Induction of Mitochondrial Biogenesis Is a Maladaptive Mechanism in Mitochondrial Cardiomyopathies

Mariangela Sebastiani, BSc,* Carla Giordano, MD, PhD,* Chiara Nediani, BSc,‡ Claudia Travaglini, BSc,* Elisabetta Borchí, BSc,‡ Massimo Zani, BSc,* Mariano Feccia, MD,§ Massimiliano Mancini, MD,* Vincenzo Petrozza, MD,† Andrea Cossarizza, MD,‖ Pietro Gallo, MD,* Robert W. Taylor, PhD,# Giulia d’Amati, MD, PhD*

Rome, Florence, and Modena, Italy; and Newcastle upon Tyne, United Kingdom

Objectives

The purpose of this study was to clarify the molecular mechanisms linking human mitochondrial deoxyribonucleic acid (mtDNA) dysfunction to cardiac remodeling.

Background

Defects of the mitochondrial genome cause a heterogeneous group of clinical disorders, including mitochondrial cardiomyopathies (MIC). The molecular events linking mtDNA defects to cardiac remodeling are unknown. Energy derangements and increase of mitochondrial-derived reactive oxygen species (ROS) could both play a role in the development of cardiac dysfunction in MIC. In addition, mitochondrial proliferation could interfere with sarcomere alignment and contraction.

Methods

We performed a detailed morphologic and molecular analysis on failing hearts from 3 patients with MIC, failing human hearts due to ischemic heart disease (IHD) or dilated cardiomyopathies (DCM), and nonfailing hearts.

Results

The MIC hearts showed marked mitochondrial proliferation with myofibril displacement. Consistent with morphologic features, increase in mtDNA content per cell and induction of genes involved in mitochondrial biogenesis, fatty acid metabolism, and glucose transport were observed. Down-regulation of these genes characterized DCM and IHD hearts. A pronounced increase in mitochondrial-derived ROS was observed in MIC hearts compared with failing hearts due to other causes. This was paralleled by up-regulation of genes encoding for uncoupling proteins and antioxidant enzymes. However, there was not a significant increase in antioxidant enzyme activity.

Conclusions

Our results suggest that besides energy deficiency, mitochondrial biogenesis per se is a maladaptive response in MIC and, possibly, in other metabolic cardiomyopathies. (J Am Coll Cardiol 2007;50:1362–9) © 2007 by the American College of Cardiology Foundation

Mutations in human mitochondrial deoxyribonucleic acid (mtDNA) cause a large variety of multi-system disorders whose main unifying feature is the altered energy homeostasis (i.e., reduced adenosine triphosphate [ATP] production). Highly energy-dependent tissues, such as the central nervous system and skeletal and cardiac muscle, are commonly involved (1). Cardiac dysfunction can occur as the sole or predominant symptom and often takes the form of hypertrophic cardiomyopathy (2).

Previous studies from our group identified 2 unrelated families with isolated maternally inherited cardiomyopathy (MIC) with a hypertrophic phenotype due to a homoplasmic point mutation (4300A>G) in the mitochondrial transfer ribonucleic acid isoleucine (mt-tRNA^{Ile}) gene (3). Heart tissue from affected family members showed a severe defect in respiratory chain enzyme activity and low steady-state levels of the mature mt-tRNA^{Ile}. Of note, comparably low levels of tRNA^{Ile} were present in skeletal muscle, which failed to show either a biochemical or a clinical defect. The illness had an early onset and an adverse clinical course in both families, with rapid progression of left ventricular hypertrophy to dilation and cardiac failure, necessitating heart transplant in 2 patients.
The molecular events linking mtDNA defects to cardiac hypertrophy are unknown. Studies of experimental models suggest that both energy derangements and increase of mitochondrial-derived reactive oxygen species (ROS) could play a role in the development of cardiac dysfunction in MICs (4,5). In addition, mitochondrial proliferation, a well-recognized compensatory mechanism in mitochondrial disease, could contribute to cardiomyopathic remodeling through mechanical dysfunction (6).

