The heart is highly dependent for its function on oxidative energy generated in mitochondria, primarily by fatty acid β-oxidation, respiratory electron chain and oxidative phosphorylation. Defects in mitochondrial structure and function have been found in association with cardiovascular diseases such as dilated and hypertrophy cardiomyopathy, cardiac conduction defects and sudden death, ischemic and alcoholic cardiomyopathy, as well as myocarditis. While a subset of these mitochondrial abnormalities have a defined genetic basis (e.g. mitochondrial DNA changes leading to oxidative phosphorylation dysfunction, fatty acid β-oxidation defects due to specific nuclear DNA mutations), other abnormalities appear to be due to a more sporadic or environmental cardiotoxic insult or have not yet been characterized.

This review focuses on abnormalities in mitochondrial bioenergetic function and mitochondrial DNA defects associated with cardiovascular diseases, their significance in cardiac pathogenesis as well as on the available diagnostic and therapeutic options. A concise background concerning mitochondrial biogenesis and bioenergetic pathways during cardiac growth, development and aging will also be provided.


La mitocondría y el corazón

Para su correcto funcionamiento, el corazón depende estrechamente de la energía oxidativa generada en las mitocondrias, principalmente a partir de la betaoxidación de los ácidos grasos, de la cadena respiratoria de electrones y de la fosforilación oxidativa. Los defectos en la estructura y función mitocondriales se asocian a enfermedades cardiovasculares, como las miocardiopatías hipertrófica y dilatada, defectos en la conducción cardíaca y muerte súbita, miocardiopatías isquémicas y alcohólicas y miocarditis. Aunque una parte de estas anomalías mitocondriales tiene una base genética definida (p. ej., los cambios en el ADN mitocondrial que conducen a una disfunción de la fosforilación oxidativa, o los defectos de la betaoxidación de los ácidos grasos debidos a mutaciones específicas del ADN nuclear), otras anomalías parecen deberse a agresiones cardiotoxicas o ambientales más esporádicas o a causas todavía no identificadas.

Esta revisión se centra en las anomalías de la función bioenergética mitocondrial y en los defectos del ADN mitocondrial asociados a enfermedades cardiovasculares, su significado en la patogenia cardíaca y las opciones diagnósticas y terapéuticas disponibles. También se describen de forma concisa los antecedentes relacionados con la biogénesis mitocondrial y con las vías bioenergéticas durante el crecimiento cardíaco, el desarrollo y el envejecimiento.

In order to fully understand the role that the mitochondrial organelle plays in cardiovascular disease, we provide in this review a brief background describing the biogenesis and function of cardiac mitochondria during normal growth, development and aging. Within this context, we then survey the interaction and characterization of mitochondria/mitochondrial abnormalities in cardiac diseases, their diagnosis, as well as therapeutic options available. While aberrations in the bioenergetic function of the mitochondria are frequently related to the cardiac dysfunction, the specific defect(s) causing the bioenergetic dysfunction often resides in a non-bioenergetic pathway e.g, signaling between the mitochondria and nucleus, or in overall mitochondrial biogenesis and/or degradation pathways. Understanding these pathways and the impact of mitochondrial defects in cardiac pathology is important to improve diagnosis and treatment of mitochondrial-based cardiac diseases.

MITOCHONDRIA AND THE NORMAL HEART

Mitochondrial bioenergetics

Mitochondria are abundant in energy-demanding cardiac tissue constituting 20%-40% of cellular volume. Mitochondrial energy production depends on both nuclear and mtDNA-encoded genetic factors which modulate normal mitochondrial function including enzyme activity and cofactor availability and on environmental factors including the availability of fuels (e.g. sugars, fats and proteins) and oxygen. Several interacting bioenergetic pathways contribute to mitochondrial energy metabolism (shown in Figure 1) including pyruvate oxidation, the tricarboxylic acid (TCA) cycle, the mitochondrial β-oxidation of fatty acids and the common final pathway OXPHOS which generates 80%-90% of cellular ATP. OXPHOS is performed by complexes of proteins located at the mitochondrial inner membrane including the electron transport chain (ETC) respiratory complexes I-IV, ATP synthase (complex V) and the adenine nucleotide translocator (ANT). Fatty acids are the primary energy substrate for the production of ATP in cardiac muscle by OXPHOS. In order to be utilized for bioenergetic production via mitochondrial fatty acid β-oxidation, fatty acids need to be effectively transported into the cardiomyocyte and subsequently into the mitochondria, a process requiring several transport proteins including the carnitine shuttle (carnitine acyltransferase and two carnitine palmitoyl transferasest as well as carnitine). Fatty acid β-oxidation and the oxidation of carbohydrates via the TCA cycle generate the majority of intramito-
Overview of mitochondrial biogenesis

Human mitochondria have their own double-strand DNA circular molecule encompassing 16,569 bp and encoding 13 proteins which constitute a portion of the 5 enzyme complexes involved in electron transport and OXPHOS. The protein-encoding mtDNA genes are transcribed into specific mRNAs which are translated on a mitochondrial specific ribosome/protein synthesis apparatus. The mtDNA also encodes part of the mitochondrial protein synthesis machinery including 2 ribosome RNA (rRNA) and 22 transfer RNA (tRNA) shown in Figure 2. In general, each cardiac cell contains multiple mitochondria (50-100) and each mitochondria contains multiple copies of mtDNA (1-10 molecules/mitochondria). Mitochondrial biogenesis is increased during cardiac hypertrophy, treatment with a variety of agents e.g. thyroxin, xenobiotics, electrical stimulation and exercise.

