several aspects of mitochondrial function. While defects in mitochondrial structure and metabolism have been widely reported in patient cells and TAFAZZIN-deficient animal models, levels of adenosine triphosphate (ATP) have been consistently found to be unchanged. This highlights the importance of evaluating deficiencies in mitochondrial function by multiple methods that are capable of capturing the many complex functions in regulating cellular metabolism, bioenergetics, ion and redox homeostasis and cell survival. Determining the mitochondrial defects responsible for BTHS has proved

challenging given the development of profound structural and functional changes that can affect a wide variety of celllar processes. For this reason, future BTHS research should focus on the impacts of non-matured cardiolipin and mono-lysocardiolipin in mitochondrial membranes and their interactions rather than solely on cellular bioenergetics or ATP perturbations. Comprehensive evaluation of bioenergetic status that includes accurate measurements of metabolic flux (e.g., respirometry with specific substrates), levels of multiple high energy phosphates (ATP, ADP, AMP, phosphocreatine), along with NADPH/NADP$^+$ and NADH/NAD$^+$ ratios will help to better integrate and understand how mitochodrial bioenergetic contributes to the pathogenesis of BTHS and may be modified to reveal new therapeutic strategies.

1. Introduction

Adenosine triphosphate (ATP) is widely known as the “energy currency“ produced by mitochondria, the “powerhouse“ of cells. Despite this, cellular levels of ATP are not necessarily a sensitive indicator of mitochondrial function (Dudek, Maack 2017) or dysfunction associated with most pathological conditions affecting cellular bioenergetics. Instead, it is more insightful to measure the metabolic flux of specific pathways (e.g., glycolysis and fatty acid oxidation), rates of oxidative metabolism and reactive oxygen species (ROS) production, and related metabolic perturbations, that may ultimately trigger the pathology, than an eventual change in ATP (Chatham, Young 2012; Nickel et al 2013). Nevertheless, changes in the size of the adenine nucleotide pool can sometimes be related to cellular bioenergetics through a slow cycle that can be stimulated by AMP deaminase, which converts adenosine monophosphate (AMP) to inosine 5′-monophosphate (IMP) depleting the adenine nucleotide pool, and increases in adenine nucleotide resynthesis, which restores it. As the ATP/ADP ratio decreases, the adenosine diphosphate (ADP) pool is consumed by adenylate kinase activity, producing AMP. If the depression persists, AMP deaminase degrades AMP to IMP, reducing the size of the adenine nucleotide pool. Conversely, when ATP/ADP is high, AMP degradation by AMP deaminase slows and the size of the pool may increase. It is impossible to determine whether an observed change in ATP levels reflects a change in mitochondrial function or an independent change in adenine nucleotide metabolism without further experimentation. Measurement of total cellular ATP is therefore a very poor indicator of mitochondrial dysfunction in the absence of these other measures, as discussed by Brault and Conway (2025) in a recent issue of the Journal of Translational Genetic and Genomic “What can ATP content tell us about Barth Syndrome muscle phenotypes?”.

2. Cardiac bioenergetics relies heavily upon mitochondria

Mitochondrial oxidative phosphorylation and, to a lesser extent, glycolysis, replenish the large amount of ATP needed for maintaining cardiac function. Because the energy demand of cardiomyocytes is constantly fluctuating, a complex network of enzymatic and signaling pathways is required to control the metabolic flow of substrates toward their oxidation in the mitochondria to provide the large amount of ATP needed.