To better clarify the molecular mechanisms linking mtDNA mutations to cardiac hypertrophy and failure, we performed a detailed morphologic and molecular analysis on failing hearts from 3 patients with tRNA\textsubscript{Ile} mutation-related MIC, comparing gene expression profiles of key regulators of mitochondrial biogenesis and cardiac energy metabolism to failing human hearts due to other causes and to nonfailing hearts. In addition, we evaluated myocardial oxidative stress by assessing myocardial superoxide production, and gene expression and activity of the antioxidant enzymes mitochondrial manganese superoxide dismutase (MnSOD) and glutathione peroxidase (GPX).

**Methods**

**Patients.** All studies conformed to Sapienza, University of Rome, Ethical Committee protocols.

The clinical profile of the 2 families with MIC has been reported previously (3). After informed consent of relatives (in the case of autopsy samples) or patients, myocardial tissue was obtained within 2 h from death (Family #1, Patient #1) or heart transplant (Family #2, Patients #2 and #3) (3). In addition, samples from patients with end-stage heart failure secondary to idiopathic dilated cardiomyopathy (DCM) (n = 4) or ischemic heart disease (IHD) (n = 9), in whom metabolic disorders (including diabetes) were excluded on the basis of clinical records, were obtained at heart transplant. Nonfailing (NF) heart samples were obtained from donor hearts which were unsuitable for transplantation for technical reasons (n = 4) and within 2 h from death in pediatric patients who died for noncardiac causes in whom mitochondrial disease was excluded on the basis of histologic, histochemical, and respiratory chain enzyme studies (n = 4). Clinical characteristics of the 3 groups are reported in Table 1.

**Tissue sample preparation.** Immediately after explant, multiple samples of myocardial tissue were snap-frozen in liquid nitrogen-chilled isopentane for molecular and biochemical studies. For histologic analysis, tissue sections obtained from formalin-fixed paraffin-embedded samples were stained with hematoxylin and eosin, Masson trichrome, and periodic acid Schiff + diastase. Ultrastructural studies were performed on samples fixed in 4% paraformaldehyde–phosphate–buffered saline and post-fixed in osmium tetroxide. Thin sections were stained with uranyl acetate and lead citrate and examined with a CM10 Philips (Eindhoven, the Netherlands) electron microscope.

**Quantitative real-time polymerase chain reaction.** EVALUATION OF MTDNA COPY NUMBER. Total DNA from left ventricular myocardial tissue was extracted by phenol–chloroform standard procedures. The mtDNA content was measured by quantitative real-time polymerase chain reaction (PCR) assays using a previously described method (7). Briefly, an mtDNA fragment (nt 4625 to 4714) and a nuclear DNA fragment (FisL gene) were amplified by multiplex PCR using TaqMan probe system and Platinum Quantitative PCR SuperMix-UDG (Invitrogen, Life Technologies, Paisley, United Kingdom). The PCR conditions, primers, and probes are as previously detailed (7). A standard curve for mtDNA and nuclear DNA was generated using serial known dilutions of a vector (provided by Genemore, Modena, Italy) in which the regions used as template for the 2 ampli-
fifications were cloned tail to tail to have a ratio of 1:1 of the reference molecules.

**GENE EXPRESSION.** The relative expression of the following genes was evaluated: atrial natriuretic factor (ANF) and myosin heavy chain α and β isoforms, as molecular markers of cardiac hypertrophy; peroxisome proliferator activated receptor (PPAR)-α, a critical regulator of cardiac oxidative metabolism, along with 3 of its targets, carnitine palmitoyl- transferase muscle isoform (mCPT-1) and medium- and long-chain acyl-CoA dehydrogenase; PPAR-γ coactivator 1α (PGC-1α), nuclear respiratory factor 1 and 2 (NRF1 and NRF2), and transcription and mtDNA maintenance factor (Tfam), important regulators of mitochondrial biogenesis; along with PGC-1β, a known homologue of PGC-1α; the glucose transporter isoforms (GLUT1 and GLUT4); heart isoforms of uncoupling protein (UCP2 and UCP3); and antioxidant enzymes MnSOD and GPX.

