MITOCHONDRIAL DISORDERS IN CHAGASIC CARDIOMYOPATHY

Nisha Garg

Departments of Microbiology and Immunology, and Pathology, Center for Biodefense and Emerging Infectious Diseases, and Sealy Center for Vaccine Development, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555 USA

TABLE OF CONTENTS

1. ABSTRACT

The mitochondria play a complex multi-factorial role in the cell. Along with their primary role in energy (ATP) production, mitochondria generate reactive oxygen species (ROS) that directly or indirectly affect several cellular functions. In this article, I review the molecular, structural and functional mitochondrial abnormalities reported in chagasic cardiomyopathy. I highlight current information about the potential etiology and the pathophysiological significance of mitochondrial dysfunction in chagasic cardiomyopathy and provide a brief background of mitochondrial biogenesis and bioenergetic pathways in cardiac growth and development.

2. INTRODUCTION

Biochemical and molecular studies have identified two primary bases for the development of cardiomyopathies; namely disorders of a) myocardial structural and contractile proteins, and b) myocardial energy metabolism. Disorders of myocardial contractile and structural proteins are usually linked to hypertrophic cardiomyopathy (HCM) (1-6). Energy metabolism disorders that may contribute to cardiac dysfunction include alterations of the mitochondrial oxidative phosphorylation (OXPHOS) pathway and/or of fatty acid (FA) β-oxidation (7-11). Current studies have advanced our knowledge of the specific genetic, molecular, and biochemical alterations that may contribute to metabolic disorders causing cardiomyopathies and have provided new tools for classification and diagnosis of patients predisposed to cardiomyopathy development (12).

In recent years, mitochondrial metabolic deficiencies have also been recognized in cardiomyopathies of infectious etiology (13-15). In particular, defects of OXPHOS pathway are recognized as the major metabolic alterations in chagasic cardiomyopathy (16, 17). In this review, I summarize the literature on cardiac mitochondrial abnormalities in response to the stress of *Trypanosoma cruzi* infection and disease development. I briefly discuss the possible consequences of mitochondrial dysfunction in the pathogenesis of chagasic cardiomyopathy.

3. MITOCHONDRIA IN NORMAL CARDIAC GROWTH AND DEVELOPMENT

3.1. Mitochondrial bioenergetics

The provision of cellular energy by mitochondria is essential for a multitude of cellular functions, including intermediary metabolism, cell mobility and cell proliferation, ion regulation, and active transport processes. The heart is a highly oxidative tissue and essentially is dependent on mitochondria for the energy required for its contractile and other metabolic activities. Mitochondria represent 30% of the total volume of cardiomyocytes and provide ~90% of the cellular energy (11, 18). The ß-

oxidation of FA, tricarboxylic acid (TCA) cycle, and respiratory chain-mediated OXPHOS pathway, the three linked metabolic pathways of energy importance, are all carried out in mitochondria (19, 20). The FA ß-oxidation and the oxidation of carbohydrates via the TCA cycle generate a majority of the intra-mitochondrial, reduced high-energy electron carrier nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine nucleotide (FADH$_2$). The OXPHOS pathway, consisting of five large-enzyme complexes, couples NADH and FADH$_2$ oxidation to phosphorylation, leading to ATP generation. All complexes are located in the inner mitochondrial membrane and designated as CI (NADH-ubiquinone oxidoreductase), CII (succinate-ubiquinone oxidoreductase), CIII (ubiquinol-cytochrome c oxidoreductase), CIV (cytochrome c oxidase), and CV (F$_1$F$_0$ ATP synthase). Respiratory complexes (CI-CIV) catalyze a series of redox reactions utilizing the reducing equivalents (NADH and FADH$_2$) generated by the degradation of energy substrates. Specifically, electrons from NAD$^+$ are transferred to the CI complex and then to CoQ. The electrons from FAD$^+$, generated from succinate oxidation in the TCA cycle, are transferred to CII complex and then to CoQ. From CoQ, electrons are transferred to the CIII, cyt C and CIV, and finally released to ½ O$_2$ to give H$_2$O. The electron energy liberated during this process is utilized to pump the protons (H$^+$) out of the mitochondrial inner membrane, resulting in the formation of an electrochemical gradient ($\Delta\Psi$) that is positive and acidic on outside and negative and basic on the mitochondrial matrix side. The electron energy stored in the proton gradient is finally captured by F$_1$F$_0$ ATP synthase to drive the condensation of ADP and Pi for ATP synthesis (19, 20). ATP is transported to cytosol in exchange for the spent ADP by adenine nucleotide translocator (ANT) (21). Consequently, a defect in any component of the three metabolic pathways could potentially impair the energy availability, and subsequently affect the cardiac performance (22-26).

3.2. Mitochondrial biogenesis

Human mitochondrial DNA (mtDNA) encompassing 16,569 base pairs is a circular double-stranded DNA and consists of a total of 37 genes (12). The 13-mt mRNAs encode for proteins essential for the assembly of the four of the five complexes involved in the OXPHOS pathway, and are translated on a mitochondria-specific ribosome/protein synthesis apparatus. The mtDNA also encodes part of the mitochondrial protein synthesis machinery, including 22 tRNAs, as well as 12S and 16S rRNAs. More than 95% of the mitochondrial proteins are, however, encoded in the nucleus, synthesized in cytosol, and transported into mitochondria. The nuclear DNA (nDNA) encodes proteins involved in mtDNA replication and transcription, protein components of mitochondrial ribosomes, and multiple structural and transport mitochondrial membrane proteins (27). In addition, nDNA encodes the pyruvate dehydrogenase enzyme complex, metabolic enzymes of the FA β-oxidation pathway and TCA cycle, and the peptide subunits of the respiratory enzyme complexes (other than the 13 mtDNA-encoded peptide subunits) (28). All four subunits of the complex II are encoded by nDNA (28). The mitochondrial respiratory chain is unique from a genetic point of view as it is under the dual regulation of the nDNA and mtDNA.

Distinct genetic features that differentiate mtDNA from nDNA include a) maternal inheritance, b) polyploidy, and c) mitotic segregation. During fertilization, mtDNA are contributed by oocytes only. As a consequence, genetic disorders of mitochondria resulting in cardiomyopathy and other diseases are transmitted in a maternal fashion (29). In general, a cardiac cell consists of 100-500 mitochondria, each containing multiple copies (2-10) of mtDNA. The mtDNA are randomly distributed to progeny cells at cell division (30). The mechanisms that regulate mtDNA levels per mitochondria or the number of mitochondria per cell have yet to be elucidated (30). It is, however, well established that pathogenic point mutations and deletions in the mitochondrial genome, generalized depletion of mtDNA, or mutations in nuclear genes, all may affect mitochondrial biogenesis/and or function (29, 31). The physiological effects of mitochondrial dysfunction are expected to be severe in tissues with high-energy demand, such as the heart that is essentially dependent on mitochondrial generation of ATP to maintain contractility and other metabolic functions.

The adult heart predominantly utilizes aerobic metabolism, with the majority of ATP energy supplied by oxidative phosphorylation (32). Under normal conditions, the heart preferentially oxidizes FA as a reduced energy source (33). At the fetal stage and just after birth, the heart functions in a relatively hypoxic environment, utilizing glucose and lactate as the predominant fuel substrates for glycolysis and lactate oxidation, respectively (34). Several factors are suggested to contribute to preferential glucose oxidation in fetal hearts. For example, extremely high concentration of lactate along with limited free FA in fetal circulation is suggested to cause inhibition of FA uptake coupled with high level of lactate oxidation. Malonyl CoA, a metabolite produced during FA biosynthesis, is a potent, non-competitive inhibitor of carnitine-palmitoyl transferase I (CPT-I), an enzyme essential for transport of long chain FAs into mitochondria (35). The sensitivity of fetal CPT-I to malonyl CoA is greater in new-born hearts than adult hearts which could lead to efficient inhibition of CPT-I and decreased long chain FA oxidation (36). L-carnitine, a cofactor of CPT-I, is also produced at lower levels in fetal hearts, thus limiting FA transport/metabolism. After birth, a switch occurs so that FAs become the primary energy substrate in the heart (37). This shift correlates with decreased levels of malonyl CoA, and increased L-carnitine expression and FA delivery to mitochondria (38, 39). Two isoforms of CPT-I, CPT-Iα and CPT-Iβ, have been identified in the heart (40). CPT-Iα is expressed in the fetal heart and declines after birth (41). The expression of CPT-Iβ, though detectable in fetal myocytes, is up regulated after birth and in later cardiac development. The specific activity of CPT-II also increases after birth (37, 41, 42). Differential expression and sensitivity of CPT isoforms to malonyl CoA is suggested to regulate cardiac fatty acid oxidation during development.

Concerning the pre-and post-natal changes in levels of cardiac-mitochondrial OXPHOS complex activities, Marin-Garcia *et al* (43) showed coordinated up regulation of the CIV and CV complex activities, polypeptide content (COX-II, COX-IV and ATP synthase-α subunits), and mtDNA copy number during early fetal cardiac development. In other studies, no changes were observed in the level of CIV and CV specific activities, COXII subunit content, and mtDNA copy number during progression from early neonatal period (<1 month after birth) to adult age (67 yrs) (44). The interpretations from these studies have, however, been limited because mitochondrial OXPHOS complexes are markedly affected by a variety of interrelated physiological, biochemical, and genetic factors during progression from early childhood to older adult.

4. MITOCHONDRIAL ABNORMALITIES IN GENETIC CARDIOMYOPATHIES

Since the early finding of a defect in mitochondrial respiratory activity associated with the accumulation of a large number of abnormal mitochondria in the skeletal muscle of a patient with hypermetabolism (45), impairment of mitochondrial function has been documented in a wide range of human diseases, including cardiomyopathies and heart failure (31, 46, 47). Mitochondrial dysfunction in dilated and hypertrophic cardiomyopathies is often due to a defect within respiratory chain complexes, each of which contain subunits encoded by mtDNA and/or nDNA. Accordingly, genetic defects in both genomes can contribute to mitochondrial cytopathies, although most of the known genetic mutations are identified in mtDNA (48). Appropriately, the diagnosis of mitochondrial defects is confirmed by biochemical assay of the respiratory chain activities and/or by molecular genetic analysis of mtDNA (49, 50).