The total amount of ATP synthesized in the heart per day is quite incredible, reaching an average of 6 kg (Yurista et al, 2021). The available energy is stored in various substrates (i.e., fatty acids, lactate, glucose, ketones, and amino acids) and converted into mechanical work. At least in the heart, up to 95 % of this energy demand is met by oxidative phosphorylation (Yurista et al, 2021). Oxidative fuel metabolism, particularly the mitochondrial tricarboxylic acid cycle (TCA) or Krebs cycle, produces NADH, which provide electrons to the mitochondrial respiratory system, and provides further electrons directly to enzymes that have its cofactor reduced to $FADH_2$. The complexes of the respiratory system ultimately transfer electrons from these reducing equivalents to molecular oxygen (O_2). Electron transfers carried out by respiratory complexes I, III, and IV are coupled with proton translocation across the mitochondrial inner membrane (mtIM), which generates a pH difference (ΔpH) that, together with the electrochemical potential ($\Delta\Psi_{mt}$), forms the proton motive force ($\Delta\tilde{\mu}_{H^+}$, pmF) used to produce ATP by the F_1F_oATP synthase.

In addition to their role in energy conversion, mitochondria play key roles in metabolism, such as the urea cycle, amino acid and lipid metabolism, and the biogenesis of heme and iron-sulfur clusters. Curiously, all of these biosynthetic pathways are energy dependent. More recently, mitochondria have been described as central to several signal transduction systems such as apoptosis, autophagy and aging.

3. Barth syndrome

Barth et al first described a family with an "X-linked syndrome involving myocardium, skeletal muscle, and neutrophils" in the early 1980s (Barth et al 1981; Barth et al 1983), which has been the subject of more recent review (Dudek, Maack 2017). A Dutch family with cardiomyopathy and high infant mortality due to infection or heart failure was described and is certainly related to the BTHS pathology. The so-called "Barth syndrome" (BTHS) is a rare X-linked genetic disease characterized by cardiomyopathy, skeletal myopathy, growth retardation, neutropenia, and abnormally high urinary 3-methylglutaconic acid excretion (Barth et al 1981; Barth et al 1983; Barth et al 2004; Saric et al 2015; Dudek, Maack 2017). Some authors (Saric et al 2015; Dudek, Maack 2017) also suggested that the pathology described by Neustein et al (1979) as "X-linked recessive cardiomyopathy with abnormal mitochondria" may be embedded in the same syndrome. Neustein et al (1979) reported

that "a trans-vascular endomyocardial biopsy from an infant with cardiomyopathy and chronic congestive heart failure showed abnormal mitochondria when examined by electron microscopy". At necropsy, similar abnormal mitochondria were found in skeletal muscle, liver, and kidney. Electron microscopy of cells and tissues from BTHS patients suggested that mitochondrial defects were central to disease pathogenesis (Barth et al 1983).

Barth syndrome is now known to be an inherited disorder linked to a defect in mitochondrial structure and function that weakens striated muscle tissue, leading to early onset cardioskeletal myopathy. More specifically, the “TAFAZZIN” trans-acylase mutated in BTHS, which is located at the mitochondrial membrane, appears to be essential in the maturation of cardiolipin (CL). CL is largely enriched at the mitochondrial inner membrane (mtIM) and at the mitochondrial contact sites between mitochondrial outer membrane (mtOM) and mtIM. CL is an essential component within the mtIM but where it binds and interacts with a large number of proteins, e.g., components of the respiratory system (Dudek, Maack 2017; Saric et al 2015). However, CL can also be localized to the mtOM, where it can interact with proteins from the cytoplasm that take part in broader cell signaling pathways (Gonzalvez et al 2005). The mechanisms that lead cells from normal CL to abnormal CL biogenesis to cardiomyopathy are not fully understood. There are many reasons for the crucial importance of CLs, which lie in their broad capabilities to interact with numerous molecules of importance (Pfeiffer et al 2003) at the mitochondrial level, but also towards cytoplasmic proteins that have landed on the mtOM surface (Gonzalvez et al 2013; Gonzalvez et al 2005; Gonzalvez et al 2008).