Total ribonucleic acid (RNA) was isolated from left ventricular myocardial tissue by using Trizol reagent and measured with NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware). One microgram of total RNA was reverse-transcribed to cDNA using random hexamer primers (AMV Reverse Transcriptase, Promega, Madison, Wisconsin). Relative expression of each gene was determined by quantitative real-time PCR using the Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen).

For each target gene, primers were carefully designed with Beacon Designer Software (Bio-Rad, Hercules, California) and checked by using Blast software (National Center for Biotechnology Information, Bethesda, Maryland) to avoid cross-homology. The specificity of the amplification was confirmed by direct sequencing using an ABI Prism 310 Genetic analyzer (Applied Biosystems, Foster City, California) following standard procedures. Nucleotide sequences for the primers are given in Table 2.

Quantitative real-time PCR was performed in triplicate using 1 μl cDNA template in a 50-μl reaction. Linearity and efficiency of PCR amplifications were assessed using standard curves generated by serial dilution of cDNA; in addition, melt curve analysis was used to confirm the specificity of amplification and absence of primer dimers. In all samples, the relative expression of each target gene with respect to 1 NF control (reference sample) was evaluated with the comparative threshold cycle (ΔCt) method, as described in the User Bulletin for the ReLIQuant UpDate (Bio-Rad). All values were normalized to the 18S rRNA housekeeping gene. Quantification was carried out with iCycler IQ_Optical System Software (Bio-Rad).

**Measurement of superoxide production by lucigenin chemiluminescence.** Superoxide (O₂⁻) production was measured by lucigenin-enhanced chemiluminescence (8). Briefly, myocardial tissue was homogenized in ice-cold 20 mmol/l Tris HCl, pH 7.4, containing 10 μg/ml leupeptin, 10 μg/ml aprotinin, 0.2 mmol/l phenylmethanesulphonylfluoride, and phosphatase inhibitor cocktail (Sigma-Aldrich, Milan, Italy). After spinning for 10 min at 3,000 rpm at 4°C, protein concentration was measured by bichoninic acid protein assay (Pierce, Rockford, Illinois). Experiments were performed on a luminometer (Lumat LB 9507, EG&G Berthold, Bundoora, Australia) using 100 μg protein/tube and a nonredox-cycling dose of lucigenin (5 μmol/l), which previous reports have indicated as a sensitive and valid probe for superoxide detection (8). Superoxide generation was measured both in the absence of substrate and in presence of 300 μmol/l nicotineamide adenine dinucleotide 3-phosphate (NADPH) or 5 mmol/l succinic acid.

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**Table 2** Primer Sequences