Excellent discussion of the mtDNA mutations identified in association with genetic cardiomyopathies can be found in recent reviews (26, 29, 31, 51). In brief, several pathogenic point mutations in mitochondrial tRNA genes have been associated with defects in mitochondrial protein synthesis and respiratory complex activities in cardiomyopathies (52-54). Other mtDNA point mutations in the protein-encoding genes are found to be heteroplasmic in DCM patients (53, 55, 56). Mis-sense mutations in cytochrome *b* have been reported in a wide spectrum of dilated and hypertrophic cardiomyopathies (57, 58). There is now a consensus view linking the mutations in mtDNA with ischemic heart disease and DCM (51, 59, 60). The multi-systemic, often maternally inherited, mitochondrial diseases present a variable cardiac phenotype, e.g., ventricular hypertrophy, cardiomegaly, and dysrhythmia, together with other syndromes (47). In addition, large-scale deletions in mtDNA that may or may not be inherited have also been detected in cardiomyopathy and cardiac conduction abnormalities. An accumulation of the mtDNA deletions in the myocardium is frequently linked to cardiac hypertrophy (61, 62), conduction block (63, 64), or heart failure (65). These abundant mtDNA deletions that may range up to 95% of total mtDNA are detectable by Southern blot analysis. Specific, less abundant but large-scale mtDNA deletions are also found by PCR analysis of cardiac tissue in many primary cardiomyopathies (66, 67).

Despite advancement in characterization of the genetic defects associated with mitochondrial disorders, the pathomechanisms in progression of clinical diseases are not well understood. In particular, the relationship between a given mtDNA mutation/deletion and the occurrence of specific clinical symptoms remains unresolved. Indeed, one is likely to observe the same clinical effects caused by different mtDNA mutations and conversely, the same genetic defect in mitochondria, leading to different clinical manifestations (50). Functional studies have also shown that biochemical defects in the same respiratory chain complex can lead to different clinical manifestations. It is likely that the physiological effects of mtDNA mutations/deletions are modulated by additional genetic, biochemical, or environmental cofactors and their identification in future studies would provide a better understanding of the outcome of the mtDNA mutations/deletions in various diseases.

5. CHAGASIC CARDIOMYOPATHY

5.1. Historical perspective

Chagasic disease is a pathological process induced by human infections with the hemoflagellate protozoan *T. cruzi* and is a major health problem in the southern parts of the American continent (68). The parasitological, serological and non-invasive cardiological diagnostic measures (electrocardiography (ECG), echocardiography (EKG), X-ray analysis) suggest that in a majority of acutely infected patients (>95%), parasitemia is controlled by the immune system and hence they exhibit no symptoms of clinical disease until several years later, when >40% of seropositive patients develop chronic heart disease (69). These observations have led to the proposal of an indeterminate phase between the acute infection and chronic disease phases during which patients remain seropositive but have no or sub-clinical cardiac involvement. Invasive ventricular angiography and light and electron microscopic analysis of myocardial biopsies or necropsies from seropositive patients have, however, detected the very early signs of myocardial damage in acute patients that progressively increase with disease severity (70, 71). In several independent studies, 40-60% of the seropositive patients exhibiting asymptomatic or subclinical left ventricular systolic dysfunction by routine clinical diagnostic methods were found to show myocardial damage by microscopic analysis of myocardial biopsies (70, 72, 73) or by invasive ventricular angiography (74, 75). The long-term clinical follow-up records of seropositive patients or patients exhibiting variable severity of disease in association with survival analysis has shown that ventricular arrhythmia most significantly increase mortality in the chagasic patients (76-78). These studies, while underscoring that an indeterminate phase clinically defined as an asymptomatic phase may actually be a progressive phase of biochemical, molecular, and functional alterations that contribute to clinical disease, also suggest that preservation of cardiac function should be the most critical measure in treating chagasic patients.

6. MITOCHONDRIAL ABNORMALITIES IN CHAGASIC CARDIOMYOPATHY

6.1. Human studies

The early insights suggesting mitochondrial alterations as an underlying cause for cardiac dysfunction in chagasic disease were provided by quantitative light and electron microscopic analysis of the myocardial biopsies obtained from seropositive chagasic patients exhibiting none to a variable degree of clinical disease symptoms (70). Microscopic examination of biopsy samples from the patients showed that degenerative myocardial changes occur very early during the indeterminate phase and excerbate with severity of clinical disease (70, 72, 73). Importantly, nuclei and mitochondria were noted to be maximally affected in indeterminate patients (70). As the disease progressed, an advanced degree of myocardial degenerative changes with increased involvement of nuclear and mitochondrial structural abnormalities was commonly noted. In experimental models of chagasic disease, the ultrastructural evaluation of the morphological alterations in the myocardium has illustrated an accumulation of large, irregular nuclei, swollen and displaced mitochondria, and myofibrillar degeneration, that becomes evident and more severe with the progression of chronic disease (79). These studies have elucidated two important observations, one, that nuclear and mitochondrial structural damage occurs much earlier than do the clinical symptoms of disease, and two, the severity of these aberrations increase with the evolution of chronic disease, thus implying a correlation between the extent of specific organelle abnormalities and clinical severity of chagasic disease.

The direct evaluation of mitochondrial metabolic functions in the myocardium of chagasic patients is limited by the ethical aspects of obtaining large tissue biopsies that are required for the mitochondrial isolation for these studies. Histochemical staining of small tissue biopsies is, therefore, the method of choice for monitoring biochemical and enzymatic changes in the myocardium. For the detection of the early signs of myocardial damage, Carrasco *et al* (70) recruited seropositive chagasic patients at various stages of infection and disease development. The severity of cardiac disease in seropositive patients was determined on the basis of abnormalities of ECG and hemodynamic parameters, extent of myocardial damage, and congestive heart failure, with the indeterminate patients showing none of these clinical signs, followed by patients exhibiting myocardial damage only, patients exhibiting abnormal ECGs and myocardial damage, leading to advanced patients exhibiting all clinical signs of cardiac damage and dysfunction. Small endomyocardial biopsies obtained from the patients were subjected to histochemical staining for lipids and polysaccharide deposits, and for a variety of enzymes involved in maintaining the myocardial structure and organelle function. They showed a reduction in the activities of succinate dehydrogenase and myosine ATPase in chagasic patients (70). The histochemical alteration index was evident in seropositive patients in the so-called "indeterminate" phase, suggesting that chagasic patients were predisposed to very early mitochondrial functional defects. The chronic chagasic patients with clinical disease symptoms exhibited highest histochemical myocardial alteration index. These results are in agreement with the ultrastructural studies (discussed above) demonstrating myocardial cellular and organelle abnormalities in indeterminate patients and the increased involvement of mitochondrial structural abnormalities with disease evolution and suggest that myocardial alterations are likely to be associated with functional defects of mitochondria in chagasic disease.

The notion of mitochondrial functional abnormalities in chagasic patients is also supported by indirect observations. Alarcon-Corredor *et al* (80) indexed the changes in the serum pattern of metabolic enzymes in chagasic patients categorized according to clinical severity of disease (as above). Blood samples from the coronary sinus, superior vena cava, and pulmonary and femoral arteries were analyzed. The main finding in this study was a substantial increase in the serum level of the enzymatic activity of glutamate-oxaloacetate transaminase (GOT) and 3-hydroxy butyrate dehydrogenase (HBDH), specifically in the blood collected at the coronary sinus, the draining site for blood metabolized by the heart. Importantly, high serum levels of GOT and HBDH were detected in indeterminate patients, and remained consistently high in patients advancing to clinical cardiac dysfunction. In comparison, an increased serum level of glutamate-pyruvate transaminase (GPT, a cytosolic enzyme) activity was found only in chagasic patients at advanced stages of heart dysfunction. It is usually assumed that an increase in activity of the metabolic enzymes in serum is related to cellular damage. Clinical studies have invariably demonstrated an increase in the serum level of these enzymes in the event of cardiac injury. Considering the site (coronary sinus) and the extent of release of GOT and HBDH in indeterminate-to-chronic patients, it was surmised that mitochondrial and cell membrane injuries are the earliest events in chagasic disease, and the degenerative mitochondrial and cellular events persist with advanced disease. The serum detection of GPT in advanced patients is then most likely due to extensive cellular injuries, also evident by the clinical documentation of abnormal ECG and severe myocardial damage.

Utilizing a similar approach to determine the extent and type of cellular damage in chagasic disease, others (81) determined the concentration or activity of a variety of electrolytes, glycoproteins, and enzymes related to cardiac metabolism in the blood of chagasic patients. The important biochemical changes observed in this study were the detection of inorganic phosphorus and isocitrate dehydrogenase at the coronary sinus in all patients. The detection of these molecules in the serum of indeterminate patients, followed by a positive coronary sinus-femoral artery inorganic phosphate gradient with advancement of chronic disease, supports the hypothesis of very early and progressive manifestations of mitochondrial metabolic abnormalities in chagasic myocarditis.