4. TAFAZZIN and cardiolipin defects in Barth Syndrome

Non-mature CLs (due to a defect in transacylation) are a key feature in BTHS, and are also central to ischemic heart disease and aging (Khuchua et al 2006; Saini-Chohan et al 2009). The processes could result in dysregulation or dysfunction associated to the mitochondrial compartment, including altered mitochondrial membrane shaping (Gawrisch 2012; Li et al 2015), protein import (Gebert et al 2009; Jiang et al 2000), oxidative phosphorylation (Bazan et al 2013; Pfeiffer et al 2003; Zhang et al 2002), fusion/fission mechanisms (Ban et al 2010; DeVay et al 2009; Joshi et al 2012), iron-sulfur cluster biogenesis (Patil, Greenberg 2013), apoptosis dysregulation (Chu et al 2014; Gonzalvez et al 2013; Heit et al 2011; Ikon et al 2015; Kim et al 2004; Li et al 2015; Manganelli et al 2015), autophagy (Chu et al 2014; Li et al 2015), and transport of protein precursors across the mitochondrial membrane (metabolites) (Brandner et al 2005; Gu et al 2004; Jiang et al 2000; Kadenbach et al 1982; Koshkin, Greenberg 2000; Noel, Pande 1986; Robinson 1993; Schlame et al 2000; Vaz et al 2003).

Ultrastructural abnormalities of mitochondria were noted in one of the first case reports describing BTHS (Barth et al 1983) (Figure 1) and have been associated with defective mitochondrial Complex III function (Barth et al 2004), but without alteration of Complex III binding (Gonzalvez et al 2013). In yeast, early reports suggested a defect in energy coupling and membrane stability (Xu et al 2003), while studies in patient-derived lymphoblasts showed abnormal proliferation, altered membrane potential, and normal ATP formation, suggesting partial uncoupling and compensatory expansion of the mitochondrial compartment binding (Gonzalvez et al 2013). Ultrastructural changes also depend on the model system and organ examined, and may be more pronounced in differentiated than in embryonic tissues. It has been hypothesized that this effect is greater in mitochondria with higher cristae stacking density (Acehan et al 2007), and since cardiac mitochondria have twice the diameter and a higher percentage of lamellated cristae (cristae increase surface area and allow for inner membrane cardiolipin assembly) than other organs, this may explain why BTHS patients predominantly exhibit cardiovascular defects (Acehan et al 2007).

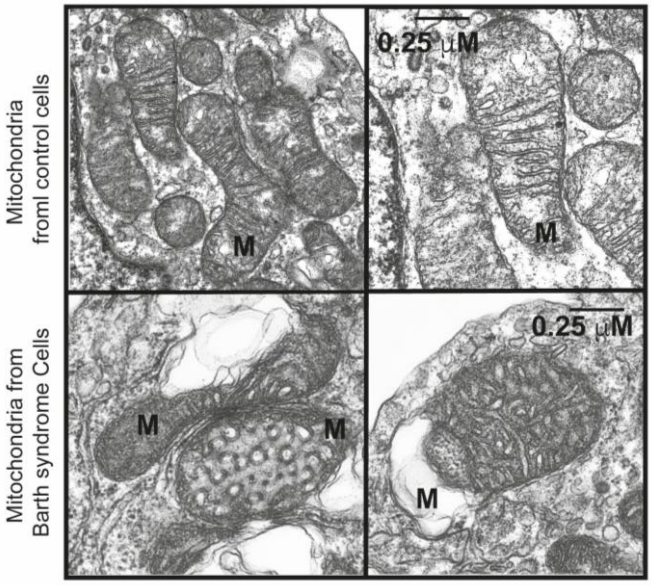


Figure 1 - Electron microscopy of BTHS lymphoblastic mitochondria. M: mitochondria; the scale bars values for the four panels are of 0.25 µM. Images are issued from partly unpublished work of Petit PX or from Gonzalvez et al, 2013, with permission of BBA.

