Serum From Pediatric Dilated Cardiomyopathy Patients Causes Dysregulation of Cardiolipin Biosynthesis and Mitochondrial Function

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Dilated Cardiomyopathy (DCM)

- Dilated cardiomyopathy (DCM) is defined as a disorder characterized by dilation and impaired contraction of the left ventricle or both ventricles.

- DCM is the most common form of cardiomyopathy and cause of heart failure in children older than 1 year of age with an annual incidence of 0.57 per 100,000 children.

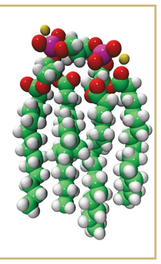
- The causes of heart failure (HF) in children differ substantially from those found in the adult population and children do not respond well to adult HF therapies.

Cardiolipin (CL)

- Cardiolipin is a mitochondrial dimeric phospholipid normally located in the inner mitochondrial membrane

- CL represent 12-15% of phospholipid mass in heart. In the heart, 70-80% is $(18:2)_4$CL.

- Total CL and $(18:2)_4$CL content are depleted in myocardium from pediatric DCM patients.



Methods

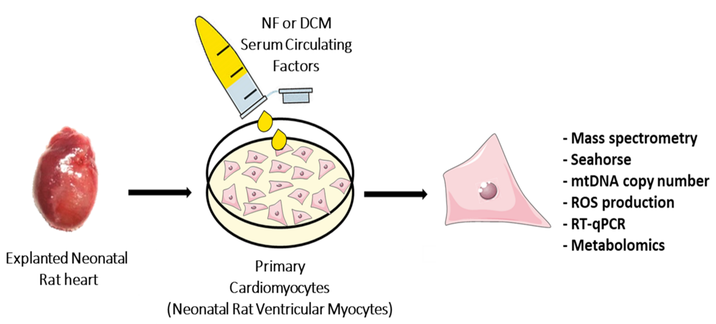


Figure 1. *in vitro* Model of Cardiomyocyte Remodeling. Ventricular myocytes (NRVMs) were isolated from the hearts of 2-day old pups, and treated with human NF or DCM circulating factors (e.g., whole sera or serum-derived exosomes) for 72 hours.

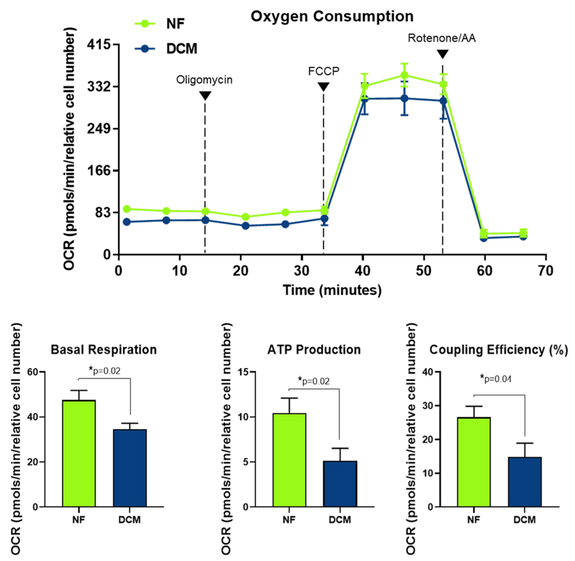


Figure 2. Treatment of NRVMs with serum from DCM patients leads to mitochondrial dysfunction. Cells were exposed sequentially to oligomycin, FCCP and rotenone/antimycin (AA). Oxygen consumption rate (OCR) was measured using a Seahorse XF Analyzer. Respiratory parameters such as basal respiration, ATP production and coupling efficiency were calculated from OCR data. Unpaired t-test: *P<0.05 significant difference between NF and DCM groups.

Hypothesis

Using a novel *in vitro* model of DCM-related cardiomyocyte remodeling that reproduces the molecular characteristics of pediatric DCM, we hypothesized that the alteration of mitochondrial function in NRVM treated with DCM pediatric sera is associate with changes in cardiolipin content and mitochondrial β-oxidation pathway.