6.2. Studies in experimental models

Experimental models have proven valuable in elucidating the molecular and biochemical alterations

associated with mitochondrial dysfunction in chagasic cardiomyopathy. Recent molecular studies have profiled the changes in mitochondrial function-related gene expression in experimental models of *T. cruzi* infection and disease development (17, 79, 82) and in the cardiac biopsies obtained from seropositive human patients (unpublished data). These studies utilized global and custom-designed arrays and confirmed the array data by traditional and real-time RT-PCR and Northern blotting approaches. The overall picture that emerged from these studies was that the myocardial transcripts encoding metabolic enzymes involved in FA β-oxidation were up regulated, while the mRNAs for a majority of the subunits of the complexes of the OXPHOS pathway were repressed in response to infection. For instance, the expression profiling with custom-designed mito-arrays showed an increased level of transcripts for long chain acyl-CoA dehydrogenase (ACADL), dodecenoyl-CoA δ-isomerase (DCI), and carnitine-o-acetyltransferase (CRAT) in the myocardium of *T. cruzi*-infected mice (17). Increased myocardial level of mRNAs for ACADL, DCI and 3-hydroxyacyl-CoA dehydrogenase were also detected by global profiling of cardiac gene expression in murine models of *T. cruzi* infection and disease development (79). Others (83) have demonstrated alterations in the protein levels of enzymes involved in β-oxidation of FA in chagasic and hypertrophic hearts. The mRNAs for the enzymes involved in the β-oxidation of FA were, in general, increased in acutely infected hearts and normalized during the chronic disease phase, thus suggesting that chagasic hearts most likely are not compromised in their capacity to generate reduced energy. In similar studies, the nDNA-encoded transcripts for subunits of the OXPHOS complexes were found to be differentially expressed, i.e. while some transcripts were shown to be increased, others were noted to be decreased in infected myocardial tissue (17, 79, 82). It is important to note that the mtDNA-encoded transcripts for the subunits of the OXPHOS complexes were diminished very early during the acute phase of infection (16), before the alterations in nDNA-encoded transcripts were detected, suggesting that the expression from the mitochondrial genome might be more severely affected in response to *T. cruzi* infection. The expression level of mtDNA-encoded OXPHOS components that were examined (9 of 13) was substantially reduced (up to 80%) with progression to chronic disease phase. A loss in mtDNA-encoded transcripts (and presumably proteins) below the threshold level is likely to result in a deficiency of respiratory complexes in chagasic hearts.

Not surprisingly then, alterations in the activities of the respiratory complexes in chagasic hearts was demonstrated. We have employed catalytic staining and spectrophotometric approaches to determine the changes in the activities of the respiratory complexes in response to *T. cruzi* infection and disease development (16). This study found the earliest and strongest repression of CI activity, i.e. a 38% loss at 3 days post-infection (dpi) and a 47-57% decline during the acute phase in infected murine hearts. The specific activity of CIII and coupled activity of CII+CIII were diminished consistently, albeit at different

levels (32-60% and 23-42% respectively) throughout the infection and disease phase. Minimal, but statistically significant, alterations in CV activity have been reported in infected murine and rat hearts (16, 84, 85). It is important to note, that along with a decline in total specific activity of respiratory complexes (determined by spectrophotometry assays), activities of the assembled complexes (determined by catalytic staining on blue-native gels) were also affected, suggesting that multiple mechanisms are likely to be involved in the inactivation of the respiratory complexes in the chagasic myocardium (16). The skeletal muscle mitochondria of the infected mice, except for a decline in CI activity, presented no statistically significant impairment of CII, CIII, and coupled CII+CIII activities at all stages of infection and disease progression (16). The observation of the heart-specific progressive and sustained deficiencies of the respiratory chain complexes implies the pathophysiological significance of mitochondrial dysfunction in CCM.

To summarize, electron microscopic, molecular, and biochemical studies discussed above suggest that abnormalities of mitochondria occur in early stages of *T. cruzi* infection and are indicative of the presence of an active, ongoing process of organelle and myocardial degeneration with progressive severity of chronic chagasic cardiomyopathy.

7. FACTORS CONTRIBUTING TO MITOCHONDRIAL DYSFUNCTION IN CHAGASIC CARDIOMYOPATHY

Considering the complexity of the information available in the literature, it is likely that a number of inter-related mechanisms may contribute to mitochondrial dysfunction in chagasic disease. Many of these pathways appear to be an outcome of constant oxidative damage to mitochondria and may also contribute to oxidative stress generation. I, therefore, focus on the source and site of action of oxidative stress as it relates to mitochondrial dysfunction in chagasic disease.

7.1. ROS generation

Infection by *T. cruzi* generally induces inflammatory cytokines (TNF-α, IL-1, and IL-6) that participate in parasite control through activation of cytotoxic agents, including ROS and reactive nitrogen species (86-89). These reactive species, while necessary in limiting *T. cruzi* replication and survival (89), also affect the host cellular and organelle function (90, 91). These observations have led to the suggestion that immune-mediated responses might be the primary source of oxidative stress in acute hearts. Others have suggested that chagasic myocardium may be predisposed to sustained oxidative stress with progressive disease severity as a consequence of mitochondrial dysfunction. This idea is based upon the fact that mitochondrial generation of ROS as a by-product of respiratory chain is the major source of free radicals in the heart (26, 92, 93). Under normal conditions, as much as 2-4% of the reducing equivalents escape the respiratory chain, leading to the formation of superoxide (O_2^-). O_2^- is dismutated by manganese

superoxide dismutase (MnSOD) to H_2O_2 that may then be converted to highly reactive and harmful hydroxyl radicals $(HO \cdot^-)$ (26, 94, 95). The CI and CIII complexes of the respiratory chain are the prime site for electron leakage to oxygen, and free radical production in mitochondria (26, 92, 93). ROS release may exponentially increase when CI and CIII function at a sub-optimal level (96). Further, CI and CIII are redox sensitive, as they contain Fe_4S_4 clusters that when oxidized; release one iron atom, resulting in the inactivation of important functional Fe-S centers and enzyme activity (97-101). The released ferrous ions, when participating in the Fenton reaction, produce highly reactive $HO \cdot^-$ radicals. The importance of these findings in chagasic disease is that an early and consistent repression of CI and/or CIII activities associated with sustained ROS production was observed in mitochondria isolated from the myocardium of mice infected by *T. cruzi* (16) (unpublished data). A consistent decline in manganese superoxide dismutase (MnSOD) activity, the major oxygen radical scavenger in the mitochondrial matrix (102), with progression of infection and disease in chagasic myocardium was also shown (103). These studies have led to a suggestion that a catastrophic cycle of mitochondrial functional decline and ROS generation, coupled with an inability to efficiently scavenge the mitochondrial ROS (due to MnSOD deficiency), predisposes the chagasic hearts to sustained oxidative stress during infection and disease development. This notion is supported by the observations of a decrease in complex I-mediated respiration and an increase in oxidative damage in MnSOD$^{-/+}$ mice (104), and the neonatal lethality associated with the development of DCM and mitochondrial dysfunction in MnSOD$^{-/-}$ mice (105). The morphologic abnormalities in mitochondrial structure associated with alterations in respiratory complex activities and increased ROS production have also been reported in ischemic hearts of experimental animals (98, 106, 107) and oxygen-depleted cardiomyocytes (99) upon reoxygenation.

7.2. Mitochondrial oxidative damage

ROS can cause damage to biological macromolecules, i.e. lipids, proteins and DNA. Lipid peroxidation (LPO) is the major biochemical consequence of an oxidative attack on unsaturated FA, abundantly present in cell and mitochondrial membranes (108). The aldehydic products of LPO, e.g. malonyl dialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), are highly reactive and able to diffuse and attack targets in the near vicinity as well as those distant from their site of origin (109). 4-HNE reacts with Cys, His or Lys residues via a Michael addition that results in irreversible alkylation and introduction of carbonyl groups into proteins (110). The direct oxidative attack by ROS on Arg, Lys, Pro, and Thr residues can also derivatize the proteins and lead to the formation of protein carbonyls (111, 112). The first indication of mitochondrial oxidative damage in chagasic disease was reported in a recent study. Utilizing an experimental model of infection and chronic disease, Wen *et al* (113) measured the changes in the mitochondrial level of LPO derivatives (i.e. MDA) by TBARS assay (114) and validated these findings by the quantitation of the triphenylphosphine (TPP)-specific lipid hydroperoxide

(LHPO) contents (115). TPP is a specific reductant of hydroperoxides and allows definitive identification of LHPO (115). The specific binding of 2,4-dinitrophenylhydrazine (DNPH) with oxidized and carbonylated proteins was exploited to detect the protein carbonyl-DNP complexes by immunoblotting with anti-DNPH antibody (116). The finding of a substantial increase in LPO and PCO derivatives in cardiac mitochondria of infected mice, compared to controls, led to the suggestion that mitochondria were exposed to oxidative stress-mediated damage in chagasic hearts. The LPO derivatives of mitochondrial membranes were detectable as early as 3 days post-infection, and gradually increased by >2-fold during the course of disease development. In comparison, the PCO content in cardiac mitochondria became evident during the acute infection phase and remained consistently enhanced throughout the chronic phase of disease progression. Given that the LPO derivatives preceded PCO formation, it was proposed that modification of protein residues by HNE/MDA-mediated cross-linking and Michael adduct formation may contribute to an elevated PCO level in cardiac mitochondria. The role of mitochondrial oxidative modifications of membrane lipids and proteins in alterations of mitochondrial integrity, increased permeability and dissipation of the mt membrane potential and protonmotive force, and decreased activity of the respiratory chain complexes (RCC, CI-CV) in chagasic hearts would likely be addressed in future studies.