Presently, the mechanisms that regulate cardiac-specific mtDNA levels and overall mitochondrial number have not yet been delineated. However, it is well established that pathogenic point mutations and large-scale deletions in the mitochondrial genome as well as generalized depletion of mtDNA levels have severe consequences for organs such as heart since ATP derived from OXPHOS is needed to maintain myocardial contractility.

Conversely, the nuclear genome encodes the entire complement of proteins involved in mtDNA replication and transcription, protein components of mitochondrial ribosomes, multiple structural and transport proteins of the mitochondrial membranes and remaining peptide subunits (presently estimated at 71) of the respiratory complexes (other than the 13 mtDNA-encoded peptide subunits. These nuclear-encoded proteins are synthesized on cytosolic ribosomes, targeted to mitochondria and imported by a complex process. Cardiac-specific regulation of a number of the nuclear genes encoding OXPHOS proteins can be mediated by variable gene expression (e.g. ATP synthase β-subunit is expressed at higher levels in heart than other tissues) sensitive to a variety of physiological and developmental stimuli, and by the presence of tissue-specific isoforms for specific peptides (e.g. cardiac specific isoforms exist for genes encoding cytochrome c oxidase subunits VIa,
VIIa and VIII). Therefore, mutations in nuclear genes involved in mitochondrial biogenesis might be expected to contribute in part to the observed mitochondrial cardiac enzyme and mtDNA defects, including increased incidence of large-scale mtDNA deletions and mtDNA depletion associated with cardiac disorders. Thus far, few mutations in nuclear genes affecting mitochondrial biogenesis leading to cardiac disorders have been identified. While the nuclear genome controls mitochondrial biosynthesis, mtDNA genes have a much higher mutation rate, lack histones, have limited DNA repair and are exposed to reactive oxygen species (ROS) generated by the ETC. The search for mutations in both nuclear and mitochondrial genomes as well as mutations that affect cross-talk between the genomes is presently expanding.

Cardiac mitochondrial changes during cardiac growth and development

In the fetal heart which functions in a relatively hypoxic environment, glucose and lactate are the predominant fuel substrates utilized by glycolysis and lactate oxidation respectively. A large endogenous supply of glycogen in the fetal and, to a lesser extent, in the neonatal heart are a significant source of glucose and myocardial ATP. Glycogenolysis is particularly important in conditions of oxygen deprivation, and may allow the fetal heart to be more resistant to the effects of hypoxia and ischemia as compared to the adult heart. Fetal hearts have lower mitochondrial content and therefore lower levels of both respiratory complex and TCA cycle activities. Fatty acid oxidation provides a small part of the energy substrate in the neonatal heart. The specific activity levels of genes encoding fatty acid enzymes (e.g. mitochondrial carnitine palmitoyltransferase [CPT] enzymes and isoforms) shows a marked change during the early postnatal period which parallels the increased utilization of fatty acids as an energy substrate in the neonatal heart. The specific activity levels of CPT-II increase during the first month of life as does myocardial carnitine levels.

Post-natally, a switch occurs so that fatty acids become the primary energy substrate in the heart. The expression of genes encoding fatty acid enzymes (e.g. mitochondrial carnitine palmitoyltransferase [CPT] enzymes and isoforms) shows a marked change during the early postnatal period which parallels the increased utilization of fatty acids as an energy substrate in the neonatal heart. The specific activity levels of CPT-II increase during the first month of life as does myocardial carnitine levels. Of the 2 tissue-specific isoforms of CPTI, CPT-Ia gene is highly expressed in the fetal heart and declines following birth while CPT-Ib, is highly expressed throughout cardiac development. Specific mitochondrial membrane-associated and cytoplasmic proteins involved in long-chain fatty acid uptake are co-ordinately regulated during development in cardiac tissue.

The level of cardiac mitochondrial creatine kinase (mtCK) which is undetectable in early fetal heart, is upregulated during neonatal development. The increased expression of mtCK within the first several weeks after birth is coupled to both the availability of ADP to mitochondria as well as to the structural re-organization of cardiac mitochondria from a random arrangement (at 1 day) to an organized network by 3 weeks postnatally. The maturation of the phosphocreatine shuttle is coordinated with the development of the contractile properties of the myocardium.

Post-natal levels of cardiac-mitochondrial OXPHOS enzyme activities and content particularly in humans, need to be clearly established. At present, the information available is limited by the small number of subjects studied with normal heart, and by the variability of mitochondrial OXPHOS enzyme activities due to dietary factors, exercise and hormonal stimuli.

A by-product of mitochondrial bioenergetic production is the generation of ROS including superoxide and hydroxyl radicals and hydrogen peroxide (H₂O₂). Side reactions of the mitochondrial ETC with molecular oxygen directly generate the superoxide anion radical. The main sites for mitochondrial ROS generation are complex I and III activities of the respiratory chain; either excessive or diminished electron flux at these sites can stimulate the auto-oxidation of flavins and quinones (including coenzyme Q) producing superoxide radicals. The superoxide radicals can be converted to H₂O₂ in the presence of the enzyme superoxide dismutase which can further react to form the hydroxyl radical.