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequences</th>
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<tbody>
<tr>
<td>rRNA18S</td>
<td>F: 5'-CCAGTATGGGTGGGCTTAAACG-3'</td>
</tr>
<tr>
<td>ANF</td>
<td>R: 5'-AACATCCAACTTGGAGTACG-3'</td>
</tr>
<tr>
<td>MHCα</td>
<td>F: 5'-GAAGGTATACACGGGAAGGAC-3'</td>
</tr>
<tr>
<td>MHCβ</td>
<td>R: 5'-GCGGCAACGGACACATCGAC-3'</td>
</tr>
<tr>
<td>GPX</td>
<td>F: 5'-GATGCCTTCTGATGGACTGC-3'</td>
</tr>
<tr>
<td>MnSOD</td>
<td>R: 5'-GTTTCCGCTTCTGAC-3'</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>F: 5'-ACACCTGACGACACACACAG-3'</td>
</tr>
<tr>
<td>PGC-1β</td>
<td>R: 5'-GGGGTGGTGGGGGAGTTAGG-3'</td>
</tr>
<tr>
<td>NRF1</td>
<td>F: 5'-GTACCTGATGCGACATGCCTC-3'</td>
</tr>
<tr>
<td>NRF2</td>
<td>R: 5'-GCTTACCTACGATGCCTGAC-3'</td>
</tr>
<tr>
<td>Tfam</td>
<td>F: 5'-AACAGGAAATATGGTTGAGAC-3'</td>
</tr>
<tr>
<td>PPAR-α</td>
<td>R: 5'-CAAGATTATGTCGACGACAATCGC-3'</td>
</tr>
<tr>
<td>GLUT1</td>
<td>F: 5'-TGCTGCTGATGCTGATCACTTC-3'</td>
</tr>
<tr>
<td>GLUT4</td>
<td>R: 5'-GGCGGACCCCTTCTGCTAC-3'</td>
</tr>
<tr>
<td>LCAD</td>
<td>F: 5'-AAGTGATGCTGATGATGATGAG-3'</td>
</tr>
<tr>
<td>MCAD</td>
<td>R: 5'-GAATTAGCTGGATGGATACG-3'</td>
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<tr>
<td>mCPT-1</td>
<td>F: 5'-GCAGCGATGTCGCTCGA-3'</td>
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<tr>
<td>UCP2</td>
<td>R: 5'-GGTTCTGCTGATGATGAC-3'</td>
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<tr>
<td>UCP3</td>
<td>F: 5'-TTTGCCTACGGCTTTACCTTC-3'</td>
</tr>
<tr>
<td></td>
<td>R: 5'-GTCCTGACGGCGGCTTCC-3'</td>
</tr>
</tbody>
</table>

GLUT = glucose transporter; GPX = glutathione peroxidase; LCAD = long-chain acyl-CoA dehydrogenase; MCAD = medium-chain acyl-CoA dehydrogenase; mCPT-1 = carnitine palmityl transferase muscle isoform; MnSOD = manganese superoxide dismutase; NRF = nuclear respiratory factor; PGC = peroxisome proliferator activated receptor γ coactivator; PPAR = peroxisome proliferator activated receptor; Tfam = transcription and mitochondrial deoxyribonucleic acid maintenance factor; UCP = uncoupling protein; other abbreviations as in Table 1.
To confirm that the measured signal was attributable to \( \text{O}_2^- \), a cell-permeable superoxide scavenger, 4,5-dihydroxy-1,3-benzene-disulfonic acid (Tiron, Sigma-Aldrich, 20 mmol/l), was used. In a subset of experiments, samples were preincubated with the following agents to assess potential sources of \( \text{O}_2^- \) production: diphenyleioidonium 20 \( \mu \)mol/l, a flavoprotein inhibitor the blocks NADPH oxidase; Nα-nitro-L-arginine methyl ester hydrochloride (100 \( \mu \)mol/l), a nitric oxide synthase inhibitor; rotenone (50 \( \mu \)mol/l), a specific inhibitor of mitochondrial respiratory chain complex I; and oxypurinol (100 \( \mu \)mol/l), an inhibitor of xanthine oxidase. A buffer blank was subtracted from each reading. Light emission was recorded and expressed as mean light units/s per mg of homogenate protein over 10 min.

**Superoxide dismutase and glutathione peroxidase activity assays.** For measurement of SOD and GPX activity, myocardiad tissue was homogenized in 20 mmol/l HEPES, pH 7.2, containing 1 mmol/l EDTA, 210 mmol/l mannitol, and 70 mmol/l sucrose, sonicated, and centrifuged at 14,000 rpm for 20 min at 4°C. Total SOD activity was measured on the supernatant by the Chemical Superoxide Dismutase Assay kit (Cayman Chemical, Ann Arbor, Michigan). This uses a tetrazolium salt for the detection of \( \text{O}_2^- \) radicals generated by xanthine oxidase and hypoxanthine. The SOD activity was expressed as units per mg of protein. One unit of SOD is defined as the amount of enzyme needed to obtain 50% dismutation of \( \text{O}_2^- \) radical. Specific MnSOD activity was measured by the addition of 5 mmol/l potassium cyanide to the tissue sample to inhibit the cytosolic copper-zinc (Cu/Zn) SOD. The GPX activity was expressed as nanomoles of reduced NADP converted to oxidized NADP per minute per milligram of protein, as previously described (9).

