



Role of Amyloid Beta in Mitochondrial Function and Dynamics in Skeletal Muscle

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ABSTRACT

Spontaneous inclusion body myositis (sIBM) is an inflammatory myopathy characterized by progressive muscle weakness, resulting in loss of function and mobility. Skeletal muscle from patients with sIBM display mitochondrial abnormalities including DNA deletions, cytochrome c oxidase deficiency, morphological deformities, and inclusion bodies. It has been suggested that amyloid β (Abeta) accumulation may be critical to pathophysiology of the disease. To understand the role of Abeta in mitochondrial function and dynamics in skeletal muscle, L6 rat and human primary skeletal muscle cells were treated with 200pg or 100nM of Abeta for 4 hours. Immediately following treatment, oxygen consumption and protein content of mitochondrial proteins were assessed. Abeta resulted in a significant decline in maximally stimulated oxygen consumption, which was observed in conjunction with a decline and increase in OPA1 and DRP1 protein, respectively, and increases in interleukin 6 and monocyte chemotactic protein 1 mRNA. These results demonstrate that Abeta causes mitochondrial dysfunction, inflammation and alters regulators of mitochondrial dynamics in skeletal muscle.

REFERENCES

- Askanas et. al. Acta Neuropathol. 116(6); 2008, 583-595.
 Askanas et. al. Proc Natl Acad Sci USA. 93(3); 1996, 1314-1319.
 Berman et. al. Cell Death Differ. 15(7); 2008, 1147-1152.

FUNDING

Virginia Tech Fralin Life Sciences Institute (MIF)

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INTRODUCTION

Spontaneous Inclusion Body Myositis (sIBM) is an inflammatory myopathy characterized by progressive muscle weakness, ultimately resulting in loss of muscle function and mobility.

sIBM is the most common muscle disease occurring in older individuals (>50y) with no known cause, cure, or enduring treatment.

Skeletal muscle from patients diagnosed with sIBM is characterized by mitochondrial abnormalities, an abnormal accumulation of amyloid beta protein, (Abeta), and inflammation.

However, the role of mitochondrial dysfunction in the pathology of sIBM is not understood. Furthermore, whether Abeta contributes to mitochondrial dysfunction has not been determined

PURPOSE

To determine whether mitochondrial dynamic proteins are altered in models of sIBM.

To understand the role of Amyloid beta on mitochondrial dynamics (fission/fusion machinery) and function in skeletal muscle.

RESULTS

- 1 Skeletal muscle from patients diagnosed with sIBM is characterized by a down regulation of mitochondrial gene expression.

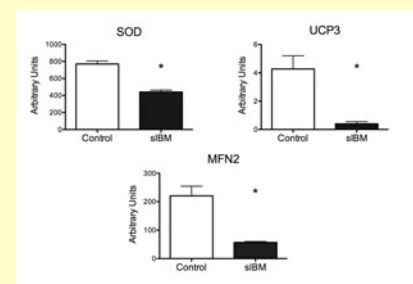


Figure 1. Skeletal muscle was obtained from the National Disease Research Interchange (NDRI) from patients diagnosed with sIBM (n=2) and healthy controls (n=2). Total RNA was extracted and mRNA levels were assessed. Skeletal muscle from patients diagnosed with sIBM have reduced expression of superoxide dismutase (SOD2), uncoupling protein 3 (UCP3), and Mitofusin 2 (MFN2) compared to controls. Values are expressed as mean \pm SEM. Different letters indicated significant differences.

RESULTS

- 2 Skeletal muscle from patients diagnosed with sIBM is characterized by a down regulation of mitochondrial proteins.

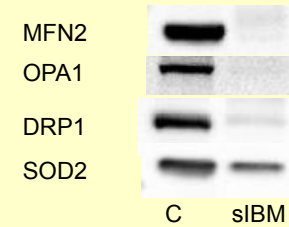


Figure 2. Skeletal muscle was obtained from NDRI from patients diagnosed with sIBM (n=2) and healthy controls (n=2). Skeletal muscle from patients diagnosed with sIBM have reduced protein content of MFN2, optic atrophy protein 1 (OPA1), dynamin related protein 1 (DRP1), and SOD2.

- 3 Exposure of L6 rat myotubes to Abeta results in abnormal substrate metabolism.