Normally, these toxic by-products which are powerful cell-damaging oxidants are neutralized by antioxidant enzymes some of which are mitochondrially located e.g. Mn superoxide dismutase (MnSOD) and glutathione peroxidase (Figure 3) and others are cytosolic e.g. Cu superoxide dismutase and catalase, or scavenged by glutathione. Increased ROS generation resulting from myocardial ischemia/reperfusion, inflammation, impaired antioxidant defenses and aging may cause profound effects on cardiac cells including increased lipid peroxidation effecting primarily membrane phospholipids and proteins. Superoxide is particularly damaging to the Fe-S...
moieties of enzymes (e.g. complex I, aconitase and succinic dehydrogenase). Oxidative damage also occurs with nucleic acids (particularly targeting mtDNA) including induction of strand breaks, a high incidence of base modification (including the formation of 8-oxoguanosine) and subsequent point mutations and deletions. Mitochondria which are the primary site of ROS generation are therefore the critical target of its damaging effects; the mitochondrial respiratory chain located in the inner membrane is often damaged resulting in a further increase in ROS generation leading to a vicious cycle of diminished mitochondrial function. In addition to the well characterized role of ROS as cell damaging, recent evidence has provided an alternative view concerning increased ROS generation and oxidative stress in its role as an important regulatory phenomena. Oxidative species (e.g. H$_2$O$_2$) can also function as a potent signal sent from mitochondria to other cellular sites rapidly, and reversibly eliciting an array of intracellular cascades leading to different physiological end-points for the cardiomyocyte (e.g. apoptosis, necrosis, cardio-protection, cell proliferation).

**Cardiac mitochondrial changes during aging and senescence**

Amongst the multiple metabolic changes that occur in cardiac muscle with advancing age are modifications in membrane fatty acid and lipid composition including increasing levels of saturated fatty acids and reduced levels of polyunsaturated fatty acids and cardiolipin. Cardiolipin, the most unsaturated cellular phospholipid, is a major component of the mitochondrial inner membrane and plays an integral role in cardiac mitochondrial membrane transport function, fluidity and stability, and as a facilitator of key mitochondrial inner membrane bioenergetic enzymes. Marked reduction in carnitine and acetyl carnitine levels has also been reported in older subjects.

Increased levels of large-scale deletions and point mutations in cardiac mtDNA, and reduced levels of

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**Fig. 3.** The mitochondrial pathway of apoptosis. Cellular signals including ROS, and UV-induced DNA damage and a variety of «death signals» trigger the apoptotic pathway by modulation of the binding of proapoptogenic proteins (e.g. BAX) to the PTP causing it to open. Elevated levels of mitochondrial calcium levels as well as excessive ROS (generated at respiratory complex I and III sites) as shown also promote the PTP opening. This is followed by the release of cytochrome c and apoptosis-inducing factor (AIF) from the mitochondria to the cytosol with subsequent activation of the caspases initiating cell self-digestion and nuclear DNA fragmentation leading to apoptotic cell death. Association of BAX with mitochondria is prevented by anti-apoptogenic Bcl family of proteins. The neutralization of ROS occurs at both intramitochondrial sites by antioxidant enzymes, manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPx) and within the cytosol by CuSOD, GPx and catalase. Also shown are the major proteins comprising the PTP at contact sites adjoining the inner and outer mitochondrial membranes including hexokinase (HK), adenine nucleotide translocator (ANT), creatine kinase (CK), cyclophosphidin D (CpD), porin and the benzodiazepine receptor (IBP) as well as the inner membrane phospholipid cardiolipin (CL).
mitochondrial enzymatic activities may occur with aging. These mitochondrial abnormalities have been proposed to be due in large part to the increased mitochondrial production of ROS noted in aging. However, the effect of aging on cardiac OXPHOS enzymatic function has been recently re-evaluated and the extent and role of mitochondrial bioenergetic decline has been put into question. The discrepancies between these findings may be based on the different methodologies employed in measuring mitochondrial activities. If one distinguishes the cardiac mitochondria as distinct populations (requiring the physical separation of the organelle populations in vitro) the interfibrillar mitochondria (IFM) located between the myofibers and the subsarcolemmal mitochondria (SSM), located below the plasma membrane, age-dependent reductions are found in IFM and not SSM respiratory complex III and IV specific activities. A host of physiological changes/parameters can also impact on levels of mitochondrial enzymatic activities. Levels of exercise and conditioning as well as increased ischemic stresses can markedly impact on mitochondrial enzyme activities.

MITOCHONDRIAL DYSFUNCTION IN CARDIOVASCULAR DISEASES

MtDNA and cardiovascular disease

Discrete mitochondrial OXPHOS defects or respiratory chain deficiency have been documented in cardiomyopathies. Both DCM and HCM are frequently accompanied by defective levels of specific OXPHOS/respiratory enzyme activities.

Cardiomyopathy (mostly HCM) often occur with specific pathogenic point mutations in mtDNA (an updated list is presented in Table 1). Pathogenic mtDNA mutations are generally located in nucleotides which are highly conserved in evolution, and frequently present in heteroplasmic fashion (a mixed population of both mutant and wildtype mtDNA genomes) albeit recent evidence has indicated that pathogenic mtDNA mutations can also be homoplasmic. These mutations are usually accompanied by reduced levels of specific respiratory enzyme activity(s).