**Statistical analyses.** All data are expressed as mean \( \pm \) SEM. Gene expression data are expressed using a natural logarithmic scale to reduce the different variability among groups and to help with their graphical representation. Analysis of variance (ANOVA) test accounting for different variability among groups followed by multiple pairwise comparison adjusted with the Bonferroni corrections, was performed. Standard ANOVA procedures were performed on enzyme activities data. Results were considered to be significant at \( p < 0.05 \). All of the reported \( p \) values are adjusted by Bonferroni correction based on 6 multiple comparisons. Statistical computing has been performed by the Statistical Software R (package nlme, Wirtschaftsuniversität, Wien, Austria).

**Results**

**Morphologic analyses.** Histologic analysis of paraffin sections from MIC hearts revealed cardiac myocyte hypertrophy associated with prominent perinuclear vacuolization (Fig. 1A). The vacuoles contained inhomogeneous granular material that stained with periodic acid Schiff and was digested by diastase, suggesting the presence of glycogen (Fig. 1B). Ultrastructural analysis confirmed this finding (Fig. 1C), revealing mitochondrial proliferation causing displacement of myofibrils (Fig. 1D).

**Gene expression profile. ANF and Myosin Heavy Chain isoforms.** The messenger RNA level of ANF was increased \( \sim \)10-fold in the 3 groups of failing hearts (FH) compared with control samples. In contrast, all FH showed a dramatic decrease of transcript levels of MHC-\( \alpha \) (up to 40-fold decrease) and MHC-\( \beta \) (up to 25-fold decrease) (Table 1). These data are consistent with the previous observation of reduced myofilament content in FH (10).

**mtDNA content and genes involved in mitochondrial biogenesis.** As shown in Figure 2A, the mtDNA content per cell increased in MIC compared with both controls and FH of other causes. The transcript levels of several key regulators of mitochondrial biogenesis (PGC1-\( \alpha \), NRF1, NRF2, and Tfam) were coordinately up-regulated (\( \sim \)15-fold increase) in MIC and down-regulated in DCM and IHD hearts (Fig. 2B). Interestingly, a similar increase in mtDNA amount, along with induction of genes involved in mitochondrial biogenesis, was observed in skeletal muscle from 2 MIC patients (Family #1, Patients #2 and #3; data not shown), despite the lack of morphologic features of mitochondrial proliferation (as previously reported [3]). As discussed previ-
ously, this is intriguing, because the biochemical defect associated with the 4300A>G mutation is not expressed in this tissue, despite a similar reduction in the steady-state levels of tRNA<sub>Ile</sub> transcript (3).

The mRNA level of PGC-1α showed a modest, though statistically significant, increase both in MIC (2-fold increase) and in IHD (1.5-fold increase) whereas it was down-regulated in DCM compared with control samples (Fig. 2C).

REGULATORS OF FATTY ACID METABOLISM AND GLUCOSE TRANSPORT. Both PPAR-α and its targets were up-regulated in MIC compared with control samples (∼10-fold increase), whereas they were down-regulated in failing DCM and IHD hearts (Fig. 2D). An increased expression of GLUT1 (6-fold increase) and GLUT4 (16-fold increase) was observed in MIC compared with control samples. In contrast, GLUT1 was markedly reduced in DCM (10-fold decrease) and showed a slight reduction in IHD (0.5-fold decrease), whereas GLUT4 showed only a slight decrease in DCM and IHD (Fig. 3A).

UNCOUPLING PROTEINS. The expression of both cardiac isoforms UCP2 and UCP3 was up-regulated in MIC with respect to controls (20- and 7-fold increases, respectively). Both isoforms were down-regulated in DCM and IHD failing hearts, with UCP2 showing the lower amount (Fig. 3B).