It should be noted that the discovery of *TAFAZZIN* was issued from the identification of the genetic cause of Barth syndrome (Xu, Malhotra et al 2006). *TAFAZZIN* gene mutations were searched by bioinformatics and *TAFAZZIN* was recognized as an acyltransferase. Xu et al showed that TAFAZZIN affects lipids, especially the lipids of mitochondria (Barth et al 2004; Xu et al 2003). This discovery confirmed the central role of mitochondria in Barth syndrome, a notion that had already been suggested by analysis of human biopsies (McKenzie et al 2006), and clearly established the importance of lipids in the disease. CL is the lipid most drastically affected in Barth syndrome. Not only does the total amount of CL decrease (Acehan et al 2007; Barth et al 1983; Barth et al 2004; McKenzie et al 2006; Xu et al 2003), but there is also a profound change in its molecular species composition (McKenzie et al 2006). In addition, “immature” CL lacking one acyl chain known as mono-

lysocardiolipin (MLCL) accumulates to high levels, which is usually below the detection limit in healthy tissues.

Abnormal CL remodeling due to *TAFAZZIN* gene mutation leads to destabilization of mitochondrial inner membrane complexes in yeast (Gu et al 2004), disrupts respiratory super-complex formation in patient lymphoblasts (Acehan et al 2007), and also interferes with super-complex formation (Xu et al 2005). Irrespective of changes in cardiolipin profiles and destabilization/alteration of mitochondrial respiratory super-complexes, metabolic flux through the TCA cycle is hardly affected in patient skin fibroblasts (McKenzie et al 2006). In a *Drosophila* flight muscle model of BTHS, the density of F_1F_0 ATP synthase dimers in the mtIM was reduced because high curvature regions in the inner membrane were less represented and dimer rows were less elongated and more scattered (Xu, Condell et al 2006). In *TAFAZZIN* knockdown mice, the mitochondrial network showed a wide spectrum of mitochondrial abnormalities (Acehan et al 2011; Phoon et al 2012).

Both the morphologic defects (i.e., mixture of swollen, honeycomb, and widened/collapsed/absent cristae) (Figure 1) and the size of the mitochondrial network vary. Depending on the publication, there are reports of increased, decreased, and unchanged mitochondrial network, depending on the stage, organs, and technical setting used with doxycycline. A comprehensive bioenergetic and lipidomic characterization of these mice revealed differential substrate utilization and a reduction in Complex III and ATP synthase activities (Zhu et al 2021). An independent assessment of mitochondrial function in the same mouse strain (Acehan et al 2011) and in human iPSC cell-derived cardiomyocytes (iPSC-CMs) from BTHS patients revealed a tissue-specific reduction in Complex II succinate dehydrogenase activity (Wang et al 2014). A separate BTHS iPSC-CM study suggested a reduction in F_1F_o ATP synthase specific activity and an overall reduction in ATP in cells cultured in galactose (which abolishes ATP generation from glycolysis) (Wang et al 2014). Interestingly, basal oxygen consumption rate is increased in BTHS iPSC-CMs, likely due to compensatory mechanisms, but overall respiratory capacity is decreased (Wang et al 2014). TAZ-deficient mitochondria generate increased ROS in yeast (Gu et al 2004), in *TAFAZZIN*-knockdown mice (Zhu et al 2021) and in human iPSC-CMs (Wang et al 2014). All these data formed a network of information that built up a picture of the complexity of Barth syndrome and also of the landscape in which an altered bioenergetic operates far from the homeostatic situation of normal tissues (Figure 2).

5. Measurement of adenosine nucleotides

Levels of ATP along with its metabolites adenosine diphosphate (ADP) and adenosine monophosphate (AMP) indicate the energy state in living cells maintained by mitochondrial function (Desousa et al 2023; Doerrier et al 2018), as well as glycolysis and other subtrate-level phosphorylation reactions. These adenosine nucleotides are fundamental molecules that provide immediate chemical energy,

facilitate signal transduction, and provide metabolic precursors (Doerrier et al 2018). ATP measurement alone is far from sufficient to access the energetic state of cells and tissues in Barth syndrome, but methods that estimate the total cellular ATP production rate may be more insightful in this context.