Direct oxidative modification of specific subunits of respiratory complexes may be an underlying mechanism in the inactivation of assembled mitochondrial complexes in chagasic hearts (113). Cardiac mitochondria from infected mice were subjected to two-dimensional blue-native gel electrophoresis to resolve the subunits of the respiratory complexes. Carbonylated subunits were then detected by immunoblotting with anti-DNP antibody and identified by N-terminal Edman sequencing. On the basis of the identity of subunits that were oxidatively modified, different mechanisms were proposed to participate in inactivation of CI and CIII respiratory complexes in chagasic hearts. Of the >42 subunits of CI, carbonyl adducts were primarily detected with NDUFS1, NDUFS2, and NDUFV1, the core subunits considered essential for electron transfer from NADH to ubiquinone and for the generation of protonmotive force (117, 118). NDUFS4, also oxidatively modified in infected murine hearts, plays an important role in regulating the enzymatic efficiency of CI (117). Considering that genetic mutations in genes encoding NDUFS1 (119), NDUFS2 (120), NDUFS4 (121) and NDUFV1 (122) in human patients and oxidation/nitration of NDUFS2 and NDUFS8 in human and bovine hearts (123) are linked to CI deficiencies, it was surmised that oxidatively modified structural subunits contribute to the inactivation of the assembled CI complex in chagasic hearts. Among the 11 components of CIII, consistent carbonylation of core proteins (UQCRC1 and most likely UQCRC2) and CYC1 was shown in the cardiac mitochondria of infected mice. Core proteins constitute the matrix portion of the CIII complex. Based upon the high-sequence similarity with soluble matrix-processing peptidases (MPP) (124), core proteins are thought to be

involved in the cleavage and processing of the targeting pre-sequence of Reiske [2Fe-2S] protein (ISP) (125) and other mitochondrial proteins (126). It was suggested that the inappropriate processing of ISP by oxidatively modified core proteins may result in incorporation of the mis-folded ISP in CIII, resulting in mis-assembly of the catalytic site and inhibition of the enzymatic activity of complex. CYC1, an essential component of the inter-membrane-associated, central catalytic domain of CIII (127), accepts electron from ISP and transfers it to soluble CYC (128). The inability to accept or transfer electrons by oxidatively modified CYC1 would disrupt the electron flow-coupled proton translocation by the protonmotive Q-cycle and thus directly affect the CIII activity. Future studies would confirm the mechanistics of oxidative stress-induced CI and CIII inactivation in CCM. Nevertheless, the observation of dose-dependent HNE-mediated inhibition of respiratory complexes in the same study supports the idea that oxidative modifications contribute to inactivation of respiratory complexes in chagasic myocardium.

In other studies, the detection of a substantial depletion of mtDNA in chronically infected murine hearts associated with compromised levels of mtDNA-encoded transcripts has led to the suggestion that a limited biosynthesis of mitochondria-encoded protein subunits may contribute to reduced assembly of respiratory chain complexes in chagasic myocardium (16). What may cause mtDNA depletion in chagasic myocardium is not known. Given the detection of similar numbers of mitochondria in cardiac sections of infected mice exhibiting increasing severity of chronic disease and in normal mice, by transmission electron microscopic analysis (79), it is likely that mitochondrial biogenesis defects may not be the probable cause of mtDNA depletion in infected mice. Instead, numerous studies strongly support reactive species as playing a prominent role in mtDNA deletions through oxidative damage. MtDNA is highly susceptible to damage by reactive oxidants due to a lack of protective histones (129). Accumulation of significantly higher levels of DNA oxidation product 8-hydroxy deoxyguanosine in mtDNA compared to nuclear DNA and increased degradation of the mutated mtDNA are shown in a variety of *in vitro* and *in vivo* conditions of oxidative stress (102, 130, 131). It was postulated that ROS-induced alterations resulting in deletions or degradation of oxidatively damaged mtDNA contribute to mtDNA depletion, and subsequently, to decreased assembly and activity of respiratory complexes in chagasic myocardium.

7.3. Structural abnormalities

The mutations in genes encoding structural and contractile proteins are frequently implicated in the pathogenesis of cardiomyopathies. Specific mutations in structural and contractile proteins, e.g. actin (132), desmin (133), sarcoglycan, and dystrophin (134), are identified in many cases of DCM and heart failure. Similarly, mutations in myofibrillar/sarcomeric proteins, e.g. β-myosin heavy chain (β-MHC), cardiac troponin T (cTnT), tropomyosin, and myosin binding protein C (MYBP-C) have been identified in cases of familial HCM (reviewed in (135)). *In vitro* and *in vivo* studies have confirmed mutations in β-

MHC, MYBP-C, actin and some other proteins are presented with excess production of mutated or normal protein that stimulate the phenotype of hypertrophy and fibrosis (135-137). In other cases, mutation in desmin and cTnT are shown to result in decreased protein expression, cardiac degeneration and impaired contractility (133, 138). Numerous ultrastructural studies have suggested potential association of intermediate filaments with mitochondria. The intracellular position and movement of mitochondria and meiosis-dependent mitochondrial rearrangement are suggested to be governed by cytoskeletal proteins (139). The interplay of mitochondrial defects with alterations in contractile and/or structural proteins is, therefore, worthy of note. For example, patients with defects in β-MHC expression are shown to exhibit a decline in respiration capacity consistent with a reduction in mitochondria number (140). Impaired energy metabolism is linked to gene mutations in β-MHC, cTnT and MYBP-C in hypertrophic cardiomyopathy patients (141). Intracellular distribution of mitochondria and respiratory function are profoundly altered with defects in desmin (142). In CCM patients, mutations in any of the structural and contractile proteins encoding genes are yet to be identified. However, numerous studies document the morphological modifications in the myofibers, and alterations in the expression of a variety of structural and contractile proteins in human patients and animal models of CCM development (79, 82) (unpublished results). The defective cellular location of mitochondria, a likely outcome of cytoskeletal organizational abnormalities, may have potential downstream effects on cardiac bioenergetic function and consequently contribute to cardiac pathophysiology in CCM patients.

8. PHYSIOLOGICAL EFFECTS OF MITOCHONDRIAL DYSFUNCTION

Three of the most important aspects of mitochondrial OXPHOS dysfunction for disease pathogenesis are i) energy depletion; ii) regulation of apoptosis or programmed cell death, and iii) oxidative stress. Whether mitochondrial dysfunction of OXPHOS pathway compromises the availability of energy for contractile and other metabolic functions in chagasic hearts, and whether mitochondrial release of ROS and/or cytochrome c contribute to apoptotic cell death in chagasic myocardium is not known and hopefully will be examined in future. I summarize the published literature addressing the sustenance of oxidative stress-induced damage in chagasic myocardium.

As discussed above, chagasic hearts are likely to be exposed to ROS of inflammatory and mitochondrial origin. To cope with free radicals, the myocardium contains high concentrations of various non-enzymatic antioxidants such as reduced glutathione (GSH) and vitamins A, C, and E, and enzymatic scavengers of ROS including glutathione peroxidase (GPx), glutathione reductase (GSR) and SOD (143). GSH, GPx, and SOD have been shown to be most critical in cardiac antioxidant defenses (144). These enzymes work in tandem to scavenge ROS. SOD is present in the cytoplasm, as well as on the endothelial cell surface

(Cu or ZnSOD) and in the mitochondria (MnSOD). The SOD catalyzes the dismutation of superoxide anion ($O_2^{\cdot-}$) to H_2O_2 that is reduced to H_2O and O_2 by GPx. GPx scavenges H_2O_2 in the presence of GSH to form H_2O and oxidized glutathione (GSSG). GSR complements the action of GPx by converting GSSG to GSH (145). Under conditions of increased ROS production, or when the antioxidant system is compromised, cells are unable to efficiently scavenge the free radicals, resulting in ROS-induced oxidative stress (90, 107, 143, 146). The myocardial cells, when oxidatively stressed, may exhibit saturation of the antioxidant defenses, loss of intracellular redox homeostasis, alterations in cellular signaling, and induction of pathological processes (90, 91, 147, 148).

In a series of recent studies, us and others have addressed the oxidative status and antioxidant defense capabilities during the course of infection and progression of chagasic disease in human patients and experimental models. The demonstration of a selenium deficiency that increased with severity of chronic disease in chagasic patients (149) was probably the first observation suggesting that antioxidant deficiencies may be related to the progression of disease pathology. Further studies in experimental CCM models showed that selenium-depletion was associated with increased susceptibility, myocarditis severity, and heart damage (150), leading to higher mortality rate (151). The myocardial damage in infected mice was arrested or reversed upon dietary supplementation with low doses of selenium (152). Considering no effect on parasite burden was observed in animals depleted or supplemented with dietary selenium, the direct beneficial effects of selenium in protecting heart from inflammatory and/or oxidative damage was concluded. In other studies, the detection of inflammatory cytotoxic mediators (TNF-α and NO) along with a reduction in plasma levels of GPx and SOD in patients led to a suggestion that an oxidant/antioxidant imbalance may drive the chagasic disease pathology (153). We have shown that when antioxidant defense responses (constituted by GPx, GSR, and GSH) were of sufficient magnitude (e.g. in skeletal muscle), *T. cruzi*-induced oxidative stress and damage was controlled (103). However, myocardium appeared to be poorly equipped with antioxidant defenses. In response to *T. cruzi*, though a transient increase in antioxidant enzyme activities (SOD, GPx, GSR) and reductant (GSH) level was noted in the myocardium of infected mice, these responses subsided or decreased with disease development. Consequently, myocardium of infected animals sustained oxidative damage evidenced by consistent increase in oxidative stress biomarkers (LPO, PCO, GSSG) during the course of infection and chronic disease (103). Altogether, these studies imply that sustained ROS generation (of inflammatory and mitochondrial origin, discussed in section 7.1) coupled with inadequate antioxidant response resulting in inefficient scavenging of ROS in the heart leads to sustained oxidative damage of the cardiac cellular components during chagasic disease. A passive antioxidant response to increased oxidative stress is shown in experimental models of ischemia/reperfusion (154) and dicroceliosis (155), and human end stage heart failure (156). Future studies would determine whether treatment with antioxidant mimics or pharmacological agents capable of enhancing the endogenous antioxidant defense response along with anti-parasite drugs would be promising avenues in preventing myocardial pathology in chagasic patients.