Pathogenic mtDNA mutations in a number of mitochondrial tRNA genes have been identified in association with cardiomyopathy. Specific tRNA genes including LEU, ILE and LYS appear to be hot spots for mutation in cardiomyopathic patients. The site of mutations within the generic tRNA cloverleaf structure may impact on the severity of phenotype and possibly to its tissue-specificity. In general, pathogenic mitochondrial tRNA gene mutations negatively affect mitochondrial protein synthesis and multiple specific respiratory enzyme activities. A cardiomyopathy-associated mtDNA mutation has also been found in mitochondrial rRNA. Other mtDNA point mutations residing at other mtDNA locations (some occurring in tRNA genes, others in mtDNA protein-encoding genes), which were not present in normal individuals have been reported in patients with DCM. These potentially pathogenic mutations are heteroplasmic, and present in highly conserved sequences. Moreover, missense mutations in cytochrome b have been reported in a wide spectrum of cardiomyopathies including DCM, HCM, histiocytoid and post-partum cardiomyopathy (Table 1). It is noteworthy that cytochrome b, the sole mitochondrial-encoded subunit of complex III, also represents a hot spot for mutations in patients with cardiomyopathy.

Multi-systemic mitochondrial diseases with cardiac involvement have been reported usually with an expanding spectrum of clinical manifestations. Some are maternally inherited (due to mtDNA mutation) and may present with a variable cardiac phenotype (ventricular hypertrophy, cardiomegaly and dysrhythmias) together with neurological syndromes including Leigh (developmental delay, muscle weakness, ophthalmoplegia and necrosis in the basal ganglia), MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) and MERRF (myoclonic epilepsy and ragged-red fibers). Specific mtDNA mutations, found in association with primary cardiomyopathy, can also be present in patients with different combinations of clinical phenotypes. The variability between mitochondrial genotype and phenotype may be explained by the involvement of unidentified genetic or environmental cofactor(s) capable of modulating the effect of mtDNA mutations. Similarly, specific syndrome(s)/phenotype(s) may be caused by entirely different nuclear and/or mtDNA mutations. For example, Leigh syndrome can be caused by mutations in ATPase-6 subunit, by point mutation in mitochondrial tRNA LYS gene, by nuclear DNA mutations in pyruvate dehydrogenase, nuclear-encoded complex II subunits or by mtDNA depletion. However, the correlation of cardiac involvement in Leigh disease and the loci effected (whether nuclear or mitochondrial) has not been established.

Sporadic large-scale rearrangements of mtDNA can also be associated with cardiac disorders. In Kearns-Sayre syndrome (KSS), cardiac conduction...
abnormalities typically co-exist with somatic large-scale mtDNA deletions.\textsuperscript{49,50} The majority of mtDNA deletions in KSS are of a single type, not inherited and are detected mostly in skeletal muscle and rarely in blood. One study showed a localized prevalence of mtDNA deletions in the tissues containing the cardiac conduction system i.e. the sinoatrial and atrioventricular nodes as compared to the surrounding myocardium.\textsuperscript{51} In contrast, a multiple mtDNA deletion phenotype associated with DCM is due to genetic defects in unidentified autosomal nuclear loci which can be either dominantly or recessively inherited.\textsuperscript{52,53} The mtDNA deletion events detected in KSS and in autosomal disorders tend to be highly abundant (reaching up to 95\% of total mtDNA) either as single-sized discrete deletions or in the aggregate and are detected by Southern blot.\textsuperscript{54,55} Moreover, less abundant, large-scale specific mtDNA deletions are frequently found in cardiac tissues of many primary cardiomyopathies by PCR analysis. These less abundant deletions reflect specific mtDNA damage (presumably a consequence of ROS), occur in an age dependent manner\textsuperscript{54,55} and their role in cardiac disease remains unclear.

Depletion in cardiac mtDNA levels has been reported in patients with isolated cardiomyopathy, ei-

### TABLE 1. Specific mtDNA point mutations in cardiac disease

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\textsuperscript{1}Ref indicates references; CM, Cardiomyopathy; HCM, Hypertrophic cardiomyopathy; CF, cardiac insufficiency.
ther DCM and HCM. In addition, depletion of cardiac mtDNA can be specifically induced by zidovudine (AZT), which inhibits both the DNA polymerase of HIV and mitochondrial DNA polymerase γ interfering with mtDNA replication. In both humans and animals treated with AZT, 5, 6 reduced cardiac mtDNA levels are found associated with a marked reduction in respiratory enzyme activities and a clinical phenotype of cardiac dysfunction. The mtDNA depletion appeared to be reversible since cessation of AZT therapy was associated with improved left ventricular function.

**Defects in nuclear-DNA encoded mitochondrial proteins and cardiovascular disease**

Mutations in a wide array of nuclear genes encoding mitochondrial proteins can cause cardiomyopathy. For example, cardiomyopathy is frequently a consequence of mutations in mitochondrial transport proteins (e.g. carnitine-acylcarnitine translocase) which facilitate the passage of critical metabolites across the inner mitochondrial membrane and also mutations in a mitochondrial transport protein, frataxin, considered to be involved in mitochondrial iron accumulation cause Friedreich ataxia (primarily HCM). 6, 7 Moreover, mutations in nuclear genes encoding factors required for the assembly and functioning of the multiple-subunit enzyme respiratory complexes have been implicated in some mitochondrial-based diseases such as Leigh syndrome and mutations in the SCO2 gene encoding a copper chaperone, involved in the assembly of complex IV (COX), may result in cardiomyopathy. 8 Interestingly, the clinical phenotype in patients with SCO2 mutations is distinct from that found with mutations in other COX assembly factors (e.g. SURF1) which typically present without cardiac involvement. 9