Myocardial O<sub>2</sub>− production. Superoxide production was minimal in myocardial homogenate from failing and non-failing hearts both in absence of substrates and in presence of succinate (not shown). After the addition of NADPH, O<sub>2</sub>− production raised significantly in all FH compared with control samples. However, this phenomenon was more striking in MIC, with a 2-fold increase in O<sub>2</sub>− production compared with DCM and IHD (Fig. 4A). Detection of superoxide was virtually abolished by Tiron.

To investigate the sources of O<sub>2</sub>− production, experiments were repeated in the presence of specific inhibitors of potential ROS-generating enzymes. In all FH, O<sub>2</sub>− generation was significantly decreased by diphenyleneiodonium, confirming the phagocyte-type NADPH oxidase as the
major source of $\text{O}_2^-$ in this setting (8). In addition, MIC hearts showed a significant decrease in $\text{O}_2^-$ production in presence of rotenone, a specific inhibitor of mitochondrial respiratory chain complex I and xanthine oxidase (Fig. 4B), whereas the presence of Nω-nitro-L-arginine methyl ester hydrochloride had no effect (not shown).

**Superoxide dismutase and glutathione peroxidase gene expression and activity.** Induction of both the MnSOD (~15-fold increase) and GPX (~5-fold increase) genes’ expression was observed in MIC compared with controls. However, the activity of both enzymes did not show any significant change in MIC hearts, despite a clear trend toward an increase for GPX (Figs. 5B and 5C). The lack of statistically significant change in GPX activity may be due to variability within the control groups.

In contrast to MIC, expression of both these genes was slightly decreased in DCM and IHD hearts (Fig. 5A), although only IHD hearts showed a significant reduction of GPX activity (Figs. 5B and 5C).

**Discussion**

Emerging evidence points to energy derangement as a major culprit for development of cardiac hypertrophy and progression to heart failure, both in acquired and in inherited disease. In recent years, numerous studies using experimental models of pressure overload and in human FH have consistently demonstrated a myocardial shift from fatty acid oxidation toward glucose oxidation for energy production (see Huss and Kelly [11] for a review). This metabolic switch is associated with the down-regulation of genes involved in mitochondrial biogenesis and fatty acid metabolism and is mediated by deactivation of the transcriptional regulator PPAR-α and its coactivator PGC-1α (10,12).

The increased reliance of hypertrophic myocytes on glycolytic pathways is likely finalized to reduce oxygen consumption; however, it may contribute to the progression of cardiac disease, possibly creating a relative energy-deficient state.
In the present study, we reveal that there is a specific metabolic gene expression profile in a cardiomyopathy caused by a significant derangement of the mitochondrial energy production pathway. In fact, we observed a marked up-regulation of PPAR-α and its coactivator PGC-1α, along with a slight increase in the expression of PGC-1β. Accordingly, we observed the induction of genes involved in fatty acid metabolism, glucose transport, and mitochondrial biogenesis. This last change is consistent with a dramatic increase in mtDNA content per cell, as well as with histologic and ultrastructural features of marked mitochondrial proliferation. In contrast, down-regulation of the PPAR-α/PGC-1α complex and their targets, as well as reduced expression of glucose transporters, was observed in DCM and IHD FH, confirming previous observations (10,12). Interestingly, the expression level of PGC-1β showed opposite behavior in DCM and IHD. These data suggest that the 2 homologues are regulated by different mechanisms in cardiac myocytes, as reported for brown adipose tissue (13).