9. SUMMARY

Several factors may contribute to the pathogenic outcome of chagasic disease. Host inflammatory and immune responses are activated to control the parasite burden. During the process of parasite destruction, inflammatory mediators may also injure the host cellular and organelle components. Mitochondria being particularly susceptible to stress-mediated damage are probably affected most, resulting in mitochondrial abnormalities at the molecular, biochemical and functional level. The deficiencies of CI and CIII enzyme complex activities is likely to create a positive feed back cycle, as these complexes in addition to being sensitive to stress, are also the primary site of oxygen radical production, leading to progressively greater levels of oxidative stress and lowered mitochondrial function. The major physiological effects of the mitochondrial disorders are energy deficit and sustained ROS production, both of which can contribute to CCM pathogenesis. The current literature suggest that oxidative damage of cellular components (DNA, lipids, and proteins) contributes to mitochondrial respiratory chain dysfunction and sustained ROS generation, and subsequently may drive cell death and tissue damage in CCM. The promising areas of research that may yield practical benefits related to treatment of CCM include a critical evaluation of the role of mitochondrial ROS in activation of the signaling cascades (e.g. MAPKs) that may be involved in instigation of cardiac hypertrophy and remodeling responses, and cellular damage. Future studies geared to testing the usefulness of therapies capable of enhancing mitochondrial function, antioxidant efficiency, or ROS scavenging in combination with anti-parasite drugs will provide convincing evidence to link the oxidative stress as a causative mechanism in the development of CCM. These studies will also provide a clue to the upstream and downstream events in redox-induced cardiac pathology and suggest the usefulness of antioxidant therapies in arresting the severity of chagasic pathology.

10. ACKNOWLEDGEMENTS

11. REFERENCES

1. Poetter, K., H. Jiang, S. Hassanzadeh, S. R. Master, A. Chang, M. C. Dalakas, I. Rayment, J. R. Sellers, L.

Fananapazir and N. D. Epstein: Mutations in either the essential or regulatory light chains of myosin are associated with a rare myopathy in human heart and skeletal muscle, *Nat Genet* 13, 63-9. (1996)

2. Rust, E. M., F. P. Albayya and J. M. Metzger: Identification of a contractile deficit in adult cardiac myocytes expressing hypertrophic cardiomyopathy-associated mutant troponin T proteins, *J Clin Invest* 103, 1459-67 (1999)

3. Schaub, M. C., M. A. Hefti, R. A. Zuellig and I. Morano: Modulation of contractility in human cardiac hypertrophy by myosin essential light chain isoforms, *Cardiovasc Res* 37, 381-404 (1998)

4. Moss, R. L. and J. S. Periera: Enhanced myosin function due to a point mutation causing a familial hypertrophic cardiomyopathy, *Circ Res* 86, 720-2 (2000)

5. Nicol, R. L., N. Frey and E. N. Olson: From the sarcomere to the nucleus: role of genetics and signaling in structural heart disease, *Annu Rev Genomics Hum Genet* 1, 179-223 (2000)

6. Gomes, A. V. and J. D. Potter: Cellular and molecular aspects of familial hypertrophic cardiomyopathy caused by mutations in the cardiac troponin I gene, *Mol Cell Biochem* 263, 99-114 (2004)

7. Antozzi, C. and M. Zeviani: Cardiomyopathies in disorders of oxidative metabolism, *Cardiovasc Res* 35, 184-99 (1997)

8. Sharov, V. G., A. V. Todor, N. Silverman, S. Goldstein and H. N. Sabbah: Abnormal mitochondrial respiration in failed human myocardium, *J Mol Cell Cardiol* 32, 2361-7 (2000)

9. Bergmann, S. R., P. Herrero, R. Sciacca, J. J. Hartman, P. J. Rubin, K. T. Hickey, S. Epstein and D. P. Kelly: Characterization of altered myocardial fatty acid metabolism in patients with inherited cardiomyopathy, *J Inherit Metab Dis* 24, 65 7-74 (2001)

10. Marin-Garcia, J. and M. J. Goldenthal: Understanding the impact of mitochondrial defects in cardiovascular disease: a review, *J Card Fail* 8, 347-61 (2002)

11. Carvajal, K. and R. Moreno-Sánchez: Heart metabolic disturbances in cardiovascular diseases, *Arch Med Res* 34, 89-99 (2003)

12. Fernandez-Moreno, M. A., B. Bornstein, N. Petit and R. Garesse: The pathophysiology of mitochondrial biogenesis: towards four decades of mitochondrial DNA research, *Mol Genet Metab* 71, 481-95 (2000)

13. Bowles, N. E. and J. A. Towbin: Molecular aspects of myocarditis, *Curr Opin Cardiol* 13, 179-84 (1998)

14. Kearney, M. T., J. M. Cotton, P. J. Richardson and A. M. Shah: Viral myocarditis and dilated cardiomyopathy: mechanisms, manifestations, and management, *Postgrad Med J* 77, 4-10. (2001)

15. Lewis, W.: Mitochondrial DNA replication, nucleoside reverse-transcriptase inhibitors, and AIDS cardiomyopathy, *Prog Cardiovasc Dis* 45, 305-18 (2003)

16. Vyatkina, G., V. Bhatia, A. Gerstner, J. Papaconstantinou and N. Garg: Impaired mitochondrial respiratory chain and bioenergetics during chagasic cardiomyopathy development., *Biochim Biophys Acta* 1689, 162-173 (2004)

17. Garg, N., V. Bhatia, A. Gerstner, J. deFord and J. Papaconstantinou: Gene expression analysis in mitochondria from chagasic mice: Alterations in specific metabolic pathways, *Biochemical J.* 381, 743-752 (2004)

18. Starling, R. C., D. F. Hammer and R. A. Altschuld: Human myocardial ATP content and *in vivo* contractile function, *Mol Cell Biochem* 180, 171-7 (1998)

19. Jafri, M. S., S. J. Dudycha and B. O'Rourke: Cardiac energy metabolism: models of cellular respiration, *Annu Rev Biomed Eng* 3, 57-81 (2001)

20. Stanley, W. C. and M. P. Chandler: Energy metabolism in the normal and failing heart: potential for therapeutic interventions, *Heart Fail Rev* 7, 115-30 (2002)

21. Portman, M. A.: The adenine nucleotide translocator: regulation and function during myocardial development and hypertrophy, *Clin Exp Pharmacol Physiol* 29, 334-8 (2002)

22. Rodrigues, B. and J. H. McNeill: The diabetic heart: metabolic causes for the development of a cardiomyopathy, *Cardiovasc Res* 26, 913-22 (1992)

23. Lopaschuk, G. D., R. B. Wambolt and R. L. Barr: An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts, *J Pharmacol Exp Ther* 264, 135-44 (1993)

24. Ferrari, R., P. Pepi, F. Ferrari, F. Nesta, M. Benigno and O. Visioli: Metabolic derangement in ischemic heart disease and its therapeutic control, *Am J Cardiol* 82, 2K-13K (1998)

25. Sack, M. N. and D. P. Kelly: The energy substrate switch during development of heart failure: gene regulatory mechanisms, *Int J Mol Med* 1, 17-24 (1998)

26. Wallace, D. C.: Mitochondrial defects in cardiomyopathy and neuromuscular disease, *Am Heart J* 139, S70-85 (2000)

27. Garesse, R. and C. G. Vallejo: Animal mitochondrial biogenesis and function: a regulatory cross-talk between two genomes, *Gene* 263, 1-16 (2001)

28. Nijtmans, L. G. J., C. Ugalde, L. P. Van den Heuvel and J. A. M. Smeitink: Function and dysfunction of the oxidative phosphorylation system, In: *Mitochondrial Function and Biogenetics*. Eds: Kohler, C. and Bauer, M. F., Springer-Verlag, Berlin 8, 149-176 (2004)

29. Suomalainen, A.: Mitochondrial DNA and disease, *Ann Med* 29, 235-46 (1997)

30. Fernandez-Silva, P., J. A. Enriquez and J. Montoya: Replication and transcription of mammalian mitochondrial DNA, *Exp Physiol* 88, 41-56 (2003)

31. Simon, D. K. and D. R. Johns: Mitochondrial disorders: clinical and genetic features, *Annu Rev Med* 50, 111-27 (1999)

32. Neely, J. R., M. J. Rovetto and J. F. Oram: Myocardial utilization of carbohydrate and lipids, *Prog Cardiovasc Dis* 15, 289-329 (1972)

33. Neely, J. R. and H. E. Morgan: Relationship Between Carbohydrate and Lipid Metabolism and the Energy Balance of Heart Muscle, *Ann Rev Physiol* 36, 413-459 (1974)

34. Lopaschuk, G. D., M. A. Spafford and D. R. Marsh: Glycolysis is predominant source of myocardial ATP production immediately after birth, *Am J Physiol Heart Circ Physiol* 261, H1698-1705 (1991)

35. Saddik, M., J. Gamble, L. A. Witters and G. D. Lopaschuk: Acetyl-CoA carboxylase regulation of fatty

acid oxidation in the heart, *J Biol Chem* 268, 25836-45 (1993)

36. Prip-Buus, C., J. P. Pegorier, P. H. Duee, C. Kohl and J. Girard: Evidence that the sensitivity of carnitine palmitoyltransferase I to inhibition by malonyl-CoA is an important site of regulation of hepatic fatty acid oxidation in the fetal and newborn rabbit. Perinatal development and effects of pancreatic hormones in cultured rabbit hepatocytes, *Biochem J* 269, 409-15 (1990)

37. Lavrentyev, E. N., D. He and G. A. Cook: Expression of genes participating in regulation of fatty acid and glucose utilization and energy metabolism in developing rat hearts, *Am J Physiol Heart Circ Physiol* 287, H2035-42 (2004)

38. Abdel-aleem, S., J. St Louis, S. C. Hendrickson, H. M. El-Shewy, K. El-Dawy, D. A. Taylor and J. E. Lowe: Regulation of carbohydrate and fatty acid utilization by L-carnitine during cardiac development and hypoxia, *Mol Cell Biochem* 180, 95-103 (1998)

39. Abdel-Aleem, S., J. D. St Louis, G. C. Hughes and J. E. Lowe: Metabolic changes in the normal and hypoxic neonatal myocardium, *Ann N Y Acad Sci* 874, 254-61 (1999)

40. McGarry, J. D. and D. W. Foster: Regulation of hepatic fatty acid oxidation and ketone body production, *Annu Rev Biochem* 49, 395-420 (1980)

41. Brown, N. F., B. C. Weis, J. E. Husti, D. W. Foster and J. D. McGarry: Mitochondrial carnitine palmitoyltransferase I isoform switching in the developing rat heart, *J Biol Chem* 270, 8952-7 (1995)