Systems capable of measuring cellular oxygen consumption rate (OCR; change in media O_2/time) and extracellular acidification rate (ECAR; change in media pH/time) can provide data for estimating rates of ATP production from oxidative phosphorylation and glycolysis (Figure 3). For example, the Agilent SeahorseXF Analyzer (Agilent, Santa Clara, CA, USA) (Gu et al 2021), enables researchers to measure OCR and ECAR of plated cells (in separate experiments), which together allow for estimation of the total cellular ATP production rate (Divakaruni , Jastroch 2022; Mookerjee et al 2017; Pelletier et al 2014). The Oroboros Oxygraph O2k can be used to simultaneously monitor OCR and ECAR in cellular suspensions during the same experiment when equipped with pH ISE-Module (Oroboros Instruments, Innsbruck, Austria), (Doerrier et al 2018). The "Real-Time ATP rate assay" report generator software provided by Agilent calculates estimations ATP produced from glycolysis (glycoATP) and oxidative phosphorylation (oxphoATP) from ECAR and OCR data, respectively, which is commonly reported as "ATP production rates" in the literature (Desousa et al 2023; Mookerjee et al 2017). However, it is important to emphasize that ATP is not directly measured by either of these instruments, but only estimated by changes in media oxygen and pH in presence of sample with and without chemical inhibitors of OXPHOS (e.g., oligomycin) or glycolysis (e.g., 2-deoxyglucose). The accuracy of these estimations is limited by wide variation in sources of extracellular protons from depending on the sample assayed (Mookerjee, Brand 2015; Mookerjee et al 2015), which could have major implications for the validity

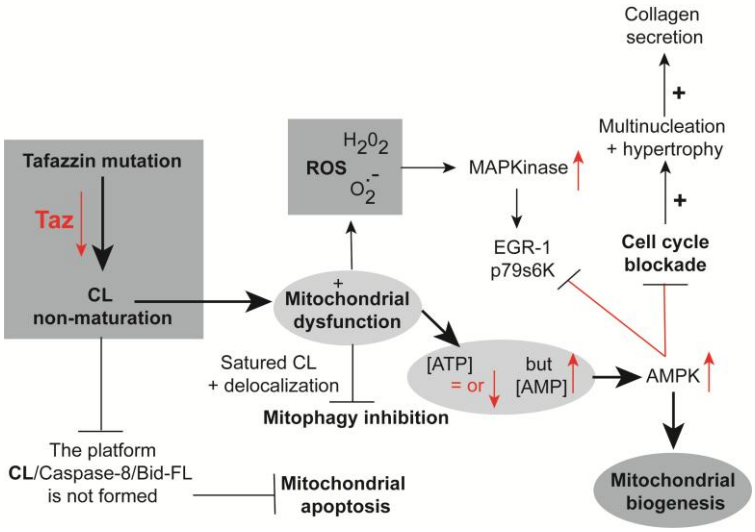


Figure 2 – Impacts of TAFAZZIN knockdown in neonatal cardiac fibroblasts). Knockdown of the TAFAZZIN gene in neonatal cardiac fibroblasts disrupts mitochondrial homeostasis through a variety of mechanisms. This scheme is mainly derived from the work of He et al (2010) and has been freely interpreted with permission of the American Journal of Physiology: Heart and Circulatory Physiology from the American Physiology Society, USA, as well as the work of Saric et al, 2015.