The coordinated induction in bioenergetic gene expression observed in MIC is likely to represent a compensatory response to energy deficiency. We previously demonstrated a global defect of oxidative phosphorylation (OXPHOS) in MIC hearts, with very low activities of respiratory chain complexes I and IV (both containing mtDNA-encoded subunits) (3). Increase of mitochondrial biogenesis (i.e., an increase in mitochondrial mass and induction of OXPHOS gene as well as enzymes of intermediary metabolism) is a well-known phenomenon in mitochondrial disorders (14). In skeletal muscle, mitochondrial proliferation has been shown to partly compensate for the respiratory dysfunction by maintaining overall ATP production (15); the histopathologic hallmark of this phenomenon is the presence of an increased number of mitochondria, mostly in the subsarcolemmal region. However, in cardiac muscle, induction of mitochondrial biogenesis has been proposed as a maladaptive response. In fact, cardiac-specific induction of PGC-1α in mice results in cardiac dysfunction with morphologic features of myocyte mitochondrial proliferation and myofibrillar disorganization and loss. Intriguingly, both mitochondrial proliferation and cardiomyopathy are reversible upon cessation of transgene expression (6). Our findings parallel the features reported in that experimental model, suggesting that mitochondrial proliferation is linked to progressive cardiac dysfunction also in the human heart. The ultrastructural features of altered sarcomere alignment suggest that mitochondrial proliferation per se may possibly interfere with contractile function; this phenomenon is limited to cardiomyocytes: In fact, in patients’ skeletal muscle, lack of morphologic evidence of mitochondrial proliferation correlates with normal muscle function (3). This observation opens new perspectives on the complex issue of tissue specificity of homoplasmic mitochondrial mutations (16).

Another interesting result of the present study is the observation of a more pronounced increase in myocardial ROS production in MIC compared with DCM and IHD. In experimental conditions, NADPH oxidase was a major source of ROS in all failing hearts, as previously reported (8,17). However, the present results implicate both the mitochondrial respiratory chain and xanthine oxidase as a source of excessive superoxide. Thus, mitochondrial dysfunction may contribute to the increased oxidative stress both by mitochondrial-derived O$_2^-$ and by a reduction of ATP/adenosine monophosphate ratio, which in turn activates the xanthine oxidase pathways. A somewhat paradoxical observation in the present study relates to the observation of high levels of ROS in the presence of increased expression of UCP2 and UCP3 in MIC. In fact, UCPs are expected to dissipate the proton electrochemical gradient formed during mitochondrial respiration and generate heat instead of ATP, thus reducing mitochondrial superoxide production (18). On the other hand, this mechanism may also lead to an increase in mitochondrial oxygen consumption, thus amplifying the energy dysfunction observed in MIC. We may speculate that the increase in UCP expression in MIC is insufficient to decrease ROS production through the mechanism of uncoupling respiration, and, therefore, high levels of ROS are maintained. Alternatively, recent studies on adipose tissue and skeletal muscle suggest that UCPs are regulated at post-translational level (19).

Increased ROS production in MIC was not reflected by a parallel induction of antioxidant enzyme activity, despite a marked up-regulation of MnSOD and GPX genes. Similar results have been previously reported in human FH (20) and in a mouse model of MIC (4) and support the hypothesis that end-stage heart muscle is unable to respond to oxidative stress by adequately increasing antioxidant countermeasures.

We believe that the present results on increased ROS production in MIC are of interest, because mounting evidence has implicated ROS signaling in cardiac maladaptive remodeling (21). However, additional work is required to fully address this issue.

A metabolic gene expression profile similar to MIC has been reported in the cardiomyopathy that develops in the context of diabetes (22,23). In that condition, increase of circulating free fatty acids may lead to activation of PPAR-α/PGC-1α complex, which in turn causes induction of mitochondrial biogenesis and fatty acid oxidation. Furthermore, induction of UCPs along with cardiac OXPHOS dysfunction and increased oxidative stress has previously been observed (24).

**Conclusions**

The present results suggest that mitochondrial biogenesis is a maladaptive response in MIC and, possibly, in other metabolic cardiomyopathies. Besides energy deficiency, mechanical interference with sarcomere alignment and con-
traction, increased oxidative stress, and uncoupled respiration are possible detrimental factors to myocyte function.

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Reprint requests and correspondence: Dr. Giulia d’Amati, Dipartimento di Medicina Sperimentale, La Sapienza, Università di Roma, Viale Regina Elena 324, 00161 Rome, Italy. Email: giulia.damati@uniroma1.it.

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