Results

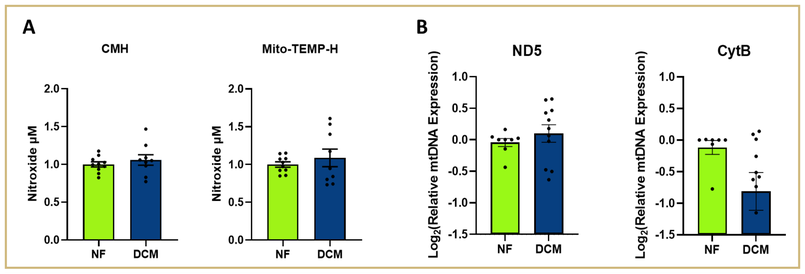


Figure 3. Mitochondrial dysfunction is not associated with ROS production or changes in mitochondrial content. A. Total and mitochondrial ROS production were measured by Electron Paramagnetic Resonance Spectroscopy using CMH or mito-TEMPO-H probe. Nitroxide concentrations were normalized to total protein. Bar equals mean ± SEM; each dot represents an individual patient serum-treated NRVM: n=10 NF, n=9 DCM samples. B. Log2 (relative mtDNA expression) of mitochondrial DNA-encoded ND5 and CytB relative to nuclear encoded Transient receptor potential melastatin 2. Bar equals Log2 (mean ± SEM); each dot represents an individual patient serum-treated NRVM: n= 8 NF, n= 11 DCM samples.

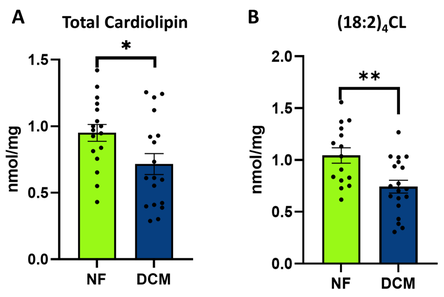


Figure 4. Total cardiolipin (CL) and $(18:2)_4$CL content are decreased in NRVMs with serum from DCM patients. A-B: quantification (nmol/mg) of cardiolipin (A) and $(18:2)_4$CL (B). Bar equals mean ± SEM; each dot represents an individual patient serum-treated NRVM: n= 19 NF, n= 19 DCM samples. Unpaired t-test: *P<0.05 and **P<0.01 significant difference between NF and DCM groups.

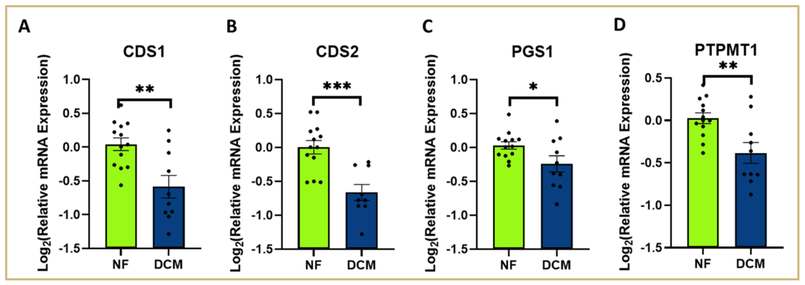


Figure 5. NRVMs treated with serum from DCM patients exhibit a decreased expression of genes related to CL biosynthesis. RT-qPCR of prototypical genes related to CL biosynthesis, including cytidine diphosphate diacylglycerol synthase 1 (CDS1; A), cytidine diphosphate diacylglycerol synthase 2 (CDS2; B), phosphatidylglycerophosphate synthase (PGS1; C), phosphatidylglycerol phosphate phosphatase (PTPMT1; D). For all groups, bar equals Log2 (mean ± SEM); each dot represents an individual patient serum-treated NRVM: n= 13 NF, n= 10 DCM samples. Unpaired t-test: *P<0.05, **P<0.01, and ***P<0.001 significant difference between NF and DCM groups.