Cardiomyopathy is the primary clinical manifestation in several inherited disorders of mitochondrial fatty acid β-oxidation. 10 Deficiencies in very long chain acyl-CoA dehydrogenase (VLCAD), 11 long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) 12 and short-chain acyl-CoA dehydrogenase (SCAD) 13 have been reported to cause cardiomyopathy in young children. Also, defects in carnitine transport into cells as well as in the carnitine-acylcarnitine shuttle mechanism, responsible for fatty acid transport into mitochondria, have been found associated with cardiomyopathy. 14 Defects in the mitochondrial trifunctional protein (MTP) that affect long chain L-3 hydroxylacyl-CoA protein are often associated with DCM. 15

The cardiac pathogenesis of these inherited disorders of fatty acid β-oxidation and carnitine metabolism likely includes both a deficient bioenergetic supply to the heart and the accumulation of toxic levels of fatty free acids with subsequent cardiac dysfunction. These disorders primarily occur in early childhood and are usually precipitated by infectious illness or fasting when the heart has an increased dependence on fatty-acid oxidation for energy. Many of the inherited fatty acid disorders described above can result in sudden neonatal death.

Cardiac conduction defects and cardiac arrhythmias are frequently present in patients with specific defects in fatty acid oxidation. 16 Both ventricular and atrial arrhythmias were associated with deficiencies in CPT-II deficiency, carnitine translocase and trifunctional protein (MTP) deficiency. In contrast, cardiac arrhythmias were absent in patients with deficiencies in CPT-I, the primary carnitine carrier and MCAD suggestive that accumulation of arrhythmogenic metabolites of fatty acids (e.g. long chain acylcarnitines) promote arrhythmias and potentially contribute to CF and sudden death. It has long been known that long chain acylcarnitines possess detergent-like properties and can extensively modify membrane proteins and lipids with toxic effects on the cardiac membrane electrophysiological functions, including ion transport and impair gap-junction channels. Moreover, the accumulation of long chain fatty acid metabolites (e.g. long chain acylcarnitines) plays a pivotal role in the production of ventricular arrhythmias that occur during myocardial ischemia. 17 The accumulation of potentially toxic long-chain esters during hypoxia/ischemia can be prevented by selective blocking of CPT-I activity with perhexilene or amiodarone reducing both the risk of membrane electrophysiologic disturbances and the incidence of lethal arrhythmias. 18

Abnormal mitochondria and severe DCM has also been reported in Barth syndrome, an X-linked disorder also characterized by cyclic neutropenia with neonatal onset. Arrhythmias and cardiac failure (CF) are frequently present. The protein tafazzin responsible for Barth syndrome 19 is encoded by the G4.5 gene and likely belongs to a family of acyltransferases involved in phospholipid synthesis. In patients with the G4.5 mutation, saturated fatty acid levels increase while unsaturated fatty acid and cardiolipin levels are markedly reduced. 20, 21 Defective acyltransferase function may result in increased fatty acid saturation, decreasing cardiac membrane fluidity and function.
Genes encoding important structural and cytoskeletal proteins have frequently been implicated in the pathogenesis of cardiomyopathy. Specific nuclear DNA mutations in structural and contractile proteins such as actin, desmin, sarcoglycan, and dystrophin have been identified as causal in many cases of DCM and CF. Similarly, cases with familial HCM are due to specific pathogenic mutations in cardiac proteins involved in generating contractile force including β-myosin heavy chain (β-MHC), cardiac troponin T, tropomyosin and myosin-binding protein C. The relationship of mitochondrial defects with mutations in nuclear-encoded structural proteins is worthy of note. Patients with specific β-MHC mutations may develop abnormal mitochondrial number and a marked reduction in mitochondrial respiratory function. A potential interaction of these nuclear pathogenic mutations with specific mtDNA mutations is also suggested by studies showing co-existence in some patients with HCM of mutations in both β-MHC and mtDNA. In addition, the intracellular distribution of mitochondria can be profoundly altered in patients with defective structural proteins (e.g. desmin) since both the intracellular position and movement of mitochondria are mediated by cytoskeletal proteins. Defective cellular location of mitochondria with potential downstream effect on cardiac bioenergetic function may play a critical role in cardiac pathophysiology.

Myocardial ischemia

When the supply of oxygen becomes limiting as occurs with myocardial ischemia, OXPHOS and mitochondrial ETC flux decline, creatine phosphate is rapidly depleted, fatty acid and pyruvate oxidation decrease and ATP production is impaired. The hydrolysis of glycolytically derived ATP and accumulation of lactate leads to a decrease in intracellular pH and intracellular acidosis, which has a direct inhibitory effect on contractile function. AMP and other intermediates accumulate with subsequent mitochondrial swelling and progressive degeneration. In addition, myocardial ischemia has been shown to result in reduced levels of respiratory complex IV, complex V and increased levels of mtDNA deletions. Sustained ischemia leads to ATP depletion and necrotic cell death.