of using energy coupling factor (ECF) measurements to estimate ATP production rates across different cell types and conditions. More direct measurements of mitochondrial ATP production (more precisely, rates of sample ATP/ADP exchange) can be accomplished with the Mg-Green fluorescence assay during respirometry experiments using the Oroboros platform with the fluorometer module (Cardoso et al 2021). However, this platform is also incapable of directly monitoring rates of intracellular ATP production from specific metabolic pathways. Determining the flux of adenine nucleotides (ATP, ADP, and AMP) and its sources may provide valuable insight to the pathogenesis of BTHS, as these molecules not only supply immediate chemical energy, but also facilitate cellular signaling and serve as metabolic precursors for several anabolic pathways (Miller et al 2019). The cellular fate of these molecules is determined in part by the pathways that produce them, which can vary widely across cell types and conditions. For example, a cell producing ATP predominantly by oxidative phosphorylation (OXPHOS) might be described as having a 'preference' for ATP produced from OXPHOS compared to glycolysis (the ratio could be 95 % / 5 % in heart). However, if the contribution of ATP from glycolysis increases, this could reflect a defect in OXPHOS or a greater reliance upon glycolytic flux to meet other metabolic demands. While overall ATP supply and bioenergetic status may be unchanged, the cellular

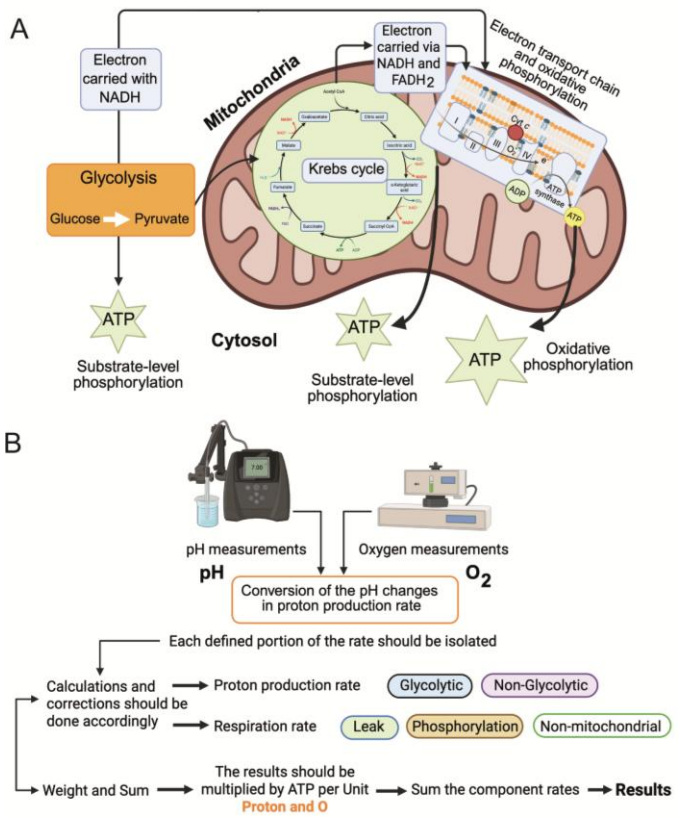


Figure 3 – Estimation of ATP production rates by extracellular Flux Measurements. (A) Schematic representation of the pathways leading to ATP production and their coupling to extracellular fluxes. **(B)** The pH measurements and oxygen consumption allows the calculation of both proton production and respiration rates. This ened into the calculation of the diffrents blocks, i.e., glycolytic or non-glycoliytic, of the leak, the phosphorylation and its non-mitochondrial counterpart. Some calculation are needed to finis the picture of the ATP production rates. The figure is freely adapted from Handel et al (2019), with permission of Trends Endocrinology & Metabolism, Elsevier B.V.

metabolic landscape may have changed significantly. Therefore, in systems where both glycolysis and oxidative phosphorylation are simultaneously at work, even indirect assessments of ATP production from one or the other pathways may provide important insight to the overall behavior of the metabolic network.