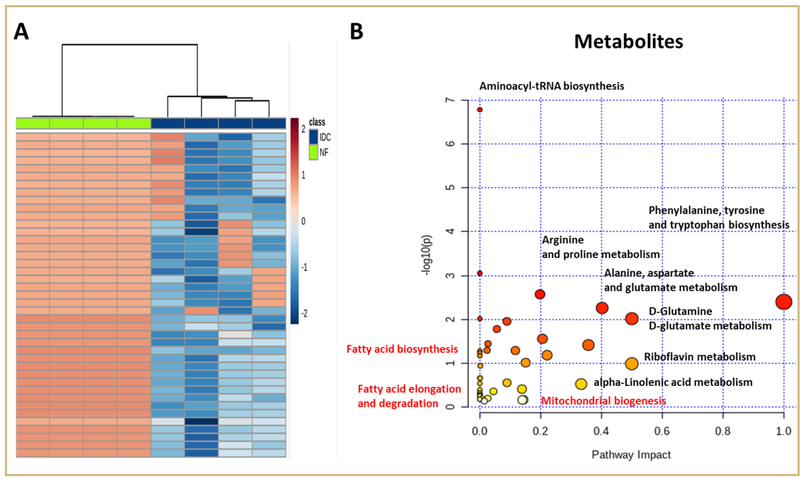


Figure 5. DCM serum induces significant changes in metabolite levels involved in fatty acid oxidation pathway in NRVMs. A. Heatmap of 41 metabolites differentially expressed in NF and DCM serum-treated NRVMs. n = 4 NF, n= 4 DCM samples, p<0.05. B. Pathway enrichment map analysis of differential metabolites between NF and DCM groups using MetaboAnalyst 5.0. The color of the circles from white to yellow to red denotes incremental fold change (−log(p)). The size of the circles from small to large indicates an increment of the impact of pathway.

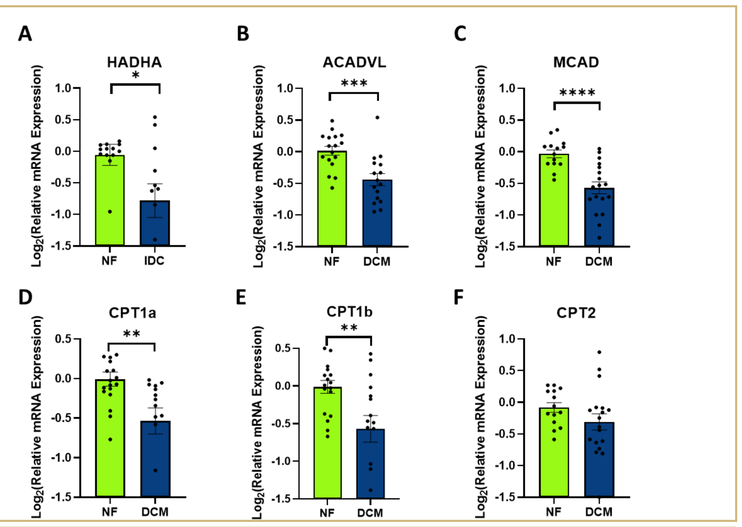


Figure 6. NRVMs treated with serum from DCM patients exhibit a decreased expression of genes related to enzymes involved in β-oxidation. RT-qPCR of prototypical genes related to β-oxidation, including monolysocardiolipin acyltransferase mitochondrial trifunctional protein subunit A (HADHA; A), Acyl-CoA Dehydrogenase Very Long Chain (ACADVL; B), Medium-chain acyl-CoA dehydrogenase (MCAD; C), Carnitine palmitoyltransferase 1a, 1b or 2 (CPT1a, CPT1b or CPT2; D-F). For all groups, bar equals Log2 (mean ± SEM); each dot represents an individual patient serum-treated NRVM: n= 13 NF, n= 10 DCM samples. Unpaired t-test: *P<0.05, **P<0.01, and ***P<0.001 significant difference between NF and DCM groups.

Conclusions

1. DCM serum treatment of NRVMs decreases:
   - Mitochondrial function
   - Mitochondrial CL content and composition
   - Gene expression of enzymes implicate in CL biosynthesis
2. Mitochondrial dysfunction is not associated with ROS production or changes in mitochondrial content.
3. DCM serum induces a significant change in the levels of metabolites involved in fatty acid metabolism and a decrease in the expression of genes related to β-oxidation.

Acknowledgements

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