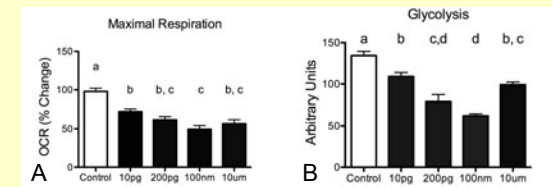


Figure 3. Rat L6 skeletal muscle cells were grown to 80% confluence and differentiated into fully differentiated myotubes. On day 7 of differentiation, cells were treated with Abeta for 4 hours. Immediately following treatment cellular respiration was measured using a Seahorse Bioscience extracellular flux analyzer (Seahorse Bioscience, Billerica, MA). Oxygen consumption and extracellular acidification rate (ECAR, a measure of glycolysis) was measured under basal conditions and following the administration of oligomycin (ATP synthase inhibitor), the uncoupler carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP) to assess maximal respiration, and rotenone (Complex I inhibitor). Abeta exposure resulted in a dose response effect on maximal respiration (A) and glycolysis. Values are expressed as mean \pm SEM. Different letters indicate significance, p<0.05.

- 4 Exposure of L6 rat myotubes to Abeta results in alterations in mitochondrial gene expression and increased inflammation.

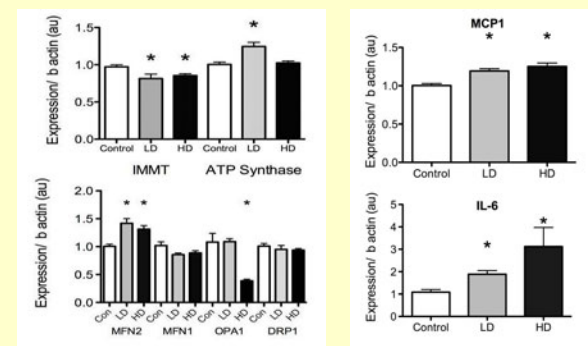


Figure 4. Rat L6 myotubes were grown and differentiated as described above. Cells were then exposed to either 200pg (LD) or 100nM (HD) of abeta for 4 hours. Immediately following treatment, cells were collected, total RNA was isolated and mRNA was assessed for inner mitochondrial membrane protein (IMMT), ATP synthase, MFN1 and 2, OPA1, DRP1, monocyte chemotactic protein 1 (MCP1), and interleukin 6 (IL-6). Data is expressed as mean \pm SEM and adjusted for β -actin. *p<0.05.

RESULTS

- 5 Exposure of L6 rat myotubes to Abeta results in alterations in mitochondrial gene expression and increased inflammation.

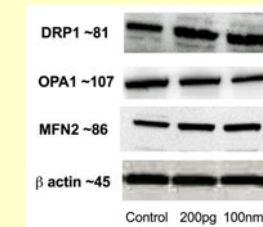
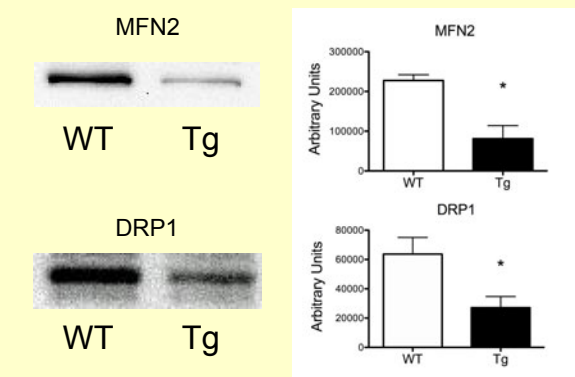


Figure 4. Rat L6 myotubes were grown and differentiated as described above. Cells were then exposed to either 200pg (LD) or 100nM (HD) of abeta for 4 hours. Immediately following treatment cells were collected and protein content was assessed for DRP1, OPA1, and MFN2.

- 6 Animals with over expression of amyloid beta precursor protein display a down regulation of mitochondrial dynamic protein content.



CONCLUSIONS

Skeletal muscle from patients diagnosed with sIBM display down regulation of mitochondrial proteins.

Exposure of skeletal muscle to Abeta results in a decline in mitochondrial function which coincides with alterations in mitochondrial dynamic proteins and increased inflammation.

Over expression of amyloid beta precursor protein leads to a reduction of mitochondria dynamic proteins in skeletal muscle.

Collectively, these data suggest a role of Abeta and mitochondrial dynamics in mitochondrial dysfunction associated with sIBM.