Paradoxically, functional mitochondria can exacerbate ischemic damage, especially at the onset of reperfusion. A pronounced increase in fatty acid influx and unbalanced fatty acid oxidation predomi-
sequent cascade of cytoplasmic changes including activation of downstream cysteine-aspartate proteases (caspases) and nuclear endonucleases leading to cell death. The release of mitochondrial peptides is directed by an opening of a megachannel, the mitochondrial permeability transition pore (PTP), a dynamic multiprotein complex located at contact sites between the mitochondrial inner and outer membranes promoted by the binding of proapoptotic proteins such as Bax (Figure 3). The anti-apoptotic protein Bcl-2 (a mitochondrial protein) prevents the association of Bax with the PTP and interferes with the release of the apoptogenic peptides (e.g. cytochrome c and AIF) from the mitochondria. Recent evidence has established that PTP opening is a critical early step of apoptosis preceding the caspase cascade. PTP opening is promoted by Ca++ influx into mitochondria and excessive mitochondrial ROS production. Other pro-apoptotic second messengers including pro-oxidants and nitric oxide also induce PTP opening. In addition to its involvement in apoptotic cell death, opening of the PTP has been implicated in the clinical cell death of cardiomyocytes induced by excitotoxins (e.g. glutamate), as well as in necrotic cell death occurring during anoxia and ischemia. This supports the emerging view that mitochondrial changes are a pivotal step in the commitment to both apoptotic and necrotic types of cell death.

PTP opening is accompanied by dissipation of the mitochondrial transmembrane potential (∆Ψm) and mitochondrial membrane depolarization. The changes in membrane potential can be either a cause or the result of PTP opening, since extensive proton influx occurs at this site. Decline in mitochondrial respiratory enzyme activities (particularly complex III) and in OXPHOS can contribute to the onset of apoptosis. The PTP represents a critical site where OXPHOS can integrate with both cell-signalling stimuli and responses. Decreased ATP content promotes the transfer of BAX to the PTP. However, there is a continual dynamic in the balance of the pro-apoptotic BAX and antiapoptotic Bcl factors in modulating the events at the PTP site. Because apoptosis requires energy, the shutdown of OXPHOS either leading or following the PTP opening is not complete. It is important to note that complete mitochondrial de-energization does not favor apoptosis but rather leads to necrosis. It should not be surprising that the early events of apoptosis involves modulation in ATP levels, given the close proximity of the PTP to the respiratory complexes in the mitochondrial inner membrane as well as its involvement in the mitochondrial loss of cytochrome c, a critical molecule in the ETC. Also present at the PTP site are a number of energy-associated mitochondrial molecules including the adenine-nucleotide exchanger, the glycolytic enzyme, hexokinase, the outer membrane voltage-dependent anion channel protein (VDAC or porin), the mitochondrial creatine kinase and the phospholipid cardiolipin.

Mitochondria and cardiotoxicity

An increasing number of drugs/toxins have been recognized as having deleterious effects on cardiac mitochondrial function. The reader is referred to two recent excellent reviews concerning mitochondria (in general) as a pharmacological target. We limit our discussion to two relatively well-characterized toxins which have cardiac-specific effects associated with mitochondria.

The anticancer anthraquinone glycoside drugs (e.g. adriamycin/doxorubicin) have long been known to affect myocardial function. Molecular and biochemical myocardial changes occur with doxorubicin-induced cardiomyopathy including striking changes in mitochondrial structure and function. Mitochondrial changes include markedly increased free radical production through abnormal NADH dehydrogenase activity; increased mtDNA damage, including accumulation of 8 OHdG adducts and large-scale mtDNA deletions; alterations in mitochondrial Ca++ homeostasis; changes in enzyme activities involved in fatty acid oxidation (e.g. CPTI) and decreased ANT activity. The majority of deleterious biochemical effects of doxorubicin can be counteracted by supplementation with any of the following: MnSOD, carnitine, adenosine, metallothionein, which provide varying levels of cardioprotection. There is also evidence that doxorubicin impacts events at the mitochondrial PTP modulating Ca++ influx, free O₂-radical generation, ANT activity, carnitine transport and metabolism and mtDNA integrity. The involvement of doxorubicin with cardiac apoptotic pathway induction is also consistent with the doxorubicin-stimulated release of cytochrome c in cardiomyocytes. In this regard, it is noteworthy that patients with viral myocarditis also tend to have decreased ANT activity and content suggesting that the mitochondrial PTP may represent a common target site for cardiac toxins/ infectious agents. Moreover, there is evidence that the PTP site of the apoptotic pathway may be a primary target for toxic...
agents (i.e. salicylate and valproate) involved in the pathophysiology of Reyes disease.\textsuperscript{88}

Intake of moderate amount of alcohol may exert beneficial effects on the cardiovascular system;\textsuperscript{102} this may be manifested by moderate increases in myocardial mitochondrial bioenergetics (i.e., increased levels of respiratory enzyme activities and mtDNA) suggesting increased cardiac-specific mitochondrial biogenesis.\textsuperscript{103} On the contrary, intake of high dosage of alcohol has been found to have detrimental effects on myocardial tissues characterized at the subcellular level by mitochondrial structural and functional abnormalities and mtDNA depletion.\textsuperscript{104-106}

**Animal models of mitochondrial-associated cardiovascular disease**

Gene ablation in mice (i.e. generation of null mutations or gene «knock-outs») targeting a relatively wide spectrum of genes encoding mitochondrial proteins results in severe cardiac dysfunction. Targeted genes include ANT,\textsuperscript{107} MnSOD,\textsuperscript{108,109} factors involved in fatty acid metabolism, e.g. PPAR, MTP subunits\textsuperscript{110,111}, the mitochondrial transcription factor mtTFA (also termed TFAM)\textsuperscript{112} and frataxin, e.g. the protein responsible for Friedreich ataxia.\textsuperscript{113} In addition to examining the specific effects on cardiac function by eliminating specific genes of mitochondrial function, tissue-specific knockout mice with mitochondrial cardiomyopathy have been used to identify modifying genes of potential therapeutic value.\textsuperscript{114} At present, there is limited information about the impact on myocardium of knocking-out nuclear genes involved directly in mitochondrial OXPHOS. A recent report described cardiac dysfunction in mice lacking cytochrome c oxidase subunit Vla-H, the heart isoform.\textsuperscript{115} Little information is currently available concerning mtDNA gene ablation since generation of mtDNA gene knock-outs represents a difficult technical challenge requiring a novel approach for the creation of a mitochondrial transgenic animal; several approaches are discussed below.