42. Park, E. A. and G. A. Cook: Differential regulation in the heart of mitochondrial carnitine palmitoyltransferase-I muscle and liver isoforms, *Mol Cell Biochem* 180, 27-32 (1998)

43. Marin-Garcia, J., R. Ananthakrishnan and M. J. Goldenthal: Heart mitochondrial DNA and enzyme changes during early human development, *Mol Cell Biochem* 210, 47-52 (2000)

44. Marin-Garcia, J., R. Ananthakrishnan and M. J. Goldenthal: Human mitochondrial function during cardiac growth and development, Mol Cell Biochem 179, 21-6 (1998)

45. Luft, R., D. Ikkos, G. Palmieri, L. Ernster and B. Afzelius: A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical, and morphological study, J Clin Invest 41, 1776-804 (1962)

46. Finsterer, J.: Mitochondriopathies, Eur J Neurol 11, 163-86 (2004)

47. Sperl, W.: Cardiomyopathies and mitochondrial defects of oxidative energy metabolism, In: Metabolic Cardiomyopathy. Eds: Bohles, H. and Sewell, A. C., Medpharm Scientific Publishers, Stuttgart 67-84 (2004)

48. DiMauro, S. and E. A. Schon: Mitochondrial respiratory-chain diseases, N Engl J Med 348, 2656-68 (2003)

49. Sewell, A. C.: Laboratory diagnosis of metabolic diseases presenting with cardiomyopathy, In: Metabolic Cardiomyopathy. Eds: Bohles, H. and Sewell, A. C., Medpharm Scientific Publishers, Stuttgart (2004)

50. Dimauro, S., S. Tay and M. Mancuso: Mitochondrial encephalomyopathies: diagnostic approach, *Ann N Y Acad Sci* 1011, 217-31 (2004)

51. Takeda, N.: Cardiomyopathies and mitochondrial DNA mutations, *Mol Cell Biochem* 176, 287-90 (1997)

52. Moraes, C. T., F. Ciacci, E. Bonilla, C. Jansen, M. Hirano, N. Rao, R. E. Lovelace, L. P. Rowland, E. A. Schon and S. DiMauro: Two novel pathogenic mitochondrial DNA mutations affecting organelle number and protein synthesis. Is the tRNA(Leu(UUR)) gene an etiologic hot spot?, *J Clin Invest* 92, 2906-15 (1993)

53. Marin-Garcia, J., M. J. Goldenthal, R. Ananthakrishnan and M. E. Pierpont: The complete sequence of mtDNA genes in idiopathic dilated cardiomyopathy shows novel missense and tRNA mutations, *J Card Fail* 6, 321-9 (2000)

54. Sternberg, D., E. Chatzoglou, P. Laforet, G. Fayet, C. Jardel, P. Blondy, M. Fardeau, S. Amselem, B. Eymard and A. Lombes: Mitochondrial DNA transfer RNA gene sequence variations in patients with mitochondrial disorders, *Brain* 124, 984-94 (2001)

55. Arbustini, E., M. Diegoli, R. Fasani, M. Grasso, P. Morbini, N. Banchieri, O. Bellini, B. Dal Bello, A. Pilotto, G. Magrini, C. Campana, P. Fortina, A. Gavazzi, J. Narula and M. Vigano: Mitochondrial DNA mutations and mitochondrial abnormalities in dilated cardiomyopathy, *Am J Pathol* 153, 1501-10 (1998)

56. Ruppert, V., D. Nolte, T. Aschenbrenner, S. Pankuweit, R. Funck and B. Maisch: Novel point mutations in the mitochondrial DNA detected in patients with dilated cardiomyopathy by screening the whole mitochondrial genome, *Biochem Biophys Res Commun* 318, 535-43 (2004)

57. Marin-Garcia, J., Y. Hu, R. Ananthakrishnan, M. E. Pierpont, G. L. Pierpont and M. J. Goldenthal: A point mutation in the cytb gene of cardiac mtDNA associated with complex III deficiency in ischemic cardiomyopathy, *Biochem Mol Biol Int* 40, 487-95 (1996)

58. Keightley, J. A., R. Anitori, M. D. Burton, F. Quan, N. R. Buist and N. G. Kennaway: Mitochondrial encephalomyopathy and complex III deficiency associated with a stop-codon mutation in the cytochrome b gene, *Am J Hum Genet* 67, 1400-10 (2000)

59. Marin-Garcia, J., M. J. Goldenthal, R. Ananthakrishnan and D. Mirvis: Specific mitochondrial DNA deletions in canine myocardial ischemia, *Biochem Mol Biol Int* 40, 1057-65 (1996)

60. Zeng, Z., Z. Zhang, H. Yu, M. J. Corbley, Z. Tang and T. Tong: Mitochondrial DNA deletions are associated with ischemia and aging in Balb/c mouse brain, *J Cell Biochem* 73, 545-53 (1999)

61. Tokoro, T., H. Ito and T. Suzuki: Alterations in mitochondrial DNA and enzyme activities in hypertrophied myocardium of stroke-prone SHRS, *Clin Exp Hypertens* 18, 595-606 (1996)

62. Arai, T., K. Nakahara, H. Matsuoka, M. Sawabe, K. Chida, S. Matsushita, K. Takubo, N. Honma, K. Nakamura, N. Izumiyama and Y. Esaki: Age-related mitochondrial DNA deletion in human heart: its relationship with cardiovascular diseases, *Aging Clin Exp Res* 15, 1-5 (2003)

63. Sato, W., M. Tanaka, S. Sugiyama, K. Hattori, T. Ito, H. Kawaguchi, H. Onozuka, H. Yasuda, K. Ito, G. Takada and *et al.*: Deletion of mitochondrial DNA in a patient with conduction block, *Am Heart J* 125, 550-2 (1993)

64. Katsanos, K. H., C. J. Pappas, D. Patsouras, L. K. Michalis, G. Kitsios, M. Elisaf and E. V. Tsianos: Alarming atrioventricular block and mitral valve prolapse in the Kearns-Sayre syndrome, *Int J Cardiol* 83, 179-81 (2002)

65. Ide, T., H. Tsutsui, S. Hayashidani, D. Kang, N. Suematsu, K. Nakamura, H. Utsumi, N. Hamasaki and A. Takeshita: Mitochondrial DNA damage and dysfunction associated with oxidative stress in failing hearts after myocardial infarction, *Circ Res* 88, 529-35 (2001)

66. Wong, L. J.: Comprehensive molecular diagnosis of mitochondrial disorders: qualitative and quantitative approach, *Ann N Y Acad Sci* 1011, 246-58 (2004)

67. Bai, R. K., C. L. Perng, C. H. Hsu and L. J. Wong: Quantitative PCR analysis of mitochondrial DNA content in patients with mitochondrial disease, *Ann N Y Acad Sci* 1011, 304-9 (2004)

68. World, Health Organization: Chagas disease: Tropical diseases progress in research, 1997-1998, WHO Technical Report Series 1, 1 (1999)

69. Rossi, M. A., S. G. Ramos and R. B. Bestetti: Chagas' heart disease: clinical-pathological correlation, *Front Biosci* 8, e94-109 (2003)

70. Carrasco Guerra, H. A., E. Palacios-Pru, C. Dagert de Scorza, C. Molina, G. Inglessis and R. V. Mendoza: Clinical, histochemical, and ultrastructural correlation in septal endomyocardial biopsies from chronic chagasic patients: detection of early myocardial damage, *Am Heart J* 113, 716-24 (1987)

71. Higuchi, M. D., L. A. Benvenuti, M. Martins Reis and M. Metzger: Pathophysiology of the heart in Chagas' disease: current status and new developments, *Cardiovasc Res* 60, 96-107 (2003)

72. Palacios-Pru, E., H. Carrasco, C. Scorza and R. Espinoza: Ultrastructural characteristics of different stages of human chagasic myocarditis, *Am J Trop Med Hyg* 41, 29-40 (1989)

73. Parada, H., H. A. Carrasco, N. Anez, C. Fuenmayor and I. Inglessis: Cardiac involvement is a constant finding in acute Chagas' disease: a clinical, parasitological and histopathological study, *Int J Cardiol* 60, 49-54 (1997)

74. Carrasco, H. A., J. S. Barboza, G. Inglessis, A. Fuenmayor and C. Molina: Left ventricular cineangiography in Chagas disease: detection of early myocardial damage, *Am Heart J* 104, 595-602 (1982)

75. Inglessis, I., H. A. Carrasco, N. Anez, C. Fuenmayor, H. Parada, J. A. Pacheco and H. R. Carrasco: [Clinical, parasitological and histopathologic follow-up studies of acute Chagas patients treated with benznidazole], *Arch Inst Cardiol Mex* 68, 405-10 (1998)

76. Espinosa, R., H. A. Carrasco, F. Belandria, A. M. Fuenmayor, C. Molina, R. Gonzalez and O. Martinez: Life expectancy analysis in patients with Chagas' disease: prognosis after one decade (1973-1983), *Int J Cardiol* 8, 45-56 (1985)

77. Pimenta, J., N. Valente and M. Miranda: Long-term follow up of asymptomatic chagasic individuals with intraventricular conduction disturbances, correlating with non-chagasic patients, *Rev Soc Bras Med Trop* 32, 621-31 (1999)

78. Leite, L. R., G. Fenelon, A. Simoes, Jr., G. G. Silva, P. A. Friedman and A. A. de Paola: Clinical usefulness of electrophysiologic testing in patients with ventricular tachycardia and chronic chagasic cardiomyopathy treated with amiodarone or sotalol, *J Cardiovasc Electrophysiol* 14, 567-73 (2003)

79. Garg, N., V. L. Popov and J. Papaconstantinou: Profiling gene transcription reveals a deficiency of mitochondrial oxidative phosphorylation in *Trypanosoma cruzi*-infected murine hearts: implications in chagasic myocarditis development, *Biochim Biophys Acta* 1638, 106-20 (2003)