An important example of this comes from examining Crabtree's hypothesis (de Kok et al 2021; Handel et al 2019) concerning the return to a homeostatic situation when a certain amount of glycolytic ATP is injected into the system, in a context where ATP is normally supplied by oxidative phosphorylation (Figure 4). Metabolic reprogramming and mitochondrial dysfunction are central elements in a variety of physiological and pathological processes (Handel et al 2019) and, therefore, may contribute significalty to the pathogenesis of BTHS. While cell culture has established itself as a versatile technique for studying physiology and disease, the study of metabolism using standard cell culture protocols profoundly interferes with the Crabtree effect due to very high and variable media glucose concentration. Therefore, care should be taken in the design and interpretation of cell metabolism studies in BTHS regarding the substrates used to maintain cell cultures, those present in the assessment of metabolic flux (OCR and ECAR), and how each may impact the cellular phenotype in the context of TAFAZZIN deficiency.

6. Direct measurement of adenine nucleotides

Direct measurement of ATP levels is often accomplished by using assay kits based on bioluminescence or fluorescence detection. Kits for ADP and AMP nucleotides are less available and could become very expensive over time (at least for routine use). As described above, use of Extracellular Flux analyzers appeared and became quite popular for estimating rates of ATP production from glycolysis and OXPHOS in living cells (Desousa et al 2023). However, this technology is expensive, has limitations discussed above, and does not provide any insight to ADP and AMP levels.

Instrumental analytical techniques are available to quantify ADP and AMP, including chromatographic methods such as ion exchange, thin layer and High-Performance Liquid Chromatography (HPLC), which have high sensitivity and efficiency (Law et al 2022; Tullson et al 1990). Our efforts need to evolve towards more complex and sensitive measurements that include the simultaneous measurement of ATP, ADP and AMP, as well as NADPH, NADH and $NADP^+$ and NAD^+ (Gendron et al 2001). We agree with our colleagues that quantifying the energetic state of tissues or cells can present some difficulties. However, it is important to remember that the difficulties are not due to the lack of techniques to solve them. In fact, if there are difficulties, they lie in the rigor of the scientific and technical approach, and the

interpretation of the data collected. ATP is highly labile and susceptible to rapid degradation during sample collection and preparation for quantification.

ATP in muscle has been measured by a variety of assays, including magnetic resonance spectroscopy (MRS) (Hancock et al 2005), fluorescent reporters (Marvin et al 2024), luciferase assays (Stanley, Williams 1969; Yang et al 2002), and high- or ultra-performance liquid chromatography (HPLC or UPLC) on tissue (Law et al 2022; Tullson et al 1990). However, only HPLC with a solid calibration system can lead to the accurate quantification of ATP in tissues or cells (Tullson, Terjung 1990). However, this measurement alone is not sufficient because any approach to the energetic state of tissues or cells requires at least an analysis of ATP and ADP in order to access the measurement of the ADP/ATP ratio.

7. What else could it be if ATP production was not the main problem?

In this review, an integrative assessment of cellular bioenergetics that incorporates metabolic flux measurements with analysis of adenine nucleotide levels has been emphasized as an important approach for better understanding the pathogenesis of BTHS. However, is it important to also consider impacts of *TAFAZZIN* mutation and cardiolipin deficiencies on cellular functions beyond those affecting bioenergetic status. For example, under normal conditions, cardiolipin associates with several inner membrane proteins to optimize mitochondrial protein transport. We have shown that inhibition of cytosolic protein synthesis either by the use of cycloheximide (in the picomolar range) or more specific gene mutation restores oxidative phosphorylation in *taz1*Δ yeast and *TAFAZZIN*-deficient human cells (de Taffin de