A recent breakthrough in the creation of a mitochondrial transgenic animal was achieved with the construction of a mouse strain containing high levels of a single largescale mtDNA deletion (4696 bp).\textsuperscript{116} Myocardial mitochondria from mice with very high levels (>85%) of the mtDNA4696 deletion tended to have abnormal cristae and marked COX deficiency;\textsuperscript{117} however, unlike human patients with KSS and its analogous mtDNA deletion, mice with high proportions of the mtDNA deletion died with kidney disease. Further studies with mice harboring the mtDNA4696 deletion also revealed that single cells (in most tissues) either harbored mutant or wild-type mitochondria; both types of mitochondria did not co-exist in the same cell.\textsuperscript{118} It was proposed that the existence of inter-mitochondrial complementation makes the expression of a deletion phenotype unlikely in the presence of full-length wildtype mtDNA. If the mouse model is relevant to human disorders with mtDNA deletions, this finding could have important ramifications for the potential treatment of deletion-based KSS using full-length mtDNA.

The use of cardiac-specific overexpression of specific genes has also been highly informative in our understanding of the role of mitochondria in cardiac dysfunction. This technique involves fusing a regulatory region from a cardiac-specific gene with a candidate gene of interest and introduction into the transgenic mice which will express the candidate gene specifically in cardiac muscle cells. Overexpression of a number of genes which mediate expression and control of cardiac energy metabolism (e.g. PGC1, PPAR, TNF-α) have been shown to lead to cardiomyopathy with severe cardiac dysfunction and marked changes in mitochondrial structure and function.\textsuperscript{119,120}

The development of animal models of mitochondrial-based cardiac dysfunction offers the possibility of direct testing for potential treatments. For example, the demonstration that MnSOD deficient animals developed ROS toxicity and DCM prompted speculation that effective treatment with anti-oxidants could ameliorate the cardiac phenotype; indeed, peritoneal injection of MnSOD-deficient mice with the anti-oxidant MnTBAP eliminated the cardiac dysfunction and reverses the ROS accumulation.\textsuperscript{121}

**Diagnosis and treatment of mitochondrial-based cardiac diseases**

The diagnosis of mitochondrial dysfunction in cardiac disease has largely come from studies of endomyocardial biopsies using histochemical, ultrastructure and OXPHOS and respiratory enzyme analysis. Recently, the diagnostic utility of skeletal muscle enzyme analysis in patients with cardiomyopathy was demonstrated\textsuperscript{122} and muscle biopsy may eventually replace the need for endomyocardial biopsy in evaluation and follow-up of mitochondrial-based cardiomyopathy. DNA analysis used to identify specific pathogenic mtDNA mutations, large-scale mtDNA deletions and evaluation of mtDNA levels

\textsuperscript{88} Rev Esp Cardiol 2002;55(12):1293-310
can be informative; however, in our experience, the overall incidence of pathogenic mtDNA mutations is low in patients with cardiac disease including those patients with definitive OXPHOS enzymatic defects. After their initial identification, the functional significance of specific mtDNA mutations can be confirmed by the use of cybrid technology. In this methodology, patient’s cytoplasts (enucleated cells containing the patient’s mitochondria and mtDNA) are fused with mtDNA-deficient recipient rho⁰ cells (enucleated cultured cells whose mtDNA has been eliminated through prolonged growth in ethidium bromide). The resulting cytoplasmic hybrid («cybrid») cell lines which can be grown under appropriate selective conditions, possess a wild-type nuclear background derived from the rho⁰ recipient cells and mtDNA derived entirely from the patient and can be studied for growth, oxygen consumption, ATP production, mitochondrial protein synthesis and evaluation of specific enzymatic activities. Further biochemical analysis can be used for the determination of levels of other key mitochondrial intermediates including coenzyme Q10, cardiolipin and carnitine.

A precise knowledge of the specific molecular and biochemical defect may allow treatment with metabolic intermediates (e.g. succinate), coenzymes and vitamins serving as electron donors, transporters and co-factors for electron transport (e.g. vitamin K, thiamine, ascorbate and riboflavin) by bypassing specific defects in OXPHOS and increasing ATP production. For instance, coenzyme Q10 has been reported to have beneficial effects in the treatment of stroke-like episodes, lactic acidosis and fatigability in MELAS, in the cardiac conduction disturbances associated with KSS and in the management of other cardiomyopathies. Recently, idebenone, a synthetic analogue of coenzyme Q has been reported to be effective in the treatment of the cardiomyopathy associated with Friedreich ataxia. Coenzyme Q10 also is potentially useful in its role as an anti-oxidant.