80. Alarcon-Corredor, O. M., H. Carrasco-Guerra, M. Ramirez de Fernandez and W. Leon: Serum enzyme pattern and local enzyme gradients in chronic chagasic patients, Acta Cient Venez 53, 210-7 (2002)

81. Carrasco, H. A., M. Alarcon, L. Olmos, J. Burguera, M. Burguera, A. Dipaolo and H. R. Carrasco: Biochemical characterization of myocardial damage in chronic Chagas' disease, *Clin Cardiol* 20, 865-9 (1997)

82. Mukherjee, S., T. J. Belbin, D. C. Spray, D. A. Iacobas, L. M. Weiss, R. N. Kitsis, M. Wittner, L. A. Jelicks, P. E. Scherer, A. Ding and H. B. Tanowitz: Microarray analysis of changes in gene expression in a murine model of chronic chagasic cardiomyopathy, *Parasitol Res* 91, 187-96 (2003)

83. Calvani, M., E. Reda and E. Arrigoni-Martelli: Regulation by carnitine of myocardial fatty acid and carbohydrate metabolism under normal and pathological conditions, *Basic Res Cardiol* 95, 75-83 (2000)

84. Uyemura, S. A., S. Albuquerque and C. Curti: Energetics of heart mitochondria during acute phase of *Trypanosoma cruzi* infection in rats, *Int J Biochem Cell Biol* 27, 1183-9. (1995)

85. Uyemura, S. A., M. C. Jordani, A. C. Polizello and C. Curti: Heart FoF1-ATPase changes during the acute phase of *Trypanosoma cruzi* infection in rats, *Mol Cell Biochem* 165, 127-33 (1996)

86. Schirmer, R. H., T. Schollhammer, G. Eisenbrand and R. L. Krauth-Siegel: Oxidative stress as a defense mechanism against parasitic infections, *Free Radic Res Commun* 3, 3-12 (1987)

87. Lima, E. C., I. Garcia, M. H. Vicentelli, P. Vassalli and P. Minoprio: Evidence for a protective role of tumor necrosis factor in the acute phase of *Trypanosoma cruzi* infection in mice, *Infect Immun* 65, 457-65 (1997)

88. Munoz-Fernandez, M. A., M. A. Fernandez and M. Fresno: Synergism between tumor necrosis factor-alpha and interferon-gamma on macrophage activation for the killing of intracellular *Trypanosoma cruzi* through a nitric oxide-dependent mechanism, *Eur J Immunol* 22, 301-7 (1992)

89. Cardoni, R. L., M. I. Antunez, C. Morales and I. R. Nantes: Release of reactive oxygen species by phagocytic cells in response to live parasites in mice infected with *Trypanosoma cruzi*, *Am J Trop Med Hyg* 56, 329-34 (1997)

90. Martindale, J. L. and N. J. Holbrook: Cellular response to oxidative stress: signaling for suicide and survival, J Cell Physiol 192, 1-15 (2002)

91. Ueda, S., H. Masutani, H. Nakamura, T. Tanaka, M. Ueno and J. Yodoi: Redox control of cell death, *Antioxid Redox Signal* 4, 405-14 (2002)

92. Ide, T., H. Tsutsui, S. Kinugawa, H. Utsumi, D. Kang, N. Hattori, K. Uchida, K. Arimura, K. Egashira and A.

Takeshita: Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium, *Circ Res* 85, 357-63 (1999)

93. Chen, Q., E. J. Vazquez, S. Moghaddas, C. L. Hoppel and E. J. Lesnefsky: Production of reactive oxygen species by mitochondria: Central role of complex III, *J Biol Chem* 278(38), 36027-31 (2003)

94. Sawyer, D. B. and W. S. Colucci: Oxidative stress in heart failure, In: *Molecular Approaches to Heart Failure therapy*. Eds: Hasenfuss, G. and Marban, E., Darmstadt : Steinkopff, 262-284 (2000)

95. Cuzzocrea, S., D. P. Riley, A. P. Caputi and D. Salvemini: Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury, *Pharmacol Rev* 53, 135-59. (2001)

96. Lesnefsky, E. J., T. I. Gudz, C. T. Migita, M. Ikeda-Saito, M. O. Hassan, P. J. Turkaly and C. L. Hoppel: Ischemic injury to mitochondrial electron transport in the aging heart: damage to the iron-sulfur protein subunit of electron transport complex III, *Arch Biochem Biophys* 385, 117-28 (2001)

97. Lucas, D. T. and L. I. Szweda: Declines in mitochondrial respiration during cardiac reperfusion: age-dependent inactivation of alpha-ketoglutarate dehydrogenase, *Proc Natl Acad Sci USA* 96, 6689-93 (1999)

98. Sadek, H. A., K. M. Humphries, P. A. Szweda and L. I. Szweda: Selective inactivation of redox-sensitive mitochondrial enzymes during cardiac reperfusion, *Arch Biochem Biophys* 406, 222-8 (2002)

99. Piper, H. M., T. Noll and B. Siegmund: Mitochondrial function in the oxygen depleted and reoxygenated myocardial cell, *Cardiovasc Res* 28, 1-15 (1994)

100. Vercesi, A. E., A. J. Kowaltowski, M. T. Grijalba, A. R. Meinicke and R. F. Castilho: The role of reactive oxygen species in mitochondrial permeability transition, *Biosci Rep* 17, 43-52 (1997)

101. Cardoso, S. M., C. Pereira and R. Oliveira: Mitochondrial function is differentially affected upon oxidative stress, Free Radic Biol Med 26, 3-13 (1999)

102. Williams, M. D., H. Van Remmen, C. C. Conrad, T. T. Huang, C. J. Epstein and A. Richardson: Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice, J Biol Chem 273, 28510-5 (1998)

103. Wen, J.-J., G. Vyatkina and N. Garg: Oxidative damage during chagasic cardiomyopathy development: Role of mitochondrial oxidant release and inefficient antioxidant defense, Free Radic Biol Med 37, 1821-33 (2004)

104. Van Remmen, H., M. D. Williams, Z. Guo, L. Estlack, H. Yang, E. J. Carlson, C. J. Epstein, T. T. Huang and A. Richardson: Knockout mice heterozygous for Sod2 show alterations in cardiac mitochondrial function and apoptosis, Am J Physiol Heart Circ Physiol 281, H1422-32 (2001)

105. Li, Y., T. T. Huang, E. J. Carlson, S. Melov, P. C. Ursell, J. L. Olson, L. J. Noble, M. P. Yoshimura, C. Berger, P. H. Chan and et al.: Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase, Nat Genet 11, 376-81 (1995)

106. Sawyer, D. B. and W. S. Colucci: Mitochondrial oxidative stress in heart failure: "oxygen wastage" revisited, *Circ Res* 86, 119-20. (2000)

107. Tsutsui, H.: Oxidative stress in heart failure: the role of mitochondria, *Intern Med* 40, 1177-82 (2001)

108. Paradies, G., G. Petrosillo, M. Pistolese, N. Di Venosa, D. Serena and F. M. Ruggiero: Lipid peroxidation and alterations to oxidative metabolism in mitochondria isolated from rat heart subjected to ischemia and reperfusion, *Free Radic Biol Med* 27, 42-50. (1999)

109. Esterbauer, H.: In: Free radicals, lipid peroxidation and cancer. Eds: McBrien, D. C. H. and Slater, T. F., Academic Press, London pp. 101-128 (1982)

110. Uchida, K. and E. R. Stadtman: Modification of histidine residues in proteins by reaction with 4-hydroxynonenal, *Proc Natl Acad Sci USA* 89, 4544-8 (1992)

111. Butterfield, D. A., T. Koppal, B. Howard, R. Subramaniam, N. Hall, K. Hensley, S. Yatin, K. Allen, M. Aksenov, M. Aksenova and J. Carney: Structural and functional changes in proteins induced by free radical-mediated oxidative stress and protective action of the antioxidants N-tert-butyl-alpha-phenylnitrone and vitamin E, *Ann N Y Acad Sci* 854, 448-62 (1998)

112. Chevion, M., E. Berenshtein and E. R. Stadtman: Human studies related to protein oxidation: protein carbonyl content as a marker of damage, Free Radic Res 33 Suppl, S99-108 (2000)

113. Wen, J.-J. and N. Garg: Oxidative modifications of mitochondrial respiratory complexes in response to the stress of Trypanosoma cruzi infection, Free Radic Biol Med 37, 2072-81 (2004)

114. Ohkawa, H., N. Ohishi and T. Kunio: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Analytical Biochem 95, 351-358 (1979)

115. Nourooz-Zadeh, J., J. Tajaddini-Sarmadi and S. P. Wolff: Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine, Anal Biochem 220, 403-9 (1994)

116. Levine, R. L., D. Garland, C. N. Oliver, A. Amici, I. Climent, A. G. Lenz, B. W. Ahn, S. Shaltiel and E. R. Stadtman: Determination of carbonyl content in oxidatively modified proteins, Methods Enzymol 186, 464-78 (1990)

117. Papa, S., A. M. Sardanelli, S. Scacco, V. Petruzzella, Z. Technikova-Dobrova, R. Vergari and A. Signorile: The NADH: ubiquinone oxidoreductase (complex I) of the mammalian respiratory chain and the cAMP cascade, J Bioenerg Biomembr 34, 1-10 (2002)

118. Carroll, J., I. M. Fearnley, R. J. Shannon, J. Hirst and J. E. Walker: Analysis of the subunit composition of complex I from bovine heart mitochondria, Mol Cell Proteomics 2, 117-26 (2003)

119. Benit, P., D. Chretien, N. Kadhom, P. de Lonlay-Debeney, V. Cormier-Daire, A. Cabral, S. Peudenier, P. Rustin, A. Munnich and A. Rotig: Large-scale deletion and point mutations of the nuclear NDUFV1 and NDUFS1 genes in mitochondrial complex I deficiency, Am J Hum Genet 68, 1344-52 (2001)