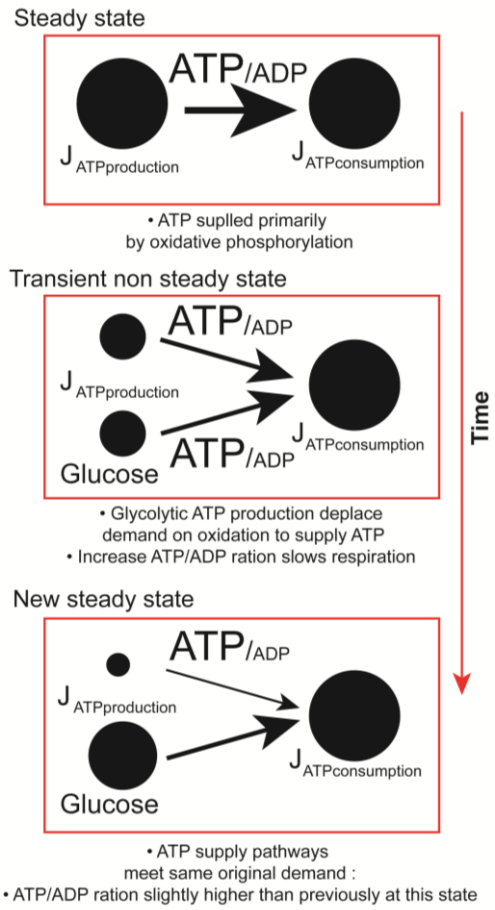


Figure 4 - The Crabtree Effect and energy Homeostasis. Adapted from Handel et al (2019) with permission of Trends Endocrinology & Metabolism, Elsevier B.V. **Top:** ATP production at cellular steady state where oxidative phosphorylation is the main support. **Middle:** An addition of sugar drives a glycolytic ATP production, that increase the ratio ATP/ADP and drive the cells towards a transient non-steady state (non-permanent). **Bottom:** The oxidative phosphorylation is slow down under the control of the new established ATP/ADP ratio.

Tilques et al 2018). Results of this study suggested that a toxic accumulation of cytoplasmic misfolded mitochondrial protein precursors secondary to impaired mitochondrial protein import may contribute to impaired mitochondrial function resulting from TAFAZZIN deficiency. This effect has been previously described in a yeast model of adPEO (autosomal dominant progressive external ophthalmoplegia) that relates to ANT mutation (adenine nucleotide translocator) (Wang, Chen 2015), inducing mitochondrial precursor over-accumulation stress (mPOS). These effects have been shown to induce the unfolded protein response activated by misfolded mitochondrial proteins (UPRam), which involves a decreased rate of cytosolic protein synthesis associated with an increased activity of the proteasome, leading to an increase in protein clearance by the proteasome (de Taffin de Tilques et al 2018). We posit that immature cardiolipin (and perhaps the accumulation of mono-lysocardiolipin) negatively impacts mitochondrial membrane protein transport, thus impacting both mitochondrial function and broader cellular protein homoestasis independent of direct effects on OXPHOS protein function.

8. Conclusion

Barth syndrome is a devastating pathology resulting from *TAFAZZIN* gene mutation that leads to defects in mitochondrial structure and function. Alteration in the biosynthesis and remodeling of CL preferentially affects tissues with high rates of energy metabolism, such as the heart and skeletal muscle. Despite decades of research, a clear view of the mitochondrial bioenergetic defects responsible for this disorder has remained elusive. However, it is clear that defects in mitochondrial function are not reflected by changes in cellular ATP levels, consistent with accumulating evidence that ATP levels alone does not accurately reflect mitochondrial function or dysfunction (Brand, Nicholls 2011). Indeed, several reports of normal cellular ATP level in models of BTHS highlight the possibility that compensatory mechanisms may contribute significantly to disease pathogenesis (Gonzalvez et al 2013; Saric et al 2015). Therefore, a more comprehensive evaluation of bioenergetic status that includes accurate measurements of OCR and ECAR, relative quantities of ATP, ADP and AMP, together with NADPH/$NADP^+$ and NADH/NAD^+ may help to integrate and understand how the bioenergetic landscape integrates with broader aspects of mitochondrial and cellular function in BTHS and other conditions associated with mitochondrial dysfunction.

Abbreviations

ADP	adenosine diphosphate	ECF	energy coupling factor
AMP	adenosine monophosphate	IMP	inosine 5′-monophosphate
ATP	adenosine triphosphate	MLCL	mono-lysocardiolipin
BTHS	Barth syndrome	TCA	tricarboxylic acid cycle
CP	cardiolipin		

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