Mitochondrial long-chain fatty acid oxidation disorders (including CPT II deficiency) are effectively treated with long-term dietary therapy, with the replacement of normal dietary fat by medium-chain triglycerides and increased carbohydrates particularly, in the acute cardiomyopathy associated with VL-CAD and LCHAD deficiencies. Prevention of these disorders also involves the strict avoidance of fasting, particularly in infancy. Patients with cardiomyopathy secondary to primary carnitine deficiency can be treated with oral L-carnitine. In a number of carnitine-treated cases, cardiomyopathy was resolved with recurrence prevented for over 5 years. There is also increasing evidence that free polyunsaturated fatty acids in the diet can provide a significant cardioprotective effect against both ischemia-related ventricular fibrillation and arrhythmias, increase mitochondrial cardiolipin content and can facilitate cardiac tolerance of ischemia and reperfusion stresses.

Deficiencies in CPT-II, carnitine acylcarnitine translocase or MTP that can result in increased incidence of cardiac conduction defects/arrhythmias can be treated with targeted drugs which would enhance glucose use and pyruvate oxidation energy, at the expense of fatty acid oxidation, in order to prevent the accumulation of long-chain acylcarnitines. Other examples of metabolic intervention include the reversal of the rise in fatty acid oxidation that occurs during reperfusion using trimetazidine and ranolazine. Dichloroacetate has shown promise in stemming the rise in lactic acidosis and reversing the decrease in pyruvate dehydrogenase activity noted in myocardial ischemia and reperfusion injury.

Carnitine therapy also can be used to lower acyl-CoA accumulation. Moreover, treatment of patients in CF with carvedilol, a β-adrenoreceptor blocker, can lead to marked improvement in myocardial energy efficiency by employing a shift of myocardial oxidative substrates from fatty acid to glucose.

The road ahead: embryonic stem cells and gene-therapy

The formation of functioning myocardial cells in mouse and human cells has been achieved with the use of embryonic stem (ES) derived from blastocyst-stage preembryos. This exciting technology has numerous potential applications in the treatment of cardiac diseases such as cardiomyopathy by augmentation, regeneration or replacement of defective cardiomyocytes. It may also be employed in pharmacological testing for the evaluation of cardiotoxic compounds. Presently, limited information con-
cerning mitochondrial structure and function in ES cells is available, nor has its use been applied to cardiac diseases with extensive mitochondrial abnormalities or for testing of mitochondrial cardiotoxicity.

Recently, mtDNAs from a respiratory-deficient chloramphenicol-resistant (CAPR) cell line from mice were introduced as enucleated cytoplasts into ES cells (treated with rhodamine 6G to eliminate ES cell mitochondria) using cell fusion; the resultant cybrids were injected into blastocysts and chimeric mice were obtained. The CAPR phenotype which is due to a mtDNA point mutation at nucleotide near the 3’ end of the 16S rRNA gene was shown to be transmitted maternally to the progeny. CAPR chimeric mice developed an array of ocular abnormalities, including congenital cataracts, and decreased retinal function as well as severe growth retardation, myopathy and dilated cardiomyopathy. Mitochondria were abnormally enlarged in skeletal and cardiac muscle of the CAPR mice and contained numerous electron-dense granules. While the cardiopathicogenic mechanism of CAPR has not yet been fully elucidated, it is noteworthy that the CAPR mutation results in marked deficiency of mitochondrial protein synthesis and shares the severity and cardiac pathogenicity manifested by other mtDNA mutations affecting mitochondrial protein synthesis (e.g. the forementioned pathogenic tRNA and rRNA mutations).

While the mouse ES cell-cybrid approach offers a potential means to generate and examine animal models of mitochondrial disease, the use of ES cells technology to study human pathogenic mtDNA mutations involved in cardiac disease has not yet been attempted. A recent report proposed the possibility of introducing «mtDNA repaired» ES cells into a patient harboring a mtDNA defect, potentially transforming a diseased myocardium into a healthy one. The «mtDNA-repaired» cell could likely be derived from the patient’s own cells (bypassing immune rejection), which have undergone treatment with ethidium-bromide to remove the endogenous defective mtDNA genomes, and which have been newly retro-fitted to contain entirely wild-type mtDNA genes. In a similar vein, treatment of a patient’s nuclear DNA defects might be envisioned as involving either a specific genetic replacement of the nuclear genetic defect (site-specific homologous recombination can be readily undertaken in ES cells) or if the precise site of the nuclear defect is not known, by replacement of the patient’s entire nucleus with a wild-type nucleus and subsequent generation of an ES cells construct. The successful development of cell engineering using ES cells therapy may have a dramatic impact on the treatment of mitochondrial-based cardiac diseases.

The effective use of gene therapy for mtDNA-encoded OXPHOS defects awaited the development of a methodology for mitochondrial gene replacement in human cells. Towards this end, several laboratories have reported progress in the creation of cationic vectors (e.g. liposomes), to carry signal peptide-targeting sequences covalently attached to mitochondrial oligonucleotides into the mitochondria of living cells. This type of mitochondrial transfection approach can be combined with an antisense strategy aimed at reducing the expression of the defective mitochondrial allele in the patient’s tissue. In addition, genetic correction of a specific mitochondrial DNA deficiency in ATP synthase has been recently demonstrated. Using directed expression of an engineered nuclear-localized version of a mtDNA-encoded polypeptide (ATPase6) in human cybrids, both the biochemical and growth phenotype resulting from a mutant 8993 allele were corrected. A similar genetic approach may prove successful in the treatment of cardiac disorders due to defined mtDNA point mutations.

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