120. Loeffen, J., O. Elpeleg, J. Smeitink, R. Smeets, S. Stockler-Ipsiroglu, H. Mandel, R. Sengers, F. Trijbels and L. Van den Heuvel: Mutations in the complex I NDUFS2 gene of patients with cardiomyopathy and encephalomyopathy, Ann Neurol 49, 195-201 (2001)

121. Budde, S. M., L. P. van den Heuvel, A. J. Janssen, R. J. Smeets, C. A. Buskens, L. DeMeirleir, R. Van Coster, M.

Baethmann, T. Voit, J. M. Trijbels and J. A. Smeitink: Combined enzymatic complex I and III deficiency associated with mutations in the nuclear encoded NDUFS4 gene, *Biochem Biophys Res Commun* 275, 63-8 (2000)

122. Schuelke, M., J. Smeitink, E. Mariman, J. Loeffen, B. Plecko, F. Trijbels, S. Stockler-Ipsiroglu and L. Van den Heuvel: Mutant NDUFV1 subunit of mitochondrial complex I causes leukodystrophy and myoclonic epilepsy, *Nat Genet* 21, 260-1 (1999)

123. Murray, J., S. W. Taylor, B. Zhang, S. S. Ghosh and R. A. Capaldi: Oxidative damage to mitochondrial complex I due to peroxynitrite: identification of reactive tyrosines by mass spectrometry, *J Biol Chem* 278, 37223-30 (2003)

124. Braun, H. P. and U. K. Schmitz: Are the 'core' proteins of the mitochondrial bc1 complex evolutionary relics of a processing protease?, *Trends Biochem Sci* 20, 171-5 (1995)

125. Iwata, S., J. W. Lee, K. Okada, J. K. Lee, M. Iwata, B. Rasmussen, T. A. Link, S. Ramaswamy and B. K. Jap: Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex, *Science* 281, 64-71 (1998)

126. Deng, K., L. Zhang, A. M. Kachurin, L. Yu, D. Xia, H. Kim, J. Deisenhofer and C. A. Yu: Activation of a matrix processing peptidase from the crystalline cytochrome bc1 complex of bovine heart mitochondria, *J Biol Chem* 273, 20752-7 (1998)

127. Robertson, D. E., H. Ding, P. R. Chelminski, C. Slaughter, J. Hsu, C. Moomaw, M. Tokito, F. Daldal and P. L. Dutton: Hydroubiquinone-cytochrome c2 oxidoreductase from *Rhodobacter capsulatus*: definition of a minimal, functional isolated preparation, *Biochem* 32, 1310-7 (1993)

128. Schagger, H., U. Brandt, S. Gencic and G. von Jagow: Ubiquinol-cytochrome-c reductase from human and bovine mitochondria, *Methods Enzymol* 260, 82-96 (1995)

129. Wei, Y. H., C. Y. Lu, H. C. Lee, C. Y. Pang and Y. S. Ma: Oxidative damage and mutation to mitochondrial DNA and age-dependent decline of mitochondrial respiratory function, *Ann N Y Acad Sci* 854, 155-70 (1998)

130. Palmeira, C. M., J. Serrano, D. W. Kuehl and K. B. Wallace: Preferential oxidation of cardiac mitochondrial DNA following acute intoxication with doxorubicin, *Biochim Biophys Acta* 1321, 101-6 (1997)

131. Serrano, J., C. M. Palmeira, D. W. Kuehl and K. B. Wallace: Cardioselective and cumulative oxidation of mitochondrial DNA following subchronic doxorubicin administration, *Biochim Biophys Acta* 1411, 201-5 (1999)

132. Goebel, H. H., K. Brockmann, C. G. Bonnemann, I. A. Warlo, F. Hanefeld, S. Labeit, H. J. Durling and N. G. Laing: Actin-related myopathy without any missense mutation in the ACTA1 gene, *J Child Neurol* 19, 149-53 (2004)

133. Goebel, H. H.: Desmin-related myopathies, *Curr Opin Neurol* 10, 426-9 (1997)

134. Finsterer, J. and C. Stollberger: The heart in human dystrophinopathies, *Cardiol* 99, 1-19 (2003)

135. Roberts, R. and U. Sigwart: New concepts in hypertrophic cardiomyopathies, part I, *Circulation* 104, 2113-6 (2001)

136. Goebel, H. H. and I. Warlo: Gene-related protein surplus myopathies, *Mol Genet Metab* 71, 267-75 (2000)

137. Goebel, H. H. and I. A. Warlo: Surplus protein myopathies, *Neuromuscul Disord* 11, 3-6 (2001)

138. Gomes, A. V., J. A. Barnes, K. Harada and J. D. Potter: Role of troponin T in disease, *Mol Cell Biochem* 263, 115-29 (2004)

139. Rube, D. A. and A. M. van der Bliek: Mitochondrial morphology is dynamic and varied, *Mol Cell Biochem* 256-257, 331-9 (2004)

140. Thompson, C. H., G. J. Kemp, D. J. Taylor, M. Conway, B. Rajagopalan, A. O'Donoghue, P. Styles, W. J. McKenna and G. K. Radda: Abnormal skeletal muscle bioenergetics in familial hypertrophic cardiomyopathy, *Heart* 78, 177-81 (1997)

141. Crilley, J. G., E. A. Boehm, E. Blair, B. Rajagopalan, A. M. Blamire, P. Styles, W. J. McKenna, I. Ostman-Smith, K. Clarke and H. Watkins: Hypertrophic cardiomyopathy due to sarcomeric gene mutations is characterized by impaired energy metabolism irrespective of the degree of hypertrophy, *J Am Coll Cardiol* 41, 1776-82 (2003)

142. Milner, D. J., M. Mavroidis, N. Weisleder and Y. Capetanaki: Desmin cytoskeleton linked to muscle mitochondrial distribution and respiratory function, *J Cell Biol* 150, 1283-98 (2000)

143. Dhalla, N. S., A. B. Elmoselhi, T. Hata and N. Makino: Status of myocardial antioxidants in ischemia-reperfusion injury, *Cardiovasc Res* 47, 446-56. (2000)

144. Marczin, N., N. El-Habashi, G. S. Hoare, R. E. Bundy and M. Yacoub: Antioxidants in myocardial ischemia-reperfusion injury: therapeutic potential and basic mechanisms, *Arch Biochem Biophys* 420, 222-36 (2003)

145. Michiels, C., M. Raes, O. Toussaint and J. Remacle: Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress, *Free Radic Biol Med* 17, 235-48 (1994)

146. Keith, M., A. Geranmayegan, M. J. Sole, R. Kurian, A. Robinson, A. S. Omran and K. N. Jeejeebhoy: Increased oxidative stress in patients with congestive heart failure, *J Am Coll Cardiol* 31, 1352-6. (1998)

147. Brener, Z. and R. T. Gazzinelli: Immunological control of *Trypanosoma cruzi* infection and pathogenesis of Chagas' disease, *Int Arch Allergy Immunol* 114, 103-10 (1997)

148. Hensley, K., K. A. Robinson, S. P. Gabbita, S. Salsman and R. A. Floyd: Reactive oxygen species, cell signaling, and cell injury, *Free Radic Biol Med* 28, 1456-62 (2000)

149. Rivera, M. T., A. P. de Souza, A. H. Moreno, S. S. Xavier, J. A. Gomes, M. O. Rocha, R. Correa-Oliveira, J. Neve, J. Vanderpas and T. C. Araujo-Jorge: Progressive Chagas' cardiomyopathy is associated with low selenium levels, *Am J Trop Med Hyg* 66, 706-12 (2002)

150. Gomez, R. M., M. E. Solana and O. A. Levander: Host selenium deficiency increases the severity of chronic inflammatory myopathy in *Trypanosoma cruzi*-inoculated mice, *J Parasitol* 88, 541-7 (2002)

151. de Souza, A. P., G. Melo de Oliveira, J. Neve, J. Vanderpas, C. Pirmez, S. L. de Castro, T. C. Araujo-Jorge and M. T. Rivera: *Trypanosoma cruzi*: host selenium deficiency leads to higher mortality but similar parasitemia in mice, *Exp Parasitol* 101, 193-9 (2002)

152. de Souza, A. P., G. M. de Oliveira, J. Vanderpas, S. L. de Castro, M. T. Rivera and T. C. Araujo-Jorge: Selenium supplementation at low doses contributes to the decrease in heart damage in experimental *Trypanosoma cruzi* infection, *Parasitol Res* 91, 51-4 (2003)

153. Perez-Fuentes, R., J. F. Guegan, C. Barnabe, A. Lopez-Colombo, H. Salgado-Rosas, E. Torres-Rasgado, B. Briones, M. Romero-Diaz, J. Ramos-Jimenez and C. Sanchez-Guillen Mdel: Severity of chronic Chagas disease is associated with cytokine/antioxidant imbalance in chronically infected individuals, *Int J Parasitol* 33, 293-9 (2003)

154. Gupta, M., K. Dobashi, E. L. Greene, J. K. Orak and I. Singh: Studies on hepatic injury and antioxidant enzyme activities in rat subcellular organelles following *in vivo* ischemia and reperfusion, *Mol Cell Biochem* 176, 337-47 (1997)

155. Sanchez-Campos, S., M. J. Tunon, P. Gonzalez and J. Gonzalez-Gallego: Oxidative stress and changes in liver antioxidant enzymes induced by experimental dicroceliosis in hamsters, *Parasitol Res* 85, 468-74 (1999)

156. Dieterich, S., U. Bieligk, K. Beulich, G. Hasenfuss and J. Prestle: Gene expression of antioxidative enzymes in the human heart: increased expression of catalase in the end-stage failing heart, *Circulation* 101, 33-9 (2000)

Send correspondence to: Nisha Garg Ph.D. Department of Microbiology & Immunology, 3.142 Medical Research Building, University of Texas Medical Branch, 301 University Boulevard, Galveston TX 775555. Tel: 409-747-6865, Fax: 409-747-6869, E-mail: nigarg@utmb.edu